Particle and Fibre Toxicology

REVIEW

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Addressing the relevance of polystyrene nano- and microplastic particles used to support exposure, toxicity and risk assessment: implications and recommendations

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Abstract

Background There has been an exponential increase in the number of studies reporting on the toxicological effects associated with exposure to nano and microplastic particles (NMPs). The majority of these studies, however, have used monodispersed polystyrene microspheres (PSMs) as 'model' particles. Here we review the differences between the manufacture and resulting physicochemical properties of polystyrene used in commerce and the PSMs most commonly used in toxicity studies.

Main body In general, we demonstrate that significant complexity exists as to the properties of polystyrene particles. Differences in chemical composition, size, shape, surface functionalities and other aspects raise doubt as to whether PSMs are fit-for-purpose for the study of potential adverse effects of naturally occurring NMPs. A realistic assessment of potential health implications of the exposure to environmental NMPs requires better characterisation of the particles, a robust mechanistic understanding of their interactions and effects in biological systems as well as standardised protocols to generate relevant model particles. It is proposed that multidisciplinary engagement is necessary for the development of a timely and effective strategy towards this end. We suggest a holistic framework, which must be supported by a multidisciplinary group of experts to work towards either providing access to a suite of environmentally relevant NMPs and/or developing guidance with respect to best practices that can be adopted by research groups to generate and reliably use NMPs. It is emphasized that there is a need for this group to agree to a consensus regarding what might best represent a model NMP that is consistent with environmental exposure for human health, and which can be used to support a variety of ongoing research needs, including those associated with exposure and hazard assessment, mechanistic toxicity studies, toxicokinetics and guidance regarding the prioritization of plastic and NMPs that likely represent the greatest risk to human health. It is important to acknowledge, however, that establishing a multidisciplinary group, or an expert community of practice, represents a non-trivial recommendation, and will require significant resources in terms of expertise and funding.

Conclusion There is currently an opportunity to bring together a multidisciplinary group of experts, including polymer chemists, material scientists, mechanical engineers, exposure and life-cycle assessment scientists, toxicologists, microbiologists and analytical chemists, to provide leadership and guidance regarding a consensus on defining what best represents environmentally relevant NMPs. We suggest that given the various complex issues surrounding

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the environmental and human health implications that exposure to NMPs represents, that a multidisciplinary group of experts are thus critical towards helping to progress the harmonization and standardization of methods.

Keywords Polystyrene, Microplastic particles, Monodispersed polystyrene microspheres, Spherical microbeads, Polystyrene manufacturing

Introduction

A growing body of research has evolved demonstrating strong evidence of the environmental ubiquity of microplastic particles (MPs) [1–8]. Furthermore, research has shown that MPs can enter the food chain either directly or indirectly [9–12] with suggestions produced from some studies implying the potential for long-term effects on human health [13–15]. It is not surprising, therefore, that both research [16, 17] and regulatory pressure [18–20] on MPs continues to grow.

Addressing the human health implications that exposure to MPs represents, however, is potentially hindered by a lack of consensus regarding the definition of microplastics. For example, the most commonly used definition of microplastics are plastic particles that are < 5 mm [21, 22]. The definition of both nano- and microplastic particles (NMPs), however, is known to be inconsistent between different research groups and regulatory bodies [23, 24]. The differences in terminology, consequently, can cause uncertainty and confusion when attempting to communicate the relationship between adverse effects observed in lab-based studies and the NMPs reported in monitoring studies. An important observation in this context is that while the majority of lab-based studies report effects on a homogeneous group of NMPs, which are typically consistent with NMPs within a physiologically relevant size range (e.g. < 10 µm), the majority of monitoring studies report MPs > 10 µm, and which are therefore likely to have toxicokinetic and toxicodynamic properties that are appreciably different from NMPs used in toxicity studies. Several reviews of the microplastic literature, for instance, have identified the obvious mismatch between the types of NMPs reported in environmental monitoring field studies from those used in laboratory-based studies [25–31].

While there have been several notable recent developments aimed at attempting to investigate the environmental fate and effect of NMPs using environmentally relevant NMPs [32], there exists a continuing need to raise awareness of the complexity of the polymer chemistry involved, and its likely impact on the relevance and reliability of laboratory-based research using commercially available NMPs [33]. While the MPs detected in the marine environment are typically characterised as predominantly a heterogeneous mixture of fragments and fibres of polyethylene (PE) and polypropylene (PP) [34–36] > 10 µm, the MPs reported in air comprise a variety of different types of polymers, among them PE, polyethylene terephthalate (PET), polystyrene (PS), polyamide (PA), polyvinyl chloride (PVC), and others [37]. On the other hand, the majority of laboratory studies have largely aimed at investigating the adverse effects of NMPs < 10 µm using monodispersed polystyrene microspheres (PSMs) [29, 35, 38]. The applicability of extrapolating results generated largely from a suite of PSMs towards the complex heterogeneous mixture of NMPs present in the environment has been questioned in the context of both environmental and human health risk assessment, with recommendations that future studies use environmentally relevant NMPs and/or provide greater information regarding the characterization of the particles used in a specific study [24, 33, 39–44]. Environmentally relevant NMPs, in this instance, refers to the use of NMPs in test studies that are known to be present in environmental systems. Consequently, recommendations regarding the use of environmentally relevant NMPs in toxicity studies would enable a more robust evaluation of the toxicological hazard that environmental exposure to NMPs represents to human health.

While there is a logical and rational basis that underlies the recommendations for the use of environmentally relevant NMPs, the generation and availability of these particles, however, is characterised by several non-trivial challenges. A key limitation that must first be overcome, for instance, relates to the current state-of-the-science regarding our understanding of what actually constitutes an environmentally relevant exposure to NMPs for human health. This includes a continuing need to strengthen the characterization and quantification of the types and physicochemical properties of NMPs present in the environment, particularly those within a physiologically relevant size range (for example, < 10 µm) [24]. Evaluating the characteristics and sources of the types and properties of NMPs representative of an environmentally relevant mixture of NMPs, however, is subject to numerous analytical challenges. For instance, plastic is subject to weathering [27, 45] and to microbial colonization in the environment. This can result in biofilm formation on the surfaces of plastic particles generated through the weathering process [6, 46], which can not only interfere with analysis but can also influence both the toxicokinetic and toxicodynamic properties of the particles.

Weathering of plastic is strongly influenced by the environmental conditions into which the plastic is released and can play an important role towards influencing the properties of NMPs generated and their environmental fate, transport, and relative toxicity [45, 47].

Recently, a number of researchers have attempted to investigate methods of artificially weathering plastic to generate NMPs, with the objective of better understanding the overall environmental and human health implications associated with weathering and aging processes [48–50]. For instance, UV degradation can result in oxidation of the surface of the plastic particle, causing changes to the surface chemistry of NMPs formed from the degradation process, whereas the formation of a biofilm can potentially inhibit surface functionality [51]. Furthermore, biofilm formation and weathering can result in modifications, such as the degree of crystallinity of the plastic particle, which can influence sorption and/or desorption of chemicals either originating from the plastic itself or from the surrounding environment [51–54]. The various changes that NMPs undergo as a result of weathering can also alter the relative density of the particle, which may result in increased rates of sedimentation in aquatic environments, for instance. For organisms ingesting NMPs colonised by microorganisms, the presence of an eco-corona as well as the relative size of the particle may strongly influence the physiological fate of the particle, including particle translocation [55, 56]. Given the stochastic nature of the various factors that can result in the generation of NMPs, which are formed from a wide variety of different types of plastic polymeric materials, combined with the influence of weathering with respect to their physicochemical properties, it is important to recognise the non-trivial challenge that defining an environmentally relevant suite of NMPs represents for the research community [57].

The objective of this review is to thus raise awareness of the challenges and limitations related to the continuing use of poorly defined NMPs used in laboratory studies, and which are being used to inform the human health implications that exposure to NMPs represents. Given current discussions related to the development of an international treaty on plastic pollution, which aims to include NMPs within the scope of the treaty [58, 59], it is thus important that effective regulation be guided by data generated from toxicity studies using environmentally relevant NMPs, and that caution be used not to overinterpret results from studies using NMPs that are inconsistent with environmentally relevant exposure. It is notable that in this review we focus on the polymer chemistry associated with polystyrene, largely because it is identified as the most frequently used type of plastic across all types of lab-based studies. In this instance, we aim to highlight the differences in the polymer chemistry between the types of PS that are commercially manufactured, summarizing their different methods of manufacture and the implications towards differences in their particle characteristics. In particular, we demonstrate that there are significant differences between the monodispersed PSMs purchased by research labs to perform various fate and effects studies, and the PS commonly used in consumer products and being detected in environmental field studies. Consistent with the observations of Wright et al. [33], we agree that an improved understanding of the adverse effects of NMPs can only arise through the generation and distribution of a suite of environmentally relevant NMPs. To achieve this goal, however, it is important to appreciate how differences in polymer chemistry and the manufacture and use of plastic articles in commerce influences the generation of NMPs most likely to represent an environmentally relevant human exposure. Consequently, we suggest that achieving the goal of generating a suite of NMPs representative of an environmentally relevant exposure for humans and ecosystems will require a framework that must include multidisciplinary expertise, including polymer chemists, material scientists/mechanical engineers, analytical chemists and toxicologists. We furthermore, suggest that this holistic approach is fundamentally critical if the significant challenge of defining the sources and characteristics of what might best represent an environmentally relevant suite of NMPs is to be achieved.

Literature review

Research aimed at assessing the environmental and human health implications that exposure to NMPs represents continues to grow in popularity [17]. Nevertheless, as noted above, there is continuing debate regarding a technical definition of microplastics, and there is currently no material standard that can be obtained which would be representative of the complex heterogeneous mixture that environmental exposure to NMPs is commonly understood to represent. To provide some insight into the types of polymeric particles that are commonly being used as a model for NMPs, we performed a literature review following Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines [60].

The PRISMA workflow was applied considering two complementary research strategies to retrieve potentially relevant studies, as follows: (i) comprehensive primary search using the PubMed-Medline electronic database, and (ii) secondary search through manual screening of the reference list of all relevant studies retrieved in the primary search.

To retrieve relevant research publications, a search strategy based on the use of relevant indexing keywords aimed at targeting studies that either generated and/or obtained NMPs from a supplier was adopted. Various descriptors and keywords were used to identify potentially relevant studies from a primary comprehensive literature database, which was created as an Endnote file from a search of the PubMed algorithm, used to obtain all studies aligned with the theme of "microplastic". To increase the scope of the search strategy, relevant studies and literature reviews were identified from the primary reference list and were manually screened to identify additional important studies that were not included in the PubMed search results. All relevant studies published from inception to 27 July 2023 were indexed and retrieved in full text to be included in the systematic review. No chronological or language limits were applied in the search strategy.

An initial keyword search of all Endnote listed fields, including the main text of the publication of the primary 'microplastic' Endnote file, which contained a total of 10,217 references, was conducted using the keywords 'Cryogenic', 'Milling', 'Generation', 'Microtome', 'Grind' and 'Cryotome. Table S1 (see Supp Info_1.pdf) summarises the results of studies identified that used and/or generated NMPs as part of their study to assess an adverse effect from either an ecotoxicological or human health perspective, to evaluate the environmental fate or translocation of NMPs across biological tissues, or to support the analytical method development regarding the analysis of NMPs in various matrices. To widen the scope of the primary Endnote file and to ensure a comprehensive search of all potentially relevant studies, a keyword search of the publication title field was included for studies not identified using the initial keywords described above and for which full text was not available. The keywords used were 'Extraction', 'Synthesis', 'Production', 'Reproductive', 'Metabolism', 'Growth', 'Inflammation', 'Oxidative', 'Polystyrene' (PS), 'Polyethylene' (PE), 'Polypropylene' (PP), 'Polyurethane', 'Nylon', 'Polyamide', 'Polycarbonate' (PC), 'polyvinyl', 'polylactic', 'effect', 'toxicity', and 'analysis'. All studies identified were manually screened for relevance, and the full text articles obtained, resulting in a total of 2607 publications. The types of polymers used in each of the studies is identified, with Table S2 (see Supp Info_1.pdf) providing a summary, where 44.5% of studies (1160/2607) used only PS as part of their study design. The majority of studies used PS either as part of their ecotoxicity or in mammalian in vivo or in vitro cell-based studies (Table S3 in Supp Info_1.pdf and Supp Info_2.xlsx).

Based on the observations from the literature review performed, which are consistent with other similar observations of the literature where the predominant use of PSMs has been observed [27, 29, 38], it appears reasonable to consider the potential differences of the PSMs used in microplastic research and its relevance towards understanding polystyrene microplastic that might be generated as a result of degradation of PS products used in commerce. The objective of the following sections is to thus consider the different chemistries involved, and the potential implications that relying on the use of PSMs represents as either facilitating understanding of the environmental and human health implications that exposure to MPs represents, or as a potential hindrance.

Polystyrene—background

From a polymer chemistry perspective, PS belongs to the group of thermoplastic polymers. Since its commercialization in 1931 by IG-Farben (BASF) [61], the production quantity of PS has increased to 18.8 million tonnes in 2018 [62], and represents about 5% of the total global plastics production, behind PP, PE, PVC, PET and polyurethane [63]. Depending on the purpose and application, PS is either manufactured unmodified, with rubber modification or foamed. Pure, unmodified styrene homopolymer, commonly referred to as general purpose polystyrene (GPPS) is characterised as a brittle material. Co-polymerization with a rubber component such as polybutadiene can significantly help to strengthen PS, resulting in the formation of "high impact polystyrene" (HIPS), which is characterised as an opaque and less brittle polymeric material [64]. A complex three-phase morphology is thus formed with polybutadiene particles, which contain polystyrene inclusions, that are dispersed in a polystyrene matrix, and which gives rise to a stronger material, as compared to GPPS [65]. Foamed PS can be produced either from expandable granules (EPS) or as extruded rigid foam (XPS), both of which can contribute to NMPs detected in the environment [66, 67]. It is notable that XPS is often misidentified as EPS, largely due to a lack of analytical protocols capable of reliably differentiating between the two types of PS [68].

Depending on the manufacturing process and/or intended functionality of the PS material, different chemical processing agents and/or chemical additives can be used to support the manufacturing process and to improve the properties or processability (see Table 1). Intentionally added substances (IAS) are added during manufacturing of the polymer. They include processing aids and additives. Processing aids either enable, facilitate or ease the production and/or processing of the polymer (e.g., polymerization catalysts, solvents, or lubricants). Most processing aids are consumed during the polymerization process and therefore do not persist in the final polymer, except possibly at trace levels. Additives are

Table 1 Common processing aids and additives used in PS, EPS and XPS grades [64, 65, 70, 76, 84–86]

Substance group	Function	Examples
Processing aids	Initiator	Peroxides (e.g. dibenzoyl peroxide)
	Chain transfer agent	Mercaptans Terpinolene Dimeric alpha-methyl styrene
	Finishing initiator	tert-butyl peroxybenzoate tert-butylperoxy-2-ethylhexyl carbonate tert- amylperoxy-2-ethyl hexylcarbonate
	Dispersant/ surfactant (Pickering stabiliser)	Poly(vinyl alcohol) Hydroxyethylcellulose Polyvinylpyrrolidone Gelatine $Ca_3(PO4)_2$ $BaSO_4$ $CaCO_3$, Sodium alkylbenzene sulfonate
	Stabilizer	MgO
	Nucleation agent	Waxes (such as paraffins, chloroparaffins, and Fischer–Tropsch waxes) Esters and amides of fatty acids Phase-incompatible polymers (e.g. low molecular weight PE)
	Blowing agent	CO_2 Hydrocarbons (e.g. pentane)
	Fast-cool agent	Mixtures of glyceride esters of higher fatty acids, preferably with carbon chain lengths of 14–20
	Anti-static coating	Esters of fatty acids and amines (e.g. glycerol monostearate) Quaternary ammonium salts Alkylphosphates Fatty alcohol condensed with ethylene oxide on to propylene oxide
	Anti-lump coating	Metal stearates SiO_2 or $CaCO_3$ powder Powders of polyamide waxes
Other polymer components	Impact modifier	Polybutadiene
Additives	UV absorber/ inhibitor	2-(2′-Hydroxyphenyl)-benzotriazole derivatives
	Primary antioxidant	Sterically hindered phenols or secondary aromatic amines (chain terminating donors for peroxy radicals) Sterically hindered amines (radical scavenger / chain terminating acceptor)
	Secondary antioxidant	Phosphites Thioesters (peroxide decomposers)
	Flame retardant	Styrene-brominated polybutadiene block copolymers Resorcin-bis(diphenylphosphat) or other phosphates + synergist (dicumyl peroxide, dicumene, azo derivatives, 2,3- dimethyl-2,3-diphenyl butane, antimony trioxide)
	Flow enhancer	White mineral oil (low molecular weight paraffins) Calcium stearate
	Mineral filler	$CaCO_3$, Talcum Wollastonite Glass fibres SiO_2
	Insoluble colorant	Inorganic pigments, e.g. TiO_2 Organic pigments
	Soluble colorant	Polycyclic anthraquinones and perinones Selected azo compounds
	Optical brightener (compensation of yellowness)	Bis-benzoxazoles Phenylcumarines Bis-(styryl)-biphenyls

substances which are "intentionally added to plastics to achieve a physical or chemical effect during processing" of the polymer "or in the final material or article"; they are "intended to be present in the final material or article" [69]. While the content of additives is typically only in the few percent level, their impact on polymer performance and stability is substantial. In addition to these intentionally used chemicals, non-intentionally added substances (NIAS) can also be present in polymers. These are not added for a technical reason during the production process but rather are formed as byproducts and degradation products in the manufacturing process or are present as contaminants in raw materials [69].

The high glass transition temperature (T_g) of PS (100 °C [70]) results in a plastic polymeric material that causes relatively low diffusivity of fillers and chemical additives leaching from the plastic [71], so that the risk of migration is generally understood to be low [64, 72, 73]. Nevertheless, given recent concerns regarding the use and potential release of chemical additives from plastic and/or NMPs, there is a need to strengthen our overall understanding of their use and diffusive exchange with the surrounding environment [74]. Consequently, in addition to summarizing the manufacturing processes associated with the different types of PS, we also provide a brief summary related to the use of both chemical processing agents and chemical additives that might be specifically associated with the manufacture and use of PS in commerce.

Manufacturing processes for GPPS

Commercially, GPPS as well as HIPS are manufactured by free radical polymerization in a continuous process [64, 65, 75]. The majority of GPPS is currently understood to be produced by solution polymerization, which has the advantage of enabling better control over the reaction temperature, which represents an important factor towards ensuring the formation of a narrow molecular weight distribution [70]. The addition of an organic solvent (e.g. ethyl benzene (EB) or toluene) as a chemical processing agent, typically between 5 to 10 wt.%, functions as a weak chain transfer agent and helps to reduce the overall molar mass [65, 75, 76]. Other chemical reactants, however, can also be used to generate a specific desired molar mass (see Table 1) [76].

A general concern raised regarding the manufacturing process and the use of chemical agents, relates to the potential presence and subsequent exposure to monomer residuals and unreacted solvent [74]. Depending on the initiation method and polymerization process, however, the types of chemicals and quantity of residual can vary. Thermal initiation, for instance, can result in a higher number of residual dimers and trimers, whereas peroxide initiated suspension polymerization results in significantly lower levels of residuals. The removal of residual monomers and solvent, particularly during the solution process, is achieved via post-polymerization devolatilization. There are several ways to remove these volatile components, such as by vacuo with degassing extruders, wiped film evaporators or flash evaporators [70, 76]. Further removal of residuals can be achieved by steam stripping [70]. Consequently, at the end of the production process, the reaction mass of the final product can still contain residual monomer and unreacted solvent (if applicable), but the inclusion levels are typically understood to be well below 1% [77]. Commercially available PS from different manufacturers may vary considerably in the levels and composition of process residues and additives. This detail is often confidential business information and not freely available.

Manufacturing processes for HIPS

For the production of HIPS, polybutadiene is dissolved in styrene. After initiating the polymerization process, polystyrene domains are formed within the continuous rubber phase. As the reaction proceeds, the amount of PS in the rubber phase increases, until an equal volume between the polystyrene and rubber phase is achieved. At this point, the application of a sufficient level of agitation causes a phase inversion to be initiated. The remaining styrene then polymerizes to a continuous polystyrene matrix. Following the phase inversion, the rubber domains cease to increase and maintain their boundaries as the polymerization in both phases continues [64, 70]. The mass polymerization of HIPS is predominantly done in a continuous process. There are various processes described in the patent literature [78–83], whereby the initiation is most commonly conducted under thermal conditions [76].

Similar to GPPS, the final polymer will contain residual monomers, oligomers and solvent (if applicable). Consequently, the application of devolatilization helps to reduce these unwanted residuals. The additives commonly present in HIPS are generally consistent with the types of chemical additives used in GPPS, see Table 1. In instances where the polymerization process is initiated using peroxide initiators, only phenolic antioxidants may be used, which is because the use of phosphite antioxidants will result in a reaction with the peroxides that causes the reaction to be slower. It is common practice to add the antioxidants prior to the polymerization process [64].

Manufacturing processes for EPS

Expandable polystyrene beads are produced by one of the following two processes [64, 65, 70, 76]:

1. Suspension polymerization of styrene into spherical beads in the presence of a blowing agent (often pentane in amounts of up to 7 wt%).
2. Extrusion of GPPS, addition of a blowing agent to the melt during extrusion, followed by underwater pelletization. In this case, the EPS particles are not perfectly spherical. This process is less common for EPS.

Suspension polymerization of styrene into spherical beads supports the production of a desired morphology of expandable particles, without the need of further modification. The blowing agent can be added during or after the polymerization. Water-insoluble monomers are dispersed in water in the presence of a suspension stabilizing agent (see Table 1). The polymerization takes place within the monomer droplets following a free radical mechanism initiated by peroxides [70]. If low residual monomer concentrations are required, a second finishing initiator is added, which disintegrates only at higher temperatures [87].

Manufacturing processes for XPS

Extruded PS foam (XPS) is produced from GPPS. In an extruder, a blowing agent, e.g. CO_2, is added to the molten PS. The melt is extruded through a nozzle with a wide slit, resulting in a closed cell foamed board [84, 88, 89]. In contrast to EPS, XPS has a closed-cell structure with the cells tightly packed together and no voids between them [84].

Manufacture of monodispersed PS microspheres

Comparable to the production of EPS particles, monodispersed PS microspheres (PSMs), which represent particles with an homogeneous suite of physicochemical properties including size, shape and polymer type, can be produced for specialised purposes. An important differentiating factor between EPS and PSMs, however, is that the suspension polymerization process for PSMs occurs without the use of a blowing agent. Furthermore, the use of suspension polymerization is only one of several different methods to generate PSMs, which can be influenced by the intended purpose and desired properties of the PSMs [90]. PSMs are commonly produced in the form of monodispersed microspheres, in well-defined sizes that range from below 1 µm up to sub-mm. Sometimes these microspheres are referred to as "uniform latex particles". These PSMs are used in research and analytics, e.g. as calibration beads for count control, filter leak testing, flow cytometry and other techniques, where their defined particle size facilitates calibration of an analytical instrument and/or research method. They were first used in medical diagnostics in the form of "latex agglutination tests" (LAT) in 1956 and are used today in a variety of strip tests in human and veterinary medicine, plant health, law enforcement, food, or the environment, with the sub-micron sized PSMs acting as a solid support platform for antigens or antibodies [91, 92]. A detailed summary of the various suppliers and types of PSMs that have been used most often in NMP research is provided in the Supplementary Information (Tables S2, S4 and S6 in Supp Info_1.pdf).

While there exists a general understanding regarding the production of PSMs, the precise conditions of their synthesis are communicated as representing confidential business information by the various manufacturers, and our attempts at disclosure requests have proved unsuccessful to date. We further note that additional product information with respect to the chemical composition and characterization of PSMs, such as might be obtained from product data sheets, is typically limited. It also proves difficult to determine if a specific supplier is the actual manufacturer of the PSMs, or if they are simply a distributor. When considering the suppliers listed in the Supplementary Information (Tables S2, S4, S5 and S6 in Supp Info_1.pdf), it is apparent that the majority of studies that report using PSMs in the context of microplastic research have obtained their materials from suppliers predominantly based in China, with other suppliers from countries, such as in the USA, Germany, the UK and Japan also being identified. Although we encountered difficulties towards obtaining a complete understanding of the manufacture and characteristics of commercially available PSMs, information available in patent applications, as well as from the scientific and technical suppliers´ literature, provides important insight regarding the fundamental components used in the manufacture of PSMs, insight which can also be used to better understand the implications regarding their physicochemical properties and ultimately to the risk of NMPs to human health and the environment. Figure S1 (see Supp Info_1.pdf) illustrates the variety of properties that can be found in PSMs.

It is commonly understood, for instance, that PSMs can be produced directly from styrene monomers, such as by suspension, emulsion or dispersion polymerization techniques, depending on the intended target size of the PSMs [90, 93]. Suspension polymerization, for instance, typically generates PSMs in the size range of between 40 and 1000 µm, emulsion polymerization provides PSMs in the size range 0.1 to 10 µm, whereas dispersion polymerization is suitable for the synthesis of particles in the range of 1–10 µm [93]. Figure 1 shows the attainable particle size ranges of the different polymerization methods in logarithmic scale.

In emulsion polymerization, a monomer that is almost insoluble in water is dispersed in an aqueous phase that

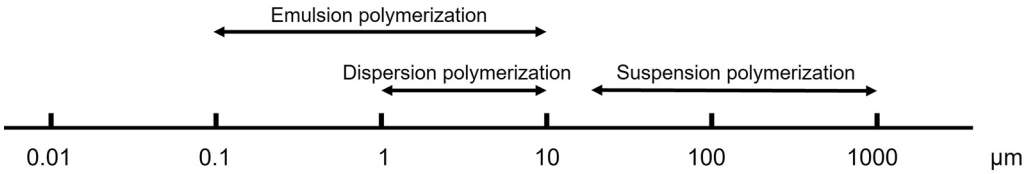

Fig. 1 Particle size ranges of emulsion, dispersion and suspension polymerization in logarithmic scale

contains a water-soluble initiator and a colloidal emulsifier or surfactant. Formation of micelles occurs if the concentration of the surfactant is high enough [94]. The initiator reacts to free radicals which first polymerize with monomer molecules in the aqueous phase until a critical chain length is achieved and the growing radical becomes hydrophobic enough to enter the micelle [95]. Monomers migrate from the monomer droplet reservoirs into the micelles, where they react with the propagating polymer chain. Figure 2a) shows a schematic representation of the emulsion polymerization process. Emulsion polymerization represents one of the most commonly used methods for the synthesis of PSMs. The resultant particles (0.1 to 10 μm), however, tend to be polydisperse (i.e., particles are of varied sizes in the dispersed phase of the dispersion system), and are therefore less appealing for applications that require a narrow particle size distribution, such as for the calibration of analytical instruments. To address this shortcoming associated with the emulsion polymerization process, adaptations can be incorporated that result in a narrower particle size distribution, e.g. applying seed methods [96].

Dispersion polymerization starts with a homogenous solution of monomers, surfactants and initiator [97]. The formed polymer itself is not soluble in the reaction medium and thus will precipitate when it is too large to be stabilized by the surfactant [98]. Figure 2b) shows a simplified scheme of the dispersion polymerization process. With this method, micron-sized PSMs (1–10 μm) can be synthesized [92, 99–102]. The method results in particles with narrow particle size distribution when monomer concentrations are not too high [101]. It is also possible to generate functionalized or crosslinked particles in dispersion polymerization processes [98].

In suspension polymerization, the monomers and initiators are insoluble in water. In the presence of surfactants, large droplets with monomers and initiator are formed in which the polymerization takes place [94]. The use of an organic solvent is possible, but not necessary. Figure 2c) shows the basic principle of suspension polymerization. Suspension polymerization typically results in the production of larger micron-sized particles (40 to 1000 μm), which are not as commonly used in the microplastic research. The width of the particle size distribution depends on the initial droplet size, which in turn depends on the stirring intensity and type and concentration of the surfactant [103].

Nanosized PSMs with a small standard deviation in particle size are typically synthesised by surfactant-free emulsion polymerization (SFEP) with potassium persulfate as the initiator [104] and coated/functionalized using surfactant-free seeded emulsion polymerization (SSEP) [105]. Consequently, understanding the nature of the polymerization technique can represent an important factor towards better understanding the sizes of particles generated as well as an expectation regarding the variability of the particle size distribution.

Functional properties of PSMs

Depending on the research question, it can be assumed that an attractive property of PSMs for microplastic researchers is their monodispersity, where it is desirable that at least 90% of the particle size distribution be within 5% of the average particle size [90]. A factor that can negatively influence the monodispersity of generated PSMs, however, is their propensity to form particle aggregates in solution. Consequently, following the generation of PSMs, it is typical for the particles to be stabilised in order to prevent their aggregation. One strategy to prevent aggregation is the application of a hydrophilic coating to the PSMs, or alternatively the introduction of reactive groups to the surface of the particles, whose functionalities induce a specific surface charge that discourages particle–particle interactions [93]. Specifically, the addition of anionic or cationic groups results in the formation of negative or positively charged surfaces, respectively, of the PSMs [106]. Functional groups include amino, carbonyl, carboxyl, hydroxy, epoxy, sulfate, sulfonate, and aldehyde groups. They can be incorporated into the polymer by intentional addition of hydrophilic comonomers, such as carboxylic acid, butyl acrylate, acrylic acid, or methacrylic acid, typical of PSMs available from Bangs and Thermofisher [107–109], but can also be formed through the use of initiators, including potassium persulfate or benzoyl peroxide, such as associated with PSMs available from Spherotech, but also from Thermofisher [102, 109]. One possibility for the stabilization of PSMs is the introduction of a negative

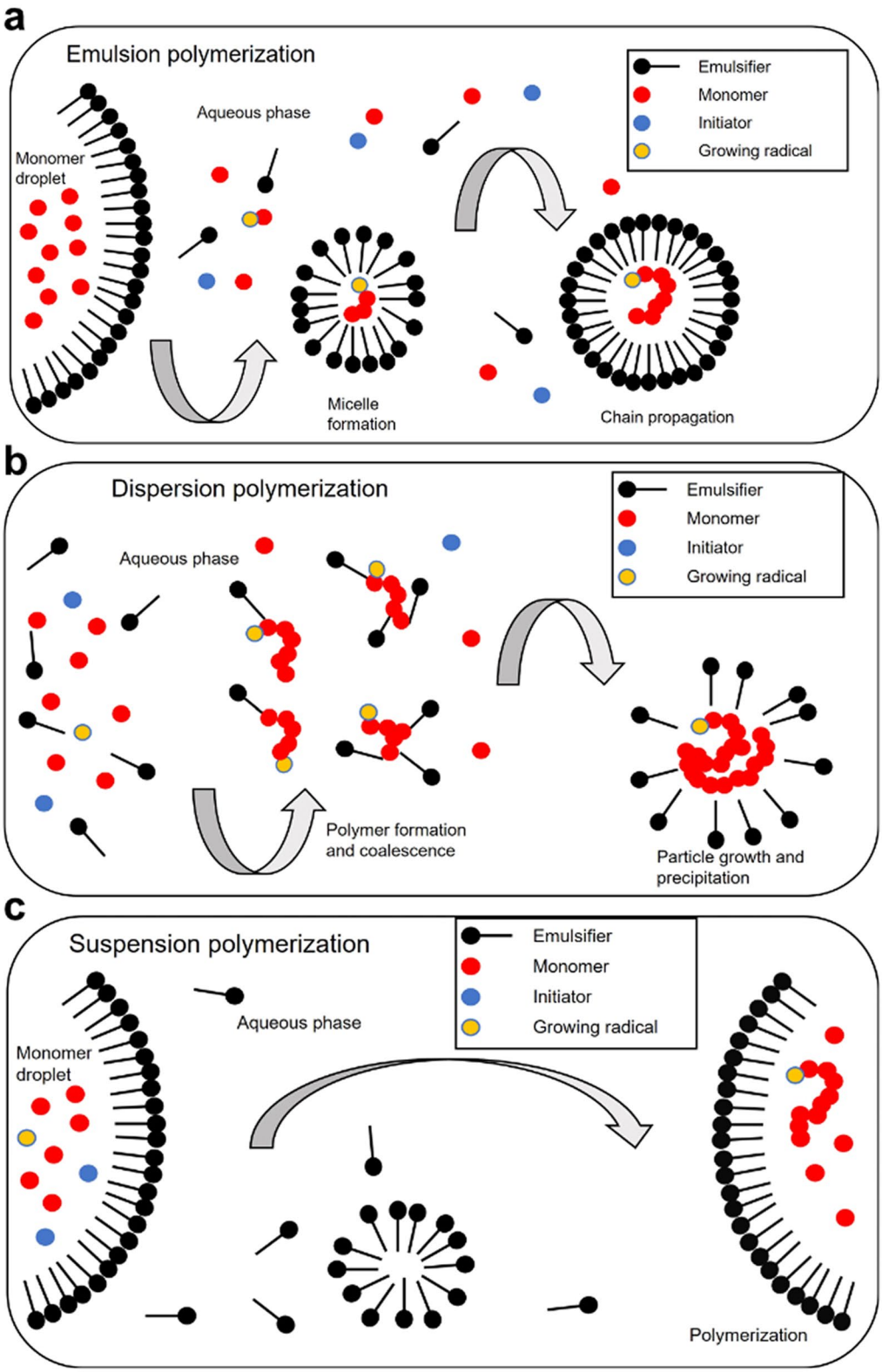

Fig. 2 Schematic representation of **a** emulsion polymerization, **b** dispersion polymerization, and **c** suspension polymerization

charge by a combination of sulfate groups, and adsorbed anionic surfactant on the surface e.g. [109, 111]. It is important to note that the preparation of PSMs without the use of a surfactant requires the inclusion of a higher number of sulfate groups, as these are necessary to support stabilization, which is lost when the surfactant is not included. The charged particle surface area is typically between 5 and 10% [109], which is understood to sufficiently prevent the aggregation of PSMs in the stock solution obtained from a supplier.

In general, the most commonly available surface functional groups in PSMs are carboxyl and amino groups. The density of carboxyl or amino groups on the surface of the microsphere can vary and can be determined using conductometric titration [108]. It is often referred to as the COOH surface titer and expressed in units of $Å^2$/COOH group, resembling the average space a reactant would have on the microsphere surface when coupling to a COOH group if all COOH groups are used. A closely packed monomolecular layer of acid groups would have an area of 20–25 $Å^2$/COOH group, therefore microspheres with an area smaller than 20–25 $Å^2$/COOH group are considered as completely coated by acid groups. PSMs with a surface titer of around 100 $Å^2$/COOH group or more are considered as having a low coverage. For amine-modified microspheres, the presence of NH_2 groups is confirmed using a ninhydrin test [108, 109, 111]. This method is also referred to as the *Kaiser* test [112] and determines free amino groups by reacting them with ninhydrin to form a chromophore [113, 114]. Measuring the COOH or NH_2 surface groups of a particle reveals more information on the particle surface as a calculation of the particles' zeta (z) potential can deliver. The zeta potential is the difference in electrical potential at the interface of a particle with its surrounding medium. It is used as an indirect estimation of the surface charge density of a particle and can be measured by electrophoretic light scattering (ELS) [115]. The zeta potential represents the parameter most typically reported in the microplastic literature as a relative indicator of the surface charge of a particle, since it is relatively easy to measure, and has consistently been proposed in guidance documents as a particle property that should be included when characterizing nanoparticles [116, 117]. Several confounding factors, however, can result in difficulties with respect to measuring, integrating and interpreting zeta potential values reported in the literature, requiring caution not to overinterpret data obtained [116, 117]. It is thus recommended that a thorough characterization of the surface functionality consider measurements of the surface titer of the carboxyl or amino groups, especially when interpreting the toxicological effects of a specific particle on a biological endpoint.

Although carboxyl and amino groups are commonly used, there are a variety of functional groups that can be added to PSMs to support their application in the context of biological, biomedical and other research areas, which include a variety of different chemical groups and biomolecules such as antigens, antibodies, oligonucleotides etc., which make them a versatile carrier material. For example, PSMs can be used to detect the presence and quantify the number of biomarkers or biological species in bodily fluids, with the PSMs used either in suspension arrays or in planar microarrays [93]. Introduction of functional groups can be done either by copolymerization with functionalized monomers or by modification after the polymerization step [102].

Consequently, the addition of functional groups to the surface of PSMs not only aims to provide a surface charge to prevent aggregation, but the functionality can also support the use of PSMs in the context of biomedical arrays and other applications, such as to support mechanistic toxicity studies aimed at evaluating the influence that a specific functional group may have on a biological receptor. The functionalized surfaces have been observed to facilitate cell adhesion [93, 118, 119]. Local electrostatic interactions between charged groups on the particle surface and cell membranes are an important factor for the particle-cell adhesion strength [120]. But functionalized surfaces can also adversely affect cell viability [121, 122]. The cellular uptake can either be enhanced or inhibited, depending on the cell type and surface charge [122], thus also influencing the toxicity of PSMs [123, 124]. Furthermore, the dispersants used for particle dispersion could be responsible for their in vitro toxicity [124].

A useful functionality that is commonly used to track PSMs in biological tissues includes the addition of a fluorophore. The incorporation of fluorophores represents an important property that enables the fate and behavior of PSMs to be traced and analytically quantified. There are a variety of PSMs that can be obtained, with various fluorescent organic dyes or other types of markers [125]. Within the microplastic research literature, these markers have been commonly used to evaluate the translocation of PSMs across epithelial tissues, as well as helping to support the development of analytical methods. Dyes or markers can be incorporated into the PSMs using a variety of methods, and which may have important implications when attempting to interpret the results of a specific study. Table 2 summarises the different methods available.

There are several different types of organic dyes used for fluorescence marking of PSMs. Interestingly, the dyes (fluorophores) are similar to the ones used for staining antibodies, proteins and other cell components, cells and tissue [132]. Some fluorescent dyes such as rhodamine

Table 2 Methods to incorporate fluorophores in PSMs

Method	Description	Comment
Internal dying with a solvent swelling/dye entrapment technique [108, 126–128]	The (non-crosslinked) polymeric microspheres swell in the solution of the dye in an organic solvent. The water-insoluble dye diffuses into the polymer matrix and is entrapped when the solvent is removed from the PSMs through evaporation or transfer to an aqueous phase. Internal dyeing produces very bright and stable particles with typically narrow fluorescence spectra. Surface groups remain available for conjugating ligands (proteins, antibodies, nucleic acids, etc.) to the surface of the PSMs	Internally dyed PSMs offer a greater resistance to photobleaching. For visibly colored (non-fluorescent) dyes, an amount of dye equal to approximately 10–40% of the microsphere weight may be entrapped within each microsphere, and up to approximately 1% of the microsphere weight for fluorophores. Depending on the surrounding solvent or medium and its Hansen solubility parameter, PS particles may swell and then degrade more easily, and also may leach the incorporated fluorescent dye into the surrounding medium [129]
Polymerizing a fluorophore in styrene in the presence of "polystyrene core" particles [130]	Copolymerization of a monomer and a dye-containing comonomer, for example, a free radical-initiated, anaerobic copolymerization of an aqueous suspension of a mono-unsaturated monomer that may or may not contain a covalent bonding group (e.g., a carboxyl, amino or hydroxyl group) and a fluorescent monomer mixture	Cross-linking of the polymeric matrix is preferred to maintain the stability and size of the microspheres in aqueous solutions and organic solvents, as this would limit the amount of leaching that might occur
Surface attachment of fluorophores [108]	The fluorophores are attached to functional groups at the surface. Surface-labeled microspheres will have fluorescence intensities in the range of labeled biological samples [131]	This method offers other unique benefits, such as environmental responsiveness of the dye and spectra much like the free dye. It is particularly useful where PSMs will be used in a solvent and will swell, as the dye will not escape

and fluorescein are often used to prepare fluorescent microspheres [133]. The development of dyes for molecular imaging primarily focused on optimizing fluorescence brightness, photostability and pH-insensitivity, e.g., leading to a series of sulfonated coumarin and rhodamine dyes (Alexa dyes) [134].

In the context of toxicological effects testing, it is important to note that some fluorescent dyes can potentially influence a toxic response [135, 136]. Table S7 summarises the toxicity of 19 organic fluorophores used in molecular imaging, as reviewed by Alford [135]. Consequently, it is recommended that the use of fluorescent dyes be thoroughly investigated before they are used in studies to determine the toxicity of PSMs, otherwise it is not clear if any observed effect is caused by the PSMs or by the fluorescent dye, particularly since the toxicity information may be unavailable for some fluorophores. One approach would be to avoid the use of fluorescent PSMs when investigating toxicological endpoints, limiting the use of these materials towards investigations with respect to mechanisms of cellular uptake or other fate processes. It is imperative to note, however, that when used to evaluate the absorption and systemic distribution of PSMs, such as from the gastrointestinal or respiratory system, that researchers carefully consider the potential for some fluorescent markers to leach from the microsphere into the surrounding medium, which has been observed and reported as a complicating factor when attempting to interpret data from such studies [129, 137]. Alternatively, tagging of the microsphere by another means, such as ^{14}C, should be explored, although in this instance there are financial implications and permitting for use and disposal associated with generating radiolabelled microspheres that may limit their feasibility.

Finally, it should be noted that while commonly used GPPS and HIPS are not produced as cross-linked polymers, PSMs are typically manufactured by copolymerization of styrene and small amounts of divinylbenzene (DVB) [93, 138]. For example, both GPPS and HIPS, typical of PS used in consumer products, are known to swell under conditions of contact with high ethanolic solutions and increasing temperature [139]. If it is intended to reduce swelling of PS, and thus improve the solvent resistance, the polymer chains can be crosslinked. Crosslinking is not typical in the production of GPPS and HIPS intended for industrial use in the packaging or construction industry, as it would prohibit typical processing of the material (extrusion, injection molding), but represents a common practice for PSMs intended for analytical purposes or for ion exchangers [102, 108, 110]. The degree of crosslinking of PSMs, therefore, represents an additional difference between PS used in consumer products for industrial use, and some PSMs, the importance of which is not reported or evaluated in the microplastic literature. Given that not all PSMs are crosslinked, it may prove beneficial to better understand the role that swelling may play in the context of a specific research question. Circumstances where solvent induced swelling can be desirable in PSMs, for instance, includes research that requires PSMs to be dosed with hydrophobic materials [140] or loading of fluorophores into the PSMs [141].

Environmentally generated polystyrene microplastic

The various types of functionalization that can be included in PSMs, and their application to support various scientific needs, using the methods summarised above, however, is notably inconsistent with the production of polystyrene used in commerce. Consequently, factors used to inhibit aggregation in PSMs, and/or the addition of functional groups aimed at a specific research need, can result in potentially significant differences regarding the toxicological effects between PSMs and environmentally relevant NMPs that might be generated from PS used in consumer products. Specifically, PSMs can be obtained in either an aqueous or nonaqueous suspension that can include various surfactants, buffer salts and preservative agents, such as thimerosal (sodium 2-(ethylmercurithio)benzoate), a mercury-containing preservative considered as causing hypersensitivity in humans and very toxic to aquatic organisms [142, 143], or sodium azide, a substance considered very toxic to aquatic life as well [102, 108, 111, 143]. Conversely, the generation of PS microplastic originating from consumer products are unlikely to contain surfactants or preservative agents typically associated with PSMs and are more likely to be characterised by a heterogeneous mixture of functionalized groups, as opposed to the relatively homogenous properties of PSMs used to support a variety of scientific research needs.

Heterogeneity of the surface groups and electric charges that are likely associated with PS microplastic generated from consumer products is further confounded by a variety of other factors not typical of PSMs. For instance, varying topography, chemical composition, and reactivity as well as the presence of IAS (Table 1) and NIAS, represent important factors that must be considered when attempting to evaluate and extrapolate differences between different types of environmentally relevant NMPs [144].

Whereas the manufacture of PSMs targets the production of a monodispersed suite of particles that can include various functionalities, the generation of NMPs that might originate from PS used in consumer products occurs via a variety of different pathways in the environment. It is widely understood, for instance, that the majority of NMPs detected in the environment, and

which comprises various types of polymers, including rubber from tire abrasion and fibres from clothing, are formed as a result of environmental degradation and fragmentation of plastic articles used in commerce [62, 145]. Environmental degradation processes include chemical reactions such as UV-light induced photooxidation and crosslinking, physical processes (abrasion, pressure, expansion, contraction) and biological processes such as metabolism by enzymes [146]. The colonization by microorganisms and subsequent biofouling also plays a major role in environmental degradation processes [125]. An initial eco-corona of biomolecules such as proteins, lipids, or carbohydrates) [57, 147] is formed very shortly after plastic is released into aquatic environments, which, over time, is substituted by biomolecules with higher binding affinity that form a hard corona [56]. On top of the hard corona, a dynamic corona can be found that is in high exchange with the surrounding environment [57]. The eco-corona may influence the movement of NMPs and its ingestion by micro- and macrofauna [148]. It is important to note that there is not just one kind of biofilm, but many different ones, depending on the polymer type and structure of the NMPs [149, 150]. The surface roughness of the particle, for instance, supports biota settlement [149]. It is important to note that the mechanisms surrounding biofilm formation on plastic and NMPs represents a specialized area of research, and for which insight from microbiologists is perceived as being greatly beneficial towards strengthening our overall understanding regarding the types of organisms present on NMPs and the role they play towards potentially influencing the fate and effects of the particles.

For polystyrene, degradation can occur at elevated temperatures in the presence of oxygen, or by photooxidation. The photodegradation is initiated by the absorption of UV radiation by the aromatic ring [151]. Peroxy radicals are formed, followed by consecutive chain scission reactions to hydroperoxides and formation of new radicals, repeating the process until finally termination takes place. Generally speaking, weathering processes enlarge the effective surface area of the plastic and increase oxygen-containing functional groups in the polymer [45]. The weathering mechanisms and rates may not only vary with the environmental conditions, but also depend on the polymer type, polymer structure and the presence of stabilizing additives [152]. Antioxidants, for instance, help to impede photooxidation weathering kinetics, but do not inhibit UV degradation [153].

The degradation reactions on the surfaces of plastic lead to a variety of products, such as PS with shorter chain length, carbonyl groups on the surface and volatile components [70, 153–156]. Specifically, aged materials are observed to have an increase in hydroxyl groups and aromatic carbon–carbon double bonds, but an associated decrease in carbon hydrogen bonds [157]. This has been confirmed by FTIR spectroscopy, which is a highly sensitive method to detect the newly created C–O, C=O and O–H bonds during oxidation [158–160]. Table S8 provides a selection of products and functional groups that are formed in the photooxidation of polystyrene along with their characteristic IR absorption bands. Photodegradation mostly occurs on the surface of the plastic and, depending on the polymer, is limited in the bulk composition of the material [152, 161]. This is because the UV radiation only penetrates a few micrometers into the polymer, and the partial pressure of oxygen is also higher at the polymer surface [162].

In the aquatic environment, the low molecular weight degradation products such as benzoic acid, acetophenone, benzaldehyde, methyl benzoate, formic acid and acetic acid [154, 160, 163] are removed from the surface by water movement and might accelerate the overall degradation of a PS particle due to the exposure of fresh surfaces [152]. Therefore, the actual photodegradation of the solid sample might be higher than measured by FTIR [152]. From controlled weathering experiments, it has been estimated that a rate of only 5% formation of defects on the surface of a PS microparticle occurs annually [164], but for deeper insights a better characterization of the particles would be necessary. Similar to considering the important insight gained from microbiologists with respect to biofilm formation on the fate and effects of plastic and NMPs, we also note the important insight that can be gained from polymer chemists, material scientists and/or mechanical engineers when helping to better understand degradation mechanisms of plastic under different life cycle scenarios.

Implications and recommendations

When considering the details discussed above, we suggest that there are significant differences between PSMs manufactured for biomedical and analytical purposes and environmentally generated PS NMPs originating from various PS products used in commerce, including GPPS, HIPS, EPS or XPS. Table 3 summarises the properties of the various PSMs and environmentally generated PS NMPs. Notable differences include the shape, surface chemistry, chemical composition of the particles and the presence of IAS and NIAS. Depending on the research question being addressed, the use of PSMs may or may not represent a suitable model particle. For example, it is reasonable to anticipate that the use of PSMs to evaluate a toxicological mechanistic cause-effect relationship between a specific particle property, such as the influence of particle size and/or surface charge in relation to a specific adverse effect, can prove insightful.

Table 3 Properties of PS pellets and particles

PS type	Typical shape & size	Typical surface functionality	Typical chemical composition of the bulk material	Typical additional components
Commercial GPPS	Pellet, > 1 mm	No	PS	Antioxidants, fillers
Commercial HIPS	Pellet, > 1 mm	No	PS and Butadiene-Styrene-Copolymer	Antioxidants, fillers
Commercial EPS	Foamed bead, > 1 mm, converted to moulded shapes and boards	No	PS	Antioxidants, fillers, flame retardants possible
Commercial XPS	Extruded board	No	PS	Antioxidants, fillers, flame retardants possible
Weathered PS MP fragments from the environment	Irregular, no specific size, broad size distribution	Carbonyl groups, adsorbed biomolecules, microbes, and abiotic materials	Depending on the source	Depending on the source: Antioxidants, fillers, flame retardants possible
Commercial spherical PS microbeads	Sphere, sizes ranging from few nm to sub-mm, with narrow size distribution	e.g. amino, carbonyl, carboxyl, hydroxy, epoxy, sulfate, sulfonate or aldehyde groups	PS, crosslinked PS, PMMA	Fluorescent dyes

Conversely, extrapolating results to imply an environmental or human health risk associated with environmentally relevant exposure to PS NMPs present in the environment we suggest is inappropriate. The challenge, for the microplastic research community, consequently, is to consider how to best evaluate the potential risks associated with human exposure to an environmentally relevant polydisperse mixture of NMPs, which consists of a heterogeneous mixture of physicochemical properties, including size, shape and polymer types [33].

The recommendation to obtain and use environmentally relevant NMPs to evaluate environmental and human health risk is not novel. Indeed, several literature reviews have highlighted the observation that the majority of lab-based studies do not use environmentally relevant plastic polymers or shapes but rather rely on the use of commercially fabricated, often fluorescently labeled PS or PE spheres, largely for convenience or for necessity of detection [29, 48, 157, 165, 166]. Intuitively, the use of either surface-functionalized particles or non-functionalized particles will result in different effects [167], the interpretation of which can be further complicated depending on whether or not the particle solutions contain surfactants or dispersants [57] which might have a toxic effect on their own [167]. There is thus a well-understood gap between the polymer types used in laboratory studies and their respective environmental representativeness [29].

Unfortunately, it is difficult to obtain a sufficient quantity of NMPs directly from the environment to support either fate and/or toxicity testing [166]. Generating NMPs in the lab may therefore currently represent the best option to help progress research towards better understanding the potential risks that NMPs represent to the environment and human health.

Particle generation methods

A large number of top-down methods have been described in the literature to generate NMPs, and which are largely based on physically damaging different types of larger plastic articles and/or larger sizes of pre-production plastic pellets [168, 169]. These methods include the application of ball milling, cryogenic milling, ultra-centrifugal milling, grinding microplastic particles with a coffee grinder, conical burr grinder, or other grinding methods, manually sanding the surfaces of plastic with sandpaper or with a kitchen grinder, homogenizing plastic with the use of kitchen blenders or generating NMPs by sawing the plastic or a combination of approaches. Application of the various methods to generate NMPs typically results in the formation of irregularly shaped particles, with the need to generate fibres requiring the application of alternative approaches, such as manually cutting, isolating fibres generated from laundering synthetic clothing, or through the use of various milling techniques, or by electrospinning. In other instances, chemical methods are used, whereby research groups generate NMPs either by bottom-up lab syntheses of the particles in the lab or through the use of solvents capable of dissolving plastic, followed by precipitation and isolation of NMPs into aqueous solutions.

Aligned with the various methods that have generated NMPs, and summarised above, are a number of strengths and weaknesses that can be identified. A key strength often referenced when applying relatively simple and inexpensive methods, such as those that grind

or cut plastic articles using commonly used appliances (e.g. kitchen blenders, coffee grinders, or manually cutting materials with scissors) is an argument that the methods represent a flexible and relatively quick and inexpensive approach that facilitates the investigation of important questions related to the properties, effects, fate and transport of NMPs. Unfortunately, the generation of NMPs using these approaches also tend to provide limited insight regarding the reproducibility of the method with respect to consistency in the generation of particles of a specific size and shape, and/or the efficacy of the method when applied to other plastic polymeric materials. An additional concern often not addressed relates to the small quantity of NMPs generated. In all methods there is a common theme that the particles generated are aimed at supporting a limited number of studies for a single research group. However, given the large number of studies being generated aimed at evaluating all aspects of NMPs in the environment (e.g. fate and transport) and their potential effects, there is a need to generate large quantities of NMPs using methods that generate particles of specific sizes and shapes in a consistent and reliable manner for a variety of different types of plastic [170]. Based on the challenges that have been encountered to date it is unlikely that a single method can be used for all types of plastic. Consequently, it is likely that a combination of techniques will be needed to generate particles of varying shapes and sizes for different types of polymers, and which can consistently and reliably generate relatively large quantities of NMPs. This would benefit from insight gained from polymer chemists, who would be better positioned to help guide the generation of NMPs with specific properties.

When considering the suitability of a specific technique aimed at generating NMPs of varying size and shape, for instance, it is important to consider how a particular method may influence the physicochemical properties of the plastic itself. For instance, the application of milling techniques applies mechanical energy that results in larger pieces of plastic to become physically damaged, resulting in its fragmentation to smaller sizes of plastic particles. Decreasing the size of MPs generated using a milling technique requires an increase in the amount of mechanical energy. Increasing the amount of energy, however, can be problematic depending on the physicochemical properties of the polymer, such as its melting point, glass transition temperature and/or degree of crystallinity. In the instance of polyethylene, which has an average melting point temperature of approximately 140 °C, attempting to generate polyethylene MPs using a milling method can result in annealing, which can result in changes to the properties of the particle, such as degree of crystallinity, that are inconsistent with the starting material. Alternatively, annealing can also significantly impact the ability to further decrease the size of the particles, whereby annealing results in the formation of larger agglomerates. In an effort to counter the issue of annealing, many researchers employ the use of cryogenic milling, aimed at keeping the polymer below its melting point temperature.

In their study using a cryogenic ball mill, which generated PS particles in the size range of 1–200 µm, Eitzen et al. [171] observed that the yield of small particles (< 100 µm) increased with increasing pre-cooling and milling durations. Based on a starting mass of 2 g, a pre-cooling period of 12 min resulted in about 50% of the particles generated to be between 1 and 100 µm. A slightly larger fraction (≈70%) was observed when using 5 × 10 min milling durations and a pre- and intermediate cooling duration of 6 min [171]. These results were in contrast to those obtained using a sieve mill, where a starting mass of 100 g of PS was possible, however, the method was reported to be difficult to control, and was therefore found to be an ineffective approach towards generating PS microplastic [171]. The importance of the number of milling cycles towards generating MPs has been observed to vary depending on the polymeric composition of the starting material. Munoz et al., for instance, report using 2, 3, 5, and 10 milling cycles of 2 min each at a frequency of 30 Hz, with an intermediate cooling time of 1 min at 5 Hz between each cycle for PS, PET, PP and high density polyethene, respectively [172]. The resulting particles were then sieved to obtain MPs in a size range of 100–250 µm, the yield of the method, however, is not reported. Nevertheless, when considering results reported by Kühn et al., the relative yield of MPs generated from weathered and aged plastic obtained directly from the marine environment is observed to be strongly influenced by the nature of the starting polymeric material [173]. Using a 30-min pre-cooling period, MPs generated from 3 mm subsamples of net, rope, foam, sheets and rigid plastic were obtained using an ultra-centrifugal stainless steel Retsch ZM 200 mill [173]. MPs in the size range of 0.5–2.0 mm accounted for ≈68% of the total mass, with particles in the 0.2–0.5 mm size fraction only accounting for about 20% and particles < 0.2 mm representing < 10%. Similarly, Astner et al. report a recovery efficiency of 2% when generating 106 µm MPs, which they then converted to nanoplastic by employing a wet-grinding method, which resulted in a binomial particle-size distribution with an average particle size of 390 nm for PE [174].

It is notable, therefore, that when reviewing the different studies that have applied cryogenic milling to generate NMPs, there is an apparent inconsistency between studies related to the application of the milling, such as

milling times, number of milling cycles and differences in relation to the use of pre- and intermediate cooling times. Consequently, the reproducibility of NMP generation methods, such as those summarized above, requires additional investigation. A specific concern, for instance, relates to inconsistencies that are observed regarding the duration of pre- and intermediate cooling times as well as the speed, duration and number of milling cycles. While the inconsistencies may be due to challenges encountered with respect to the efficacy of cryogenic milling to generate NMPs for different types of polymeric materials, the end result are concerns regarding the efficiency of the method to reliably and reproducibly generate MP that might reflect particles < 150 µm.

Acknowledging that there may be important differences related to how NMPs are generated between the lab and in the environment [51], some methods have attempted to generate NMPs using simulated weathering and aging techniques [175–177]. However, limited quantities of NMPs are generated using such approaches and are unlikely to be sufficient to support the development of a large repository of model NMPs that might support the research community. Nevertheless, having access to weathered NMPs directly or guidance with respect to best practices that research groups could adopt would be very useful.

In their review of weathering pathways and experimental protocols that have been employed in relation to microplastic research, Alimi et al. report that the majority of studies have mostly aimed at evaluating the influence of weathering on the sorption and desorption of contaminants, with a subset of studies evaluating the toxicity of leachates from weathered NMPs [178]. When considering all studies that have directly weathered NMPs, only about 10% of them have used the particles to evaluate the toxicological effects associated with weathering. Based on the limited number of studies, however, the results imply that weathering does significantly alter the properties of NMPs and can increase the potential for adverse effects to occur [178]. Consequently, having protocol that simulates the natural weathering and aging of NMPs should represent an important research need, since environmental exposure to weathered NMPs most likely represents the dominant environmentally relevant scenario, as opposed to exposure to NMPs originating from 'virgin' plastic. The majority of effect studies, however, continue to rely on NMPs generated from 'virgin' plastic, with only a small number of studies simulating natural UV radiation (UVR) to produce weathered NMPs [178, 179].

Given that there are a multitude of weathering processes that can occur in the environment, research is warranted in order to better understand the relative implications of the various weathering processes on the fate and effects of NMPs, and which would benefit from inputs from a diverse group of experts, including polymer chemists, material scientists, mechanical engineers, microbiologists, and analytical chemists. It is generally well understood, however, that photo-oxidation represents the primary trigger in the weathering process of plastic articles. Natural solar UVR is largely dominated by UVA (λ = 315–400 nm). The UVR dose that arrives at the surface of plastic is the product of the irradiance, expressed as energy per unit surface area, and the time of exposure. The longer the exposure time the greater the UVR dose, which represents the most likely environmentally relevant scenario. When attempting to simulate environmental UVR doses in the lab, there is a tendency to increase the irradiance intensity, such as through the use of UVC (λ = 100–280 nm), largely because it would be impractical to simulate environmental exposure times on the order of months to years in the lab. Alimi et al., however, note that the simulated process is only sufficient to generate enough energy to initiate photodegradation at the surface of the plastic [178], and it remains unclear if the simulated process sufficiently replicates the natural weathering process over longer periods of time, representing an important research need to better understand the relative importance of irradiance versus exposure time [161].

When considering the various properties of NMPs that may be encountered in the environment, it becomes obvious that the current practice of relying on the use of PSMs is insufficient to effectively characterise and quantify the environmental and human health implications that exposure to NMPs represents [33]. Similarly, there is a lack of evidence that the adoption of various ad-hoc approaches recently employed to generate NMPs provide efficient and reproducible methods that are capable of generating a consistent, reliable and relevant source of NMPs that are representative of environmentally relevant exposure. The aspirational recommendation emphasised here and in other studies, which strongly urges research groups to use NMPs that are representative of environmentally relevant exposure, however, requires specific guidance with respect to the various aspects that should be considered if a repository of an environmentally relevant suite of model NMPs is to be achieved. Given the various complex aspects that need to be considered, we suggest the need to adopt a holistic strategy that would benefit from the active involvement from a multidisciplinary group of experts [33, 180].

Addressing the 'relevant' in environmentally relevant NMPs

To enable an assessment of the risks that NMPs may pose to human health and the environment, it is first necessary

to assess the fate and effects of relevant particles [33]. As summarised above, several research groups have applied various approaches to generate their own NMPs, such as through the use of cryogenic-milling. Concerns, however, regarding the reproducibility of the method(s) and the efficiency to generate NMPs of a toxicological relevant particle size (e.g. < 10 μm) in sufficient quantities to support the microplastic research community have been raised. It seems reasonable, therefore, to conclude that the generation and characterisation of NMPs representative of an environmental exposure for human health would represent an improvement on the overall reliance of PSMs. Moving forward, however, there remains a need to evaluate the influence of weathering and/or the formation of an eco-corona on the fate and effects of NMPs [147]. Parallel to the development of guidance with respect to best practices that could be adopted to generate NMPs, there is thus the need to also develop best practices aimed at artificially weathering NMPs.

While recommendations, such as those proposed above, may be perceived as representing reasonable and rationale advice, several questions remain regarding how to turn these recommendations into tangible outcomes. For instance, which particle shapes and sizes and for what types of plastic polymers should be prioritised when working towards the development of best practices? Who will provide the resources and leadership necessary to support the development of best practices? On these questions, unfortunately, there is no simplistic solution, since our understanding of the complexity of the characteristics and quantities of human exposure, which the heterogeneous nature of NMPs represents, is poorly defined. Furthermore, identification of which types of NMPs to prioritise will most likely require multidisciplinary expertise, which we suggest would be necessary to address the various factors that likely influence the sources of NMPs to the environment, as well as to help guide which exposure scenarios and properties of the particles might require prioritisation. A complementary approach is thus envisioned, which could be visualized as a holistic strategic framework that is consistent with a "One Health" approach and which aims to bring together various experts who could consider the various factors that are most likely important throughout all stages of the life cycle of plastic used in commerce. Specifically, there is a need to bring together polymer chemists, material scientists, mechanical engineers and analytical chemists to collaborate with exposure and life cycle assessment scientists with the aim of better characterizing the primary sources of exposure for humans to NMPs under environmentally relevant conditions. Insight from an improved understanding of exposure would thus benefit the strategic design of toxicity studies necessary to both characterise toxicological mechanisms of action and quantify reliable and relevant dose–response relationships for use in evaluating human health risks.

Figure 3 presents a conceptual framework aimed at communicating the connectivity between different components related to assessing the environmental and human health implications that exposure to NMPs represents. It is suggested that this can best be achieved through a multidisciplinary approach, whereby communication between experts is supported through the establishment of a community of practice. At the core of the framework is the need for transparency in the context of problem formulation, and which emphasises the need to ensure the acquisition of reliable and relevant data obtained from quality assured and quality-controlled experiments. Considering the current need to better understand the human and environmental risks associated with exposure to NMPs, prioritising work on those model NMPs that are most relevant to the problem being addressed must represent the primary driver.

Key elements of the conceptual framework

The overall goal of the framework presented in Fig. 3 is to provide a summary of the key elements necessary to support progress on generation of NMPs that are relevant from an environmental exposure perspective. In this context, problem formulation represents the central element that must be robustly defined. Identifying the most relevant model NMPs to be used to address the problem under investigation is both fundamental and critical. It is thus important to acknowledge that there are potentially several different research questions that can emerge. Here we summarise five different themes that would benefit from access to NMPs that are capable of supporting the generation of high-quality data that are relevant, reliable and are thus, fit-for-purpose. These include:

- Exposure Assessment: There is currently a mismatch between the types of NMPs used in toxicity studies and those which are understood to be present in the environment. Nevertheless, characterization and quantification regarding what represents an environmentally relevant exposure to NMPs remains a critically important data need. For example, it is currently not possible to accurately quantify either the dietary or inhalation exposure of NMPs for humans, neither is it possible to provide an accurate description of the physicochemical properties of the NMPs present in air or in food and beverages. Given the wide range of different types of plastic used in commerce, identifying the most significant sources of exposure represents a potentially overwhelming task. Here we suggest that there is a potential opportunity to consider

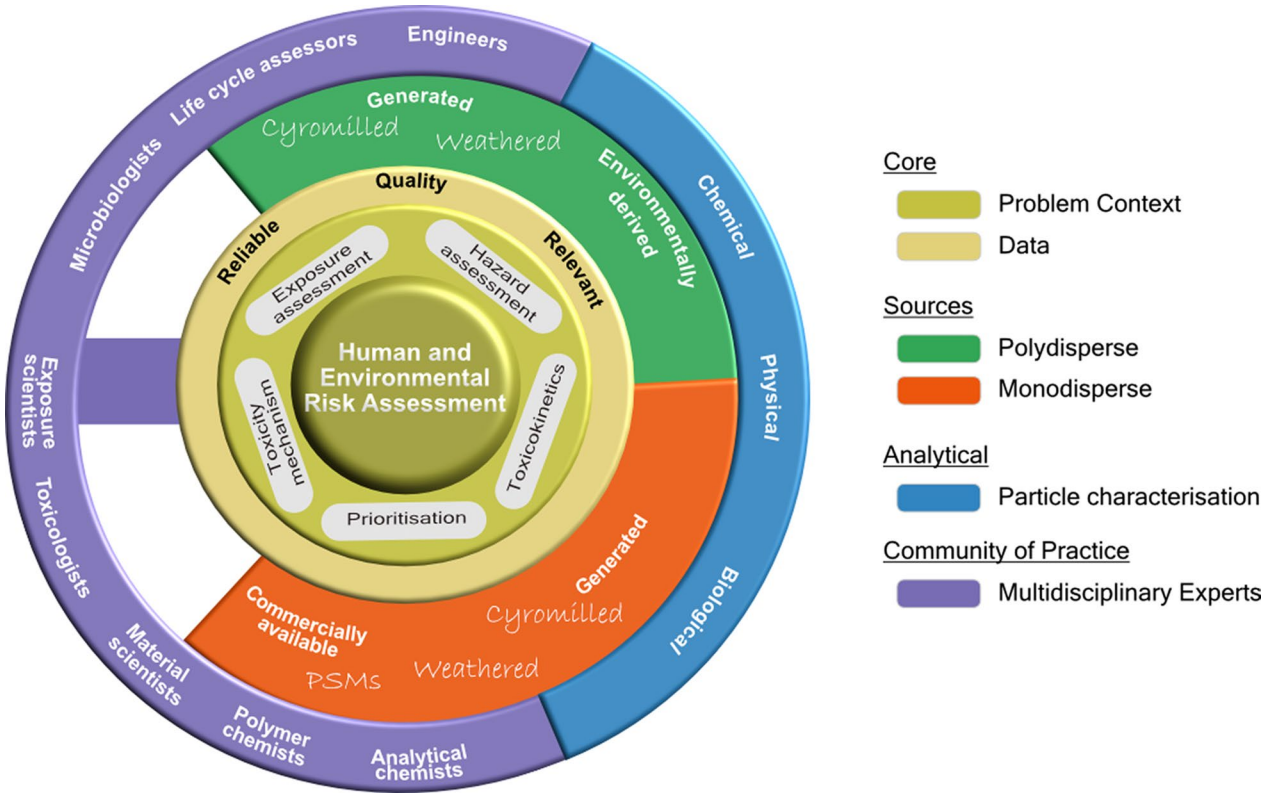

Fig. 3 Conceptual framework to set priorities in the context of prioritizing the acquisition and properties of the best model NMPs necessary to advance the ability to evaluate their human and environmental risks. Each shell represents a key element, and spokes illustrate direct connectivity between key elements

a strategic approach towards more efficiently prioritising consumer use and exposure scenarios that may result in exposure to NMPs, which we propose would benefit from active collaboration between a multidisciplinary group of experts. Specifically, this should consist of a group that would bring together polymer chemists and material scientists who could collaborate with exposure and life-cycle assessment scientists. The aim of bringing together the different groups of experts would represent a first step in achieving a consensus on the definition of which NMP are most abundant in the environment. Once this has been established, and since NMPs are subject to microbial colonization in the environment, insight gained from microbiologists could contribute to stablishing a consensus on the definition of what is the most environmentally relevant NMP. During this process clarification could be sought on a variety of open questions, including those related to weathering, the presence of an eco-corona, their size, shape and polymer composition.

- Hazard Assessment: When considering the large number of studies that have been conducted to date, and which have reported a variety of observed adverse effects associated with exposure to NMPs, it is concerning that the majority of studies have used PSMs from a large number of different commercial suppliers (as discussed above). Data are summarised for both in vitro and mammalian in vivo studies in Supp Info_2.xlsx, which report adverse effects on several endpoints, with adverse effects typically observed at high test concentrations. A general observation is that the most commonly reported toxicological response appears to be associated with oxidative stress and/or inflammation biomarkers. Depending on the study, adverse effects on a variety of systems have also been reported and include effects on the liver, kidney, reproductive organs, neurobehavioral effects, immunity, intestinal health, pulmonary and genotoxicity. Given the reliance on the use of poorly characterised PSMs it is, however, currently not possible to extrapolate these results with sufficient confidence to human exposure to NMPs. In this regard, an improved understanding of human exposure to NMPs would support the generation of a more relevant suite of NMPs that would strengthen

the relevance of future hazard assessments. In the absence of relevant and reliable data from either an assessment of exposure or hazard, it is presently not possible to perform a risk assessment. Given the expertise of particle and fibre toxicologists, it is critical that they help support the prioritisation of NMPs regarding the most likely relationships between their physicochemical properties and the implications regarding cellular interactions and responses in health and disease [33].

- Toxicity mechanism: Whereas significant concerns have been raised regarding the relevance of data generated from hazard studies using PSMs for risk assessment, it could be argued that if hazards were identified from the use of environmentally relevant NMPs then the use of PSMs may represent an important tool for the understanding of the toxicological mechanisms of action of MPs. For instance, the ability to produce PSMs of different sizes and surface chemistry, can support an understanding regarding the relative differences between different physicochemical properties of monodisperse particles with respect to a toxicological endpoint. To support mechanistic understanding, however, there is a need for the particles to be robustly characterised with respect to their physicochemical properties. Most current publications have little to no particle characterization reported [43]. Table 4 summarises several important physicochemical properties and methods of analysis, which are identified as representing a base set of information necessary for the characterization of particles. It is important to note that commercially available PSMs do not typically include all data reporting on the characteristics listed in Table 4, which would imply that research groups should perform their own analysis on each batch of particles obtained. Similarly, any NMPs generated should also be fully characterised. Generally, the practice of fully characterizing particles for toxicity testing represents a fundamental principle in particle and fibre toxicology (see for instance [181]). Furthermore, additional guidance with respect to the safety testing and assessment of manufactured nanomaterials should be leveraged, since we note that there already exists various OECD guidance documents that would be relevant to the microplastic research community [182–184]. Indeed, the challenges identified with respect to NMPs are comparable to the challenges faced in the early stages encountered by the toxicity testing of manufactured nanomaterials, with many of the recommendations presented here being similar to those expressed by Bouwmeester et al. [185]. Consequently, opportunities to leverage expertise and learnings gained from advances in the testing of manufactured nanomaterials should also represent an important consideration for the group of experts included within a community of practice.
- Toxicokinetics: While there are likely specific physicochemical properties associated with NMPs that cause them to have novel hazards, the direct risk they represent to human health will be influenced by their potential to enter the body and reach a toxicological

Table 4 Frequently used analytical methods to characterise microparticles

Purpose	Methods	References
Particle detection	Optical microscopy	[187–189]
	AFM	[187, 188]
Particle morphology (size and shape)	DLS	[190]
	SEM	[187–189, 191]
	TEM	[187, 188]
Composition (polymer type, additives, fillers, surface functional groups)	μFTIR	[187–189]
	μRAMAN	[187–189]
	Py-GC–MS	[187, 189, 192]
	LDIR	[193]
	XRF	[67]
	EDX	[191, 194]
	^{1}H-NMR	[194, 195]
Detection of surfactants and dispersants	HPLC–MS	[7]
Particle surface area, surface charge, porosity	Electrophoretic light scattering (Zeta potential)	[120, 196]
	BET	[190]
	Potentiometric titration	[197–199]
Molecular weight distribution	GPC	[194, 196]

site of action. Generally, the overall understanding with respect to uptake and translocation pathways for NMPs remains an ongoing research need, with available studies limited to a relatively small group of monodisperse PSMs to characterise uptake and translocation. Largely unknown are the translocation rates as well as accumulation and retention in critical target sites and their underlying mechanisms. The uptake, translocation and potential accumulation of NMPs in the body will largely be influenced by their physicochemical properties, and which are similar to those summarised when working towards an improved understanding of their toxicological mechanism of action [186]. Consequently, advancing an improved understanding of the toxicokinetics of NMPs will require the same factors required in advancing toxicological mechanisms of action. Specifically, studies aimed at characterizing and quantifying the uptake, translocation and accumulation of NMPs will benefit from the use of both monodisperse and environmentally relevant NMPs, and will facilitate the development and parameterisation of physiologically based toxicokinetic models [186].

- Prioritisation: Finally, prioritisation represents an important issue that needs to be addressed in the context of plastic pollution and NMPs. If one of the goals of regulatory measures targeting NMPs, such as the current discussions on an international treaty on plastic pollution, for instance, is to help reduce environmental and human health risks associated with exposure to NMPs, then it is critically important to generate reliable and relevant data that can be used to prioritise the primary sources of NMPs identified as representing the greatest risk to human health and the environment. In the absence of hazard data for NMPs that are consistent with environmentally relevant exposures, coupled with an insufficient understanding of human exposure to NMPs, prioritising actions on NMPs cannot be judged to be proportionate and efficiently progressed. There is thus a danger that actions limiting and/or banning the use of some types of plastic used in commerce may result in regrettable substitution when they are based on an absence of scientific evidence that can demonstrate a causal relationship. We suggest that a multidisciplinary group of experts would be best positioned to direct the prioritisation of actions based on robust, reliable and relevant science.

The primary objective for expressing a need for a community of practice that is comprised of a multidisciplinary group of experts is to ensure the generation of high-quality data that are both reliable and relevant. Although the amount and type of data will vary depending on the problem definition, the data generated must be both transparent and robust. This will necessitate the need to develop and apply best practices, which would ideally result in the development and acceptance of standardized methods with respect to the generation, weathering, analysing and evaluation of the exposure and hazards associated with NMPs present in the environment. Aligning the different experts, however, represents a non-trivial challenge and will require top-down support from leadership in the regulatory and research communities.

Conclusions

Key policy initiatives targeting plastic pollution should be based on scientific evidence, but the main focus of research has been, through necessity, on the hazardous properties of a relatively small group of readily available monodisperse PSMs. Not surprisingly, therefore, there remains considerable uncertainty as to the actual harm being caused by plastic pollution [200]. Using a polydisperse group of NMPs representative of an environmentally relevant exposure when investigating the impacts of NMPs in vitro and in vivo has been suggested as an important research need that would address this uncertainty and lead not only to better targeted initiatives and regulations, but also set a sound foundation for future policy making and avoid regrettable decisions [33]. To start this process we have identified an urgent need to establish a coordinated multidisciplinary group of experts, supported within a community of practice that could be charged with the task of developing a consensus on not only what best constitutes an environmentally relevant suite of NMPs, but also what are the best practices that could be adopted for their generation and weathering. Given the multitude of challenges that the research community currently face, the ambitions of the community of experts should possibly aim to start simple and build a repository of model NMPs for which a consensus can be made regarding their environmental relevance. This should perhaps be swiftly followed by the development of guidance documents with respect to best practices for use by the research community. It is important to acknowledge, however, that establishing a multidisciplinary group, or an expert community of practice, is not trivial and will require significant resource in terms of expertise and funding. An open question, therefore, relates to who will take a leadership role in supporting the organisational and logistical structure of a community of practice? In this regard, we recognize that there are already several groups that have attempted to establish community of practices that might help to address the

concerns raised in this review. These include the group of projects supported through the European Horizon 2020 initiative, known as CUSP [201], as well as PRIORITY [202], and those supported by industry including the Plastics Europe Brigid project [203] and the International Council of Chemical Associations, i.e. the Microplastics Advanced Research and Innovation Initiative (MARII) [204]. While there are elements of the conceptual framework presented here in each of the projects listed above, to the best of our knowledge there is, however, no recognizable effort being directed towards establishing a consensus on what is an environmentally relevant NMP.

Mindful of the wide range of plastic polymers used in commerce [205] and considering the complex chemistry surrounding each of them, it is not unreasonable to anticipate that insight gained from several different groups of experts be necessary when generating a suite of NMPs representative of those that may actually occur in the environment. Here we suggest as a minimum, but not limited to, the need to consider including polymer chemists, materials scientists, mechanical engineers, analytical chemists, toxicologists, exposure and life-cycle scientists and microbiologists, given a general perception regarding the valuable input that each could provide towards (1) Arriving at a consensus regarding what best represents an environmentally relevant NMP, and (2) Developing guidance on best practices on the generation, characterization and toxicity testing of NMPs.

Abbreviations

DVB	Divinylbenzene
EB	Ethyl benzene
ELS	Electrophoretic light scattering
EPS	Expanded polystyrene foam
FTIR	Fourier transformed infrared spectroscopy
GPPS	General purpose polystyrene
HIPS	High impact polystyrene
IAS	Intentionally added substances
LAT	Latex agglutination test
MP	Microplastic particles
NIAS	Non-intentionally added substances
NMP	Nano- and microplastic particles
PA	Polyamide
PC	Polycarbonate
PE	Polyethylene
PET	Polyethylene terephthalate
PMMA	Polymethyl methacrylate
PP	Polypropylene
PS	Polystyrene
PSM	Polystyrene microspheres
PVC	Polyvinyl chloride
SFEP	Surfactant-free emulsion polymerization
SSEP	Surfactant-free seeded emulsion polymerization
T_g	Glass transition temperature
UVR	Ultraviolet radiation
XPS	Extruded polystyrene foam

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12989-024-00599-1.

Additional file 1.

Additional file 2.

Acknowledgements
Not applicable.

Author contributions
All authors contributed to the original draft and revisions of the manuscript. All authors reviewed the final manuscript.

Funding
Todd Gouin acknowledges funding from the American Chemistry Council. Mark A Pemberton received payment from Plastics Europe for researching and reviewing the relevant literature and for preparation of this manuscript.

Data availability
No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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Received: 13 May 2024 Accepted: 10 September 2024
Published online: 27 September 2024

References

1. Cózar A, Echevarría F, González-Gordillo JI, Irigoien X, Úbeda B, Hernández-León S, Palma ÁT, Navarro S, García-de-Lomas J, Ruiz A, Fernández-de-Puelles ML. Plastic debris in the open ocean. Proc Natl Acad Sci. 2014;111:10239–44. https://doi.org/10.1073/pnas.1314705111.
2. Eriksen M, Lebreton LCM, Carson HS, Thiel M, Moore CJ, Borerro JC, Galgani F, Ryan PG, Reisser J. Plastic pollution in the world's oceans: more than 5 trillion plastic pieces weighing over 250,000 tons afloat at sea. PLoS ONE. 2014;9: e111913. https://doi.org/10.1371/journal.pone.0111913.
3. Eerkes-Medrano D, Thompson RC, Aldridge DC. Microplastics in freshwater systems: a review of the emerging threats, identification of knowledge gaps and prioritisation of research needs. Water Res. 2015;75:63–82. https://doi.org/10.1016/j.watres.2015.02.012.
4. van Sebille E, Wilcox C, Lebreton L, Maximenko N, Hardesty BD, van Franeker JA, Eriksen M, Siegel D, Galgani F, Law KL. A global inventory of small floating plastic debris. Environ Res Lett. 2015;10(12): 124006. https://doi.org/10.1088/1748-9326/10/12/124006.

5. Lebreton L, Van Der Zwet J, Damsteeg JW, Slat B, Andrady A, Reisser J. River plastic emissions to the world's oceans. Nat Commun. 2017;8(1):15611. https://doi.org/10.1038/ncomms15611.
6. Lacerda AL, Rodrigues L, van Sebille E, Rodrigues FL, Ribeiro L, Secchi ER, Kessler F, Proietti MC. Plastics in sea surface waters around the Antarctic Peninsula. Sci Rep. 2019;9(1):3977. https://doi.org/10.1038/s41598-019-40311-4.
7. Li J, Liu H, Paul CJ. Microplastics in freshwater systems: a review on occurrence, environmental effects, and methods for microplastics detection. Water Res. 2018;137:362–74. https://doi.org/10.1016/j.watres.2017.12.056.
8. Chen J, Wang W, Liu H, Xu X, Xia J. A review on the occurrence, distribution, characteristics, and analysis methods of microplastic pollution in ecosystems. Environ Pollut Bioavail. 2021;33(1):227–46. https://doi.org/10.1080/26395940.2021.1960198.
9. Li D, Shi Y, Yang L, Xiao L, Kehoe DK, Gunko YK, Boland JJ, Wang JJ. Microplastic release from the degradation of polypropylene feeding bottles during infant formula preparation. Nature Food. 2020;1(11):746–54. https://doi.org/10.1038/s43016-020-00171-y.
10. Enyoh CE, Shafea L, Verla AW, Verla EN, Qingyue W, Chowdhury T, Paredes M. Microplastics exposure routes and toxicity studies to ecosystems: an overview. Environ Anal Health Toxicol. 2020;35(1): e2020004. https://doi.org/10.5620/eaht.e2020004.
11. Mohamed Nor NH, Kooi M, Diepens NJ, Koelmans AA. Lifetime accumulation of microplastic in children and adults. Environ Sci Technol. 2021;55(8):5084–96. https://doi.org/10.1021/acs.est.0c07384.
12. Milne MH, De Frond H, Rochman CM, Mallos NJ, Leonard GH, Baechler BR. Exposure of US adults to microplastics from commonly-consumed proteins. Environ Pollut. 2024;343:123233. https://doi.org/10.1016/j.envpol.2023.123233.
13. Park JW, Lee SJ, Hwang DY, Seo S. Recent purification technologies and human health risk assessment of microplastics. Materials. 2020;13(22):5196. https://doi.org/10.3390/ma13225196.
14. Ragusa A, Svelato A, Santacroce C, Catalano P, Notarstefano V, Carnevali O, Papa F, Rongioletti MCA, Baiocco F, Draghi S, D'Amore E, Rinaldo D, Matta M, Giorgini E. Plasticenta: first evidence of microplastics in human placenta. Environ Int. 2021;146: 106274. https://doi.org/10.1016/j.envint.2020.106274.
15. Jenner LC, Rotchell JM, Bennett RT, Cowen M, Tentzeris V, Sadofsky LR. Detection of microplastics in human lung tissue using μFTIR spectroscopy. Sci Total Environ. 2022;831: 154907. https://doi.org/10.1016/j.scitotenv.2022.154907.
16. Zhou M, Wang R, Cheng S, Xu Y, Luo S, Zhang Y, Kong L. Bibliometrics and visualization analysis regarding research on the development of microplastics. Environ Sci Pollut Res. 2021;28(8):8953–67. https://doi.org/10.1007/s11356-021-12366-2.
17. Yu Y, Wang S, Yu P, Wang D, Hu B, Zheng P, Zhang M. A bibliometric analysis of emerging contaminants (ECs) (2001–2021): evolution of hotspots and research trends. Sci Total Environ. 2024;907: 168116. https://doi.org/10.1016/j.scitotenv.2023.168116.
18. Gago J, Booth AM, Tiller R, Maes T, Larreta J. Microplastics pollution and regulation. In: Rocha-Santos T, Costa M, Mouneyrac C, editors. Handbook of microplastics in the environment. Cham: Springer Nature; 2020.
19. Mitrano DM, Wohlleben W. Microplastic regulation should be more precise to incentivize both innovation and environmental safety. Nat Commun. 2020;11(1):5324. https://doi.org/10.1038/s41467-020-19069-1.
20. Commission Regulation (EU) 2023/2055 of 25 September 2023 amending Annex XVII to Regulation (EC) No 1907/2006. https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32023R2055&qid=1705310558548. Accessed Jan 2024.
21. Thompson RC, Olsen Y, Mitchell RP, Davis A, Rowland SJ, John AW, McGonigle D, Russell AE. Lost at sea: where is all the plastic? Science. 2004;304(5672):838. https://doi.org/10.1126/science.1094559.
22. Arthur C, Baker J, Bamford H. Proceedings of the international research workshop on the occurrence, effects, and fate of microplastic Marine debris. Sept 9–11, 2008. In: NOAA technical memorandum NOS-OR&R-30; 2009.
23. Hartmann NB, Hüffer T, Thompson RC, Hassellöv M, Verschoor A, Daugaard AE, Rist S, Karlsson T, Brennholt N, Cole M, Herrling MP, Hess MC, Ivleva NP, Lusher AL, Wagner M. Are we speaking the same language? Recommendations for a definition and categorization framework for plastic debris. Environ Sci Technol. 2019;53(3):1039–47. https://doi.org/10.1021/acs.est.8b05297.
24. The World Health Organization. Dietary and inhalation exposure to nano- and microplastic particles and potential implications for human health. Geneva; 2022.
25. Huang J, Chen H, Zheng Y, Yang Y, Zhang Y, Gao B. Microplastic pollution in soils and groundwater: characteristics, analytical methods and impacts. Chem Eng J. 2021;425: 131870. https://doi.org/10.1016/j.cej.2021.131870.
26. Phuong NN, Zalouk-Vergnoux A, Poirier L, Kamari A, Châtel A, Mouneyrac C, Lagarde F. Is there any consistency between the microplastics found in the field and those used in laboratory experiments? Environ Pollut. 2016;211:111–23. https://doi.org/10.1016/j.envpol.2015.12.035.
27. Rozman U, Kalčíková G. Seeking for a perfect (non-spherical) microplastic particle—the most comprehensive review on microplastic laboratory research. J Hazard Mater. 2022;424: 127529. https://doi.org/10.1016/j.jhazmat.2021.127529.
28. Heinrich P, Hanslik L, Kämmer N, Braunbeck T. The tox is in the detail: technical fundamentals for designing, performing, and interpreting experiments on toxicity of microplastics and associated substances. Environ Sci Pollut Res. 2020;27(18):22292–318. https://doi.org/10.1007/s11356-020-08859-1.
29. Leistenschneider D, Wolinski A, Cheng J, ter Halle A, Duflos G, Huvet A, Paul-Pont I, Lartaud F, Galgani F, Lavergne É, Meistertzheim A-L, Ghiglione J-F. A critical review on the evaluation of toxicity and ecological risk assessment of plastics in the marine environment. Sci Total Environ. 2023;896: 164955. https://doi.org/10.1016/j.scitotenv.2023.164955.
30. Brehm J, Ritschar S, Laforsch C, Mair MM. The complexity of micro- and nanoplastic research in the genus Daphnia—a systematic review of study variability and a meta-analysis of immobilization rates. J Hazard Mater. 2023;458: 131839. https://doi.org/10.1016/j.jhazmat.2023.131839.
31. Pradel A, Catrouillet C, Gigault J. The environmental fate of nanoplastics: what we know and what we need to know about aggregation. NanoImpact. 2023;29: 100453. https://doi.org/10.1016/j.impact.2023.100453.
32. Redondo-Hasselerharm PE, Rico A, Koelmans AA. Risk assessment of microplastics in freshwater sediments guided by strict quality criteria and data alignment methods. J Hazard Mater. 2023;441: 129814. https://doi.org/10.1016/j.jhazmat.2022.129814.
33. Wright S, Cassee FR, Erdely A, Campen MJ. Micro- and nanoplastics concepts for particle and fibre toxicologists. Part Fibre Toxicol. 2024;21(1):18. https://doi.org/10.1186/s12989-024-00581-x.
34. Nunes BZ, Huang Y, Ribeiro VV, Wu S, Holbech H, Moreira LB, Xu EG, Castro IB. Microplastic contamination in seawater across global marine protected areas boundaries. Environ Pollut. 2023;316(P1): 120692. https://doi.org/10.1016/j.envpol.2022.120692.
35. Kukkola A, Krause S, Lynch I, Sambrook Smith GH, Nel H. Nano and microplastic interactions with freshwater biota—current knowledge, challenges and future solutions. Environ Int. 2021;152: 106504. https://doi.org/10.1016/j.envint.2021.106504.
36. Burns EE, Boxall ABA. Microplastics in the aquatic environment: evidence for or against adverse impacts and major knowledge gaps. Environ Toxicol Chem. 2018;37(11):2776–96. https://doi.org/10.1002/etc.4268.
37. O'Brien S, Rauert C, Ribeiro F, Okoffo ED, Burrows SD, O'Brien JW, Wang X, Wright SL, Thomas KV. There's something in the air: a review of sources, prevalence and behaviour of microplastics in the atmosphere. Sci Total Environ. 2023;874: 162193. https://doi.org/10.1016/j.scitotenv.2023.162193.
38. Pencik O, Durdakova M, Molnarova K, Kucsera A, Klofac D, Kolackova M, Adam V, Huska D. Microplastics and nanoplastics toxicity assays: a revision towards to environmental-relevance in water environment. J Hazard Mater. 2023;454: 131476. https://doi.org/10.1016/j.jhazmat.2023.131476.
39. The World Health Organization. Microplastics in drinking-water. Geneva; 2019.
40. SAPEA, Science advice for policy by European academies. A scientific perspective on microplastics in nature and society. Berlin: SAPEA; 2019. https://doi.org/10.26356/microplastics.

41. Hale RC, Seeley ME, La Guardia MJ, Mai L, Zeng EY. A global perspective on microplastics. J Geophys Res Oceans. 2020;125(1):1–40. https://doi.org/10.1029/2018JC014719.
42. De Ruijter VN, Redondo-Hasselerharm PE, Gouin T, Koelmans AA. Quality criteria for microplastic effect studies in the context of risk assessment: a critical review. Environ Sci Technol. 2020;54(19):11692–705. https://doi.org/10.1021/acs.est.0c03057.
43. Gouin T, Ellis-Hutchings R, Thornton Hampton LM, Lemieux CL, Wright SL. Screening and prioritization of nano- and microplastic particle toxicity studies for evaluating human health risks—development and application of a toxicity study assessment tool. Microplast Nanoplast. 2022;2(1):2. https://doi.org/10.1186/s43591-021-00023-x.
44. Thornton Hampton LM, Bouwmeester H, Brander SM, Coffin S, Cole M, Hermabessiere L, Mehinto AC, Miller E, Rochman CM, Weisberg SB. Research recommendations to better understand the potential health impacts of microplastics to humans and aquatic ecosystems. Microplast Nanoplast. 2022. https://doi.org/10.1186/s43591-022-00038-y.
45. Duan J, Bolan N, Li Y, Ding S, Atugoda T, Vithanage M, Sarkar B, Tsang DC, Kirkham MB. Weathering of microplastics and interaction with other coexisting constituents in terrestrial and aquatic environments. Water Res. 2021;15(196):117011. https://doi.org/10.1016/j.watres.2021.117011.
46. Yang Y, Liu W, Zhang Z, Grossart HP, Gadd GM. Microplastics provide new microbial niches in aquatic environments. Appl Microbiol Biotechnol. 2020;104(15):6501–11. https://doi.org/10.1007/s00253-020-10704-x.
47. Andrady AL, Barnes PW, Bornman JF, Gouin T, Madronich S, White CC, Zepp RG, Jansen MAK. Oxidation and fragmentation of plastics in a changing environment; from UV-radiation to biological degradation. Sci Total Environ. 2022;851: 158022. https://doi.org/10.1016/j.scitotenv.2022.158022.
48. Lehtiniemi M, Hartikainen S, Näkki P, Engström-Öst J, Koistinen A, Setälä O. Size matters more than shape: ingestion of primary and secondary microplastics by small predators. Food Webs. 2018;17: e00097. https://doi.org/10.1016/j.fooweb.2018.e00097.
49. Sarkar AK, Rubin AE, Zucker I. Engineered polystyrene-based microplastics of high environmental relevance. Environ Sci Technol. 2021;55(15):10491–501. https://doi.org/10.1021/acs.est.1c02196.
50. Bridson JH, Masterton H, Theobald B, Risani R, Doake F, Wallbank JA, Maday SDM, Lear G, Abbel R, Smith DA, Kingsbury JM, Pantos O, Northcott GL, Gaw S. Leaching and transformation of chemical additives from weathered plastic deployed in the marine environment. Mar Pollut Bull. 2024;198: 115810. https://doi.org/10.1016/j.marpolbul.2023.115810.
51. He W, Liu S, Zhang W, Yi K, Zhang C, Pang H, Huang D, Huang J, Li X. Recent advances on microplastic aging: identification, mechanism, influence factors, and additives release. Sci Total Environ. 2023;889: 164035. https://doi.org/10.1016/j.scitotenv.2023.164035.
52. Luo H, Tu C, He D, Zhang A, Sun J, Li J, Xu J, Pan X. Interactions between microplastics and contaminants: a review focusing on the effect of aging process. Sci Total Environ. 2023;899: 165615. https://doi.org/10.1016/j.scitotenv.2023.165615.
53. Fajardo C, Martín C, Costa G, Sánchez-Fortún S, Rodríguez C, de Lucas Burneo JJ, Nande M, Mengs G, Martín M. Assessing the role of polyethylene microplastics as a vector for organic pollutants in soil: ecotoxicological and molecular approaches. Chemosphere. 2022. https://doi.org/10.1016/j.chemosphere.2021.132460.
54. Bridson JH, Abbel R, Smith DA, Northcott GL, Gaw S. Impact of accelerated weathering on the leaching kinetics of stabiliser additives from microplastics. J Hazard Mater. 2023;459: 132303. https://doi.org/10.1016/j.jhazmat.2023.132303.
55. Jasinski J, Wilde MV, Voelkl M, Jérôme V, Fröhlich T, Freitag R, Scheibel T. Tailor-made protein corona formation on polystyrene microparticles and its effect on epithelial cell uptake. ACS Appl Mater Interfaces. 2022;14(41):47277–87. https://doi.org/10.1021/acsami.2c13987.
56. Ramsperger AF, Narayana VK, Gross W, Mohanraj J, Thelakkat M, Greiner A, Schmalz H, Kress H, Laforsch C. Environmental exposure enhances the internalization of microplastic particles into cells. Sci Adv. 2020;6(50):eabd1211.
57. Laforsch C, Ramsperger AFRM, Mondellini S, Galloway TS. Microplastics: a novel suite of environmental contaminants but present for decades. In: Reichl F-X, Schwenk M, editors. Regulatory toxicology. Berlin Heidelberg: Springer; 2021. p. 1185–210. https://doi.org/10.1007/978-3-642-36206-4_138-1.
58. Hettiarachchi H, Meegoda JN. Microplastic pollution prevention: the need for robust policy interventions to close the loopholes in current waste management practices. Int J Environ Res Public Health. 2023;20(14):6434. https://doi.org/10.3390/ijerph20146434.
59. Rognerud I, Hurley R, Lusher A, Nerland Brate IL, Hovland Steindal E. Addressing microplastics in a global agreement on plastic pollution. Nordisk Ministerråd. 2023. https://doi.org/10.6027/temanord2022-566.
60. PRISMA. http://www.prisma-statement.org/. Accessed Jan 2024.
61. Laage E, IG Farbenindustrie: Verfahren zur Darstellung schmelzbarer Massen durch Polymerisation von Styrol, DE Patent 547383.
62. Ryberg MW, Hauschild MZ, Wang F, Averous-Monnery S, Laurent A. Global environmental losses of plastics across their value chains. Resour Conserv Recycl. 2019;151: 104459. https://doi.org/10.1016/j.resconrec.2019.104459.
63. PlasticsEurope. Plastics—the fast facts 2023. https://plasticseurope.org/knowledge-hub/plastics-the-fast-facts-2023/. Accessed Jan 2024.
64. Maier RD, Schiller M. Handbuch Kunststoff-additive. 4th ed. München: Hanser; 2016.
65. Asua JM, editor. Polymer reaction engineering. Oxford: Blackwell Publishing Ltd.; 2007.
66. Turner A. Foamed polystyrene in the marine environment: sources, additives, transport, behavior, and impacts. Environ Sci Technol. 2020;54(17):10411–20. https://doi.org/10.1021/acs.est.0c03221.
67. Turner A. Polystyrene foam as a source and sink of chemicals in the marine environment: an XRF study. Chemosphere. 2021;263: 128087. https://doi.org/10.1016/j.chemosphere.2020.128087.
68. Feld L, da Silva VH, Strand J. Characterization of foamed plastic litter on Danish reference beaches—pollution assessment and multivariate exploratory analysis. Mar Pollut Bull. 2022;180: 113774. https://doi.org/10.1016/j.marpolbul.2022.113774.
69. Commission Regulation (EU) 10/2011 of 14 Jan 2011. https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32011R0010&qid=1705310436255. Accessed Jan 2024.
70. Scheirs J, Priddy D, editors. Modern styrenic polymers: polystyrenes and styrenic copolymers. Chichester: Wiley; 2003.
71. Welle F. Diffusion coefficients and activation energies of diffusion of organic molecules in polystyrene below and above glass transition temperature. Polymers. 2021;13(8):1317. https://doi.org/10.3390/polym13081317.
72. Guazzotti V, Hendrich V, Gruner A, Fiedler D, Störmer A, Welle F. Migration of styrene in yogurt and dairy products packaged in polystyrene: results from market samples. Foods. 2022;11(14):2120. https://doi.org/10.3390/foods11142120.
73. Guazzotti V, Hendrich V, Gruner A, Störmer A, Welle F. Styrene monomer levels in polystyrene-packed dairy products from the market versus simulated migration testing. Foods. 2023;12(13):2609. https://doi.org/10.3390/foods12132609.
74. United Nations Environment Programme and Secretariat of the Basel, Rotterdam and Stockholm Conventions. Chem Plast—A Tech Rep. 2023. https://doi.org/10.59117/20.500.11822/42366.
75. Niessner N, Wagner D. Practical guide to structures, properties and applications of styrenic polymers. Shawbury: Smithers Rapra; 2013.
76. Keim W. Kunststoffe—Synthese, Herstellungsverfahren, Apparaturen. Weinheim: Wiley; 2006.
77. Zhang Y, Paul T, Brehm J, Völkl M, Jérôme V, Freitag R, Laforsch C, Greiner A. Role of residual monomers in the manifestation of (cyto)toxicity by polystyrene microplastic model particles. Environ Sci Technol. 2023;57(27):9925–33. https://doi.org/10.1021/acs.est.3c01134.
78. Roper AN, Kite DM, Shell Int. Res. The manufacture of polyvinyl-aromatic compounds, and the resulting polymers. GB Patent 1 175 262 (1969).
79. Kii T, Suka M, Mitsui Toatsu Chem. Inc: Method of polymerizing styrene. US Patent 3 679 651 (1972).
80. Bronstert K, Buchholz K, Echte A, BASF AG: Production of rubber-modified vinylaromatic polymers. US Patent 3 679 651 (1972).
81. Finch CR, Kuntzsch JE, Dow Chem. Co. Process for the production of alkenyl aromatic polymers containing a reinforcing polymer therein. US Patent 3 660 535 (1972).

82. Carter DE, Simon, RHM, Monsanto Co.: Continuous mass polymerization process for polyblends. US Patent 3 903 202 (1975).
83. Morita T, Shimazu K, Dainippon Ink and Chemicals: Production of styrene resins by continuous bulk polymerization. EP Patent 0 307 238 (1989).
84. Mountassir A, Tirri T, Sund P, Wilén CE. Sulfenamides as standalone flame retardants for polystyrene. Polym Degrad Stab. 2021;188: 109588. https://doi.org/10.1016/j.polymdegradstab.2021.109588.
85. Fink JK. Handbook of engineering and specialty thermoplastics. Polyolefins and styrenics, vol. 1. Hoboken: Wiley; 2010.
86. Höfer R. Processing and performance additives for plastics. In: Matyjaszewski K, Möller M, editors. Polymer science: a comprehensive reference, vol. 10. Amsterdam: Elsevier; 2012. p. 369–81.
87. Gausepohl H, Gellert R. Kunststoffhandbuch Bd. 4: Polystyrol. München: Hanser; 1996.
88. Munters CG, Tandberg JG: Heat insulation. US Patent 2 023 204 (1935).
89. McIntire OR, Dow Chem. Co.: Manufacture of cellular thermoplastic product. US Patent 2 450 436 (1948).
90. De La Vega JC, Elischer P, Schneider T, Häfeli UO. Uniform polymer microspheres: monodispersity criteria, methods of formation and applications. Nanomedicine. 2013;8(2):265–85. https://doi.org/10.2217/nnm.12.210.
91. Ugelstad J, Berge A, Ellingsen T, Schmid R, Nilsen TN, Mork PC, Stenstad P, Hornes E, Olsvik O. Preparation and application of new monosized polymer particles. Prog Polym Sci. 1992;17:87–161. https://doi.org/10.1016/0079-6700(92)90017-S.
92. Bangs LB. New developments in particle-based immunoassays: introduction. Pure Appl Chem. 1996;68(10):1873–9. https://doi.org/10.1351/pac199668101873.
93. Vafajoo A, Rostami A, Foroutan Parsa S, Salarian R, Rabiee N, Rabiee G, Rabiee M, Tahriri M, Vashaee D, Tayebi L, Hamblin MR. Multiplexed microarrays based on optically encoded microbeads. Biomed Microdevice. 2018;20(3):66. https://doi.org/10.1007/s10544-018-0314-4.
94. Nesvadba P. Radical polymerization in industry. In: Chatgilialoglu C, Studer A, editors. Encyclopedia of radicals in chemistry, biology and materials. Hoboken: Wiley; 2012. p. 2012. https://doi.org/10.1002/9781119953678.rad080.
95. Chern CS. Emulsion polymerization mechanisms and kinetics. Prog Polym Sci. 2006;31(5):443–86. https://doi.org/10.1016/j.progpolymsci.2006.02.001.
96. Bradford EB, Vanderhoff JW, Alfrey T. The use of monodisperse latexes in an electron microscope investigation of the mechanism of emulsion polymerization. J Colloid Sci. 1956;11(2):135–49. https://doi.org/10.1016/0095-8522(56)90033-2.
97. Sudol ED. Dispersion polymerization. In: Asua JM, editor. Polymeric dispersions: principles and applications. Dordrecht: Springer; 1997. p. 141–54. https://doi.org/10.1007/978-94-011-5512-0.
98. Kawaguchi S, Koichi I. Dispersion polymerization. Adv Polym Sci. 2005;175:299–328. https://doi.org/10.1007/b100118.
99. Wiley RM, Dow Chem. Co.: Method of polymerizing a monoethylenically unsaturated vinylidene compound in aqueous suspension. US Patent 2, 875–185.
100. Lok KP, Ober CK. Particle-size control in dispersion polymerization of polystyrene. Can J Chem. 1985;63:209–16. https://doi.org/10.1139/v85-033.
101. Tseng CM, Lu YY, El-Aasser MS, Vanderhoff JW. Uniform polymer particles by dispersion polymerization in alcohol. J Polym Sci, Part A: Polym Chem. 1986;24:2995–3007. https://doi.org/10.1002/pola.1986.080241126.
102. Spherotech, Inc. 27845 Irma lee circle, lake forest, IL 60045, USA. https://www.spherotech.com/. Accessed 14 Jun 2023.
103. Vilchis L, Ríos L, Villalobos MA, Guyot A. In situ syntheses of a suspension agent based on a styrene–acrylic acid copolymer for the suspension polymerization of styrene. J Appl Polym Sci. 2002;86(13):3271–85. https://doi.org/10.1002/app.11122.
104. Tauer K, Deckwer R, Kühn I, Schellenberg C. A comprehensive experimental study of surfactant-free emulsion polymerization of styrene. Colloid Polym Sci. 1999;277(7):607–26. https://doi.org/10.1007/s003960050433.
105. Thickett SC, Abdelrahman AI, Ornatsky O, Bandura D, Baranov V, Winnik MA. Bio-functional, lanthanide-labeled polymer particles by seeded emulsion polymerization and their characterization by novel ICP-MS detection. J Anal At Spectrom. 2010;25(3):269–81. https://doi.org/10.1039/b916850h.
106. Paul-Pont I, Tallec K, Gonzalez-Fernandez C, Lambert C, Vincent D, Mazurais D, Zambonino-Infante J-L, Brotons G, Lagarde F, Fabioux C, Soudant P, Huvet A. Constraints and priorities for conducting experimental exposures of marine organisms to microplastics. Front Mar Sci. 2018;5:252. https://doi.org/10.3389/fmars.2018.00252.
107. Liu Y, Hu J. Investigation of polystyrene-based microspheres from different copolymers and their structural color coatings on wood surface. Coatings. 2021;11(1):1–14. https://doi.org/10.3390/coatings11010014.
108. Bangs Laboratories, Inc., 9025 Technology drive, fishers, Indiana 46038-2886, USA. https://www.bangslabs.com/support/technical-support. Accessed 22 Apr 2024.
109. Thermo Fisher Scientific, 168 Third Avenue, Waltham, MA 02451, USA. https://www.thermofisher.com/de/de/home/life-science/cell-analysis/qdots-microspheres-nanospheres/idc-surfactant-free-latex-beads/latex-bead-technical-overview.html. Accessed 22 Apr 2024.
110. Polysciences, Inc. 400 Valley road, Warrington, PA 18976, USA. https://www.polysciences.com/media/attachment/2022%20Poly%20catalog.pdf. Accessed 22 Apr 2024.
111. JSR Life Sciences, LLC, 1280 N. Mathilda avenue, Sunnyvale, CA 94089, USA. https://www.jsrlifesciences.com/research-reagents-ivd/immutex. Accessed 22 Apr 2024.
112. Kaiser E, Colescott RL, Bossinger CD, Cook PI. Color test for detection of free terminal amino groups in the solid-phase synthesis of peptides. Anal Biochem. 1970;34(2):595–8. https://doi.org/10.1016/0003-2697(70)90146-6.
113. Esko K, Karlsson S, Porath J. A method for determining free amino groups in polymers with particular reference to the merrifield synthesis. Acta Chem Scand. 1968;22:3342–4. https://doi.org/10.3891/acta.chem.scand.22-3342.
114. Sarin VK, Kent SBH, Tam JP, Merrifield RB. Quantitative monitoring of solid-phase peptide synthesis by the ninhydrin reaction. Anal Biochem. 1981;117(1):147–57. https://doi.org/10.1016/0003-2697(81)90704-1.
115. Ramaye Y, Dabrio M, Roebben G, Kestens V. Development and validation of optical methods for zeta potential determination of silica and polystyrene particles in aqueous suspensions. Materials. 2021;14(2):290. https://doi.org/10.3390/ma14020290.
116. Lowry GV, Hill RJ, Harper S, Rawle AF, Hendren CO, Klaessig F, Nobbmann U, Sayre P, Rumble J. Guidance to improve the scientific value of zeta-potential measurements in nanoEHS. Environ Sci Nano. 2016;3(5):953–65. https://doi.org/10.1039/C6EN00136J.
117. Gao X, Lowry GV. Progress towards standardized and validated characterizations for measuring physicochemical properties of manufactured nanomaterials relevant to nano health and safety risks. NanoImpact. 2018;9:14–30. https://doi.org/10.1016/j.impact.2017.09.002.
118. Jennings TL, Rahman KS, Fournier-Bidoz S, Chan WCW. Effects of microbead surface chemistry on DNA loading and hybridization efficiency. Anal Chem. 2008;80(8):2849–56. https://doi.org/10.1021/ac7026035.
119. Lerman MJ, Lembong J, Muramoto S, Gillen G, Fisher JP. The evolution of polystyrene as a cell culture material. Tissue Eng Part B Rev. 2018;24(5):359–72. https://doi.org/10.1089/ten.teb.2018.0056.
120. Wieland S, Ramsperger AFRM, Gross W, Lehmann M, Witzmann T, Caspari A, Obst M, Gekle S, Auernhammer GK, Fery A, Laforsch C, Kress H. Nominally identical microplastic models differ greatly in their particle-cell interactions. Nat Commun. 2024;15(1):922. https://doi.org/10.1038/s41467-024-45281-4.
121. Banerjee A, Billey LO, Shelver WL. Uptake and toxicity of polystyrene micro/nanoplastics in gastric cells: effects of particle size and surface functionalization. PLoS ONE. 2021;16(12): e0260803. https://doi.org/10.1371/journal.pone.0260803.
122. Patiño T, Soriano J, Barrios L, Ibáñez E, Nogués C. Surface modification of microparticles causes differential uptake responses in normal and tumoral human breast epithelial cells. Sci Rep. 2015;5(1):11371. https://doi.org/10.1038/srep11371.
123. Schultz CL, Bart S, Lahive E, Spurgeon DJ. What Is on the outside matters—surface charge and dissolve organic matter association affect the toxicity and physiological mode of action of polystyrene nanoplastics to C elegans. Environ Sci Technol. 2021;55(9):6065–75. https://doi.org/10.1021/acs.est.0c07121.

124. Stock V, Böhmert L, Coban G, Tyra G, Vollbrecht M-L, Voss L, Paul MB, Braeuning A, Sieg H. Microplastics and nanoplastics: size, surface and dispersant—what causes the effect? Toxicol In Vitro. 2022;80: 105314. https://doi.org/10.1016/j.tiv.2022.105314.
125. Yokota K, Waterfield H, Hastings C, Davidson E, Kwietniewski E, Wells B. Finding the missing piece of the aquatic plastic pollution puzzle: interaction between primary producers and microplastics. Limnol Oceanogr Lett. 2017;2(4):91–104. https://doi.org/10.1002/lol2.10040.
126. Zhang Q, Han YX, Wang W, Zhang L, Chang J. Preparation of fluorescent polystyrene microspheres by gradual solvent evaporation method. Eur Polymer J. 2009;45:550–6. https://doi.org/10.1016/j.eurpolymj.2008.11.016.
127. Sun F, Feng C, Wan Y, Yang X, Zhao H. Preparation of fluorescent polystyrene microspheres by improving swelling performance based on molecular weight and surface carboxyl group content. Química Nova. 2021;45(2):145–51.
128. Brinkley JM, Haugland RP, Singer VL, Molecular probes Inc.: Fluorescent microparticles with controllable enhanced Stokes shift. EP Patent pp. 596–098.
129. Schür C, Rist S, Baun A, Mayer P, Hartmann NB, Wagner M. When fluorescence is not a particle: the tissue translocation of microplastics in daphnia magna seems an artifact. Environ Toxicol Chem. 2019;38(7):1495–503. https://doi.org/10.1002/etc.4436.
130. Rembaum A, Cal. Inst. of Technol.: protein specific fluorescent microspheres for labelling a protein. US Patent 4, 326–008.
131. Kohri M. Development of HRP-mediated enzymatic polymerization under heterogeneous conditions for the preparation of functional particles. Polym J. 2014;46:373–80. https://doi.org/10.1038/pj.2014.13.
132. Tsien RY, Ernst L, Waggoner A. Fluorophores for confocal microscopy: photophysics and photochemistry. In: Handbook of Biological Confocal Microscopy. Boston: Springer; 2006. pp. 338–352. https://doi.org/10.1007/978-0-387-45524-2_16.
133. Yu L, Wang Q, Li T, Chen L. Preparation of a pH-sensitive polystyrene fluorescent microsphere based on a cyanine dye. J Chem Res. 2012;36(11):632–4. https://doi.org/10.3184/174751912X13466874476971.
134. Panchuk-Voloshina N, Haugland RP, Bishop-Stewart J, Bhalgat MK, Millard PJ, Mao F, Leung WY, Haugland RP. Alexa dyes, a series of new fluorescent dyes that yield exceptionally bright, photostable conjugates. J Histochem Cytochem. 1999;47(9):1179–88. https://doi.org/10.1177/002215549904700910.
135. Alford R, Simpson HM, Duberman J, Hill GC, Ogawa M, Regino C, Kobayashi H, Choyke PL. Toxicity of organic fluorophores used in molecular imaging: literature review. Mol Imaging. 2009;8(6):341–54. https://doi.org/10.2310/7290.2009.00031.
136. Cavaco M, Pérez-Peinado C, Valle J, Silva RDM, Correia JDG, Andreu D, Castanho MARB, Neves V. To what extent do fluorophores bias the biological activity of peptides? A practical approach using membrane-active peptides as models. Front Bioeng Biotechnol. 2020;8: 552035. https://doi.org/10.3389/fbioe.2020.552035.
137. Weis JS. Improving microplastic research. AIMS Environ Sci. 2019;6(5):326–40. https://doi.org/10.3934/environsci.2019.5.326.
138. Ding Z, Kriz D, Aklonis JJ, Salovey R. Model filled polymers. IX. Synthesis of uniformly crosslinked polystyrene microbeads. J Polym Sci B Polym Phys. 1992;30(11):1189–94. https://doi.org/10.1002/polb.1992.090301102.
139. Guazzotti V, Gruner A, Juric M, Hendrich V, Störmer A, Welle F. Migration testing of GPPS and HIPS polymers: swelling effect caused by food simulants compared to real foods. Molecules. 2022;27(3):823. https://doi.org/10.3390/molecules27030823.
140. Zhu H, McShane MJ. Loading of hydrophobic materials into polymer particles: implications for fluorescent nanosensors and drug delivery. J Am Chem Soc. 2005;127(39):13448–9. https://doi.org/10.1021/ja052188y.
141. Srivastava P, Tavernaro I, Scholtz L, Genger C, Welker P, Schreiber F, Meyer K, Resch-Genger U. Dual color pH probes made from silica and polystyrene nanoparticles and their performance in cell studies. Sci Rep. 2023;13(1):1321. https://doi.org/10.1038/s41598-023-28203-0.
142. Thermo Fisher Scientific, 168 Third Avenue, Waltham, MA 02451, USA. Particle technology technical notes and reference guide; 2018. https://www.thermofisher.com. Accessed Oct. 2022.
143. European Chemicals Agency (ECHA). https://echa.europa.eu/. Accessed Jul 2023.
144. Metze FK, Sant S, Meng Z, Klok H-A, Kaur K. Swelling-activated, soft mechanochemistry in polymer materials. Langmuir. 2023;39(10):3546–57. https://doi.org/10.1021/acs.langmuir.2c02801.
145. Li Y. Legislation and policy on pollution prevention and the control of marine microplastics. Water. 2022;14(18):2790. https://doi.org/10.3390/w14182790.
146. Letcher TM, editor. Plastic waste and recycling—environmental impact, societal issues, prevention, and solutions. London: Elsevier Academic Press; 2020.
147. Dawson AL, Bose U, Ni D, Nelis JLD. Unravelling protein corona formation on pristine and leached microplastics. Microplast Nanoplast. 2024;4(1):9. https://doi.org/10.1186/s43591-024-00086-6.
148. Galloway TS, Cole M, Lewis C. Interactions of microplastic debris throughout the marine ecosystem. Nat Ecol Evolut. 2017;1(5):0116. https://doi.org/10.1038/s41559-017-0116.
149. Dąbrowska A, Gniadek M, Machowski P. The proposal and necessity of the numerical description of nano- and microplastics' surfaces (plastisphere). Polymers. 2021;13(14):2255. https://doi.org/10.3390/polym13142255.
150. Debroy A, George N, Mukherjee G. Role of biofilms in the degradation of microplastics in aquatic environments. J Chem Technol Biotechnol. 2022;97(12):3271–82. https://doi.org/10.1002/jctb.6978.
151. Waldman WR, De Paoli MA. Photodegradation of polypropylene/polystyrene blends: styrene-butadiene-styrene compatibilisation effect. Polym Degrad Stab. 2008;93(1):273–80. https://doi.org/10.1016/j.polymdegradstab.2007.09.003.
152. Masry M, Rossignol S, Gardette J-L, Therias S, Bussière P-O, Wong-Wah-Chung P. Characteristics, fate, and impact of marine plastic debris exposed to sunlight: a review. Mar Pollut Bull. 2021;171: 112701. https://doi.org/10.1016/j.marpolbul.2021.112701.
153. Ward CP, Armstrong CJ, Walsh AN, Jackson JH, Reddy CM, Ward ACP, Armstrong CJ, Walsh AN, Jackson JH, Reddy CM, Ward CP. Sunlight converts polystyrene to carbon dioxide and dissolved organic carbon. Environ Sci Technol Lett. 2019;6(11):669–74. https://doi.org/10.1021/acs.estlett.9b00532.
154. Kaczmarek H, Felczak A, Szalla A. Studies of photochemical transformations in polystyrene and styrene-maleic anhydride copolymer. Polym Degrad Stab. 2008;93(7):1259–66. https://doi.org/10.1016/j.polymdegradstab.2008.04.011.
155. Singh B, Sharma N. Mechanistic implications of plastic degradation. Polym Degrad Stab. 2008;93(3):561–84. https://doi.org/10.1016/j.polymdegradstab.2007.11.008.
156. Yousif E, Haddad R. Photodegradation and photostabilization of polymers, especially polystyrene: review. SpringerPlus. 2013;2(1):398. https://doi.org/10.1186/2193-1801-2-398.
157. Larue C, Sarret G, Castillo-Michel H, Pradas del Real AE. A critical review on the impacts of nanoplastics and microplastics on aquatic and terrestrial photosynthetic organisms. Small. 2021;17(20):2005834. https://doi.org/10.1002/smll.202005834.
158. Sandt C, Waeytens J, Deniset-Besseau A, Nielsen-Leroux C, Réjasse A. Use and misuse of FTIR spectroscopy for studying the bio-oxidation of plastics. Spectrochim Acta Part A Mol Biomol Spectrosc. 2021;258: 119841. https://doi.org/10.1016/j.saa.2021.119841.
159. Ranby B, Lucki J. New aspects of photodegradation and photooxidation of polystyrene. Pure Appl Chem. 1980;52(2):295–303. https://doi.org/10.1351/pac198052020295.
160. Gardette JL, Mailhot B, Lemaire J. Photooxidation mechanisms of styrenic polymers. Polym Degrad Stab. 1995;48(3):457–70. https://doi.org/10.1016/0141-3910(95)00113-Z.
161. Zvekic M, Richards LC, Tong CC, Krogh ET. Characterizing photochemical ageing processes of microplastic materials using multivariate analysis of infrared spectra. Environ Sci Process Impacts. 2022;24(1):52–61. https://doi.org/10.1039/d1em00392e.
162. Bottino FA, Di Pasquale G, Pollicino A. Studies on polymer surface photooxidation. Recent Res Devel Polym Sci. 1998;2:203–10.

163. Rivaton A, Gardette JL, Mailhot B, Morlat-Therlas S. Basic aspects of polymer degradation. Macromol Symp. 2005;225:129–46. https://doi.org/10.1002/masy.200550711.
164. Mauel A, Pötzschner B, Meides N, Siegel R, Strohriegl P, Senker J. Quantification of photooxidative defects in weathered microplastics using 13C multiCP NMR spectroscopy. RSC Adv. 2022;12(18):10875–85. https://doi.org/10.1039/d2ra00470d.
165. Karakolis EG, Nguyen B, You JB, Rochman CM, Sinton D. Fluorescent dyes for visualizing microplastic particles and fibers in laboratory-based studies. Environ Sci Technol Lett. 2019;6(6):334–40. https://doi.org/10.1021/acs.estlett.9b00241.
166. Kefer S, Friedenauer T, Langowski HC. Characterisation of different manufactured plastic microparticles and their comparison to environmental microplastics. Powder Technol. 2022;412: 117960. https://doi.org/10.1016/j.powtec.2022.117960.
167. Chen J, Xu Z, Liu Y, Mei AX, Wang X, Shi Q. Cellular absorption of polystyrene nanoplastics with different surface functionalization and the toxicity to RAW264.7 macrophage cells. Ecotoxicol Environ Safety. 2023;252:114574. https://doi.org/10.1016/j.ecoenv.2023.114574.
168. Kefer S, Miesbauer O, Langowski H-C. Environmental microplastic particles vs. engineered plastic microparticles—a comparative review. Polymers. 2021;13(17):2881. https://doi.org/10.3390/polym13172881.
169. von der Esch E, Lanzinger M, Kohles AJ, Schwaferts C, Weisser J, Hofmann T, Glas K, Elsner M, Ivleva NP. Simple generation of suspensible secondary microplastic reference particles via ultrasound treatment. Front Chem. 2020;8:169. https://doi.org/10.3389/fchem.2020.00169.
170. Glais M, Falher T, Deniau E, Chassenieux C, Lagarde F. Elaborating more realistic model microplastics by simulating polypropylene's environmental ageing. Ecotoxicol Environ Saf. 2024;283: 116769. https://doi.org/10.1016/j.ecoenv.2024.116769.
171. Eitzen L, Paul S, Braun U, Altmann K, Jekel M, Ruhl AS. The challenge in preparing particle suspensions for aquatic microplastic research. Environ Res. 2019;168:490–5. https://doi.org/10.1016/j.envres.2018.09.008.
172. Munoz M, Ortiz D, Nieto-Sandoval J, de Pedro ZM, Casas JA. Adsorption of micropollutants onto realistic microplastics: role of microplastic nature, size, age, and NOM fouling. Chemosphere. 2021;283: 131085. https://doi.org/10.1016/j.chemosphere.2021.131085.
173. Kühn S, van Oyen A, Booth AM, Meijboom A, van Franeker JA. Marine microplastic: preparation of relevant test materials for laboratory assessment of ecosystem impacts. Chemosphere. 2018;213:103–13. https://doi.org/10.1016/j.chemosphere.2018.09.032.
174. Astner AF, Hayes DG, O'Neill H, Evans BR, Pingali SV, Urban VS, Young TM. Mechanical formation of micro- and nano-plastic materials for environmental studies in agricultural ecosystems. Sci Total Environ. 2019;685:1097–106. https://doi.org/10.1016/j.scitotenv.2019.06.241.
175. Wang L, Zhang J, Huang W, He Y. Laboratory simulated aging methods, mechanisms and characteristic changes of microplastics: a review. Chemosphere. 2023;315: 137744. https://doi.org/10.1016/j.chemosphere.2023.137744.
176. Sun Y, Yuan J, Zhou T, Zhao Y, Yu F, Ma J. Laboratory simulation of microplastics weathering and its adsorption behaviors in an aqueous environment: a systematic review. Environ Pollut. 2020;265: 114864. https://doi.org/10.1016/j.envpol.2020.114864.
177. Martinho SD, Fernandes VC, Figueiredo SA, Vilarinho R, Moreira JA, Delerue-Matos C. Laboratory studies about microplastic aging and its effects on the adsorption of chlorpyrifos. Polymers. 2023;15(16):3468. https://doi.org/10.3390/polym15163468.
178. Alimi OS, Claveau-Mallet D, Kurusu RS, Lapointe M, Bayen S, Tufenkji N. Weathering pathways and protocols for environmentally relevant microplastics and nanoplastics: What are we missing? J Hazard Mater. 2022;423: 126955. https://doi.org/10.1016/j.jhazmat.2021.126955.
179. Born MP, Brüll C. From model to nature—a review on the transferability of marine (micro-) plastic fragmentation studies. Sci Total Environ. 2022;811: 151389. https://doi.org/10.1016/j.scitotenv.2021.151389.
180. Borgatta M, Breider F. Inhalation of microplastics—a toxicological complexity. Toxics. 2024;12(5):20–3. https://doi.org/10.3390/toxics12050358.
181. Oberdörster G, Maynard A, Donaldson K, Castranova V, Fitzpatrick J, Ausman K, Carter J, Karn B, Kreyling W, Lai D, Olin S, Monteiro-Riviere N, Warheit D, Yang H. Principles for characterizing the potential human health effects from exposure to nanomaterials: elements of a screening strategy. Part Fibre Toxicol. 2005;2(1):8. https://doi.org/10.1186/1743-8977-2-8.
182. Organisation for Economic Co-operation and Development (OECD). (2012). Guidance on sample preparation and dosimetry for the safety testing of manufactured nanomaterials. ENV/JM/MONO. 2012; 40. http://www.oecd.org/officialdocuments/publicdisplaydocumentpdf/?cote=ENV/JM/MONO(2012)40&docLanguage=En.
183. Organisation for Economic Co-operation and Development (OECD). Series on testing and assessment: guidance document on inhalation toxicity studies, second ed. Paris. 2018; 39. https://www.oecd.org/officialdocuments/publicdisplaydocumentpdf/?cote=env/jm/mono(2009)28/rev1&doclanguage=en.
184. Organisation for Economic Co-operation and Development (OECD). Test no. 413: Subchronic inhalation toxicity: 90-day study. 2018. https://www.oecd-ilibrary.org/environment/test-no-413-subchronic-inhalation-toxicity-90-day-study_9789264070806-en%0Ahttps://www.oecd-ilibrary.org/environment/test-no-413-subchronic-inhalation-toxicity-90-day-study_9789264070806-en%0Ahttps://www.oecd-ilib.
185. Bouwmeester H, Lynch I, Marvin HJP, Dawson KA, Berges M, Braguer D, Byrne HJ, Casey A, Chambers G, Clift MJD, Elia G, Fernandes TF, Fjellsbø LB, Hatto P, Juillerat L, Klein C, Kreyling WG, Nickel C, Riediker M, Stone V. Minimal analytical characterization of engineered nanomaterials needed for hazard assessment in biological matrices. Nanotoxicology. 2011;5(1):1–11. https://doi.org/10.3109/17435391003775266.
186. Chen CY, Lin Z. Exploring the potential and challenges of developing physiologically-based toxicokinetic models to support human health risk assessment of microplastic and nanoplastic particles. Environ Int. 2024;186: 108617. https://doi.org/10.1016/j.envint.2024.108617.
187. Mariano S, Tacconi S, Fidaleo M, Rossi M, Dini L. Micro and nanoplastics identification: classic methods and innovative detection techniques. Front Toxicol. 2021;3: 636640. https://doi.org/10.3389/ftox.2021.636640.
188. Kalaronis D, Ainali NM, Evgenidou E, Kyzas GZ, Yang X, Bikiaris DN, Lambropoulou DA. Microscopic techniques as means for the determination of microplastics and nanoplastics in the aquatic environment: a concise review. Green Anal Chem. 2022;3: 100036. https://doi.org/10.1016/j.greeac.2022.100036.
189. Geiss O, El Hadri H, Giechaskiel B, Al Wasif Ruiz T, Putaud J, Barrero J. Micro- and nanoplastics in the air: sources, transport & analytical methods for their determination. Luxembourg: Publications Office of the European Commission, Joint Research Centre; 2024. https://data.europa.eu/doi/https://doi.org/10.2760/028991, JRC135818.
190. Blancho F, Davranche M, Fumagalli F, Ceccone G, Gigault J. A reliable procedure to obtain environmentally relevant nanoplastic proxies. Environ Sci Nano. 2021;8(11):3211–9. https://doi.org/10.1039/D1EN00395J.
191. Gniadek M, Dabrowska A. The marine nano- and microplastics characterisation by SEM-EDX: the potential of the method in comparison with various physical and chemical approaches. Mar Pollut Bull. 2019;148:210–6. https://doi.org/10.1016/j.marpolbul.2019.07.067.
192. Singh B, Kumar A. Advances in microplastics detection: a comprehensive review of methodologies and their effectiveness. TrAC Trends Anal Chem. 2024;170: 117440. https://doi.org/10.1016/j.trac.2023.117440.
193. Ourgaud M, Phuong NN, Papillon L, Panagiotopoulos C, Galgani F, Schmidt N, Fauvelle V, Brach-Papa C, Sempéré R. Identification and quantification of microplastics in the marine environment using the laser direct infrared (LDIR) technique. Environ Sci Technol. 2022;56(14):9999–10009. https://doi.org/10.1021/acs.est.1c08870.
194. Ramsperger AFRM, Jasinski J, Völkl M, Witzmann T, Meinhart M, Jérôme V, Kretschmer WP, Freitag R, Senker J, Fery A, Kress H, Scheibel T, Laforsch C. Supposedly identical microplastic particles substantially differ in their material properties influencing particle-cell interactions and cellular responses. J Hazard Mater. 2022;425: 127961. https://doi.org/10.1016/j.jhazmat.2021.127961.
195. Peez N, Janiska M-C, Imhof W. The first application of quantitative 1H NMR spectroscopy as a simple and fast method of identification and quantification of microplastic particles (PE, PET, and PS). Anal Bioanal Chem. 2019;411(4):823–33. https://doi.org/10.1007/s00216-018-1510-z.
196. Hildebrandt J, Thünemann AF. Aqueous dispersions of polypropylene: toward reference materials for characterizing nanoplastics. Macromol Rapid Commun. 2023;44(6):2200874. https://doi.org/10.1002/marc.202200874.

197. Graillat C, Pichot C, Guyot A. Preparation and characterization of low size polystyrene latex particles with various strong acid surface charges. Colloids Surf. 1991;56:189–200. https://doi.org/10.1016/0166-6622(91)80120-D.
198. Dong Y, Gao M, Song Z, Qiu W. As(III) adsorption onto different-sized polystyrene microplastic particles and its mechanism. Chemosphere. 2020;239: 124792. https://doi.org/10.1016/j.chemosphere.2019.124792.
199. Turner BF, Fein JB. Protofit: a program for determining surface protonation constants from titration data. Comput Geosci. 2006;32(9):1344–56. https://doi.org/10.1016/j.cageo.2005.12.005.
200. Nielsen MB, Clausen LPW, Cronin R, Hansen SF, Oturai NG, Syberg K. Unfolding the science behind policy initiatives targeting plastic pollution. Microplast Nanoplast. 2023;3(1):3. https://doi.org/10.1186/s43591-022-00046-y.
201. European research cluster to understand the health impacts of micro- and nanoplastics (CUSP). https://cusp-research.eu/. Accessed Aug 2024.
202. Plastics monitoRIng detectiOn RemedIaTion recoverY (PRIORITY). https://ca-priority.eu/. Accessed Aug 2024.
203. Bridging the knowledge gap between microplastics and human health (BRIGID). https://plasticseurope.org/sustainability/plastics-health/microplastics/brigid/. Accessed Aug 2024.
204. Microplastics Advanced Research and Innovation Initiative (MARII). https://icca-chem.org/focus/microplastics-advanced-research-and-innovation-initiative-marii/. Accessed Aug 2024.
205. Pilapitiya PNT, Ratnayake AS. The world of plastic waste: a review. Clean Mater. 2024;11: 100220. https://doi.org/10.1016/j.clema.2024.100220.

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