MICROPLASTICS: ADDRESSING ECOLOGICAL RISK THROUGH LESSONS LEARNED

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MICROPLASTICS: ADDRESSING ECOLOGICAL RISK THROUGH LESSONS LEARNED

Running title: Microplastics Ecological Risk

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Abstract: Plastic litter is an environmental problem of great concern. Despite the magnitude of the plastic pollution in our water bodies there is still limited scientific understanding about the risk for the environment, particularly for microplastics. The apparent magnitude of the problem calls for quickly developing sound scientific guidance on the ecological risks of microplastics. We suggest future research into MP 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 MP risk assessment. Finally, we advocate an expansion of the “vector effect” hypothesis in regards to microplastics risk to help focus research of MP environmental risk at different levels of biological and environmental organization. This article is protected by copyright. All rights reserved

Keywords: Microplastics, Nanoparticles, Mixture toxicity, Vector effects, Ecological risk
INTRODUCTION

There is a growing concern over the ecological risk of microplastics (MPs) from regulators, the scientific community and public [1, 2]. The use of plastics has gradually increased since the middle of the last century and yearly production volumes have now surpassed 200 million tons [3]. This intensive use has led to a widespread distribution of plastics in the aquatic environment [4], where a significant part is present as MPs, referring to 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 half a million particles per square kilometer, \(10^2-10^4\) times greater than outside these zones [7]. However, MPs 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 MPs, 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 MP 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 following consumption [8].

Microplastics can be grouped into primary and secondary materials (Figure 1), each with several subcategories. Primary MPs are plastic 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 MPs are micro-sized fragments derived from the breakdown of larger plastic debris by processes such as bio- and photodegradation and physical wave-action [11]. Although it is not known yet which form dominates in the environment, the
balance is likely location-dependent, primary MPs might be more important in close proximity to waste water effluents sites, whereas secondary MPs may dominate in the open sea [4].

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

Despite the paucity of information on ecological risks posed by MPs in personal care products, some major corporations have pledged to phase out primary MPs and, in addition, regulations are being enacted that mandate phase outs in coming years [12]. These eliminations will eventually reduce the number of MPs entering the environment. However, the high amounts of recalcitrant MPs currently in the environment, the fact that other MPs will continue to be produced, and that large plastic debris in the environment breaks down to secondary MPs, generates continued ecological exposures in the future. This reemphasizes the need to increase our understanding of the fate and ecological effects of MPs in the aquatic environment.
Science is lacking to support evidence-based decision making with respect to ecological exposure, effects, and the risks posed by MPs. There is a critical need to better understand the range of likely exposures and their temporal and spatial variability and the likely ecological receptors in order 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 two areas can help guide the development of hypotheses in the area of MP risk. A key question in the risk assessment of ENPs relates to particle characterization. Similar to MP, 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 MPs are important to consider when addressing their environmental impact, since they will influence their fate and result in varying degrees of chemical sorption to the MPs from ambient waters, wastewaters and sediments where they reside. The study of the combined toxicological effects of MPs 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 mode-of-action). When addressing risk from chemical mixtures the aim is to quantify the combined effects of more than one stressor and to assess whether interactions between the stressors involved causes mixture-specific effects that deviate from additivity (i.e. synergy or antagonism).
Towards better characterization of ecological risk of MP – lessons from engineered nanoparticles and mixtures

In order to conduct risk assessments of MPs in the environment, further progress in both hazard and exposure assessment is needed. In the following section we highlight current understandings and point towards future challenges for hazard and exposure studies. Parallels to the ENPs and mixture area are made in order to suggest possible focuses for future research.

Microplastic exposure assessment

For exposure assessment to be useful for risk assessment purposes, the quantification of MPs should be related to the observed hazard, which requires quantification with regards to volume of the specific water body. Indeed, a U.S. EPA 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 in order to relate adverse effects to risk. Most MP studies have focused on crudely quantifying the abundance of MPs in the environment [15]. Many studies have simply been presence-absence measures based on tows without determination of the number of MP 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 MPs and natural food [16, 17]. It is essential to know the size and number of MPs 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 MPs is an artifact of our methodological limitations, but does not inherently reflect size classes
of the greatest ecological concern or potential effects. Microplastic sampling from sediment and sandy beaches have typically involved manual selection by picking MPs identifiable by the naked eye and is biased towards 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 MPs of all sizes and shapes per environmental unit for exposure assessments. However, this method requires multiple samples per unit area in order to assess spatial heterogeneity in MP exposure, a sampling approach which 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 data of MPs from a much larger volume than the bulk sampling method. On the other hand, 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 account for the effects of wave action that often prevents the mouth of the net from being full through the course of a surface tow, which will thus underestimate actual concentrations of the measured MP sizes. Further, net size has most often been in the range of 0.30-0.39 mm, meaning nano-sized plastic particles have not been sampled. A knowledge gap, with implications for future decisions regarding sampling methods used, concerns the relative effects of different sized MPs: i.e., how hazardous are larger particles (typically 1-5 mm) compared to smaller MPs, down to the nano size range (<100 nm). Certain nano sized particles has been shown to produce stress response in pelagic organisms. For example Zhao & Wang [18] found reproductive effects of AgNPs in daphnids which they attributed to particle effects. Besseling et al. [19] have shown that MPs in the nano-sized range
can affect daphnid growth and reproduction but a direct comparison between ENP and MPs has yet to be made. This knowledge gap (i.e., how particle size affect toxicity) was highlighted several years ago for ENPs [20], and improving understanding of this has since then been one of the important research aims. A similar focus regarding MP particle effects could aid in the effort to reach consensus on whether particular attention should be paid on the collection of the smallest size classes that are missed with current sampling approaches. A better understanding of the relationship between occurrences of different size fractions in environmental matrices could allow for expanding exposure scenarios based on current data regarding the larger MP fractions. If such a relationship could be established it would allow for computation of smaller size fractions based on data for larger fractions and thus expand the use of existing monitoring studies greatly.

Important MP exposure metrics include key physical characteristics that could impact their hazard potential. A myriad of physical properties affect the ecological fate and toxicity of MPs—both directly and indirectly—by their interaction with other contaminants and the biosphere. Thus, it is critical that future research both quantify and characterize MPs in the context of ecological risk. For instance, as with ENPs, the interaction between MPs and the surrounding environment and biota largely depend on their surface properties. Stone et al. [21] 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 to what extent such considerations can be extrapolated to the field of MP environmental risk. If smaller MPs are more hazardous than larger ones due to their higher surface/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,
since particle number and surface area can differ among treatments with similar weight but different sizes. Microplastic density provides useful information for fate modeling, as some fraction of the MPs will settle into depositional sediments. Their density may however change through time in the environment due to the formation of biofilms on the particles [22], 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 [23] and toxicity [24]. An analogous process is degradation of MPs 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 [16,25]. An important qualifier for the potential adverse effects that MPs may have on biota and where researchers should focus their efforts is the transformations MPs undergo in their environment. Chemical analyses with FT-IR have been used within conservation science for many years [26], and show promise for the identification of environmental pollution of plastics [27]. Both fouling and degradation pose analogous concerns as 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 instance, quantitative descriptions of how MPs and ENPs partition between the different compartments is still needed.

Microplastic hazard assessment

As described above, most data concerning MPs in the environment are biased towards 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, e.g., Cu ENP versus micron-sized
Cu particles [28]. Other ENP studies have shown that form can be a driver for toxicity, such as for carbon nanotubes (CNT) with needle-like features that enable them to “spike” cells and provoke inflammatory responses. These types of effects mimic those of asbestos [29]. Few studies of MP cellular effects have been conducted but von Moos et al. [30] reported cellular uptake and the subsequent decrease in lysosomal membrane stability. Whereas some properties might thus be shared between MPs and ENPs, other such as asbestos like properties might not be shared by MP fibers. Even though they resemble CNT in form MP fibers originating from e.g., fishing nets might not share the needle like properties of CNT and asbestos.

A range of biota is known to feed on MPs, 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. [31] showed that ingestion of MPs by the sea urchin larvae *Tripneustus 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 days of exposure to 300MP/ml. The authors discussed the importance of MP shape both in regard to preference for feeding and in regard to physical stress [31]. Apart from indicating possible effects on marine larva the study illustrates the importance of obtaining exposure estimates that can be related to hazard assessments, as discussed above. MPs effects might however not be restricted to impairment and pseudo-saturation. von Moos et al. [30] found that nano-sized MPs (0-80µm) 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 [30]. 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 MPs hazardous effects. Apart from these types of direct effects, MPs can have indirect hazardous effects by serving as carriers for other contaminants that adhere to the surface of the MPs. Several studies have reported elevated concentrations of plastic-derived chemicals such as flame retardants in birds [32] and phthalates in filter feeding whales and sharks [17] indicating that MP might be an important route of exposure for other contaminants. However, another study by Koelmans et al [33] used the biodynamic model to calculate the likely exposure concentrations of nonylphenol and bisphenol A in Lugworms and Cod, as a function of MP ingestion. The authors of the latter study concluded that nonylphenol and bisphenol A risk as a function of MP ingestion are expected to be limited for the two species. Finally there is the possibility that MPs 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 MPs as a vector for other contaminants.

The next steps therefore require research in both direct effects, where parallels to ENP research could provide meaningful hypotheses on particle interactions on both organismal and cellular level, and research in combined effects of MPs and adhered contaminants. Due to the heterogenic nature of MPs and their complex interaction with the environment and other stressors, it may be appropriate to invoke methods of mixture toxicity assessment when evaluating the effect of MPs in the environment, as described by e.g. Greco et al [34]. Even though such mixture toxicity studies in themselves do not provide mechanistic understanding, they can help focus research by revealing whether there are interactions influencing the toxicity of the mixture to ultimately quantify risk. Oliveira et al. [35] studied the combined effects of
MPs and pyrene to the teleost fish *Pomatoscistus microps*. They found that MPs altered the toxicity and fate of pyrene in some aspects whereas other endpoints were unaffected. The authors found that MPs delayed pyrene-induced mortality possibly due to altering the metabolism of pyrene [35], suggesting a change in uptake patterns when MPs are present. They further assessed toxicity with several biomarkers and found that some mixture effects differed from single treatment exposures whereas others did not. The authors discussed possible interactions between MPs and pyrene in the light of these findings [35]. Their discussion illustrates how a mixture toxicity experimental set up might be evoked to address the complex environmental risk of MPs.

In order 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 in order to compute the mathematically derived additive effect, which subsequently can be used to assess whether there are 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 decades [36]. Several different categories of mixture effects have been proposed and parallels from these concepts with interactive effects of MP can provide useful insights that can help quantify risk of MPs (table 2). However, any lacking information, such as if there are hazardous phthalates added to the plastic, would blur the assessment and possibly lead to a false conclusion about the type of mixture toxicity.

In order to improve the information for risk assessments the next stages of MP research can therefore be divided into two phases (figure 2). First of all there is a need for better understanding of the basic hazardous properties of MPs, which is the aim of phase one. 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, shapes etc. in order 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 stage one studies and run partly in parallel, include interactions between MPs 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 MPs and other contaminants. While, trophic transfer of MPs has been observed [37], 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 MP hazard.

Similar to ENPs, MPs 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 expected to float on surface waters, whereas MPs 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 should ultimately sink to sediments [11]. Furthermore, the colonization of MP surfaces by periphyton can increase particle density and cause them to sink [3]. This means that MPs 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 MPs by planktivores (i.e., the Trojan-horse effect) [11], but has also been used to describe elevated intracellular stress as a function of ENP-facilitated transport across cell membranes [38]. To facilitate future research, we propose focusing on multiple levels of vector-effects, as discussed below.

EXPANDING THE “VECTOR EFFECT” FRAMEWORK

The “Trojan-horse” effect [11] or “vector effect” refers to scenarios where other pollutants, such as hydrophobic persistent organic pollutants (POPs and/or persistent, bioaccumulative and toxic, PBTs) or metals adhere to the MP and are transported into the gut of e.g., planktivores [39] or detritivores via MP ingestion. As mentioned above the importance of such a “vector-effect” is still debatable but should be an important focus of future studies, both due to the magnitude of plastic debris in the aquatic environment [4] and since plastics have been shown to both sorb and bind organic contaminants to a much greater degree than natural sediments [13]. To foster a more efficient and comprehensive research trajectory, we propose expanding the definition of vector effects to uniquely recognize (1) the vector/Trojan-horse effect [39], to be termed “Organismal-vector effect”, and to differentiate it from (2) the transport of MP-adhered contaminants between environmental compartments and geographical locations, to be termed “Environmental-vector effect” and finally (3) “Cellular-vector effect”, i.e. the transport of MPs across the cell membrane by endocytosis, as an important third and final “vector-effect” for, e.g., metals (figure 3).

Apart from the “organismal-vector effect” already addressed above there are studies indicating that the two other levels are equally relevant. Model calculations reveal that more than 90% of the 5 trillion plastic pieces in the oceans might be MPs [4]. A significant part
of these MPs are accumulated in the five marine gyres illustrating that horizontal transport is very important for environmental distribution of MPs. Microplastic transport is however not confined to a horizontal vector. Model estimates indicate that a significant part of MPs are removed from the sea surface indicating a vertical vector transport [4]. This hypothesis is supported by samples of deep-sea sediment, where MPs were found in samples taken at depths up to 5000 meters [40]. These findings illustrate that MPs are transported to all parts of the ocean and due to the magnitude of plastic pollution might thus serve as an important environmental-vector for other contaminants. Delineating these discreet transport mechanisms may greatly influence which organism are exposed and therefore change the ecosystem impacts.

If hydrophobic chemicals adhere to MPs with densities lower than water, these might stay longer in the water column to be picked up by pelagic species. Furthermore, MPs transported by currents over large distances could serve as vectors for otherwise locallyconstrained contaminants, resulting in changes in geographical distribution of 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 MPs and subsequent intra-cellular effects, as observed by Von Moos et al [30], indicate that such transport could, in addition to direct MP effects, result in intra-cellular effects. They concluded that this was most likely due to endocytosis. Such transport could, in addition to the direct MP 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 MP adhered contaminants. The three effect levels establish a coherent and comprehensive research framework essential to more fully understanding MP risks across
three relevant levels of ecological and biological organization (i.e., environmental, organismal and cellular).

The final note of this paper relates to the current discussions on ecological risk assessment. The three scientific committees under the European Commission have stressed that future risk assessment must have a higher degree of environmental realism [41]. 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 where mechanisms are not yet well understood. However, for MPs, 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 MPs to inform risk management as precisely as possible and in a more ecologically realistic manner.

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Data availability—No specific data that are not published in the scientific literature have been used for this paper.
REFERENCES


Figure 1. Primary and secondary microplastics. 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 MPs, such as the polyethylene beads (10-106 µm) pictured, are typically uniform in shape and composition. Secondary microplastics are micron-sized following the degradation of larger plastic debris. They are typically much more diverse in shape, size, color and composition than primary MPs, as can be seen in a sample trawled from the Mediterranean Sea.

Figure 2. The next steps of research into microplastics (MPs) hazardous effects should be divided into two tiers. The first round of research should focus on gaining a better understanding of MPs hazardous properties, both physical and chemical. These types of studies could lend themselves to the hypothesis tested for engineered nanoparticles, where a substantial amount of studies have been published concerning particles interactions with biological systems. In the second tier more complex studies on MPs hazard in combination with other contaminants should be conducted. The importance of MPs as vectors for other contaminants is currently debated and hypothesis from mixture toxicity might prove useful for addressing this issue. Finally there is a need for generation of data that address population level effects, both of MPs alone and in
This last focus area is however not addressed in the current paper.

Figure 3. Expanding the “vector effect” framework: the three levels of vector effects in which microplastics (MPs) transport other contaminants into new locations. The environmental-vector effect sees MPs with adhered contaminants 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 occurs when organisms inadvertently feeds on the MPs so that adhered contaminants now enter the organism through the diet and are transported into the gut of the animals. In this scenario, the MP ingestion serves to deliver the contaminant into the organism resulting in a change in exposure route and potentially dose. Once in the gut MPs, depending on their size, and the contaminants they carry could be transported into cells, potentially, via endocytosis or phagocytosis. In this cellular-vector effect contaminants achieve cellular entry with the MP resulting in elevated intracellular concentrations.
### Tables
Table 1. Possible parallels from engineered nanoparticles to microplastics

<table>
<thead>
<tr>
<th>Exposure</th>
<th>ENPs research area</th>
<th>Parallel to MP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Factors related to the particle itself</td>
<td>Importance of</td>
<td>Importance of</td>
</tr>
<tr>
<td></td>
<td>Metal composition</td>
<td>Plastic constituent</td>
</tr>
<tr>
<td></td>
<td>Shape</td>
<td>Shape</td>
</tr>
<tr>
<td></td>
<td>Size (ENP v micro-sized)</td>
<td>Size</td>
</tr>
<tr>
<td>Environmental behavior</td>
<td>Aged NP studies,</td>
<td>Changes to surface properties through degradation and</td>
</tr>
<tr>
<td></td>
<td>ion release for Me NPs</td>
<td>weathering leading to increased absorption of other pollutants or</td>
</tr>
<tr>
<td></td>
<td>Formation of protein coronas and thus ‘environmental identity’</td>
<td>release of constituent material.</td>
</tr>
<tr>
<td></td>
<td>Weathering of NPs</td>
<td>Formation of surface biofilms</td>
</tr>
<tr>
<td></td>
<td>Aggregation/agglomeration vs disaggregation</td>
<td>lead to change in environmental</td>
</tr>
<tr>
<td></td>
<td>Adsorption of other contaminants (e.g. to carbon black)</td>
<td>distribution</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Potential to sorb contaminants</td>
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<tr>
<td></td>
<td></td>
<td>and serve as vector</td>
</tr>
<tr>
<td>Organism interactions</td>
<td>Endocytosis, Trojan horse effect</td>
<td>Cellular uptake and intracellular</td>
</tr>
<tr>
<td></td>
<td>Intracellular effects (e.g. ROS)</td>
<td>effects</td>
</tr>
<tr>
<td></td>
<td>Reduced feeding behavior,</td>
<td>Physical damage following</td>
</tr>
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</table>
The areas where feasible parallels from engineered nanoparticles (ENP) to microplastics (MPs) research are possible are grouped within three overall categories. Factors relating to particle itself, environmental behavior, and organism interaction. Within each of these areas there are several fruitful parallels to 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.
<table>
<thead>
<tr>
<th>Type of mixture effect</th>
<th>Type of interaction</th>
<th>Possible parallels to MP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Greater than predicted</td>
<td>True synergy: both components are stressors alone and enhance the effect when combined</td>
<td>e.g. MP with toxic property in itself and adhered toxicant, that is transported via vector-type mechanism.</td>
</tr>
<tr>
<td></td>
<td>Potentiation: One component does not cause harm in itself, but enhance the effects of the other component, which produce a stress response alone.</td>
<td>e.g. Inert MP without hazardous properties, than enhance the bioavailability of environmental contaminants via vector type effects</td>
</tr>
<tr>
<td>Equal to predicted</td>
<td>No interaction between two components, which individually cause stress response</td>
<td>e.g. MPs blocking gills without interacting with chemical that cause apoptosis in gill cells, both leading to death of the organism.</td>
</tr>
<tr>
<td>Less than predicted</td>
<td>True antagony: Both components cause stress response alone and elicit a reduced response in combination</td>
<td>e.g. Toxic MP interacting with metal ions and thus reducing their toxicity by reducing direct exposure to gills.</td>
</tr>
<tr>
<td></td>
<td>Inhibition: One component does not cause harm in itself, but reduce the effects of the other component, which produce a stress response alone.</td>
<td>e.g. inert MP that decreases bioavailability of other chemical similar to carbon black</td>
</tr>
</tbody>
</table>

*Overview of relevant concepts from mixture toxicity that could help focus research on interactions between microplastics (MPs) and other contaminants. First column: Three overall categories of mixture effects that are relevant for MP environmental risk research (synergy, additivity and antagony). Second column: Types of interactions within the field of mixture toxicity under the three overall categories of mixture effects. Third column: examples of scenarios where the application of mixture toxicity methodologies might facilitate a better understanding of environmental risk associated with MPs.*
Figures

Figure 1. Primary and secondary microplastics

Primary MPs

Secondary MPs

100 μm
Figure 2. Recommendation for future research strategy into microplastic environmental risk

Phase 1

Hazardous properties of MPs

Phase 2

Interactions of MPs and other contaminants

Population level effects of MPs
Figure 3. Three levels of microplastic vector effects