PERSPECTIVE

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Research recommendations to better understand the potential health impacts of microplastics to humans and aquatic ecosystems

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Abstract

To assess the potential risk of microplastic exposure to humans and aquatic ecosystems, reliable toxicity data is needed. This includes a more complete foundational understanding of microplastic toxicity and better characterization of the hazards they may present. To expand this understanding, an international group of experts was convened in 2020–2021 to identify critical thresholds at which microplastics found in drinking and ambient waters present a health risk to humans and aquatic organisms. However, their findings were limited by notable data gaps in the literature. Here, we identify those shortcomings and describe four categories of research recommendations needed to address them: 1) adequate particle characterization and selection for toxicity testing; 2) appropriate experimental study designs that allow for the derivation of dose-response curves; 3) establishment of adverse outcome pathways for microplastics; and 4) a clearer understanding of microplastic exposure, particularly for human health. By addressing these four data gaps, researchers will gain a better understanding of the key drivers of microplastic toxicity and the concentrations at which adverse effects may occur, allowing a better understanding of the potential risk that microplastics exposure might pose to human and aquatic ecosystems.

Keywords: Microplastic, Research recommendations, Hazard characterization, Aquatic organisms, Human health, Environmental management

Introduction

Researchers are finding microplastics almost everywhere they look. Microplastics, defined as solid, polymeric particles with at least three dimensions greater than 1 nm and < 5 mm in size [19], contaminate marine [133], freshwater [53], and terrestrial [138] habitats, and more recently, have been detected in drinking water [69, 102], food [12], and the atmosphere [13, 135]. Given their ubiquity, most organisms, including humans, are frequently exposed to microplastics. Studies in aquatic organisms show that microplastics can cause inflammation and tissue damage [63, 87], reduced growth [145], altered development [42], and reductions in reproductive success [26, 57]. Though the possible effects in humans are less well-defined, initial studies in rodent models suggest that exposure to some forms of microplastics may impact endocrine signaling [2, 3, 54], initiate oxidative stress and inflammation [77, 136, 141], and reduce gamete viability [4, 54, 80].

These findings have captured the attention of the public and increased societal concern for ecosystem and human health, prompting legislators, environmental managers, and other organizations to take action to better

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understand the risks of microplastic exposure. Within the past decade, the United Kingdom Parliament, the European Chemicals Agency, the European Food Safety Authority, and the World Health Organization have all released comprehensive reports and specific recommendations to assess the impact and potential risks of plastic and microplastic pollution [33, 37, 44, 100, 132]. Effective management of microplastics requires an understanding of the potential adverse health effects on humans and the environment, as well as the key drivers of toxicity (e.g., particle size, composition, etc.) and concentration thresholds at which these effects begin to manifest. However, developing health-based thresholds for microplastics is challenging because they represent a diverse suite of physical and chemical characteristics [111]. Toxicological effects may be initiated via a variety of mechanisms rather than a single molecular initiating event, and some of these mechanisms are poorly elucidated. In support of legislative mandates to develop microplastics management strategies for aquatic habitats and drinking water for human consumption [146, 147], the State of California convened a group of international experts in microplastics research to identify and characterize the hazards associated with microplastics. Specifically, experts were tasked with identifying which microplastic characteristics

(e.g., size, morphology, polymer, etc.) contribute most to toxicity [49] and developing health-based thresholds for both the aquatic environment [92] and drinking water [25]. These efforts were limited by critical gaps in knowledge, or a lack of studies the experts deemed fit for the purpose of risk assessment [25, 45, 92]. Here, we identify those shortcomings and the research initiatives needed to address them, which can be grouped into four categories: 1) improved particle selection and characterization for toxicity testing; 2) experimental designs that allow for establishing dose-response curves; 3) the connection of microplastics to established or novel adverse outcome pathways (AOPs); and 4) a clearer understanding of exposure (Fig. 1). Each of the four research gaps are discussed in depth below and recommendations for future study designs are postulated (Table 1).

I. Improved particle characterization and selection for hazard identification and characterization

Assessing potential microplastic toxicity in aquatic organisms has been achieved primarily via laboratory studies in which biota are exposed to microplastics at a given concentration or concentrations and physiological responses are measured. Most exposure studies have been conducted using a single particle type

Fig. 1 Visual summary of key research recommendations for microplastic hazard characterization

Table 1 Summary of priority research initiatives to advance microplastic research

Category	Recommendation	Priority Research Initiatives
Particle Characterization	Identify microplastic characteristics that best predict hazards through extensive particle characterization and toxicity screening	• Comparative studies to determine the relative toxicities of different types of microplastics • Detailed particle characterization beyond size, polymer, and morphology (e.g., volume, surface area, associated chemicals, charge, functionalization, particle behavior, biomolecular corona) to allow potential correlations between particle metrics and observed effects
	Better characterize microplastic hazards by conducting toxicity tests using polydisperse, environmentally relevant distributions of microplastic particles	• Toxicity studies using well characterized, environmentally relevant mixtures of particles (e.g., diverse morphologies, polymers, sizes, etc.) • Toxicity studies using understudied but environmentally prevalent and well characterized microplastic types (e.g., fibers, tire wear particles, paint, recycled plastics) • Toxicity studies using weathered particles obtained using well-described methods
Threshold Development	Design experiments to generate robust dose/concentration-response data for health-based threshold development	• In vivo and in vitro toxicity studies with a sufficient number (3 minimum) and range of concentrations (including environmentally detected) to determine dose/concentration-response relationships
Toxicological Pathways	Connect microplastics to existing or novel adverse outcome pathways	• More fully describe key events across levels of biological organization leading to negative impacts following microplastic exposure
	Increase the relevance of in vitro studies for hazard characterization by developing a framework for extrapolating in vitro results to in vivo effects	• Development of quantitative in vitro to in vivo extrapolation (QIVIVE) approaches to connect in vitro concentration-effect relationships to in vivo dose-response relationships • Development of physiologically based kinetic (PBK) models to predict particle kinetics in vivo
Exposure Characterization	Characterize understudied microplastic exposure routes	• Describe microplastic exposure and toxicity in sediment-dwelling aquatic organisms • Comprehensive microplastic exposure assessment for human health

(e.g., polystyrene spheres of a single size), but if particles are well characterized, these studies can provide important information on the potential hazards of specific microplastic types and characteristics (e.g., size, morphology, polymer type, etc.). In addition, there is also a need for studies in which organisms are exposed to combinations of microplastics as close as possible to what they would be exposed to in the ambient environment. For instance, fibers and spheres respectively make up 52–73% and 1–3% of anthropogenic particles detected in the environmental water samples [7, 17, 142], but roughly only 7% of studies published through 2020 use fibers whereas 62% use spheres [50]. Similarly, 82% of studies are conducted with polystyrene or polyethylene polymers, which make up only 5–28% of what is reported in the aquatic environment [17]. Only 12% of aquatic organism tests used weathered particles [50], which are likely to present greater risks to biota due to increased ingestion probability, leachates, biofilm formation, particle roughness, increased surface area, and potentially other mechanisms [51, 66, 83]. For studies focused on the potential human health impacts of microplastics, similar biases regarding particle selection were observed as 77% of the rodent in vivo studies used polystyrene spheres [50]. In addition, microplastic particles were often limited to a single size (69% of studies), and no studies used weathered particles. This lack of particle diversity is also reflected in in vitro studies.

If particles are comprehensively characterized (see [28, 45] for guidance on minimum particle characterization), experiments that employ a single particle type may provide insight regarding specific relationships between microplastic characteristics and biological effects. Thus, it is recommended that future toxicity tests address one of two experimental objectives. The first is to determine how specific microplastic particle types (e.g., polyester fibers, tire wear particles) and characteristics (e.g., size, surface area, volume) may present a hazard to aquatic organisms and/or humans. Identification of the most harmful microplastic types is important for the development of monitoring programs, as there are numerous measurement techniques that can be used to quantify microplastics, with some being more appropriate and cost-effective for different sizes, morphologies, and polymer types [27]. The second objective is to determine concentrations at which environmentally relevant distributions of microplastics cause adverse effects. The limited particle diversity and incomplete particle characterization in most existing studies are impediments to achieving either objective.

Recommendation 1: Identify microplastic characteristics that best predict hazards through extensive particle characterization and toxicity screening

Results from studies using singular particle types can be extrapolated to more relevant mixtures of microplastics found in the natural environment so long as particles are extensively characterized, and the relative importance of different particle characteristics to toxicological outcomes are understood [70]. For example, Zimmerman et al. [145] exposed *Daphnia magna* to polyvinyl chloride, polyurethane, or polylactic acid with or without extractable chemical additives. The particle morphology (i.e., fragments) and size (i.e., 20–40 μm) were held constant. Using this experimental design, Zimmerman et al., could discern which effects were driven by polymer type and which were driven by additive chemicals. Perhaps most importantly, the size, polymer composition, and morphology of the microplastics used were all extensively characterized. These findings provide much-needed insight into which microplastic characteristics may cause toxicity. More similarly designed studies are needed for other types of microplastics to identify which particle characteristics and polymer types are of greatest toxicological concern. Study relevance may be further increased by using particle types frequently most detected in the environment.

Particle size is a critical factor influencing microplastic toxicokinetics and toxicodynamics [25, 49]. In aquatic organisms, smaller microplastics may be taken up via the gills and ingested, while larger plastics may interfere with motility through entanglement [40, 58]. Once a particle is ingested, its size also influences the likelihood for translocation beyond the gut or gills to other tissues [91, 130], as well as their retention and excretion [68]. Current evidence indicates that size strongly affects the observed adverse outcomes, including differential effects on growth [116], immune function [81], oxidative stress [140], and mortality [46]. Many studies suggest that toxic effects are more likely to be observed following exposure to smaller particles [15], although larger particles may be more harmful to aquatic species in specific scenarios. For example, larger particles take up more volume in the gut once ingested, possibly leading to reduced food assimilation and food dilution [49, 70].

In humans, size determines the extent to which particles may be taken up and distributed within the body. For instance, particles < 10 μm may be inhaled [104] and those < 1 μm may be taken up by cells [9, 43]. As the size of inhaled particles decreases, translocation efficiency increases [73]. Smaller, orally ingested microplastic particles are also expected to translocate from the gut more efficiently [134]. For instance, 50 and 100 nm polystyrene nanospheres were detected in the liver, spleen, blood, and

bone marrow of female rats after 10 days of exposure via gavage. In contrast, particles larger than 100 nm were not detected in the bond marrow and those larger than 300 nm were not detected in the blood [59]. Yet despite these observations, there is insufficient data to reliably model the particokinetics of microplastics for humans [25] or other organisms [92], thus increasing uncertainties of risk assessments.

Experiments that disentangle the relative effects of different microplastic morphologies are also needed as particle morphology likely influences retention, translocation, and toxicity. In aquatic organisms, a fiber, defined as having a length to width ratio of three or greater, may be retained in the gut for extended periods of time [137] or more likely to translocate via its smallest dimension [91] compared to fragments or spheres with similar particle lengths. Several studies report that fibers or irregularly shaped particles (e.g., fragments) are more toxic than uniform particles such as pellets or spheres [11, 108]. In some instances, specific morphologies may elicit unique adverse effects as fibers have been shown to cause respiratory stress [118]. Similar findings have been described in mammalian studies as fibers have been found to persist in airways in humans [96], and fragments were found to induce hemolysis in human-derived cells at rates proportionate to their roughness [23]. However, mammalian toxicity studies that use diverse particle morphologies are limited, with most ingestion-based studies using spheres, several using fragments, and none using fibers [25].

At the interface of size and shape are particle volume and surface area, which were identified as being the primary drivers of food dilution and oxidative stress in aquatic species [49, 70] and used as the basis for thresholds in the ambient environment [92]. Though food dilution is not relevant for human health, similar relationships between surface area and oxidative stress and other adverse effects have been detected in mammalian models. For example, Schmid and Stoeger [114] found that nanoparticle surface area was highly correlated with acute lung inflammation when in vivo studies in mice and rats were retroactively analyzed. Surface area also influences the formation of the particle corona, which can include toxicants and antigens which influence both uptake and toxicity in humans and other organisms [38, 89]. To date, most studies focused on the influence of surface area on toxicokinetics and toxicodynamics use small, spherical particles (typically less than 1 μm). Additional studies are needed to determine if the previously described relationships between surface area and toxicity persist across larger size ranges and other particle types with high surface area to mass ratios (e.g., fragments, fibers).

In laboratory studies, volume and surface area particle characteristics may be estimated using equations based upon the shape (e.g., volume of a sphere = 4/3 πr^3). To estimate these parameters in environmental particles, modelling techniques may be used [71]. However, particle volume and surface area are not typically measured, estimated, or reported in microplastic occurrence or toxicity studies. Measurement or estimation of such parameters in laboratory studies is crucial to understanding the relevant exposure metric for specific types of toxicological effects. Thus, it is important that studies not only report these characteristics but that they are considered as potential drivers of toxicity in future experiments using aquatic species or rodents.

Finally, experiments designed to decouple particle-driven effects from those caused by chemical leachates (i.e., monomers, additive mixtures) and sorbed chemicals (e.g., [145]) are critical to understanding the toxicological drivers of microplastics. Adverse effects have been attributed to chemical additives following the inhalation of nylon fibers [104, 126] and polyvinyl chloride particles [139] in humans; and in aquatic organisms, leachates from tire wear particles [120, 123] and single-use food packing [144] have been demonstrated to be toxic. Disentangling physical and chemical particle characteristics causing toxicity will facilitate more targeted, efficient management and mitigation strategies for reducing environmental and human health risks from microplastics (e.g., prioritizing assessment of alternatives for chemical additives in plastics).

Though methods for microplastic analysis and particle characterization are still emerging, techniques for quantifying particles as well as determining size, morphology, and polymer type are readily available for most particle types excluding nanoplastics (<1 μm) [14, 106]. Microplastics are most often enumerated by manually counting particles via visual light microscopy, which may be facilitated by staining particles with nile red. For smaller particles <20–50 μm, other microscopic or light scattering techniques (e.g., scanning electron microscopy, transmission electron microscopy, dynamic light scattering) are often preferrable. Particle size is most often assessed by manual measurements via microscopy, but other techniques such as dynamic light scattering can be used to generate size distributions. Polymer confirmation and identification are most commonly achieved via Fourier-transform infrared spectroscopy (FTIR) or Raman spectroscopy, but polymers may also be identified using pyrolysis-gas chromatography/mass spectrometry. Other particle characteristics such as surface area and volume may be estimated in some cases [70, 72], but currently, there are no widely used techniques for gathering empirical data describing these characteristics in microplastics.

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Future methodological studies should seek to develop methods for better particle characterization, particularly for characteristics hypothesized to drive toxicological effects (e.g., surface area, volume).

Recommendation 2: Better characterize microplastic hazards by conducting toxicity tests using polydisperse, environmentally relevant distributions of microplastic particles

To fully characterize the hazards of microplastics, it is important to understand how environmentally relevant mixtures of particles may cause toxicity [48]. Assessing the integrated effects of multiple plastic types from exposures conducted with a single type of microplastic is challenging as some evidence suggests organisms respond differently to diverse mixtures of microplastics (i.e., polydisperse) than each type of microplastic alone. For instance, Ziajahromi et al., [143] found polyester fibers or polyethylene beads to be more toxic to *Ceriodaphnia dubia* when presented alone than when presented as a mixture [143]. There are few studies that have tried to mimic naturally occurring mixtures of microplastics by exposing organisms to more than one particle type at the same time (i.e., ~5% of aquatic organism studies, 0% of human health studies [50]), but more studies are needed to definitively identify the primary hazards of microplastics.

Microplastic distributions vary greatly depending on the environmental matrix [72, 142]. However, some patterns have emerged from studies aimed at describing mixtures of microplastics in the real-world [71]. In drinking water, studies have shown that most samples are typically a mixture of relatively small (<10 μm) fragments and fibers [102, 103], whereas in the aquatic environment, most surface water and sediment samples, and thus biota, appear to be dominated by fibers and a diverse array of fragments, films, and foams [17, 142]. Toxicity evaluations reflective of these particle distributions would be useful in bridging the gap between laboratory studies and realistic exposures, ultimately leading to better hazard characterization.

Predicting microplastic toxicity may be further complicated by the influence of environmental weathering. Most organisms will encounter microplastics that are weathered [121], fouled with life (including pathogens [1]), and that include a sorbed mixture of ambient chemical pollutants (organics and metals [112]). There is some evidence that particles aged in the natural environment have different bioavailability and toxicity compared to the effects observed from pristine microplastics [16, 21]. Studies have found enhanced effects from microplastics that had been soaked in ocean or lake water compared to virgin microplastics [16, 110]; though in some cases,

weathering has been shown to decrease toxicity [115]. Other studies have found increased chances of translocation [109]. Despite this, there is a lack of toxicity data for weathered particles as only roughly 12% of studies used microplastics that were collected from the environment or artificially weathered prior to toxicity tests [50]. The use of weathered particles in future studies will provide more realistic assessments of microplastic toxicity, though it is important that researchers fully describe approaches used for particle weathering to ensure that studies are representative of environmental conditions and repeatable.

Approaches for generating polydisperse, environmentally realistic distributions of microplastics may include the acquisition or generation of the most prevalent particle types typically found within the habitat and matrix of interest. These particles may then be combined in toxicity tests in similar proportions observed in the environment. Artificial weathering can also increase environmental relevance as has been demonstrated in previous studies [84]. Alternatively, some studies have also used field-collected microplastics in toxicity testing [16, 74, 101]. However, if this approach is taken, it is essential that particles are well-characterized as described in Recommendation 1.

II. Inform the development of health-based thresholds for microplastics

Most microplastic toxicity studies are focused on determining if physiological or behavioral effects can be detected, rather than developing robust dose-response data. Though exploratory, hypothesis-driven studies have supplied the field with a foundational understanding of microplastic toxicity effect mechanisms (i.e., hazard identification), studies which generate robust dose-response data are needed to identify critical concentrations at which those effects manifest (i.e., hazard characterization). Thus, future studies should aim to generate robust dose-response data from which critical effect metrics can be derived. Below, we discuss why this is important and provide specific recommendations for future studies seeking to inform health-based thresholds for aquatic organisms and humans.

Recommendation 3: Design experiments to generate robust dose-response data for health-based threshold development

Health-based guidance values are traditionally derived from chronic undefined laboratory studies [10], though in vitro data may also be used for hazard characterization (see Section III, Recommendation 5). However, it was challenging for the experts in the California Health Effects Workshop to derive health-based thresholds for drinking water and the aquatic environment due to the

availability of few fit-for-purpose studies [25, 92]. Of the in vivo studies in the Toxicity of Microplastics Explorer (ToMEx) database, only 52% of human health studies ($n = 14$) and 44% aquatic organism studies ($n = 73$) included three or more exposure concentrations in their experimental design [50]. Robust dose-response data is essential to threshold development because it captures the critical points at which contaminant concentrations elicit adverse health effects.

To analyze and describe dose-response relationships, different approaches should be used depending on the specific aims of the study. In environmental toxicology, no observed effect concentrations (NOECs) and lowest observed effect concentrations (LOECs) are often used to inform threshold development for the aquatic environment. Here, it is important to consider that NOECs and LOECs are entirely dependent on the dose selection and experimental design of the study from which they are extracted [36, 75]. For instance, if the LOEC is also the lowest test concentration, it is possible that even lower concentrations not included in the design will induce an adverse biological response. This could result in an underestimation of risk. Conversely, if no effects are observed in a study, the highest observed effect concentration (HONEC) may overestimate risk. Therefore, approaches that consider the whole dose-response curve such as effect concentrations (e.g., ECX) are preferred. Theoretically, only a minimum of three distinct test concentrations are required to derive lethal or effect concentrations of certain percentages (i.e., LCX or ECX, respectively), but a greater number of test concentrations is strongly recommended when possible to ensure that an adequate dose-response relationship may be observed [97]. Of the 162 studies in the ToMEx aquatic organisms database, only 16 report ECX or LCX for distinct species, most of which are cladocerans [50]. This represents a lack of robust dose-response data for aquatic species, particularly for organism groups of regulatory interest such as bivalves and fish.

To understand dose-response relationships for human health, approaches similar to those used for ecological health are often employed. Here, NOEC and LOEC values (referred to as No/Lowest Observed Adverse Effect Levels, NOAELs/LOAELs for human health applications) are often used as a starting point for threshold development [64, 124]. Alternative approaches like Benchmark Dose (BMD) modelling make use of all the data to describe dose-response relationships for a particular endpoint rather than only using discrete experimental concentrations [124]. A significant advantage of the BMD approach is that it provides an estimate of uncertainty via a confidence interval [34]. Another major benefit is that in vitro data may be incorporated into a BMD analysis,

so long as quantitative in vitro to in vivo extrapolation models are available (see Section III, Recommendation 5). Yet, despite these advantages, only 53% of evaluated in vivo studies were identified as having acceptable dose-response data appropriate for BMD modelling (i.e., at least three microplastic treatment groups with a concentration range ≥ 3, including control accompanied by estimates of uncertainty such as standard deviation) [25]. Thus, it is recommended that future studies ensure they use a sufficient number and spacing of exposure concentrations as close as possible to the linear range of the dose-response curve, and adequately report uncertainties associated with effects (e.g., standard deviation, 95% confidence intervals).

III. Increase understanding of toxicological pathways induced by microplastics for improved Hazard characterization

Numerous studies have demonstrated the potential for microplastics to cause a wide array of biological effects in aquatic organisms, including oxidative stress [60], reduced growth [6, 86], tissue damage [63], reduced reproductive output [26, 57] and behavioral alterations [24, 41, 90]. In rodents, microplastic exposure has been shown to impact endocrine signaling [2, 3, 54], initiate oxidative stress and inflammation [77, 136, 141], and negatively affect reproductive potential [4, 54, 80]. Yet, the specific mechanisms and pathways by which microplastics cause adverse effects are not yet well understood. Many studies provide evidence of altered molecular or cellular-level responses following microplastic exposure [20, 39, 88], but it is often unclear if these observations are indicative of adverse effects at the organism or population level, or if they are merely adaptive and healthy responses (e.g., increased levels of antioxidative enzymes) to a stressor with no significant impact on overall health over longer time periods. In addition, it is unknown if effects observed on apical endpoints such as sperm count in male rodents [136] are due to general inflammation or more specific mechanisms targeting sensitive tissues such as testis [25]. To address these uncertainties, it is recommended that future studies aim to characterize AOPs by assessing endpoints across multiple levels of biological organization in both humans and aquatic species. Initial efforts to identify AOPs for microplastics have indicated a need for further elucidation of mechanisms linking molecular and whole organism adverse effects [61]. This effort may be accelerated using in vitro systems and a framework for linking in vitro results to in vivo effects [113]. Development of AOPs may also help to understand the interplay of physical and chemical effects from microplastics but should be based on realistic exposure levels [62].

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Recommendation 4: Connect microplastics to existing or novel adverse outcome pathways

Workshop participants agreed that, while not necessary for developing risk-based regulatory thresholds, scientific confidence in thresholds expands when the mode of action of the microplastic related effects and pathways of effect are understood. Such knowledge would facilitate read across attempts which are of importance for microplastics due to their extreme diversity. AOPs provide a powerful conceptual mechanism for creating this linkage, often starting with a molecular initiating event ultimately leading to an effect at the organism level [5, 128]. For aquatic organisms, there is demonstrable evidence ingestion of microplastics can cause food dilution [29, 40, 70, 129] and experts agreed that there is at least partial evidence for the induction of oxidative stress responses following particle translocation [49]. In turn, these pathways were used to form the basis for thresholds for the aquatic environment [92]. However, experts also agreed that these pathways need further development and experimental validation to increase confidence in the derived thresholds [92]. For human health, biomarkers suggestive of effect mechanisms (e.g., oxidative stress, inflammation, reactive oxygen species formation, etc.) have been identified at varying levels of biological organization, however confirmatory linkages to apical endpoints (e.g., sperm reduction in testis) are absent [25].

Though most toxicity mechanisms for microplastics are only partially understood or have yet to be explored, some recent studies have proposed partial AOPs or hypothesized which existing AOPs may be applicable to microplastics [55, 61, 62, 67, 85]. For instance, following a systematic literature review, Jeong and Choi identified several putative AOPs to which nano- and microplastics could be connected, leading to adverse outcomes on growth, reproduction, and survival following oxidative stress [61]. Similarly, Coffin et al. [25] noted that several rodent studies found that microplastic ingestion induced oxidative stress responses in conjunction with impacts to reproductive biomarkers (e.g., [4, 136]), and that some responses had similarities with key events described in AOPs characterizing generalized inflammatory responses [127]. While it may be reasonable to assume that these observations are directly related, these effects have yet to be linked by distinct key event relationships and experimentally observed within the same network of events. Furthermore, uncertainties with regards to particle characterization (e.g., verification of the absence of chemical additives or impurities) in these studies prevent direct linking of molecular endpoints to apical endpoints [25]. Future studies should aim to identify and develop AOPs for microplastics using one or more strategies (summarized by [128]). An example of this might be top-down

development where researchers may begin with a well-defined adverse outcome at the organismal level and work their way down biological levels of organization. Researchers should also draw upon existing AOP knowledge, for example by using the AOP-wiki (aopwiki.org) or AOP knowledgebase (aopkb.oecd.org), as these pathways are not contaminant-specific and multiple contaminants may share the same AOP. Thus, it is likely that some existing AOPs may inform microplastic effect mechanisms and require only experimental validation. Even if the primary goal of the study is outside the scope of AOP development, researchers should always strive to describe cascades of specific biological responses and include endpoints across biological levels of organization. This is particularly important for aquatic organisms, and making mechanistic linkages between the cellular, organismal, population, and community levels can be achieved with carefully designed mesocosm or macrocosm approaches.

Recommendation 5: Increase the relevance of in vitro studies for hazard characterization by developing a framework for extrapolating in vitro results to in vivo effects

In vitro approaches in toxicology have become more widespread as new applications are developed and reductions in animal testing are encouraged [94, 98]. However, the use of such data for developing management thresholds is currently limited due to unclear methods for reliably extrapolating in vitro results to potential in vivo effects for particles [113], though strategies for soluble chemicals have been previously developed [117]. If reliable methods for extrapolating in vitro results to in vivo effects are established, researchers may take advantage of the cost, resource, and time benefits often provided by in vitro systems while generating meaningful data that can be used to characterize the hazards of microplastics. In vitro approaches could also be used as part of a tiered system, with the use of cell lines as a screening tool to prioritize which particle sizes, morphologies, etc. should be studied in costlier in vivo models. This strategy has been used and is recommended for soluble chemicals such as endocrine disruptors (e.g., [47]). In the United States, development of a quantitative in vitro to in vivo extrapolation model for microplastics and other contaminants may be necessary to conduct risk assessments due to the mandate phasing out the use of in vivo studies by the United States Environmental Protection Agency by 2035 [125].

Microplastics are different from many other contaminants because they are comprised of both chemical and physical constituents and behave as colloid particles that can settle, diffuse, and agglomerate differentially. This presents a challenge in seeking to develop a tool for the extrapolation of in vitro data. For instance, buoyant

microplastics may rapidly move away from the cell surface in an unagitated system, resulting in an exceedingly low effective concentration [119]. Thus, in addition to the concentration and exposure duration, particle (e.g., size, density, buoyancy, surface chemistry) and media characteristics (e.g., viscosity, density, presence of proteins) must be extensively described to fully understand differences between in vitro and in vivo test systems. A second, unique challenge is that the organ partitioning of microplastics in vivo is not dictated by hydrophobicity as is the case for many chemical contaminants, but rather the phagocytic capacity of the organ [32, 105]. There are some interesting developments that may help in addressing the challenges associated with microplastic exposures in vitro, including the use of semi-wet [76, 95], inverted culture systems [18, 119, 131], or dynamically flowing systems such as cell-on-a-chip models, which may facilitate cell-particle contact.

Novel computation approaches need to be further developed to support the incorporation of in vitro data from microplastic studies into risk assessment exercises. This firstly includes the use of dosimetry models, specifically tailored to capture the particle dynamics in vitro such as the In vitro Sedimentation, Diffusion and Dosimetry, In vitro Sedimentation, Diffusion, Dissolution, and Dosimetry, and Distorted Grid models [30, 31, 52, 122] which provide time-dependent particle and aggregate concentrations at any given height in the media column. Secondly, efforts have also been made to predict in vivo microplastic concentrations based on results from in vitro studies using Physiologically Based Kinetic (PBK) models which take into account the partitioning of particle-based on phagocytic capacity as described earlier [78]. Thus far, PBK models have been developed for quantum dots (20 nm) [82], metallic nanoparticles (Bachler et al., 2013), titanium dioxide (15-150 nm) [8], nanocrystals and some polymers such as PLGA (50-135 nm) [22, 79]. In a final step, PBK models can be used in quantitative in vitro to in vivo extrapolations of observed effects [65, 107]. Though these efforts demonstrate the possibility of in vitro to in vivo extrapolation for microplastics, research investments for improving estimates of dosimetry and generating reliable data describing the transport and partitioning of microplastic particles in vivo are warranted.

IV Improved exposure assessment for microplastics
Recommendation 6: Characterize understudied microplastic exposure routes
The primary purpose of this working group was hazard identification and characterization for the purposes of health-based threshold development. However, there is also a need for better microplastic exposure assessment

to improve future assessments of risk. Most microplastic toxicity studies have focused on a limited number of exposure routes. For aquatic organisms, most studies have added microplastics to water [15, 56]. However, effective risk assessment and management require a holistic understanding of relative contributions from multiple sources with a similar route of exposure (e.g., ingestion, dermal, inhalation). While this working group largely focused on aqueous exposures, aquatic organisms, particularly those species associated with the benthos, are likely to be exposed to microplastics via the sediment, which generally have higher microplastic concentrations than the water column [35]. Thus, it is recommended that future studies aim to evaluate microplastic exposure and toxicity in aquatic organisms in sediment and conduct depth-integrated risk assessments.

Here, ingestion-based studies where microplastics were added to food [77] or drinking water (Hou et al., 2020, [4]) or administered via oral gavage (e.g., [99]) were the primary focus. However, humans are exposed to microplastics via a wide variety of sources including food, and air [93]. The relative contribution of these sources to microplastic exposure, uptake, and toxicity are not well characterized in humans. Relative source contribution from drinking water was identified as the most sensitive parameter in the derivation of a health-based guidance level for drinking water [25]. As such, it is recommended that future studies aim to evaluate microplastic exposures such that a comprehensive exposure assessment through all relevant sources may be conducted. Having a comprehensive understanding of exposure for both will allow for more reliable estimations of risks that microplastics may pose to humans.

Conclusions
The field of microplastics research has reached the point where there is no longer any doubt of widespread exposure of animals and humans to plastic particles. This has led the management community to seek advice regarding whether there is a need to set limits, and what those limits should be, for microplastics in drinking water, foods, and the natural environment. Research into the bioavailability and effects of microplastics have demonstrated that microplastics can cause harm, but it is often the case that these studies cannot readily inform risk assessments. Here, we have discussed the research gaps that need to be filled to increase our understanding of the risk microplastics pose to biota and humans and best advise managers on setting health-based thresholds in a more accurate and relevant way. Such data are essential for researchers to understand the extent to which microplastics, varying in size, shape, and chemical profile, at environmentally relevant concentrations, and capturing myriad exposure

pathways, pose a risk to human health and the health of aquatic species, biodiversity, and ecosystems. With increased understanding, we can adapt management strategies and risk assessments to help effectively and efficiently manage this novel contaminant.

Declarations

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