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Production, labeling, and applications of micro- and nanoplastic reference and test materials†

Guillaume Crosset-Perrotin, ‡[a] Angélique Moraz, ‡[b] Raquel Portela, ‡[c]
Victor Alcolea-Rodriguez, ‡[c] David Burrueco-Subirà, [d] Casey Smith, [e]
Miguel A. Bañares, [c] Hosein Foroutan [f] and D. Howard Fairbrother *[e]

Challenges inherent to the extraction of micro- and nanoplastics (MNPs) from the environment, combined with the limited range of commercially available MNPs, have prompted an increasing number of researchers to generate in-house reference and test MNPs. The first part of this review provides a comprehensive overview of existing MNP production methods, including top-down and bottom-up fabrication techniques. Strengths and weaknesses of different methods are compared and contrasted, and the potential for optimization and control over MNP properties is discussed. Methods to label and to artificially weather MNPs before, during, or after production, as well as appropriate dispersion protocols for introducing MNPs into different media, are also covered. The second part of this review focuses on how reference and test MNPs have been implemented in different types of studies, categorized as toxicity, uptake, fate, and monitoring. Given the wide range of properties needed to fully define MNPs, we propose a set of essential properties that need to be characterized depending on the study type. Looking forward, we suggest future needs, not only in the creation of reference MNPs, but also in experimental protocols that would help to better understand the behavior and impacts of MNPs. Overall, this review aims to provide the necessary information to guide researchers in decision-making regarding which reference MNPs are most appropriate to answer their specific research questions and to serve as a framework that will contribute to obtaining reliable, benchmarked data urgently needed to develop consensus on the fate and risk posed by MNPs.

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Environmental significance

The number of studies on the potential hazard of MNPs is surging, but the lack of representative, traceable and well-characterized reference and test MNPs hampers quality control and inter-study comparability and limits the scope and reliability of the extracted conclusions. This study evaluates the main fabrication methods for such particles, and their respective strengths, weaknesses, scalability, ease of use, cost, and availability. It also examines the techniques used to label and to weather reference and test MNPs prior to, during, or after production. Based on the research question investigated, the key MNPs properties to characterize are identified. This study aims to provide a baseline for the standardization of production and characterization of reference MNPs, which will ultimately facilitate the assessment of the risk associated with MNPs.

1 Introduction

As an essential material in modern life, plastics have a wide range of applications, particularly polyethylene (PE, accounting for 27% of the global plastics production), polypropylene (PP, 19%), polyvinyl chloride (PVC, 13%), polyethylene terephthalate (PET, 6%), polyurethane (PU, 6%), and polystyrene (PS, 5%).[1] Polyolefins (*e.g.* PE and PP) are typically used for food packaging, extrusion, or molding[2] while PVC finds use in furniture, piping and in buildings.[2] In contrast, PU has a wide range of applications such as building insulation, car seats and footwear. PET is employed in the production of plastic bottles and clothing. Native PS is

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[a] *Eawag, Swiss Federal Institute of Aquatic Science and Technology, Ueberlandstrasse 133, 8600 Duebendorf, Switzerland*
[b] *Agroscope, Swiss Federal Research Station for Agroecology and Agriculture, Reckenholzstrasse 191, 8046 Zürich, Switzerland*
[c] *CSIC – Instituto de Catalisis y Petroleoquimica (ICP), C/Marie Curie 2, 28049 Madrid, Spain*
[d] *Leitat Technological Center, C/de la Innovació 2, 08225 Terrassa, Barcelona, Spain*
[e] *Department of Chemistry, Johns Hopkins University, Baltimore, MD, USA. E-mail: howardf@jhu.edu*
[f] *Department of Civil & Environmental Engineering, Virginia Tech, Blacksburg, VA, USA*

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‡ Contributed equally to this work.

used in packaging, while expanded PS foams are common insulation materials. With increasingly stringent environmental concerns and regulations there is also a push to use more naturally sourced and/or biodegradable plastics such as polylactic acid (PLA).

In some specific applications plastics need to be manufactured in the micro- and nanometer size range, including microfibers for the textile industry, millimetric commodity pellets for manufacturing, micro, and nanoparticles for additive manufacturing, biomedical applications, paints, and personal care products.[3–6] These small-sized plastics, referred to as primary microplastic (MP, >1 μm and <5 mm) and nanoplastic (NP, <1 μm) particles (referred to hereafter as MNPs)[7,8] can enter different environmental matrices through various pathways.[9–12] However, plastics usually enter the environment as much larger macroscopic objects either discarded at the end of their use phase or as debris from waste mismanagement. Due to their generally poor biodegradability, these macroscopic plastics persist in the environment and are embrittled and fragmented into secondary MNPs by weathering (*e.g.* by mechanical wear or photodegradation).[13,14] It is these secondary MNPs that account for a majority of MNP pollution, as primary MNPs are becoming increasingly regulated.[15,16]

Due to their persistence and mobility, MNPs have been detected in almost every environmental compartment, including remote areas such as deep-sea trenches,[17] mountains,[18] and the Arctic.[19] Further, recent reports show the presence of MNPs in plants[20] and animals,[21] as well as human blood,[22] lungs[23] and placenta.[24] However, the accuracy of these studies is sometimes questioned. Indeed, one of the biggest reasons for the lack of clarity regarding the environmental fate and impact of MNPs stems from the fact that the identification and quantification of MNPs in different environments is extremely arduous. Due to the extremely low concentrations of MNPs in the environment, the first step in identification and characterization requires them to be concentrated and/or separated from the environmental or biological matrix following protocols that often comprise several digestion and filtration steps, which can lead to particle losses and contamination.[25,26] The inherent challenges associated with these processes, combined with the lack of procedural standardization ranging from sampling to identification, make comparing results from different studies extremely challenging.[26,27] Consequently, it is unclear whether the high variability in MNP concentrations reported in a given environmental compartment (*e.g.* water bodies, soil), in potential sources for human-exposure (*i.e.* food, beverages, personal care products, *etc.*), or the human body itself, reflects the inconsistency amongst analytical methods or actual MNP concentration variations.[26] These considerable analytical difficulties result in a lack of quantitative information on MNP exposure and thus the risks posed by MNPs, although there is a widespread consensus that their concentration in the environment is increasing.[15]

These concerns have been responsible for a rapid increase in the number of studies evaluating the fate and effects of MNPs, including their toxicity, a topic which is intensely debated. On the one hand, the physical properties of the MNPs may be a key factor of their toxicity, for example in the flock worker's lung disease, similar to other hazardous non-plastic micro- or nanoparticles such as asbestos or silica dust.[28–30] On the other hand, the chemical components within the plastics or adsorbed onto their surface, such as chemical additives or metals, also have the potential to significantly contribute to the hazards of MNPs.[31,32] Indeed, it is unclear whether physical or chemical characteristics, or both dictate the toxicity of the MNPs because often some or most of them are neither controlled nor reliably reported.[15,33] This example of toxicity is illustrative of the current challenges in MNP research. Moreover, variability is also observed in the results of ecotoxicity studies, which may be indicative of real differences, but also reflect inconsistent quality control or method validation.

One of the most significant difficulties in accurately measuring MNP exposure, fate and hazard is the limited accessibility to reference and test MNPs, because well-defined and varied MNPs provide an opportunity to ensure data quality and conduct controlled studies to identify the role that different physicochemical properties (*e.g.* size, shape, composition, surface properties) play in regulating key behaviors relevant to their environmental fate and effects. According to the terminology of the National Institute of Standards and Technology (NIST) and ISO Guide 30, the majority of MNP research is undertaken using research grade test materials, because MNP reference materials (sufficiently homogeneous and stable with respect to one or more characterised properties) are not readily available. Ideally, these MNPs should represent the main polymer types, particle sizes, shapes, surface properties and polymer compositions encountered in the environment. To effectively deploy reference and test MNPs in scientific studies, it is also important that a well-defined dose of these materials is homogeneously distributed into relevant environmental matrices, where they can serve either as a quality control (to exactly define recovery rates, detection limits, and analytical quality of a method)[26] or as a tracer to study MNPs transport, fate, and effects. A survey of the literature reveals that a majority of studies employ commercially available, spherical, monodisperse, pristine particles, typically made of PS or PE, which may behave differently than fragmented, and aged particles found in the environment.[34,35] Moreover, these materials are often costly and their manufacturers sometimes provide limited information regarding the homogeneity, stability, and presence of surfactants or additives in the plastics. These types of existing limitations have driven an increasing number of research groups to develop and use their own test and reference MNPs.

The importance of developing reference and test MNPs to improve the research quality in the field was recognized by a workshop of the American Chemistry Council in 2022. The stakeholders identified and summarized the needs and knowledge gaps for standardizing MNPs production and characterization methods,[36] acknowledging that suitable production methods must be reproducible and with high yields. The two main methods of obtaining test and reference MNPs are chemical bottom-up and physical top-down approaches, each of which have innate strengths and weaknesses with respect to the range and properties of MNPs they can produce. To date, the majority of in-house produced MNPs are fragments, predominantly obtained by milling, a poorly defined process with a generally low yield for MPs of smaller sizes and NPs.[37] Bottom-up methods generally offer better control over MNP properties and labels can be incorporated into MNPs during production to facilitate their detection or quantification, particularly in more complex matrices. In some MNP production methods uncontrolled aging can accompany the MNP manufacturing processes.

Despite the growing in-house production of reference and test MNPs, the capabilities, advantages, and limitations of the diverse existing methods have received limited attention.[37,38] Besides, there is also a lack of appropriate characterization of the produced MNPs, tailored to the specific needs of different research studies. This deficiency is in part because the range of characteristics that fully describe a MNP is large, which poses a challenge given the limited range of analytical methods available to most researchers.

This review article assesses the current state of efforts directed towards the production, modification, and applications of reference MNPs and is a result of a 2023 workshop organized as part of The International Network For Researching, Advancing, and Assessing Materials for Environmental Sustainability (INFRAMES) program.§ Specifically, this paper aims to review: i) the main options to obtain test and reference MNPs, focusing on top-down and bottom-up production methods, ii) the possibility of mimicking the characteristics of the environmental MNPs by weathering, iii) the techniques for MNPs labeling to improve their detection, iv) the use of test and reference MNPs in the scientific literature, and v) the key properties of reference and test MNPs that should be characterized and how these depend on the specific research question. Although some discussion of MNP dispersion and the analytical techniques for MNP characterization and detection is included, these important topics are not the focus of this review. For more details on MNPs identification and quantification methods, the reader is directed towards several notable recent publications and review articles.[39–42]

2 Methods to obtain MNPs

Fig. 1 is a schematic of the principal routes researchers have used to obtain reference and test MNPs. Notably, reference and test MNPs can be obtained by: 1) direct purchase from a commercial supplier (commercial MNPs), including a few MNPs certified by metrology institutes; 2) collection and extraction from the environment; and 3) in-house production through top-down or bottom-up approaches in the laboratory. Commercial MNPs are the easiest to obtain, although this is the most expensive approach, and the MNPs are limited to specific polymer types, sizes, surface chemistries and shapes (ESI† 1). The most direct and representative approach, although challenging, is to extract MNPs from the environment. In controlled laboratory conditions, MNPs can be generated through size-reduction of macroscopic materials such as pellets, sheets, or environmental debris by physical means, typically mechanical (top-down methodologies, see section 2.3), or through chemical reactions and solvent separation processes (bottom-up, see section 2.4). Before, during or after production, lab-made MNPs can also be labeled to improve their traceability and quantification and/or weathered to improve their environmental relevance. In the following sections, we review the main methods that have been used to produce and modify reference and test MNPs. It should be noted that in this review the term “yield” is used to denote the mass of MNPs produced per unit mass of the initial material, whereas “production rate” is the mass of MNPs produced per unit time.

2.1 Commercial MNPs

In the last decade a number of commercial MNPs have become available and they have found widespread use in environmental studies.[35,36,43,44] Despite their relative popularity, the choices of MNPs are often limited to monodisperse and spherical particles, obtained through reproducible, high-yield chemical methods (see section 2.4), though a few manufacturers offer fragmented variants. ESI† 1 contains a current list of commercially available MNPs, including information on the manufacturer, quantity, size, shape, polymer type, label if there is one, type of dispersion (suspension or powder), and price. This table is accessible in an online repository and intended to be continuously updated with new manufacturers and reference materials (see Data availability) The currently available MNP sizes range from tens of nanometers up to hundreds of micrometers, typically with low polydispersity values (variation coefficients less than 1%). The majority of commercial MNPs are restricted to a few polymer types, most notably PS and PE, which collectively represent over approximately one third of total global plastic production.[1] In contrast, PP, PVC, PU, and PET which also constitute an important fraction of the total plastic production, are far harder to obtain as commercial MNPs. The range of commercially available MNPs available for use as test materials could be extended if primary MNPs were made accessible and their characteristics were known

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§ https://inframes.pratt.duke.edu/about.

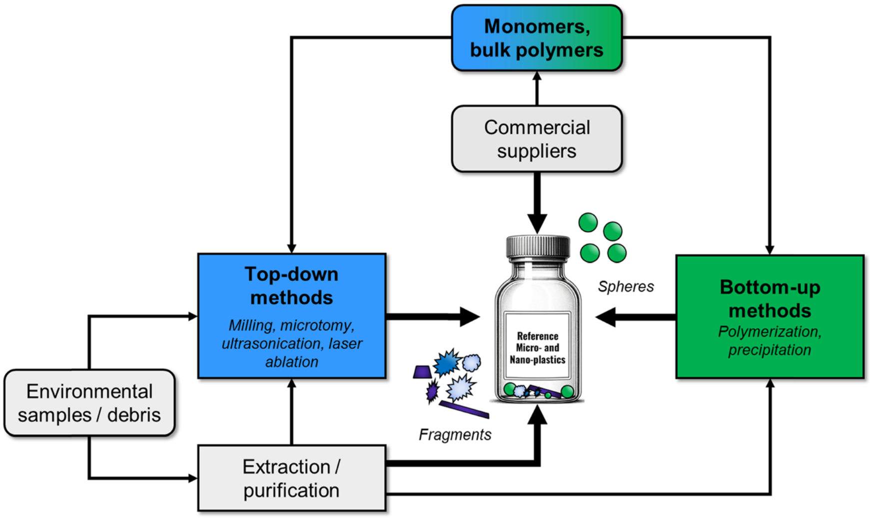

Fig. 1 Schematic overview of the main routes to obtain reference micro- and nanoplastic materials (MNPs).

and reproducible. To aid in detection, some MNPs can be purchased with an embedded fluorescent, phosphorescent label, or enriched with a stable isotope (*i.e.* ^{13}C or ^{2}H).

Unfortunately, most MNPs cannot be considered reference materials according to NIST or ISO terminology. However, some new reference MNPs are currently under development as part of different projects and initiatives, so it is expected that in the near future the number of commercially available reference MNPs will grow. A very small number of commercial MNPs can be purchased from metrology institutes with certified properties; for example, standard reference materials (SRMs) can be obtained from the National Institute of Standards and Technology (NIST) consisting of spherical PS NPs with certified size (SRM 1964 – 60 nm ± 0.63, SRM 1963a – 101.8 ± 1.1 nm, SRM 1691 – 269 ± 4 nm, SRM 1690 – 895 ± 5 nm, and SRM 1961 – 29.64 ± 0.06 µm). Several companies offer NIST-traceable size standards of PS MNPs in the range of sizes from 20 nm to 160 µm (for example, Applied Physics). Interestingly, the German Federal Institute for Materials Research and Testing (BAM) offers micrometric cryogenically milled PS and PET fragments, and even cryogenically milled artificially UV-aged PE fragments. These particles are accompanied by a certificate of analysis of their morphology, size distribution, crystallinity, and surface chemistries, but are typically expensive, with prices reaching several hundred dollars per gram even for the cheapest ones (ESI† 1).

Certain characteristics of commercially available MNPs, such as the presence and concentration of organic or inorganic additives, surface properties, and stability are typically unknown or not reported, which complicates efforts to elucidate how these factors influence their behavior.[42] Small-sized commercial MNPs are often sold as suspensions wherein the particles' stability has been enhanced by functionalization (*e.g.* carboxylated or aminated) or the addition of surfactants. The use of surfactants is strongly discouraged for test and reference MNPs because their presence could lead to surface interactions that differ from environmental MNPs. Indeed, Wieland *et al.*[45] recently determined that nominally identical PS MPs acquired from different manufacturers differ significantly in their physicochemical properties, particularly zeta potential. This difference was likely due to the presence of various surfactants, initiators, and catalysts used during the synthesis, affecting particle–cell interactions. This study highlights the importance of characterizing MNPs "in-house" regardless of their nominal or reported physicochemical properties such that their behaviors can be meaningfully compared to other studies. One of the biggest differences between commercial MNPs and those encountered in the environment is that MNPs found in nature are invariably composed of polydisperse and irregularly shaped, non-spherical particles.[34] As a result, there is no guarantee that the behavior of commercial MNPs is an accurate reflection or predictor of real-world MNP environmental fate and effect.

2.2 MNPs extracted from the environment

MNPs can be extracted from environmental samples and repurposed as reference and test MNPs in subsequent experiments.[38,46,47] For instance, Waldschläger *et al.*[47] collected floating particles from a river using a 5 mm mesh net and selected those that visually appeared plastic for further analysis with FTIR and Raman spectroscopy to confirm polymer types. To the best of our knowledge, this is the only study that collected actual MNPs (down to 580 µm) for subsequent use as test materials without subsequent size-reduction (*e.g.* milling of larger sized fragments).

In studies aiming at reusing environmental MNPs as reference MNPs, extraction steps for purification and concentration will almost certainly be required because of the heterogeneous matrix and the likelihood that MNPs only constitute a very small fraction of the particles present. For example, MNPs may be intermixed with sand or sediment particles and/or be coated with an eco-corona. These coatings will likely need to be removed and the MNPs separated from the rest of the environmental constituents. Based on existing protocols, coatings are removed by oxidation with hydrogen peroxide, or alkaline, acidic, or enzymatic digestion, while mineral particles such as sand and sediment can be removed by sieving, density-based flotation, oil-based extraction,[48–52] and electrostatic[53] or magnetic[54] particle extractions.[40,48,55] To optimize purification and collection of environmental MNPs, these steps can be combined in different sequences depending on the complexity of the matrix. Regardless of the extraction chosen, it is crucial to preserve the integrity of the particles during the process and prevent unintentional aging or damage due to harsh chemical treatments. One possible solution to address this issue would be to use commercial MNPs as an internal control, subjecting them to the equivalent extraction process, and then characterizing the product to ensure that no alterations occurred during processing.[51]

In principle, extracted MNPs offer significant advantages as reference materials, because the particles are realistic and represent the complexity and diversity of particulate plastic pollution in the environment in terms of shapes, sizes, polymer types, and properties. However, given the complexity of these real-world samples, MNPs first require identification with spectroscopic methods. Unfortunately, the trace concentrations of MNPs in the environment necessitate the processing of large sample masses to obtain sufficient material. Moreover, given the complexity of these real-world samples, MNPs must first be identified and characterized with time-intensive spectroscopic methods. As a result, extracting and characterizing MNPs from environmental samples can take days to weeks,[48] in contrast to in-house production methods, which usually require just a few hours. Moreover, the extraction processes have the real possibility of producing unintended artificial weathering of the MNPs. For these reasons, the extraction of MNPs as reference and test materials from realistic environmental samples is extremely challenging, severely limiting the overall utility of this methodology. This is reflected in the lack of popularity for this approach.[56]

2.3 Top-down production

Top-down approaches encompass mechanical and thermal methods that physically reduce the size of plastics to generate MNPs. The principal top-down techniques used by researchers, shown in Fig. 2, are (cryo-)milling, sonication, laser ablation, sanding, and (cryo-)microtomy. These will be described in more detail in the following sections. Table 1 summarizes the main reports using top-down methods to produce MNPs as well as the key parameters that define the method (procedure, origin of the plastic, polymer type, size, morphology, pre- or post-production labeling).

2.3.1 Grinding or milling. Grinding and milling are machining processes where physical size reduction of macroscopic plastics is achieved, for example with stainless-steel balls in a hermetic chamber[66] or rotary blades.[60] As a result of the random directionality of the stresses that fracture the plastic, the resulting MNPs are irregularly shaped and polydisperse. Additional sieving steps or sequential fractionations may be required to obtain a specific size range. Incomplete fractionation may occur, as dry MNPs may aggregate due to electrostatic charges; however, this can be minimized using a wet sieving process.[62]

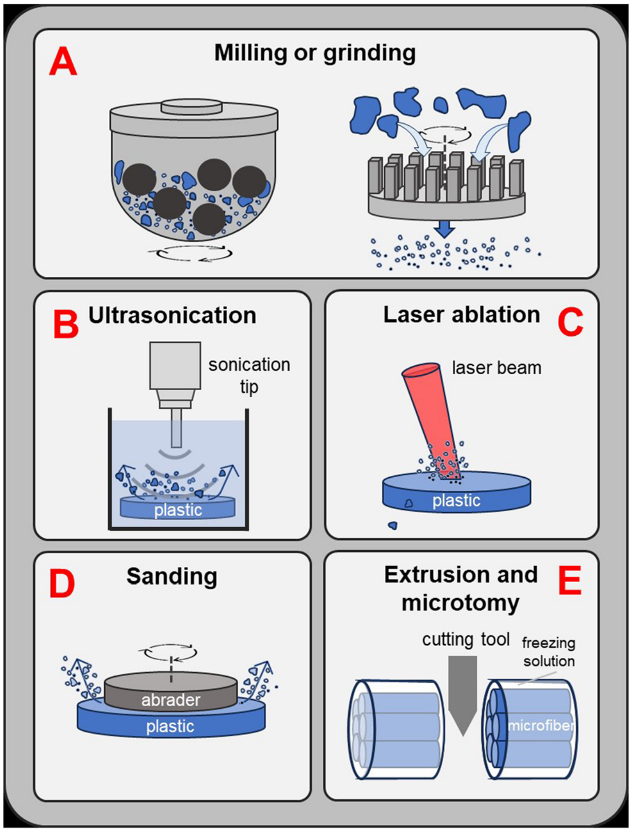

Fig. 2 Schematic of top-down approaches used to produce MNPs, including (A) milling and grinding, (B) sonication, (C) laser ablation, (D) sanding, (E) extrusion and microtomy.

A wide range of plastics can be milled using readily available household instruments. For example, PE, PS, PES, PVC, PET, and PP fragments of hundreds of micrometers in size were generated using a kitchen grinder.[72,79] An immersion blender was used for between 10–120 min to grind PP, PS, and PE fragments into similar sizes of a few hundred micrometers.[80,81] Similarly, Ekvall *et al.*[82] used an immersion blender for 5 min, followed by a <0.45 μm filtration step, and observed PS nanoplastics in the 125–437 nm range. Although the yield was not reported, it is reasonable to assume that only a very small quantity of NPs was produced. Relevant experimental parameters such as the initial material size, the blade rotation per minute, or the blending time are generally not provided, making it challenging to reproduce and optimize these methods. While not reported in the literature, it is worth mentioning that contamination of the MNP surface due to the various plastic

Table 1 Reference and test MNPs produced by top-down methods

Production method	Labeling	Starting material	MNPs morphology	MNP size	Characterization techniques	Advantages	Limitations	Ref.
Blade grinder and planetary ball mill		PS (Goodfellow) PE (Total)	Fragments	0.07 µm (PE) 0.3 µm (PS)	ζ, DLS, AF4, ATR-FTIR	• Method reproducibility	• High affinity for common polymeric membrane filters	57
Cryomilling and cascade sieving		PBAT, Grade EF04P (Mater-Bi) LDPE (Dow Chemical)	Fragments	5.9–500.7 µm (PBAT) 591.0–43.2 µm (LDPE)	DLS, TGA, FTIR, DSC, GPC	• Simple and effective • Extended to other plastic families • Employed to investigate kinetics and time course of size reduction, salinity	• Small yield (5%) on the later fraction (45 µm)	58
Blade milling in liquid nitrogen		PET (Indorama, Polymers Europe)	Fragments	~60 µm	FE-SEM, Raman	• Fast • High yield • Simple • Accessible	• Loss of crystallinity during milling	59
Blade milling in liquid nitrogen		PLA (ErcrosBio LL 650)	Fragments	240 ± 65 µm	FE-SEM, Raman	• Fast • High yield • Realistic MPs		60
Cryomilling		PP, PMMA (LG Chem.) PET (LOTTE Chemical)	Fragments	50–300 µm	FE-SEM, Raman, DSC	• Fast • High yield • Realistic MPs	• Bulk material may be affected during synthesis	61
Cryogenic grinding and wet-sieving		PVC (Flexfilm)	Fragments	75–2000 µm	LD	• Fast • Extended to other plastic families	• Broad size distribution • Large particles only	62
Cryogrinding with ball mill and sieving		PE PP (ocean)	Fragments	Polydisperse	Coulter counter, LD, SEM	• Fast • High yield • Realistic MPs	• Reproducibility is limited	63
Centrifugal milling		PP (Lyondell Basell)	Fragments	1–180 µm	SLS, SEM-EDX, TGA, XRF	• Many monodisperse size fractions	• Possible talc contamination	64
Cryomilling with a ball mill and sieving		PVC (Sigma-Aldrich Merck)				• Stable particles	• Only for plastics with density >1	
Cryomilling, sieving, oxidation with ozone in water		PS	Fragments	1–100 µm	SEM, Coulter counter	• Extended to other plastic families	• Broad size range • Specialized equipment • PS oxidation • Generation of dissolved organic compounds	65
Cryomilling with a ball mill		PS (culture test tubes, Fisherbrand)	Fragments	250 nm	ATR-FTIR, SEM, Coulter counter, NTA	• No aging during cryomilling • Fast	• Low yield (<0.1%)	66
Cryomilling	NR, NB staining	PA (Lanxess) PET (TPL) PLA (Nature Works) PS, LDPE, PVC (Ineos) PMMA (Röhm) PP (Borealis)	Fragments	90–125 µm	Optical microscopy, DLS	• Fast • High yield • Realistic MPs	• Size and shape heterogeneity	67
Milling and sieving		PET	Fragments (flakes)	5–60 µm 61–499 µm 500–3000 µm	ATR-FTIR, FE-SEM	• Fast • High yield		68
Mechanical abrasion *via* stirring and ultrasonication		PP, PE (environmental debris)	Fragments	120–180 nm 600–800 nm	DLS, TEM, XPS, BET, Py-GC/MS, ATR-FTIR	• Simple • High yields (80–90%)		69

Table 1 *(continued)*

Production method	Labeling	Starting material	MNPs morphology	MNP size	Characterization techniques	Advantages	Limitations	Ref.
Crushing, grinding, milling, and aging with UV and ozone sonication		PS (single-use forks)	Fragments	90–100 µm 1–5 µm (after aging)	SEM-EDX, ATR-FTIR, ζ, DSC, XRD, BET	• High concentration (~400 mg·L^{-1}) • Fast and simple aging protocol •Monodisperse • Artificially and naturally aged particles similarity	• Size distribution limited to 1–5 µm or ~100 µm • Only PS	70
Alkaline ultrasonication		PLA PS PET PVC PE PP PA (daily life products)	Fragments Fibers Films	30.2 ± 12.1 µm (PS) 32.0 ± 6.5 µm (PLA) 26.6 ± 5.2 µm (PET)	Raman, ATR-FTIR, µ-FTIR, SEM-EDX	• Size and shape reproducibility for PLA and PET • Efficient to produce aged particles • High yield and production rate	• Slight color change • Potential alteration with KOH • Poor reproducibility for PS, PE, PP, PVC and PA • Poor interlab reproducibility • PLA and PS are oxidized	71
Fragments ground with household blender		PE	Fragments		ATR-FTIR	• Fast	• Large particles (>100 µm)	72
Fibers cut with a scalpel and encapsulated in methylcellulose or gelatine		PP PS PES (daily life products)	Fibers	100–500 µm		• Simple • Affordable	• ATR-spectra suggest modification of the plastics	
Paraffin wax embedding and microtomy	NR staining	PET PA6,6 (Goodfellow)	Fibers	14.7 ± 4.8 µm 28.4 ± 3.6 µm 41.3 ± 3.5 µm 11.8 ± 5.1 µm 30.2 ± 2.9 µm 108.3 ± 24.3 µm	Optical microscopy	• Fast • Target size is precise, repeatable and modulable	• Microtome availability	73
Extrusion and microtomy or cryomilling		HDPE, PP, PS (LG Chem) PET (Lotte Chemical) PA6 (Hyosung TNC Co.)	Cylinders Fragments	74–133 µm (cylinders) ~10–100 µm (fragments)	Optical microscopy, FE-SEM, FTIR, DLS, Py-GC/MS	• Simple method • Suitable for any plastics	• High polydispersity • Filtration • Cylinders not environmentally relevant	74
Cryotomy	NR staining	Nylon (PA) PET PP (Goodfellow)	Fibers	40, 70, 100 µm (length) Aspect ratio >1 : 3	Optical microscopy, SEM	• Monodisperse • Targeted size • Cheap and quick (~60k fibers per h)	• Limited to fibers <100 µm • Requires −80 °C • Freezing might modify the properties	75
Microtomy		PA6 PA6.6 PET PP (Aquafil S.)	Fibres	200 µm (length)	ATR-FTIR	• Suitable for fibers from different origins	• Possible variability of fiber length should have been considered in the detection probability	76

Table 1 *(continued)*

Production method	Labeling	Starting material	MNPs morphology	MNP size	Characterization techniques	Advantages	Limitations	Ref.
Laser ablation and filtration		PC PET (Goodfellow)	Spheres with irregular surface	50 nm	DLS, TEM, XPS	• Monodisperse nanoplastics • High production rate • Suitable for any plastics	• Formation of carboxyl and hydroxyl group during ablation • Change of surface charge	77
Laser ablation and rotavap		PET (Goodfellow)	Fragments Spheres	26.7 ± 14.2 nm	TEM, DLS, AF4, ζ, XPS, spectrophotometer for CCC and CSC evaluation	• Extended to other plastic families • Controllable polydispersity and size	• Oxidation • Laser needed • Low yield • Small size	78

or metal parts of the kitchenware may also occur, although this potential issue has not been explicitly addressed.

Due to the viscoelastic properties of plastics, grinding results in the deformation of the material and inhibits complete breakdown.[59] To overcome this limitation, milling is often carried out under cryogenic conditions using liquid nitrogen to impart brittleness to the plastic. This process, referred to as cryogenic milling or grinding, facilitates size reduction by preventing melting caused by the frictional heat released during milling.[58,64,66] Cryomilling also reduces the size polydispersity of the resulting material in comparison to room-temperature conditions, and the size distribution can be further modulated by varying the parameters used to cryomill the MNPs.[64,66] Thus, Eitzen *et al.* (2019) evaluated the effect of liquid nitrogen cooling time prior to milling on the size distribution of PS granules.[65] Milling without pre-cooling resulted in 90% of the milled PS mass being composed of particles larger than 100 μm. When the pre-cooling time was increased to 12 min, only 45% of the particle mass was composed of particles larger than 100 μm, the rest being smaller than 100 μm. The same study also evaluated the impact of the number of milling cycles on the mass distribution of PS MNPs.[65] After one 10 min cycle at 30 Hz, more than 95% of the PS MNP mass was in excess of 100 μm. Increasing the number of milling cycles to more than three resulted in a mass fraction dominated by particles of sizes between 50 and 100 μm.[65] Overall, these results highlight the potential to control size distributions of milled MNPs, although the impact of other milling parameters (*e.g.* rotation speed, time, or quantity of material under milling, *etc.*) on particle size distribution has yet to be investigated systematically. These efforts would also benefit from more thorough reporting of mass, volume, and size distribution of resulting MNPs.

In addition to size distribution, it is important to characterize the physicochemical properties of ground or cryomilled MNPs. Although Smith *et al.* (2024) did not observe any measurable changes in the surface chemistry or chemical bonds in cryomilled PS, PMMA, PE and PVC MNPs compared to the nascent polymers, as determined by XPS and ATR-FTIR, some changes in the physical properties of MNPs after milling have been reported.[83] For example, Jiménez-Arroyo *et al.* (2023) observed a change in intensity of the 397 *vs.* 410 cm^{-1} Raman bands for cryomilled micrometric PLA, indicative of a higher amorphicity and a broader crystallinity distribution compared to the as-received PLA millimetric pellets.[60] Similarly, Tamargo *et al.* (2022)[59] noted a slight loss in crystallinity for cryomilled PET compared to the initial material due to particle cracking, embrittlement, and surface fracturing associated with compression during cryogrinding. Cuthbertson *et al.* (2024)[84] also reported changes of both crystallinity and molecular weight due to cryomilling. Unfortunately, the impact of milling/cryomilling on MNP properties is rarely reported. More systematic data and understanding is certainly needed in this area, particularly given the widespread use of this technique to create MNPs.

The popularity of grinding, blending, milling and cryomilling as means to create MNPs can be attributed to the apparatus being fast, widely available, inexpensive, and capable of generating MNPs for a variety of polymer types. However, the substantial polydispersity of the resulting material and low particle yield[58,66] are limiting factors, particularly for nanoplastics generation.

2.3.2 Ultrasonication. Ultrasonication is a relatively simple method for generating MNPs, where pieces of plastics are exposed to sound energy using a sonication probe or in an ultrasonic bath.[69,71,85,86] The formation and implosion of cavitation bubbles during ultrasonication generate high-frequency sound waves, thereby producing mechanical energy that breaks down plastic. Free radicals generated during the process also contribute to the fragmentation process.[71] The resulting MNPs can then be concentrated through filtration or centrifugation. For example, Von Der Esch *et al.* (2020) sonicated several types of plastics under alkaline conditions (pH = 13) for 15 h; the high pH, coupled with sonication created a harsh oxidative environment, leading to a higher rate of particle formation compared to the situation when sonication was conducted in circumneutral (pH ≈ 7) water. This same protocol was also successfully applied to PLA, PS, PP, PE, and PET to produce fragments with sizes ranging from ~1 to over 100 μm. For PLA, PE, and PS, the size distribution among replicates was similar, demonstrating a high reproducibility for this procedure,[71,86] although sonicators may differ from one lab to the other and result in some variability. Other advantages of this approach are that 50% of particles were smaller than 20 μm, with a comparatively high (10^4–10^5 particles) yield of 5 to ~100 μm sized particles as compared to other top-down methods. However, there are some limitations with regards to polymer types that can be processed by this approach; for example, under basic conditions polyamides form gels and therefore cannot fragment. While Raman spectroscopy measurements revealed no significant post-sonication modification of the plastics,[71] this could be because changes are restricted to the outermost surface as would be revealed by surface sensitive techniques such as XPS or confocal depth profile Raman.

Blancho *et al.* (2021) agitated and sonicated beach and sea plastic debris made of PE and PP, which generated nanosized fragments (~150 to 500 nm) reaching a concentration of 400 mg L^{-1} in the resultant solution.[69] The yield, which ranged from 80 to 90% of the mass of particles <1 mm, was remarkably high for nanoplastic production using a top-down technique, and was likely due to the use of weathered and potentially embrittled plastics. Similarly, Enfrin *et al.* (2020) extracted PE nanoplastics from facial scrub products (~400 nm) and subjected them to ultrasonication, generating nanosized particles (~50 nm).[86] The amount of NPs produced (10^{13} to 10^{14} particles per g of initial material) correlated with the energy input of the sonicator, demonstrating the potential to optimize the generation of NPs.

Given the common availability of ultrasonic baths in many laboratories, this is an ideal and somewhat underutilized method for producing reference and test MNPs, albeit ones that are possibly aged.

2.3.3 Laser ablation. Laser ablation is an established method for producing nanoparticles.[87] In brief, a plastic is placed in a liquid, typically Milli-Q water, and irradiated with a continuous or pulsed laser. Light absorption produces a temperature increase resulting in melting, vaporization, and, ultimately, fragmentation of the plastic.[88] The fragmentation efficiency depends on the laser wavelength, fluence (energy per unit area), pulse duration, and number of pulses. Since most polymers absorb predominantly in the UV range, lasers with low wavelengths (below 400 nm) are used.[88] For example, Tolardo *et al.* (2022)[77] and Magrì *et al.* (2018)[78] utilized laser ablation to produce relatively monodispersed spherical-shaped PET and PC MNPs with mean diameters ranging from 26.7 to 57.7 nm. By adjusting the fluence, the particle size was controlled across a wide range from 20 nm to ~1 μm.[89,90] This particle size tunability stems from the ability of higher fluences to promote the formation of larger particles.[89,90] The size and structure of the produced particles are also influenced by the viscosity of the liquid in which the polymer is placed,[90] and ablation rates can decrease with higher molecular weight polymers.[88] These considerations make it clear that parameter optimization is necessary to achieve the desired particle properties. However, regardless of the parameters applied, the ablated particles generally tend to be spherically shaped. Laser ablation has also been applied to other polymers such as PA, PE, PMMA, or PTFE,[88,91] and with suitable modifications it is reasonable to expect that this method can be applied to any plastic. In terms of yield, Magrì *et al.* (2018)[78] reported the generation of ~10 μg of MNPs per laser cycle, which only takes a few seconds.

Similar to ultrasonication, laser ablation may well modify the surface properties of the plastic. For example, an increase in the O/C ratio of PET and PC MNPs, and the formation of carboxyl and hydroxyl groups have been reported, likely a consequence of photooxidation.[77,78,88,92] Given the nature of the production method, which in some sense amounts to a form of accelerated aging, laser ablation can be considered a viable means to produce spherical, weathered MNPs. Overall, laser ablation can produce monodisperse MNPs in the nanosized range in a relatively short time, but the specialized nature of the equipment as compared to milling or grinding for example means that this method has yet to find widescale implementation.

2.3.4 Sanding. Sanding with diamond rotary burrs[93] or polishing paper discs[94] can also achieve size-reduction of macroscopic plastics and thereby generate MNPs with heterogeneous shapes representative of those encountered in the environment, particularly in the low micro- to nanosize regime.[93] Unfortunately, few studies have reported details of the sanding process or the effect of sanding parameters on the MNPs making it hard to develop as a standardized approach. One notable exception is a study by Grigoriadi *et al.* (2023) who examined the influence of sandpaper grit, abrasion time, and pressure applied to the plastic during sanding.[94] For sanding times of less than 5 min, particle size distributions were monodisperse, although absolute sizes were strongly polymer dependent; PP (~10 μm) = PE (~10 μm) > PET (~1 μm) > PS (~0.5 μm). Each sanding cycle produced between 0.03 and 0.3 grams of MNPs, representing a reasonably high yield. The largest particle sizes were obtained with the coarsest sandpaper (grit size ~125 μm), with a systematic decrease in particle size as the grit density of the sanding paper increased, down to a minimum particle size at grit sizes of ~5 μm.[94] The same group also proposed a mathematical expression based on the mechanical properties of the plastics to predict the particle size generated as a function of the energy input.[94,95] The development of these types of parameterized relationships is of great value to the standardization of MNP production. However, the MNPs formed during sanding are often poorly characterized and there is the real possibility of surface contamination arising from material transfer from the sanding paper, so it remains unclear if and under what conditions the physicochemical properties of MNPs prepared in this way differ from those of the bulk plastic.

2.3.5 Extrusion followed by microtomy. The previous top-down methods described in this section produce spherical or irregularly-shaped particles. However, a considerable quantity of MNPs in the environment are present in the form of synthetic fibers, a consequence of their widespread use in the textile and clothing industries and released, for example, during laundering.[96] The definition generally accepted for fibers is particles of an aspect ratio larger than 1 : 3.[7] While fibers can be more easily distinguished from other particles in the environment due to their characteristic shape, micro- and nanofibers are not to the best of our knowledge commercially available. Therefore, there is a need to develop laboratory-based methods to create this class of MNPs. The production of micro- and nanofibers usually is a two-step process where first a long polymer filament is generated through extrusion, such as melt-spinning,[97,98] which is then cut at regular intervals.

The general approach to produce long polymer filaments is to melt plastic pellets or powders before extruding them. This high throughput technique has been applied to PHBV, PLA, and PET and produced kilogram quantities of filaments per hour with a diameter of ~30 μm.[97,98] Electrospinning is another extrusion process, where a liquid polymer (either dissolved or melted) passes through a spinneret and is elongated by an electric field, forming a thin filament. It is important to note that the filaments are typically collected as complex non-woven networks and rarely as a single filament.[99] This process can be performed with a simple apparatus, enabling the fabrication of fibers typically as small as a few hundred nanometers in diameter for multiple polymer types – including PS, PVC, PLA, PP, PE, PU, nylon 6,6, PMMA, and PET.[99] Furthermore, the characteristics of the filament are easily controllable.[99] For instance, the diameter of the fiber can be tuned by adjusting specific parameters such as the voltage applied or the flow rate of the polymer solution. The throughput of about 1 g h^{-1} is reasonably high, although the resulting filament must still be cut to obtain fibers. While electrospinning demonstrates significant potential, particularly for nanofiber production, the generation of reference fibers with such a method has not yet been reported.

Common approaches to cutting the resulting filament into micro- and nanofibers involve ball-milling or manual cutting with a kitchen knife.[98,100,101] However, the fibers produced are typically longer than 100 μm, polydisperse, and exhibit a high degree of sample-to-sample variability. Alternatively, a microtome, a cutting tool that prepares thin sample sections, has been used to more precisely reduce the length of the microfibers.[75] To implement this tool, the thin plastic filaments are aligned before being placed in the microtome and sliced at regular intervals. At room temperature, alignment of the filaments can be accomplished by placing them in a wax such as paraffin that can subsequently be dissolved in an organic solvent to leave behind an aligned bundle of fibers.[73] Another option is cryomicrotomy, which involves aligning the plastic filaments by using a freezing agent at −80 °C, before the filaments are cut with the microtome.[75] Cryomicrotomy and microtomy can successfully cut most common plastics found as microfibers (PET, PP, and nylon 6,6) to targeted lengths, from 10 to 100 μm.[73,75] The cutting process allows for the production of fibers at rates ranging from 10^5 to 10^6 fibers per h. Although microtomy has only been applied to cut microfibers down to lengths as small as 10 μm,[73,75] there is no reason *a priori* why this approach could not be extended to produce nanofibers, provided that plastic nanofilaments are available. As a final note, we have classified the generation of fibers as a top-down method because the main challenge is to cut the resulting filament at very small intervals rather than the extrusion process, which is a well-established industrial bottom-up process.

2.3.6 Summary of top-down approaches. Grinding and milling are the most commonly used techniques due to their wide availability in labs, while the other top-down methods are significantly less common, and sometimes relatively niche. In some instances, the lack of popularity for a particular method is apparent; laser ablation requires specialized instrumentation and training. But some methods appear underutilized; thus, it remains unclear why simple techniques such as sanding and ultrasonication are not more widely used. Apart from laser ablation, which tends to produce well-defined, spherical-shaped fragments, most top-down methods generate irregular, jagged particles. Grinding and milling generate highly polydisperse fragments, although sieving can narrow the size distribution if desired. Sanding and ultrasonication appear to produce fairly monodisperse particles, though further investigations are required to assess the size characteristics of the resultant MNPs. Despite limited instrument availability, microtomy is a commonly used technique to fabricate fibers, likely because it can achieve high throughput and reproducibility.

The yields and production rates of top-down methods, though not always reported, are reasonable for particles larger than ~1 μm, but unfortunately low for nanosized particles. All top-down techniques can be applied to both pristine and aged plastics. There is a suggestion that weathered plastics are more amenable to fragmentation by strong mechanical (such as milling and sanding) or chemical (sonication) stress as compared to their pristine counterparts,[70,102] potentially opening up a route to higher MNP yields.

Alteration of MNPs as a consequence of using top-down methods, due to contamination or oxidation of the plastic surface, has sometimes been reported, highlighting the need to systematically investigate the unintentional changes that can occur during fragmentation. Indeed, some top-down methods do produce weathered MNPs, and metal contamination, including Zr and Y with milling,[64] Al with sanding[93] or Pb, Ti, Fe, and Al with ultrasonication.[70] This contamination is due to material transfer from the apparatus, so the use of metal-free parts must be prioritized, when possible, because this type of surface contamination may be critical, for example in artificially skewing MNP toxicity measurements. In contrast, induced surface changes (excluding metal transfer) can help to mimic real MNPs characteristics, since the majority of MNPs in the environment are secondary MNPs formed by the physical breakdown of plastic debris. Therefore, top down approaches seem to be the most relevant to produce reference and test MNPs for environmental and human impact studies. However, it must be noticed that nanoplastics generated by mechanical routes have been reported not only to be oxidised, but also to have a low surface charge, facilitating their stable dispersion in water.[103] One of the biggest challenges in creating true-to-life MNPs is understanding how to mimic as closely as possible the environmental processes. In this line, a very recent publication explores the effects, advantages and limitations of three distinct cryogenic grinding techniques (ultracentrifugal mill, immersion blender, mixer mill).[104]

In conclusion, further investigation of ultrasonication and sanding methods are warranted due to their efficient production of monodisperse low micron-sized and nanosized particles. Given that most studies utilizing grinding and milling often lack a clear description of the methodology employed, we encourage future studies to publish a detailed procedure and to attempt fine-tuning milling parameters in order to obtain specific size distributions and reduce polydispersity. This additional information could enable the modeling of the size distributions of the resulting material as a function of the instrument parameters (*i.e.* precooling time, milling frequency, *etc.*). More easily accessible top-down methods that can create reference and test nanofibers would be welcome as the study of these high aspect ratio MNPs is severely lacking.

2.4 Bottom-up production

Bottom-up methods involve the controlled production of MNPs from 1) smaller building blocks, typically by polymerization of monomers, or 2) by solvent removal or precipitation of MNPs from solutions of dissolved macroscopic plastics (Fig. 3). Table 2 summarizes the main reports using bottom-up methods to produce MNPs along with the key information related to their production and properties (technique used, origin of the plastic, polymer type, size, morphology, labeling). These chemical processing approaches offer significantly more control over the size and composition of the resulting MNPs, typically in the nano- or low micron-sized range as compared to top-down methods (Table 2). The high yields and reproducibility associated with these methods are well suited to scale-up MNP production. Moreover, there is no uncontrolled MNP aging associated with bottom-up methods, and in some instances, controlled surface functionalization can be performed during or post-production, for example, with carboxyl groups introduced by the addition of acrylic acid during the polymerization. The presence of these surface functional groups can mimic MNP aging[105] or provide anchoring points for subsequent metal labeling.[106] Labels can also be incorporated into the bulk of MNPs during bottom-up production (*e.g.* metal or fluorescent cores), as described in the next section. Bottom-up

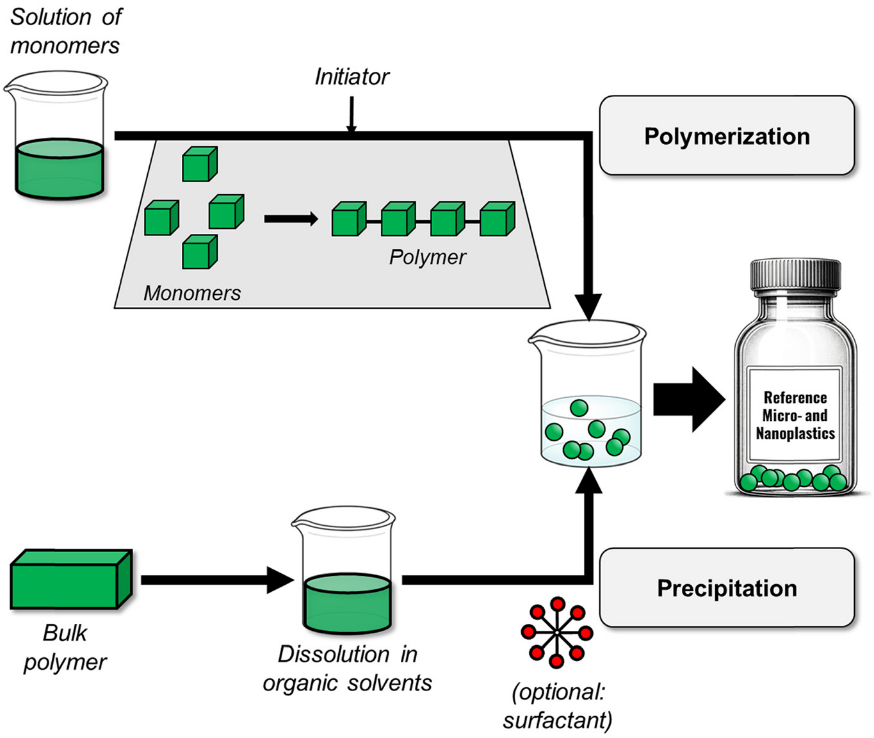

Fig. 3 Schematic representation of the two main routes for the bottom-up production of micro and nanoplastics (MNPs).

Table 2 Reference and test MNPs produced by bottom-up methods

Production method	Labeling	Starting material	MNP morphology	MNP size	Characterization techniques	Advantages	Limitations	Ref.
Emulsion polymerization and conjugation with functionalized gold-containing nanoparticles	Au	PS, (Polysciences, Warrington, PA)	Spherical	15 ± 5 nm (Au)	SP-ICP-MS	• Detection and quantification of nanoplastics in aqueous media	• Approach not directly applied in more complex matrices presenting negatively charged species (organic dissolved matter and/or other particles)	106
				759 ± 23 nm, 990 ± 20 nm, 3030 ± 121 nm (PS)	SEM	• Carboxyl groups at MNPs surface mimic plastic aging • Conjugation with functionalized positively charged AuNPs		
One-step polymerization and direct radiolabeling of a sulfonate end-capped nano-sized PS	^{14}C	PS	Spherical	20.0–47.5 nm	Biomolecular imaging (autoradiography)	• NPs trackable in simulated environmental settings	• Low accessibility to autoradiographic equipment	110
					ATR-FTIR	• Yield of production of 84 ± 28%	• Unrealistic scenario with synthesized nanoPS • Necessity to study the behavior of synthetic nanoPS *vs.* PS in the environment • Limited characterization possible because of radioactive label	
Synthesis of a polyacrylonitrile core containing Pd followed by addition of a cross-linked PS shell	Pd entrapment	PS	Spherical (raspberry-like if synthesis conditions are modified)	150–190 nm	SEM	• Techniques pre-developed for measuring inorganic nanoparticles can be used	• Metal tracer alone cannot provide size, shape, or polymer identity information	111
					TEM STEM-EDX DLS ICP-MS	• Lack of leaching of metal tracer indicates suitability for ecotoxicity tests		
Synthesis PS and P2VP copolymer intercalated with metal nanoparticles and capped with polymeric shell	Au, Pt, Pd entrapment	PS-P2VP	Spherical	300–500 nm	DLS	• Techniques pre-developed for measuring inorganic nanoparticles can be used	• Metal tracer alone cannot provide size, shape, or polymer identity information	112
		PMMA-P2VP			AFM HAADF-STEM SEM-EDX FFF ζ FTIR TGA Raman spICP-MS	• Lack of leaching of metal tracer indicates suitability for ecotoxicity tests		

Table 2 *(continued)*

Production method	Labeling	Starting material	MNP morphology	MNP size	Characterization techniques	Advantages	Limitations	Ref.
In situ growth of Au nanoparticles on NPs surface	Au nanoparticle adsorption	PS	Spherical	50–1200 nm (PS)	TEM			113
		PE		0.2–9.54 μm (PE)	SEM-EDX			
		PVC		0.21–1.54 μm (PVC)	XPS			
		PET		0.12–53.63 μm (PET)	NTA			
		PP		160–582.5 nm (PP)	spICP-MS			
		PMMA		400 nm (PMMA)				
Free-radical polymerization		PE	Spherical	30–110 nm	DLS TEM NMR	• Works without surfactant • Lower polydispersity without surfactant than with	• Lower yield without surfactant	114
Free-radical polymerization		PE	Spherical	10–110 nm	DLS Cryo-TEM SEC NMR DSC	• Fast process (4 h) and important production possible (grams) • Yields up to 30%	• Surfactant (SDS)	115
Nickel(II)-catalyzed emulsion polymerization		PE	Disc-shaped	30–300 nm	DSC DLS TEM AFM	• Monodisperse	• Rather low molecular weight (~10^3 g mol^{-1}) • Potential nickel contamination	116
Polymerization with Ziegler–Natta catalyst		PP	Spheres	<500 nm, 1 μm, 5 μm, 10 μm, 20 μm	SEM TEM AFM XRD FTIR DSC	• Monodisperse particles in the micrometer size range	• Potential metal contamination (Al, Ti)	117
Dissolution and emulsification with a surfactant or biosurfactant	NR added to solution	PE (CTTM)	Fragments	200–800 nm	TGA DSC	• Simple, cheap and fast for NP • Labelling possible High yield with surfactant	• Density of final product is higher than the initial ones • High polydispersity Use of a surfactant	118

Table 2 *(continued)*

Production method	Labeling	Starting material	MNP morphology	MNP size	Characterization techniques	Advantages	Limitations	Ref.
Dissolution and precipitation in water	Rhodamine B added to solution	PET (IZO HomeGoods)	Spheres	95 ± 14 nm (no fluorescence) 88 ± 14 nm (with fluorescence)	DLS ζ ATR-FTIR ^{19}F-NMR TEM SEM UV-vis Raman Py-GC/MS XPS	• Stable particles • Monodisperse • Simple method	• Produced nano PET different than the initial one • Only valid for PET	119
Dissolution and reprecipitation with surfactants		LDPE HDPE PVC PP PS PET (Goodfellow)	Spheres	40 nm (LDPE) 50 nm (PVC, PET) 70 nm (HDPE, PP) 200 nm (PS)	FPA-FTIR NTA SEM	• Simple protocol using common reagents • Monodisperse	• Could not recognize IR spectra of PET • Surfactants might affect surface properties	120
Dissolution in different solvents and nanoprecipitation with DMSO, water/DMSO or water/ethanol		LDPE pellets (Sumitomo Seika Chemicals) LDPE sheets (KOKUGO) HDPE rods (Alfa Aesar and ESCO) PP pellets (Sigma-Aldrich) PVC powder (FUJIFILM Wako Pure Chemical Corporation) PS sheets (Goodfellow)	Spheres	426–463 nm (LDPE, HDPE, PP) 180 nm (PVC) 226 nm (PS)	Optical microscopy GPC DSC SEM	• >90% of NPs • Properties generally similar to commercial polymers	• Crystallinity of reference particles lower than commercial polymers	121
Impinging jet mixing process for nanoprecipitation or nanoemulsion, followed by cryomilling	NR, fluorescein, rhodamine B added to solution	PET granules (Goodfellow) PS granules (Sigma-Aldrich) PE flakes (Sigma-Aldrich) PP granules (Sigma-Aldrich)	Spheres Fragments	PET (31 nm) PS (71 nm) PE (32 nm) PP (31 nm)	AFM SEM DLS ζ	• High yields • Stable NPs • Small-sized particles • Easy-to-add fluorescent tags and	• Surfactant needed	107

Table 2 *(continued)*

Production method	Labeling	Starting material	MNP morphology	MNP size	Characterization techniques	Advantages	Limitations	Ref.
					DSC TGA	chemical additives • Simple setup-up		
Dissolution of ball-milled plastics and nanoprecipitation		PET (plastic bottles)	Spheres	90 nm	DLS TEM XPS	• Monodisperse • No surface oxidation during synthesis	• Surfactant • Ball-milling before dissolution	77
Dissolution and nanoprecipitation		PET (plastic bottles) PET films (Goodfellow)	Spheres	69, 79, 80, 85, 108, 126 and 154 nm	DSC DLS FE-SEM	• Controlled size-distribution • No surfactant	• Not environmentally relevant shapes	122
Dissolution and nanoemulsion	Au, Pt, octaethylporphyrin, quantum dots added to solution	PP (Sigma-Aldrich)	Spheres	80–350 nm	SEM EDX spICP-MS DLS Raman	• 90% yield • High production rate • Can easily add different tags • Reproducible • Monodisperse • Good colloidal stability • Signal of NPs is similar to bulk polymer	• Shapes not environmentally relevant	123
Dissolution and nanoprecipitation with SDS	NR staining	PET (drink bottle)	Fragments	224 nm	TEM DLS ATR-FTIR	• Monodisperse • Large production rates	• Surfactant (SDS)	124
Dissolution of PET in TFA, stirred and centrifuged. Precipitate resuspended in SDS and ultrasonicated		PET (bottles) PET (synthesized) PS (Sigma-Aldrich)	Spheres (PS) Fragments (PET)	200, 500, 1000 nm (PS) <200 nm (PET)	AFM Raman NTA SEM	• High sensitivity and reliability in the trace nanoplastics detection		125
Dissolution and precipitation		PA-6 (pellets) LDPE (Lupolen) PET PU	Fragments	0.102 μm (PA-6) 11–50 μm (LDPE) 1–7 μm (PET) 1.03 μm (PU)	DSC Analytical centrifugation FTIR SEM He-pycnometry Electron paramagnetic resonance ζ Headspace-GC	• High yields and production rate • Irregular morphologies		126

methodologies also offer the possibility of incorporating specific additives or producing additive-free MNPs.[107] One disadvantage of bottom-up methods is that the shape of the MNPs is typically spherical (Table 2), in contrast to the irregularly shaped MNPs encountered in the environment. Another important issue may arise from the possible incomplete removal of surfactants, solvents, precursors, or initiators employed in some bottom-up approaches. These species may lead to erroneous conclusions[38,108,109] about MNP properties, so a detailed analysis of the liquid phase wherein the MNP is suspended at the end of the synthesis is strongly encouraged, but this is rarely reported.

To some extent, the bottom-up synthesis of MNPs leverages existing knowledge on creating polymeric NPs for drug delivery in biomedical applications.[3,4] At the same time, significant current efforts are being directed towards developing new innovative methods and refining existing techniques to achieve better control over MNP properties such as particle size, shape, or crystallinity. Fig. 3 shows the two main bottom-up strategies for creating reference MNPs that are summarized in the next sections: polymerization reactions and solvent removal/precipitation, both in emulsions or suspensions.

2.4.1 Polymerization. MNPs of any polymer type can in principle be synthesized directly from their respective monomers, which can be obtained commercially or synthesized from precursors. Various free-radical or catalytic polymerization techniques can be used, such as micro-emulsion, mini-emulsion, surfactant-free emulsion, and interfacial polymerization.[127] In these methods, dispersed monomers, often in water, are emulsified by stirring, generally in the presence of surfactants, before a water-soluble initiator is added to trigger the polymerization. Soap-free emulsion polymerization has also been successfully employed, for example, to obtain water-stable monodisperse PS NPs with different concentrations of surface carboxyl groups and roughness using ammonium[128] or potassium persulfate as initiators.[105] These nanoplastics were free of additives and their metal content was below 100 ppt, as demonstrated by inductively coupled plasma-mass spectrometry ICP-MS. One challenge of the polymerization approach is that the produced particles can exhibit lower molecular weight (<5000 g mol^{-1}) (*e.g.* by free-radical polymerization of PE under mild conditions[114]), crystallinity (*e.g.* free radical emulsion polymerization of PE[115]), or melting temperature (*e.g.* nickel(II)-catalyzed emulsion polymerization of PE[116]) compared with commercial polymers. In this respect, it should be noted that high molecular weight PP ($>10^5$ g mol^{-1}) with sizes as low as 60 nm has been obtained by polymerization with the Ziegler–Natta catalyst,[117] although this requires very harsh conditions including a final purification step to remove the metallic catalysts and the residual propylene monomer.

Polymerization has also been used as part of a 3D printing process to produce MP fibers of different sizes, shapes and aspect ratios. In this process, an acrylic resin is polymerized with spatial specificity into MP fibers using a two-photon lithography technology. The density of the resultant fibers is similar to that of many common MNPs (PP, PS, HDPE).[129]

2.4.2 Solvent removal methods. In solvent removal methods, preformed plastics dissolve and the solvent is then displaced or removed by evaporation or by the addition of a second solvent, in which the polymers are less soluble, to form the MNPs. Starting materials are typically millimeter-sized pre-production pellets, but films or other plastic pieces from the environment that have previously undergone size reduction have also been used (Robles-Martín *et al.*, (2023),[122] Table 2). Acetone is a typical solvent, for example in the synthesis of PMMA and PVC NPs,[130] due to its volatility and miscibility in common aqueous solvents such as water. However, other organic solvents, often more toxic and expensive (*e.g.* fluorinated or aromatic solvents as hexafluoroisopropanol or phenol), are needed to dissolve polymers such as PET.[119,122,130] Regarding the strategies used to induce MNP formation, these may include: 1) organic solvent evaporation after emulsification, which can be rendered surfactant-free by employing a salting-out method; 2) organic solvent displacement into a miscible antisolvent, which can be controlled by dialysis; and 3) supercritical fluid solvent expansion in air or a liquid.[131] For example, Balakrishnan *et al.* (2019) produced PE NPs (200–800 nm) by dissolving PE in toluene followed by emulsification in water and subsequent evaporation of both water and toluene.[118] The yield increased substantially by adding a surfactant (Tween 60 or 80), which improved the fraction of emulsified toluene. An interesting variant was to use a biosurfactant – a solution of microalgae exudates – to mimic the presence of an eco-corona at the surface of the PE MNPs. Interestingly, it was this biosurfactant that resulted in the highest yield among the three tested surfactants.[118] A similar approach can be used with other polymers that dissolve in a volatile solvent, as Merdy *et al.* (2023) did for PVC, PS, and PP with toluene.[120] The same authors were unable to apply the method to PET due to the lack of a suitable volatile solvent. However, MNPs from PET have been successfully prepared by solvent displacement, wherein the PET is first dissolved in hexafluoroisopropanol[119] or trifluoroacetic acid.[124] The solution is then slowly injected into a continuously stirred aqueous phase, leading to the formation of nanospheres as the organic solvent is displaced. Precipitation methods were also used by Tanaka *et al.* (2021) to prepare NPs of polyolefins (PE and PP), PVC, and PS from pellets dissolved in xylene, cyclohexanone, and toluene, respectively.[121] Each solution was added to a corresponding antisolvent (dimethyl sulfoxide, 35% water/dimethyl sulfoxide, and 30% water/ethanol, respectively), causing precipitation of NPs that were separated when the resultant dispersion was filtered. Finally, the filtrate was dispersed in *tert*-butyl alcohol and freeze-dried at room temperature to produce a dried powder of NPs.[121] In another example, Cassano *et al.* (2021) obtained PP NPs of 80–350 nm *via* fast oil-in-water emulsion precipitation in the presence of the biosurfactant sodium cholate.[123] They were also able to integrate labeling moieties such as fluorescent dyes, plasmonic Au nanoparticles, quantum dots, or metal tags (platinum octaethylporphyrin) into the core by simply adding these moieties into the organic phase prior to emulsification, without changing the shape or size of the final MNP. In a recent, relatively simple approach, Peller *et al.* (2022) partially solubilized PE, PET, PC, and PS in water with a very low volume of long-chain alkane solvents to obtain MNPs directly in aqueous media.[132] The MNP particle size distribution depended on the starting material, the solubilizer, and the type of mixing. Vigorous shaking yielded average sizes of 1.3–4.4 μm, which were reduced to 338–724 nm after sonication. Moreover, some starting materials led to more irregularly shaped and rougher-surfaced particles that are more environmentally relevant.[132] An advantage of solvent removal is that tunability over the final MNP properties can be achieved by varying the experimental parameters such as concentration, agitation rate, and injection rate. For example, the mean diameter of PET nanospheres increased from 69 to 154 nm as the injection rate increased from 0.05 to 2 mL min^{-1},[122] and the density of porous PE particles could be modified by varying the initial PE concentration in toluene.[118] Advances such as the use of microfluidics[133] or flash nanoprecipitation[127] may provide additional control over the process.

2.4.3 Summary of bottom-up approaches. Bottom-up approaches in many ways offer complementary advantages to top-down approaches, including high reproducibility, high yields and production rates, and tunability, particularly for small MPs and NPs. The particle sizes produced range from a few tens of nanometers up to a few micrometers and are essentially monodispersed. Most of the common polymer types can be generated through these chemical procedures, although detailed synthetic protocols are polymer specific. Therefore, creating a heterogeneous range of particle sizes and/or polymer types would require particles produced in different batches to be combined. In bottom-up methods the majority of the MNPs are pristine, but their surfaces are sometimes modified with the surfactants used for the polymerization or the emulsification that were not removed. Monomers and precursors that are part of the synthesis may unintentionally also remain in the MNPs or their suspensions. For these reasons, the final composition of the solution should be determined and surfactant-free bottom-up syntheses should be encouraged. Importantly, bottom-up approaches offer the possibility to incorporate various labels or specific chemical additives directly in the plastic bulk. A drawback of bottom-up approaches is that they require some knowledge of polymer chemistry, often use harsh organic solvents, and are often more labor intensive than typical top-down approaches.

3 MNP labeling

One of the biggest challenges in MNP studies lies in the difficulty associated with their identification and quantification in realistic, heterogeneous environmental matrices where the overwhelming majority of species are not MNPs (*e.g.* silica, clays, or natural organic matter). For this reason, several methods have been developed to prepare reference and test MNPs that are easier to identify and quantify. Specifically, these methods typically involve the addition of a label or tag to improve the ability to detect and differentiate test MNPs from other naturally occurring species without the need for large sample collection or complex extraction procedures. Furthermore, labeled reference materials can also be applied as internal standards, positive controls or controls for method validation. These labels, tags, or tracers (Fig. 4) are typically incorporated either after MNP production, by direct application of the label to the surface of existing MNPs, or during MNP production, by incorporation of the label onto the surface or into the bulk during the bottom-up preparation of the MNPs. An alternative would be to use a labelled plastic as the starting material, although this is very rare. Ultimately, the selection of which labelling approach to use will depend on the particular application and properties of the label (*e.g.*, limit of detection associated with a particular label) and the accessibility of each labelling method to a particular researcher. In this section, current lab-based labeling techniques applied to MNPs will be discussed alongside their respective advantages and disadvantages.

3.1 Fluorescent labeling

Fluorescent dye labeling is one of the easiest and most cost-efficient techniques to prepare labeled MNP materials, and thus is the most widely used. Fluorescent tags are added in one of two ways: by physical adsorption of a fluorescent dye onto the MNP surface (staining) or by addition of a fluorophore to the polymer matrix during bottom-up preparation of MNPs. When a MNP is fluorescently labelled the fluorescent signal enables the visualization of the MNP, typically by fluorescent microscopy, but also by flow cytometry[134] and photoluminescence-based techniques. Although certain plastics do naturally fluoresce, the degree of fluorescence is rarely sufficient to enable visualization. The staining procedure can also be directly applied to environmental samples; however it is insufficient for the quantification of MNPs in environmental samples because visualization of fluorescent particles does not guarantee that these particles are plastic.[135,136]

One commonly used and straightforward method for fluorescently-labelling MNPs is *via* surface staining with Nile red (NR). This hydrophobic dye enables the visual differentiation of MNPs from interfering hydrophilic particles such as sand in environmental samples. A recent review has summarized the dyes used for detection of MNPs.[135] While NR is the common choice, Rose Bengal[135] and pink textile dyes[137] are less expensive alternatives. It should be noted that some polymers including PC, PU, PET, and PVC, have been shown to fluoresce only weakly after staining with NR, limiting its applicability to lower density (and generally more hydrophobic) plastics. A typical protocol involves adding the stain to MNPs on filter paper followed by washing and drying to remove excess dye.[138] Careful consideration of the carrier solvent for the dye must be applied to ensure compatibility with the MNPs. Additionally, samples must be pre-treated to remove undesired natural organic matter that could bind to the dye and cause false MNP signals. The advantage of this method is that it provides a high throughput means to prepare easily identifiable MNPs of lower-density polymers such as PS, PP, and PE.[139] In an alternative approach, Caponetti *et al.* (2021) have developed a detection system using a probe based on perylene-diimide to label different types of MPs, including PVC, PE, PET, PP, PS, PMMA, and PTFE. The probe exhibited high selectivity for PVC, with intense red emission after labeling, providing an environmentally friendly, sensitive, low-cost approach for MNP detection.[140] Since staining is restricted to the MNP surface, the fluorophore concentration, and thus the fluorescence signal of each particle, is limited. Further, many fluorophores are not photostable, which compromises the labeled MNP storage and their application in photolysis studies designed to examine MNPs behavior in the presence of light. Moreover, MNP surface properties could be altered by adsorption of the dye, which would cause the measured properties to not reflect those of the unamended MNPs. An alternative to adsorption is to infuse the fluorophores into the MNPs matrix using a dye-organic solvent solution, such as NR dissolved in THF.[141] This simple approach allows for incorporation of different dyes simultaneously, and provides great control over dye concentration.

Compared to stained MNPs, embedded fluorescent dyes obtained by bottom-up approaches are generally brighter and more photostable. These embedded dye MNPs are typically purchased from commercial sources and despite their advantages they suffer from a limited range of polymer types (*e.g.* PS, PMMA, ESI† 1) high costs and are often sold as suspension stabilized by surfactants.

A key advantage of fluorescent labels is in qualitative tracking and imaging of MNPs, for example in bioaccumulation studies. However, imaging dyes such as NR require excitation with UV light that is also absorbed by biological material, making MNP differentiation difficult. Moreover, the use of UV could damage tissues and living organisms. A possible solution to overcome this problem is the incorporation of a fluorophore requiring lower excitation energy. For example, Yakovenko *et al.* (2022) demonstrated top-down synthesis of MNPs embedded with lanthanide-based upconverting nanoparticles that can be excited by near-infrared radiation and emit in the visible region, facilitating benign tracking and imaging of MNP accumulation in organism tissues.[142] Overall, when used appropriately, fluorescent labeling is well suited to direct identification and tracking MNPs. However, sizing of MNPs using fluorescence intensity is qualitative, and there is the potential interference from background fluorescence, risk of false positives, underestimation of polymers with weak fluorescence (*e.g.*, PVC), possible quenching by color pigments and the need to properly select the fluorophores.[143,144]

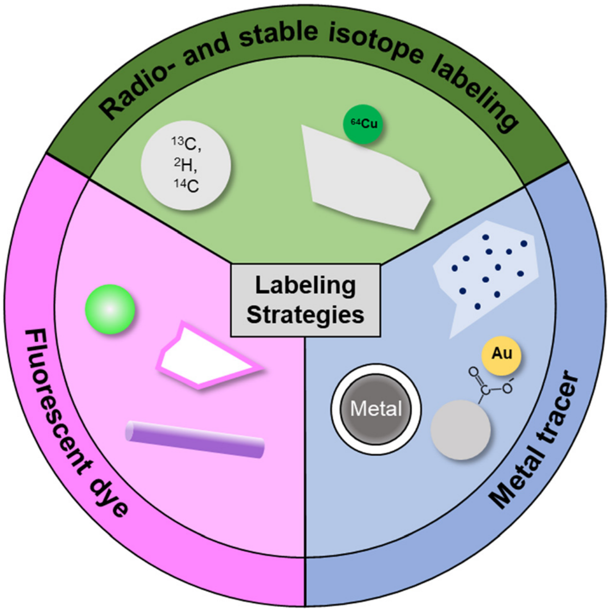

Fig. 4 Summary of the main MNP labeling strategies.

3.2 Metal tagging

Tagging MNPs with metals is an alternative and in many ways complementary approach to fluorescence-based labeling. The strength of metal-tagging is the ability to detect and quantify MNPs by means of analytical techniques such as ICP-MS, as opposed to the optical imaging capabilities afforded by fluorescent tags. Given the high analytical sensitivity of techniques such as ICP-MS, metal tagged MNPs can be detected at much lower and environmentally relevant concentrations as compared to traditional detection methods such as Raman or UV-vis.[41] For metal-labeled MNPs, the lower detection limit for particle size is determined by the mass of the metal. Moreover, if the metal tag is from an element with low natural environmental abundance (*e.g.* Pd, Ta) the MNPs can be discriminated from carbon-based background interference. The metal label itself can take many forms, such as a nanoparticle, an ion, or an organometallic compound.[106,113,145] Consequently, there are a wide variety of procedures that can be employed to incorporate metal labels into different polymer types. In addition to ICP-MS, metal labeled MNPs can also be analyzed by single particle ICP-MS (spICP-MS), where each metal-labeled MNP is detected as an individual pulse in the plasma. Analysis of the pulse height distribution and frequency enables determining the size distribution as well as the number concentration of MNPs.[83]

A common approach to preparing metal-tagged MNPs is *via* surface attachment of the metal tag through physical adsorption or chemical bonding. One method proposed by Jiménez-Lamana *et al.* (2020)[106] utilized the presence of carboxylate groups at the surface of PS MPs to electrostatically attach positively charged gold nanoparticles for detection by spICP-MS. The MNP size was then determined by the generated metal signal intensity, due to the proportional relationship between the number of attached gold nanoparticles and the surface area of the MP. Despite detection of MNPs with sizes up to 1 μm in water samples, it is unlikely that this attachment strategy can be applied in more complex matrices containing other negatively charged species, such as dissolved organic matter, that will likely compete for the positively charged gold nanoparticles. Similar surface attachment strategies using the ability of MNPs to adsorb trace metal ions[146] and hydrophobic organic compounds have also been employed.[145] In the case of direct metal ion labeling, high concentrations of tagged MNPs must be used to generate enough metal mass for ICP-MS detection, and sorption is metal-dependent.[145] Use of a hydrophobic organometallic probe (metal phthalocyanine) also enabled MNP detection by spICP-MS. However, multiple recovery steps to separate the MNPs from water-insoluble probe aggregates could lead to an underestimation of MNP concentrations.[145]

Unlike fluorescence staining, surface attachment of metal tags allows for application in photochemical weathering studies and quantitative analysis. However, these surface alterations could potentially modify MNP interactions and environmental behavior, much like the fluorescence staining strategies discussed above. Additionally, changes in concentration, pH, or temperature could cause desorption and a decrease in the surface concentration of the metal tag, thereby lowering detection sensitivity and limiting the potential matrices in which the MNPs could be deployed.

To avoid desorption, an alternative and more robust approach to a surface-attached metal tag is fabrication of a MNP containing an entrapped metal. In a method developed by Mitrano *et al.* (2019) metal-tagged MNPs were synthesized by first preparing a PAN core containing Pd followed by addition of an outer PS shell.[111] This metal core–polymer shell structure allows for larger quantities of metal atoms to be contained within each MNP as compared to surface attachment, significantly improving detection and quantification by ICP-MS. The outer shell also minimizes the leaching potential of the metal tracer, thus allowing for use in ecotoxicity studies. Additionally, metals like Pd, not found at high environmental concentrations, improve selectivity in complex, heterogeneous matrices. The outer shell can also be modified to a different polymer-type for direct assessment of multiple MNP surface interactions with aquatic or terrestrial environments. For example, Rauschendorfer *et al.* (2021) prepared core–shell MPs containing intercalated metal chloride salts of gold, platinum, or palladium and PMMA or PS shells.[112] Tracking of the unique metal signature can be used to identify reference MNPs of different polymer types. Importantly, these types of labeled reference MNPs are well-suited for fate studies in complex systems and environments, such as soils, plants, and wastewater treatment plants.

One of the disadvantages of metal core–polymer shell MNPs is that they require a somewhat complex synthesis process with multiple reaction steps and solvents. In principle, polymer shells are synthesized through emulsion polymerization.[147] As a result, a number of polymers, including polyamides, polyesters (PET, PBT, PLA), polycarbonates, and silicones cannot be produced with metal cores.[147] Polyolefins (PE, PP) can theoretically be polymerized by emulsion; however, this requires the application of high pressure.[148] Additionally, the metal tracer cannot directly provide MNP size or shape information, thereby limiting its application in studies aiming to examine particle-size changes or size-dependent MNP properties. These core–shell syntheses also yield spherical particles of monodisperse sizes which are not representative of realistic environmental MNPs. Another disadvantage is that incorporation of the metal core modifies the density of the tagged-MP compared to an untagged-MP;[112] therefore, these materials are unable to accurately probe the colloidal stability of MNPs.

An alternative to the fabrication of metal core–polymer shell MNPs is the distribution of a metal tag homogeneously throughout the polymer matrix. In a method developed by Smith *et al.* (2024), MNPs containing uniformly distributed organometallic additives were prepared using solution casting and cryomilling.[83,149] Similar to the detection method of core–shell MNPs, metal-tagged MNPs were detectable by spICP-MS in a variety of environmental matrices at low μg L^{-1} concentrations. This approach is relatively straightforward and the synthetic flexibility enables the preparation of a suite of metal-tagged NPs for a variety of commercial polymers including PS, PMMA, PVC, LDPE, and PVP. In contrast to the core–shell method, the metal-tag is distributed throughout the entire volume of the microplastic and thus any changes to the size of the MNPs during an experiment can be examined. The metal-tags are present at a sufficiently low concentration that they do not modify the surface characteristics of the MNPs, in contrast to the surface bound metal-tags that rely on electrostatic attractions.

The incorporation of metals can also be combined with other labelling approaches as demonstrated by Luo *et al.* (2022) who entrapped a metal–luminophore complex (*i.e.* tris(thenoyltrifluoroacetonato)europium) in PS using an emulsion polymerization reaction.[150] The resulting MNPs were metal-tagged and luminescent when UV-irradiated, which enabled not only their visualization but also their quantification.

3.3 Isotope labeling

Isotope labeling is a technique where isotopes, stable or non-stable, are incorporated into MNPs to facilitate their detection. To enable isotopically labelled MNPs to be clearly distinguished and identified in realistic environmental media and limit the potential for signal interference, the isotope concentration in the labelled MNP should be much higher than the natural abundance of the isotope found in the environment.

3.3.1 Stable isotope labeling. Ready-to-use, stable isotopically labeled MNPs, mainly ^{13}C or deuterium (^{2}H), are commercially available but expensive, usually hundreds of dollars per gram for deuterium-enriched particles to few thousand dollars for ^{13}C-enriched particles[151,152] (ESI† 1). As an alternative, lab-scale bottom-up synthesis performed with commercially-available isotopically-labeled monomers or building blocks,[153,154] or a combination of bottom-up and top-down methods, can drastically reduce the cost. For example, Mauel *et al.* (2022) synthesized ^{13}C-enriched PS, followed by centrifugal milling to obtain weathered MPs, which could be quantified by NMR.[155]

Since stable isotope enrichment affects only the molecular weight of the polymer backbone while retaining its structural and elemental composition, labeled and non-labeled particles are expected to elute chromatographically at the same time in analytical techniques, such as Py-GC/MS or TED-GC/MS. However, since they differ in terms of mass isotopically labelled MNPs can be distinguished by mass spectrometry. In this way deuterated MNPs are frequently employed as internal standards, as they are more affordable than ^{13}C-labeled MNPs.[156–158] For example, the recovery of deuterated standards spiked before analysis with Py-GC/MS has enabled the quantification of environmental MNPs to be internally calibrated.[158] However, at high temperatures, there is the potential for inorganic compounds to react with the labeled MNP and trigger hydrogen–deuterium exchanges, compromising this analytical approach.[157]

To-date, ^{13}C labeled MNPs have been primarily utilized to assess the degradation rates of biodegradable macroplastics such as PBAT used in mulch films[159] as well as MNPs made of more conventional, persistent polymers like PE.[151,152,160] In these scenarios, the use of GC-MS isotope ratio mass spectrometry allows the tracking of $^{13}CO_2$ or $^{13}CH_4$ and an accurate assessment of the biotransformation kinetics.[159,161] Indeed, some researchers have claimed that using ^{13}C isotopically labeled plastics is the only reliable method to evaluate MNP biodegradation.[161] ^{13}C-labeled MNPs are also well-suited for fate studies, as ^{13}C can be easily distinguished from other carbonaceous compounds, although as far as we are aware this application has never been reported. One reason for the lack of applications for fate studies could be the high prices of isotope labeled-particles, which favor the use of other labels (*e.g.* metals) instead. Nonetheless, stable isotope labeling is a promising tool for MNPs research because the environmentally relevant properties of isotopically labeled particles will almost always be identical to non-labeled particles, in contrast to issues that can arise with other labeling techniques.[153]

3.3.2 Radioisotope labeling. Radiolabeling of MNPs can be achieved by derivatizating the particle's surface to incorporate a radioisotope (*e.g.* ^{64}Cu, ^{65}Zn, or ^{131}I). For example, ^{64}Cu was complexed to amine-functionalized PS MNPs, modified by the addition of the chelating agent DOTA.[162] Alternatively, the surface of PS MPs was sulfonated to enable the complexation of ^{65}Zn.[163] In a different method, ^{64}Cu was complexed to a porphyrin and dissolved in THF, facilitating diffusion of the radioisotope into the bulk of PE, PA, PET, PS, and PVDC.[164] According to the authors, this method could potentially be applied to a wide range of radiolabels. PVC has also been labelled with ^{131}I by using the Conant–Finkelstein reaction where some of the chlorine atoms in PVC are replaced with ^{131}I *via* nucleophilic substitution using $Na^{131}I$ in solvents such as acetone, THF, and PBS.[165] In contrast, the incorporation of organic radioisotopes is typically performed by polymerization of enriched monomers as described in the bottom-up section. So far, this approach has been limited to ^{14}C-containing PS, which was synthesized by emulsification of ^{14}C-labeled styrene.[110,166,167]

The detection of radioisotope-labeled MNPs can be performed by non-invasive imaging techniques such as positron emission tomography or single photon emission computed tomography techniques which measure the radiation emitted as a result of the isotope decay (positron or gamma rays).[168] These instruments are sensitive to very low concentrations of isotopes with limits of detection and quantification on the order of ∼1 ng particles per g per sample.[110] Furthermore, the field of view of these techniques is large, enabling imaging of large surfaces (*i.e.* a whole body); however, the resulting image resolution is relatively poor.[168] Another advantage is that the emitted radiation can pass through matter, allowing for labeled particles to be tracked *in vivo* without destructive sample preparation.[169] As long as the radionuclides have not decayed, samples can be reanalyzed multiple times. Conversely, the short half-life times of metallic radionuclides, including ^{64}Cu, ^{131}I, or ^{65}Zn ($t_{1/2} < 10$ days), limit their use for long-term fate experiments.[168] The impact of the ionizing radiation emitted by the labeled material must also be considered. For example, the radiation may induce additional toxicity, rendering radiolabeling potentially unsuitable for certain toxicity studies. Perhaps the biggest limitation of using radioisotopes is the necessity of dedicated facilities for radioactive substances,[170] specialized instrumentation, and measures to protect workers. The implementation of rigorous safety protocols can impede the comprehensive characterization of labeled materials, as exemplified by Al-Sid-Cheikh *et al.* (2020), who could not image their ^{14}C-labeled PS with TEM due to the aforementioned constraints.[110]

4 Simulation of environmental weathering

Plastics in the environment may undergo various biotic or abiotic processes that alter their properties.[14,171,172] Weathering or aging is therefore a key aspect to consider when preparing environmentally-relevant MNP test and reference materials. Abiotic environmental weathering processes include oxidation, induced by sunlight or exposure to oxidants and physical transformations caused by wear or abrasion. Microbial activity can also alter MNPs through biofilm formation. To mimic the weathering processes mentioned above, pristine MNPs can be transformed, either in the laboratory or outdoors under natural conditions. Aging MNPs by exposing them to natural conditions is cheap, simple, and realistic, but usually requires long exposure times to observe significant transformations. Alternatively, laboratory-based studies allow for controlled accelerated weathering of particles and significantly reduce the exposure times compared to the equivalent natural process.[14,173] In general, artificial weathering promotes uniform aging of the material, which may not be achievable in the environment due to variable weather, an important issue when the goal is to produce a well-defined reference and test material.[174] However, artificial weathering can require relatively expensive equipment. Moreover, accelerated weathering may not mimic the processes taking place in the environment, resulting in differently aged particles.[175] This is because various weathering mechanisms occur in the environment at the same time and these may need to be combined to produce realistic MNPs. For example, plastics found on a beach are likely to undergo both oxidation due to sunlight exposure and physical weathering caused by erosion from particles in the sea surf.

Regardless of the weathering method applied, it is essential to adequately characterize the resulting particles. Alimi *et al.* (2022) proposed a list of properties to evaluate weathered MNPs, mainly physicochemical surface and bulk properties such as surface morphology (roughness and presence of cracks),[171] with two purposes: comparing the properties of artificially and naturally aged MNPs and benchmarking studies with different materials. Furthermore, studies that compare the fate and ecotoxicological effects of artificially-aged and environmental MNPs would be useful to determine if MNPs weathered by these two means and have similar characteristics behave similarly. One the challenges to these comparative studies is that obtaining large amounts of environmental MNPs is extremely challenging as discussed in section 2.2.

The following sections provide a concise overview of the effects of weathering on the properties of MNPs and how to reproduce or simulate weathering. More detailed discussions of materials' weathering processes can be found in ref. 171, 173 and 175–177.

4.1 Oxidative weathering

Several studies have shown that, as for macroscopic plastics, exposing MNPs to oxidative conditions created either by UV irradiation from sunlight or as a result of exposure to ozone,[70] can affect a wide range of their properties, including surface morphology, surface chemical composition (due to the formation of oxygen-containing functional groups such as carbonyls[178]), and consequently, the particle's hydrophilicity[173,178] and color.[16,179] Such oxidative conditions may also cause MNP fragmentation, a reduction in the molecular weight of the constituent polymers,[180] as well as an increase in crystallinity which may cause material brittleness.[16,171,181,182] In addition, as has been discussed previously oxidative weathering can be an unintended consequence of MNP production, particularly for top-down approaches.

To date, most studies on lab-simulated environmental aging of MNPs were designed to mimic photooxidation processes.[171,172] For example, filtered UV light is often applied in a temperature-controlled weathering chamber to simulate daylight conditions[70,183,184] and can provide much higher radiation doses than in the environment. ISO and ASTM protocols[171] have been established for testing the physicochemical properties of plastics and for accelerated weathering procedures describing how to simulate photooxidation in different environmental compartments. In brief, these protocols provide a general description of the apparatus, the parameters to monitor (*i.e.* relative humidity, temperature), and the methods for characterizing weathered materials. However, these protocols must be considered with care. For example, some suggest the use of xenon lamps for photooxidation, but those may produce excessive radiation in the UVC region (specifically <300 nm) and may not accurately depict the broad spectrum of sunlight.[171,174]

4.2 Mechanical weathering

Although the aging of MNPs due to oxidative conditions is relatively well documented, studies on physically induced weathering of MNPs are much more scarce.[16,182,185,186] Historically, mechanical aging processes have been overlooked, as the chemical industry had little interest in assessing the fragmentation of plastics.[16] In one example of a laboratory experiment, the effects of wind entrainment and transport in sediment revealed that physically induced weathering generated cracks on the surface of the plastics and tended to produce more rounded particles over time.[185,186] Physically induced weathering can be performed with a simple setup such as sand-filled bottles on a roller mixer,[182] or in a more complex manner employing a continuously aerated glass test-tube chamber.[185] The application of mechanical energy affects the surface morphology of plastics and is generally viewed as the key step to fragment particles into smaller pieces, including NPs.[86,185] There is clearly a need for more systematic studies of mechanical weathering and how this relates to MNP formation.[187]

4.3 Biological weathering

While biodegradable polymers, such as PLA biodegrade quite rapidly in many environments (*e.g.* soils), biological transformations of MNPs made of conventional petroleum-based polymers such as PE occur over many decades in most microbial populations. As a result, MNPs from these polymer types are considered to persist indefinitely on the timescale of most laboratory studies.[14,171,177] It is important to note that several ISO and ASTM protocols have been established to measure the effects of microbial activity on MNPs (*e.g.* CO_2

production to measure biodegradation – ASTM D5988). However, despite the absence of biological transformations to the bulk of many MNPs, their surface properties may be modified due to the eco-corona coating or biofilm formation, which could significantly influence their fate or ecotoxicological effects. For example, the coating may alter the effective density and hydrophilicity of the MNPs, or favor the leaching of additives.[177,178] Approaches to extract MNPs from biological media without damaging the biofilm coating and to accurately measure and quantify their transformed properties have yet to be developed due to the inherent challenges. There have been attempts to incubate MNPs to grow biofilms,[171] for example in wastewater,[188] microalgae culture,[189] or lake water,[189] to then characterize the resulting biofilm (see Alimi *et al.*[171] for more references). Standardization of these protocols would be valuable, as coating of MNPs with biofilm or organic matter in a reproducible manner would make it easier to compare the results of studies conducted in different laboratories.

5 Preparation of liquid and solid MNP dispersions

Reference and test MNPs are produced and utilized in three forms: powder, suspension, and embedded in a solid matrix. When MNPs are obtained as dry powder, they may be directly added to relevant media. However, establishing dispersion protocols is often required to spike in an experiment or to accurately mimic their form in relevant aqueous media.[38] Furthermore, successful dispersion is often needed to ensure the accurate characterization of the MNP's size distribution and concentration. The dispersibility of MNP particles within a liquid medium is a reflection of the particle's colloidal stability, which in turn is influenced by MNP size, composition, and surface properties such as hydrophobicity and surface charge.[190] For micron-sized MNPs, density and size also determine whether a particle will remain suspended, float, or sediment in a liquid.[190] All these characteristics are crucial determinants of MNP homogeneity and stability within aqueous media.

In reality, when added to water as powders, MNPs often fail to disperse and rapidly form aggregates or adhere to the walls of the vessel because of their hydrophobicity.[65] To overcome this issue, ultrasonication is sometimes employed to facilitate disaggregation of individual particles and enhance the dispersion in the medium.[66] In toxicology, a standard method for dispersing nanomaterials is the NanoGenoTox protocol, which involves applying ultrasound at low temperatures for 16 min at 400 W and 10% amplitude.[191] Originally developed for other nanomaterials, this protocol has been adapted to generate highly stable nanoplastic solutions.[192] However, ultrasonication can result in oxidation and fragmentation, as described in section 2.3.2 and so this needs to be assessed. For hydrophobic MNPs (*e.g.* PVC) less polar solvents such as ethanol, isopropanol or 1-propanol have proven to be effective in promoting dispersion, although solvent selection criteria must align with the research question. For example, Parker *et al.* (2023) selected 1-propanol over ethanol and isopropanol for suspending PVC and PP particles (0.1 wt%) due to its superior dispersion efficiency and reduced cytotoxicity.[64] Alternatively, surfactants[38] or biosurfactants[118] can be added to water to stabilize reference MNPs and minimize agglomeration. However, surfactants modify surface properties by binding to the hydrophobic surface of MNPs, thereby rendering them hydrophilic.[38] Indeed, the use of surfactants has led to numerous erroneous results regarding the properties of MNPs, such as toxicity and transport.[38] To avoid the use of surfactants, Eitzen *et al.* (2019) enhanced the dispersion of PS particles by applying ozone (O_3) to promote the oxidative modification of the surface.[65] Ozone reacts with aromatic and aliphatic carbon atoms at the MNP surface and forms carbonyl, carboxyl and hydroxyl groups, thus increasing the hydrophilicity of the PS. In essence, ozonation serves as a form of accelerated weathering. As such, this process achieves the stated goal of improving MNP dispersion with the caveat that MNPs dispersed in such a fashion can no longer be classified as pristine MNPs.

Instead of being used in suspension or as a pure powder, MNPs have also on occasion been incorporated into a solid matrix. Solid dispersions are preferred as the means to prepare MNP kits containing several polymer types, and also in situations where the size or polymer type precludes the formation of stable MNP suspensions. Another advantage of these solid matrices is that the use of a physical diluent makes it easier to accurately weigh a low amount of MNPs incorporated within a larger sample mass. In one example of a solid matrix, Seghers *et al.* (2023) proposed freeze-drying a solution of NaCl containing MNPs to form a carrier.[193] Similarly, gelatin or soda capsules have been used as solid matrices to encapsulate MNPs, selected because they dissolve easily in water and under moderate heating (40 °C) to release the particles.[194] Since the capsule-to-capsule mass variability, even for particle sizes down to 50 μm, was low, the capsules could be used to perform an interlaboratory comparison of analytical methods. Another approach is where MNPs are dispersed in an inert particulate matrix (*e.g.* SiO_2,[195] alumina,[196] $CaCO_3$ (ref. 197)). Dispersing MNPs into a solid matrix is particularly important for mass-based quantification methods such as Py-GC/MS, as it facilitates the preparation of calibration standards where the MNP concentration is comparable to values expected in real-world environmental samples. Thus, as part of the development of calibration kits for Py-GC/MS where MNPs from 12 polymer types were dispersed in a solid diluent, Frontier Laboratories reported that SiO_2 was suitable for the analysis of every polymer type, except PU because of uncontrolled reactions with PET in the kit during pyrolysis.[195] This occurred because the pyrolysis of PET creates an acidic environment that alters the products of PU pyrolysis and consequently the reliable quantification of PU is compromised. Dispersion of PU in alumina failed to address this issue;[196] however, the use of

$CaCO_3$ was found appropriate for all polymer types. This was ascribed to the ability of $CaCO_3$ to act catalytically to transform reactive products generated during the pyrolysis of PU into stable compounds that can serve as markers for the identification of the parent PU.[197] These kits are important because they can serve as the basis for inter-laboratory referencing of analytical methods used for MNP quantification. While there has been some progress in this area, the lack of MNP characterization in the matrix, particularly the size, poses a challenge.

6 MNP characteristics and their relevance in MNP research

Ensuring that reference and test MNPs are appropriately characterized before use is a critical step in improving the reliability and comparability of research outcomes. While commercially available MNPs provide a convenient starting point, their physicochemical characteristics – such as additive content, surface properties, and stability – are often unknown or poorly documented, complicating efforts to elucidate the relationships between the physicochemical properties of MNPs and their fate and interactions in environmental and biological systems. As highlighted earlier, in-house characterization is essential to verify the physicochemical properties of MNPs and assess alterations to these properties that may occur intentionally or inadvertently during their production. The following sections summarize the various characteristics that collectively describe MNPs. They are presented and ordered by the frequency with which they are reported and/or characterized in studies using reference and tests MNPs (see Table 3). These physicochemical properties of MNPs link synthesis to their applications, as the research endpoint determines the properties of interest in the produced reference and test MNPs. The aim is to describe those MNP key properties and provide relevant examples of biological effects, environmental fate, or implications for detection to illustrate the importance of characterizing specific MNP properties in different types of research studies. The most widely used analytical techniques are also introduced, without providing an exhaustive discussion of the analytical techniques themselves, which is beyond the scope of this review. A concise summary on the analytical techniques used to interrogate MNPs is provided in Fig. 5 and Table 5, while more detailed information can be found in several recent reviews on the characterization of MNP properties.[41,198–202]

6.1 Polymer type

While polymer type is often the baseline characteristic used to describe MNPs, it is usually not a stand-alone predictor of MNP properties and needs to be considered alongside other MNP properties such as size and shape. Moreover, although polymer type defines the surface properties of pristine MNPs', they can be altered, sometimes significantly, by weathering. In some instances, however, differences in polymer type may provide a logical explanation for MNP behavior. For example, Ma *et al.* (2024) observed a higher toxicity to human cells of pristine PET and PVC NPs compared with PS NPs,[219] although each of the three polymer NPs had a similar size, shape and surface charge. They hypothesized that this polymer type dependent effect was due to the variations in chemical structure and hydrophobicity which impacted the nature of the adsorbed species and the resultant NP toxicity. It should be noted that knowledge of the polymer type can provide insights into the likely sources, distribution, and entry pathways of MNPs into the environment.[261] For example, Deng *et al.* were able to clearly link the high concentration of PET fibers in water and sediment to a nearby textile industry.[262] Identifying the polymer type of MNPs (*e.g.*, FTIR, Raman, or Py-GC/MS) typically relies on matching the measured spectra with the characteristic spectra of known samples, either included in commercial libraries or collected using in-house produced reference MNPs. However, this approach is not always straightforward because polymers can contain more than one polymer type as well as other components (see 6.5 Bulk MNP composition).

6.2 Size

Particle size has well documented effects on MNP fate, bioavailability, and impacts on the environment and living organisms.[263,264] Indeed, MNP size is a key parameter in determining the behavior of MNPs in any study type. For example, Ward *et al.* (2019), demonstrated that bivalves such as oysters and mussels selectively ingest smaller particles while rejecting larger ones in the size range from 19–1000 μm, indicating a size cut-off for ingestion.[228] Smaller sized MNPs were also preferentially taken up by earthworms, demonstrating the general importance of MNP size for uptake and distribution.[230] In addition to preferential uptake, MNPs smaller than approximately 1 μm can cross biological barriers[265] and accumulate in tissues,[266] although aggregation may decrease NP toxicity by limiting bioavailability and reducing the exposed surface area.[267] This size dependent behavior highlights the need to determine MNP dispersion characteristics in uptake and toxicity studies, as discussed in section 5 Preparation of liquid and solid MNP dispersions. Environmental fate of MNPs is also determined by the size; for example, Shaniv *et al.* (2021) showed that PS particles of 50 nm presented higher mobility in soil compared to 110 nm PS,[233] while at the air–water interface, Harb *et al.* (2023) reported that 0.5 and 2 μm PS particles are aerosolized two orders of magnitude more easily by sea spray than 10 μm particles.[239] These observations demonstrate that smaller sized MNPs have increased mobility and will transport further in the environment.

Table 3 Application, characterization and detection of reference MNPs as a function of the research question addressed and classified by type of studies comprising monitoring, uptake, toxicity, and environmental (aquatic, terrestrial, atmospheric) fate. If the material was purchased, the manufacturer or company is indicated and the nominal size is reported when available. If not, the production method is specified. The key properties were identified based on their impact on the outcome of the study

Study type	Research question	Matrix	Reference or test MNP	Use	Characterized property (technique)	Key property	Detection technique	Ref.
Ecotoxicity	Impact of NPs on mortality, riboflavin secretion and iron reduction	Bacterial cell	Metal doped PS, PS (30 μm thick) and PE (25 μm thick) films (Goodfellow)	Tracer	Size (DLS, SEM, STEM-EDX), ζ (ELS)	Polymer type	ICP-MS	203
Ecotoxicity	Chemical toxicity of leachates of MPs	Bacteria, algae, larval stage of echinoderms	PET (milling)	Tracer	Polymer type (ATR-FTIR), shape (FE-SEM)	Polymer type, size	FTIR	68
Ecotoxicity	Impact of MPs on cell aggregation	Freshwater microalgae cell	UCNPs-labelled and UCNPs-free HDPE (grinding)	Tracer	Size (granulometry and NTA), shape and texture (AFM), crystallinity (DSC), chemical structure (SAXS), ζ (ELS), luminescence, hydrophobicity (FluidFM)	Hydrophobicity	Optical microscopy, AFM imaging	204
Ecotoxicity	Bioaccumulation, toxicity and transfer of NPs	Periphyton, aquatic snail	Metal doped PS (polymerization)	Tracer	Pd concentration (ICP-MP), location of Pd (TEM-EDX), leaching of Pd, size (NTA), ζ (ELS)	Size	ICP-MS	205
Ecotoxicity	Uptake and impact of NPs on mortality and growth	*Gammarus pulex*	Metal doped PS (polymerization)	Tracer	Location of Pd (TEM-EDX), leaching of Pd, size (DLS), shape (SEM)	Shape	ICP-MS	206
Ecotoxicity	Impact of protein corona on the regulation of NPs-induced autophagy	Mouse cell	PS (300 nm, BaseLine ChromTech Research Centre), fluorescent PS (Tianjin Da'e Technology)	Tracer	Shape (TEM)	Size, ζ, protein corona	Flow cytometry, confocal microscope, ELS	207
Ecotoxicity	Intestinal toxicity of MP fibers	Zebrafish	PP microfibers (cryomicrotomy)	Tracer	Polymer type (Raman), shape (UV-vis)	Shape	Raman, fluorescence microscopy	208
Ecotoxicity	Impact of MP fibers and particles on feeding, lipid accumulation, growth, and moulting	Crustaceans	PA granules and microfibers (Goodfellow, cryomicrotomy)	Tracer	Bulk plastic composition (GC-MS)	Shape	Fluorescence microscopy	209
Ecotoxicity	Shape-dependent accumulation and toxicity of MPs	Zebrafish	Fluorescent and pristine PP and PS (grinding)	Tracer	Polymer type (Raman), shape (fluorescence microscopy)	Shape	Raman, fluorescence microscopy	210
Ecotoxicity	Comparison of toxicity of MPs, MP fibers and NPs	Mussels	PA microfibers (PA filament, Goodfellow, microtomy), PS (20 μm, Sigma-Aldrich), and fluorescent PS (50 nm, Magsphere)	Tracer	Size and dispersion stability (TEM and DLS), ζ (ELS)	Size, shape	Optical microscopy, fluorescence spectrometry	211
Ecotoxicity	Toxicity of MPs and MP fibers	Waterflea	Fluorescent PET fiber (orange fluorescent fleece, chopping), PE (1–4 μm, Cospheric)	Tracer	Polymer type (ATR-FTIR), size (fluorescence microscopy)	Shape, size	Stereo and fluorescent microscopy	212

Table 3 *(continued)*

Study type	Research question	Matrix	Reference or test MNP	Use	Characterized property (technique)	Key property	Detection technique	Ref.
Ecotoxicity	Toxicity of MP fibers	Algae	Polyester (polyester monofilament fiber, Huixiang Fiber, microtomy)	Tracer	Shape (optical microscopy), length distribution (counting chamber)	Size	Optical microscopy, SEM	213
Ecotoxicity, aquatic fate	Environmental fate, ingestion and impact of NPs on physiology and structure of the organisms	Cyanobacterium, green algae, *Daphnia magna*	Metal doped PS (polymerization)	Tracer	Mass concentration (TOC-VCSH), size (DLS and TEM), shape (TEM), ζ (ELS)	Size	ICP-MS	214
Ecotoxicity, aquatic fate	Environmental impact and toxicity of MPs	Simulated seawater, ultrapure water, marine bacteria	PP, PS, PE, PET and PLA (dissolution–reprecipitation of conventional plastic products)	Tracer	Polymer type and surface oxidation (FTIR), size (DLS)	Polymer type, surface oxidation	FTIR	215
Ecotoxicity, human toxicity	Toxicity of NPs	Bacteria, yeast, algae, protozoans, mammalian cells, crustaceans, midge larvae	Carboxylated PS (26 and 100 nm, Bangs Laboratories)	Tracer	Size (DLS, SEM) shape (SEM), dispersion (ELS), ζ (ELS), concentration (SLS)	Additives, particle adherence on the organism	SLS	216
Ecotoxicity, human toxicity	Toxicity of MPs	Mice, human cornea and conjunctival cells	Fluorescent PS (2 μm and 50 nm, Baseline ChromTech Research Center and Beijing Dk Nano Technology)	Tracer	Size, shape (TEM), polymer type (FTIR)	Size	Fluorescence microscopy	217
Ecotoxicity, human toxicity	Effect of inhalable MP fibers on lung	Human and murine lung organoids	PET and PA microfibers (Goodfellow and commercially available fabrics, cryomicrotomy)	Tracer	Concentration, shape and size (SEM), elemental composition (EDX), polymer type (FTIR)	Polymer type, leachate	—	218
Human toxicity	Impact of gastrointestinal digestion and colonic fermentation on biodegradable MPs	Gastrointestinal digestion fluid, human colonic microbiota	PLA (milling)	Tracer	Shape and texture (FESEM), crystallinity (Raman)	Crystallinity, polymer type	FESEM	60
Human toxicity	Polymer dependent toxicity of NPs	Human cells	PET, PVC, PS (solvent removal)	Tracer	Concentration (gravimetry), shape (SEM), size (DLS), ζ (ELS), polymer type (FTIR)	Concentration, exposure time, polymer type, density, hydrophobicity	Confocal microscopy	219
Human toxicity	Toxicity of MPs	Human cells	ABS, PVC (ball milling)	Tracer	Size, shape, texture (SEM and optical microscopy)	Polymer type, shape, texture	—	220
Human toxicity	Toxicity of MPs	Human cells	HDPE (1–10, 50 and 100 μm, Cospheric), LDPE (cryomilling)	Tracer	Size, shape, texture (SEM), polymer type (Raman)	Concentration, shape, texture	—	221
Human toxicity	Toxicity of NPs used in food packaging/beverage bottles production	Intestinal epithelial barrier and liver cells	PC and PET (laser ablation)	Tracer	Size (DLS), surface chemistry (XPS), shape (TEM)	Size, polymer type and surface chemistry	—	77
Human toxicity	Uptake and toxicity of NPs	Human hematopoietic cell	Fluorescent metal doped PET (dissolution–reprecipitation)	Tracer	Size and shape (TEM), Ti content (EDX and MS), polymer type (FTIR), size (DLS), ζ (ELS)	Polymer type, size and shape	Confocal microscopy	222

Table 3 *(continued)*

Study type	Research question	Matrix	Reference or test MNP	Use	Characterized property (technique)	Key property	Detection technique	Ref.
Human toxicity, uptake	Toxicity of MPs of different polymer type, size and concentration	Human cells	PS (50 nm, 200 nm and 1.1 μm, Polysciences), fluorescent PS (44 nm, 190 nm and 1.04 μm, Polysciences), aminated fluorescent PS (55 nm, Magsphere) and PMMA (70 nm, 400 nm and 1.1 μm, Polysciences)	Tracer	Size (DLS), ζ (ELS)	Size, surface charge, shape	Confocal microscopy	223
Human toxicity, uptake	Uptake, distribution and toxicity of MNPs	Human small intestinal epithelium model	PS (25 and 100 nm, Phosphorex)	Tracer	Size (SMPS and APS)	Size	Confocal microscopy	224
Human toxicity, uptake	Uptake and toxicity of NPs	Human intestinal epithelial cells	PET (laser ablation)	Tracer	Shape (TEM), size (DLS), ζ (ELS), surface chemistry (XPS), dispersion (ELS)	Size	Flow cytometry, confocal microscopy	78
Human toxicity, uptake	Uptake and toxicity of NPs	Human macrophage and epithelial cells	Fluorescent functionalized PS (polymerization)	Tracer	Size and shape (TEM, SEM, DLS), ζ (ELS)	ζ	Confocal microscopy	225
Uptake	Uptake, distribution, accumulation and elimination of NP	Rainbow trout	Metal doped PS (polymerization)	Tracer	Pd concentration (ICP-MS), size (NTA)	Size	ICP-MS	226
Uptake	Uptake, distribution, accumulation and elimination	Oyster	Metal doped PS (polymerization)	Tracer	Size (TEM and DLS), ζ (ELS)	Size	ICP-MS, TEM	227
Uptake	Uptake and elimination of MPs	Oyster, mussel	Pristine (113, 287, 510, and 1000 μm), and fluorescent (19 μm) PS (Polysciences and Cospheric), PA fibers (A.C. Moore)	Quality control	Size and shape (optical microscopy), polymer type (Raman, FTIR)	Size, aggregation	Fluorescence microscopy	228
Uptake	Uptake, effect of surface charge and wettability	Oyster, mussel	Pristine and carboxylated fluorescent PS (10 μm, Polysciences)	Tracer	ζ (ELS), hydrophobicity (contact angle)	ζ, hydrophobicity	Flow cytometry	229
Uptake	Uptake, accumulation and elimination of NPs and MP fibers	Soil, earthworm	Metal doped PS (polymerization) and metal doped PET fibers (extrusion and cutting)	Tracer	Pd concentration (ICP-MS), size (DLS)	Size, shape	ICP-MS	230
Uptake, terrestrial fate	Transport of NPs in soil	Soil, earthworms	Metal doped PS (polymerization)	Tracer	Pd concentration (ICP-MS), size (DLS) and ζ (ELS)	Size, shape	ICP-MS	231
Terrestrial fate	Transport of NPs and MP fibers	Simulated soil (unsaturated porous media)	Metal doped PAN and metal doped PS (polymerization)	Tracer	Pd concentration (ICP-MS), size (DLS), shape (SEM, STEM-EDX)	Size, aggregation	ICP-MS	232
Terrestrial fate	Transport of NP	Saturated natural porous media	Pristine, carboxylated and aminated fluorescent PS (191 nm, 114 nm and 51 nm; 52 nm; 57 nm, Magsphere)	Tracer	ζ (ELS), size (DLS)	Size, surface chemistry, ζ	Fluorescence spectrophotometry	233
Terrestrial fate	Plant uptake and translocation of NPs	Wheat, lettuce	Lanthanide fluorescent chelates doped PS (polymerization)	Tracer	Size (DLS), shape (STEM), elemental composition (EDX), ζ (ELS), stability and leaching of tracer (fluorescent spectroscopy), concentration (ICP-MS)	Size, shape, aggregation, ζ	ICP-MS, fluorescent spectroscopy, SEM	150

Table 3 *(continued)*

Study type	Research question	Matrix	Reference or test MNP	Use	Characterized property (technique)	Key property	Detection technique	Ref.
Aquatic fate	Fate and physicochemical modification of NPs in drinking water treatment process	Deionized and lake water	Metal doped PS (polymerization)	Tracer	Size (DLS), ζ (ELS), Pd distribution (STEM), Pd concentration (ICP-MS)	Size, ζ	ICP-MS	234
Aquatic fate	NPs depolymerization	Water	PET (dissolution–reprecipitation)	Tracer and quality control	Shape (FE-SEM), size (DLS)	Polymer type	HPLC	122
Aquatic fate	Fate of MP fibers	Sludge	Metal doped PET fibers (extrusion and cutting of PET granules, Serge Ferrari Tersuisse Multifils)	Tracer	Shape and size (SEM, optical microscopy), In concentration (ICP-MS)	Size, shape, concentration, density, polymer type	ICP-MS	98
Aquatic fate	NPs and MP fibers flux	Sludge and effluent from a pilot WWTP	Metal doped PET (polymerization)	Tracer	Size (DLS and optical microscopy), ζ (DLS), shape (SEM), In distribution (STEM-EDX), In concentration (ICP-MS) solid content of the stock dispersion (TGA)	Aggregation	ICP-MS	235
Aquatic fate	Degradation of MPs	Seawater	PET and PA fibers (Helly Hansen and Pierre Robert Group, microtomy)	Tracer	Shape and size (optical microscopy and SEM)	Size, polymer type, additives	SEM, GC-MS and LC-UV/LC-MS/MS	236
Aquatic fate	Environmental degradation of MPs including additives leaching	Seawater, freshwater	PET (Helly Hansen), PAN (Varner) and PA (PRG) microfibers (microtomy)	Tracer	Polymer type (ATR-FTIR), size and shape (optical microscopy and SEM)	Polymer type, matrix salinity, additives	GC-MS and UPLC-MS/MS	237
Aquatic fate, atmospheric fate	Impact of aging on chemical structure, surface shape and crystallinity of MPs	Water, air	LDPE (≤300 nm), PET (≤300 nm) and PVC (≤250 nm) (Goodfellow)	Tracer	Size distribution (Coulter counter), size (SEM)	Polymer type, shape, crystallinity	Raman and ATR-FTIR, SEM, XRD	238
Aquatic fate, atmospheric fate	Aerosolization of MNP *via* sea spray	Water, air	Fluorescent PS (0.5, 2, 10 μm, Polysciences), PE (1–10 μm, Cospheric)	Tracer	Concentration and size (fluorescence microscopy, Raman)	Size, density, concentration	Fluorescence microscopy, Raman	239
Atmospheric fate	Impact of shape in long-range transport of airborne MP fibers	Air	Acrylic resin fibers (3D printing)	Tracer	Size and shape (laser microscopy)	Size, shape	High speed camera	129
Monitoring	Detection of MPs	Water	PS, PET, PMMA, PVC, PTFE, PE and PMMA (Sigma-Aldrich and plastic objects grinding)	Tracer	Concentration, size and shape (confocal fluorescence microscopy)	Size	Confocal fluorescence microscopy	240
Monitoring	Identification of single NP particles and agglomerates	Water	PS (800, 300 and 100 nm, Sigma-Aldrich)	Tracer	Polymer type (SERS)	Size	SERS	241
Monitoring	Quantification of NPs	Tap, mineral, and river water	PS (100, 300, 600 and 800 nm, Sigma-Aldrich)	Tracer	Texture and thickness (AFM)	Size	SERS	242
Monitoring	Detection and identification of NP	Milli-Q and river water	PS (600–1000 nm, Aladdin and 5 μm, ChromTechResearch Center)	Quality control	Size (DLS), concentration (NTA, UV-vis)	Size, shape, density	SEM	243
Monitoring	Identification of NPs	Commercially bottled drinking water	PS (50 nm, Phosphorex, 200, 500 and 1000 nm, Sigma-Aldrich), PET (dissolution–reprecipitation and	Tracer	Surface and shape (AFM), size (NTA)	Size	SERS	125

Table 3 *(continued)*

Study type	Research question	Matrix	Reference or test MNP	Use	Characterized property (technique)	Key property	Detection technique	Ref.
			sonication)					
Monitoring	Detection and quantification of NPs	Deionized, bottled, tap and river water	PS (Zhongkeleiming Technology)	Quality control	Polymer type (Raman)	Size	Raman	244
Monitoring	Detection and quantification of NPs	Drinking, tap, and river water	PS (polymerization)	Positive control	Size (SEM), polydispersity, ζ (ELS), Au label (SEM)	Size, surface charge	ICP-MS	106
Monitoring	Detection and quantification of NPs	Water, radish, mussel	PS (100 and 300 nm, Sigma-Aldrich), pristine and carboxylated PS (49 nm, Polysciences)	Tracer	Size, dispersion (DLS)	Size, surface functionalization	Fluorescence spectroscopy	245
Monitoring	Impact of MPs on gut microbiota and polymer degradation	Gastrointestinal digestion fluids	PET (cryomilling)	Tracer	Shape (FESEM), structure, crystallinity and polymer type (Raman)	Crystallinity, surface properties	FESEM, Raman	59
Monitoring	Extraction of MPs	Soil	Metal doped PET (milling) and PET fibers (extrusion and cutting)	Quality control	Metal concentration (ICP-MS), size, shape, staining (microscopy)	Polymer type, size	FTIR-ATR, microscopy, ICP-MS	246
Monitoring	Fabrication of nylon NPs	Mammalian cells	PA (dissolution–reprecipitation)	Tracer	Fluorescence (fluorescence spectroscopy), polymer type (FTIR), size and shape (DLS and FE-SEM)	Shape	Fluorescence microscopy	247
Monitoring	Identification and quantification of MPs	Human sputum	PS and mix of PE, PP, PC, PA6, PET, PU, PVC, PS, PMMA (Shanghai Microspectrum Company)	Quality control	Polymer type (LDIR)	Polymer type	LDIR	248
Monitoring	Detection of NPs	Lab equipment, hot water-contained commercial paper cup, coastal seawater	PS (300 nm, Samchun Chemicals and 200 nm, Polysciences), PMMA	Quality control	Polymer type (SERS), size and shape (FE-SEM), size of synthesized PMMA (TEM)	Polymer type, size, dispersion	SERS	61
Monitoring	Extraction and quantification of NPs	Soil, sediment	PS and PMMA (50, 100 and 500 nm, Janus New-Materials)	Quality control	Composition (Py-GC/MS and ICP-MS), shape (TEM)	Size	Py-GC/MS	249
Monitoring	Quantification of NPs	Plants	PE and PMMA (120 and 100 nm, China Petrochemical Corporation)	Quality control	Size distribution (laser diffraction), polymer type (FTIR), shape (SEM)	Thermal properties, size	Microcombustion calorimetry	250
Monitoring	Extraction, detection and quantification of NPs	Influent and effluent from WWTP, river water	PS (50, 100 nm, NIST and 500 nm, Janus New-Materials), PMMA (50, 100 nm, Phosphorex, Hopkinton and 500 nm, Janus New-Materials)	Quality control	Polymer type (Py-GC/MS), size (DLS and SEM), shape (TEM)	Size, polymer type, protein corona	Py-GC/MS	251
Monitoring	Identification and quantification of MNPs	Water, river water	PVC, PP (Sigma-Aldrich) and PS (900 mm, Goodfellow)	Quality control	Polymer type (Py-GC/MS)	Size	Py-GC/MS	252
Monitoring	Detection and identification of MPs	Tap water	PA (1–315 μm. Carl Roth GmbH), PE, PMMA (1–315 μm, 15–150 μm. Sigma-Aldrich), PP, PS (150 μm, 106–125 μm, Polysciences)	Quality control	Polymer type, size and shape (Raman)	Size, dispersion	Raman	253
Monitoring	Identification of MPs	Sediment, nail polish	PA, PET, PS, PP, PE (milling)	Quality control	Polymer type (Raman)	Size	Raman	254

Table 3 *(continued)*

Study type	Research question	Matrix	Reference or test MNP	Use	Characterized property (technique)	Key property	Detection technique	Ref.
Monitoring	Identification and quantification of MPs (interlaboratory study)	Deionized water	PE, PS, PVC and PET (various size ranges, NIVA and Cospheric)	Quality control	Color, shape, concentration (optical microscopy)	Size, shape, polymer type, color	Optical microscopy, FTIR, Raman	255
Monitoring	Extraction, identification and quantification of MPs	Seafood	PS, PMMA, PVC (Sigma Aldrich), LDPE (500 μm. ThermoFisher Scientific), PET (20–110 μm. NIVA) and PP (20–120 μm. Lyondellbasell)	Quality control	Polymer type (Py-GC/MS), solubility in DCM (Py-GC/MS)	Solubility	Py-GC/MS	256
Monitoring	Identification and quantification of NPs	Surface water, groundwater	PS (200 nm, Zhongkeleming Technology), PVC, PMMA, PP, PS, PE, PET (Macklin Biochemical)	Quality control	Size (DLS), polymer type (Py-GC/MS)	Size	Py-GC/MS	257
Monitoring	Extraction of MPs	Fishmeal	PET, PVC, HDPE, LDPE, PP, PS (commercial products, grinding and cutting)	Quality control	Polymer type (ATR-FTIR)	Color, density	Optical microscope	258
Monitoring	Quantification of MPs	Wastewater influent	HDPE, PP, PS (LG Chem), PET (Lotte Chemical), PA (Hyosung TNC), HDPE, PS, PET (20–100 μm. Korea Testing and Research Institute)	Quality control	Size (optical microscopy and DLS), shape and texture (FE-SEM), density (density gradient)	Size, mass, polymer type	FTIR, Py-GC/MS	259
Monitoring	Detection of MPs	Wastewater, sludge	PS, LDPE (Ineos), PET (TPL), PLA (Nature Works), PA (Lanxess), PMMA (<125 μm Röhm), PS (40 μm, BS-Partikel)	Quality control	Size (DLS), staining (fluorescence microscopy)	Size, stability of dispersion, NR staining efficiency and robustness	Fluorescence microscopy, FTIR	260
Monitoring	Quantification of MPs	Blood	PMMA (Cospheric), PP (Sigma-Aldrich), PS (Cospheric), PE (Cospheric), PET (Goodfellow)	Quality control	Polymer type (Py-GC/MS)	Size, polymer type	Py-GC/MS	22
Monitoring	Extraction of MPs	Wastewater effluents	LDPE, PVC, PS, PP, PET, PLA and PA (sonication)	Quality control	Size (fluorescence microscopy)	Size	Fluorescence microscopy	67

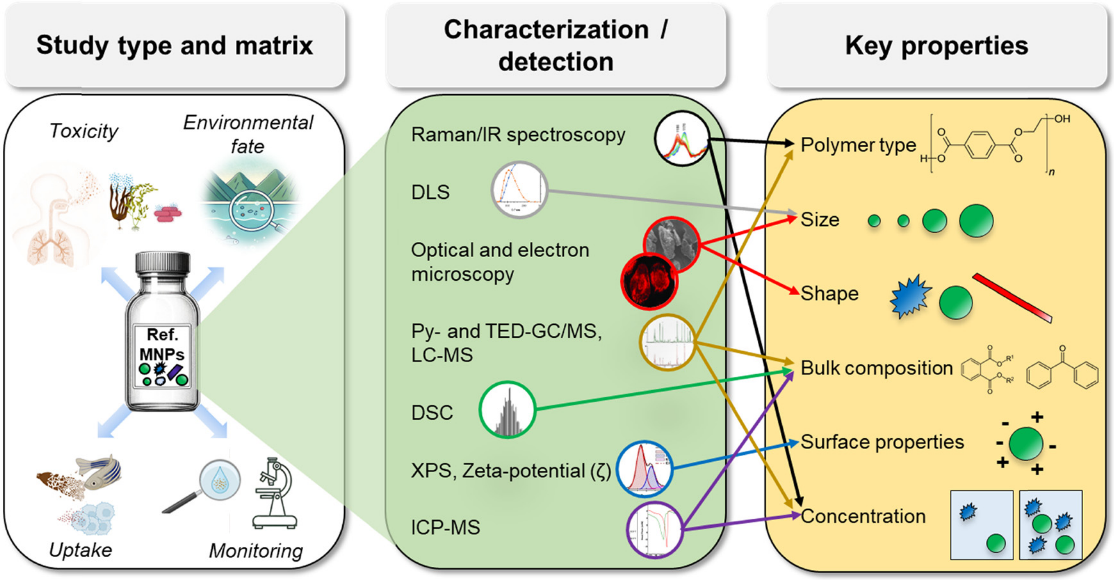

Fig. 5 Schematic summary of the type of studies where intentionally produced MNPs are used as test or reference materials (left) and the most common analytical techniques (middle) employed to characterize relevant MNP properties (right).

The size of MNPs also needs to be known to ensure it falls within the analytical window of the detection method selected for the study.[255,268] Similarly, when a new method is validated through spike-recovery experiments, the range of reference MNPs sizes for which the method has been validated should be reported, as recovery rates are also size-dependent. Smaller-sized MNPs pose particular challenges as it has been shown that detection errors increase as particle size decreases.[269] In addition, Le Juge *et al.* (2023) has shown that the quantification of NPs by Py-GC/MS may be size-dependent.[270]

The importance of MNP size is reflected in it being ubiquitously reported in the studies described in Table 3. For spherical MNPs or MNP fragments generated by top-down methods, the size of an individual particle is measured by its (effective) diameter, while the size characterization of the particle ensemble should be reported in terms of the overall size distribution. For fibers, the dimensions should be reported in terms of the distributions in both length and diameter, as the aspect ratio is a critical parameter as discussed in section 6.3. Particle size and particle size distribution can be evaluated by (electron or optical) microscopy[271] or by electrophoretic methods, which measure the hydrodynamic diameter.[272] The most common method for the characterization of micro/nanopowders is SEM, while for dispersed nanoparticles dynamic light scattering (DLS) is by far the most preferred technique.[223,243] However, DLS struggles with polydisperse samples. Nanoparticle tracking analysis (NTA) is an alternative to DLS for polydisperse MNP suspensions that analyzes particles individually. For metal-doped MNPs, ICP-MS and spICP-MS has proven to be an effective means of determining both particle size concentration and particle size distribution.[273]

6.3 Shape and aspect ratio

MNP shape, including aspect ratio, can also be a significant factor in controlling MNP properties and environmentally relevant behavior. Keller *et al.* (2020) have demonstrated that the mobility of spherical MNPs and organic solids is similar, whereas MNP fibers exhibit less mobility, emphasizing the role of shape in environmental particle dynamics and transport in terrestrial media.[232] Similarly, shape is also recognized as a key parameter in the transport of MPs in water[274] and the atmosphere.[129,275] There are some conflicting results regarding the uptake of fibers by marine organisms compared to lower aspect ratio particles, although it is clear that shape plays an important role in the ingestion of MNPs.[221,228,230] In a related MNP accumulation study, shape was found to play a role in the time required for MNPs to be expelled from the bivalves' gut, with ingested fibers being retained longer in the gut compared to spherical particles.[210,228]

Interestingly, in a study about the impact of PE spherical beads and PET fibers on freshwater zooplankton, Ziajahromi *et al.* (2017) observed uptake of spherical beads but not fibers, although the external physical impact (carapace and antenna damage) caused by the fibers made them even more toxic to daphnia than the spherical beads.[212] In a different study, Han *et al.* (2020) were able to link the shape and sharpness, as defined by a 2D-local curvature equation, of PVC and ABS resulting from milling with their toxicity to human cells.[220] The fragments were not sharp enough to induce physical damage to cell wall but did trigger immunotoxicity. However, the accumulation or toxicity effects of MNPs on marine isopods were independent of the shape of MNPs (beads, fragments or fibers), suggesting that marine isopods might be less sensitive than other marine invertebrate species.[276] In contrast, a study with PE MNPs supported the idea that irregular shapes help to trigger cytotoxicity and inflammatory responses, in marked contrast to the lack of toxicity observed for smooth spheres.[221] Other studies have reported that the length and rigidity of fibers has also been reported to affect their toxicity;[213,277,278] however, considering the contradictory results on the importance of length (*i.e.* longer fibers leading either to more or less damage than shorter ones), this parameter alone is not a sufficient descriptor of a fibers' toxicity, and rigidity is difficult to assess.

It should be noted, however, that there is a lack of studies where a direct comparison of shape effects on MNP's behavior is the central focus. Moreover, the overwhelming majority of studies focus on spherical MNPs or MNP fragments generated by top-down methods. An analysis of the literature highlights the importance of conducting more studies using fibrous reference MNPs. Indeed, fibers are reported to be the most or second most prominent shape of MNPs in terrestrial environments, water bodies and biota.[279–282] For both characterization and detection, shape is most readily determined by using either optical or electron microscopes, depending on the MNP size.

6.4 Surface properties

Surface properties such as charge, functionalization, oxidation state, texture, adsorbed chemical or biological contaminants, *etc.* represent another key characteristic that define MNPs. Since surface properties are often affected by the production and labelling method (see MNP production section), for many study types it is crucial that they are well characterized to avoid data misinterpretation. Surface properties can also be altered by ageing (*e.g.* by irradiation) or by protocols used to extract MNPs from complex matrices, as they may require corrosive chemicals (*e.g.* H_2O_2). Further, changes in the MNP surface may impact their reliable identification and quantification. In this context, reference MNPs have been used to assess the impact that modifications to particle surfaces caused by weathering or extraction protocols, can have on the misidentification of MNPs by techniques such as FTIR, mass spectrometry, or chromatography.[67] For example, Philipp *et al.* (2022) observed that PLA turned from transparent to milky and became more brittle after a one-day Fenton reaction.[51] Lusher *et al.* (2017) provides a compilation of chemical resistance of seven individual polymers to different solvents, acids, and bases.[283] More importantly, MNP surface properties profoundly influence their interactions with solvents and biological media, contaminants, natural particles, and organisms, which has a direct effect on processes such as uptake, transport, adsorption and biofilm or eco-corona formation.[41,177,284]

Using zeta-potential (ζ) as a proxy, surface charge is the most widely reported surface characteristic. Notably, it was identified as an important MNP property in determining cellular uptake.[285] In fact, cell membranes, which are negatively charged, have a stronger interaction with positively charged particles, which are thus more easily internalized than negatively charged MNPs. Silva *et al.* (2014) proposed using the magnitude of the difference in ζ between MNPs and cells or organisms as a metric of toxicity for charged MNPs,[286] although the influence of other properties like aggregation and biofilm formation cannot be ignored.[225] In addition, Rosa *et al.* (2013) reported that both oysters and mussels exhibited selective feeding, preferentially ingesting or rejecting MNP particles based on their surface charge and hydrophilicity.[287] Similarly, the mobility of PS NP tracers in soil was found to be associated with their surface functional groups (among carboxyl, hydroxyl, and amino groups) as well as the resulting charge and hydrophobicity/hydrophilicity.[233] Indeed, the surface properties of MNPs will influence their tendency to undergo heteroaggregation with other particulate species as well as their propensity to adsorb organic and inorganic chemicals onto their surfaces.[190,288,289] Thus, Rowenczyk *et al.* (2020) demonstrated that the surface oxidation of plastic debris greatly influences the sorption of organic pollutants and metals, likely due to the alteration of the surface charge and hydrophobicity.[290] Variabilities in surface properties could also rationalize studies that showed nominally identical particles sourced from different manufacturers which exhibit significantly different toxicity towards cells.[45,291]

Surface morphology is sometimes evaluated, and in a few studies it has been observed to impact MNP behavior. Thus, rougher surfaces have been seen to be more prone to pollutant adsorption or trapping[292,293] due to their higher surface area-to-volume ratios; similarly, rougher surfaces also favor biofilm growth and the formation of an eco-corona which can alter the surface properties and density of the particles.[177,294]

The MNP surface properties that play important roles in governing MNP interactions with the environment can be characterized by various techniques. For example, XPS can provide information about the surface chemistry and composition of MNPs, while ELS is used to determine zeta potential, a proxy for MNPs surface charge.[295] Surface texture is best examined by microscopic techniques such as SEM or TEM. One characteristic that has sometimes been cited as playing a central role in determining MNP properties is hydrophobicity/hydrophobicity,[290] although no analytical method has emerged as being able to reliably and accurately quantify this parameter for nanoparticles.[296] Finally, it should be mentioned that MNP test materials should be screened for surface contamination before use, and particularly for microbial/endotoxin contamination when biological effects are under evaluation. In this regard, typical thermal, chemical or physical treatments used to ensure particles sterility may damage MNPs, so clean production and handling, when possible, is highly desirable. MNPs can be analyzed for endotoxin contamination using limulus amebocyte lysate (LAL) test[119,126] or HEK toll-like receptor (TLR) reporter cells.[297,298]

6.5 Bulk MNP composition

MNPs sometimes consist of more than just a single polymer. For example, plastics used to produce reference MNPs by a top-down approach may be co-polymers or polymer blends (*i.e.* a mixture of many polymers),[299,300] or contain different types of additives.[31] This type of species includes not only intentionally added substances, principally organic chemicals, but also inert elements such as metals or carbon fibers,[84] added to modify certain polymer characteristics (*e.g.* resistance to UV aging) and occasionally even to identify the manufacturer. Indeed, plastics contain over 300 different additives, known as PoTSs (potentially toxic substances), which are typically not bound to the polymer matrix and can thus migrate to the surface and subsequently desorb, or leach from the plastic and produce toxic effects.[301–303] Moreover, the adsorption of pollutants onto the MNP surface may be altered by the presence of these additives.[288] The importance of additive leaching also increases as the surface area to volume ratio of the MNPs increases, so smaller particles are likely to pose increased risks in this respect. Some additives will improve the photostability of MNPs, while other additives cause color changes which may influence visual identification or the uptake of MNPs by living organisms, if they confuse the particles with food: according to de Sá *et al.*, a higher amount of white PE than red and blue PE was ingested by fish due to its similarity with their main natural preys.[304] Unfortunately, the details of the composition and concentration of these various additives in MNPs is often proprietary, although their presence has the potential to profoundly impact MNP behavior, particularly toxicity. Indeed, the impact of MNP composition on behavior is typically overlooked, likely because the challenges in determining additive speciation and concentration are considerable.

Details on the MNP components and their concentration can be obtained by chromatography- and MS-based techniques such as ICP-MS, HPLC or Py-GC/MS.[305] To differentiate between the effects of additives/contaminants and the particles themselves in determining the impact of MNPs, most notably in toxicity studies, it is necessary to determine additive leaching rates from MNPs generated from bulk polymers in top-down methods to avoid misinterpretation of toxicity results.[306] In contrast, MNPs generated by bottom-up methods typically use polymer feedstocks that are relatively well characterized and free of additives, since these are incorporated during the manufacture of macroscopic plastics/polymers, but the absence of chemicals residual from the synthesis, particularly surfactants, must be verified.

6.6 Bulk MNP physical properties

Characterization of MNPs' bulk physical properties such as crystallinity or density, is missing in most studies reported in the literature, but these properties are important to explain certain findings related to MNP transformations, fate and toxicity.

For example, crystallinity influences a plastics' mechanical properties. A higher crystallinity is correlated with strength, but also with brittleness, and can thus be considered a proxy for the likelihood of fragmentation. While crystallinity itself may have a limited impact in many MNP-related studies, its characterization is important in understanding how MNPs become embrittled and fragment in the environment, as a consequence of chemical (*e.g.* photooxidation) or physical (*e.g.* erosion) weathering, and thus change in size and shape.[16] Embrittled MNPs are also more likely to fragment inside the human body when they encounter chemical and/or physical stress, such as those that occur during digestion.[59] Crystallinity is also an important factor regarding the biodegradability and depolymerization of MNPs.[59,60] Crystalline particles are less easily broken down by enzymes than amorphous ones, and, therefore, the polymer is less bioavailable for microorganisms.[307]

Density depends on the composition, molecular weight, and degree of crosslinking, and is positively correlated with crystallinity.[308] Consequently, a weathered, more crystalline plastic is denser than its pristine counterpart, although the difference is typically small. Density can influence MNP fate in the environment,[16] because it controls the settling velocities of particles in a fluid, and is typically calculated with Stokes' law.[190] These rates moderate the transport and distribution of MNPs in water or the atmosphere,[16] and therefore influence, for example, which aquatic organisms come into contact with the MNPs. However, ingestion experiments performed with MNPs of various densities suggest that density may be a negligible parameter for particle selection by marine organisms like oysters and mussels in comparison with other MNP characteristics, such as surface properties.[287]

MNP crystallinity is typically determined by DSC,[204] whereas MNP density is rarely characterized (Table 3). This may be because density is typically not considered relevant, or that referring to densities provided in polymer textbooks or by the manufacturer is considered sufficient. This lack of reporting is also a consequence of the difficulty in measuring MNP density, although it can be estimated by determining the buoyancy of MNPs in a range of solutions with different densities.[309]

6.7 Concentration and dose

The effects of MNPs often depend on the concentration and/or dose, and these parameters are usually reported across all study types, although the way in which they are reported varies considerably. If the MNPs are obtained from commercial sources as a dispersion in a liquid or solid matrix, then the concentrations are almost always provided by the vendor. However, in experimental studies these values need to be checked to ensure that they have not been influenced by how the MNPs were dosed or by matrix effects (*e.g.* MNP aggregation). MNP concentration can also be reported in terms of particle number concentration (PNC). For polydispersed MNPs, particle size distribution (PSD) is another extremely important parameter that should be measured. Optical microscopies, micro-FTIR and micro-Raman can determine PNC and PSD for MPs, due to their larger size. In contrast, laser-based techniques[310] such as dynamic light scattering (DLS) can measure PSD for both MPs and NPs, while nanoparticle tracking analysis (NTA) can determine PSD and PNC for NPs. For metal-doped MNPs, ICP and sp-ICPMS have been used effectively to determine PNC and PSD at extremely low, environmentally relevant MNP concentrations.[83,232] Although electron microscopy (EM) techniques such as SEM and TEM are capable of measuring PSDs at micro- and nanoscale dimensions, respectively, these are low-throughput techniques and drying effects during sample preparation may cause unwanted particle aggregation leading to PSD distortion. Polymer mass concentration can be determined by mass spectrometry (*e.g.* Py-GC/MS, TED-GC/MS),[311–313] and if the MNP concentration is high enough, gravimetric methods are also an option. However, in the absence of PSD information mass concentration is an ambiguous metric because a given gravimetric amount of MNP can be associated with a small or large number of MNP particles.

There are a number of different scenarios where it is important to know the concentration/dose of reference or test MNPs; for example, as internal standards (*e.g.* in Py-GC/MS) to correct for matrix effects and/or instrumental drifts, or in method validation MNP dose/concentration should be quantified to determine recovery rates. For MNPs used to evaluate toxicological effects it is important to determine not only concentration, but also exposure time, because toxicity can be dose and concentration-dependent.[77,314] Moreover, the PSD is a critical piece of information for interpreting toxicity data because it can be used to estimate the exposed surface area of MNPs that will come into contact with organisms in the environment.

6.8 MNP solution chemistry

In addition to the profound influence that electrolyte composition plays in determining MNP stability and aggregation state, any MNPs in suspension will constantly interact with other possible components (*e.g.* antimicrobial agents or surfactants). Thus, the composition of MNP-containing solutions or solid matrices should be detailed. In addition to the typically unwanted and confounding effects of surfactants, the potential presence of deliberately included biocides should also be considered in commercial NP suspensions.[315,316] For example, Heinlaan *et al.* (2020) observed that the toxicity of commercially available PS NP was caused by the antimicrobial additive sodium azide and not by the particles themselves, emphasizing the need for careful consideration of additional compounds in toxicity assessments.[216] In the case of bottom-up MNP production, it must be determined if surfactants, unreacted monomers, and residual polymerization by-products, including initiators, catalysts and solvents, have inadvertently remained in the final solution.[16,31,41,317,318]

7 MNP detection

In many studies, it is necessary to detect and characterize MNPs after they have been introduced into a matrix or subjected to various treatments, and this can be challenging. The literature provides a number of examples on the detection and quantification of MNPs across diverse matrices ranging from marine organisms,[319] human[23,24] and animal tissues,[320] air,[321] water,[322] dust,[323] and soil.[324] The selection of suitable analytical techniques for MNP detection and characterization post-experiment depends on several factors, including particle size range and concentration (which must be above the detection limits), matrix, label, state of the MNPs (powder, liquid suspension, or dispersion in an organic matrix), and the key properties to be examined (Table 5). After extraction from aqueous media or air filters, spectroscopic techniques such as FTIR and Raman as well as mass spectrometry techniques like Py-GC/MS can be used to detect MPs, although quantification of NPs is more challenging due to their smaller size. In biological media, detecting MNPs is essential for understanding cellular uptake and transport mechanisms; this can be accomplished with electron microscopy techniques like TEM, evaluating samples *in vitro* that have been fixed, or by *in vivo* analysis of pre-treated models.[325–327]

Labelled MNPs are often used to facilitate the detection and localization of MNPs using methods like fluorescence microscopy, flow cytometry, or photoluminescence spectroscopy,[149,328] without the difficulties inherent in MNP extraction. However, the label can modify the particle's surface properties and bias the results of the study.[329] This has led to an increase in label-free, and rapid detection methods for MNPs.[200,245,330–332]

8 Application of reference and test MNPs

The previous sections detailed existing methods to produce, characterize, and detect the reference and test MNPs that are indispensable as tracers, to study their behavior and effects in controlled experiments, and as calibration and internal standards, to develop and validate analytical methods to detect and quantify MNPs. Table 3 compiles information on the various applications of reference and test MNPs retrieved from 72 research papers published between June 2013 and February 2024 identified by in the Web of Science core collection, Scopus database and Google scholar by using "microplastic*", "nanoplastic*", "characterization", "reference", "standard", "material*", "tracer", "control", "internal standard", "labelling", "test", "impact", "polymer type", "size", "shape", "detection", "quantification", "spik*" keywords. Entries are organized according to study type, divided into toxicity (both human toxicity and ecotoxicity), uptake by organisms (entry and distribution), environmental fate (including terrestrial, aquatic, and atmospheric), and monitoring (quantification and identification). A single report can feature in more than one study type; for example, studies may feature both uptake and fate or uptake and toxicity, as in the works of Rubin *et al.* (2023) or Heinze *et al.* (2021), respectively.[215,231] For each publication, Table 3 reports the research question being addressed, the type of sample or matrix into which the MNPs were intentionally spiked or introduced, the use (as a tracer or for quality control), the polymer type and state (pristine, doped, or functionalized), the physicochemical properties characterized and the methods used for characterization. If the material was purchased, the manufacturer or company is indicated; otherwise, the top-down or bottom-up production method is indicated. As a minimum requirement, the studies selected for our review report the characterization of at least one MNP property. This is notable because a significant fraction of the literature purporting to use reference and test MNPs do not characterize any of their properties[34] and were excluded from our analysis. For each of these selected studies, the properties of the reference MNPs characterized and the analytical techniques used to measure these properties are indicated. In addition, the key property/properties responsible for the outcomes or conclusions of the study are identified. Polymer type (*i.e.*, chemical structure) and particle size are the most frequently characterized properties. In Table 3, size stands out as a key parameter in *circa* 70% of the studies. Shape (sphere, fragment, fiber) follows in relevance, with 19 mentions. Surface and bulk properties are reported at significantly lower frequencies. Similarly, the properties of the media where MNPs are stored or spiked are also frequently underreported, often being limited to the type of solvent/media and the MNP concentration (particle- or mass-based).

Reference and test MNPs are indispensable for advancing research by enabling controlled experiments. They facilitate systematic investigations into the fate and effects of MNPs while ensuring experimental reproducibility. It is important to recognize that reference and test MNPs are used differently depending on the study type: in our classification, toxicity studies assess the impact of MNPs on humans[219,220] and other living organisms[210,211] using *in vitro* and *in vivo* models. In these experiments, reference MNPs and their detailed characterization are critical for controlling the concentration, type, and properties of particles used as tracers, allowing researchers to correlate observed toxicological outcomes with specific physicochemical properties. Proper characterization of reference MNPs is essential to avoid artifacts caused by contaminants, such as surfactants used in particle suspensions, or unintended surface modifications introduced during production. Uptake studies differ from toxicity studies in that they focus on the entry (ingestion, inhalation, cellular uptake) and distribution of MNPs within organisms, whereas fate studies focus on the distribution, transport, accumulation, and elimination of MNPs in a given environmental matrix (*i.e.* water or terrestrial environments, or atmosphere). In uptake and fate studies, MNPs are often used to track the translocation of the particles, so MNPs need to be detected or visualized in the matrix. Monitoring studies aim to identify and/or quantify MNPs in the environment and living organisms, so they typically employ MNPs as a quality control[333] to validate or demonstrate the feasibility of sample preparation methods,[240] extraction protocols,[249,251] detection techniques,[61,67] or quantification methods.[253,334,335] In this context, MNP recoveries, assessed by adding and recovering known quantities of MNPs from test media, provide a metric for the under- or overestimation of MNPs. This allows the efficacy of the method to be gauged as a function of the MNP characteristics and allows results to be reported more quantitatively and with a higher degree of confidence. Similarly, reference MNPs can also be used as internal standards to correct for matrix effects and instrumental drifts.[198,336] Moreover, reference MNPs are also needed to calibrate or to obtain reference spectra of MNPs with different characteristics. This information improves the accuracy of polymer identification/detection when considering possible effects of, for example, color, opacity, texture, crystallinity, presence of additives or contaminants, the matrix, *etc.*[337] The use of well-characterized reference and test MNPs ultimately allows researchers to assess the extent to which different studies can be meaningfully compared, thereby promoting a better understanding of the relationships between MNP properties and their environmental or biological fate and impact.

9 Summary

In the past 5–10 years there has been an awareness that the quantity of MNPs entering the environment is increasing rapidly, fueling an explosion in research efforts directed towards understanding their behavior and effects. However, the inherent challenges of MNPs collection, identification and quantification in different environmental compartments has led most researchers to focus on analytical method development and lab-based studies where reference and test MNPs are either purchased from commercial vendors or produced in house. This review was motivated by the need not only to compare, contrast and assess the existing MNPs production methods, but also to evaluate the properties and applicability of the existing MNPs to answer diverse research questions.

The initial part of this review overviews existing commercial MNPs (a detailed list is included in ESI† 1) and their limitations, as well as the means of producing "in house" reference and test MNPs. There are a wide variety of top-down methods that create MNPs by applying an external force to fragment larger sized plastics (Table 1); grinding and milling the most common methods, often under cryogenic conditions. Top-down methods are particularly well-suited to produce MNPs of random shapes and sizes that reflect those encountered in the environment. In contrast, bottom-up methods rely on the formation of MNPs from molecular building blocks (Table 2). These methods provide greater opportunities to control key MNP properties, particularly size, and can be adapted to include different labels that facilitate the detection and quantification of MNPs in complex environmental matrices. However, bottom-up methods are invariably restricted to the synthesis of spherical particles whose surface properties can sometimes be compromised, most notably by the adsorption of surfactants. Table 4 provides a concise summary of the characteristics, advantages and limitations of the two types of production methods.

The wide range of properties that collectively describe MNPs can present a daunting challenge for most researchers, who do not have an unlimited array of characterization/detection techniques available. The second part of this review has surveyed the literature to ascertain the applications of reference and test MNPs to answer key research questions, divided into four study types (fate, toxicity, uptake and monitoring), identifying properties that are ubiquitously important to characterize regardless of the study type (*e.g.* size), as well as those that are mostly relevant to certain types of studies (*e.g.* bulk polymer composition for toxicity and monitoring). Table 5 provides an overview of the MNP properties that need to be characterized depending on the study type (toxicity, uptake, fate, and monitoring) and the most common techniques that are appropriate to determine them depending on the particle size.

Table 3 showed that most studies with characterization data provide information about at least 2 or 3 properties of MNPs, with a significantly smaller number of studies characterizing 4 or more properties. Toxicological studies are the most characterization intensive, due to the wide range of MNP properties that can contribute to adverse effects in humans and environments. In conclusion, regarding in MNPs studies:

- Polymer type, size, and shape of reference and test MNPs should always be characterized. These properties are relevant in all types of studies, improve the comparability across studies and are relatively easy to measure using available techniques.
- Surface properties are important to determine in almost all study types, as they are responsible for particle stability and strongly influence MNP interactions with other species.
- Bulk plastic composition, especially identification and concentration of additives and residual monomers, should be assessed in monitoring studies and is critical to toxicity studies, but not for other study types.
- Bulk physical properties are generally not needed with some exceptions. For example, density should be considered in specific cases like fate and transport studies, or monitoring studies where the density influences the efficiency of MNPs extraction from the sample matrix. Crystallinity should be reported when the study involves the potential for MNP biodegradation and/or fragmentation.
- Particle concentration and dose as well as solution chemistry are not MNP properties, but these parameters are key for some study types. Thus, knowing MNP dose is crucial when spiking for detection method validation or for *in vitro* toxicity studies, and solution chemistry must be well-defined for toxicity studies.

10 Future directions and needs

This review concludes by discussing some of the future directions and open issues related to the production, characterization and use of reference and test MNPs, including several research opportunities, the need for more of both commercially available and in-house, and MNPs suggestions best practices for MNP studies, highlighting the need for quality assurance and quality control. The hope is

Table 4 Comparison of top-down and bottom-up methods to obtain reference and test MNPs

Parameter	Top-down	Bottom-up
Shape	Fragment	Sphere
MPs production rate	High	High
NPs production rate	Low	High
Surface properties	Irregular, oxidation possible	Texture can be controlled, surfactants may be present
Reproducibility	Poor	High
Labeling options	Only surface, post-synthesis	Bulk and surface
Advantages	• Instrumentation is often widely available • Generally simple procedure • Applicable to most polymer/plastic types • Higher environmental relevance	• Faster and scalable • Controllable size • Labels can be incorporated • Shape is mostly limited to spheres
Limitations	• Sieving or filtration is required for size fractionation • Potential metal contamination • Labelling is difficult • No control over additive content	• Use of non-environmentally friendly polymer-specific solvents • Requires some experience in polymer chemistry

Table 5 Characterization requirements in studies using reference and test MNPs and possible analytical techniques as a function of the property and the particle size. XX = needed, X = may be needed/useful depending on the research question, — = generally not needed

Study type					Analytical techniques	
Toxicity	Uptake	Fate	Monitoring	Property	NP	MP
XX	XX	XX	XX	Polymer type	Py- and TED-GC/MS Raman and FTIR	
XX	XX	XX	XX	Size	DLS, NTA, spICP-MS Electron microscopy	Optical microscopy
XX	XX	XX	XX	Shape	Electron microscopy	Optical microscopy
XX	XX	XX	—	Surface charge	Zeta potential measurement (ζ)	
X	X	X	—	Surface chemistry	XPS	
—	—	X	X	Density		Density gradient
X	—	X	—	Crystallinity	DSC and Raman	
XX	—	—	X	Bulk composition[a]	GC/MS, LC/MS	

[a] Includes the characterization of additives, residual monomers and co-polymers.

that the implementation of these practices will help to improve the overall data quality in the field of MNP research as a route to providing a better understanding and an improved, more quantitative assessment of the risks (or not) posed by different MNPs.

10.1 Commercial MNPs

Commercially available MNPs are dominated by PS and PE, usually spherical and pristine. There is therefore an obvious need to expand the range of commercially available MNPs to encompass a wider range of polymer types and shapes. Commercial MNPs should also include irregular fragments in the low microsized and nanosized ranges to mimic the shape and size range of environmental MNPs. Finally, disclosing the details of the polymer composition (*e.g.* the type and concentration of any chemical additives) and/or the composition of the matrix/suspension containing the MNPs as standard practice, would be extremely valuable for researchers.

Besides these technical considerations, discussions between academic researchers and plastics manufacturers should be strengthened to foster the production of large quantities of MNP particles with controlled chemical and physical properties. Similarly, encouraging interdisciplinary interactions between environmental scientists and analytical chemists, polymer chemists or materials scientists would be invaluable, as the former have specific needs that could be addressed with the expertise of the latter. The involvement of institutions such as the Joint Research Center (JRC) of the European Commission, metrology institutes (*e.g.* NIST), standardization bodies and technical committees will be crucial to this endeavor. Once the proof-of-concept and suitability of a production method have been demonstrated, the aim should be to produce certified reference materials (CRMs) of varying characteristics following the validation and certification processes outlined in guides such as ISO 17034:2016[47], ISO Guide 35:2017[48], and JJF1343-2012[49]. Although we acknowledge that certification is expensive, commercially available reference MNPs with a certificate of analysis of the essential properties highlighted in this review would be highly beneficial for interlaboratory comparisons, standardization, and validation of analytical procedures.

An underutilized benefit of well-controlled and well-characterized commercial MNPs (ideally certified) is their facilitation for inter and intra laboratory comparisons. There are already an enormous number of studies describing the behavior and effects of MNPs, but results obtained in one study are often hard to compare with other studies even if the research question and matrix are nominally similar. If a commercial MNP is included as part of a study, then this data will provide a benchmark to compare results from these studies with other studies where the same commercial MNP was employed.

One challenge with commercial MNPs is the lack of consensus as to the ability of these well-defined MNPs to mimic the behavior of MNPs produced by natural processes. One issue is the different shapes, with commercial MNPs being usually spherical due to their bottom-up production, in contrast to the irregular shapes of naturally-occurring MNPs. Thus, it would be valuable to determine if the behavior of spherical MNPs can be directly extrapolated to MNPs generated naturally or by top-down means with equivalent spherical diameter.

10.2 "Realistic" MNPs

"Realistic" MNPs can be obtained by extraction from the environment, an option largely underexplored at the moment due to the intensive labor involved and the low rate of recovery. The harsh chemicals sometimes used to perform extraction from complex matrices may also unintentionally alter MNP properties. For monitoring studies, this may make the detection of the MNPs with spectroscopic and spectrometric instruments challenging as the polymer fingerprint(s) may have changed. Using reference MNPs as an internal control, subjecting them to the same extraction process, and then characterizing the MNPs produced and comparing these results to the pristine reference MNPs is a useful test to ensure no measurable alterations occurred during the extraction process. One interesting alternative might be to collect MNPs through airborne samples.

The production of commercial MNPs almost exclusively involves bottom-up methods. In contrast, more "realistic" MNPs representative of the heterogeneous shapes and polydispersed sizes found in the environment are currently produced predominantly in house using top-down approaches. One overarching challenge in the fabrication of realistic MNPs is the low-throughput of top-down methods which makes larger-scale, multi-laboratory and round robin type studies difficult. Top-down production typically has high yields for fragments of sizes above a few microns, but these yields drop dramatically for nanosized particles ($\ll$1%).[37] Therefore, developing high-yield methods to fabricate nanosized fragments is essential to produce kilogram quantities of MNPs in a reasonably rapid and cost-effective manner, suitable for widespread use. Herein, we note that in the most common top-down methods, (*e.g.* milling) the production yields (mass of MNP divided by the mass of starting material) and the production rates (amount of product per unit time) are rarely detailed, despite being essential to allow a particular method to be scaled-up. We suggest systematically reporting throughput values clearly, ideally as a function of the MNP size range(s) produced. One promising approach to increase the yield of MNPs would be to use artificial weathering as a means to embrittle bulk plastic before the top-down method is used to break the plastic down and create MNPs.[102] Some relatively under-utilized top-down methods appear amenable to producing reasonable quantities of weathered MNPs directly, such as ultrasonication, where fragmentation and oxidation occur simultaneously.[71,77] However, care should be taken to determine the extent to which the artificial weathering of the bulk plastic alters the properties of the MNPs.

In some specific cases, production hurdles have hampered the ability to create reference MNPs. One example is in the production of MNP fibers, which is being hindered by two major interconnected challenges. First, extrusion/spinning processes with a reasonable throughput must be developed or adapted to obtain polymer filaments with diameters ranging from nanometers to a few micrometers, representative of the MNP fiber diameters found in the environment, but significantly smaller than the 10 to 40 μm range produced by the textile industry. In this respect, electrospinning was identified as a potentially viable process to produce polymer filaments down to about 100 nm in diameter, something which is not achievable with traditional extrusion processes such as melt-spinning. The second, and perhaps most challenging aspect is to develop efficient methods to cut the filaments at small and regular lengths (<1 μm) capable of producing batches of very small MNP fibers. For almost all top-down methods, however, there is a need to harmonize the level of detailed reporting of experimental parameters so that greater control and reproducibility can be exerted over the characteristics of the MNPs produced, particularly particle size distributions.

A detailed discussion on the development of unique types of reference MNPs, such as tire wear particles made of rubber, was beyond the scope of this review. However, we would like to highlight that some options do exist to obtain these rubber particles, including direct collection on the road and top-down approaches such as, abrasion of tires in the laboratory or cryomilling a mixture of tires from different manufacturers.[338,339] The ISO/TS 22638:2018 protocol describes a method for the standardized production of representative tire and road wear particles without any contamination issues that could arise from the use of a road simulator. However, the main challenge in the production of reference tires MNPs lies in the varying composition of tires caused by the different manufacturing processes.[340]

10.3 Storage stability of MNPs

The stability of both commercially available and in-house generated MNPs can change over time. This is because storage conditions may alter the properties of MNPs, resulting for example in their agglomeration. For this reason, it is necessary to assess the optimal storage conditions of MNPs to provide recommendations to the community. This same information can also be used as the basis to improve the colloidal stability of test and reference MNPs. Various approaches have been developed to improve MNP colloidal stability, including the use of organic solvents, surfactants or the surface oxidation of MNPs (*e.g.* with ozone). However, these strategies may compromise the environmental relevance of the particles, potentially skewing the outcome of the studies. There is therefore a need to effectively disperse MNPs while not compromising their environmental relevance. Moreover, the stability of reference and test MNPs, whether stored in a solution, as powder, or embedded in a solid matrix, is often overlooked for both commercially-available or in-house produced materials. It is essential that stability assessment becomes part of the standard characterization of MNPs and is reported consistently. To this end, the ISO 33405:2024 protocol provides some guidelines for the characterization of the stability and homogeneity of reference materials.

10.4 MNP labelling

Multilabeled or multifunctional test and reference MNPs, benefitting from the advantages of different labels (*i.e.* for MNP imaging, quantification, sizing), would be useful to expand our understanding of transport, degradation, fragmentation and uptake mechanisms, particularly in complex matrices. Notably, the combination of labels that enable both quantitative assessment (*i.e.* metal or isotope tags) as well as visualization (*i.e.* fluorescent, colored) of MNPs offers the most interesting synergies. For example, Luo *et al.* (2022) synthesized metal-tagged fluorescent MNPs, which allowed the *in situ* visualization of the particles and their further quantification by ICP-MS.[150] Furthermore, multifunctional MNPs could enable the comparison and performance validation of different analytical techniques. For example, MNPs with a color tag visible to optical microscopy/spectroscopy techniques and a stable isotope label identifiable by thermo-analytical techniques could be used as internal standard for both instruments, opening the door to method cross-validation and harmonization. For these reasons, the commercial availability of these more complex, multi-modal MNPs is needed.

The labelling of macroscopic sized plastics would also help MNP research efforts. This is a consequence of the low production rates of MNPs from macroscopic plastics due to natural chemical or physical degradation processes in the environment.[180] Moreover, quantification of these MNP production rates tends to be obscured by the complex, heterogeneous media into which they are released. Accurate, quantitative information on MNP release rates in realistic environments and the factors that influence these release rates is therefore difficult to obtain. This knowledge gap could be addressed by designing metal-doped or stable isotope labeled macroscopic plastics that facilitate the detection and quantification of MNPs released as plastics break down in different release scenarios (*e.g.* sand abrasion, natural sunlight). Information gained from these types of studies would also be valuable for developing emission inventories for various sectors and compartments, which are essential inputs for environmental models that inform policy. For instance, sources such as tire abrasion, agricultural dust, and ocean spray have been identified as contributors to atmospheric MNPs. However, current estimates of emission rates from these sources carry up to 100% uncertainty.[341]

10.5 MNP weathering

In the environment MNPs often transform from the initial state in which they enter the environment because of natural weathering, but protocols for weathering MNPs are often lacking and incompletely described and therefore difficult to replicate in the absence of more widely accepted, standardized procedures. In part, this reflects the wide variety of ways in which MNPs can be naturally weathered in the environment, either by chemical (*e.g.* oxidation), physical (*e.g.* abrasion) or biological (*e.g.* microbial activity) processes. Moreover, even for a specific type of weathering experimental parameters may vary (*e.g.* UV light sources of different spectral range or power for photochemical weathering). Given this variability, the properties of particles that have nominally been weathered under the same conditions will inevitably differ amongst studies, hampering meaningful cross-comparisons. So, while we encourage the accurate reporting of the detailed procedures used to effect weathering, reporting the properties of the weathered MNPs as compared to the pristine MNPs before weathering is the critical information needed to compare results across different studies and to understand the relationships between accelerated and natural weathering. For example, it can help determine the timescale relationship between MNPs artificially weathered in a lab and those weathered by natural sunlight to achieve similar changes in physicochemical properties. Indeed, the importance of reporting particle characterization and experimental protocols for MNP weathering has been highlighted in a recent review as the key knowledge gaps in this area.[171]

10.6 Establishing property–function relationships for MNP studies

The enormous range of polymer types places a significant burden on researchers. In this vein, if property–function relationships could be established that are independent, or at least largely independent of polymer type, then this would reduce the number of MNP studies needed. For example, if similarly sized NP spheres of different polymer types (*e.g.* PVC, PE, PET) could be differentially weathered in such a way that they all possessed the same surface charge and were then found to exhibit the same behavior in situations where size, shape and surface chemistry are determinant properties (*e.g.* transport), this would allow results from one MNP study to be meaningfully extrapolated to other polymer types. In a similar vein, there is a reasonable expectation that as MNPs are increasingly weathered in the environment, polymer type will become less important in determining behavior. Unfortunately, to-date most MNP studies have been conducted for one polymer type with a unique set of MNP characteristics as opposed to either multiple polymer types with similar characteristics or a MNPs of the same polymer type each with different characteristics (*e.g.* differently weathered MNPs of the same polymer type). This absence of internal diversity of MNP polymer types and characteristics has hindered the development of cross-cutting MNP relationships of this type.

Another strategy that could be employed to reduce the experimental burden would be to categorize MNPs based on both their properties (*e.g.* size, shape) and reactivity (*e.g.* ROS production) and then to only study the most representative MNP. This approach would enable “grouping” and read-across strategies, similar to those adopted in other fields, such as nanotoxicity.[342,343]

10.7 MNP database

One important step towards the standardization of reference materials for MNPs would be the creation of a FAIR (findable, accessible, interoperable, reusable) and open database or data repository for the storage and reuse of relevant information regarding reference and test MNPs. Indeed, this was a specific recommendation of the American Chemistry Council in 2022 tasked with establishing guidelines for reference MNPs.[36] This information should encompass, at a minimum, the production method or the manufacturer (and batch), the physicochemical properties that have been characterized and by what method(s), the outcome of studies performed, as well as the experimental protocols, including details of the matrix where the MNP study was conducted (*i.e.* pH, conductivity, *etc.*).[344,345] The establishment of such a database would significantly facilitate the comparison of reference and test MNPs produced by different means. This information in turn could open up the opportunity for meta-analysis and modelling studies aiming at data gap identification and predictions, insights that would be particularly useful in disentangling the multiplicity of MNP variables that are involved in toxicity studies.[346] A number of analogous databases have been developed for nanomaterials, and it may be feasible to reuse similar structures for reference MNPs.[345,346]

10.8 MNP toxicity studies

Results from MNP toxicity studies are often contradictory and inconsistent, even when the studies are nominally conducted on the same organism or cell line, using the same nominal MNPs at similar concentrations and exposures times, *etc.* One of the difficulties arises from the variable stability, homogeneity and composition of the MNP suspensions used in toxicity studies. The use of surfactants to produce stable MNP suspensions is strongly discouraged. If surfactants were necessary for MNP production (as in some bottom-up methods) or dispersion, they must be removed prior to MNP use, as they modify surface properties (*e.g.* surface charge) and, thus, significantly distort the characteristics (*e.g.* stability) and behavior (*e.g.* uptake) of the MNPs. In situations where surfactants are needed to create stable MNP suspensions toxicity studies have no environmental relevance because the MNPs would not be exposed to the organism in the environment and therefore pose no risk, at least as a colloidal particle (risk = hazard × exposure). We hypothesize that another reason for the discrepancy in reported toxicity of MNPs is the variability in plastic composition. This includes the presence of chemical additives, which can leach from MNPs, and play a determinant role in regulating the measured MNP toxicity. For example, plasticizers are a significant component in many plastic materials (*e.g.*, typically about 30–35 wt% for PVC) and many plasticizers, such as di(2-ethylhexyl) phthalate and some of its metabolites, are endocrine disruptors. Top-down methods of generating MNPs use commercial plastics often of unknown composition, while bottom-up approaches offer much better control over the composition of MNPs. Creating identical MNPs but with differing concentrations of additives will help to discern if the physical or the chemical properties of the particles control their toxicity. As an extension of this idea, the creation of MNPs with a single additive or with multiple additives would allow the toxicity of additives to be studied both in isolation, while also serving as a platform to identify synergistic or antagonistic effects when they are present in more complex, but industrially relevant mixtures. In addition, the effect of possible microbial/endotoxin contamination must also be evaluated, as it may be a cause of divergent results.

10.9 MNPs as internal standards and positive controls for QA/QC

Well-characterized MNPs, either commercial or lab synthesized, should be used as internal standards or positive controls in studies designed to estimate the degree of analytical uncertainty (accuracy), as well as identify and quantify instrumental drift and thereby improve data quality, particularly when multi-step sample processing is needed as a prerequisite to analysis. While the QA/QC measures applied in MNP research mainly consist of analyzing different types of blanks and preventing contamination during the sampling collection and in the lab,[26] an evaluation of MNP losses during both sample preparation and detection is not systematically carried out. In situations where spectroscopic methods are used for MNP detection, a known number of easily identifiable and clearly distinguishable MNPs (*e.g.* colored spheres) could be added as MNP standards to the sample before processing.[51] Recognizing that some or all of characteristics associated with the MNPs being quantified will not be known, the operator should still be able to make an informed guess to ensure that the MNP standards are as similar as possible to the environmental MNPs expected in the samples in terms of polymer type, size, *etc.* By determining the recovery of the MNP standards at the end of the sample preparation this approach offers the best means to estimate MNP extraction efficiency. This information would then indicate whether the native MNPs in samples have been quantitatively extracted and thereby provide a means to estimate sample-specific recoveries, helping to identify poorly prepared samples. Although this information is a useful gauge of the MNP loss rate during the whole analytical process, these values should not be used to correct results in an absolute quantitative fashion, as the analytes and the standards are not strictly identical.

10.10 Standardization of MNP characterization/detection

A significant gap in the characterization and detection of MNPs is the lack of harmonized and standardized operating procedures, which are crucial for ensuring quality control and comparability of results, minimizing errors and misunderstandings. Many initiatives are ongoing, promoted by different research projects and technical committees of standardization bodies, but only a few standards exist, and these are all relatively new. The existing standards have been compiled into a table available in an online repository (see Data availability) ISO has two documents, 24187:2023 and 5667-27, about principles for the analysis of microplastics present in the environment and water, respectively, one with a method to determine mass concentration of tire and road wear particles, 21396:2017, and two complementary drafts, 16094-2 and -3,[347,348] for the analysis of microplastics in water, covering sampling, vibrational spectroscopy analysis, and thermos-analytical methods, respectively. ASTM has already three complementary standards: D8332-20 for the collection of aqueous samples with suspended solids for identification and quantification of microplastic particles and fibers, D8333-20 for preparation of these type of samples for analysis by microspectroscopy or Py-GC/MS, and D8489-23e1, a complementary test method for determination of size, distribution, shape, and concentration in waters using a dynamic image particle analyzer. ASTM also suggests how to prepare reference samples to calibrate and assess the efficiency of these collection, preparation, and identification methods described in D8402-23. These efforts will hopefully provide more guidance for MNP characterization/detection soon. Crucially, recommendations arising from these efforts should equally by both the scientific and industrial communities, fostered by regulation.

Abbreviation

ABS	Acrylonitrile butadiene styrene
AFM	Atomic Force Microscopy
AF4	Asymmetric Flow Field Flow Fractionation
APS	Aerodynamic Particle Size Spectrometry
ASTM	American Society for Testing and Materials
ATR	Attenuated Total Reflectance
BAM	Federal Institute for Materials Research and Testing (German: Bundesanstalt für Materialforschung und -prüfung)
BET	Brunauer–Emmett–Teller
CCC	Critical coagulation concentration
CRM	Certified reference materials
CSC	Critical stabilization concentration
DLS	Dynamic light scattering
DOTA	Dodecane tetraacetic acid or tetraxetan
DSC	Differential Scanning Calorimetry
EDX	Energy-Dispersive X-Ray Spectroscopy
ELS	Electrophoretic Light Scattering
EM	Electron Microscopy
FE-SEM	Field Emission-Scanning Electron Microscopy
FFF	Field-Flow Fractionation
FluidFM	Fluidic force microscopy
FPA	Focal plane array
FTIR	Fourier Transform Infrared Spectroscopy
GPC	Gel Permeation Chromatography
HAADF	High-angle annular dark-field imaging
HPLC	High-performance Liquid Chromatography
ICP-MS	Inductively Coupled Plasma-Mass Spectrometry
JRC	Joint Research Center
LC-UV-MS/MS	Liquid Chromatography-Ultraviolet-Mass Spectrometry/Mass Spectrometry
LD	Laser Diffraction
LDIR	Laser Direct Infrared Spectroscopy
LEXRF	Low-Energy X-Ray Fluorescence
MADLS	Multi Angle Dynamic Light Scattering
MIR	Mid Infrared
MP	Microplastic particle
MNP	Micro- and nanoplastic particle
NB	Nile Blue
NIST	National Institute of Standards and Technology
NMR	Nuclear Magnetic Resonance
NP	Nanoplastic particle
NR	Nile red
NTA	Nanoparticle tracking analysis
PA	Polyamide
PA6	Nylon 6 or polyamide 6 or polycaprolactam
PA6,6	Nylon 6,6 or polyamide 6,6
PAN	Polyacrylonitrile
PBAT	Polybutylene adipate terephthalate
PBS	Phosphate-buffered saline
PC	Polycarbonate
PDI	Polydispersity index
(HD-/LD-)PE	(High density-/low density) polyethylene
PES	Polyethersulfone
PET	Polyethylene terephthalate
PHBV	Poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate)
PLA	Polylactic acid
PMMA	Polymethyl methacrylate
PNC	Particle number concentration
PP	Polypropylene
PS	Polystyrene
PSD	Particle size distribution
PTFE	Polytetrafluoroethylene (Teflon)
PU	Polyurethane
PVC	Polyvinyl chloride
PVDC	Polyvinylidene chloride
PVP	Polyvinylpyrrolidone
Py-GC/MS	Pyrolysis-Gas Chromatography-Mass Spectrometry
P2VP	Poly(2-vinylpyridine)
QA/QC	Quality assurance/quality control
ROS	Reactive oxygen species
SAXS	Small-Angle X-Ray Scattering
SDS	Sodium dodecyl sulphate
SEC	Size Exclusion Chromatography
SEM	Scanning Electron Microscopy
SERS	Surface-Enhanced Raman Spectroscopy
SLS	Static Light Scattering
SMPS	Scanning Mobility Particle Sizer
spICP-MS	Single Particle Inductively Coupled Plasma Mass Spectrometry
SRM	Standard reference material
SRS	Stimulated Raman scattering
STEM	Scanning Transmission Electron Microscopy
STXM	Scanning Transmission X-Ray Microscopy
TED-GC/MS	Thermal Extraction Desorption-Gas Chromatography/Mass Spectrometry
TEM	Transmission Electron Microscopy
TGA	Thermogravimetric Analysis
THF	Tetrahydrofuran
TPEF	Two-Photon Excitation Microscopy (fluorescence)
UCNP	Upcon® upconverting nanoparticle

UPLC-MS/MS	Ultra-High Performance Liquid-Chromatography-Mass Spectrometry/Mass-Spectrometry
UV	Ultraviolet Light
UV-vis	Ultraviolet-Visible Spectroscopy
WWTP	Wastewater treatment plant
XPS	X-Ray Photoelectron Spectroscopy
XRD	X-Ray Diffraction
ζ	Zeta-potential

Data availability

The sources of the data retrieved in ESI† 1 are listed in the document with a URL link. The list of the commercially-available MNPs (Supplementary Material 1) can also be accessed using the DOI: **https://doi.org/10.5281/zenodo.14969091** and referred to as: Crosset-Perrotin G, Moraz A, Portela R, Alcolea-Rodriguez V, Burrueco-Subirà D, Smith C, *et al.* Commercially available microplastics and nanoplastics (MNPs) for use as test or as reference materials. Zenodo; 2025. The list of standards related to microplastics can be accessed using the DOI: **https://doi.org/10.5281/zenodo.15086119** and referred to as: Portela R. List of standards related to microplastics. Zenodo; 2025.

Conflicts of interest

There are no conflicts to declare.

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