



Understanding Human Health Impacts following Microplastic Exposure Necessitates Standardized Protocols

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Abstract

Microplastics (MPs; 1 μm - 5 mm) are a persistent and pervasive environmental pollutant of emergent and increasing concern. Human exposure to MPs through food, water, and air has been documented and thus motivates the need for a better understanding of the biological implications of MP exposure. These impacts are dependent on the properties of MPs, including size, morphology, and chemistry, as well as dose, and route of exposure. This overview offers a perspective on the current methods used to assess the bioactivity of MPs. First, we discuss methods associated with MP bioactivity research with an emphasis on the variety of assays, exposure conditions, and reference MP particles that have been used. Next, we review the challenges presented by common instrumentation and laboratory materials, the lack of standardized reference materials, and the limited understanding of MP dosimetry. Last, we propose solutions that can help increase the applicability and impact of future studies while reducing redundancy in the field. The excellent protocols published in this issue are intended to contribute to standardization in the field so that the MP knowledgebase grows from a reliable foundation.

Keywords

Microplastics; reference materials; bioavailability; exposure; dosimetry

Introduction

Although natural and synthetic polymers have been used by humans for millennia, the development and introduction of modern plastics into everyday life did not occur until

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the early 1900s (Andrady & Neal, 2009; Williams & Rangel-Buitrago, 2022). Plastics rapidly became entrenched in every aspect of modern life due to their high versatility and low production costs (Andrady & Neal, 2009). The increasing reliance on plastic products coupled with a lack of infrastructure for plastic waste management (Browning et al., 2021) has resulted in plastic pollution becoming a significant global problem (Kurtela & Antolović, 2019). Large macroscopic plastic litter can take decades to degrade in the natural environment, resulting in persistence of mismanaged plastic waste, and this introduces numerous microplastic particles into the environment over time (Shahul Hamid et al., 2018).

Microplastics (MPs) are commonly defined as plastic particles measuring between 1 μm and 5 mm on their longest dimension (Moore, 2008; Thompson et al., 2004) and can be further classified into primary and secondary MPs. Primary MPs are produced as nurdles, pre-production plastic pellets, and microbeads, the latter of which were commonly found in personal care products prior to the U.S. ban in 2015 (Germanov et al., 2018). Secondary MPs result from the environmental degradation of macroplastics, such as plastic bags and bottles, as well as microfibers released from synthetic textiles (Germanov et al., 2018). MPs can further degrade into nanoplastics (NPs < 1 μm). Environmental MPs are often complex mixtures, containing additives from the manufacturing process such as plasticizers, dyes, and flames retardants (Campanale et al., 2020) and adsorbed toxins from the environment including polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs) (Rochman et al., 2013), heavy metals (Turner & Filella, 2021) and bacteria (Junaid et al., 2022). These findings highlight challenges related to the heterogeneity of environmental MPs. Other variables to consider include particle size, morphology, and surface roughness (i.e., weathering or surface oxidation) (P. Liu et al., 2020; Rosal, 2021). MPs are therefore a diverse and persistent class of chemical mixtures.

MPs have been detected in a wide variety of geographical features and locations including all continents (Barnes et al., 2009) and major oceans (Avio et al., 2017). Additionally, MPs have been identified in lakes (Eriksen et al., 2013), rivers (Hurley et al., 2018), soils (S. Zhang et al., 2018), and both indoor and outdoor air (Ageel et al., 2022; Li et al., 2020; Mizuguchi et al., 2023; Wright et al., 2019; Q. Zhang et al., 2020). Human MP exposures occurs directly via the environment, but it can also occur through ingestion of contaminated water and foodstuffs. Both bottled and tap water (Gambino et al., 2022), as well as table salt and spices (Afrin et al., 2022; Kosuth et al., 2018; Kwon et al., 2020), some fruits and vegetables (Oliveri Conti et al., 2020), seafood (Rochman et al., 2015; Van Cauwenberghe & Janssen, 2014), meat and other processed dietary protein sources (i.e., chicken nuggets and vegan meats) (Kedzierski et al., 2020; Milne et al., 2024), beer (Diaz-Basantes et al., 2020; Kosuth et al., 2018), milk and honey (Diaz-Basantes et al., 2020; Kwon et al., 2020) have been documented to contain MP particles.

Evidence regarding human exposures to MPs comes from studies that, e.g., demonstrated that they can be isolated from human feces (Schwabl et al., 2019; Yan et al., 2022; J. Zhang et al., 2021), placentas (Garcia et al., 2024; Ragusa et al., 2021), breast milk (Ragusa et al., 2022), testes and semen (Hu et al., n.d.; Zhao et al., 2023), and blood (Leslie et al., 2022). More recently, MPs have been found in human heart tissues (Yang et al., 2023) and carotid artery atheromas, which correlated with a higher rate of cardiovascular events, such as heart

attack, stroke, and death from any cause (Marfella et al., 2024). Studies have also observed tissue accumulation of MPs in the human lung and intestine (Amato-Lourenço et al., 2021; Jenner et al., 2022; Pauly et al., 1998; Zhu et al., 2024).

Because of the ubiquity of MP exposures, it is imperative that the impact of exposures on humans are thoroughly studied. It is reasonable to predict that measurable adverse outcomes will occur following human exposure to these particles. As the MPs research field is still emerging, there is a limited but increasing number of studies investigating the effects of MP exposure in organisms, many of which have focused on invertebrates and fish (Prokić et al., 2021). Observed effects vary with exposure and organism studied (Foley et al., 2018; Maity & Pramanick, 2020). Some outcomes include neurotoxicity in *Caenorhabditis elegans*, reproductive toxicity in *Daphnia magna*, and oxidative stress in *Brachionus calyciflorus* (rotifer) (Maity & Pramanick, 2020). While these studies are imperative for understanding the ecotoxicological effects of MP exposure, the anatomy, metabolism, physiology, genetics, and routes of exposure differs between invertebrates, fish, and humans, necessitating additional studies (Morgan & DeLouise, 2020). Studies in rodents and human cell lines are emerging and informing future studies to maximize understanding (da Silva Brito et al., 2022; Yong et al., 2020). Another factor that needs to be accounted for to fully understand MP toxicity, is the relationship between the composition of the particles and their bioactivity (Verla et al., 2019; Wang et al., 2018). Interestingly, MPs have been reported to both aggravate (Huang et al., 2021) and alleviate (Y. T. Zhang et al., 2021) chemical toxicity. The diversity of biological effects supports the need for foundational principles and common methods and controls.

This overview will discuss current methods for investigating MP toxicity, highlighting differences in methodology with biological models and the lack of consistency between studies with respect to exposure conditions and reference MP particles used. We emphasize key challenges that currently prevent accurate comparison between studies, such as instrumentation and a lack of relevant reference materials, and incomplete understanding of the relationships between the real-world exposures and experimental doses. Lastly, we propose solutions that can help increase the applicability and impact of future studies while reducing redundancy in the field. The practicable protocols published in this issue are intended to contribute to standardizing the field so that the MP knowledgebase grows from a reliable and consistent foundation. These protocols include best practices to prevent field and workplace contamination, isolation and characterization of environmental MPs, and performance of toxicological effects studies.

Current Methods

Biological Models for Microplastics Toxic Effects Studies

MP investigations have been focused both on physiochemical characterization and quantitation of environmental MPs to understand exposure and the effects following MP exposures. There is a great deal of variability in effects studies due to the use of different model organisms, toxicological endpoints, exposure conditions, and the MPs tested. Most testing thus far has been performed using invertebrates, fish, rodents, and human cell lines (Prokić et al., 2021), with common endpoints including survival, behavior, oxidative stress,

and reproductive function (Maity & Pramanick, 2020). Table 1 lists some methods that have been commonly used in toxicological investigations, but this is not exhaustive. One key point to highlight is that the methods employed should be biological model dependent.

Exposure Conditions for Microplastics Toxic Effects Studies

Additionally, there is also significant variability in the exposure conditions used between studies. Wide ranges in concentrations (10^4 - 10^{15} particles/L) and particle sizes (50 nm to 50 μ m) (Lenz et al., 2016) appear in the literature. Studies also include exposures with MPs alone, in mixtures, or in combination with other pollutants (i.e., endocrine disrupting chemicals) (Heinrich et al., 2020; Ziccardi et al., 2016), and those that include additional pollutants vary in terms of pre-exposure or mixture incubation times. Studies involving mixtures rather than pure MPs are certainly environmentally relevant. A key trade-off to be considered when designing studies is environmental relevance versus reproducibility in terms of material properties and exposure conditions.

Reference Microplastic Particles for Toxic Effects Studies

Another important factor in experimental design is considering what type or source of MPs to test. Different types of reference MPs can be produced in-house by methods such as cryomilling (i.e., to produce MP fragments), cryoslicing (i.e., to produce MP fibers), dissolution and reprecipitation. Furthermore, MPs can also be sourced from the environment or purchased commercially. While commercial MPs may be convenient, the choices in polymers and morphologies are limited. Many commercially available MPs are made of either polystyrene (PS) or polyethylene (PE). Commercial MPs are uniform in morphology (i.e., microsphere) and polymer type as they were historically developed as standards for use in instrument calibration. However, they are not sufficiently characterized by the manufacturer for use in toxicological studies. This means important characteristics such as zeta potential, inorganic contaminants, surfactant or dispersant information, density, surface area and molecular weight need to be evaluated prior to use in toxicological studies (Ramsperger et al., 2022). To date, the detailed characteristics of commercial MPs used in toxicological effects studies are lacking in the literature (Deng et al., 2018; Hou et al., 2021; Wei et al., 2021; Xie et al., 2020). Illustrating the significance of in-house characterization, 3 μ m PS MPs that have identical manufacturer specifications have shown to have different surface charges which correlate with different adhesion and internalization of these particles by murine macrophage cells (Ramsperger et al., 2022; Wieland et al., 2024).

There are numerous characteristics of environmental MPs that can make them far more complex than commercially available MPs including the potential for advanced surface oxidation (aging), adsorbed environmental toxins, and/or an ecocorona with microbial colonization. Figure 1 compares the commercial PS microsphere characteristics to the characteristics of environmental MPs. Commercial microspheres, although not usually well characterized, may have varying surface charge, contaminants from the production processes, and/or surfactants and additive chemicals, that tend to be absent or in addition to other acquired factors from the environment that are usually found on environmental MPs. Therefore, producing reference MPs that are more comparable to environmental MPs will be important in studying their effects on human health.

Production of Reference Microplastic Particles

Cryomilling of plastics has become a popular in-house method to produce MP fragments, shapes that closely resemble those found in the environment (Tewari et al., 2022). The cryomill is an ideal instrument to produce secondary MPs (plastics formed by the breakdown of larger plastics or primary plastics) as it grinds down primary plastics in the presence of liquid nitrogen, thereby, preventing the polymer from heating up or melting during processing and preserving the polymeric structure (Tewari et al., 2022). Many publications cite this method for creating MP reference materials (e.g., Jungnickel et al., 2016; Scircle et al., 2020; Tewari et al., 2022). Matthew Cole first described a method to produce reference MFs and this protocol has been highly utilized in the MPs field (Cole, 2016). Briefly, fibers are wrapped taught around a spool, glycol freezing solution is applied and allowed to freeze and frozen fiber sections are sliced using a cryostat at determined lengths based on aspect ratios. Glycol is dissolved and fibers are collected via filtration. Although, another fiber isolation technique is using ultracentrifugation, however, this technique is viable for more dense polymers as the less dense fibers will not pellet after centrifugation.

Producing in-house MPs via cryomilling and cryoslicing offers more versatility in terms of the polymers that can be processed; however, these methods must be optimized for each polymer type. For cryomilling, optimization factors include the number of milling cycles and the grinding frequency (McColley et al., 2023) and, for cryoslicing, the section thickness may need adjustment based on aspect ratios and is size limited based on the instrument that is used (Cole, 2016). Other potential drawbacks to cryomilling include time-intensive size fractionation of particles via sieving due to electrostatics, limited control over the size distribution, uniformity of the morphologies of the particles produced, and potential contamination from the components of the mill (McColley et al., 2023).

Microfibers are more consistent with environmental MPs than spherical particles, as fibers make up a substantial portion of the larger MPs present in the environment (Acharya et al., 2021). Microfiber cryoslicing is a promising method that achieves precise control over the fiber length, but it involves a tedious isolation process involving glycol dissolution and fiber capture (Cole, 2016). MPs found in the environment are the most relevant to study in terms of understanding exposure and effect; however, given current isolation techniques (trawl nets, grab samples, sieves, size-selective filters, etc.), their collection and isolation are resource- and time-intensive and require methods that manipulate the original state of the samples (i.e., using oxidizing agents to digest natural debris in collected samples) (Razeghi et al., 2021).

Current Challenges

Based on the status of the literature, persistent challenges have emerged including the size limitations on certain analytical instruments, a lack of relevant reference materials, and incomplete understanding of the relationships between the real-world exposures and experimental concentrations. Many of the ideal analytical instrument choices for characterizing MPs may have some combination of limited availability to investigators, size detection limits, and high purchase/operating costs. Fourier Transform Infrared (FTIR)

spectroscopy and Raman spectroscopy are ideal choices for identifying polymer composition of MP particles; however, these methods have size detection limits: FTIR requires $> 20\ \mu\text{m}$ sized particles and Raman requires $> 2\ \mu\text{m}$ sized particles for accurate identification. Thus, these techniques are unable to identify the smallest MPs and NPs which account for more than 99% of the total particles found in the environment by count concentration (Kooi & Koelmans, 2019). Another limitations of these methods is their difficulty in identifying chemical mixtures. Technologies such as pyrolysis-GC-MS, where particle size is not a limiting factor, and instruments with much smaller detection limits, such as μ -FTIR, can be cost prohibitive for many research groups. When available, Pyr-GC-MS, as well as liquid chromatography mass spectrometry (LC/MS) and High-Performance Liquid Chromatography (HPLC), are great choices for identifying and quantifying polymeric mixtures, additives (i.e., plasticizers), and co-pollutants that may be found in environmental samples.

A more accessible methodology, staining with Nile Red dye and analysis via optical microscopy, has been highly utilized in the field because it has been shown to stain many plastic polymer types (Erni-Cassola et al., 2017; Shim et al., 2016). However, this requires a large input of personnel time for particle quantitation and is more prone to human bias. Automation, including machine learning for image analysis, may eventually overcome these issues (Madejski et al., 2020). This is nevertheless limited to optical methods that are size-limited. Another consideration is that Nile Red is a lipophilic and hydrophobic stain, which can also stain particles of biological origin, creating the possibility of false positives, especially if methods of polymer identification are not accessible or applicable to the particle size. The journal, Science of the Total Environment (STOTEN), has recently published their minimum requirements for characterizing MPs in environmental samples, stating that visual and microscopic methods alone do not meet the minimum requirements for polymer identification. Spectroscopic techniques such as Pyr-GC-MS, FTIR, and Raman do satisfy their requirements (“STOTEN’s Minimum Requirements for Measurement of Plastics in Environmental Samples,” 2024). Thus currently, there are no widely-accessible techniques to accurately identify MPs that are smaller than $2\ \mu\text{m}$ in size. Optimistically, as the field grows, more technologies and methodologies will be developed to overcome these limitations.

The ubiquitous nature of plastic consumables in the laboratory creates a need for stringent controls to prevent sample contamination and these add considerable costs in terms of time and resources (Kutralam-Muniasamy et al., 2023). An example of a common material used in MPs research that can also be a source of plastic contamination is the filters used for MPs isolation. Many filter substrates are themselves polymeric. Other options include metal sieves, glass or quartz fiber filters, aluminum oxide filters, and silicon nanomembranes. Metal sieves have minimal plastic contamination risk and are reusable; however, they are available at very specific pore sizes that create larger size fractions that are biased towards the higher end of the MP size range (Saboor et al., 2022), rendering them unsuitable for NP isolation. Polymeric filters present a real plastic contamination concern and pore size can vary widely between filters, but they are more accessible in terms of cost and availability (Cai et al., 2020). Glass and quartz fiber filters are commonly used for particulate matter sampling and recently been employed in the use of Pyr-GC-MS to analyze airborne MPs,

but these, as well as the metal sieves, polymeric filters, and aluminum oxide filters are not optically transparent unlike the silicon nanomembranes, which may be an important feature when investigating particle count vs mass. Some filters can also be cost prohibitive (i.e., aluminum oxide filters). Silicon nanomembranes avoid many of the disadvantages of the other filter types as they have smaller consistent pore sizes (increased porosity), a wide range of pore selection and are an inert, inorganic material, however, they can be cost prohibitive and fragile (Carter et al., 2023; Madejski et al., 2020).

The MPs available for inclusion in toxicological studies, whether commercial or produced in-house, are varied. Unfortunately, this high degree of heterogeneity precludes comparisons between studies. This is worsened by the lack of standardized reference materials available for MP toxicological effects research. Toxicological studies have long employed reference materials to study the effects of novel stressors. The same could be done to better understand MPs toxicity. Reference materials are clearly needed, but a consensus as to what properties these particles should have and how they should be incorporated into studies is a matter of debate. Furthermore, commercially available MPs, limited by polymer type and morphology, are likely not developed for the purpose of toxicity studies. However, due to their availability, researchers still utilize these particles in toxicological effects studies, which can lead to variable outcomes. For instance, Ramsperger et al tested supposedly identical MP particles and showed that they differed substantially in characteristics and their responses to cell interactions (Ramsperger et al., 2022), demonstrating a clear disparity in the results from between seemingly identical particles purchased from two different manufacturers. More data is needed to evaluate the toxicological effects of MPs as the quantity of current published data is not comparable between studies due to the lack of standardized materials, protocols, and ultimately reproducibility.

Recommendations

Here, we propose solutions that can help increase the applicability and impact of future studies while reducing redundancy in the field. As the field is still relatively new, standard practices are being developed as research proceeds, creating the challenges described above. To overcome these pitfalls, development of standard particle characterization information needs to be included in all studies. The American Chemistry Council (ACC) hosted a multi-stakeholder workshop in May 2022 in Atlanta, Georgia, to discuss opportunities to develop such standardized reference particles and methods. The consensus relies on cryomilling particles of the most in demand polymer type, characterized and sold by the National Institute of Standards and Technology (NIST); however, these particles will not be available for some time.

Standardized methods, accepted by the field, for reference MPs production (i.e., cryomilling) is essential as well as a total comprehension of human exposure from environmental sources. Although, understanding of the effects of environmental MPs will change over time, having standardized procedures to estimate human exposure to environmental MPs will allow for more accurate dosimetry data for *in vitro* toxicological effects studies. Ideal studies will investigate the toxicity of isolated environmental MPs; however, this is a distant possibility due to the difficulty of isolating substantial amounts

of these particles without sample manipulation and the lack of reproducibility between samples. One way to circumvent this issue is to characterize a subset of environmental particles and use this information to develop specific environmental MP mimetics for use as novel reference materials.

To gain better mechanistic understanding of adverse responses to MPs in studies that employ cultured cells or model organisms, it is necessary to have a complete understanding of the physicochemical properties of the MPs to which humans are exposed. This should include, at minimum, confirmation of the polymer identity, chemical composition of any additives, and elemental analysis of possible inorganic contaminants, particle size distribution, surface charge, morphology, and concentration. Representative methods for assessing these properties are listed in Table 2.

In addition to a standard set of reported characteristics of the experimental MPs, the field could benefit from a more consistent approach to reference MP production. Because commercial sources are not a viable option in the near future, the existence of reproducible procedures for creating reference MPs would facilitate the shift from the less environmentally relevant PS microbeads to the more environmentally relevant fragments and fibers of other polymers, such as PE, PP, and PVC (Andrady 2011). Highly utilized techniques such as cryomilling (McColley et al. 2023), cryoslicing (Cole 2016), and dissolution-precipitation (Tanaka et al. 2021) present promising methods for creating a large variety and quantity of various MPs for use in MPs toxicological studies. These methods involve optimization for different polymer types and MP sizes, so standardized procedures should include parameters that serve the investigators ideal test conditions.

The first step in reference MP/mimetic design is selecting the polymer type, size, and morphology for the study. Figure 2 summarizes the best approach for reference MP production for in vitro toxicological effects studies based on these three criteria. For example, if the shape of particle being investigated is a fiber, the common Cole 2016 method can be used to create cryosliced fibers to an aspect ratio of 3:1 (*l:w*). Common feedstocks to this method are purchased from Goodfellow (Song et al., 2022). However, if a specific particle type is being investigated, then the cryomill approach to produce reference MPs may be considered. Common feedstocks of plastic polymer pellets include those found in the Polymer Kit 1.0 offered through Hawaii Pacific University Center for Marine Debris Research (Gao et al., 2022). Many studies have published protocols for cryomilling plastics to certain size distributions and these protocols can be referenced based on desired size (McColley et al., 2023).

An important aspect to understanding MPs toxicology is to investigate where they become localized within cells and organisms. A series of cellular internalization studies utilizing fluorescent MPs varying in size, charge, and polymer type, in combination with studies investigating whether MPs cross biological barriers, such as the blood-brain barrier, placenta, and gut epithelial barriers, would aid in understanding the absorption and distribution of MPs following exposure. Previous work in nanoparticles found that some of their behaviors were due to their nanosize (Lead et al. 2018). Additionally, studies that investigated the effects of nanoparticle properties on internalization found that

variables such as size, surface, and shape, impacted cellular internalization (Kettler et al. 2014) while studies investigating nanoparticle biological barrier crossing identified size, charge, and shape impacted the extent to which nanoparticles could cross barriers (Jia et al. 2020). Consistent with these findings, Wieland et al recently tested the influence of particle surface charge on the adhesion to and internalization into mouse macrophages and showed that adhesion and internalization were strongly correlated with zeta potential of commercially available PS particles (3 μm) of varying surface charge (Wieland et al 2024). The trends learned in nanoparticle research could be extrapolated to MP research to accelerate understanding of how MP variation may impact their absorption.

As is true for any other particle types that are used in cell culture model systems, the relationship between the applied concentration of MPs and the dose that reaches the cells will need to be measured and/or modeled in order to make inferences about relevance to real-world exposures in intact organisms. *In vitro* dosimetry models were developed for NPs (Deloid et al., 2017; Hinderliter et al., 2010) that can be used as a starting point for developing similar models for MP particles. MP buoyancy/density hydrodynamic size have been shown to influence particle settling amount and speed (Elagami et al. 2022). Similarly, studies investigating the variables associated with absorption and distribution of MPs *in vivo* would improve understanding of exposure-dose-response relationships.

When working with environmental MP samples, a considerable concern is contamination from the field, the work environment, experimental reagents, and laboratory supplies. Best practices to reduce MP contamination are advised and not always elementary. Some examples of best practices in the field are collecting field and blank samples, using proper controls for all known variables (including collection containers and mediums), and reducing MP contamination include working in a laminar flow hoods and wearing a 100% cotton lab coat. The first protocol in this series will outline these best practices for reducing MP contamination. Subsequent protocols will include isolating environmental and experimental MPs from environmental and biological samples.

Concluding remarks

MPs are an emerging contaminant of concern with unknown implications for both human and ecosystem health. Standard methods for creating and characterizing experimental and reference MPs, and for testing the biological effects of MP exposure in various organisms are instrumental for being able to interpret results in the context of MP toxicity as a whole. In addition to methodological standardization, the completion of studies of biodistribution, disposition (i.e., tissue and subcellular localization), the relationship between exposure and experimental dose, and the diversity in the physiochemical properties of environmental MPs will maximize the impacts of any new findings on the field as a whole. The MPs research field is currently in desperate need of the kinds of standardized methods and approaches described herein. Following the published protocols in this issue will contribute to this effort, which will increase reliability and reproducibility between studies, ultimately moving the field forward.

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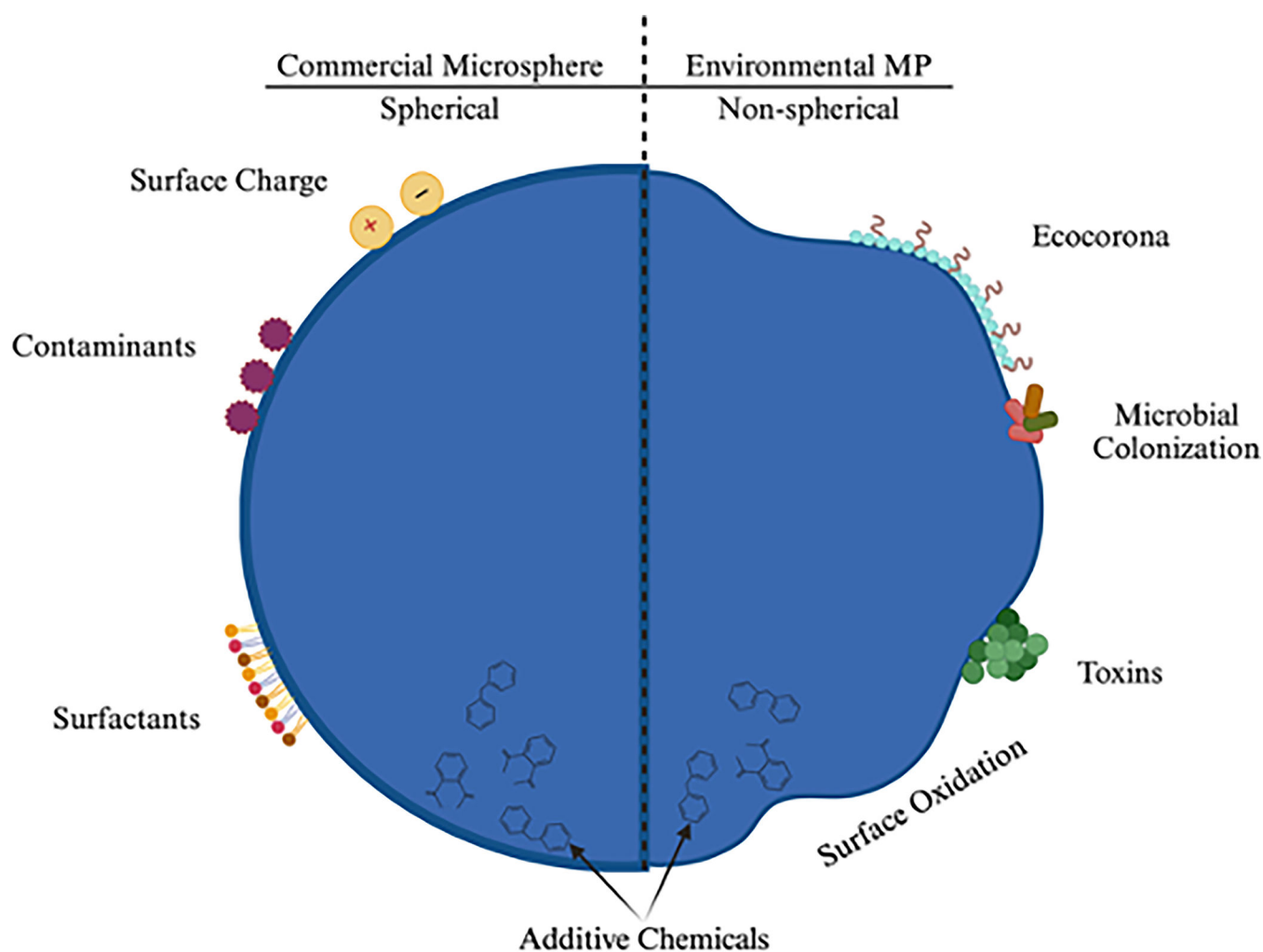


Figure 1. Schematic of commercial microsphere compared to environmental microplastic. Commercial microspheres usually have varying surface charge, contaminants from the production processes, and/or surfactants and additive chemicals, which can be absent or in addition to other acquired factors from the environment that are usually found on environmental MPs, such as surface oxidation, adsorbed environmental toxins, and/or an ecocorona with microbial colonization.

Reference MP Production

Morphology	Particle	Fiber
	Cryomill (McColley et al., 2023) 	Cryoslicing (Cole 2016) 
Polymer Type	Plastic Pellets as feedstock (i.e., Polymer Kit 1.0) (Gao et al., 2022) 	Plastic Fibers as feedstock (i.e., Goodfellow) (Song et al., 2022) 
Size	Refer to published protocols to obtain desired size distribution of particles. (McColley et al., 2023)	Usually at least a 3:1 (<i>l:w</i>) aspect ratio. (Song et al., 2022)

Figure 2. Example of best approaches for reference MP production.
The morphology of particle of interest for examination in toxicological studies will determine the best approach for in house production. This is not an exhaustive example of approaches for reference MP production, but stages the workflow for cryogenic milling or slicing for particles and fibers, respectively. The polymer type will determine the feedstock for each approach and size(s) of particles is dependent on feedstock.

Table 1.

Example methods used to study a specific endpoint in model organisms or cells.

Model Organism	Endpoint			
	Survival	Behavior	Oxidative Stress	Reproductive Toxicity
Invertebrates	<i>Daphnia magna</i> – Daily mortality (Y. Liu et al., 2022)	<i>Corbicula fluminea</i> – Siphoning & burrowing behavior (Fu et al., 2022)	<i>Daphnia magna</i> – Superoxide Dismutase (SOD) & Catalase (CAT) activity, glutathione (GSH) & Malondialdehyde (MDA) levels (Y. Liu et al., 2022)	<i>Daphnia magna</i> – Number of broods & offspring (Y. Liu et al., 2022)
Fish	<i>Danio rerio</i> – Daily mortality (Cormier et al., 2022)	<i>Danio rerio</i> – Novel tank diving test (Cormier et al., 2022)	<i>Cyprinus carpio</i> – CAT activity, GSH & MDA levels (Yedier et al., 2023)	<i>Danio rerio</i> – Number of eggs per fertilization attempt (Cormier et al., 2022)
Rodents	Mortality has not been observed	Mouse – Open-field and light-dark preference tests (Gaspar et al., 2023)	Mouse –SOD, GSH & MDA protein levels (Z. Liu et al., 2022)	Mouse –Follicle-stimulating hormone (FSH), luteinizing hormone (LH), estradiol (E2), and progesterone (Z. Liu et al., 2022)
Human Cells	Caco-2, A549, U937, THP-1, HaCaT and Jurkat cells – Changes in cell number (Gautam et al., 2022)	N/A	Caco-2, A549, U937, THP-1, HaCaT and Jurkat cells –Nitric Oxide levels & H2DCF-DA for Reactive Oxygen Species levels (Gautam et al., 2022)	N/A

Table 2.

Methods for measuring MP properties.

Property	Analytical Method
Polymer Identity	FTIR, Raman spectroscopy (Fu et al. 2020; Rocha-Santos and Duarte 2015; Tirkey and Upadhyay 2021)
Chemical Composition	(pyr)-GC-MS, HPLC (Fu et al. 2020; Rocha-Santos and Duarte 2015)
Elemental Composition	ICP-MS; SEM/EDS (Ivleva, 2021)
Particle Size	DLS, NTA (Fu et al. 2020); SEM (Ivleva, 2021)
Surface Charge and Characterization	DLS (Fu et al. 2020), AFM (Fu et al. 2020); ESEM-EDS, SEM (Rocha-Santos and Duarte 2015); TEM (Ivleva, 2021)
Particle Morphology	Optical microscopy, SEM-(EDS) (Rocha-Santos and Duarte 2015), LDIR (Dong et al. 2023)
Concentration	Optical microscopy (Rocha-Santos and Duarte 2015), LDIR (Dong et al. 2023)