Dietary and inhalation exposure to **nano- and microplastic particles** and potential implications for human health

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ABBREVIATIONS AND ACRONYMS

ABS	acrylonitrile butadiene styrene
bw	body weight
EFSA	European Food Safety Authority
FAO	Food and Agriculture Organization of the United Nations
GEMS/Food	Global Environmental Monitoring System – Food Contamination Monitoring and Assessment Programme
LOD	limit of detection
LOQ	limit of quantification
MMAD	mass median aerodynamic diameter
MOE	margin of exposure
MP	microplastic particles
NMP	nano- and microplastic particles
NP	nanoplastic particles
OECD	Organisation for Economic Co-operation and Development
PBPK	physiologically based pharmacokinetics
PCB	polychlorinated biphenyl
PET	poly(ethylene terephthalate)
PM	particulate matter
PM10	particulate matter ≤ 10 µm
PM2.5	particulate matter ≤ 2.5 µm
PVC	polyvinyl chloride
QA/QC	quality assurance and quality control
QIVIVE	quantitative in vitro to in vivo extrapolation
TAS	total assessment score
WHO	World Health Organization
wt	weight
ww	wet body weight

EXECUTIVE SUMMARY

The number of reports of the presence of microplastic particles (MP) in the environment has increased significantly during the past few years. MP have been detected in air, water, soil, food and beverages, indicating that exposure of humans to these particles is ubiquitous. In 2019, the World Health Organization (WHO) commissioned a report to evaluate the evidence for risks to human health associated with exposure to nano- and microplastic particles (NMP) in drinking-water. The report was based on literature reviews of studies published up to December 2021 in which original data on the occurrence of NMP in air, water, food and beverages were reported and also experimental studies on their toxicity. WHO experts evaluated the quality of the studies of environmental monitoring and of toxicity, particularly with regard to the reliability and relevance of the data for characterizing risk. The possible role of NMP as vectors of chemicals and pathogens was also assessed, and clinical observations from occupational epidemiology are summarized. A key observation is that MP are ubiquitous in the environment and have been detected in environmental media with direct relevance for human exposure, including air, dust, water, food and beverages.

There is increasing awareness of the occurrence of NMP in air and their implications for human health. Studies of the inhalation of NMP should include consideration of their biokinetics, as their intake depends on their size, shape, density and surface chemistry, which influence their deposition in the alveolar regions of the lungs. Observations from occupational epidemiology suggest that acute and chronic exposure to elevated concentrations of NMP, such as polyvinyl chloride dust and nylon flock, can result in harm to the respiratory tract. Better characterization is necessary of the properties of NMP in air, such as the fractions that contribute to (regulated levels of) particulate matter and their absolute concentrations. The current lack of such data limits characterization and quantification of the impact of human inhalation of NMP.

Ingestion of MP has been reported in a variety of foods and beverages, including fish and seafood products, salt, sugar, honey, rice, milk and drinking-water. Limited characterization of the hazard of NMP due to dietary exposure suggests the possibility of adverse outcomes similar to those of other well-studied insoluble particles, as they have similar modes of

action, including generation of reactive oxidation species and stimulation of an inflammatory response.

A number of difficulties obviated an assessment of overall human exposure to NMP, including the limited availability of data on the occurrence of NMP measuring < 10 µm in water, food and beverages. Observations from particle and fibre toxicology indicate that particles < 10 µm are probably taken up biologically. Most of the available studies on the occurrence of NMP in water, food and beverages reported particles measuring > 10 µm, which are unlikely to be absorbed or taken up. As most of the toxicity studies were conducted with a monodisperse group of plastic particles, typically measuring < 10 µm, studies of effects and of exposure are mismatched, obviating extrapolation of data on toxicity for use in a quantitative risk assessment.

For this report, the quality, reliability and relevance of data on both exposure and effects were assessed for their possible contribution to a risk assessment of NMP. The assessment scores indicated that the available data are of only very limited use for assessing the risk of NMP to human health. Several shortcomings were identified, the most important of which was the heterogeneity of the methods used, including use of “bespoke” methods for analysing data obtained by environmental monitoring and inconsistencies in observations of adverse effects. Assessment of the quality of the studies should promote best practices in experimental design to be used in future studies. It is generally recommended that standard methods be developed and adopted to ensure that the research community can reduce uncertainties, strengthen overall scientific understanding and provide more robust data for assessing the risks of exposure to NMP to humans.

Research to improve exposure assessment, for instance, should be designed to complement research on the dosimetry and biokinetics of environmentally relevant NMP. Quantitative data on the rate of translocation of NMP and on their size distribution, shape, polymer composition and surface chemistry in air, food and beverages, including drinking-water, are necessary to determine which properties are most relevant for studies of biokinetics and adverse effects. This will require methods for characterizing and quantifying NMP < 10 µm, depending on the media in which they are dispersed.

The selection of in-vitro and in-vivo test systems should be guided by better understanding of exposure. Testing

of the toxicity of environmentally relevant NMP should represent a priority, and it is recommended that a series of well-characterized reference NMP be generated and made available to the research community. Furthermore, the applicability of tools for extrapolating results obtained in vitro to the situation in vivo and physiologically based pharmacokinetics models should be assessed and new models developed as necessary.

Little is known about the adverse effects of MP-associated biofilms, although the available data provide no evidence of a risk to human health. NMP constitute only a fraction of the particles that occur in the environment on which microorganisms can colonize and form biofilms. Additionally, little is known about exposure to NMP in air, food and beverages, particularly with respect to the polymer composition of NMP < 10 µm, the specific mass and the identity of associated plastic additives, including factors that might influence their bioavailability. It is therefore not currently possible to characterize or quantify the potential role of NMP in the transport of chemicals. The possibility of enrichment of antimicrobial-resistance genes in MP-associated biofilms and the role of NMP as vectors for pathogens and chemicals should be studied further. Such research would benefit from better measurement of exposure made possible by the development and application of standard methods.

Recommendations

Although the limited data provide little evidence that NMP have adverse effects in humans, there is increasing public awareness and an overwhelming consensus among all stakeholders that plastics do not belong in the environment, and measures should be taken to mitigate exposure to NMP. This should include better management of plastics throughout their product life-cycle and reducing the use of plastics, when possible, to move towards a more sustainable plastics economy. In addition to measures to better manage plastic, such as innovations in waste treatment and initiatives to reduce the use of plastics, innovations in materials science should be supported, particularly to ensure substantial reductions in the release of NMP from plastic products used in commerce.

The weight of the scientific evidence provided by current data on adverse effects of NMP on human health is low, because of substantial limitations of the available information.

Strengthening of the evidence necessary for reliable characterization and quantification of the risks to human health posed by NMP will require active participation by all stakeholders in developing and making available standard methods. Standard methods should be developed to improve the quality and reliability of data from both environmental monitoring and studies of effects. Researchers should ensure that studies on the sources and occurrence of NMP in air, water, food and beverages are based on appropriately designed, quality-controlled protocols.

Of particular interest are NMP measuring < 10 μm. Better understanding of environmentally relevant exposure to these particles could be used to generate reference NMP for use in testing biokinetics and toxicity. Better understanding of the toxicological effects of environmentally relevant NMP at environmentally relevant concentrations, combined with use of standardized toxicity testing methods will provide more accurate dose–response relationships, from which threshold effect concentrations can be derived and assessed for risk.

1. INTRODUCTION

Both the intentional use and unintentional generation of nano- to micro-sized plastic particles and their release to the environment have implications for human and ecosystem health and are emerging public concerns. Increasing numbers of studies have demonstrated the presence of nano- and microplastic particles (NMP) in drinking-water, air, food and beverages, indicating possible risks to human health associated with exposure to the particles and to chemical toxicants and biological agents vectored by NMP *(1–9)*.

WHO previously reviewed scientific information on microplastic particles (MP) in drinking-water, drinking-water sources and wastewater *(1, 2)* to evaluate the potential risks for human health. This report extends the assessment by including evaluations of exposure to NMP from other sources, including the air and diet. Recent reports on MP in food and the environment, such as those of the European Food Safety Authority (EFSA) *(10)*, the Science Advice for Policy by European Authorities *(11)*, the Norwegian Scientific Committee for Food and Environment *(12)*, the Government of Canada's Science Assessment of Plastic Pollution *(13)*, the Committee on Toxicity of Chemicals in Food, Consumer Products and the Environment in the United Kingdom of Great Britain and Northern Ireland *(14)* and the State of California (United States of America) *(15)*, show that the available data on exposure to MP and its effects are insufficient to conduct a full quantitative risk assessment. This report summarizes current scientific understanding of exposure to and the effects and potential risks of NMP in relation to human health and provides guidance and recommendations for future research. The report:

- summarizes data on human exposure to NMP in food, beverages, drinking-water and air and on the pathways specific to human health;
- examines the implications for human health on the basis of data on occurrence, toxicology and exposure;
- when possible, identifies opportunities for mitigating exposure to NMP; and
- identifies gaps in the data and proposes topics for research.

1.1 Background and scope

As discussed in the WHO report on MPs in drinking-water *(1)*, plastic is considered to provide various social benefits. For instance, it is a relatively inexpensive, flexible, robust, lightweight, waterproof material that is easy to maintain and sterilize and has insulating properties. The possibility for moulding plastic into a wide range of shapes and forms has led to its use in many commercial products, such as packaging and building and construction materials, and in the automotive and aerospace industries. Direct benefits of the use of plastic to human health and the environment include their role in extending the shelf-life of perishable

food items, their use in sterile medical devices, such as examination gloves, syringes and intravenous tubes, and as an inert material for prosthetics. The lightweight plastic used in packaging and the automotive and aerospace industries helps to reduce fuel consumption during the transport and shipping of commercial goods and products. Furthermore, the insulating properties of plastic used in construction and electrical applications improve the energy efficiency of housing and appliances, and the combined lightweight and insulating properties of plastics help to reduce the emission of greenhouse gases. Nevertheless, there is growing awareness that unintended release of plastic to the environment due to mismanagement of solid waste or lack of infrastructure for efficient reduction, reuse or recycling of plastic material at the end of its commercial life or daily use can harm organisms, the environment and, potentially, human health. By drawing attention to the prevalence of macro- and micro-litter in the marine environment, researchers have raised awareness of the negative effects of the release of mismanaged solid plastic waste on marine organisms and ecosystems.

Human exposure to NMP is intuitively linked to the widespread use of plastic; however, identification of the sources of NMP is a non-trivial exercise. Current understanding is that humans are exposed to a complex mixture of NMP of various shapes, sizes and polymer composition from both primary and secondary sources. Data for quantifying and characterizing exposure are limited. For this report, we reviewed the available information on the occurrence of NMP in food, beverages, drinking-water and air. In view of the advancements in scientific understanding of the effects of exposure to particles on human health, the literature on the biokinetics and toxicity of particles in general is also summarized.

Given the speed at which research on NMP is emerging and the difficulty of conducting quantitative analyses in the absence of standardized methods for assessing exposure and effects, this report indicates how the available evidence might be used to assess the implications of exposure to human health by assessing the quality of studies on exposure to and the effects of NMP, while also summarizing the occupational and epidemiological data on the biokinetics of exposure to particles. These various lines of evidence are used to evaluate the implications of NMP for human health, to recommend future research and to provide guidance for mitigating exposure.

Several reports on the effects of NMP on human health published recently *(1, 10–14)* were key sources of information for this report, as were results from the USA of a Southern California Coastal Water Research Project workshop organized in coordination with the State of California Water Resources Control Board and the California Ocean Protection Council to evaluate the human and ecological effects of MPs in water *(15–18)*. Data from several reviews were also used, including on exposure to MP in food, beverages, drinking-water and air *(3, 5, 7–9, 19–23)*, epidemiological data from studies of occupational exposure and the results of experimental studies conducted in vitro and in vivo *(24–27)*. Although every effort was made to ensure that the literature reviewed and evaluated for this report was as comprehensive as possible, it is not possible in this rapidly emerging field to guarantee that every study was retrieved. Additional information was obtained by literature searches with various online tools, including PubMed, Scopus and Google Scholar, for studies published up to December 2021, and from external experts who constructively peer-reviewed the report.

Studies with implications for human health on experimental effects and environmental monitoring that contained original data were evaluated and scored with respect to the information they provided on various fundamental criteria for quality assurance and quality control (QA/QC) *(2, 3, 16, 28)*. The scores summarized in the report were not used for screening or for prioritization but to provide a relative indication of the reliability and relevance of the data for determining whether exposure to NMP affects human health and the results obtained contributed to recommendations for strengthening the quality, reliability and relevance of future studies. Details of the scoring of any individual study are available upon request.

1.2 Definitions

The definitions, composition and properties of NMP have been debated for several years. Below, we briefly summarize the terms commonly used in research on the implications of exposure to NMP for the environment and human health. A common definition of “microplastics” is plastic particles that are < 5 mm in diameter *(11, 29, 30)*. This definition is perceived as a pragmatic approach for differentiating crudely between macro- and microparticles in the marine environment *(30–33)*.

A definition that is appropriate for assessing the potential effects of exposure to NMP on the environment and human health, for both scientific and regulatory purposes, remains, however, an area of debate *(11, 29, 34–39)*. Contentious components of defining NMP include the polymer composition and dimensions for differentiating

nano-, micro-, meso- and macroplastic particles. Nevertheless, regulatory bodies have recently provided or proposed a number of definitions for regulatory decision-making. The list in Box 1 is not exhaustive and is presented to illustrate various perspectives and challenges associated with defining NMP. For a more thorough discussion, see, for instance, references *37* and *38*.

Box 1 Definitions of nano-, micro-, meso- and macroplastic particles and related terms

Agglomerate – a collection of weakly bound particles or aggregates of which the resulting external surface area is similar to the sum of the surface areas of the individual components *(40)*

Aggregate – a particle comprising strongly bound or fused particles *(40)*

Airborne fibre – object with a length (L) > 5 µm, a diameter (D) < 3 µm and an aspect ratio L:D > 3:1 *(41)*

Elastomer – a macromolecular material that returns rapidly to its initial dimensions and shape after substantial deformation by a weak stress and release of the stress *(42)*

Gas – a substance that (i) at 50 °C has a vapour pressure > 300 kPa (absolute) or (ii) is completely gaseous at 20 °C at a standard pressure of 101.3 kPa *(43)*

Liquid – a substance or mixture that (i) at 50 °C has a vapour pressure of ≤ 300 kPa (3 bar), (ii) is not completely gaseous at 20 °C and at a standard pressure of 101.3 kPa and (iii) has a melting-point or initial melting-point of ≤ 20 °C at a standard pressure of 101.3 kPa *(43)*

Macroplastic – plastic ≥ 1 cm *(25)*; plastic ≥ 5 cm *(44)*; plastic litter ≥ 5 mm *(45)*

Microbead – plastic bead ≤ 5 mm used in products such as bath and body products, skin cleansers and toothpaste *(46)*; a microplastic used in a mixture as an abrasive, i.e., to exfoliate, polish or clean *(35)*

Microfibre – a fibre of linear density approximately ≤ 1 dtex and > 0.3 dtex (Note: dtex is a direct measure of the linear density of a fibre, as the mass of fibre (g) per length (10 km) with a cross-sectional width of 5–10 µm *(47, 48)*)

Microplastic – a material consisting of solid polymer-containing particles, to which additives or other substances may have been added, and in which ≥ 1% w/w of particles have (i) all dimensions 1 nm ≤ x ≤ 5 mm, or (ii), for fibres, a length of 3 nm ≤ x ≤ 15 mm and a length to diameter ratio of > 3. Polymers that occur naturally and have not been chemically modified (other than by hydrolysis) are excluded, as are polymers that are (bio)degradable *(35)*; plastic particle < 5 mm in diameter; 1 – < 1000 µm *(37)*

Nanofibre – a fibre with a cross-sectional dimension within a range of nm, i.e., < 0.1 dtex or < 1 µm *(47)*

Nanomaterial – a material with any external dimension in the nanoscale or with an internal structure or surface structure in the nanoscale *(49)*; a natural, incidental or manufactured material containing particles, in an unbound state

or as an aggregate or an agglomerate, in which ≥ 50% of the particles in the number size distribution have one or more external dimensions in the size range 1–100 nm *(40)*

Nanoplastic – plastic particle measuring 1 to < 1000 nm *(37, 50)*

Particle – a minute piece of matter with defined physical boundaries and a defined physical boundary as an interface *(40)*

Plastic – a material that contains as an essential ingredient a high relative molecular mass polymer and which, at some stage in its processing into finished products, can be shaped by flow *(42)*

Polymer – a substance within the meaning of Article 3(5) of Regulation (EC) No. 1907/2006 *(51);* a molecule of high relative molecular mass, the structure of which essentially comprises the multiple repetition of units derived, actually or conceptually, from molecules of low relative molecular mass *(52)*

Primary microplastic – intentionally produced plastic particles in the size range 1 to < 5000 µm *(36)*

Secondary microplastic – microplastic particle in the size range 1 – < 5000 µm formed by fragmentation in the environment during use *(36)*

Solid – a substance or a mixture that does not meet the definition of a liquid or gas *(43)*

Synthetic plastic microbead – any solid plastic particle ≤ 5 mm intended to be used to exfoliate or cleanse the human body or any part thereof *(53)*

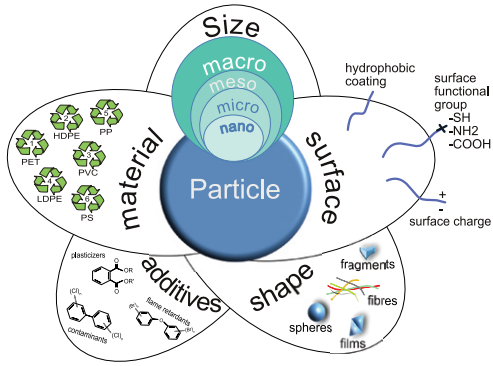
Thermoplastic – material that can be softened repeatedly by heating and hardened by cooling through a temperature range characteristic of the plastic and, in the softened state, of being shaped repeatedly by flow into articles by moulding, extrusion or forming. Note: Many thermoplastic materials can be thermoset by appropriate treatment to induce cross-linking, e.g., by the addition of a suitable chemical cross-linking agent or by irradiation *(42)*.

Thermoset – plastic that, when cured by heat or other means, changes into a substantially infusible, insoluble product *(42)*

1.3 Composition and properties of particles

Assessment of human exposure to NMP requires characterization of several parameters, summarized in Fig. 1. In addition to particle size, shape and polymer composition, information on chemical additives and the physicochemical properties of the polymer, such as surface activity and particle density, is important for understanding their effects on human health.

Fig. 1 Attributes of NMP to be considered in assessing both exposure and hazard



1.3.1 Properties

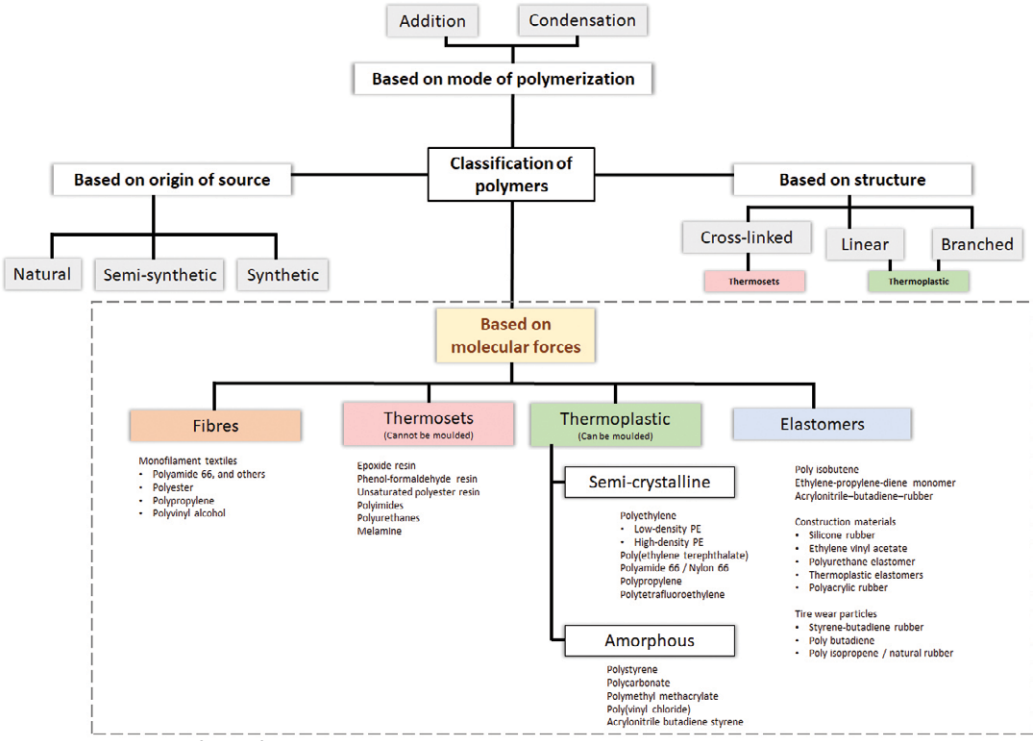
The heterogeneity of NMP, which have various polymer compositions, sizes and shapes, significantly complicates assessment of human exposure. The difficulty is increased by the inconsistency in reporting of the properties of NMP in the scientific literature. Furthermore, reporting of accurate data on each of the properties of NMP is limited by analytical capability, which may vary significantly among research groups *(2, 3, 28, 54–60)*. Depending on the sample matrix, the sampling method may be limited by the pore sizes of filters, which determine the lower size limit of particles that can be sampled. When extraction and isolation of particles for analytical verification are required, digestion methods may physically alter the size of the particles, influencing quantification of exposure.

The analytical methods for verifying polymer composition are evolving constantly, with the introduction of methods such as Fourier transform infrared spectroscopy, Raman spectroscopy and pyrolysis–gas chromatography–mass spectroscopy. The sensitivity of analysis with respect to both size and shape has improved, although characterization of the polymer composition of fibres is still difficult *(37, 39)*. Laboratory studies on the effects of exposure to NMP on human health are often limited to a single type of polymer and shape in a relatively narrow size range and may not represent human exposure.

1.3.2 Composition

All polymeric materials can be divided into subclasses according to the method of synthesis or some characteristic of the material. For instance, manufactured synthetic polymers can be classified into four main groups according to their structure, origin or source, molecular force and mode of polymerization (Fig. 2). In polymer science, for

Fig. 2 **Classifications of manufactured and commonly encountered plastic materials**



Source: adapted from references *64* and *65*

instance, classification is based on structure *(61–63)*. Other aspects, such as the relative strength of the molecular forces characteristic of the polymer, can strongly influence its stability and its potential degradation and fragmentation to NMP *(64, 65)*.

Natural polymers such as cellulose, starch, proteins, rubber, silk and wool, derived from plants and animals, may have complex sequences of repeat units and physical properties equivalent to those of synthetic polymers *(62)*. A potentially important question is whether the properties of synthetic polymers make them more hazardous than natural polymers and, if so, what those properties are.

The types of manufactured synthetic polymers to be included as NMP is perhaps one of the most contentious issues in defining NMP. There is no consensus on the inclusion

of elastomers, such as rubbers, or modified natural or semi-synthetic polymers, such as rayon and cellophane, in the definition *(37, 39)*. A pragmatic approach has been adopted for this report, which accounts for the complex, heterogeneous nature of exposure to NMP. Consequently, data on materials classified as synthetic polymers, which include thermoplastics, thermosets, elastomers and fibres, and have been reported in the literature as representative of NMP are included. Fig. 2 lists plastic materials within each polymer category that are typically encountered in commerce and reported in the literature as contributing to NMP *(12, 39, 62, 63, 66)*.

Synthetic polymers are used in plastic products because of their properties *(62)*. For instance, polystyrene has a low thermal coefficient as a foam and has been widely used in the production of coffee cups; poly(methyl) methacrylate is a tough, transparent polymer and is used as artificial glass; polyethylene is waterproof and provides a thin, often clear, material for use in plastic films and food or beverage containers; polyamide 66 (often referred to as nylon 66) resists abrasion and degradation and is a semi-crystalline thermoplastic produced as a monofilament and used in textiles; poly(ethylene terephthalate) (PET) has low permeability to carbon dioxide and is often used in soft-drink containers; thermoset epoxies provide good adhesion and strength and are thus used as adhesives; polyolefins have low dielectric constants and are used in wire and cable insulation; polyimides are resistant to high temperatures and are used in electronics; and polyisopropene self-reinforces upon extension and is used for its elastomer properties.

The composition of a polymer also influences its particle density, which in turn influences exposure dosimetry in toxicity testing. As discussed in section 4, the density of a particle, with its size and shape, determine its deposition in the respiratory tract. The densities of common polymeric materials range between 0.8 and 2.3 g/cm^3 (Table 1).

1.3.3 Additives

Few synthetic polymers are used commercially in the “pure” state. Some polyethylenes and polystyrenes are sold as homopolymers without additives; however, chemical additives and other materials are added to most polymers to improve their properties, which can result in a range of densities (see Table 1) *(62, 67, 68)*. On a weight basis, fillers represent > 50% of all additives used, followed by plasticizers, reinforcing agents, flame retardants and colouring agents *(67)*. For instance, fillers such as finely ground rubber are added to brittle plastic to add strength; composites of glass, carbon

Table 1. Average densities of commonly used polymers, the applications of representative additives and the estimated typical percentage added (weight/weight) of commonly used polymers

Polymer	Density (g/cm^3)	Application			
		Anti-oxidant	Flame retardant	Plasticizer	Ultraviolet stabilizer
Typical amount (% wt/wt)		0.05–3	0.7–25	10–70	0.05–3
Thermoplastics					
Acrylonitrile butadiene styrene	0.98	✓			✓
Polyamide 66 (nylon 66)	1.24	✓	✓	✓	✓
Polycarbonate			✓		✓
Polyethylene (amorphous)	0.85				
• Low-density polyethylene	0.89	✓	✓		✓
• High-density polyethylene	0.96	✓	✓		✓
Polyethylene (crystalline)	1.00				
Polypropylene	0.99	✓			✓
Polystyrene	1.04	✓	✓	✓	✓
Poly(vinyl chloride) (PVC)	1.39	✓	✓	✓	✓
Thermosetting					
Epoxide resin	1.2	✓	✓	✓	✓
Phenol–formaldehyde resin	1.36		✓	✓	
Unsaturated polyester resin	1.23–2.3	✓	✓	✓	✓
Polyurethanes	1.2	✓	✓	✓	✓

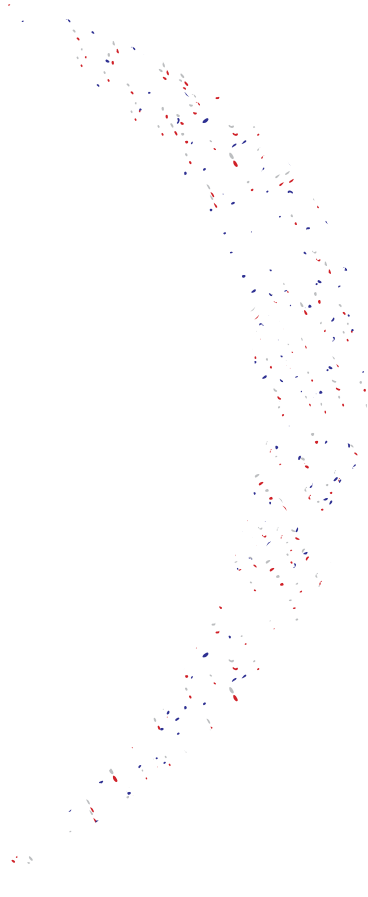
Sources: references *1*, *62*, *67* and *68*

or boron fibres are made for high-modulus and high-strength applications; and carbon black and silicas are added to synthetic rubber formulations to resist tearing and raise the modulus. Various plasticizers are added to lower the glass transition or reduce crystallinity to soften the final product, such as in PVC. Polymeric properties can be improved by adding silanes and other agents to improve bonding between the polymer and other solid phases, such as glass fibres; both glass and rubbery polymers can be cross-linked to improve elastomer behaviour or to control swelling.

Chemical plasticizers, an important group of plastic additives, can be added externally to a polymer or internally, whereby they are chemically bonded. It has been estimated that 80–90% of all plasticizers are used in a single polymer – PVC *(67, 68)*. In the absence of any plasticizer, PVC is a rigid solid, with limited commercial application. Addition of a plasticizer helps to soften the polymer, and levels > 50% (wt %) have been reported in applications such as shower curtains and vinyl upholstery *(67)*. Table 1 summarizes the levels of additives used in common polymeric materials.

The risks associated with use of chemical additives in commercial products are usually assessed by the regulatory authorities responsible for authorizing their use in commerce, including possible migration of additives into food from packaging *(51, 69)*. Studies of the leaching of chemical additives into food from plastic packaging were reviewed by Hahladakis et al. *(68)*. Inhalation or ingestion of NMP after degradation and fragmentation of plastic packaging, however, may represent a new exposure pathway for chemicals of potential concern *(68, 70–76)*.

1.3.4 Surface chemistry



The wide variety of solid plastic polymers results in differences in the surface chemistry of particles. Depending on the nature of the polymer, NMP are either charged (positively or negatively) or neutral. In some applications, the plastic may have a hydrophobic or hydrophilic coating, which will further influence the fate of fragmented particles that enter the environment. As the size of a particle decreases, its surface area increases, increasing the number of surface-expressed molecules and thus increasing the surface activity of the particle *(77)*. Reactive surface sites on a particle can interact with protons and other ions in the environment, which may also influence the toxicity of the particle. The surface charge of a given particle is strongly influenced by pH and ionic strength (in aqueous systems), which may influence the extent of aggregation with other particles in the atmosphere or water *(78)* – a consideration for toxicity testing.

Fotopoulo and Karapanagioti *(79)* observed significant alteration of functional groups on the surface of eroded polyethylene pellets. The formation of aldehydes, esters, carbonyls, ketones and ketocarbonyls caused the surface of weathered MP of polyethylene to be largely negatively charged, whereas particles of weathered polypropylene were reported to remain neutral. Differences in surface chemistry may therefore occur according to the nature of the weathering process and the composition of the polymer *(64)*.

1.3.5 Particle size

The degradation and fragmentation of manufactured plastic materials to form micro- and potentially nano-sized plastic particles are key factors in their implications for human health. There is, however, no consensus on the upper and lower size limits for differentiation of particles as microplastics and nanoplastics *(36, 37)*. In the definitions summarized in Box 1, MP are commonly defined as particles measuring < 5 mm on the basis of a suggestion by Arthur et al. *(29)*, which has been adopted by the European Chemicals Agency *(35)* and referred to in the reports on microplastics of the Science Advice for Policy by European Authorities *(11)* and WHO *(1)*. Nevertheless,

several groups have recommended that additional detail be added to the definitions of particle size ranges, such as a lower size limit for MP of 1–20 µm *(45, 50, 80)* and an upper size limit ranging from 500 µm to either 1 mm or 5 mm *(45)*.

The size ranges of nanoplastic particles (NP) have also been debated. Gigault et al. *(50)*, for instance, suggested that NP are produced unintentionally by degradation and fragmentation of plastic objects and show colloidal behaviour within the size range of 1–1000 nm. Another common definition of NP is that of the European Commission for nanomaterials *(40)*, with an upper size of 100 nm. Alternatively, Hartmann et al. *(37)* suggested an approach whereby nano- (1 – < 1000 nm), micro- (1 – < 1000 µm), meso- (1 – < 10 mm) and macroplastic particles (> 1 cm) are differentiated, with "particles" defined according to the definition of the European Commission in Box 1.

In view of the inconsistent terminology for specific particle size ranges, the term "NMP" in this report refers to plastic particles measuring 1 nm to 5000 µm. Thus, MP are particles measuring ≤ 5 mm, whereas NP measure ≤ 1 µm. In the environment, the size of all plastic debris is distributed continuously, and characterization of the distribution will simplify prioritization of the particle size categories that most strongly influence human exposure *(81)*. Particle size distribution and the limit of detection are the basic parameters for interpreting information from environmental monitoring or toxicity testing in assessing the implications of exposure to NMP on human health.

1.3.6 Particle shape

MP are typically reported to occur as spheres, irregular particle fragments, fibres and films *(1, 25)*. As the shape of particles may be an important toxicological property for human health effects, this information is reported when available. In their assessment of particle shapes in environmental samples, Burns and Boxall *(82)* reported that the most abundant category in water and sediment is fibres (48.5%), followed by fragments (31%), spherical beads (6.5%), films (5.5%) and foams (3.5%). Similar distributions of particle shapes were reported in fresh and drinking-water *(2)*. Kooi and Koelmans *(81)* estimated that, like particle size distributions, particle shapes are also distributed continuously, indicating the importance of characterizing the shapes of NMP to ensure environmentally relevant testing.

Key messages

- NMP are a heterogeneous mixture of particles and fibres of various shapes, sizes, polymer composition, surface chemistry and associated chemicals.
- In this report, a pragmatic definition of microplastics is used, in which synthetic polymeric particles are < 5 mm in diameter, while NP are particles < 1 µm in diameter.
- The properties and composition of NMP change during their life-cycle in the environment.

2. HUMAN EXPOSURE

Human exposure to NMP is widely recognized as occurring predominately through the diet or by inhalation *(1, 4, 10, 11, 19, 83, 84)*. The possibility of human exposure to MP was raised by the observation of MP in seafood, such as mussels, intended for human consumption *(85, 86)*. Other studies have demonstrated the occurrence of MP in food and drinking-water, food packaging and both indoor and outdoor air *(1, 83, 87–94)*. Thus, MP occur in drinking-water, a variety of foods and beverages and air, although insufficient quantitative data are available for a full exposure assessment *(95)*.

This section summarizes the data available for assessing human exposure to NMP. A continuing challenge to characterizing and quantifying concentrations is, however, the lack of standard analytical methods for identifying NMP of varying polymer composition, size and shape in foods, beverages and air *(2, 3, 11, 59, 60, 96–99)*. Recommendations for improving sampling and analysis from the previous WHO report *(1)* are shown in Box 2.

2.1 Occurrence in drinking-water

In the first WHO report *(1)*, human exposure to MP in drinking-water was summarized for both tap and bottled water. In a critical review of nine studies of MP in fresh and drinking-water, the concentrations ranged from 0 to 10^4 particles/L *(1)*. As mentioned above, characterization and quantification of MP in drinking-water are limited by the lack of standardized methods, which also limits comparison of studies, even by the same group *(2, 60, 100–103)*.

Koelmans et al. *(2)* proposed a scoring system for assessing the quality of studies on concentrations of MP in drinking-water. The system is based on eight criteria developed for evaluating studies of MP in samples *of* biota *(28)* according to how samples are collected, handled and analysed. A score of 0, 1 or 2 is given to each criterion. The criteria are:

- description of the sampling method used,
- sample volume and number of samples,
- sample processing and storage,
- mitigation of contamination of samples during preparation and handling,
- use of clean air conditions in the laboratory,
- use of negative controls to quantify and characterize laboratory contamination,
- use of positive controls to quantify rates of recovery of particles and

- analytical verification of polymers associated with particles isolated from samples.

Box 2 Recommendations for improving sampling and analytical methods

- Investigators should provide complete information about the method used for sampling so that it can be reproduced.
- Sample volumes will depend on the nature of the matrix being sampled and the size of the particles being analysed, which in turn are determined by the filter or mesh size used. Sample volumes should be sufficiently large to detect low MP concentrations reliably.
- Wherever possible, plastic material should not be used in sampling and analysis. If plastic material must be used, it should be characterized and reported.
- Materials should be rinsed with filtered water to avoid contamination.
- Sampling and sample processing should be done by trained professionals, or the quality of samples collected or processed by volunteers should be (quantitatively) validated against results obtained by professionals.
- If preservatives are used, their effect on polymer mass or particle shape should be evaluated, either in the study or from the literature.
- Laboratory surfaces should be thoroughly (wet) cleaned with filtered water to avoid contamination.
- All samples should be handled in a laminar-flow hood or in a clean-air laboratory.
- Blanks should be run, per day or per series, at least in triplicate, and results should be corrected against blanks.
- Positive controls should be used to verify the recovery of particles during digestion, density separation and filtration.
- Digestion should be used when necessary. Usually, digestion is not necessary for drinking-water from a treated source. For more complex matrices, such as foods and aerosol particles, however, in which high concentrations of organic matter hamper the selection and (visual) identification of particles, a digestion step is required.
- Polymers should be identified in a representative subsample of the entire sample.
- Data should be reported as consistently as possible, such as number of particles/L and mass/L for liquid matrices and particles/mass for solids, with limits of detection for both number and mass concentrations and minimum and maximum particle size. When possible, morphology should be specified. Exposure concentrations must be reported consistently for risk assessment.

- Standard methods should be developed for sampling and analysis, which may depend on the media being sampled. For example, methods for drinking-water will differ from those for foods and other beverages, as will those for indoor and outdoor air. As far as possible, the same principles should be followed.

The scoring system of Koelmans et al. *(2)* also includes an assessment of sample treatment methods. As drinking-water samples contain negligible amounts of organic matter, they do not always require treatment, and a score of 2 is given for sample treatment in such studies. A maximum total assessment score (TAS) of 18 can therefore be attributed to studies of drinking-water. The average TAS reported by Koelmans et al. *(2)* are 13.7 (13–14) for studies of bottled water, 12.5 (11–14) for those of drinking-water sampled at treatment plants and 11.5 (8–15) for studies of treated tap water. The study considered most relevant by Koelmans et al. *(2)* for assessing human exposure is that of MP in bottled water by Mason et al. *(104)*, in which the average concentration was 10.4 particles/L, consisting of larger particles that could be confirmed as MP spectroscopically. This study was chosen because it achieved a high score on the criteria and reported the highest average spectroscopically confirmed particle concentration; when these elements are combined with other assumptions on particle characteristics, the data represent a conservative exposure scenario. Only four of the eight criteria, however, were assigned a maximum score of 2, and Koelmans et al. *(2)* recommended caution in using the data for a robust risk assessment. Higher-quality data are necessary to improve understanding of exposure to MP in drinking-water, a recommendation echoed by Brachner et al. *(96)*.

Since publication of the review by Koelmans et al. *(2)* and the WHO report on MP in drinking-water *(1)*, further studies have reported on the presence of MP in drinking-water (Table 2). These were obtained in a literature search in PubMed with the keywords used by Koelmans et al. *(2)*: microplastic AND (bottled water OR tap water OR drinking-water).

Table 2. Recent studies on the numbers and characteristics of microplastic or microplastic-like particles in drinking-water

Reference	Water type	Lower size bound (µm)	Particles/L in sample (average)	Particles/L in blanks (average)	Particle size (µm)	Predominant particle shape	Predominant polymer type	Quality score (TAS)[a]
105	Bottled (mineral water) Glass Single-use PET Reusable PET	1	 3074–6292 2649 4889	384	Most particles < 5 (> 75% in glass and > 95% in plastic bottles)	No discussion of shapes	PET in plastic bottles, polyethylene and styrene butadiene copolymer in glass	13
106	Drinking-water treatment plant from surface sources (3 sites): raw, treated	1	628 338 369	< 5% of counts in samples	≤ 95% of particles 1–10	Fragments, closely followed by fibres	PET but also polypropylene, polyethylene, polyacrylamide	11
107	Bottled Single-use Returnable Glass Beverage carton	5–20	 14 118 50 11	14 ± 13	Typically, 40–50% in 5–10 range; > 80% < 20	No discussion of shape; described as fragments	PET but also polypropylene, polyethylene	14
104	Bottled	6.5–100 lower bound microscopically and in software	315	23.5	Not further specified		No characterization	14
102	Tap from groundwater sources	10–100	0.2, 0.8 and 0.0 (LOD, 0.3)	Unknown	Mainly 20–100	Fragments	PET, polypropylene, ABS, polyurethane	14
108	Tap from groundwater sources	20	0.0007	0.67 particles/L 0.3 fibres/L	50–150	Fragments	Polyester, PVC, polyethylene, polyamide, epoxy resin	15
103	Tap from 24 sources	60	Average not reported, as only one result (5.5) > LOQ	0.5 (LOQ = 4.1 LOD = 0.9)	Not specified	Not specified	No characterization	9
104	Bottled	> 100	10.4	4.15	Not further specified	Fragments (66%), fibres (13%), films (12%)	Polypropylene (54%)	14
102	Tap from groundwater sources	> 100 (10-µm sieve size)	0.312 (LOD, 0.58)	0.26	Not further specified	Fibres (82%), fragments (14%), films (4%)	PET, polypropylene, polystyrene	14
91	Tap from unspecified sources	100 (lowest reported)	5.45	0.33 particles/L (5 particles in 30 500-mL blanks	Fibre lengths, 100–5000	Mainly fibres (98.3%)	No characterization	8
109	Bottled	0.5	5.42×10^7	1×10^7 (estimated)	Range, 1.28–4.2	Not reported	Not reported	10

Reference	Water type	Lower size bound (µm)	Particles/L in sample (average)	Particles/L in blanks (average)	Particle size (µm)	Predominant particle shape	Predominant polymer type	Quality score (TAS)[a]
110	Tap water	1	440	2.4	3–4453; Mean, 66	> 50% fragments	Polyethylene and polypropylene	13
111	Drinking-water treatment plant: raw and treated	1	6614 ± 1132 (raw) 930 ± 72 (treated)	< 5% of samples	84.4–86.7% 1–5	> 50% fibres	PET, polyethylene, polypropylene	12
112	Bottled	3	148 ± 253	0	≥ 3 µm	Not reported	Not reported	15
113	Bottled	6.5	118 ± 88 14 ± 14 50 ± 52 81.0 ± 3.0 26.0 ± 2.0 12.0 ± 1.0	 15 8 6	≥ 5 µm (returnable bottles) ≥ 5 µm (single-use bottles) ≥ 5 µm (glass bottles) 6.5–20 µm 20–50 µm ≥ 50 µm	62.8% fibres, 37.2% fragments	Polyethylene, polypropylene, PET	14
100	Drinking-water treatment plant: raw and treated	25	4.9 (raw) 0.0011 (treated)	Depending on polymer composition. LOD, 1.1–65; LOQ, 3.3–197	> 25	Not reported	ABS, polystyrene	16
114	Tap water	500	18 ± 7 (range, 5–91)	1	50% < 0.5 mm; 25% 0.5–1 mm	Fibres	Poly (trimethylene terephthalate); epoxy resin	14
115	Tap water from groundwater wells	Not defined	2.8	Not reported	Not reported	Fibres	Polyethylene	6
116	Tap water	10	0.7 ± 0.6 (range, 0.3–1.6)	Not reported	≥ 500 µm (> 50%)	Fibres (99.2%)	PET and rayon	11
117	Tap and bottled water	25	2.1 ± 5.0 (range, 0.99–26)	Not reported	25–500	Not reported	Polyethylene, polystyrene, PET	10
118	Tap water	50	0.6 (range, 0.24–1.00)	Not reported	≥ 100 µm (> 80%)	Fragments (77.5-93.6%)	Polyethylene, PET, polypropylene	11
119	Tap water	1	343.5	1-7	1–10 (85%)	Fragments	Polyethylene, polypropylene, PET	11
120	Bottled	15	6–58	Not reported	40–723	Fragments and Fibres	Not reported	6
121	Tap water	Not defined	39 ± 44 (range, 1.9–225)	Not reported	19.2–4200	Fragments	polystyrene, styrene-ethylene-butylene, polypropylene, polyester	11
122	Groundwater	Not defined	38 ± 8 (range, 16–97)	Not reported	18–491	Fragments 94%)	Polyethylene, polypropylene, PVC, nylon, PET	15
123	Tap water		13.23	< 5%	100–200	Fragments	Nylon, polyester	9

ABS, acrylonitrile butadiene styrene; LOD, level of detection; LOQ, limit of quantification

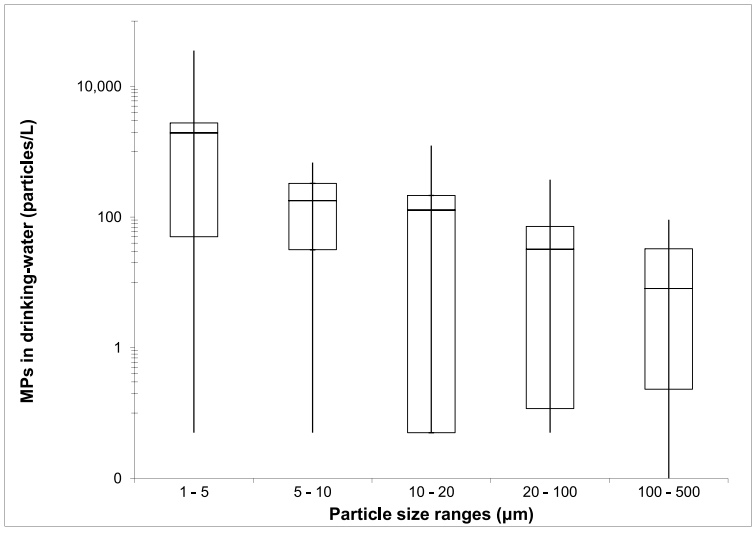
[a] TAS, total accumulated score. The maximum score is 18. The score is calculated by adding the scores for nine quality criteria; for each criterion, a score of 0, 1 or 2 is assigned. TAS values are shown in bold when all the underlying scores are non-zero.

Ball et al. *(100)* reported use of a robust protocol for quality assurance and quality control when measuring MP in drinking-water (i.e., tap water) at several sites in the United Kingdom, and the data were evaluated as being of relatively high quality, with a TAS of 16. Consistent with concerns raised by Koelmans et al. *(2)* regarding potential laboratory contamination of samples during handling and preparation, Ball et al. *(100)* reported relatively high, variable contamination of blanks, particularly for MP ≥ 25 µm. In line with the recommendations of the Association of Official Agricultural Chemists, an internationally recognized body that makes recommendations for quality assurance and quality control, both a limit of detection (LOD) and a limit of quantification (LOQ) are calculated for each polymer verified as representing MP ≥ 25 µm. The LOD ≥ 25 µm is defined as the mean of blank samples plus 3.3 × the standard deviation of the blank, whereas the LOQ ≥ 25 µm is calculated as the mean of the blank samples plus 10 × the standard deviation of the blank. In estimating both the LOD ≥ 25 µm and LOQ ≥ 25 µm, 10 blanks were analysed with drinking-water samples. The concentrations were reported to be typically below the LOQ ≥ 25 µm, with only three observations at 0.0008–0.002 particles/L; 20 samples were above the LOD ≥ 25 µm, at 0.0004–0.0041 particles/L. The polymer types identified most commonly as MP ≥ 25 µm in drinking-water were polystyrene and acrylonitrile butadiene styrene (ABS). In the assessment of raw surface water, sampled before treatment for use as drinking-water, the density of MP ≥ 25 µm was reported as about 15 particles/L, the highest concentration being 113 particles/L *(100)*. The concentrations in surface waters are consistent with the range of values reported by WHO *(1)* and are significantly higher than those in drinking-water, indicating efficient removal of MP ≥ 25 µm during treatment of raw surface water (> 99.99%). The types of polymers in raw surface water and drinking-water differ, MP ≥ 25 µm in raw surface waters consisting mainly of polyethylene, PET and polypropylene and that in drinking-water being mainly polystyrene and ABS. This observation suggests a source of MP after treatment, i.e., from within the drinking-water treatment, storage and distribution system *(100, 108, 118, 119, 123)*.

Although most studies of MP in drinking-water continue to be conducted in Europe, observations have also been reported from China *(110, 111, 116, 119, 123)*, Mexico *(114)*, Thailand *(113, 118)*, Saudi Arabia *(117)*, Australia *(122)* and various other locations, such as Japan and the USA *(121)*. The concentrations in tap water were generally consistent at all locations, ranging from below the LOD to 1247 particles/L (Table 2). Exposure from bottled water may be more variable, concentrations as high as 5.4 × 107 having been reported *(109)*. In view of the variation in concentrations, the data reported in Table 2 could be used in a probabilistic quantitative assessment of human exposure in relation to drinking-water *(124)*. When appropriate, exposure could be reduced by use of technologies to treat both wastewater, to limit the release of MP into the environment, and drinking-water, to reduce the concentration of particles *(1, 100, 111, 119, 125)*.

As noted above, the previous WHO report *(1)* concluded that the data of Mason et al. *(104)*, with other assumptions on particle characteristics, represent a conservative exposure scenario, and they were used to estimate exposure to chemicals that might be associated with MP in drinking-water *(1)*. Studies published more recently provide additional information on the concentrations of MP in drinking-water according to particle size distribution, allowing re-evaluation of the previous, conservative exposure scenario. Kankanige and Babel *(113)*, for instance, reported concentrations of MP measuring 6.5–50 µm in 10 brands of single-use PET-bottled water in Thailand. The particles were reported to consist mainly of fibres measuring 6.5–20 µm at concentrations of 29–127 MP/L. Their observation that the number of particles increases with decreasing particle size is consistent with those of others, such as Ossmann et al. *(105)*, Schymanski et al. *(107)* and Winkler et al. *(112)*. Consequently, studies that include particle concentrations in relation to particle size distribution, with verification of the polymer composition of MP, are perceived as informative for exposure assessment (Box 2). Fig. 3 summarizes the concentrations of MP in drinking-water found in the studies listed in Table 2, with the approximate concentrations of the particle sizes reported.

Fig. 3 Concentrations of MP in drinking-water according to particle size in studies with a total assessment score ≥ 11 and in which particles were verified as plastic



The lower box indicates the 25th percentile, the black line indicates the mean, and the upper box indicates the 75th percentile. The whiskers above and below the box indicate the maximum and minimum values, respectively. The numbers of data points for each particle size range are: 3 (1–5 µm), 5 (5–10 µm), 5 (10–20 µm), 6 (20–100 µm) and 5 (100–500 µm).

Fig. 3 illustrates the general trend to increasing concentration with decreasing particle size, with relatively high, variable concentrations of MP in drinking-water reported to measure between 1 and 5 µm. The data do not include concentrations of particles not confirmed as MP, including those < 10 µm reported by Mason et al. *(104)* and Zuccarello et al. *(109)*, as concern has been raised about whether all these particles are MP *(112, 126)*.

The analytical difficulty of verifying the polymer content of particles < 5 µm should be emphasized, particularly in communicating results for use in assessing human exposure *(126)*. Assessment of human exposure to MP should therefore be based only on studies that transparently and robustly adhere to the quality criteria defined by Koelmans et al. *(2)*. Research to ensure robust analysis of NMP < 5 µm in drinking-water is thus critical, as these particles may be of greater concern for human health than particles measuring > 5 µm.

2.2 Occurrence in air

2.2.1 Particulate matter in air

Particulate matter (PM) in air is a complex mixture of particles from various sources, generated both naturally and by human activity *(127, 128)*. NMP in air are thus one component of a heterogeneous mixture of particles. The concentrations can be characterized in various ways, most commonly as mass per volume but also as particle number counts or total surface area per volume of air. "Total suspended particulate concentration" was once a routine metric for monitoring PM in air, covering a broad particle-size range distribution of 0 to about 40 µm. For the purposes of human health risk assessment, respirable PM are often defined as particles with an aerodynamic diameter of < 2.5 µm (fine particles) *(127)*. The "inhalable fraction" refers to coarse particles with an aerodynamic diameter > 2.5 µm, which are usually defined as the fraction between 2.5 and 10 µm, although references to the "inhalable fraction" can include sizes ≤ 100 µm *(127–129)*. Aerodynamic diameter is used as a surrogate for particle size. Ultrafine particles have a mobility diameter < 0.1 µm and do not usually contribute significantly to the total mass of particles; however, when expressed as particle number counts, particles < 0.1 µm dominate the entire respirable size range. The contributions of different sizes of PM to airborne particle mass (or number) vary substantially, as do the emission factors, including gaseous precursors, physical characteristics and chemical composition. Studies of the contribution of particles due to tyre and road wear to PM ≤ 10 µm (PM_{10}), for instance, indicated an average contribution of about 1.9%, with a range of 0.42–2.48%,

Coarse particles (i.e., 2.5–10 µm) are usually formed by mechanical degradation of larger solid particles, the amount of energy required to degrade the particles into smaller sizes increasing with decreasing particle size *(132, 133)*. Thus, in urban environments, coarse particles are usually associated with dust from roads and industrial activity but may also include biological material such as pollen grains and bacterial fragments. In coastal areas, evaporation of sea spray can contribute to the composition of coarse particles. Fine and ultrafine particles, i.e., < 2.5 µm aerodynamic diameter, are formed mainly from gases and are generated during combustion and degradation of material.

2.2.2 Nano- and microplastic particles in air

While concern about human exposure to MP in drinking-water, foods and beverages has increased, awareness is also emerging of the presence of MP in air *(20, 25, 134-141)*. Like those in surface waters, the concentrations of MP in the atmosphere are probably subject to spatial and temporal variation, as emissions to the atmosphere are influenced by differences in the size of sources (such as between industrial or urban and remote locations) and in processes that influence atmospheric transport and mobility (i.e., wet and dry deposition, air speed and direction) *(20, 142–147)*.

The main sources of MP in air have been considered to be a combination of degradation and fragmentation of textiles in indoor air *(87, 88)*. Tyre and road wear particles are common in urban outdoor air and are generally characterized as agglomerations of smaller particles from tyre wear and road dust *(131, 143, 145, 146, 148)*. In the context of human health, however, atmospheric particles (section 2.2.1) are complex, heterogeneous mixtures of solid particles and liquid droplets of varying shapes and sizes that are present in the air we breathe, both indoors and outdoors.

In studies of human exposure by inhalation, particle size should be characterized in order to differentiate between respirable and inhalable fractions. As discussed in section 2.2.1, the fraction of tyre and road wear particles that contributes to PM_{10} can range from 0.42 to 2.48% on a particle mass basis and that of $PM_{2.5}$ (respirable fraction) may be < 1%. Particle size is currently used for regulatory purposes to control PM mass concentrations on the basis of statistical relations between the size of PM and implications for human health and is also the metric usually used in both epidemiological and toxicological studies. Therefore, given the uncertainty about the effects of exposure to NMP on human health, regulatory guidance on PM might be considered to address this concern *(9)*. Such guidelines could be used in a preliminary default position, as regulatory values such as those set by WHO *(133)* are based on the total mass of PM and do not differentiate the potential toxicity of the components of PM. Characterization of the size and shape of NMP in both indoor and outdoor air could then guide the design of mechanistic studies of toxicity, preferably supported by appropriate data on biokinetics and biodynamics.

Characterization of NMP in air can thus contribute to overall understanding of the heterogeneous mixture of PM. The chemical characteristics of the main constituents of both PM_{10} and $PM_{2.5}$ are known to be dominated by organic carbon and nitrate *(129, 132, 133, 149, 150)*. PM in air can be characterized and quantified by a variety of methods in which flow rates can be actively pumped from low to high volume. As reviewed and summarized by Enyoh et al. *(20)*, the methods depend on the target contaminant and research question. Importantly, for NMP, none of the methods currently available allows online monitoring for discriminating and differentiating NMP from other types of PM. Air samples must therefore be collected and analysed retrospectively.

The available methods for sampling air for PM are based on gravimetric measurements, which may be the most appropriate for assessing the concentrations (on a mass or particle count basis) of MP in air. They include low- and high-volume air samplers fitted with a size-selective inlet head (i.e., PM_{10}), a filter substrate and a regulated flow controller (e.g., Kleinfiltergerat and Partisol samplers). Another widely used sampling instrument is the "tapered element oscillating microbalance", in which a sample is collected onto a filter and real-time short-resolution gravimetric data are provided, although this feature is useful only if microplastics concentrations are to be assessed for short-time resolution. Standard guidelines are available for mass-based PM measurements, such as the European Committee for Standardization EN 12341, which could be used to contextualize the concentrations of microplastics in air and determine their relative mass contribution to total PM or on a particle count basis. Other instruments for characterizing and quantifying MP in air include cascade impactors, which are used to collect and separate PM on impaction substrate filters or discs by aerodynamic size distribution, and cyclone samplers, which give results that can also be interpreted on a mass or particle count basis. The sampling duration in the various methods depends on the volume of air sampled and the level of PM in the air. When sensitivity is not a concern or longer sampling is required, low-volume air samplers (~ 17 L air/h) are appropriate; however, when sensitivity is important, larger volumes of air are to be sampled or shorter temporal resolution is required, high-volume air samplers (~ 70 m^3 air/h) are more appropriate *(3)*.

Complementary use of outdoor air monitoring and modelling is effective for deriving estimates of the exposure of the general population to PM. Exposure estimates can be refined by use of portable personal samplers, which indicate individual exposure from both outdoor and indoor air, or use of low- and high-volume air samplers with conventional impactor or cyclone technology, which provide integrated daily, or longer, measurements of size-separated PM fractions *(3)*.

All sampling instruments risk being contaminated by their plastic components and by external variables of background contamination and human error. Systematic collection of blanks is therefore recommended, such as those collected with samples in the field, travel blanks to assess contamination during transport and blanks to evaluate background contamination during analysis and handling of samples *(3, 139)*. Filter-based sampling requires downstream compositional analysis. For MP, this is usually done with operator, semi-automated or automated micro-spectroscopy (Raman or Fourier transform infrared) *(3, 139)*. Whether samples are analysed directly on the filter or extracted and processed first, the filter substrate must be carefully

selected to avoid analytical signal interference or disintegration during processing, such as in the case of organic substrates. Liquid impingers eliminate the requirement for a sample substrate, reducing the handling time of samples. A disadvantage, however, is that evaporation may occur during sampling and should be assessed. Viscous, non-evaporative liquids such as mineral oil are thus recommended for collecting samples, although these types of liquids might interfere with downstream analysis.

In view of recent interest in characterizing and quantifying MP in air, several uncertainties in method development should be addressed, such as reporting on the aerodynamic size distribution of MP in air and the most appropriate concentration units for reporting data. The size distribution of MP must be evaluated, for instance, in order to choose the appropriate tools, as some instruments are suitable for measuring total suspended particulate and others for differentiating particle size fractions. If the size fractions of the MP have implications for human health, larger monitoring programmes may be warranted to evaluate their spatial and temporal variation or to assess personal exposure. Differences in exposure to microplastics during different activities and in different environments, such as indoors and outdoors, should be considered. The concentration units used can significantly affect how samples are collected, processed and analysed. If data are to be reported as particle counts, the particles must be isolated from samples and examined visually without destroying them. Mass concentrations from particle size impactor samplers might allow quantification of the particle size distribution of NMP in air but would probably not allow characterization of particle shapes.

Most counts of MP in air have been made by analysing bulk deposition *(3, 139)*. These results do not, however, necessarily correspond to the inhalable fraction relevant for assessing human exposure or to estimated exposure, which is in a volume of air. Exposure to MP by inhalation was studied by Liu et al. *(151)*, who estimated that inhabitants of Shanghai, China, were exposed to 21 particles/day by inhalation. The size of the particles 1.7 m above the ground was reported to be 23.07–9955 µm, with an average of 597.5 µm. While particles > 30 µm in aerodynamic diameter are less likely to enter the nasal passages *(152)*, these data provide an indication of human exposure to MP in air. Ingestion may also be relevant. For instance, particles > 30 µm, which are less likely to be inhaled, may be deposited on food or be ingested with dust. Studies with better differentiation and quantification of exposure to MP < 30 µm, and particularly $PM_{2.5}$ and PM_{10}, would refine assessments of exposure by inhalation.

Wright et al. *(3)* recently reviewed and evaluated 27 studies reporting MP in air and atmospheric deposition for their reliability by the approach described by Koelmans et al. *(2)* for drinking-water. Briefly, the studies were scored for 11 criteria. As for studies of drinking-water, a value of 2 (reliable), 1 (limited reliability) or 0 (unreliable) was assigned for each criterion. The final score is expressed as a TAS, calculated as the sum of each score, to a maximum value of 22. According to Hermsen et al. *(28)* and Koelmans et al. *(2)*, a reliable study should have no 0 scores for any criterion. The only study for which there were no 0 scores is that of Wright et al. *(153)*, who reported MP in atmospheric deposition collected in London, United Kingdom, during January and February 2018. They reported a predominance of fibres with diameters of 5–75 µm, most being 400–500 µm long. The most abundant non-fibrous particles measured

75–100 µm, the smallest particle being 25 µm in diameter, the smallest particle that could be identified with the method used. The deposition rates were estimated to be 575–1008 MP/m^2 per day. The particles reported by Wright et al. *(153)* were > 25 µm, which are likely to be deposited in the upper airways and be swallowed, implying ingestion.

Summaries of the TAS for all studies are reported by Wright et al. *(3)*. In the interest of brevity, the information presented in this report (Table 3) is thus limited to that from studies of MP in air with a TAS > 10, which are relevant to human exposure via inhalation and in which concentrations in air of MP < 20 µm are reported. It should be noted that discrimination of particles < 50 µm was poor in all the studies reviewed by Wright et al. *(3)*, most studies reporting a predominance of particles > 50 µm. For instance, the average size of non-fibrous particles was 164 ± 167 µm, and particles measuring 75–100 µm were the most abundant. Gaston et al. *(154)* reported that 30% of fibres measured 100–300 µm, with an average particle fragment size of 104 µm in outdoor air and 58.6 µm in indoor air; Wang et al. *(155)* reported an average particle size of 851 µm; Liu et al. *(151, 156)* reported averages of 582 µm and 246 µm; and Dris et al. *(135)* reported that most of the fibres detected were 200–400 µm in length. Some authors, such as Allen et al. *(142)* and Bergmann et al. *(143)*, found that particles < 50 µm predominated; however, the distribution of particles < 50 µm remains uncertain, and additional data are necessary to estimate the abundance of particles most relevant for inhalation, i.e., < 20 µm (Table 3). Generally, the distributions reported are limited by the analytical method used, and the microscopic magnification used strongly influences the results. Use of higher magnification and an appropriate analytical method, such as Raman microscopy, would allow detection of smaller particles. The variations in particle size reported therefore do not necessarily reflect the actual particle size distribution in air but may be artefacts of the analytical method used.

In some studies, fibres with diameters of 5–75 µm predominated, while in others mainly fragments measuring < 10 µm to > 2 mm were found, and mainly particles < 50 µm were found in others (Table 3). The shapes and sizes are influenced by factors including inconsistencies in sampling, sample preparation and analysis. Standard methods should be developed for accurate characterization and quantification of MP in air *(3)*.

To characterize human indoor exposure by inhalation, Vianello et al. *(159)* collected air samples from three apartments in Aarhus, Denmark, with a "breathing thermal manikin", which simulates the presence of a human occupant and has an inlet at the mouth connected to a low-volume air pump. Samples were collected on filters that were then analysed by micro-Fourier transform infrared spectroscopy, with a lower instrument size LOD of 11 µm. The results suggested that MP were ubiquitous in the air, at concentrations of 1.7–16.2 particles/m^3, consistent with Gaston et al. *(154)* but significantly lower than those reported by Liao et al. *(157)*. The most abundant polymers were polyester (59–92%), polyethylene (5–28%), nylon (0–13%) and polypropylene (90.4–10%); the concentrations of non-synthetic particles were one or two times higher *(159)*. In contrast to studies that reported a predominance of fibres, the studies summarized in Table 3 suggest the relative importance of fragments in human inhalation exposure. As most people spend much of their time indoors,

Table 3. Studies with a total assessment score > 10 of microplastic particles in indoor and outdoor air at urban and rural sites

Reference	Sample type	Lower size bound (µm)	Particle concentration (average)	Number of particles in blanks (average)	Particle size (µm)[a]	Predominant particle shape	Predominant polymer type	Quality score[b]
157	Air (indoor and outdoor)	10	1583 ± 1181 MP/m³ (indoor); 189 ± 85 MP/m³	3.3 ± 1.8 MP/filter	> 90% < 100 µm, the 5–30 µm fraction representing 54.1–65.2% of total	Fragments > 80%	Indoor: Polyester, polyamide, polypropylene Outdoor: Polyethylene, polystyrene, polyester	18
158	Air (outdoor)	10	282 ± 127 MP/m³ (range, 104–650)	3.9 ± 2.2 MP/filter	73.5–96.6% represented by 5–30-µm fraction	Fragments (88.2%)	Polyethylene, polyester, polystyrene	18
159	Air (indoor)	11	9.3 ± 5.8 particles/m³	7.7 ± 3.8 MP/blank, data not corrected	36 and 21 for the major and minor dimensions	13% fibres, 87% fragments	81% polyester, 6% polyethylene, 5% polyamide, 2% polypropylene and 6% other polymers; non-synthetic, 95% protein, 5% cellulose; MP > 4% total particles	15
154	Air (indoor and outdoor)	20	3.3 fibres and 12.6 fragments/m3 indoors; 0.6 fibres and 5.6 fragments/m³ outdoors	2.4 fibres and 12.2 fragments per filter indoors; 0.4 fibres and 6.3 fragments per filter outdoors	Fibre lengths: indoors 641 µm; outdoor 616 µm; approx. 30% of fibres 100–300 µm Fragments: outdoors 104 µm; indoors 58.6 µm	Fragments	Polystyrene, PET, polyethylene	15
156	Air (outdoor)	12	0.41 MP/m³ (0–2 MP/m³)	No MP in one blank collected	246.52 µm (12.35–2191.32 µm)	Fibres (43%), fragments (48%), beads (9%)	PET (51%), epoxy resin (19%), polyethylene (12%), alkyd resin (8%); fibrous PET (87%)	13
142	Deposition	10	365 ± 69 particles/m² per day (range, 29–462)	3 ± 1 fibres, 1 ± 1 film and 8 ± 1 fragments per filter	Most MP < 50 µm, fibre lengths predominantly 100–200 µm and 200–300 µm (max, 3000 µm); films, 50–200 µm	Fragments > fibres > films	Polystyrene (as fragments) followed by polyethylene	12

Additional information presented by Wright et al. *(3)* and in Annex 1

[a] Geometric mean

[b] Maximum, 22

inhalation of indoor air is probably an important exposure pathway; however, in view of the LOD of 10 µm, inhaled particles are unlikely to reach the alveolar regions of the lungs and are more likely to be swallowed (section 4). Thus, methods should be developed for monitoring MP < 10 µm.

The data summarized in Table 3 indicate the ubiquity of MP in both indoor and outdoor air; however, differences in sampling methods and in the reporting of results prevent comparison of the data with air quality guidelines for PM or with effect thresholds reported for polymeric particles in occupational exposure. Inconsistent units and lack of standardized sampling methods obviate comparisons of studies and extrapolation. The data do imply, however, that MP in air are a potentially important source. As none of the methods used allows quantification of concentrations < 10 µm, limited information is available on exposure to the respirable fraction of NMP, < 2.5 µm. The particle sizes in deposition studies reviewed by Wright et al. *(3)* indicate whether exposure occurred via ingestion, directly, as dust or as dust settled on food during its collection, manufacture, packaging, distribution and preparation. Limited information is available on contamination of food with MP *(160)*. Given the ubiquity of MP in atmospheric deposition, research should be conducted to determine overall exposure in air and the stages at which NMP are introduced into food.

Because of the lack of data on the contribution of MP with PM_{10} and $PM_{2.5}$, human exposure to the respirable fraction of MP cannot be estimated reliably. In view of the quality score for characterizing deposition of MP in the study of Wright et al. *(153)*, the value of 771 MP/m^2 per day could be used as a conservative estimate of deposition of particles measuring 100–500 µm onto surfaces. The value of 9.3 ± 5.8 MP/m^3 reported by Vianello et al. *(159)*, which is generally consistent with the observations of Gaston et al. *(154)* (TAS = 15 for both studies) for particles measuring 20–100 µm, could be used to estimate exposure to the particles that are most likely to be trapped in the upper airways during respiration and subsequently swallowed. Alternatively, the study of Liao et al. *(157)* (TAS = 18) suggests an upper limit of 1583 ± 1181 MP/m^3 for particles measuring 5–30 µm, which could be used to estimate daily human exposure via inhalation of approximately 3000 MP/day on the assumption of daily inhalation of air of 15 m^3. Consistent with the data on drinking-water (section 2.1), the variation in the concentrations reported in air could be used for a probabilistic quantitative assessment of human exposure *(124)*.

2.3 Dermal exposure

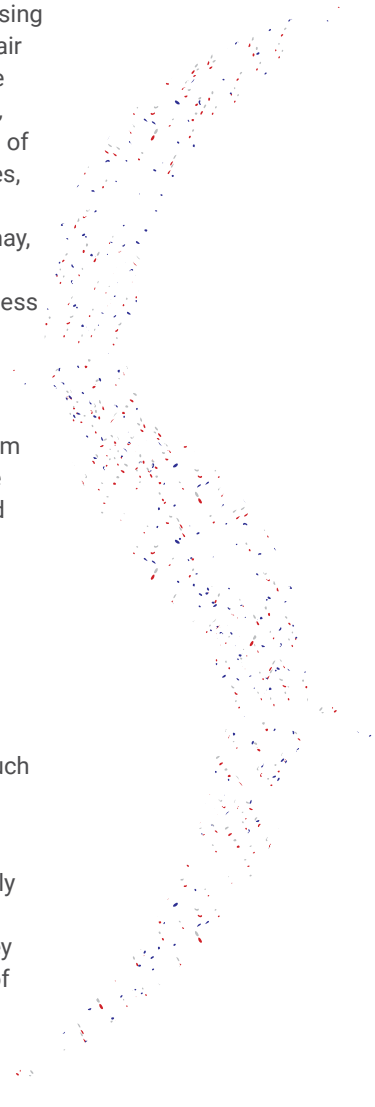
Human exposure to NMP is largely dominated by ingestion in food and beverages and by inhalation. Dermal exposure to MP > 1 µm is limited by the barrier of the stratum corneum *(161)*, which comprises several layers of corneocyte cells and individual packing of these cells in a "bricks-and-mortar" structure of proteins and intercellular lipids. To reach the systemic circulation, chemicals and other foreign agents, such as particles, bacteria and viruses, must penetrate this barrier.

Transdermal delivery of pharmaceuticals to optimize permeation has been studied for centuries, including investigation of the mechanisms by which chemicals and particles with various physicochemical properties interact with the stratum corneum *(161–163)*. The strategies include use of chemical permeation enhancers, biochemical manipulations and physical disruption of the barrier function *(162, 163)*. Innovation in nanoscience has also included evaluation of the conditions in which nanoparticles penetrate the stratum corneum *(162, 164, 165)*. Research is being conducted on passive transcutaneous or lipid systems for delivering NMP to avoid use of methods that disrupt or compromise the integrity of the skin barrier. The methods are based on delivery of antigens by passive diffusion through the intact skin by establishing concentration gradients, increasing the hydration of the skin by occlusion and diffusion into hair follicles *(165)*, which represent shunt pathways across the stratum corneum that can be penetrated by particles *(162, 166)*. Mahe et al. *(166)*, for instance, observed penetration of 40- and 200-nm polystyrene nanoparticles into hair follicles, and Vogt et al. *(167)* also observed penetration of 40-nm nanoparticles. Penetration of particles into hair follicles may, however, be influenced by particle size, those measuring ~ 600 nm being optimal, as this corresponds to the thickness of the overlapping cuticular hair surface cells *(167, 168)*. Negatively charged, hydrophobic surface properties also enhance follicular absorption *(169)*.

While physiological variation in the thickness of the stratum corneum and the density of hair follicles can influence the results of experimental studies, three trends may be noted *(164)*.

- Experimental studies demonstrate consistently that healthy human skin is a formidable barrier to penetration of NP.
- Hair follicles are important collection sites for nanoparticles, especially when skin is massaged or flexed.
- The physicochemical properties of nanoparticles, such as surface charge and hydrophobicity, significantly influence permeation.

Kohli and Alpar *(170)* investigated permeation of negatively charged 50- and 500-nm latex particles in a study of their potential use for transcutaneous delivery of vaccines. They observed no difference in the behaviour of the two sizes of particle, < 0.1% of which permeated the stratum corneum. Permeation was attributed to the repulsive force between



the negatively charged lipids in the skin and the particles. Generally, limited permeation of nano-sized latex particles is consistent with negligible exposure to nanoparticles in cosmetics and personal care products across normal skin *(171)*. Indeed, one of the limiting factors for trans-follicular vaccination is the relatively low follicular penetration of nanoparticles, which is < 10% of the total applied amount *(168)*. Data on permeation of nanoparticles, however, vary, and research is continuing on a dermal drug delivery system based on "smart" nanoparticle systems that can release active pharmaceutical ingredients at specific times and locations by penetrating deep into hair follicles *(169)*.

Observations on permeation of NMP across the stratum corneum are, however, relatively limited, variable and inconclusive *(172)*, and no information is available on dermal uptake of NMP during typical environmental exposure. This information would be useful for overall assessment of risks for human health. For instance, assessments should be conducted of the safety of NMP in direct contact with the skin, such as in application of nanotechnology in drug delivery and the use of cosmetic and personal care products *(171)*. Research on dermal uptake of ambient NMP by healthy intact and by damaged skin would also be useful. Research on dermal exposure to NMP should build on the decades of research on the application of nanotechnology in the pharmaceutical sciences. Although MP in atmospheric deposition and dust have been reported *(3)*, the prevalence of particles measuring < 10 µm is unknown, and methods should be developed to characterize such exposure in order to determine the importance of dermal exposure in environmentally relevant scenarios.

2.4 Occurrence in food

The presence of plastic debris ingested by marine organisms was reported in a number of studies conducted in the late 1960s and early 1970s *(173–181)* and now includes observations of a broad range of MP in marine, freshwater and other organisms *(80, 182–192)*. MP may be ingested either directly from the water column or sediment or indirectly by consumption of lower trophic prey that have recently ingested MP *(193)*. The evidence indicates that MP released into the environment can enter the food chain, with implications for human exposure through consumption of seafood and fish *(7, 56, 85, 194–200)*.

In a review of studies of ingestion of plastic debris, including MPs, by > 800 species and approximately 87 000 non-human organisms, the average concentration was four particles per individual *(201)*. Depending on the study, the concentration of MP > 500 µm was measured in the stomach and intestines, with limited data on

concentrations in the tissues of fish consumed by humans. MP are therefore ingested by non-human organisms, and laboratory studies indicate that egestion in faeces represents an important elimination process *(202)*. Thus, while MP are ingested at all levels of biological organization, their detection may represent a snapshot in time and space. Most (80%) of the organisms sampled did not contain MP at the time of sampling, consistent with the estimate that 16.6% of organisms contained MP by Hermsen et al. *(28)*. Furthermore, the frequency of occurrence varied significantly, ranging from 0 to 100%, depending on species, time of sampling, location and sample size *(28, 200, 201)*. The lack of standard methods for extracting and analysing MP in organisms results in differences in reported data, such as on particle size, shape and polymeric composition *(200, 201)*. For instance, studies on the occurrence of fibres in organisms began to appear only after 2011, when the release of synthetic textile fibres was first reported *(203)*. Since then, fibres and fragments have tended to be the dominant shapes of MP observed; however, some studies included semi-synthetic polymers in their analysis, while others excluded them. Natural, semi-synthetic and synthetic fibres should be reported separately in the future, although natural textile fibres tend to dominate the total number of fibres detected *(204)*. Data on MP < 100 µm in biological samples are limited, 5 µm being the lowest size reported so far. Additional research is thus required to characterize and quantify the environmental and biological fate of particles < 100 µm. MP have generally been studied only in the gastrointestinal tract, and their presence in other biological tissues and organs is usually either not studied or not reported *(56, 200, 201, 205, 206)*.

Studies of commercial fish and seafood provide comparable results *(56, 201, 205)*, with significant variation in the sample size and reporting of particles in the gastrointestinal tract. As MPs are analysed only in the gastrointestinal tracts of fish, which are usually removed before the fish are sold for human consumption, exposure to MP in fish tissues is not certain and has been perceived to be negligible *(10, 56, 206)*. Some laboratory studies have, however, found uptake of MP into tissues of fish that are likely to be consumed by humans. This route of exposure should be better characterized and quantified *(83, 207–209)*.

Human exposure from consumption of seafood such as bivalves, crustaceans and small fish, from which the gastrointestinal tract is not removed, may be more relevant. Quantification of exposure from seafood depends, however, on the rate of consumption and differences in the concentrations of MP. Estimated concentrations are influenced by various factors, including the analytical methods, which may or may not verify the polymeric composition of particles, and are directly influenced by the inherent variation in environmental concentrations. Table 4 summarizes the concentrations of MP reported in seafood and in other foods and beverages for human consumption from studies identified in a search in PubMed with the keywords “microplastic” AND “food” OR (“seafood” OR “human exposure” OR “beverages”). The review was performed up to December 2021, and only those publications that provided original concentrations were reviewed. Of the 87 studies identified, 58 reported concentrations in seafood or fish tissues, 18 reported concentrations in salt products and four reported MP in beer; two reported MP in honey and milk and one on MP in rice, sugar and *nori* seaweed. Each study was assessed for reliability according to the 10 criteria of Hermsen et al. *(28)*. The maximum possible TAS was 20, with

an average for all studies of 10.5. In the interests of transparency, details of all the studies reviewed in this report are summarized in Table 4. No study received a non-zero score on all criteria.

Seafood is the food product that has been studied the most often to date. Normalization of the concentrations of MP in seafood per wet body weight (ww) reported in Table 4 results in an average concentration of approximately 3 ± 4 MP/g. In several studies, the concentrations in various food and beverages were combined with estimates of per capita consumption to estimate dietary exposure (Table 5); seafood represented the largest source of exposure. The importance of commercial bivalves as a source of exposure to MP was first highlighted by Van Cauwenberghe and Janssen *(85)*, who estimated that the annual dietary exposure of European consumers of molluscs was 1800 and 11 000 MP/year for the lowest and highest consumers, respectively. Estimates based on meal portions of seafood by EFSA *(10)* and the Food and Agriculture Organization of the United Nations (FAO) *(83)* and the finding of 4 MP/g (ww) by Li et al. *(273)* indicate that an average portion of 225 g of mussels would result in consumption of 900 MP per meal of mussels (Table 5). In a study of cultural and regional differences in consumption of mussels, the exposure of a consumer in the United Kingdom was estimated to be 123 particles/year, while that of a consumer in Japan was estimated to be 56 210 particles/year *(19, 160)*.

Table 5 summarizes estimates derived in studies of human exposure to MP based on consumption of contaminated food. Table 4 shows significant variation in the quality of the data reported and therefore in estimates of daily and annual human ingestion of MP, so that it is difficult to compare the values with those in Table 5, and the difficulty is compounded by differences in the particle sizes used to estimate consumption. Caution should therefore be exercised in extrapolating the data for assessing the implications of exposure to MP for human health.

While the values reported in Table 5 for seafood suggest that it may be an important source of MP, only a limited range of types of food and beverages has been studied so far, and they do not necessarily represent the major sources of daily caloric intake by humans. For instance, the WHO Global Environment Monitoring System – Food Contamination Monitoring and Assessment Programme (GEMS/Food) shows a maximum per capita consumption of fish and seafood of 78 g/day in the cluster G17 (consisting of Samoa and São Tome and Principe) and a minimum of 9 g/day in cluster G1 (consisting of Afghanistan, Algeria, Azerbaijan, Gaza Strip and West Bank, Iraq, Jordan, Libya, Mauritania, Mongolia, Morocco, Pakistan, Syrian Arab Republic, Tunisia, Turkmenistan, Uzbekistan and Yemen). As illustrated in Fig. 4, cereals, grains, fruits and vegetables (including roots) account for approximately 50% of the foods ingested daily, although limited data are currently available on MP in these food categories. The foods listed in Table 5 represent about 25% of the food categories ingested daily. More data are required on food categories that better represent the human diet for a more robust assessment of human exposure to MP.

Table 4. Reported numbers of microplastic or microplastic-like particles and particle characteristics in studies of their presence in food and beverages for human consumption

Reference	Sample type	Lower size bound (µm)	Particle concentration (average)	Number of particles in blanks (average)	Particle size (µm)	Predominant particle shape	Predominant polymer type	Quality score
91	Beverages (beer)	100	4.05 particles/L	< 2 particles/ sample	Average fibre length, 0.98 mm (0.1–5 mm)	98.4% as fibres	Not specified	8
210	Beverages (beer)	0.8	28 ± 9 MP/L	5 fibres/ blank	Not specified	Fibres and fragments	Not specified	6
211	Beverages (beer)	0.8	21.3 MP/L	16.7 particles/L	Not specified	Fibres and fragments	Not specified	7
212	Beverages (beer, cold tea, soft drinks, energy drinks)	11	Maximum values (MP/L): 6 (cold tea); 7 (soft drinks); 6 (energy drinks); 28 (beer)	Laboratory blank contained no MP	0.1–1 mm	Fibres	Polyamide, poly(ester-amide), PET and ABS	8
213	Beverages (milk)	11	6.5 ± 2.3 MP/L	Laboratory blank contained no MP	40% fibres < 0.5 mm; 60% > 0.5 mm	Fibres (97.5%), fragments (2.5%)	Polyether sulfone and polysulfone	8
214	Beverages (milk)	5	2040–10 040 MP/L	44 ± 24 MP/filter	5–7 µm	Fibrous fragments	Polyethylene, polyester, polytetrafluoroethylene, polypropylene	17
89	Fish (dried)	149	1 particle/fish	None observed in procedural blanks	> 149 µm	Fragments (85.7%), films (10%), filaments (4.1%)	Polypropylene (47.2%), polyethylene (41.6%), polystyrene (5.6%), PET (2.8%), nylon-6 (2.8%)	11
215	Fish (dried)	20	0–1.92 ± 0.12 MP/fish	None observed in procedural blanks	195–5780 µm	Fibres	Polyethylene, PET, polystyrene, PVC, polypropylene	10
216	Fish (tinned sardines and sprat)	149	1–3 particles/tin	None observed in procedural blanks	190–3800 µm	Fragments (46.6%), films (26.6%), filaments (26.6%)	Polypropylene (33.3%), PET (33.3%), polyethylene (16.6%)	14
217	Fish (tinned sardines, tuna, salmon)	Not specified	Not reported	Not specified	Not specified	Not specified	Nylon, 1,2-polybutadiene, ethylene vinyl alcohol, wool	7
218	Fish (anchovy)	10	6.78 ± 2.7 MPs/ fish	None observed in procedural blanks	100–1000	Fibres	Polyethylene, polypropylene, polyamide, polyester, polystyrene	15

Reference	Sample type	Lower size bound (µm)	Particle concentration (average)	Number of particles in blanks (average)	Particle size (µm)	Predominant particle shape	Predominant polymer type	Quality score
219	Fish (seabass, muscle tissue)	20	No MP	0.095 MP/ sample	Size of non-plastic fibres not reported	Non-plastic fibres	Not specified	13
220	Fish (various, fillets)	20	0.47 ± 0.84 MPs/ fish	0.25 ± 0.43 MPs/fish	300–1000	Not specified	Polyethylene and Polypropylene	11
221	Fish (various, fillets)	Not specified	2.47 ± 2.99 to 0.47 ± 0.86 MP/ fish	0.40 ± 0.54 fibres/ blank	54–765	Fragments	Polyethylene, polypropylene	13
222	Fish (various, fillets)	20	< LOD	0.4 MP/ blank	Not specified	Fibres	Not specified	10
223	Fish (various, fillets)	Not specified	0.12–0.51 MP/g	Not specified	52.2% 100–250 µm	Fragments	Polyethylene, polypropylene, nylon	11
224	Fish (various, fillets)	Not specified	Average, 5.7 ± 1.7 and 18.5 ± 4.6 particles/10 g fish muscle	Not specified	> 100 µm	Fibres	Not specified	8
225	Fish (various, fillets)	Not specified	0.74 ± 0.57 MP/g	None observed in procedural blanks	25–1000 µm	Fragments	Polyethylene, polystyrene	15
226	Fish (various, skin)	8	Range, 4.23–9.3 MP/individual (skin)	0.57 ± 0.17 MP/blank	Average size 973 ± 803 µm (skin)	97.9% fibres	Cellophane (33.5%), polypropylene (15%), polyethylene (13%), nylon (8%), polyester (PET, 4.5%)	14
227	Fish and seafood	5	2 MP/g (ww) (maximum)	0.8 ± 0.131 items/ filter	5–25 µm	90% fibres	PET, polyethylene	11
228	Fish and seafood	0.1	8.66E4 ± 2.43E4 to 9.50E4 ± 6.64E4 MP/g	Not specified	1.6–2.8 µm	Not specified	Not specified	7
229	Fish and seafood	30	0.26 ± 0.16 to 4.46 ± 3.72 MP/g	Not specified	38.2–820 µm	Fragments	Polypropylene, polyethylene, polystyrene, PET	8
230	Honey	40	Fibres: 87 ± 73/500 g Fragments: 4 ± 4/500 g	Not specified	40 µm to 9 mm (fibres); 10–20 µm (fragments)	Fibres and fragments	Not specified	4
231	Honey	30	Not specified	Not specified		Fragments and fibres	Mainly soot or char; fibres mainly cellulose and PET	10
232	Nori (seaweed)	5	1.8 ± 0.7 MP/g (dry weight)	0.1 ± 0.2 MP/g (dry weight)	0.1–4.97 mm; media, 1.13 mm	Fibres	Polyester (18.9%), rayon (6.6%), polypropylene (4%) polyamide (2%), cellophane (2%); cotton and natural cellulose fibres (61%)	12

Reference	Sample type	Lower size bound (µm)	Particle concentration (average)	Number of particles in blanks (average)	Particle size (µm)	Predominant particle shape	Predominant polymer type	Quality score
92	Rice	Not specified	45–317 µg/g (dry weight) (polyethylene); 105 µg/g (dry weight) (polypropylene); 17 µg/g (dry weight) (PET)	LOD reported for individual polymers	Not specified	Not specified	Polyethylene, polypropylene, PET	14
90	Salt	149	2 particles/kg (estimated)	Collected but results not specified	Average size, 515 ± 171 µm	Fragments and filaments	Polypropylene (40%), polyethylene (33%), PET (6.7%), polyisoprene/ polystyrene (6.7%), polyacrylonitrile (10%), polyamide-6 (3%)	12
91	Salt	100	212 particles/kg	< 2 particles/ sample	Average fibre length, 1.09 mm; range, 0.1–5 mm.	99.3% as fibres	Not specified	8
233	Salt	5	550–681 MP/ kg (sea salt); 43–364 MP/ kg (lake salt); 7–204 particles/ kg (rock and well salts)	4.4 ± 2.1 particles/ filter or 18 particles/ kg	45 µm to 4.3 mm; particles < 200 µm represented 55% of total	Fragments and fibres	PET, polyester, polyethylene, polypropylene, cellophane, poly(1-butene)	7
234	Salt	5	127 ± 51.6 MP/ kg	6 particles/ filter	30 µm to 3.5 mm	Fibres	PET (83.3%), polyethylene (3.3%), polypropylene (6.7%)	7
235	Salt	45	367 ± 154 to 2133 ± 153 MP/kg	Not specified	Not specified	Fibres	Nylon, polyethylene	7
236	Salt	100	120–580 MP/kg	None observed in procedural blanks	100–500 µm	Fibres	Polyethylene	7
237	Salt	50	1.68 ± 1.83 MP/ kg	Not specified	3.3–4660 µm	Fragments	Polyvinyl acetate, polypropylene, polyethylene	10
238	Salt	65	11–193 MP/kg	None observed in procedural blanks	65–2500 µm	Fibres	Polyethylene	16
239	Salt	Not specified	2 ± 1 to 72 ± 40 MP/kg	Not specified	55–2000 µm	Fibres	Polyethylene, polypropylene, polyester	7
240	Salt	20	8–102 MP/kg	2.03 ± 1.01 MP/salt type	20–5000 µm	Fibres	Polyethylene, polyurethane, polypropylene	10

Reference	Sample type	Lower size bound (µm)	Particle concentration (average)	Number of particles in blanks (average)	Particle size (µm)	Predominant particle shape	Predominant polymer type	Quality score
241	Salt	10	1570–31 680 MP/kg	Not specified	10–4628 µm	Fragments and fibres	Polypropylene, polyamide, PET, PVC	7
242	Salt	100	672 ± 2560 MP/kg (median = 82)	LOD, 0.72 MP/kg (PET fibres)	100–5000 µm	Fragments	Polyethylene, polypropylene, PET	16
243	Salt	390	6.7–53.3 MP/kg	Not specified	390–9360 µm	Not specified	Polyethylene, polyvinyl acetate, polystyrene	1
244	Salt	5	< 700 MP/kg	Not specified	5–3800 µm	Fragments and fibres	Cellophane, polystyrene, polyamide, polyarylether	6
245	Salt	Not specified	275 ± 25 to 1832 ± 40 MP/kg	Not specified	20–5000 µm	Fragments	Polyethylene, polypropylene, PET, nylon and polystyrene	5
246	Salt	500	56 ± 49 to 103 ± 39 MP/kg	Not specified	500–2000 µm	Fragments and fibres	PET, polyamide, polyethylene, polystyrene	9
247	Salt	1	140.2 MP/kg	None observed in procedural blanks	89.7–1474.9 µm	Fragments	Polypropylene, polyethylene, PET	9
58	Salt	20	2395 MP/kg	None observed in procedural blanks	63–100	Fragments	Polypropylene, polyethylene	12
248	Seafood (clams)	1.2	0.0–5.47 particles/g (ww)	5.8 ± 2.2 particles/filter	Not specified	Fibres (90%)	Not specified	16
249	Seafood (clams)	20	0.06-5.17 MP/g (ww)	Not specified	62.3% < 500 µm	Fibres	Rayon, polyester	12
250	Seafood (clams)	10	23 ± 20 MP/clam	None observed in procedural blanks	39% < 500 µm	Fibres	PVC, polyethylene, polypropylene, polyester	12
251	Seafood (clams, oysters)	100	3 MP/individual	Not specified	Not specified	Fibres	Polyethylene	4
252	Seafood (crab)	0.5 mm	Not specified	Not specified	0.5–5 mm	Fibres and fragments	Not specified	3
253	Seafood (mussels)	Not specified	34–178 items/mussel	Not specified	Not specified	Fibres	Not specified	11
254	Seafood (mussels)	Not specified	6.2 ± 7.2 items/g (ww)	None observed in procedural blanks	750 µm to 6 mm	Fibres	Not specified	8
160	Seafood (mussels)	Not specified	Average, 0.09 ± 0.03 MP/g (ww) to 3.0 ± 0.9 MP/g (ww)	6.5 ± 0.95 MP/blank	Fibre length, 0.2–2 mm; thickness, 1–5 µm	Fibres	Polyester, PET	13

Reference	Sample type	Lower size bound (µm)	Particle concentration (average)	Number of particles in blanks (average)	Particle size (µm)	Predominant particle shape	Predominant polymer type	Quality score
255	Seafood (mussels)	5	2.2 items/g (ww); 4 items/ mussel	0.67 ± 0.82 items/ filter	33 µm to 4.7 mm fibres)	65% fibres	PET, polyester, cellophane	16
256	Seafood (mussels)	5	0.13 ± 0.14 MP/g (ww)	1 fibre/ blank	77% < 50 µm	Fibres and fragments		9
257	Seafood (mussels)	10–20 µm	3.5 fibres/10 g (ww)	LODs of 2.3, 4.7 and 1.5 fibres/ sample for black, blue and red fibres, respec- tively	200–1500 µm	Fibres		8
258	Seafood (mussels)	1.2	0.9 ± 0.2 MP/g (ww)	< 10% of total MP detected in blanks	100–500 µm	77.8% fragments; 22.2% fibres	Polyethylene, PET, polystyrene	17
259	Seafood (mussels)	5	1–5.4 MP/g (ww)	0.4 ± 0.5 items/ filter	250 µm to 1 mm (48–76% of particles)	Fibres (80%)	PET (74%), polyethylene, PVC, polyethylene, rayon	12
260	Seafood (mussels)	25	1.05–4.4 MP/g (ww)	19 fibres/ tape strip	Median length, 1.2 mm	Fibres and fragments	Polyamide, PET	13
261	Seafood (mussels)	1.2	37 MP/g (dry weight)	Reported as minimal	Median length, 200 µm	Fibres	Not specified	9
262	Seafood (mussels)	0.7	Not specified	Not specified	20 µm to 5 mm	Fragments	Not specified	14
263	Seafood (mussels)	5	0.7–2.9 MP/g (ww)	0.67 ± 0.75 items/ filter	5–250 µm	Fibres	Polyester, polypropylene, polyethylene, rayon, cotton	11
264	Seafood (mussels)	1.6	0.76 ± 0.40 MP/ individual; 0.15 ± 0.06 MP/g (ww)	Not specified	32.6% 100–500 µm	Fibres and fragments	Polyethylene, polypropylene, polystyrene, ABS, PET, styrene–butadiene rubber copolymer	15
265	Seafood (mussels)	8	0.86 ± 0.82 MPs/g (ww)	0.67 ± 0.58 items/ filter	890 µm (average)	Fibres	Cellophane, PET	9
266	Seafood (mussels)	500	0.04 MP/g (ww)	Not specified	72% > 500 µm	Fragments and filaments	PET	7
267	Seafood (mussels)	500	0.87 ± 0.55 to 10.02 ± 4.15 MPs/mussel	Not specified	39% 500–1000 µm	Fibres	Polyethylene	15
268	Seafood (mussels)	Not speci- fied	8.72 ± 5.30 MP/ mussel	Not specified	> 100 µm	Fibres	Not specified	11
269	Seafood (mussels)	53	1.53 ± 2.04 MPs/g (ww)	Not specified	Not specified	Fragments	Ethylene/ propylene copolymer	11

Reference	Sample type	Lower size bound (µm)	Particle concentration (average)	Number of particles in blanks (average)	Particle size (µm)	Predominant particle shape	Predominant polymer type	Quality score
270	Seafood (mussels)	30	0.08 to 8.6 MP/g (ww)	1.33 ± 0.58	41.7–4679 µm	Fragments	Polypropylene, Polyethylene, PET	16
271	Freshwater Mussels		2.85 ± 1.27 MP/g	None observed in procedural blanks	44.8% < 100 µm	Fragments	Not specified	13
85	Seafood (mussels, oysters)	5	Average, 0.24 ± 0.07 to 0.35 ± 0.05 particles/g (ww)	None observed in procedural blanks	5–25 µm	Fragments	Not specified	8
272	Seafood (mussels, oysters)	20	0.61 ± 0.56 (mussels) and 2.1 ± 1.7 (oysters) MP/ individual or 0.2 ± 0.2 MP/g (ww)	1.2 ± 0.8 particles/ filter, none verified as plastic	50–100 µm (52%); 20–50 µm (37%); > 100 µm (11%)	Fragments (80%)	Polypropylene and polyethylene	18
273	Seafood (mussels, scallops, clams)	5	Average, 2.1–10.5 items/g (ww)	0.5 ± 0.55 items/ filter	5 µm to 5 mm; 33–84% < 250 µm	Fibres and fragments	Polyethylene, PET and polyamide	10
274	Seafood (mussels, scallops, clams, oysters)	20	Average, 0.15 ± 0.2 MP/g (ww) or 0.97 ± 0.74 MP/individual	LOD, 0.24/ sample (polyethylene, polypropylene, polystyrene) and 0.51/ sample (polyester and polyethylene vinyl acetate)	Fibre lengths, 43 µm to 4.7mm; MP < 300 µm represented 65% of total	Fragments (76%); fibres (24%)	> 80% polyethylene, polypropylene, polystyrene, polyester and expanded polystyrene	17
275	Seafood (oysters)	5	0.37– 0.57 MP/g (ww)	Not specified	Not specified	Fibres and fragments	Polyamide, PET	12
276	Seafood (oysters, clams, snails)	25	Average, 0.2–20 particles/g (ww) and 3.5–17.7 particles/ individual	None observed in procedural blanks	37–58% were 10–25 µm	58% fibres, 26% fragments, 14% films and 2% pellets	Polyethylene, PET and nylon	10
277	Seafood (oysters, mussels, clams)	10	4.0 ± 2.1 MP/g (oysters); 3.2 ± 1.8 MP/g (mussels); 0.7 ± 0.3 MP/g (clams)	Not specified	10–428 µm	Fibres	Ethylene vinyl alcohol	11
278	Seafood (Clams, mussels, crabs)	5	0–5 MP/ individual (mussels); 0 MP (crab soft tissue and clams)	Fibres excluded from analysis due to contamination	Not specified	Not specified	Not specified	12

Reference	Sample type	Lower size bound (µm)	Particle concentration (average)	Number of particles in blanks (average)	Particle size (µm)	Predominant particle shape	Predominant polymer type	Quality score
279	Seafood (Prawn, oysters, clams)	74	0.31 ± 0.10 MP/g (oysters/ clams); 0.25 ± 0.08 MP/g (prawns)	Not specified	74–2000 µm	Fibres	Cellulose, polyamide, acrylonitrile, polyethylene, polypropylene, PET	10
280	Seafood (oysters)	5	3.24 ± 1.02 MPs/g (ww)	None observed in procedural blanks	53% < 100 µm	Fragments	PET (69.4%)	11
281	Seafood (oysters)	Not specified	64 MP/g (ww)	Not specified	30–5000 µm	Fibres	Not specified	8
282	Seafood (oysters)	Not specified	0.07 ± 0.04 MP/g (ww)	Limited to a few polyamide fibres	75% > 500 µm	Fibres	PET, polyacrylonitrile, rayon	14
198	Seafood (Pacific oyster)	500	0.6 ± 0.9 MP/ oyster	Not specified	0.1–4.5 mm	Fibres	Not specified	8
194	Seafood (prawns)	Not specified	1.5 MP/g (ww)	Not specified	> 100 µm	Fibres	Not specified	11
283	Seafood (prawns)	10–20	0.68 ± 0.55 MP/g (ww) or 1.23 ± 0.99 MP/ prawn	Reported as < LOD (not assessed)	200–1000 µm	96.5% fibres	Not specified	11
284	Seafood (prawns)	100	6.78 ± 2.8 MPs/ prawn	Not specified	100–1000 µm	Fibres	Polyethylene, polypropylene, polyamide, nylon, polyester, PET	12
285	Seafood (prawns)	100	1.02 MP/g (ww)	Not specified	87% > 100 µm	Fibres	PET, polypropylene, polystyrene	8
230	Sugar	40	Fibres: 217 ± 123/500 g Fragments: 32 ± 7/500 g	Not specified	40 µm to 9 mm (fibres); 10–20 µm (fragments)	Fibres and fragments	Not specified	4

ww, per wet body weight

Additional information on the total assessment score (TAS) for each study available upon request. The maximum TAS was 20.

Table 5. Estimated daily and annual per capita ingestion of microplastic particles

Reference	Sample	Portion size (g)	Country or region	Daily per capita consumption (g/day)	Particle concentration (MP/g)[a]	Per capita ingestion (MP/day)	Per capita ingestion (MP/year)	Particle size (µm)
10	Mussels	225	France		4	900[b]		25
268	Mussels			18.16 g/year			70.82	> 100
274	Mussels		Republic of Korea	0.67	0.12	0.08	29	4–300
263	Mussels	100	United Kingdom			100		5–250
160	Mussels		United Kingdom	0.23	1.5	0.3	123	0.2–2 mm
160	Mussels		Belgium, France, Spain	8.75	1.5	12.7	4620	0.2–2 mm
270	Mussels			836–5672 g/year		21-458	176–10 380	> 40
228	Mussels			0.21		237		1.6–2.8
277	Mussels			3109.3 g/year			8084.1	
254	Mussels (cooked)	225	Italy			1395		> 750
249	Clams						1088.64	
274	Manila clams		Republic of Korea	1.25	0.35	0.44	155	43–300
274	Oysters		Republic of Korea	0.84	0.07	0.06	21	43–300
274	Scallops		Republic of Korea	0.25	0.08	0.02	7	4–300
283	Prawns		Belgium	1.4	1.92	0.3	100	> 200
19	Seafood			37.82	1.48	55	20 430	
227	Fish and seafood		United Kingdom[c]	20.74	0.77	16	5828	> 5
227	Fish and seafood		United Kingdom[d]	57.2	0.77	44	16 076	> 5
224	Fish muscle (bartail flathead; Platycephalus indicus)		Persian Gulf	45	1.85	80	29 200	> 100
228	Fish sea (bream)		Mediterranean Sea	22.5			25 500	
223	Fish		India	43		65		
225	Fish		Islamic Republic of Iran	43			174 MP/kg bw per year	
216	Canned fish		Global	0.25	0.03	0.008	2.7	
89	Dried fish		Bangladesh	1.01	0.7	0.7	246	> 149
215	Dried fish		Sri Lanka	3700 g/year			851–1147	
19	Salt			5	0.11	0.55	200	> 30
91	Salt		USA	5	0.21	1.05	380	> 100
234	Salt		Spain	5	0.28	1.4	510	> 30
233	Salt		China	5	0.68	3.4	1 241	> 45
90	Salt		Malaysia	10	0.002	0.02	7.3	500
238	Salt		Sri Lanka	8.3			158	
239	Salt		India	10.98			48–216	
240	Salt		Turkey	14.8–18.01			63.7–302.4	
58	Salt		Republic of Korea	10.06			12 000	> 20

Reference	Sample	Portion size (g)	Country or region	Daily per capita consumption (g/day)	Particle concentration (MP/g)[a]	Per capita ingestion (MP/day)	Per capita ingestion (MP/year)	Particle size (µm)
242	Salt		Global	10.06		0–117	0–42 600	> 100
19	Sugar			66.81	0.44	30	10 730	> 10
19	Honey			2	0.1	0.2	73	> 10
92	Rice	100	Australia		3.7 mg ± 1.4 (unwashed rice); 2.8 mg ± 0.3 (washed rice); 13.3 mg ± 2.5 (microwaved)			
213	Milk		Mexico	0.36 L/day	6.5 ± 2.3 MP/L	2.4	858	10–500
91	Beer	0.35L	USA		4.05 MP/L	1.42	520	> 100
19	Alcohol			0.04	32.27 MP/L	1.3	470	> 100
19	Bottled water			0.44 L/day	94.37 MP/L	40	15 155	> 5
19	Tap water			3.26 L/day	4.23 MP/L	13.8	5 030	> 100
1	Drinking-water			2L/day	10.4 MP/L	20.8	7 592	150
19	Inhalation					170	62 050	> 50

[a] Maximum or average value

[b] Concentration per meal, not per day

[c] Based on per capita consumption derived by the United Kingdom Department for Environment, Food and Rural Affairs *(227)*

[d] Based on per capita consumption derived by FAO *(227)*

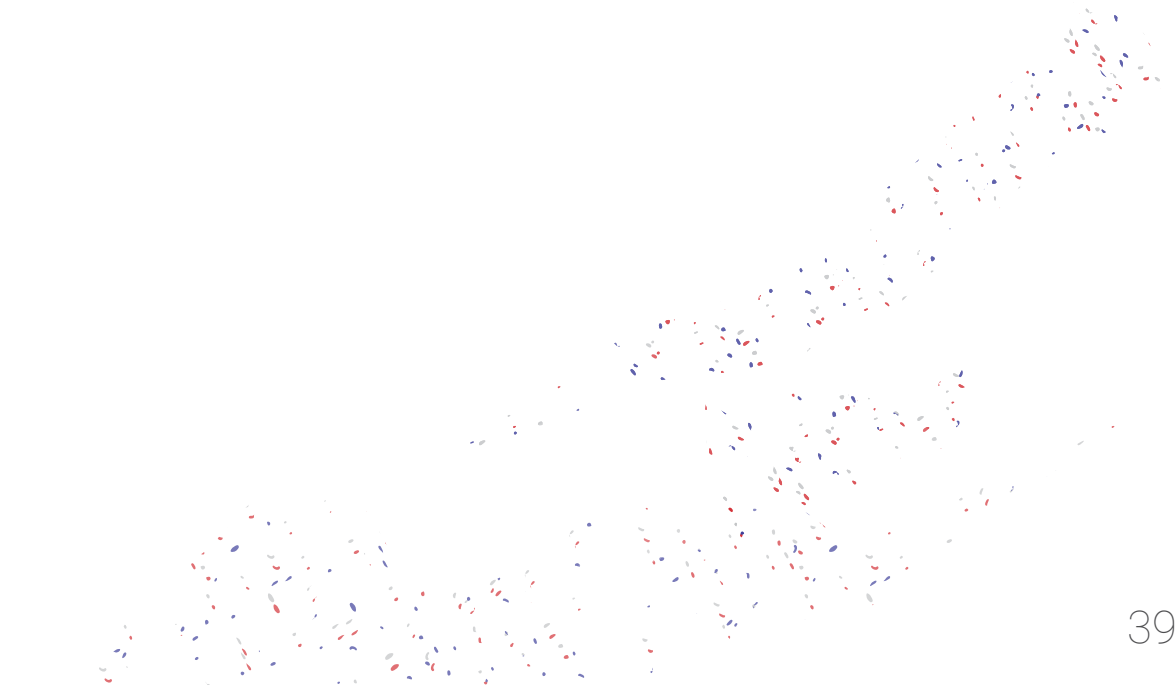
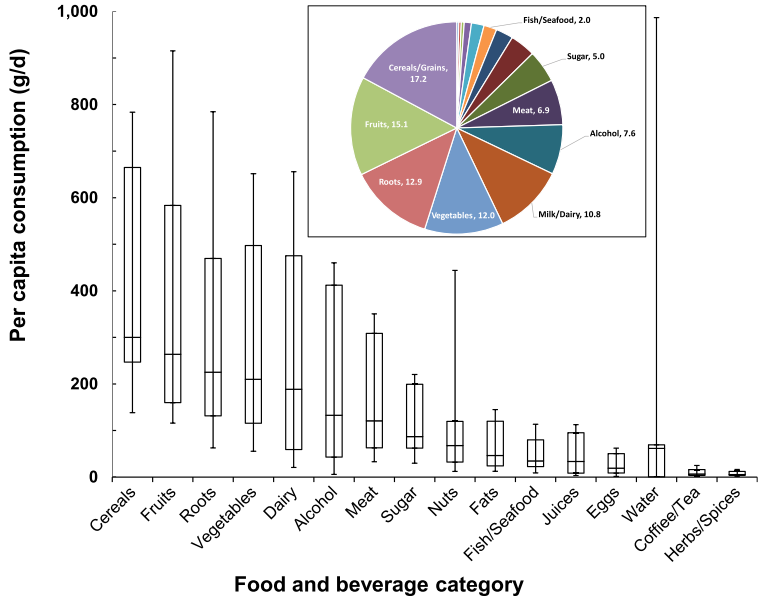


Fig. 4 Dietary consumption from 16 food categories derived from all 17 GEMS/Food clusters



Source: https://www.who.int/teams/nutrition-and-food-safety/databases/global-environment-monitoring-system-food-contamination, accessed 16 May 2020.
Inset summarizes the average percentage of each food category in total per capita dietary consumption of foods in all 17 GEMS/Food clusters

To address the lack of data on different food categories, standard methods should be developed for consistent, robust assessment of exposure. Table 4 summarizes the TAS of studies on MP in food and beverages. The only matrix for which substantial resources have been invested in developing an analytical method is seafood, although quality assurance and control are limited, with variation in sample sizes, reporting of concentrations in individual organisms, whether and how many samples were pooled, different methods for extracting MP from tissues, particularly with respect to tissue digestion, variation in processing of procedural blanks, filter substrates and pore sizes and different approaches to categorizing particles as MP, including both visual inspection and analytical verification of polymer composition.

Food categories other than seafood have been analysed by methods for which the performance has not been verified, with low TAS for studies of MP in sugar, honey, salt, beer and other beverages. Most of the reports did not provide details of the efficiency of recovery of MP of various shapes, sizes and polymer composition, which could result in underestimation of the concentrations. Furthermore, the samples of food items tended to be small (i.e., < 25 individual items) purchased on a single date from a limited number of suppliers, usually without production lot numbers or dates, raising concern about their use for extrapolating concentrations of MP in a specific food category. To assess dietary exposure, both the sample sizes and the temporal

and spatial trends should be substantially increased for a robust statistical analysis of variations in concentrations of MP.

The availability of standard analytical methods, consistent with the elements summarized in Box 2, is fundamental for assessing human dietary exposure to NMP and particularly quantification and characterization of particles < 10 µm. As summarized by the EFSA Panel on Contaminants in the Food Chain *(10)* and by FAO *(83)*, MP > 150 µm are unlikely to be absorbed, and uptake of smaller MP is expected to be < 0.3%. As discussed in section 3, particle size and shape influence systemic uptake, distribution and elimination. Particles > 10 µm that are inhaled, for instance, are generally understood to be trapped in the upper airways and subsequently swallowed. NP < 0.1 µm in the gastrointestinal tract can potentially be taken up systemically (7% estimated by FAO *(83)*), and the probability of egestion increases with size. Quantification of human exposure to NMP < 10 µm is essential, as the effects on human health increase with decreasing particle size. Table 5 indicates, however, that most studies have addressed exposure to MP > 10 µm, which are probably excreted directly *(1, 10, 83)*.

Research should also be conducted on the sources and characteristics of NMP in food and beverages in order to introduce effective, efficient measures to reduce exposure. In the characterization and quantification of MP in seafood, for instance, it appears to be assumed that the environment is the main source of contamination, whereby filter feeders, such as mussels, ingest and accumulate MP from contaminated seawater and sediment *(85, 254)*. Other studies suggest contamination during processing of food and beverages and from packaging. Li et al. *(263)*, for instance, reported significantly greater contamination of processed than of live farmed mussels, suggesting that MP were introduced during de-shelling and cleaning rather than by ingestion and accumulation from the environment. Karami et al. *(216)* suggested that tinned fish are contaminated during preparation and packaging. Kutralam-Muniasamy et al. *(213)*, Kosuth et al. *(91)* and Iñiguez et al. *(234)* suggested focusing on steps in the processing of milk, beer and salt at which contamination with MP might occur. Liebezeit and Liebezeit *(210)* suggested that MP and other anthropogenic debris are introduced into German beer during manufacture, whereas Lachenmeir et al. *(211)* proposed that a microfiltration step to remove yeast cells from processed beer would be sufficient to also remove MP. They further suggested that observations of MP in beverages such as beer are an artefact due to poor quality assurance and quality control, with contamination occurring by deposition of MP onto samples in the laboratory.

Deposition of MP from the air has been suggested as a further source of dietary exposure. In a comparison of direct exposure to MP from the consumption of mussels and exposure in household dust, Catarino et al. *(160)* estimated that exposure to MP due to deposition was more than two orders of magnitude greater than that from ingestion of contaminated mussels. As foods can be contaminated by deposition from air and/or in processing and packaging, additional research is necessary to characterize and quantify relevant sources of contamination. A number of studies have recently addressed the relative importance of food packaging (see for instance *92–94, 286–295*). The observations provide valuable preliminary insight into the role of plastic packaging; for example, heating plastic containers appears to

increase the release of NMP *(92, 93, 287, 289–291, 293)*, with pH also possibly playing a role *(94)*. Concern has also been expressed about the lack of standardized methods required for robust assessments *(292, 296, 297)*. More research on the role of plastic packaging should be conducted for quantitative assessment.

Methods for determining the polymeric composition of particles should be included in future studies. Of the 87 studies in Table 4 on MP in food, 27 did not provide confirmation that the particles were plastic, and 29 provided limited verification. Thus, only about one third of studies that reported the occurrence of "microplastic" provided satisfactory analytical verification. Widespread reporting of MP in food in the absence of verification is an obvious problem for estimating human exposure. For instance, in their analysis of MP in honey, Liebezeit and Liebezeit *(230)* reported an average concentration of 87 ± 73 items/500 g of honey and suggested that the honey was contaminated by plastic fibres attached to pollen, during processing of the honey or from packaging. They did not, however, provide analytical verification that the fibres were plastic, a concern raised by Mühlschlegel et al. *(231)*, who included analytical verification and reported limited contamination of honey by MP.

Estimates of dietary exposure to MP will require significant advances in:

- development of standard analytical methods appropriate for characterizing and quantifying various foods and beverages, with application for particles < 10 µm;
- targeted sampling to identify sources of contamination throughout manufacture, processing and packaging of foods and beverages; and
- characterization of the contamination of food and beverages by deposition during preparation and consumption.

Given the limited data on exposure to contamination in important food categories, illustrated in Fig. 4, an intelligent sampling strategy is necessary to clarify the differences among various sources of contamination and for robust quantification of human exposure. Contamination of cereals, grains, fruits and vegetables with NMP should be characterized, as some research suggests contamination from agricultural soils *(298, 299)*. Agricultural practices include use of a variety of plastic products (e.g., plastic film for mulching, vinyl tunnels, fertilizer bags) that may introduce NMP into agricultural soils and into products *(300–302)*. Application of biosolids to soil is also an important source of MP in the terrestrial environment *(300, 303)*.

Although concern has been expressed here about the quality of the reporting of MP in food and beverages, recent research by Mohamed Nor et al. *(124)* suggests a probabilistic approach to estimating human exposure. For instance, they estimated that the total daily median MP mass intake from nine media (fish, mollusc, crustacean, tap water, bottled water, salt, beer, milk and air) was 0.2 (0.0001–7500) µg/child per day and 0.6 (0.0003–17000) µg/adult per day *(124)*. Comparison with the estimate by the World Wildlife Fund that potential exposure to MP is 700 mg/person per day *(304)* suggests that the latter estimate represents the 99th percentile intake of an average person.

Current approaches to assessing human exposure to MP from food are all based on combining data on dietary absorption rate with data on the amount of MP in food components. In several studies, exposure was assessed by deterministic estimation of the total intake from all dietary components, *(19, 124, 304, 305)*. A major limitation

of these studies is use of databases with different definitions of MP and use of different analytical techniques. As a result, the data are inconsistent, including different ranges of MP size, obviating confident quantification of human exposure. In addition, exposure estimates based on average exposure rates do not reflect the actual distribution of global MP intake rates. These issues could be resolved in probabilistic approaches, such as that of Mohamed Nor et al. *(124)*. In this approach, lifetime exposure is assessed for intake of eight food types that represent 20% of the human diet by weight. Intestinal absorption, biliary excretion and plastic-associated chemical exposure were also estimated (section 5.2), in both MP number and mass concentrations. The probabilistic model provided estimates of MP concentrations in body tissues, gut and stool, the last of which could be compared with empirical data. To ensure the comparability of data on microplastic abundance, re-scaling was done to account for the different size ranges and types of particles targeted by the different methods used *(306–308)*. The simulated microplastic concentrations in stool were 15% of those in the available empirical data, which would appear to be consistent, as only 20% of the human diet was taken into account. This demonstrates the usefulness of probabilistic approaches for estimating human exposure.

2.5 Summary and recommendations

Human exposure to MP has been characterized in air, drinking-water, food and beverages, and research should be conducted to differentiate the sources of contamination. Given the ubiquitous exposure to MP, standard methods should be used to characterize and quantify NMP of the sizes considered most relevant to human health, i.e., < 10 µm. Most studies to date have addressed MP > 10 µm, and the results are incomplete for assessing risk, as the implications for human health increase with decreasing particle size.

Assessment of exposure by inhalation will require methods for quantifying and characterizing NMP associated with the respirable fraction of atmospheric particulates. Methods for sampling particles with aerodynamic diameters associated with $PM_{2.5}$ and PM_{10} could be used. Data on atmospheric deposition of MP indicate the potential sources and processes that influence environmental fate and transport and could be used to characterize the fraction of MP associated with ingestion of dust.

Studies of the presence of MP indicate that exposure occurs via inhalation and by ingestion of drinking-water, food and beverages, although the relatively small number of samples, food categories and populations limits a comprehensive assessment of exposure for the implications for human health *(4, 13, 141, 309, 310)*. Concern has also been raised about the quality of studies. Standard methods are required, with investigations of laboratory contamination, and which

ensure consistency in the analysis of particles within the relevant size range, such as 1–5000 µm, to allow comparison of studies and to estimate human exposure. A key challenge is characterization and quantification of NMP measuring < 10 µm. Significant analytical challenges include extracting, isolating and verifying the polymeric composition of particles of such sizes, and it is also likely that the background contamination increases with decreasing particle size. A robust quality assurance and quality control protocol is therefore necessary.

Key messages

- Human exposure to NMP is ubiquitous and occurs by all routes.
- Information on exposure from air, drinking-water, food and beverages is limited. Data on the characteristics of NMP and their quantification in each of these media are necessary, with better understanding of their sources.

3. OBSERVATIONS FROM EPIDEMIOLOGY

Within the Global Burden of Disease programme *(311)*, it was estimated that, in 2015, 4.2 million people had died prematurely due to exposure to airborne PM. The components of PM that represent the greatest risk to human health, however, are poorly understood, although a contribution of NMP cannot be excluded *(111)*. At present, exposure estimates and routine measurements for environmental epidemiological studies are lacking. Such studies should include exposure to NMP and to other types of particles (e.g., from combustion sources) and gases, with consideration of confounding factors. Effects due to long-term exposure can be measured only from exposure estimates over periods from years to decades, which would exclude retrospective studies. Effects of short-term exposure may be foreseen in the near future, with observation of spatial and temporal variation in the exposure of a population for whom sufficient information on health is available. Until then, the best information is from occupational epidemiology, in which subgroups of the general population who often experience exposure well above ambient levels are studied. As such studies usually do not include vulnerable people, the findings cannot be extrapolated to the general population; however, the findings can provide insight into pathology related to NMP exposure. Most regulatory jurisdictions define limits of exposure to particulates in the workplace, such as those in the United Kingdom of 10 mg/m^3 as an 8-h time-weighted average for inhalable dust and 4 mg/m^3 for respirable dust *(76)*. Like the guidelines set for PM, these exposure limits are for dust in general and are not specific for NMP. Studies of controlled exposure of humans to synthetic fibres and particles to simulate occupational exposure are extremely rare because of ethical considerations *(312, 313)*. Studies have, however, been conducted on people exposed occupationally to mixtures of various fibrous and non-fibrous plastic particles at high concentrations over extended periods *(314)*.

One of the earliest documented outbreaks of disease related to exposure to synthetic fibres by inhalation was reported in 1975 among workers in the textile (nylon, polyester, polyolefin, acrylic) industry (reviewed in *315*), in which workers showed symptoms of allergic alveolitis. Outbreaks of occupational interstitial lung disease have since been reported in the manufacture of nylon "flock" *(316, 317)*, which are short fibres produced for making velvet-like textiles and upholstery. When they are produced in a rotary mill, for instance, a substantial amount of respirable nylon dust is generated, leading to an average exposure concentration of 2.2 mg/m^3 *(314)*. Otherwise healthy, often young workers in this industry develop respiratory symptoms, including chest pain, shortness of breath and cough *(318)*. The bronchoalveolar lavage fluid of such workers shows an abnormal cellular profile. Nonspecific interstitial pneumonia is established, with accumulation of lymphocytes, lymphocytic inflammation of the bronchioles and in some cases proliferation of lymphocytes in alveolar tissues. Flock workers' lung is, however, a rare disease; for example, it has been diagnosed in 24 workers in North America *(317, 319–322)*. Although the condition is debilitating, it is usually reversible, and the respiratory symptoms stabilize

and ultimately improve after removal from exposure *(317, 320)*, which also suggest that the NMP are cleared from the lungs over time, unlike some other particles or fibres. In some cases, however, the condition evolves to fibrosis and respiratory failure *(317, 320, 321)*.

Similar pathological presentation and symptoms have been reported in workers exposed to other synthetic flock, such as polyethylene *(323)*, polypropylene *(324)* and rayon *(325)*, raising concern that exposure to high levels of non-specific polymeric organic fibres increases the risk of interstitial lung disease. Long-term occupational exposure to respirable cotton (and flax and hemp) fibres is also associated with lung disease, respiratory symptoms and loss of pulmonary function, and asthma and chronic obstructive pulmonary disease have been documented *(326)*. Although the adverse effects of synthetic fibres appear to be due mainly to their physicochemical properties and high concentrations, the adverse effects triggered by occupational exposure to respirable cotton fibres are suggested to be due to an endotoxin secreted by Gram-negative microbes on the surface of cotton fibres *(326)*.

It has been suggested that exposure in the nylon flocking industry increases the risk of lung cancer. In a retrospective study of 162 workers in a nylon flocking plant, the risk was three times higher than that of controls *(327)*. Moreover, workers in a polyester and polyamide fibre factory in France were found to be at statistically significantly greater risk of death from various cancers (n = 79; relative risk, 1.42; 95% confidence interval, 1.06 ; 1.89 for high exposure; and n = 105; 1.38, 1.05; 1.81 for previous exposure to polymer dust), irrespective of the level or duration of exposure *(328)*. These findings should be corroborated in a larger cohort study, with adjustment for confounding by individual smoking histories and other lifestyle factors to allow extrapolation to other exposure scenarios. A study of female textile workers in China, however, found no association between exposure to synthetic fibres and lung cancer risk *(329)*. It is therefore difficult to draw conclusions about the carcinogenic risk of exposure to microplastics.

Histopathological analyses of lung biopsy samples from synthetic textile workers exposed to various polymers showed not only interstitial fibrosis but also granulomatous lesions containing foreign bodies considered to be acrylic, polyester and nylon dust *(315)*.

Exposure to PVC particles has also been linked to disease, predominately in occupational settings. Inhalation of airborne PVC dust has been associated with interstitial lung disease, as determined by chest radiographic abnormalities *(330, 331)*. In a cross-sectional study of 818 PVC workers, forced expiratory volume in 1 s and forced vital capacity were inversely related to exposure to dust, after adjustment for age, height and smoking. The response was observed mainly in cigarette smokers, suggesting an interaction between smoking and PVC dust. The authors concluded that, while an average dust index (age × mg/m^3) of 12.9 caused a partial decrease in lung function (a loss of 53 mL over 20 years, in addition to losses due to age and smoking), workers exposed to higher levels might suffer an important loss of lung function *(331)*. Exposure for 60 days to total dust (of which PVC particles < 1 µm were the predominant component) at a concentration of 0.3–42 mg/m^3 (median, 2 mg/m^3) resulted in severe dyspnoea, a decrease in transfer factor (diffusing capacity), profuse

accumulation of macrophages with some haemorrhage and increased numbers of elastic and collagenous fibres in one individual *(332)*. The patient had had minimal exposure to low concentrations (0–3 mg/m^3) of airborne vinyl chloride monomer, for which pulmonary effects usually occur after exposure to higher concentrations over a longer period, and had not been exposed to asbestos or other known fibrogenic agents. The presence of PVC particles in the lung indicated that they were probably responsible.

Epidemiological studies have also been conducted on the potential relation between occupational exposure to plastic dusts and cancer. Demers et al. *(333)* reported an association between current exposure to plastic dusts and lymphocytopenia; however, later sequential analysis was not predictive, implying no clear association. Earlier studies identified a risk of cancer among women working in the plastics industry, and later studies investigated possible cause–effect relations *(334–337)*. Sorahan and Nichols *(336)* found no relation between occupational exposure to amine catalysts, non-flammable and flammable solvents, polyurethane dust, latex, rubber, curled hair or coir fibre, feathers or foam handling and the risk for lung cancer among female workers. Limitations to the studies have, however, been identified, including lack of data on smoking, uncertain exposure estimates, estimates only for exposure by inhalation and, in some instances, limited cohort size.

3.1 Summary and recommendations

Numerous epidemiological studies have been conducted among workers exposed to various NMP in the plastics and textiles industries. Studies of occupational exposure to particulates do not, however, reflect the exposure of the general population, and caution is warranted in extrapolating results for various types and concentrations of particles associated with occupational activities to indoor and outdoor environments. Data obtained from occupational epidemiology may, however, be helpful in identifying hazards and pathological adverse effects after long-term, elevated exposures to specific NMP. There is some evidence of specific lung pathology in occupational settings. There is inadequate evidence of the carcinogenic risk of exposure to NMP.

Key messages

- Evidence in the literature that inhalation or oral uptake of NMP can affect the gastrointestinal tract or other organs apart from the lung is limited and of inadequate quality.
- Better estimates are required of exposure of the general population to NMP and co-pollutants by inhalation and in the diet.

4. DOSIMETRY AND BIOKINETICS

Assessment of the risks posed by xenobiotics in studies in experimental animals usually requires extrapolation from high to low doses and extrapolation of data from animal species to humans. Extrapolation may require clarification of the exposure pathway. For example, if exposure to airborne particles results in deposition only in the upper respiratory tract, most, if not all, of the particles will be swallowed, resulting in oral exposure. The toxicity of those particles can thus be tested directly by oral exposure of experimental animals.

This section summarizes studies on how exposure, dose and biokinetics influence the uptake of NMP by the body, their distribution among organs and their clearance. Considerable research has been conducted on the biokinetics of particulates, including NMP of specific shape, size and polymeric composition. An important consideration is extrapolation of observations on well-defined particles to the concentrations and properties of NMP in the environment. This section provides summaries of studies on various types of particles. While caution should be exercised in extrapolating laboratory results to the heterogeneous mixture of NMP in the environment, the data can inform future studies and provide a perspective of the implications for human health.

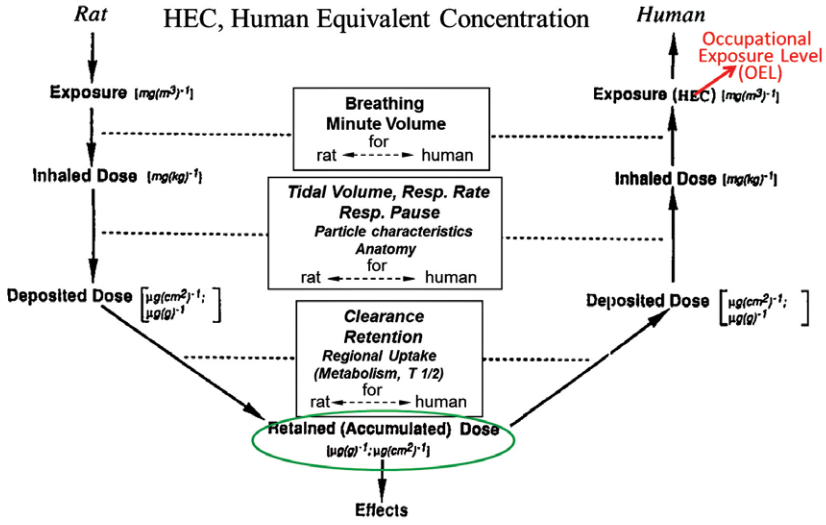
4.1 Dosimetry: extrapolation from external to internal exposure

4.1.1 Dosimetry: inhalation

Exposure-dose–response relationships are the basic model for assessing the effects of stressors, activity patterns and exposure on various biological end-points in assessing risks to human health. As toxicity is related to the dose of a substance, it is important to determine whether an external exposure can provide an internal dose capable of causing an adverse effect. This information is also required in extrapolating results obtained in vitro or in experimental animals to humans. The challenge is to simulate human physiology realistically, to evaluate the kinetics of particles in experimental animals and cells and to use biokinetic models to extrapolate measured doses in cells to humans in vivo *(129, 338–341)*. Fig. 5 provides an example of extrapolation of concentrations and doses from rats to humans.

The dose of particles required to elicit an adverse effect at the most critical biological target depends on factors that influence their deposition and clearance. “Particle deposition” refers to deposition onto tissues in the respiratory tract, which

Fig. 5 Attributes of NMP to be considered in assessing both exposure and hazard



Concept: *The same normalized retained lung dose in rats after acute, subchronic or chronic inhalation of poorly soluble particles is reached by humans exposed to the HEC. Effects may be different for both species.*

Source: reference *342* (https://creativecommons.org/licenses/)

is influenced by aerodynamic behaviour (inhalation) and thermodynamic or electrostatic interactions *(340)*. Although various metrics can be used (e.g., mass, number, surface area, volume), deposited mass is most frequently used to describe the relationship with a toxicological response *(308, 340)*. Clearance of inhaled deposited particulates depends on the initial site of deposition and the physicochemical properties of the particles, which determine the relative importance of specific biokinetics and biodynamics.

Considerable research has been conducted on the dosimetry of inhaled particles, resulting in clear understanding of the biokinetics of the deposition and clearance of insoluble particles such as carbon black, coal, diesel soot, talc and titanium dioxide *(341–343)*. Particle size, shape, density, surface properties (i.e., surface charge, area and chemistry) and the distribution of those properties influence the deposition of all types of particle, whereas clearance is related not only to the properties of the particle but also to its physiological location in vivo *(342, 344)*.

The epithelial surfaces of the respiratory tract are the largest interface between humans and the environment. For instance, the respiratory tract is estimated to cover > 100 m^2, while the gastrointestinal tract, the next most important interface, covers 30–40 m^2 *(345, 346)*. The epithelium of the respiratory tract is constantly exposed to a heterogeneous mixture of particles suspended in inspired air *(346, 347)*, and the fractions of respired particulates deposited in various regions of the respiratory tract have been investigated and modelled for a range of particle sizes and ventilatory patterns *(129, 131, 348)*. Many of these aspects are included in particle dosimetry models, such as the multiple-path particle dosimetry model *(349)*. The latter can be used to model particle deposition and clearance in humans (age-specific) and experimental animals (rat, mouse, rabbit, pig and monkey) for extrapolation of results from one species to another (Fig. 5).

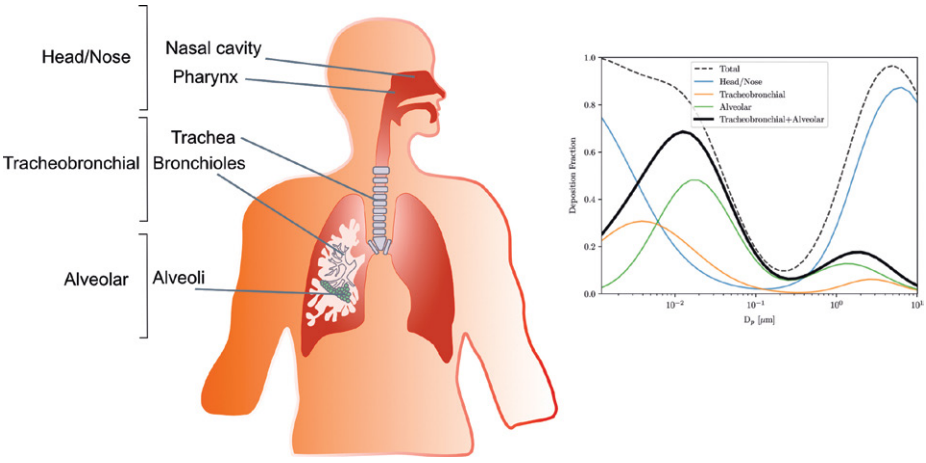
4.1.2 Particle inhalation models

The mechanisms of particle deposition in the respiratory tract include settling or sedimentation, in which the density of particles is an important factor; inertial impaction, in which changes in the direction of flow can determine whether particles deposit on surfaces; and diffusion, which is influenced by Brownian forces between gaseous molecules and particles, which cause particles to collide and deposit onto surfaces. Secondary processes of deposition include interception (typical for fibres) and electrostatic interactions between predominately positively charged particles and the negatively charged surfaces of the respiratory tract *(347)*.

Fig. 6 illustrates the relations among particle sizes deposited in different regions of the respiratory tract. Particles ≤ 10 µm (mass median aerodynamic diameter, MMAD, the diameter above and below which 50% of particles in the aerodynamic size distribution lie, according to mass) deposit mainly in the nasopharyngeal region. Smaller particles, including those measuring nanometres, are more likely to deposit in the alveolar pulmonary regions due to diffusion (Fig. 6) *(347)*.

In view of the heterogeneity of size, shape, density and surface charge of NMP, models may be useful for screening and identifying the most hazardous combinations of properties. The properties relevant for particle inhalation include a neutral or negatively charged surface and a relatively narrow distribution of densities, centred on about 1 g/cm^3. It is likely, for instance, that positively charged NMP interact with the larger fraction of naturally occurring negatively charged particles, causing them to aggregate into a larger, heterogeneous mixture of particles, whereas neutral NMP can be inhaled directly or undergo weathering to a predominately negatively charged surface. The material density of the most commonly encountered plastic materials (Table 1), such as polyethylene, polypropylene, nylon and polyester, is usually 0.85–2.3 g/cm^3, although the effective density in a matrix or an agglomerate may be lower. Particles of the same geometric diameter but with different (effective) densities

Fig. 6 Main regions of particle deposition in the human respiratory system and modelled deposition of a 1-g/cm³ spherical particle in relation to the diameter of the particle



Source: Adapted from references *347* and *349*

D, diameter

have a different deposition pattern: lower densities result in smaller aerodynamic diameters.

All the mechanisms of deposition are strongly influenced by the pattern of breathing, with significant differences between breathing patterns at rest and during exercise in individuals of different ages and sex *(347)*. Fig. 6 shows important differences in the deposition pattern of particles. For particles > 2 µm MMAD, there is a stronger (but not exclusive) likelihood of deposition in the upper respiratory tract (head, nasopharynx) and upper airways (tracheobronchial), while particles measuring 0.01–1 µm are deposited deeper in the lung (pulmonary, alveoli, gas exchange area). The deposition of particles in relation to their size distribution can be compared with the typical particle size distribution of PM in an urban area.

The dose of particles in the respiratory system therefore depends on their (aerodynamic) size, micrometre-sized particles being deposited mainly in the upper respiratory tract and particles > 10 µm MMAD being less likely to move beyond the nose and mouth. Most deposited particles with a diameter > 2.5 µm MMAD will be transferred to the digestive system, as they tend to be swallowed, whereas smaller particles are more likely to interact with tissues of the alveoli, where they can be engulfed by alveolar macrophages and transported to the mucociliary escalator or become senescent. The main mechanism for deposition of nanoparticles is diffusion, indicating that slow air speed such as in alveolar spaces and near the olfactory epithelium will result in a higher likelihood of deposition. Typically, there is little deposition when diffusion and impaction are less dominant, which is the case

for particles of 0.2–1.0 µm MMAD. Monitoring of the physicochemical properties, particle size distribution, density and surface properties of NMP in air would add important data for models of particulate inhalation and support characterization and quantification of human inhalation exposure.

Particle shape should also be considered, as experimental data indicate that fibres reach the lower airways more efficiently than spheres *(350–352)*.

4.1.3 Particulate inhalation: experimental studies

Inhalation of particulates, including NMP, by experimental animals in vivo has been studied for several decades. For instance, Stemmer et al. *(353)* administered a single dose of 5 mg polyurethane dust to weanling and 9-month or older rats to assess the pulmonary inflammatory response. The dose was based on the total amount of dust estimated to be inhaled by rats exposed for 8 h per day for 30 days; the MMAD was < 5 µm for 52% of the particles and < 10 µm for 93.5%. Exposure was by intratracheal instillation, a commonly used method for introducing materials into the lungs of test animals because of its simplicity, relatively low cost, delivery of a well-defined dose, delivery to the rodent lung of particles that are not respirable by rodents but are relevant to humans (such as long fibres) and for practicality and safety *(354)*. This method of exposure does not, however, represent physiological exposure, limiting extrapolation for evaluating pulmonary toxicity. As summarized by Driscoll et al. *(354)* in a critical review, there are inconsistencies between dosing by instillation and by inhalation, and data based on instillation should be interpreted cautiously for evaluating respiratory toxicity; limiting assumptions should be stated and guidelines followed carefully. These caveats should be kept in mind in interpreting the toxicological data summarized in section 5.

More recent studies have involved approaches for better understanding the adverse effects of exposure dose and specific stressors. For instance, Xu et al. *(355)* compared the adverse effects of spherical PVC particles measuring 0.2–2.0 µm and crystalline fragments of silica measuring 0.5–3.0 µm. Crystalline silica particles were used as a positive control because of their strong association with silicosis, lung cancer and autoimmune diseases, the crystalline structure of the particles accounting for their pathogenicity *(356)*. Xu et al. *(355)* administered the particles to male Wistar rats by intratracheal instillation at 10 or 50 mg/kg body weight (bw). The differences in the adverse effects of PVC and silica particles were attributed to differences in their biokinetics, PVC particles being cleared more efficiently than those of

crystalline silica. The mechanism of the slow clearance and biopersistence of silica particles is poorly understood; however, it would appear that the surface-dependent properties of silica particles, such as the reactivity of silanol groups and changes in surface area that increase surface reactivity due to particle fracture, influence clearance rates *(356–358)*.

Translocation of NMP from the respiratory tract epithelial surfaces has been proposed, with concern about the ultimate fate and potential accumulation of NMP in the body. The size of insoluble particles influences their translocation into interstitial spaces, decreasing size being associated with greater potential translocation. Ferin et al. *(359)* observed that particles measuring 20–30 nm penetrated the interstitial space more easily than those of 200–500 nm. In a study of polystyrene nanoparticles (average diameter, 56.4 nm and 202 nm), Chen et al. *(360)* gave male Sprague-Dawley rats 0.6 mg of radioiodinated polystyrene particles by intratracheal instillation and found that only a small fraction passed rapidly into the systemic circulation but that translocation was markedly increased after infusion of lipopolysaccharides to induce pulmonary inflammation. The use of radiolabelled particles helps to characterize and quantify the fate and transport of particles and thus strengthens understanding of the mechanisms of translocation, including physiological processes (e.g., pulmonary inflammation) in relation to the size and shape of particles.

The shape of NMP is important *(361)*. Although atmospheric monitoring indicates exposure to a wide range of fragments and fibres *(136)*, most experimental studies of the adverse effects of NMP in animals have been conducted with spherical particles; a few studies involved exposure to respirable fibres. Warheit et al. *(362)* exposed male rats (Crl:CD(SD)IGS BR) to aerosols of nylon-66 respirable fibres at 0, 4, 15 or 57 fibres/cm^3 by nose-only inhalation for 6 h/day for 20 exposures. The nylon-66 fibres were described as white, 18-denier, trilobal, chopped and ground fibres > 99% pure with a mean length of 9.8 µm and a diameter of 1.6 µm. The results showed rapid lung clearance over 12 months, with a no-observed-effect level of 57 fibres/cm^3, the highest concentration tested.

In general, complementary use of dosimetry models and careful study design, including controls, are required to understand the mechanisms of effects on human health of inhalation of NMP (see section 5). NMP that are representative of environmentally relevant exposure should be characterized and quantified.

4.1.4 Dosimetry: ingestion

The physicochemical properties of particles, such as size, shape, surface area and chemistry, are usually measured in a dry state and then used to compare a biological response to an oral dose *(363, 364)*. In the same way as for inhalation, the relation between particle properties and an adverse effect may, however, be influenced by interactions between particles and between particles and the physiological fluids or food suspension used to deliver the dose *(364–368)*. Dispersion of particles in the dosing medium is therefore an important factor to consider; homogeneous dispersion allows robust interpretation of results *(363, 365, 368)*. The fate of particles in the dosing medium influences various dose metrics, including the actual delivered mass and particle number *(364, 366, 369)*. Delivery of particle stressors to cell systems in vitro or to animals in vivo to evaluate their fate after oral ingestion may vary, and standardized methods are necessary for comparisons and evaluations, not only between in-vivo test systems but also for quantitative in vitro to in vivo extrapolation (QIVIVE).

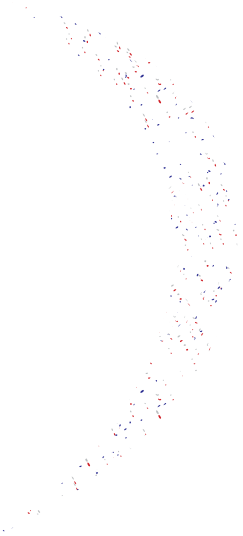
4.1.5 Dosimetry: in vitro

Cell culture systems, which are simple models of complex biological systems, have provided valuable insight into the biological, physiological and pathological responses of cells exposed to chemical and physical stressors; however, these systems have limitations, including uniform distribution of a dose, particularly for particles *(363, 364, 368, 370, 371)*. Administered particle doses are usually reported as an initial mass or number concentration, which does not represent the dose actually delivered to cells over time *(371)*. Actual exposure is to particles that come into direct contact with cells and can trigger an adverse effect, which is not necessarily equivalent to the nominal concentration of particles in the medium *(370)*. The mass, surface area or number of particles delivered to receptor sites on the cell surface or the corresponding area under the time–concentration curve reflect the dose at the site of action better than the nominal media concentration *(338, 371)*. The delivered dose is also determined by interactions between the physicochemical properties of the particles, the properties of the medium in which the particles are suspended and the duration of the test. Particles are subjected to dispersive forces in the test medium, which can result in formation of larger agglomerates. This in turn influences the effective density of the particles, further affecting deposition kinetics. Standard methods are thus required for dispersion and dosing of NMP in in-vitro systems to ensure adequate analysis of adverse effects *(369, 371–373)*. The properties of the cell systems themselves should also be considered, as

the presence of a sticky mucus layer, such as in co-cultures of epithelial and goblet cells, or the presence of macrophages can influence dose deposition and uptake kinetics, whereas monoculture cell systems are devoid of mucus *(364)*.

Interpretation of data from in-vitro models often depends on the nature of the exposure and the complexity of the models *(367)*. Most systems involve submerged cultures. To ensure quality and for extrapolation to an equivalent human dose, several critical parameters should be reported to allow computational modelling of the dose and dose rate, essential for extrapolating data to in-vivo models:

- the biologically effective dose *(369–371)*, i.e., the number or mass of particles in direct contact with a certain number or surface of cells (cm^2);
- the dose rate and duration of exposure *(371)*;
- the size (and size distribution) of the primary particles and the agglomerates or aggregates *(16, 371, 374)*;
- sample preparation (e.g., use of proteins to disperse the plastics stably and sonication, which may generate reactive oxygen species) *(16, 374)*; and
- the presence of contaminants, such as chemical toxicants or endotoxins *(16, 374)*.



Other requirements may be added, according to the study hypothesis. For example, a study of the relevance of size for uptake into cells would require at least two sizes of plastic of the same composition and appropriate controls for assessing the influence of surface properties such as area, charge and reactivity *(374)*. A special case is use of "air–liquid interface" exposure systems, in which cells are exposed to NMP via an aerosol under static or dynamic flow conditions, resulting in a more realistic but also more complicated system to assess the deposited dose. Some exposure systems include a microbalance in parallel to the exposed cell surface, which gives a fair indication of the dose delivered over an exposure period.

An appropriate dose range is particularly important for in-vitro studies, because cell monocultures or co-cultures differ from cells in an organ and from physiologically relevant oral and inhalation exposure *(364)*. There is currently no standardized method for determining the dose to be administered in vitro that is equivalent to published estimates of human exposure.

Table 6. Criteria for evaluating the reliability and quality of in-vivo and in-vitro studies on the effects and biokinetics of nano- and microplastic particles

Criterion	Comment
Human exposure characterization	See section 2. Exposure must be characterized to identify environmentally relevant concentrations and properties of NMP. Specifically, size, shape, polymer composition and surface properties should be quantified and relevant exposure pathways (inhalation, oral, dermal) characterized.
Characterization of NMP biokinetics	• particle size distribution • agglomeration state • particle shape • chemical composition • particle surface area • surface chemistry and charge
Characterization of NMP after administration	Important to increase the quality of data on the relation between dose and an adverse effect, as the physicochemical properties and dose may change during administration in either in-vivo or in-vitro test systems.
Method of administration	Details of how particles are introduced into an in-vivo or an in-vitro test system, i.e., type of inhalation exposure, such as intratracheal or nose-only, solvent or delivery vehicle used, method of aerosolization, sample volume, cell surface
Duration of exposure	Times of observation and, ideally, particle properties at each time
Use of negative and positive controls	Necessary to evaluate the performance of the study by demonstrating that the adverse effects associated with particles, such as crystalline silica (positive control), are consistent with other observations and that negative controls, such as a solvent or vehicle, provide an appropriate baseline for interpreting adverse effects.

4.1.6 Dosimetry: summary and implications

This section addresses the dosing of NMP for assessing the adverse effects of their inhalation and ingestion and characterizing and quantifying the biokinetics. Several methods for the dosimetry of particles are summarized, with their challenges and implications. Dosimetry is critical to the quality of a study. A testing strategy will contribute to hazard identification and risk assessment only if the dose, route of exposure and particles are fully characterized *(374, 375)*. The principles are summarized in Table 6. Many are included in tools for evaluating toxicity screening methods, such as the modified version of the ToxRTool *(374)* and the NMP toxicity study assessment tool *(16)*, which was used to evaluate the quality of studies cited in section 5.

4.2 Biokinetics

Once a foreign substance enters the body by inhalation or ingestion, it may or may not cross the biological barriers and be distributed in the body. Some substances accumulate in lipid-rich tissues, for instance, while others are readily eliminated via the urine or bile, transported to the gastrointestinal tract and excreted in faeces. The process that

influences physiological uptake, distribution and elimination throughout the body is known as biokinetics. Metabolism, another important biokinetics mechanism, is not expected to influence the physiological fate of NMP *(83, 86, 376)*.

4.2.1 Inhalation

After deposition of NMP in the respiratory system (Fig. 6), they are cleared by various mechanisms that effectively eliminate inhaled particles. These include mechanical mechanisms, such as sneezing, mucociliary clearance, phagocytosis by alveolar macrophages and lymphatic transport *(312)*.

The basic clearance processes are solubility, macrophage function, mucociliary transport, cellular endocytosis, intercellular sieving and lymph and capillary blood flow. The site of deposition in the respiratory tract and the physicochemical properties of particles, such as size, shape and surface reactivity, influence the clearance mechanism *(129, 131, 347)*.

Numerous studies have been conducted to characterize the clearance of insoluble particles deposited on surfaces in the lower respiratory tract (see, e.g., *342, 377*). Clearance is generally understood to occur in two phases: an initial phase with a half-life of 3–12 h in the tracheobronchial region and a second, alveolar phase that may last several months or longer *(347)*. In experiments with whole-body exposure of rats to PVC particles at 8.3 mg/m^3 for 25 h/week for 7 months, the average mass of PVC retained in the lungs 1 month after cessation of exposure was 2 mg/lung *(378)*. Marked differences in clearance kinetics at high dose levels have been observed between rodents and humans, and caution should be taken in extrapolating observations from experimental animals.

Mucocillary clearance of particles depends on the mobility of mucus, a viscoelastic secretion that protects the mucosa from dehydration and provides a medium for inhaled particles. As a result of constant ciliary action, the mucus flows and is eventually cleared from the airway and transferred to the digestive system, from which particles are egested *(341, 347, 379)*. Inhaled particles that reach the alveolar region of the lung can be transferred into the interstitium. Their presence at the epithelial surface can also stimulate chemotactic signals that attract alveolar macrophages to the site of particle deposition, where phagocytosis by the macrophages initiates particle clearance from the alveolar region. Alveolar macrophages, however, have a finite lifespan and decay, releasing any undissolved particles for phagocytosis by another alveolar macrophage. Particles that cross the alveolar epithelium into the interstitium may encounter interstitial macrophages, initiating a process similar to that for alveolar macrophages. A fraction of particles can also be transferred from the interstitium to lymph nodes. Modelling of this process is informative for assessing risks for human health of inhaled particulates *(341, 379)*. Another fraction of particles may be retained in the interstitium.

Kevlar para-aramid fibrils have been shown to be biodegradable in the lung, as the recovered fibres appeared to be “shorter” than the original fibres *(380)*. The Kevlar fibres were reported to have a half-life in the lung of 30 days. The mean length of

Kevlar fibres recovered from digested lung tissue decreased from 12.5 µm to 7.5 µm over 6 months after exposure, and the mean fibre diameter decreased from 0.33 µm to 0.23 µm. Warheit et al. *(362)* assessed mucociliary clearance of nylon fibres in a 4-week nose-only study in rats and measured the recovery and dimensions of fibres several times after exposure. They reported a rapid decrease in the number of recovered nylon fibres at 3, 6 and 12 months after exposure (from an exposure concentration of 57 fibres/cm^3). In this study, the fibre lengths did not change up to 180 days after exposure, indicating that biodegradability does not affect lung clearance of nylon fibres. Rapid lung clearance of inhaled nylon fibres was, however, reported, with an estimated half-time clearance of inhaled fibres of about 2 months at the high exposure and 1 month at the medium exposure.

Recently, MP were detected in lung tissue collected during routine coroner autopsies of 20 non-smoking adults aged 48–94 years *(381)*. A total of 31 synthetic polymer particles and fibres were observed in 65% of individuals, dominated by fragments with a mean particle size of 3.92 ± 1.96 µm. Polyethylene and polypropylene were the main plastic polymers detected, and 16% of particles were identified as cotton. Although inhalation is understood to be the most likely exposure route for particles observed in the lung, Amato-Lourenço et al. *(381)* did not rule out the possibility that some particles may reach the lungs by systemic translocation.

4.2.2 Ingestion

Particles that are ingested are considered to be available systemically only when they are absorbed by the intestinal epithelium, pass through the liver and are distributed via the bloodstream throughout the body. A number of physiological barriers significantly limit the absorption and systemic bioavailability of particles from the gastrointestinal tract, although local absorption may occur. As discussed in the previous WHO report *(1)*, microplastics > 150 µm ingested from drinking-water are expected to pass through the gastrointestinal tract without being absorbed.

A fundamentally important physiological barrier in the gastrointestinal tract is mucus, a selectively permeable hydrogel that acts as a physical barrier to particle diffusion across the epithelial tissues. The main structural component of the mucus layer is mucin, a highly glycosylated protein with oligosaccharide side-chains that include terminal sialic acid and sulfate residues, resulting in a net negative charge *(382)*. The average pore size of the mesh-like structure formed by the interactions of mucins is 10–500 nm. The mucus layer significantly impedes the diffusion of small particles by interaction filtering (i.e., electronic and hydrophobic interactions) and can fully block the penetration of larger particles by both steric (i.e., size) and interaction filtering. The rate of passage of particles along the gastrointestinal tract

is usually effective in ensuring that most ingested particles are excreted. Szentkuti *(383)* studied the rate of diffusion of carboxylated fluorescent latex NP of various sizes across the mucus layer to the enterocyte surface and found that 14-nm particles passed through the mucus layer within 2 min and 415-nm particles within 30 min; however, micrometre-size particles did not diffuse through the mucus. Although the author observed permeation of both 14- and 415-nm particles, none of the particles was endocytosed by the enterocytes but appeared to move in the opposite direction with mucus. Therefore, while particle size is an important factor in permeation of mucus, the accumulation of particles on the enterocyte surface is insufficient to trigger phagocytic events, and the mechanism probably does not represent a significant translocation pathway.

O'Hagan *(384)* identified several physiological sites of particle uptake according to their size, by ordinary enterocytes, intestinal macrophages, the epithelium of Peyer's patches and the villus tips. The main function of the epithelial cells (enterocytes) is to absorb and transport nutrients for systemic distribution *(385)*; however, they can also endocytose particles in the nanometre range, such as partially digested dietary ferritin (~12 nm in diameter) from meat and plant foods *(386)*. In an early study, Sanders and Ashworth *(387)* suggested that ordinary enterocytes are responsible for the uptake of 200-nm polystyrene particles in rats, although Jani et al. *(388)* proposed an upper size limit of 100 nm for uptake by enterocytes. Garrett et al. *(389)* also observed uptake of polymeric nanoparticles by enterocytes in the mouse gut in vivo, using 30–50-nm ammonium palmitoyl glycol chitosan particles. The particles were then transported to the liver through the circulatory system and were recirculated through the bile to the small intestine and excreted in the faeces. According to Yoo et al. *(390)*, the upper particle size limit for endocytosis is about 500 nm.

It has been proposed that particles > 500 nm are absorbed by intestinal macrophages *(390)*, as observed for 1-µm polystyrene microplastics in dogs and rats *(391)*. Uptake of particles > 1 µm is, however, currently considered to occur most frequently across specialized "microfold cells" in Peyer's patches, which are domed regions that form gastro-associated lymphoid tissue *(384, 392)*. There are, however, important differences in the rate of uptake by enterocytes and by Peyer's patches in the large and small intestine *(393–395)*.

Microfold cells sample lumenal microparticles such as viruses and bacteria (1–10 µm) and transport them to

the underlying lamina propria by a process known as "transcytosis" *(396)*. Most studies on transcytosis have been conducted with latex microplastics. As latex has no pathogenic activity, observation of their absorption suggests that microfold cells are "particle agnostic" and are possibly influenced by the physicochemical properties of particles, such as size and surface charge *(396)*.

Translocation and absorption of microparticles < 10 µm across microfold cells has been observed after active phagocytic transport of various inert materials from the gut lumen, with the particles migrating to the blood via mesentery nodes and the thoracic lymph duct *(397)*. Microfold cells therefore not only represent a conduit through which particles can permeate Peyer's patches but also play a key role in the body's immune surveillance, actively sampling the contents of the gastrointestinal tract for potentially harmful particulate antigens. This implies that particles transported by microfold cells first enter the lymphatic system and that this immunological barrier facilitates clearance of foreign substances by immune cells *(394, 398)*.

The rate of uptake across Peyer's patches depends on the size of particles. After a single oral dose (12.5 mg/kg) of polystyrene microspheres to female Sprague–Dawley rats, uptake was rapid for those measuring 50 nm, moderate for 500-nm and slow for 1-µm microspheres *(388)*. After the Peyer's patches, particles are translocated towards the mesentery node via mesentery blood and lymph vessels (10 days after daily dosing) *(399)*, and 50-nm fluorescent polystyrene microspheres were found in the mesentery networks, their concentration peaking 12 h after administration; the levels of 500-nm microspheres peaked between 12 and 18 h after administration. It is hypothesized that particles enter the mesentery network and vessels via phagocytes and open lymphatic tubules in the Peyer's patches. After 18 h, particles were present in liver and spleen *(388)*. While 1-µm spheres were present at lower levels, they persisted in Peyer's patches, the mesentery network and nodes for 36 h, and most of the 50- and 500-nm spheres were transported to the liver and spleen within 24 h.

Another mechanism of translocation of microplastics of the size of those in foods and beverages is villous uptake. Evidence of this mechanism was obtained over a century ago in studies of various microparticle types. A phenomenon referred to as "persorption" has been described, which is passive absorption of microparticles measuring 5–150 µm from the intestinal lumen through gaps in the mucosa that

may result from mechanical kneading of single-layered intestinal epithelium resulting from cell shedding, particularly at desquamation zones of the villi *(383, 400–402)*. Volkheimer *(400, 401)* conducted many experiments in animals and in humans and observed that starch particles up to 130 µm in diameter could be detected in blood after ingestion. Following persorption, particles are immediately removed from the intestinal wall into the lumen of blood and lymph vessels and are finally excreted; 12 h after ingestion of a starch suspension, for instance, only a few starch granules were observed in peripheral blood, and negligible numbers were present after 24 h *(402)*. The phenomenon of persorption and its influence on uptake of MP should be studied further with standardized methods and materials. Generally, however, absorption of particles > 10 µm is considered to be negligible, whereas absorption of particles < 10 µm increases with decreasing size. In a review in 2012, Carr et al. *(403)* concluded that the mechanism of uptake of small microparticles (1–5 µm) is almost entirely villous, at least after a single exposure.

Overall intestinal absorption of MP is reported to be low. Carr et al. *(403)* conducted experiments in various rodent species and observed that only 0.04–0.3% of latex MP measuring 2 µm was absorbed. Pathological tissue, e.g., tissue from patients with inflammatory bowel disease, may transport more particles than healthy tissue (0.45% as compared to 0.2%), as shown by Schmidt et al. *(404)* in human colon tissue in vitro in an Ussing chamber. The reason was suggested to be greater permeability of gut tissue in inflammatory bowel syndrome than in healthy tissue. Stock et al. *(405)* investigated the uptake of 1-, 4- and 10-µm polystyrene MP in vitro in the Caco-2 monolayer, the microfold cell model with specialized cells and the mucus model, and in vivo in HOTT mice. Consistent with the observations of Carr et al. *(403)*, negligible numbers of particles were found in cells of the jejunum and duodenum of mice in vivo *(405)*, and no particles were found in any other organ. Caco-2 cells took up more 1- and 4-µm particles (≤ 0.8% and 3.8% of total particle recovery, respectively) in vitro, whereas few 10-µm particles were found (0.07% recovery). The authors suggested that fewer 1-µm particles were absorbed because they were taken up only by phagocytosis, while the 4-µm particles may have been absorbed by both phagocytosis and micropinocytosis, although the 1-µm particles may also have lower settling rates, reducing their availability for absorption. Furthermore, the surface chemistry of the two particle sizes differed, and greater absorption of 4-µm particles might have been due

to the presence of a sulfate group. Both the 1-µm and the 4-µm particles were recovered at significantly higher rates in co-cultures than in the Caco-2 monoculture. The authors concluded that only a minor fraction of small MPs enters the intestinal wall *(405)*.

The potential for NMP to cross the intestinal barrier is thus understood to occur in a size-dependent manner whereby the uptake and transport of particles measuring up to 5–10 µm into intestinal cells is possible and intracellular uptake of larger particles is unlikely, because of incompatibility with the size of intestinal epithelial cells (about 10 µm) *(310)*. Consistent with observations by EFSA *(3)*, FAO *(66)* and the previous WHO report *(1)*, NP may thus be absorbed, although the rate of absorption appears to decrease with increasing particle size, becoming negligible for particles > 150 µm. Caution should, however, be exercised in extrapolating from the results of the available studies, which are limited to a homogeneous group of polymers and sizes that do not necessarily represent the heterogeneous mixture of NMP encountered in the environment.

Polystyrene NP, for instance, have been used in numerous studies of toxicity in mammals in vivo and in vitro (reviewed in section 5). The oral bioavailability of 50-nm and 100-nm neutral and positively and negatively charged particles was investigated by Walczak et al. *(406)* in vitro. Size was a major determinant of translocation of NP, up to 7.8% of 50-nm NP and 0.8% of 100-nm NP being translocated. Surface charge and chemistry were also found to influence translocation. A study in rats in vivo by the same group indicated ≤ 1.7% particle uptake in kidney, heart, stomach wall and small intestine wall, which was less than that in the in-vitro study *(407)*. Sinnecker et al. *(408)* added fluorescent latex NP of various sizes to isolated perfused rat small intestine. They found no particles in the vascular or lymphatic systems but a significant amount in lumen samples. The results indicate that intestinal tissue provides a sink function for NP. Most particles were detected in the mucus lining and did not permeate the epithelium. The authors concluded that a healthy small intestine provides an effective barrier against NP uptake and is strongly influenced by the health of the mucus film layer.

The rates of uptake and translocation depend not only on size but also on intestinal location and time. Oral exposure of male Sprague-Dawley rats to a single dose of polystyrene microspheres (1.6 µm; 1.65×10^9 microspheres per animal) resulted in maximum concentrations (representing 1.4%

of the initial dose) in the proximal segment of the Peyer's patches 0.5 h after administration. The percentage of microspheres taken up by Peyer's patches decreased with both location (i.e., towards the distal regions) and time *(409)*. In a study with polymethyl methacrylate nanoparticles, 95% of the dose administered to rats was eliminated within 48 h *(410)*.

In a study of eight individuals, a median of 20 MPs/10 g of stool was observed, and the particles detected ranged in size from 50 to 500 µm *(411)*. More recently, Zhang et al. *(412)* reported detection of MP in the stool of 96% of participants (n=26) at concentrations ranging from 1 to 36 MP/g and sizes from 20 to 800 µm. These two studies provide only preliminary assessments of human exposure to MP and subsequent elimination and recommend that more robust mass balance studies be conducted to characterize and quantify excretion as an effective elimination pathway and possibly for use in biomonitoring to improve understanding of human exposure. Additional research should be conducted on NMP and particularly on their retention and translocation.

4.3 Biokinetics: summary and recommendations

To better understand exposure to and the main mechanisms of biodistribution of NMP from experimental results in animals, consideration should be given to use of experimental data, such as from in-vitro bioassays, in biokinetic models, such as physiologically based pharmacokinetic (PBPK) models and their use to complement understanding of how NMP are retained, cleared, translocated and distributed in the body *(367, 413, 414)*. PBPK models that have been developed for engineered nanomaterials could be used to estimate the retention, clearance and translocation of NMP. Use of existing models would indicate similarities in behaviour and also, perhaps more importantly, differences. By understanding the physicochemical properties of NMP and the physiological processes that might influence NMP and nanomaterials differently, research can be directed to assessing the implications for human health.

Whether NMP are inhaled or ingested, their biokinetics is strongly influenced by their size, shape, density and surface chemistry. The experimental data summarized above suggest that caution should be exercised in extrapolating from the limited data available, but they are sufficient to conclude that MP > 150 µm are unlikely to be absorbed and that absorption increases with decreasing particle size, via oral exposure. MP < 1 µm, which include the nano-sized fraction, are most likely to be absorbed, but characterization and quantification of uptake are limited.

Research to strengthen exposure assessment would complement dosimetry and biokinetics. Specifically, quantitative data on the (rate of) translocation of NMP and on size distribution, shape, polymer composition and surface chemistry in air, food and beverages, including drinking-water, are necessary to determine the properties

of NMP that are most relevant for use in studies of biokinetics and effects. This will require different measurement techniques according to the media in which the NMP are dispersed.

- Physiological mechanisms for the uptake, distribution and elimination of MP minimize tissue exposure. The probability of uptake into the body increases with decreasing particle size.
- There is insufficient information to assess biodistribution (uptake, retention, clearance, rate of translocation), including the likelihood that NMP will cross biological barriers after deposition on the epithelium tissue or after reaching the circulation
- Dosimetry models are available for extrapolation of results on particle inhalation obtained in experimental animals to humans, but they have not been evaluated or validated for NMP.
- Data on the biokinetics of NMP obtained in models in vitro cannot currently be extrapolated to the situation in vivo.

5. TOXICOLOGICAL EFFECTS

The sections above show that MP are ubiquitous in the environment but that the data on exposure in the diet and by inhalation are insufficient for quantitative assessment of exposure. Furthermore, there is concern about the use of non-standard methods for generating the data and the fact that monitoring has been mainly of particles measuring > 10 µm (section 2). There is thus great uncertainty about human exposure to biologically relevant NMP measuring < 10 µm. We recommend that research be conducted on the adverse effects of NMP in studies which account for their dosimetry and characterization, with quantification of the properties of particles, such as size, shape, surface properties and polymer composition, and biokinetics (section 4).

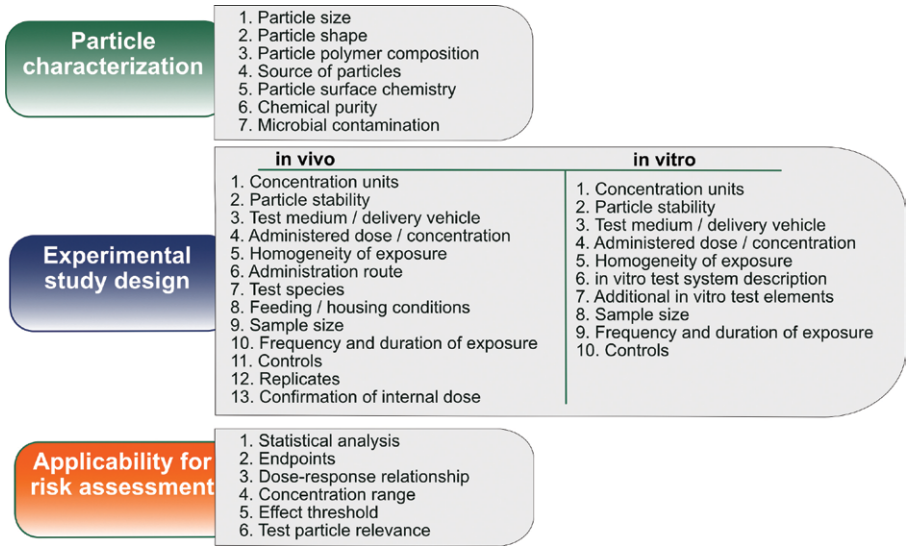
Although uncertainty about human exposure to NMP is a significant barrier to assessing risks to human health, several studies have reported adverse effects both in vivo and in vitro, and occupational epidemiological data are available *(11, 12, 26, 195)*. Better understanding of the toxicological effects of NMP will require studies of the relations between particle properties and their toxicity. The physicochemical properties of particles, such as their size, shape and surface chemistry, are understood to contribute to some toxicological end-points. Thus, the toxicity of a fragment or fibre can be attributed to interaction of the particle with tissues, the effect of a chemical or biological contaminant on the particle, including desorption of chemicals or pathogens on the surface, and the complex interaction of several factors *(415)*. This section summarizes studies on the toxicological effects of plastic particles and fibres, particularly those due to physical interaction with particles.

The studies were identified in a literature review in PubMed with the keywords “microplastic”, “microplastics” AND “toxicity” and were supplemented by studies referenced in published reviews on the toxicity of NMP. Additional references on synthetic fibres were obtained from the reference list in a report by the Health and Safety Executive in the United Kingdom *(416)* on the hazards and risks of fibres, supplemented by a search with the keywords “synthetic fibre” AND “toxicity” OR “health” in PubMed up to December 2021. As noted in the introduction, although every effort has been made to ensure that the literature reviewed and evaluated for this report is as comprehensive as possible, it is not possible to guarantee that every study has been captured in this rapidly emerging field.

All studies identified were evaluated with a recently published NMP toxicity study assessment tool, the purpose of which is to screen and prioritize studies for risk assessment according to their reliability, which is scored on a number of criteria of quality assurance and quality control (QA/QC) *(16)*, summarized in Fig. 7, which include consideration of:

- identification of the test substance,
- characterization of the test system,
- description of the study design,
- documentation of the results and
- the plausibility of the design and the results for risk assessment purposes.

Fig. 7 Approach used to evaluate studies of effects in vivo and in vitro for use in assessing human health risks due to exposure to nano- and microplastic particles



Source: from reference *16* (https://creativecommons.org/licenses/by/4.0/)
All the criteria have equal weight. Thus, studies with non-zero scores against all criteria ideally represent those that should be prioritized for risk assessment.

The approach is based on previous methods for evaluating study designs and reporting details, such as the principles of the Klimisch score *(417)*, guidance and criteria used in a modified version of the ToxRTool *(374)* and those proposed by de Ruijter et al. *(418)* for assessing the quality of ecotoxicological studies. The objective of the NMP toxicity study assessment tool is to provide a standard procedure for evaluating and scoring the quality of toxicity studies of relevance to human health in a transparent approach *(16)*. The results can thus be used to screen and prioritize a study for the purposes of risk assessment and can also be used to provide guidance for strengthening the quality of future studies, which is perceived to be a principal factor for assessing human health risks.

5.1 Literature review and experimental study evaluation

A total of 109 studies with data from in-vivo or in-vitro test systems, representing a variety of exposure pathways and toxicological end-points, were identified and evaluated *(315, 353, 355, 362, 405, 419–522)*. Nearly all provided results for a monodisperse group of plastic particles: polystyrene

spheres were tested in 67% of the studies in mammals in vivo and 80% of bioassays in vitro, in 57% of which NMP < 1 µm were used. The second most common plastic particle tested was polyethylene, with results in 14% of in-vivo and 12.5% of in-vitro studies. Limited numbers of studies provided results for polyurethane, polypropylene, PVC, PET, acrylic ester copolymer and nylon. While the common use of monodisperse NMP in the studies may strengthen understanding of toxicological mechanisms of action for specific polymeric particles of well-defined shapes and sizes, environmental monitoring implies that human exposure is dominated by exposure to a heterogeneous mixture of particles of varying shape, size and polymer composition. It is difficult therefore to extrapolate the results for elevated concentrations of monodisperse particles to environmentally relevant exposure scenarios *(16, 523–525)*.

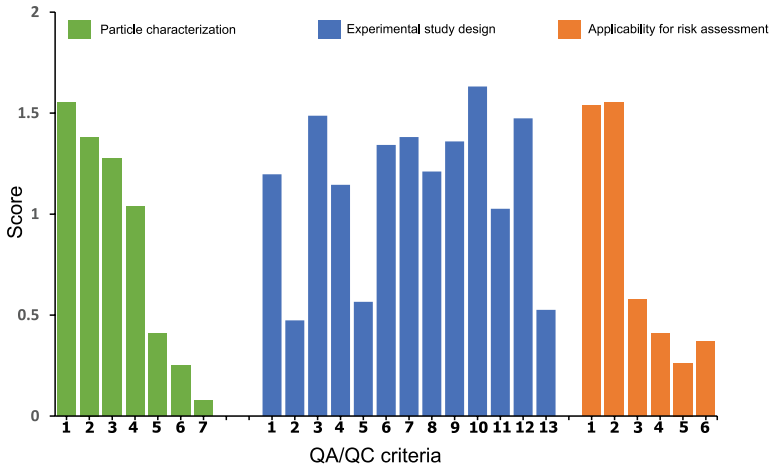
5.1.1 Experimental studies in vivo

The results of scoring with the quantitative quality assessment scoring tool developed by Gouin et al. *(16)* for all studies in mammals in vivo are summarized in Figs 8 and 9. As not all the studies were designed for quantitative assessment of human health risk, they should be scored against each criterion for comparison with other studies rather than simply evaluated according to their rank on the total score. This approach is consistent with evaluations proposed by de Ruitjer et al. *(418)* and Gouin et al. *(16)*, who argued that the scores presented should not be considered value judgements but a guide for screening and prioritizing studies for further interpretation, as well as providing guidance to improve the design and execution of future studies.

Several experimental studies have been conducted of the effects of exposure to NMP by inhalation (Fig. 9), most of them in rats. Five polymers have been tested, with variable results. Intratracheal instillation of PVC measuring 0.2 to < 5 µm[1] at doses of 10–125 mg/kg bw induced various biochemical and histopathological changes in rats *(355, 488)*. A dose of 50 mg/kg bw PVC had acute effects on biochemical and cytotoxic parameters and lung weight, comparable to those of crystalline silica at 10 mg/kg bw, 2 days after exposure, with recovery to normal by day 28 *(355)*. The effects were milder than those of quartz particles *(483)*, and the authors concluded that PVC powders are not cytotoxic, fibrogenic or pathogenic in rats *(355)*. Polyurethane foam particles (< 10 µm) induced progressive inflammation in the airways of rats after intratracheal instillation (5 mg per animal), leading to fibrosis of the lower respiratory tract 12 months after exposure, formation of a few scars and papillary adenomas in four rats 18 months after exposure *(353)*. The authors noted that the response was typical of exposure to PM. Acrylic ester polymeric particles (10–1500 nm; +/- 1.2 µm MMAD) were not toxic in rats after inhalation at 3 and 10 mg/m^3 for 6 h/day for 5 days, with a no-observable-adverse-effect level of

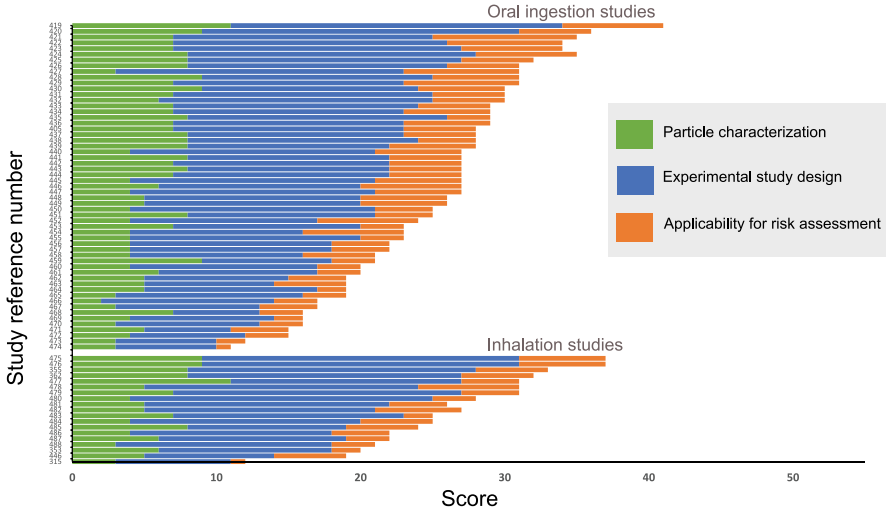
[1] Note that this is the geometric diameter. In an aerosol, the MMAD of these particles might be different, which would influence the dose and location of deposition in the respiratory tract.

Fig. 8 QA/QC evaluation scores for 76 studies in mammals in vivo



Average scores per criterion for three elements: particle characterization, experimental study design and applicability for risk assessment. Individual criteria are summarized in Fig. 7 and in reference *16*.

Fig. 9 QA/QC evaluation scores for 76 studies in mammals in vivo exposed by ingestion or inhalation



Average scores per criterion for three elements: particle characterization, experimental study design and applicability for risk assessment. See references *15* and *16* for further information.

10 mg/m^3 *(475)*. Ethylene oxide–propylene oxide polymer particles (1.1 µm MMAD) were highly toxic when administered to rats by inhalation, the toxicity increasing with chain length and molecular weight. The median 4-h lethal concentration of the most toxic compound, U-1500, was 106 (range, 4–245) mg/m^3. This compound induced pulmonary haemorrhage after repeated exposure to aerosols (6 h/day, 5 days/week) for 2 weeks at concentrations as low as 5 mg/m^3 *(484)*, although there were significantly fewer lesions after a 2-week recovery period. U-1500 ethylene oxide–propylene oxide also induced biologically significant focal (0.3 mg/m^3) or multi-focal (5.2 mg/m^3) fibrosis in rats after inhalation for 6 h/day, 5 days/week for 13 weeks, with no change during a 5-week recovery period *(482)*. Long-term exposure to U-1500 particles thus caused irreversible pulmonary fibrosis at concentrations as low as 0.3 mg/m^3. Other ethylene oxide–propylene oxide polymer particles were not toxic, causing only slight alveolitis after inhalation for 6 h/day, 5 days/week for 2 weeks at 100 mg/m^3 *(480)*.

The occurrence of "frustrated phagocytosis"[2] in the distal lung has not been reported in occupational epidemiology studies on NMP (section 3), and no association has been found with mesothelioma, as the diameter and aspect ratios of synthetic fibre dust are different from those of certain asbestos fibres and some carbon nanotubes. Flock dust was found to contain fibres 10–15 µm in diameter and ~ 1000 µm long, consisting of respirable particles and elongated shreds of nylon (aerodynamic diameter, 4–8 µm) *(314)*. While a proportion of the shreds were characterized as fibres, with an aspect ratio > 3:1, they were morphologically distinct from high-aspect ratio asbestos and carbon fibres, with heterogeneous width and surface. The main properties common to asbestos and carbon fibres and nylon flock dust are durability and biopersistence. It might therefore be hypothesized that the structure–toxicity relation for synthetic fibres shifts above a certain diameter to physicochemical pathogenicity influenced by (positive) surface charge. Thus, it is essential to determine the surface charge of environmental synthetic fibres and how it changes with fibre "age" and weathering, both indoors and outdoors.

The few studies of inhalation exposure suggest that the toxicity of some polymers – but not of others of similar size – is due to properties such as relative molecular mass, whereas other studies indicate that size proportionately influences responses. After intratracheal instillation of polystyrene beads (0.125 or 1 mg), the smallest particles (64 nm) caused more lung inflammation *(487)*, indicating that surface area is also important. Fig. 9 indicates that the studies with the highest scores and which met the minimal number of QA/QC criteria are those of Warheit et al. *(362)* and Ma-Hock et al. *(475)*. Most of the studies of inhalation effects were assigned a score of "0" (i.e., of "unacceptable quality" for extrapolating the results for use in risk assessment), as only one or two doses were tested and a threshold effect concentration was not used. Additional concerns are lack of information on the presence of impurities and on surface area and surface charge, which can influence toxicity *(15, 16)*. More than half of the 19 studies evaluated were conducted before 2000, which may indicate that they are not pertinent for assessing the toxicity of NMP to which populations are exposed today . Standardized methods and reference materials representative of

[2] Frustrated phagocytosis occurs when phagocytic cells present in the lung cannot remove NMP, e.g., because they are fully loaded already or the NMP are substantially larger than those that the cell can remove.

environmentally relevant NMP (section 2) should be made available for use in toxicity studies to ensure robust analyses of implications for human health and assessment of risk. In the interim, study reports should include thorough characterization of the test particles, which is important for understanding the relations between the properties of particles and toxicological end-points, and measures to ensure that the observations are not influenced by an artefact, such as a chemical contaminant or endotoxin.

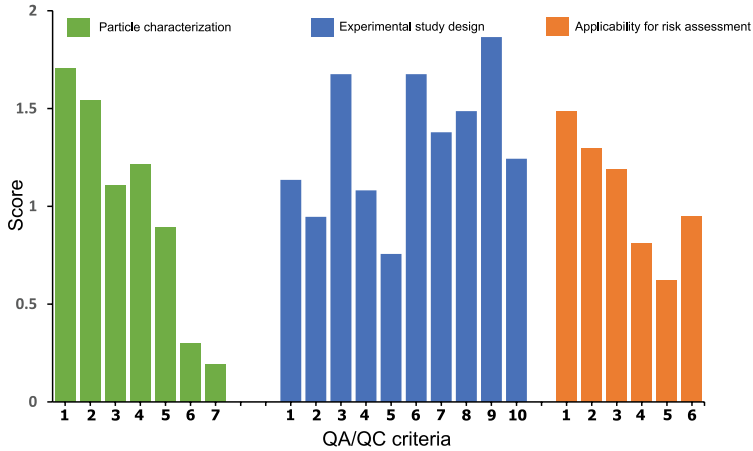
The number of studies on the toxicity of NMP after oral exposure has increased recently, allowing comparison with the adverse health effects of nanoparticles such as titanium dioxide, which is widely used as a food colourant. Effects such as changes in inflammatory response have been summarized *(526)*, and Pinget et al. *(527)* reported a significant effect of titanium dioxide on immune cells, with increased macrophages, and effects on gut microbiota that could trigger diseases such as inflammatory bowel disease and colorectal cancer. Results for other particle types, including NMP, have been both similar and contrasting. Grouping and read-across approaches for comparing the physicochemical properties of nanoparticles of different composition, shape, size and surface chemistry have been used *(447, 508, 528–532)*. In view of the uncertainties of both exposure to and the toxic effects of NMP, however, findings on the toxicity of other nanoparticles cannot be extrapolated to NMP. Nevertheless, understanding how the properties of microparticles influence adverse health effects could be important and should be considered in studying toxicity and in risk assessment *(533)*.

In its report on MP in drinking-water *(1)*, WHO noted that there were no epidemiological studies on ingested MP and that the data, at that time, from studies in animal models in vivo were limited and inadequate for a risk assessment of ingested MP. Currently, data on the absorption and toxicity of plastic particles are available for only a few polymeric particles, i.e., polystyrene, polyethylene and PET, and the reliability of some of the studies is doubtful *(1)*, as discussed below. The relevance and reliability of current data for evaluating the implications of exposure to NMP for human health should therefore be evaluated.

Since the WHO report *(1)*, several relevant publications on toxicity in vivo after short-term exposure of the gastrointestinal tract to NMP have been published and reviewed, (see, for instance, *15, 16, 27, 523, 524, 534–536)*. For this report, the studies were evaluated with regard to characterization of particles, study design and applicability for risk assessment (Figs 9 and 10). The NMP used in toxicity tests should be characterized better to improve understanding of their toxicological mechanisms of action *(537)*, such as by use of standardized reference materials *(15, 16, 538)*.

Fig. 9 summarizes the studies evaluated. In most, polystyrene particles of different sizes, mainly in the micrometre range, were tested, and the species used were limited to mice and rats. The toxic effects observed were mainly pathological changes in the gut and liver and disorders of energy metabolism *(27)*; reduced mucus secretion and intestinal inflammation were also observed *(448, 454)*. Dysfunction of the gut barrier was reported by Jin et al. *(455)*, and changes in the composition of the caecal microflora were also found *(448, 454, 455)*. Liver inflammation, lipid accumulation and changes in the lipid profile were reported *(455, 527)* as well as changes in

Fig. 10 QA/QC evaluation scores for 37 studies of effects in vitro



Average scores per criterion for the three categories of particle characterization, experimental study design and applicability for risk assessment. Individual criteria are summarized in Fig. 7.

markers of lipid metabolism *(422, 539)*. Disorders in energy metabolism *(422, 539)* and in bile acid metabolism *(455)* were described. In a study of co-exposure to organophosphorus flame retardants and NMP, Deng et al. *(436)* found that MP aggravated oxidative stress, neurotoxicity and metabolic disorder. Rafiee et al. *(437)* observed no significant change or abnormality in neurobehaviour after exposure to NMP, and Stock et al. *(405)* found no or no significant inflammatory response or any histologically detectable lesion in mice fed polystyrene NMP.

In a study on MP, about which there is some controversy, Deng et al. *(422)* exposed groups of 5-week-old male ICR mice to 5 or 20 µm pristine polystyrene MP at a dose of 0.01 mg/day (1×10^5 5-µm and 2×10^3 20-µm particles), 0.1 mg/day (1×10^6 and 2×10^4 particles) or 0.5 mg/day (5×10^6 and 1×10^5 particles), respectively, by oral gavage for 4 weeks. No adverse effect was reported on body weight; however, the relative liver weight increased at the high dose of each size of MP, with histological evidence of inflammation and lipid droplet accumulation in affected livers. Biological parameters of energy metabolism (ATP and lactate dehydrogenase), lipid metabolism (total cholesterol and triglycerides), oxidative stress (glutathione peroxidase, catalase and superoxide dismutase) and neurotoxic responses (acetylcholinesterase) in the liver were affected in response to all doses of MP. Metabonomic analyses also implied adverse effects on energy metabolism, lipid metabolism, response to oxidative stress and response to neurotoxicity.

Braeuning *(540)* raised a number of concerns with respect to the study of Deng et al. *(350, 422)*, noting that the histological evidence of liver inflammation and hepatic lipid accumulation in treated mice does not provide unequivocal evidence of an effect owing to the quality of the histopathological analyses. Thus, Braeuning considered

that the small variations in biochemical measurements (Fig. 4 of Deng et al. *(422)*) might be due to the biological variance expected from five animals per group. Concern has also been raised about the particle mass balance, with the tissue burden of kidney, liver and gut combining to exceed the administered dose *(541)*.

Several studies reported adverse effects on mammalian reproductive health. Luo et al. *(458)* studied the reproductive effects of pristine 5-µm polystyrene MP administered in drinking-water to groups of pregnant ICR mice at a concentration of 100 or 1000 µg/L throughout gestation and lactation, with some of the female offspring at the high concentration mated with untreated males to produce an F2 generation. The actual concentrations, however, were not verified by analytical quantification. Exposure to MP was reported to have no effect on body weight. The relative liver weight increased in F1 offspring at both concentrations but not in dams, and hepatic triglyceride and total cholesterol levels increased in dams and decreased in F1 mice at both concentrations. Exposure to the high concentration resulted in changes in the caecal microflora in both dams and F1 offspring. Changes in colon mucus secretion and ion transporter transcription profile were also observed in exposed dams. Transcriptomic and metabonomic analyses of liver and plasma indicated that exposure to MP can cause metabolic disorder in offspring and that some consequences are still evident in F1 (280 days) and F2 offspring.

Luo et al. *(452)* studied the developmental effects of pristine 0.5-µm and 5-µm polystyrene NMP administered in drinking-water to groups of pregnant ICR mice at a concentration of 100 or 1000 µg/L throughout gestation. The F1 offspring were maintained until postnatal day 42, when they were terminated for examination. Exposure to MP had no effect on the sex ratio or survival of offspring, and no statistically significant adverse effects were reported on body weight or liver to body weight ratio. Liver and serum cholesterol and triglyceride levels were altered in male offspring of exposed groups, and metabonomic analyses of serum, verified by transcriptomic analysis of liver, indicated that exposure to MP in utero could cause disordered fatty acid metabolism after birth, particularly with larger diameter particles. Lack of information on dose, however, makes it difficult to interpret the significance of some of the findings.

Several limitations should be considered when using these results for risk assessment. Inadequate characterization of chemical impurities that may be associated with the monodisperse type of particles used in the studies reduces the usefulness of the results for assessing the implications for human health of exposure to the complex, heterogeneous mixture of NMP expected to occur in the environment. Thus, standard reference materials representative of environmentally relevant ingested NMP should be made available, which will be possible only with better characterization of NMP in food and beverages representative of human diets, and methods are required to verify the dose actually delivered. As discussed in section 4, uncertainty in dosimetry poses challenges to interpretation and extrapolation of in-vivo data in experimental animals to humans.

There is continuing debate about how the size of particles influences their intestinal absorption and systemic biodistribution (section 4), with subsequent adverse effects at the cellular level. Information on the biokinetics of particles could be combined with

quantitative structure–activity models to estimate their toxicity for risk assessment *(323)*.

In their review, the Norwegian Scientific Committee for Food and Environment *(5)* suggested that the quality of current data on MP is insufficient to reach a conclusion about the risk to human health. The conclusion was based on a systematic search of literature published up to February 2019 and reports from EFSA *(10)*, FAO *(83)* and the European Commission's Science Advice for Policy *(11)*. In an analysis of the latest studies, the German Federal Institute for Risk Assessment *(344)* concluded that plastic particles in food cannot be assumed to pose a risk to human health, although understanding of the toxicity of NMP after ingestion is still limited and largely influenced by particle properties; however, the adverse effects of realistic exposure concentrations in relation to tissue and individual susceptibility require further research. These observations are consistent with those of other bodies *(2, 4–6, 15)*. There is thus a general awareness that the relevance and reliability of the available data are insufficient for a quantitative assessment of risk.

5.1.2 Experimental study design

As mentioned above, a number of studies have reported adverse effects on mammalian reproductive health in both males and females. The studies addressed adverse effects of NMP throughout the reproductive cycle, including on the number of viable sperm in the epididymis *(470)*, sperm deformities and disruption of the blood–testis barrier *(467)*, translocation of NMP to the placenta and fetal tissues *(464, 471)* and apoptosis of sperm cells with dose-related expression of cytokines *(467)*, which can serve as biomarkers of underlying inflammation.

In their evaluation of 12 studies of mammalian toxicity, Coffin et al. *(15)* identified a number of shortcomings, including some mentioned here, that made them inadequate for deriving a threshold value of NMP in drinking-water for effects on human health. As noted above, in most studies only one polymer type, usually polystyrene spheres, was tested, and the observed adverse effects cannot be extrapolated to those of the heterogeneous mixture of particles to which humans are exposed. Another concern is the limited characterization of the particles tested, and it is unclear whether the adverse effects observed were due to the particles themselves or to other factors, such as a chemical or endotoxin contaminant. Other factors that could also influence the results were not always well documented, making it difficult to attribute the effects to exposure to the test particles.

To address these concerns, Coffin et al. *(15)* strongly recommended use of the standard guidelines that have been

Table 7. Experimental study design criteria related specifically to animal husbandry

Criterion	Comment
Breeder, supplier, species and strain	• Use of outbred animals is recommended when the aim of the study is to assess responses in a heterogeneous population of individuals. • Use of inbred animals, which are genetically identical, is recommended to control introduction of population variance due to heterogeneity among individuals. • Age is particularly important in the context of studies of reproductive effect, as it is an indicator of maturity in relation to specific reproductive end-points such as fertility, sperm counts and pregnancy.

Housing conditions	• Acclimatization period: Researchers must demonstrate that the animals have been acclimatized to the experimental conditions in order to evaluate stress-related factors, which are known to influence reproductive end-points strongly.
	• Environmental conditions: Light cycles, humidity and temperature must be reported.
	• Use of any environmental enriching materials must be reported, as they may be a source of NMP and chemicals such as phthalates that could influence interpretation of any observed adverse effect. Reports should include:
	  • diet supplier and analysis of the diet;
	  • analytical composition of drinking-water;
	  • composition of water and food containers; and
	  • composition of materials used for bedding, including the source and analysis.
	• Animal group size and group housing, when groups consist of more than one individual.
	• Details of the availability of food. In most studies, it was reported that food and water were available ad libitum, but none of the studies considered the influence of food and water intake on the biokinetics and biodynamics of NMP.

Route of exposure	• For instance, reports of studies of oral gavage did not indicate whether stomachs were devoid of food and/or the time since the previous feeding. • Vehicle • How the particles are dispersed in the vehicle
Measurements	• Body weight • Body temperature • Food and water consumption • Animal behaviour • Clinical and blood chemistry • Specifics regarding when blood samples were taken, including collection of blood samples before exposure to demonstrate an experimental baseline • Historical data on mating and pregnancy specific to the species and strain used should be made available and referenced.

developed to study reproductive effects in mammals, such as OECD 421, 422 and 443 *(542–544)*. These guidelines list key points to be considered in studies for evaluating the effects of NMP on reproductive toxicity, from fertility to effects on the fetus, birth and weaning, which strengthen the reliability, relevance and comparability of the data obtained. The systematic approach of the OECD guidelines enables

quantitative analysis of mammalian reproductive effects, such as those on sperm counts and the impact of a stressor on ovarian histology.

Consistent with the observations of Coffin et al. *(15)*, we raise concern about the reporting of animal husbandry with respect to data interpretation. To ensure consistency and to strengthen the comparability of future studies, it is recommended that the study design criteria shown in Fig. 7 and the criteria specific to animal husbandry summarized in Table 7 always be reported.

Consistent with the observations of Coffin et al. *(15)*, we emphasize the importance of reporting aspects of study design. Robust, transparent reporting of the details of animal husbandry is strongly recommended. In their review of studies on bisphenol A, Thigpen et al. *(545)* showed that external factors, such as the materials used (bedding, caging, water bottles and standard rat chow), can strongly influence interpretation of the observed effects *(546)* by introducing estrogenic chemicals into the experimental test system. Given the presence of plastic additives such as bisphenol A and phthalates in a variety of consumer products and their potential impact on reproductive health *(547, 548)*, the potential role of any other chemical stressors in the test system that may influence the results should be thoroughly addressed.

Stress on test animals is another important consideration. Stress is influenced by a variety of environmental factors and also experimental design *(549–554)*. Test animals must therefore be adequately acclimatized before the start of a study. In their study on the effects of NMP on sperm in male mice, Hou et al. *(445)* reported that the animals were acclimatized before starting exposure; however, the mice were housed singly during the acclimatization period, which is a form of stress *(555)*. As Hou et al. *(445)* did not state how the animals were housed during the experimental phase of the study, it is not possible to fully evaluate the potential influence of the study design on the results. Housing animals singly or in groups can influence hormonal levels, a particular concern in studies of female reproduction *(556–558)*. Any change in housing conditions can cause stress in animals, with negative effects on endocrine pathways, thereby introducing uncertainty in interpretation of test results.

The route of exposure should be stated clearly. Exposure by ingestion or inhalation should adhere to recommendations,

and standard methods should be used (e.g., OECD 39 for inhalation studies *(559)*), particularly in extrapolating observed adverse effects to humans. The route of exposure should ideally be consistent with exposure anticipated in humans for extrapolation purposes. In several studies of reproductive effects, NMP were introduced in drinking-water. Very few studies, however, provided satisfactory information on the homogeneity of the exposure or the stability of NMP in drinking-water, with respective average scores of 0.57 and 0.47 (Fig. 8). For example, in their study of the adverse effects of 10-µm polystyrene spheres on testicular tissues in Sprague-Dawley rats, Ijaz et al. *(470)* used saline as a control but used a different culture medium to disperse the particles that were then administered by oral gavage. The authors provided no analysis of the composition of the medium, the dispersion of particles in the culture medium or the stability of the particles in the exposure vehicle. Furthermore, they gave no explanation for using a different vehicle for control and test animals. Inconsistency among studies also includes the reporting on the water used. Use of pure, distilled or tap water as the vehicle to disperse NMP was reported, with no analysis of the water for possible contaminants. The examples given here are representative of issues that arise when attempting to interpret study results, as various indirect factors can influence adverse effects.

None of the studies reported the timing of administration of NMP. This is particularly important in the context of oral gavage, as the presence or absence of food in the stomach of animals can influence biokinetics *(15)*. Furthermore, details of when and how physiological measurements are taken should be provided, including those for body weight, behaviour and food and water consumption. When clinical biochemical samples are taken, details of how and when samples were taken and how they were stored, including details of the sample containers, should be reported, as these can indicate whether adverse effects occurred before termination of the study.

Ideally, animals should be necropsied one by one in a separate room to avoid stress to other animals; furthermore, the order of necropsy should be counterbalanced among groups, and both physiological and biochemical samples should be processed in a randomized manner *(560, 561)*. Blood samples should be collected during a defined period (e.g., 09:00–13:00) to avoid diurnal variation in hormonal levels, as recommended in the endocrine disruptor testing guidelines of the United States Environmental Protection

Agency *(562, 563)*. Additionally, when blood samples are collected for hormone measurements, animals should be moved to a holding room the day before necropsy to minimize stress-related effects due to cage transport, which has been known for some time to affect thyroid hormone levels *(564)*.

In some studies, such as that of Ijaz et al. *(470)*, blood samples were collected and stored in sterile tubes at the end of the live phase for further analysis; however, there was no mention of how the blood was taken, the volume of blood or the storage conditions, which raises concern about the interpretation and therefore the validity of the results reported. It is common practice, for instance, to collect blood before initiating a study to characterize a baseline for biochemical analysis for comparison with levels observed at the end of the experiment. This helps to address variation in the study population and strengthens data interpretation.

Ijaz et al. *(470)* used diethyl ether to anaesthetize the animals in their experiment; however, this is not permitted in many other countries and can influence the biochemical analysis of, e.g., hepatic enzyme activity. As a general rule, experiments in which animals are used for the purpose of advancing scientific understanding must be designed on the basis of a harm–benefit analysis *(561)*. Particularly in studies in mammals in vivo, researchers are ethically required to adhere to standard test methods or to provide appropriate justification of why standard practices were not followed.

We note that the studies evaluated were designed to consider various questions and consequently differ in duration, exposure dose and types of NMP used, toxicological end-points, species and sample sizes. Nevertheless, adoption of the three segments of the OECD test guidelines is strongly recommended for any study of reproductive toxicity:

> segment I: Fertility and general reproductive performance, applicable to studies of both male and female rats;
>
> segment II: Teratology or embryo-fetal toxicity, applicable to studies in rats and rabbits; and
>
> segment III: Perinatal and postnatal development, applicable to studies in rats on the effects of active pharmaceutical ingredients during the last trimester of pregnancy and during lactation.

In the study of Ijaz et al. *(470)*, who used a 60-day exposure, it is unclear whether that duration corresponds to a relevant reproductive segment. It is likely that the duration was

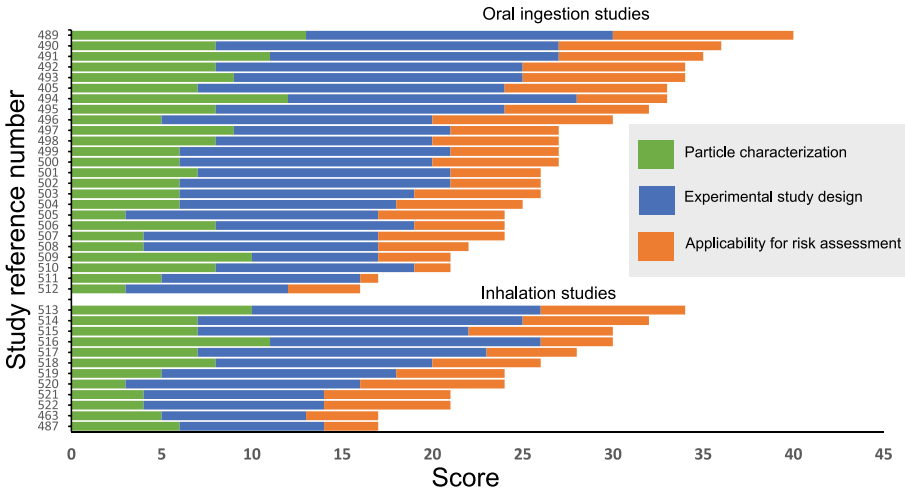
selected to correspond to the length of one of the four cycles of the seminiferous epithelium during the development of mature sperm. The duration of spermatogenesis from type A spermatogonia of stage VIII tubules is estimated to be 35 days in mice and 56 days in Sprague-Dawley rats *(565–567)*. As Ijaz et al. *(470)* used Sprague-Dawley rats, the 60-day exposure was presumably meant to correspond to the duration of spermatogenesis. To reduce potential ambiguity, future study reports should clarify important elements of the study design *(560)*.

Non-standard methods were usually used for assessing sperm and their motility and histological examination of the testes and related structures, and the results are often semi-quantitative rather than quantitative. Some of the methods used were simply inappropriate, such as use of a haemocytometer to count sperm *(440)*. More accurate methods for testicular histology are necessary to evaluate reliably whether effects on testicular function represent a direct response to exposure to a test material.

It is further recommended that measurements such as those obtained from immunoassays be validated against negative and positive controls. Validation is important for interpreting toxicological responses, as use of controls can rule out cross-reactivity and ensure comparison of matrix samples with the reference standard *(568)*. Negative and positive control chemicals should be included in assay validation, as recommended in guidance from the European Medicine Agency *(569)* and the US Food and Drug Administration *(570)*.

Interpretation of the results of most of the studies on the toxicity of NMP with regard to human health is therefore limited *(15)*. Concerns other than those raised above include insufficient statistical power due to small sample sizes, the methods used to section tissues, lack of understanding of the development of reproductive tissues and lack of histopathological evidence of the presence of particles and pathological processes, such as inflammation. Strengthening QA/QC of both particle characterization and experimental study design consistent with the recommendations in this report are therefore critical for improving overall understanding of the toxicity of NMP and the implications of exposure for human health.

Fig. 11 QA/QC evaluation scores for 37 studies of effects in vitro designed to reflect exposure by ingestion and by inhalation



Score per study for particle characterization, experimental study design and applicability for risk assessment. See references *15* and *16* for additional details.

5.1.3 Experimental studies in vitro

In experimental studies in vitro, biological and mechanistic pathways are studied under controlled conditions that cannot be achieved in vivo. The assays include those of portal-of-entry toxicity in models of the lungs and gastrointestinal tract, non-cellular assessment of the durability of NMP, protein interactions, complement activation and pro-oxidant activity. In-vitro studies can be used to assess the effect of exposure to particles on various physiologically relevant end-points, including cell viability, inflammatory response, proliferation, necrosis and apoptosis; changes in these parameters imply adverse effects on tissue homeostasis *(571)*. Well-designed studies can indicate the effects of the physicochemical properties of NMP on intercellular uptake and toxicological mechanisms *(572)*.

In-vitro studies are usually performed in monocellular systems. This, however, excludes intercellular communication, whereas signalling among cells is central to tissue and organ homeostasis. Caution is therefore warranted in extrapolating the effects of particles in vitro to in-vivo systems *(571)*. Toxic effects observed in vivo, for instance, are not restricted to the expression of signalling molecules but include the migration of inflammatory cells, changes in the vascular compartment, tissue injury and fibrotic alterations. A combination of in-vitro and in-vivo data is therefore necessary to elucidate the toxicological mechanisms of action of particles *(537, 572)*. Complex organotypic human models, such as organoids, 3D tissue culture and organ-

on-chip appear to be promising for assessing the toxicity of NMP and would also reduce the use of animals in testing. A variety of tools will be required for QIVIVE to determine the implications of human exposure to NMP *(572)*.

Figs 10 and 11 summarize the results of in-vitro studies on the toxicity of NMP and the models used, which include the airways *(516, 519)*, intestine *(493, 501, 512)*, placental epithelium *(493)* and skin *(573)*. The models of airways included both bronchial *(516)* and alveolar epithelium. Nanosized polystyrene beads were used in most studies, except in one, in which bronchial epithelial cells were exposed to polycarbonate and ABS particles generated from 3D printer emissions *(574)*. The particles used in studies of the airways measured 0.025–2.0 µm, which are substantially smaller than the MP detected and quantified in environmental samples (section 2) but relevant to those implicated in adverse effects on human health (< 0.1 µm). Ultrafine particles trigger inflammatory mechanisms in vitro that may play a role in chronic pulmonary inflammation *(517, 574)*. Particle surface area and the oxidative potential of ultrafine particles appear to be critical parameters in the inflammatory response *(533, 571)*. Particle interactions with lipid mediators, such as COX2 protein, play a central role in interference with regulatory pathways after exposure to ultrafine particles.

Confounding factors such as the presence of impurities (e.g., endotoxins) or chemicals added to plastic should be considered when assessing the effects of NMP in vitro. Xu et al. *(513, 575)* concluded that much of the toxicity of PVC in their tests was due to leaching of chemical additives by assessing the toxicity of the original particles and those from which chemical additives had been removed and comparing them with silica particles at a similar dose and particle size distribution *(513)*. The PVC particles were less toxic than silica, which was considered to be due partly to faster clearance from the lung. These observations are consistent with those of Pigott and Ishmael *(483)*, who assessed PVC powders (1–250 µm) obtained from an industrial source, α-quartz (median diameter, 33 µm) as a positive control and polymethylmethacrylate powder as a non-cytotoxic material. Aliquots of PVC dust suspensions were added to culture vessels to a final concentration of 0.5 mg dust/10^6 cells and exposed for 2 h. Comparison of alcohol-washed and unwashed powders indicated a cytotoxic effect of a surfactant associated with the PVC dust, as the toxic response was mitigated when it was removed *(483)*. Separation of the effects of the particles and of the chemical thus supported results obtained in vivo, in which minimal tissue damage was observed.

In-vitro studies relevant to oral toxicity are based on human intestinal cell models (mainly Caco-2 cells), as reviewed by Yong et al. *(27)*. In most studies, cellular uptake of NMP was observed, but they generally had insignificant toxicity, except at high concentrations. No significant effects on cell viability were observed after exposure to 5-µm polystyrene beads *(501, 512)*. Stock et al. *(405)* reported significant loss of cell viability only at very high concentrations of the smallest particles tested (1 µm); no cytotoxicity was observed at any concentration of the larger particles. Similar findings were reported by Hesler et al. *(493)*, who found a significant increase in metabolic activity after exposure to 46-nm polystyrene beads at 100 µg/mL and significantly decreased metabolic activity after exposure to 0.01 µg/mL of 446-nm polystyrene beads. Wu and colleagues *(501)* reported low toxicity after exposure to 0.1- and 5-µm polystyrene particles but observed depolarization of mitochondria and inhibition of the toxicant efflux pump, ATP-binding cassette transporter, which increased the toxicity

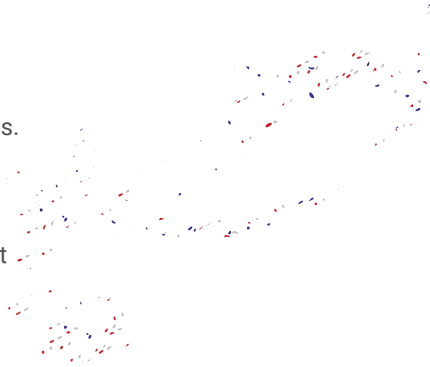
of arsenic. Exposure to 0.1- and 5-µm polystyrene beads at 200 µg/mL induced significant generation of reactive oxygen species. In another study by Wu et al. *(512)*, 5-µm polystyrene beads had no significant effect on superoxide dismutase, catalase or glutathione activity or on malondialdehyde levels after exposure to 12.5–50 µg/mL for 48 h.

Polystyrene beads (0.046–5 µm) did not adversely affect membrane integrity, even at the highest concentrations *(493, 501)*. Genes attributed to tight junction pathways were found to be differentially expressed after exposure to 50 µg/mL polystyrene beads (5 µm) *(512)*, which suggested an effect on membrane integrity. Hesler et al. *(493)* did not observe translocation, although polystyrene beads were observed in intestinal cells and more 0.446-µm polystyrene beads were internalized than the smaller 0.046-µm beads; no explanation was provided. Wu et al. *(512)* reported that inflammatory and immune pathways were affected by exposure to 5-µm polystyrene beads, although their conclusions are not supported by the data presented. Stock et al. *(405)* found that uptake of microplastics did not affect the polarization of macrophages or the release of chemokines.

Although all the in-vitro studies give insights into the potential toxicity of NMP, the results are inadequate for risk assessment because of the use of unrealistically high concentrations and testing predominantly of polystyrene beads, which are not considered to be representative of environmental exposure. Moreover, as in the reports of in-vivo studies, the properties of the particles tested were not adequately described. Nevertheless, the factors that appear to determine dose-dependent relations are particle size, surface chemistry (NH_2-polystyrene was generally more potent than neutral and COOH particles) and exposure duration *(518)*. As most of the studies were of nano-sized polystyrene particles, the results for environmentally relevant MP of irregular or fibrous shape and different polymer composition could be used to identify potentially hazardous particles. The in-vitro study with the highest quality score was that of Choi et al. *(489)*, who observed that differences in the shape of polyethylene particles result in significant differences in toxicity. They found that irregularly shaped particles with a rough surface structure have effects on cells that include pro-inflammatory cytokine release and haemolysis, whereas spherical particles were not severely cytotoxic at the concentrations tested.

5.1.4 Summary

Concern about human exposure to airborne NMP is increasing, and characterization of their contribution to the concentrations of atmospheric particles is important for assessing the implications for human health (section 2). In the Global Burden of Disease programme *(311)*, it was estimated that 4.2 million people had died prematurely in 2015 due to exposure to airborne PM. The components of PM that represent the greatest risk to human health, however, are poorly understood, although a contribution of NMP cannot be excluded *(111)*.



Dietary exposure implies potential concern about a variety of end-points; however, the reliability and relevance of the available data make it difficult to reach definitive conclusions. Furthermore, contradictory results have been obtained for monodisperse NMP, which are not representative of environmentally relevant human exposure. Observations both in vivo and in vitro are generally consistent with respect to various biochemical responses, such as those related to reactive oxygen species and various inflammatory biomarkers. More research is required, however, on whether mechanistic responses are direct physical effects in which particles overwhelm cellular tissues by a particle overload effect or are indirect effects, such as leaching of a chemical contaminant or an indirect immune response.

A number of studies in which rodents were exposed to polystyrene spheres (0.2–20 µm) showed adverse effects in the digestive, hepatic, renal, thyroid, cardiac and reproductive systems. In all the studies, the adverse effects consistently included oxidative stress, altered metabolic profile and lipid metabolism and chronic inflammation *(419, 422)*.

The findings with various biomarkers suggest an association with particle-mediated toxicity. If these biomarkers are relevant to adverse outcomes, they will provide additional insight into the mechanisms of the effects initiated or aggravated by exposure to NMP. The in-vitro studies evaluated, for instance, provide evidence that exposure to NMP results in a pattern of inflammation and reactive oxygen species. While the results of a number of studies suggest a trend to inflammation and oxidative stress, which might be associated with a molecular event, the QA/QC of study design must be strengthened, particularly when different organ and cell systems and different types of NMP are tested. Inconsistent QA/QC, discussed above and elsewhere *(15, 16)*, limits definitive conclusions.

5.2 NMP as vectors of chemical exposure

NMP may present a hazard in various ways: because of their physical form; as vectors of chemicals, including monomers, additives and sorbed chemicals; and as vectors of microorganisms in biofilms (section 6). As risk is a function of both toxicity and exposure, reliable, relevant characterization of both components is essential. The leaching of chemicals from NMP has been identified in studies both in vivo and in vitro as a factor in the observed adverse effects; therefore, attribution of adverse effects to the particles themselves is difficult to establish if the concentrations of chemical contaminants are not measured. The particles tested in most studies

were virgin polystyrene spheres. These could leach monomer residues or various chemical additives, which, at high concentrations, could trigger adverse effects such as inflammation and oxidative stress. To assess the implications to human health of NMP as vectors of chemicals associated with MP, as occurs during environmentally relevant exposure, each pathway of exposure to the associated chemicals should be assessed, including in the diet, by inhalation and due to leaching from NMP. The toxicity of chemicals associated with plastics does not necessarily present a risk in drinking-water or food if the exposure is sufficiently lower than a defined margin of exposure (MOE) or if the exposure resulting from leaching is negligible or minor as compared with that from other sources. Quantification of exposure to both NMP and associated chemicals is thus critical for risk assessment.

Plastic commodities contain a variety of chemical additives and unbound monomers that can leach into water, air or, in the case of plastic packaging, into food before consumption *(576–578)*. As shown in Table 1, the application and use of chemical additives varies widely according to the polymer composition and the intended use of the plastic product. Consequently, evaluation of the role of NMP as a vector for human exposure to chemical additives will require characterization and quantification of their concentrations in NMP and in the diet *(124)*. In the absence of this information, risk has been estimated mainly by applying conservative assumptions and exposure scenarios for qualitative assessment of the relative implications for human health *(1, 10, 11, 83, 124, 579)*. For instance, EFSA *(10)* and FAO *(83)* adopted a conservative approach in estimating that a meal of mussels could result in exposure to 4 MP/g (Table 5). For MP with a diameter of 25 µm and a density of 0.92 g/cm^3, EFSA *(10)* estimated an intake of 7 µg on the basis of typical consumption of a 225-g portion of mussels. In a conservative scenario in which chemical additives and other sorbed chemical contaminants are assumed to be present in MP at the maximum concentrations reported and that the total mass of chemical is bioavailable after ingestion, EFSA estimated that exposure to sorbed chemicals represents a negligible fraction of total intake, with increases of < 0.006% in polychlorinated biphenyls (PCBs), < 0.004% in polycyclic aromatic hydrocarbons and about 2% in bisphenol A over those seen in other exposure pathways.

Using the approaches of EFSA and FAO in relation to mussels and estimates of human exposure to MP in drinking-water, WHO *(1)* also evaluated exposure to chemicals from ingestion of water contaminated with MP. Assumptions were made that would result in very high exposure to total MP on a mass basis: spherical MP with a diameter of 150 µm, a density of 2.3 g/cm^3 and an exposure of 10.4 µg/L. At a default water consumption of 2 L/day, daily intake of MP was estimated to be 85 µg. In this highly conservative scenario, exposure to MP would be to 1.4 µg/kg bw per day for an adult with a default body weight of 60 kg. The report noted that a more realistic estimate would be about 0.03 µg/kg bw per day. The plastic-associated chemicals evaluated for their implications for human health were bisphenol A, cadmium, chlordane, di(2-ethylhexylphthalate), dichlorodiphenyltrichloroethane, hexachlorobenzene, polycyclic aromatic hydrocarbons, polybrominated diethyl ethers and PCBs. If these plastic-associated chemicals are present at the maximum reported concentrations in MP and the chemicals are 100% bioavailable after ingestion, the MOEs for each chemical suggest that their levels are of little concern for human health.

Section 2 summarizes data on human exposure to MP in food, beverages, drinking-water and air. In view of the significant difficulty of quantifying human exposure to NMP, it would be inappropriate to estimate total exposure to chemicals leached from NMP in food, beverages and air as was done by EFSA *(10)* and FAO *(83)* for seafood and by WHO *(1)* for drinking-water. Unlike previous estimates, based on specific exposure scenarios, the inherent uncertainties associated with estimates of total human exposure would be highly speculative and inconclusive. We thus propose tiered approaches based on more relevant, reliable data to quantify the concentrations of both NMP and associated chemicals for assessing total exposure from all relevant pathways.

For example, 80–90% of all chemical plasticizers are used in a single polymer, PVC *(67, 68)*. Further, metal stabilizers are added to PVC, including those based on cadmium and lead *(580)*, which may be present at toxic levels in plastic products. Turner and Filella *(580)* observed that, at the regulatory concentration limits in electrical and electronic plastic commodities of 100 mg/kg for cadmium and 1000 mg/kg for lead, the concentrations in recycled plastic and plastic debris in the environment could result in non-compliant levels. For example, maximum concentrations of 6760 mg/kg cadmium and 23 500 mg/kg lead have been reported in plastic litter *(581)*. Quantification of exposure to NMP characterized as PVC should be a priority, as this polymer may be the largest source of chemical additives for humans and the environment *(582)*. While PVC is found in about 10% of consumer plastic products, it was also detected at 2% in plastic litter in the environment *(580)*. The relatively small proportion of MP from PVC is also consistent with data presented in section 2 on potential exposure via drinking-water, food and beverages.

Turner and Filella *(583)* estimated a threshold limit of 8 mg/day of metal-contaminated PVC and simulated mobilization of cadmium and lead from PVC in simulated gastric fluids of seabirds, finding maximum exposure to 20 mg/kg cadmium and 1800 mg/kg lead *(583)*. Mohamed Nor and Koelmans *(584)* simulated mobilization of a number of PCBs in artificial gut fluid and also uptake of chemicals by MP from contaminated food, including the effect of digestion *(585*). Biphasic kinetics was observed, which allowed derivation of kinetic rate parameters for each of the two sorption reservoirs. Bioavailability was defined as the fraction of chemical desorbed from these reservoirs according to the human gut retention time, estimated from the calibrated model. Bioavailability was thus shown to depend on gut retention time.

There is growing interest in evaluating the bioavailability (or at least bioaccessibility) of plastics-associated chemicals *(582, 584, 586–593)*. Although use of simulated gastric fluids, such as by Turner and Filella *(583)*, allows quantification of bioaccessibility, additional methods would be necessary to characterize the bioavailable fraction *(583, 591)*. In their assessment of the weight of evidence for MP as vectors of plastic-associated chemicals, Koelmans et al. *(591)* observed that, while many studies reported bioaccessible amounts or the leached fraction of chemicals from MP in simulated gastric fluids, most did not consider possible interactions with other components of the digestive system in vivo. Thus, according to the physicochemical properties of a chemical, the leached fraction might be partitioned into other compartments in the gut; for example, indigestible fractions of the natural diet would be eliminated by excretion. While the bioaccessible fraction would be a

refinement of highly conservative assumptions derived from screening, which are based on 100% bioavailability, evaluation of bioavailability in a more realistic exposure scenario would improve assessment. The approach would be consistent with standards for assessing the migration of chemical additives from consumer products, such as EN 71-3:2019 for the migration of certain elements from toys in the European Union *(594)* and methods that have been proposed for assessing the bioaccessibility of chemicals *(586, 595)*.

A possible framework for quantifying the bioaccessible fractions of chemicals was proposed by Mohamed Nor et al. *(124)* and Mohamed Nor and Koelmans *(584)*, who used a probabilistic kinetics exposure model for plastic-associated chemicals, with three components. The first is a probabilistic exposure model for 1–5000 µm MP (see section 2.4). In the second component, given a known average particle retention time in humans, desorption or resorption of plastic-associated chemicals in the gut is modelled according to particle size-specific adsorption and desorption rate constants measured independently in artificial desorption experiments of intestinal fluid in vitro *(584, 585)*, with dynamically changing concentration gradients. Bioavailability was thus modelled over time. The background concentrations and fluxes of the same chemicals in common foods were included in quantification of the concentration gradients in the gut. The third component is a traditional PBPK model that simulates subsequent uptake and biodistribution of bioavailable plastic-associated chemicals in the body. Simulations were performed with and without the inclusion of NMP in food and drinking-water, with probabilistic account of the multidimensionality of NMP and uncertainty in model parameters. Four representative chemicals were investigated: benzo[*a*]pyrene, di-(2-ethylhexyl)phthalate, PCB126 and lead. The integrated, realistic chemical modelling approach demonstrated that, at the 50th percentile of exposure, the concentrations of chemicals leached from NMP resulted in a negligible change in the tissue concentrations of the four chemicals *(124)*. This conclusion is case-specific, and it is recommended that the framework be applied to other chemicals of concern.

The MOE can be estimated according to the extremely conservative assumption that 100% of a chemical is bioavailable on a mass/mass basis or as the total mass of NMP equivalent to the MOE of concern. The most sensitive MOE from the estimates for drinking-water reported by WHO *(2)* was for cadmium. With this approach, it can be assumed that concern about exposure to cadmium would increase as the mass of NMP increases if all NMP contain the maximum concentration of cadmium. For instance, an MOE > 10 000 can be derived from the adult median mass of 0.6 µg/person day and an assumed maximum level of cadmium in NMP of 6760 µg/g *(581)*. Performing the calculation in reverse, an assumption of a maximum mass of NMP of 17 mg/person day can be derived that would result in an MOE of cadmium of < 1, whereas the MOEs for other plastic-associated chemicals remain > 100. Thus, given the relative toxic potency of cadmium and its known use in plastic products, evaluation of its bioavailability after exposure to NMP should be a priority. Ideally, such studies should be accompanied by monitoring to quantify the polymer composition of NMP to which humans are exposed and the amounts of cadmium associated with environmentally relevant exposure, with appropriate methods to evaluate both bioaccessiblity and bioavailability. As noted above, PVC may be

the greatest source of metal additives. Li et al. *(582)*, however, using non-targeted analysis, found that most organic chemical leachates that migrated into simulated gastric fluids were from PVC. An additional concern was organic chemicals leaching from NMP originating from recycled plastic. This is an important observation, as in most conservative approaches it is assumed that all NMP contain the maximum amounts of various plastic-associated chemicals. Li et al. *(582)*, however, suggest that conservative approaches should be revised to account for differences in relative mass and the types of chemicals in polymers. If PVC, for instance, represents a relatively small fraction of total exposure, estimates of chemical-specific MOE that approach values of concern should be refined to obtain more accurate estimates of implications for human health.

5.3 Summary and recommendations

Many studies have been conducted in the past few decades to improve scientific understanding of the toxicity and implications for human health of exposure to a variety of natural and synthetic particles. Adverse effects associated with exposure by both inhalation and ingestion have been investigated. A limited subset of NMP have been assessed, including epidemiological data on the adverse effects of occupational inhalation of synthetic fibres, such as nylon and plastic dusts generated from PVC and polyurethane foam. While adverse effects, including accumulation of macrophages, frustrated phagocytosis, decreased lung function, interstitial lung disease and lung cancer, have been reported, the studies have substantial limitations, such as limited cohort size and insufficient accounting for confounding factors. The data are also contradictory, as several studies found no significant relation between exposure and adverse effects. Furthermore, occupational exposure to particles is not representative of the exposure of the general population. Caution is thus warranted in extrapolating results for different types of particles and exposure concentrations associated with occupational activities to indoor and outdoor environments.

Controlled tests of the toxicity of NMP in vivo and in vitro after inhalation or ingestion indicate that high concentrations of some types of NMP elicit various biochemical effects, some of which depend on the physical characteristics of the particle (e.g., size, shape) and others on their chemical characteristics (e.g., solubility, surface chemistry, composition) *(508, 596, 597)*. Only a few types of particle have been studied, however, consisting mainly of polystyrene particles of various sizes and surface chemistry. Caution should be exercised in extrapolating observations on a homogeneous test particle to the heterogeneous mixture of

particles that comprise NMP to which the general population is exposed.

To better interpret the results of toxicity testing of NMP, studies both in vivo and in vitro of exposure by inhalation and ingestion have been evaluated with respect to various QA/QC criteria *(16)*. The results show that the majority of the reports do not provide sufficient information on the test particles, which is critical for understanding the mechanisms of observed adverse effects and various toxicological end-points and the implications of NMP for human health. Inadequate characterization of test particles can also obviate comparison and replication of studies. For instance, adverse effects may not be due to the test particle itself but to a chemical contaminant or endotoxin, the relative importance of which may differ from one supplier to another *(15, 16)*. Therefore, a series of reference NMP should be made available that, ideally, represent the NMP to which humans are exposed.

Advances in characterizing and quantifying human exposure (section 2) are essential for future toxicity testing. For instance, most of the data currently available on exposure are limited to MP measuring > 10 µm, while systems to test the properties of MP to which human exposure is most relevant are required for risk assessment. While some studies have investigated effects in vitro with cells representative of internal organs (liver or brain), the results should be analysed in quantitative extrapolation models specific to NMP. There is significant uncertainty about the absorption and systemic bioavailability of NMP, and integrated tools are necessary to guide and prioritize research.

Uncertainty about exposure to NMP must be reduced. Thus, data are required to characterize and quantify the properties (size, shape, polymer composition, surface chemistry) of NMP in air, drinking-water, food and beverages to be used in a probabilistic exposure assessment *(124)*. The selection of in-vitro and in-vivo test systems must be guided by accurate data on exposure, with a series of well-characterized reference NMP in relevant, robust dosimetry models. The applicability of existing QIVIVE and PBPK models to interpretation of test data is uncertain, and these tools should be assessed and new models developed as necessary that are consistent with the principles of replacement, reduction and refinement (the “3Rs”) for humane testing in animals.

Key messages

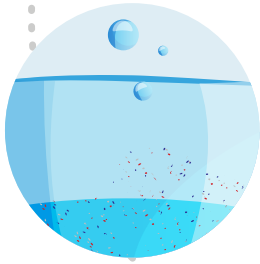
- Data on toxicity after inhalation or dietary exposure for characterizing the hazard of NMP are limited to studies with polystyrene beads. Information is required on the effects of particle size, shape, polymer composition and other factors representative of environmentally relevant NMP.
- The limited hazard characterization of NMP suggests that they may have adverse effects similar to those of other well-studied solid and insoluble particles through similar modes of action.
- The available data are insufficient to determine whether exposure to NMP is associated with any direct or indirect characteristic pathology, as concern about QA/QC has been poorly accounted for in published studies.

6. NANO- AND MICRO-PLASTICS AS VECTORS OF PATHOGENS

Microorganisms can populate numerous surface types by forming biofilms, which contain diverse bacteria, algae, protozoans and fungi. In the environment, plastic can provide a new surface substrate for biofilm-forming microbial communities, often referred to as "plastispheres" *(598–600)*. The propensity of microorganisms to populate plastic particles depends on physical, chemical and biological factors, which have been studied mainly in marine environments. A conditioning film consisting of organic and inorganic substances forms within seconds around submerged surfaces by adsorption, and this material-specific alteration of surface properties strongly influences the composition of the colonizing microbial community *(601–603)*. Environmental conditions, including high nutrient concentrations (nitrogen and phosphorus), salinity, temperature, high ultraviolet radiation and oxygen content, influence the formation of plastics and microplastics biofilms *(604–608)*. Material properties such as hydrophobicity and surface roughness also affect microorganism attachment and propagation *(609–611)*. The increasing number of plastic surfaces available for biofilm colonization on aquatic ecosystems is a topic of increasing concern and research. A limited number of studies suggest that plastispheres can disperse over longer distances than other particles, potentially introducing invasive species into vulnerable ecosystems *(612–614)*.

6.1 Microplastic-associated biofilms in water

Biofilms can protect microorganisms from external stressors such as ultraviolet light and toxic substances and facilitate nutrient accumulation and horizontal gene exchange *(615)*. While most microorganisms in biofilms are thought to be non-pathogenic, they may harbour pathogens that multiply only after they have infected a host *(616)*.



The WHO report on MP in drinking-water *(1)* discussed the hazards and potential risks associated with biofilms, which may attach to and colonize MP and find their way into drinking-water or drinking-water sources. Although MP may serve as vectors for harmful organisms, including enteric viruses and protozoa, the significance of microplastic-associated biofilms is probably negligible because the much larger surface area of other particles in drinking-water and drinking-water distribution systems can attract more biofilms than MP. A limited number of studies of fresh water suggest that MP might function as vectors for long-distance transport of pathogens and thus increase the transfer of organisms

with antimicrobial resistance. Plastic-mediated transport of pathogens should not, however, be overestimated, as there are significantly larger sources of opportunistic and obligate pathogens in surface waters used as sources of drinking-water. In addition, clarification and membrane treatment of drinking-water remove most plastic particles, and disinfection, including in distribution systems, can inactivate pathogens and control their growth. The possibility that non-pathogenic microorganisms could acquire and spread antimicrobial resistance genes is an issue of concern and should be studied further. WHO *(1), w*hile acknowledging substantial lack of data, concluded that there was no evidence of a risk to human health of microplastic biofilms in drinking-water. Research should, however, be conducted on horizontal transfer of antimicrobial resistance genes in plastisphere microorganisms and in other biofilms, such as those in wastewater treatment plants.

Since publication of the WHO report in 2019, Wu et al. *(617)* conducted a comparison of biofilms associated with MP and those on rock and leaf microparticles in river water using high-throughput sequencing. Microplastic biofilms were found to have not only a distinctive community structure but also distinct patterns of enrichment of antibiotic-resistance genes, including in two opportunistic human pathogens (*Pseudomonas monteilii* and *P. mendocina*). This finding underlines the importance of further research on drivers of antimicrobial resistance associated with MP *(618)*.

MP in the marine environment also provide a novel surface substrate for microbial communities, including typical aquatic colonizers such as Rhodobacteraceae and Gammaproteobacteria *(619, 620)* and also a few microplastics-specific colonizers such as Hyphomonadaceae and Erythrobacteraceae *(604)*. It is unclear, however, whether the diversity of microplastic-associated microbial communities is different from that of assemblages that colonize natural particles and are present normally in water *(611)*. While some studies indicate less diversity on plastic than on non-plastic substrates *(617, 621)*, others found no difference in microbial composition on natural and artificial surfaces in the ocean but rather that the assemblage is influenced by environmental factors *(622)*. Most of the microorganisms reported to be associated with MP are non-pathogenic; however, several studies have shown that microplastics in the marine environment can harbour opportunistic pathogens, in particular *Vibrio* spp., which were found enriched on a polypropylene particle sampled in the

North Atlantic Gyre *(598)*, on plastic particle samples from the Bay of Brest, France *(623)*, and on MP from the North Sea and the Baltic Sea *(612)*. Other studies did not confirm enrichment of potential pathogens associated with plastic *(624–626)*. *Vibrio* spp. are known to form biofilms on various substrates, including glass, wood and other natural materials. A study of the bacterial assemblages on polyethylene, polystyrene and wood microparticles in the Baltic Sea showed that *Vibrio* were present on MP but were more abundant on wood particles. Environmental factors and nutrient availability appeared to be the major drivers of microbial assemblage, rather than the substrate itself *(620)*. A meta-analysis of studies in marine environments concluded that the abundance of potential human pathogens on MP and on naturally occurring particles might be comparable *(622)*.

Several studies found enrichment of antimicrobial resistance genes in microplastic-associated biofilms *(627–630)*. Gene transfer and metabolic functions are more extensive in biofilms than in free-living microorganisms; however, no well-controlled comparison has been made of antimicrobial resistance gene enrichment in biofilms on microplastic and on natural particles. It has been suggested that not only the composition of the microorganisms associated with MP but also heavy metals adsorbed on MP drive enrichment of antimicrobial resistance genes *(631)*.

6.2 Microplastic-associated biofilms in food

Although exposure to MP in seafood, including to any associated biofilms, is expected to be very low (see section 2.4), many pathogens efficiently establish infection with a very small inoculum. It is not known whether exposure to microplastic-associated pathogens can result in established infection of aquatic species and, if they do, through what route of exposure, and it is also unknown whether humans could subsequently be exposed to the pathogens by ingestion of contaminated seafood. There is no experimental evidence that microplastic-associated pathogens can establish infection in seafood. One study showed direct transfer of *Escherichia coli* tagged with green fluorescent protein from MP to the gut tissue of a coral species in a laboratory experiment, which provides preliminary proof that MP are vectors for pathogens *(632)*. No alteration in microbial composition was observed, however, on MP as compared with natural chitin microparticles after passage through the gut of the marine mussel *Mytilis edulis (633)*. This raises the question of whether exposure to microplastics-associated biofilms represents a greater risk of infection than exposure to biofilms associated with naturally occurring microparticles, which are more abundant in aquatic environments.

Although studies are lacking on potential infection due to exposure to microplastics, food safety regulations and risk management strategies in many countries mitigate

the risk of exposure to pathogens in food. Cooking of seafood inactivates pathogens, including those associated with MP.

Little is known about the adverse effects of microplastic-associated biofilms. The available data provide no evidence of a risk to human health of such exposure. MP constitute only a fraction of the particles in aquatic environments that provide the surface area for biofilm formation. The possibility of enrichment of antimicrobial resistance genes in microplastic-associated biofilms and that MP might be vectors for pathogen transmission should, however, be studied further.

Key messages

- It is unclear whether the diversity of microplastic-associated microbial communities, including pathogens, is different from that of assemblages on other types of particles.
- Research should be conducted on whether exposure to microplastic-associated pathogens can result in established infection (and, if so, through what route of exposure) and whether humans could subsequently be exposed to microplastic-associated pathogens.

7. SUMMARY AND RESEARCH TOPICS

7.1 Summary

Environmental monitoring of air, water and biota provides convincing evidence that NMP are distributed across the planet. The concentrations are, however, highly variable and are influenced by human activity. In most of the studies conducted to date, MP have been characterized and quantified in marine and freshwater systems, and wastewater treatment effluent has been identified as an important source of NMP in the aquatic environment *(1)*. Characterization and quantification of NMP in air raise awareness about the importance of the atmospheric fate and transport of NMP as a source for both marine and freshwater systems, for human exposure by inhalation and for contamination of food and beverages (section 2). The available studies of concentrations of NMP in air, food and beverages were conducted in only a few locations for only a few food categories, resulting in only crude estimates of human exposure. The data on foods and beverages are limited to a few product types, which are not necessarily the main foods in human diets, and limited quantitative data are available on exposure to the inhalable fraction of particles. Although one objective of this report was to assess risks to human health, the available data are insufficient for a quantitative assessment of total human exposure, as estimated intake is based on limited data with well-known analytical limitations. As observed previously *(634)*, the evidence is insufficient to determine risks to human health

The findings cited in this report do not, however, imply that exposure to NMP is "safe", as concluded by some stakeholders *(635)*. The limits to the reliability and relevance of the available data for quantifying exposure to and the effects of NMP on human health and the environment and how those uncertainties should best be addressed in attempting to determine the presence or absence of risk have been discussed elsewhere *(634, 636–638)*. The constructive momentum built by widespread public awareness and an overwhelming consensus among stakeholders that plastics do not belong in the environment should be leveraged for transformation to a more sustainable plastics economy. In addition to measures for better management of plastic, such as better waste treatment, and initiatives to reduce the use of plastic, innovations should also be encouraged in materials science, particularly with regard to the substantial releases of NMP from plastic products used throughout commerce. As it is clear that human exposure to NMP is ubiquitous, a reduction in exposure can only have widespread benefits for humans and the environment.

In order to assess the risk and the implications of exposure to NMP on human health, we collected and evaluated all the available data for an assessment of the overall weight of evidence for a risk to human health. The shortcomings that obviated a risk assessment included inconsistencies in the data, such as in sampling and the experimental design of studies, and the absence of clear approaches to extrapolate the adverse effects observed in experimental test systems with monodisperse particles to those of the complex mixture of heterogeneous NMP present in the

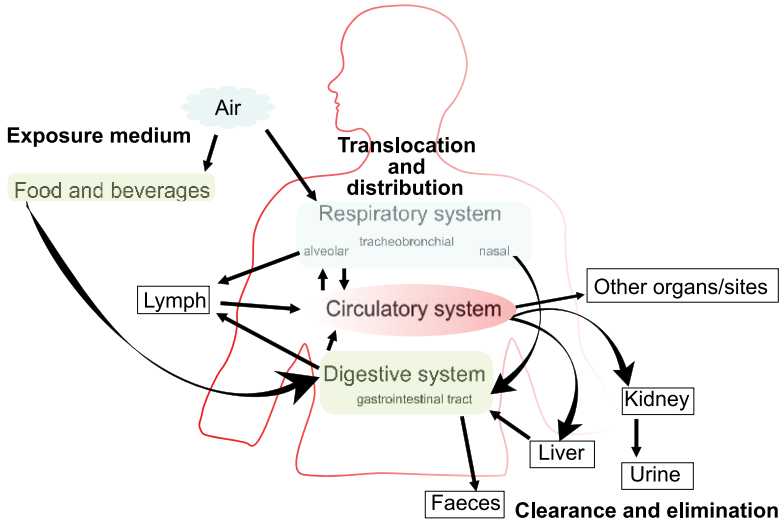
environment. The recommendations made in this report should be perceived as guidance to decision-makers in advancing scientific understanding and reducing the barriers to risk assessment. For instance, better understanding of the sources of, exposure to and effects of NMP could assist prioritization of measures for mitigation.

Exposure to NMP can occur by both inhalation and in the diet. Fig. 12 summarizes the main routes of exposure and biokinetics that are considered important for assessing the probable effects of exposure to NMP on human health. The data on contamination of air with MP imply that atmospheric contamination should be better characterized and quantified. The physicochemical properties of particles, such as size, shape and density, influence their potential deposition in the alveolar regions of the lungs, where their biopersistence can have adverse effects (section 5). Positively charged NP, such as NH_2-polystyrene nano-sized particles, appear to be more potent than neutral particles of similar size. The human health effects of exposure to NMP such as PVC dust and nylon flock are well documented in occupational epidemiological studies (section 3), and mitigation of acute and chronic exposure to elevated concentrations of these particles is strongly recommended. The properties of NMP in air, the fraction that contributes to PM_{10} and $PM_{2.5}$ and their absolute concentrations should be better characterized in order to assess the effects of inhalation of NMP on human health.

Characterization and quantification of NMP in air would improve understanding of potential deposition onto food and beverages, which could influence estimates of the amounts ingested. Information is required on the probability distributions of the physicochemical properties of deposited and ingested particles, such as size, shape, composition and surface chemistry. Section 4 summarizes the biokinetics of distribution, translocation, clearance and elimination and indicates that particle size determines whether particles are absorbed across gastrointestinal epithelial tissue and where they can be distributed in the circulatory system, as the fraction of absorbed particles increases with decreasing size. Understanding of human exposure to particles of different sizes in the atmosphere, food and beverages could guide research on quantifying the extent of absorption after environmentally relevant exposure. Particles that are not absorbed are excreted directly in faeces, but limited research has been conducted on the passage and elimination of particles through the gut. With better data on concentrations in air, food and beverages, mass balance models could be developed for better understanding of human exposure to NMP.

The adverse effects of inhalation of NMP (section 5) include oxidative stress, inflammation, lipid peroxidation, DNA damage and aggravation of underlying effects such as asthma and chronic obstructive pulmonary disease. Experimental studies, both in vivo and in vitro, show that adverse effects are triggered at high concentrations. Particle size, shape and surface chemistry are important properties, but it is not known which are the most important in determining potency. For instance, does the effect observed represent an intrinsic property of the particle or does the concentration-dependent response indicate physical stress on the cell or organism? If it is the latter, are the effects reversible after elimination of exposure, or are NMP intrinsically biopersistent, resulting in long-term effects? Better understanding is required of the factors that influence the biodynamics and biokinetics of NMP after exposure. The current knowledge base is insufficient to differentiate adverse effects associated with exposure to NMP from those of particles occurring naturally in the

Fig. 12 Uptake and biokinetics that influence the effects on human health of exposure to nano- and microplastic particles



diet or inhaled. Although it is known that exposure to high concentrations of PM is associated with respiratory effects, limited quantification of NMP in air obviates a robust risk assessment. Thus, research to identify adverse effects that are intrinsic to NMP would provide guidance for an NMP-specific human health risk assessment. The available data do not allow firm conclusions on the risks to human health of inhalation or ingestion of NMP, but, as NMP are part of the PM mixture, the health impacts will not exceed those of PM.

The role of NMP as a vector for chemicals associated with plastics and for other contaminants and pathogens is summarized in sections 5 and 6. Estimates based on highly conservative assumptions suggest that exposure would have to be several orders of magnitude higher than that from drinking-water before the MOEs of concern for plastic-associated chemicals would be exceeded. The available data are insufficient to conclude whether leaching of plastic-associated chemicals from NMP represents a risk for human health. Although transport of pathogens may be minimal, exposure to pathogens and other harmful microorganisms in food and beverages due to inadequate hygiene or improper food handling is a well-understood risk; hence, precautions to minimize and protect humans from exposure to pathogens in food and beverages should also protect against contaminated NMP.

Although there are several sources of uncertainty, it is recommended that risk management strategies for mitigating exposure to NMP be considered, as reducing exposure is key to reducing any of the potential risks considered in this report.

7.2 Options for reducing exposure

As briefly summarized in section 2 and in reports from various regulatory bodies, microplastics originate from many sources, including by degradation of larger plastic items, and are ubiquitous. As there are only limited data on the numbers and composition of NMP in air, water, food and beverages, the most important sources of NMP cannot be identified.

Given the importance of degradation of discarded plastic into NMP, strategies for better management and use of plastics are critical to minimize exposure to NMP. As noted by WHO *(1)*, even simple, low-cost measures can reduce the input of plastics into the environment. The Rio Declaration *(639)* includes a statement about the precautionary approach, which includes cost–effectiveness: "Where there are threats of serious or irreversible damage, lack of full scientific certainty shall not be used as a reason for postponing cost-effective measures to prevent environmental degradation".

As outlined in the WHO report *(1)*, consistent with the European Union Plastics Strategy *(640)*, the following measures are proposed to prevent entry of plastics into the environment.

- Improve the economics and quality of plastic recycling.
- Curb plastic waste and littering.
- Drive innovation and investment towards circular solutions and sustainable manufacturing practices to decrease the input of waste to the environment.
- Engage in international initiatives to minimize and eliminate plastic waste.

Key messages are summarized at the end of each section of this report. They are brought together here.

Introduction:

- NMP are a heterogeneous mixture of particles and fibres of various shapes, sizes, polymer composition, surface chemistry and associated chemicals.
- In this report, a pragmatic definition of microplastics is used, in which synthetic polymeric particles are < 5 mm in diameter, while NP are particles < 1 µm in diameter.
- The properties and composition of NMP change during their life-cycle in the environment.

Human exposure:

- Human exposure to NMP is ubiquitous and occurs by all routes.
- Information on exposure from air, drinking-water, food and beverages is limited. Data on the characteristics of NMP and their quantification in each of these media are necessary, with better understanding of their sources.

Observations from epidemiology:

- Evidence in the literature that inhalation or oral uptake of NMP can affect the gastrointestinal tract or other organs apart from the lung is limited and of inadequate quality.
- Better estimates are required of exposure of the general population to NMP and co-pollutants by inhalation and in the diet.

Dosimetry and biokinetics:

- Physiological mechanisms for the uptake, distribution and elimination of MP minimize tissue exposure. The probability of uptake into the body increases with decreasing particle size.
- There is insufficient information to assess biodistribution (uptake, retention, clearance, rate of translocation), including the likelihood that NMP will cross biological barriers after deposition on the epithelium or after reaching the circulation.
- Dosimetry models are available for extrapolation of results on particle inhalation obtained in experimental animals to humans, but they have not been evaluated or validated for NMP.
- Data on the biokinetics of NMP obtained in models in vitro cannot currently be extrapolated to the situation in vivo.

Toxicological effects:

- Data on toxicity after inhalation or dietary exposure for characterizing the hazard of NMP are limited to studies with polystyrene beads. Information is required on the effects of particle size, shape, polymer composition and other factors representative of environmentally relevant exposure to NMP.
- The limited hazard characterization of NMP suggests that they may have adverse effects similar to those of other well-studied solid and insoluble particles through similar modes of action.
- The available data are insufficient to determine whether exposure to NMP is associated with any direct or indirect characteristic pathology, as concern about QA/QC has been poorly accounted for in published studies.

NMP as vectors for pathogens:

- It is unclear whether the diversity of microplastic-associated microbial communities, including pathogens, is different from that of assemblages on other types of particles.
- Research should be conducted on whether exposure to microplastic-associated pathogens can result in established infection (and, if so, through what route of exposure) and whether humans could subsequently be exposed to microplastic-associated pathogens.

Several themes can be identified in the key messages and are used to define the necessary research. Generally, the characterization and quantification of exposure to NMP and the associated human health effects are incomplete and insufficient for an assessment of risk, although the potential effects of NMP on human health should continue to be monitored. As more data become available for better understanding of mechanisms of action and subsequent effects, it may be possible to characterize and quantify human health risk in the future. The basic research requirements necessary to advance scientific understanding are listed below.

- **Standard methods**: Sampling and analysis of NMP in air, water, food and beverages require robust, quality-assured methods and suitable reference standards representative of environmentally relevant NMP.
- **Particle characterization**: Quality-assured environmental monitoring studies should be conducted to characterize the distributions of size, shape and composition of NMP in the environment for studies of the effects of exposure on human health and to prepare reference standards for environmentally relevant testing of toxicity.
- **Sources of NMP**: Although NMP are ubiquitous in the environment, their sources cannot currently be accurately defined. They include tyre and road wear particles, textiles, degradation and fragmentation of plastic, but it is not known which source predominates. The contributions of different factors would guide strategies for mitigating exposure.
- **Uptake and fate of both inhaled and ingested NMP**: Information on the absorption and systemic uptake of NMP is available from only a few studies with a limited number of plastic polymers. More information is required on the absorption, distribution and elimination of NMP. More research should be conducted on the influence of the food matrix on the bioavailability of ingested particles and the efficiency of their absorption and elimination.
- **Toxicology**: Quality-assured experiments suitable for risk assessment should be conducted, with adequate characterization of exposure to the types of NMP to which humans are most commonly exposed.

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ANNEX

Quality assurance and quality control criteria to be met to achieve each score

The criteria are considered to have been met if they are referred to in a publication. The table below summarizes the scores for quality, with the categories and criteria described in the literature.

Activity		Score 2	1	0
Sampling	Sampling methods	**Dust:** • Location • Date • Apparatus • Mass/area collected **Atmospheric deposition:** • Sampler description (including collection surface area) and whether bulk or wet deposition collected • Location • Date • Height (of sampler and site, if appropriate) • Sampling duration (per sample and per campaign) • Materials used (e.g., filtered water) in sample collection **Suspended particulate (air):** • Location • Sampling instrument (make, model) • Aerodynamic size fraction • Flow rate • Height (of sampler and site for atmospheric air) • Filter substrate • Sampling duration (per sample and campaign) • Date and meteorological conditions	Only a subset of the required criteria are reported (e.g., date, location, materials used); however, the data are reproducible.	Insufficient reporting of sampling methods
	Sampling duration	**Atmospheric deposition:** typically coarse resolution (e.g., 1 week or 1 month) **Suspended particles (air):** 24 – 72 h* for low volume (16.7 L/min) sampler Sampling duration may differ according to the nature of the sample (e.g., if highly polluted, high organic content), use of a high- or a low-volume sampler and research question (e.g., if interested in a specific activity) *72 h defined as optimum by Liu et al. *(1)*, while 24 h is typical for collecting PM_{10} sample (EN 12341)	Application of consistent sampling resolution to the best of the authors' ability, which is appropriate to address the research question	Inconsistent sampling duration unrelated to research question or sample type, or insufficient reporting

Activity		Score 2	1	0
	Sample processing and storage	**Atmospheric deposition:** Sample collection in filtered water. Store sample shortly after collection in the dark at 4 °C, or filter, dry and store in a cool, dark place **Suspended particles (air):** Transfer filter to a petri dish. Store in cool, dark place.	Insufficient storage at room temperature and/or or storage Unnecessary exposure or contamination risk during transportation	Insufficient reporting
Mitigation of contamination	**Laboratory preparation**	• Cotton laboratory coat or non-synthetic clothes • Equipment and laboratory surfaces wiped and rinsed • Plastic avoided in the protocol when appropriate • All apparatus used is rigorously cleaned with ultrapure water and/or filtered solvents. • All reagents and solvents used are filtered.	Criteria met only partially, e.g., only wiping laboratory surfaces and equipment, not wearing a cotton laboratory coat	No precautions or insufficient reporting
	Clean air conditions	• Clean room or laminar flow cabinet • A clean room should be classified in accordance with ISO 14644 and/or with an indication of the maximum permitted airborne particle concentration.	Mitigation of airborne contamination by keeping samples closed as much as possible if negative samples were run in parallel and examined for contamination	No regard for airborne contamination, use only of a normal fume hood or insufficient reporting
	Negative control (blanks)	• Field controls collected either in parallel to samples (paired) or throughout the sampling period (at least in triplicate), but without with no exposure to air or /deposition • Laboratory (procedural) controls (at least in triplicate) treated and analysed in parallel to with actual samples Sample concentrations reported should account for controls, i.e., deduct on of baseline microplastic count, shape and polymer type	Insufficient type of a control, e.g., fewer than three replicates, reporting of negative control results with no indication of whether sample data were blank corrected	No negative controls or insufficient reporting
Sample purification and handling	**Positive control**	Controls (at least in triplicate) with added microplastic particles treated at the same time as the samples and for which the particle recovery rates are determined	Insufficient type of a positive control (e.g., only part of the protocol is tested)	No positive controls or insufficient reporting
	Sample treatment (if necessary; if not necessary, a score of 2 is assigned)	**Dust only:** Sieving **All sample types:** Digestion of sample in a protocol such as wet peroxide oxidation and/or enzymes. If another chemical was used, the effects on different polymers should be tested before application and reported All sample treatments should be onducted at < 50 °C to prevent damage to microplastics or changes in glass transition temperature	If wet peroxide oxidation is carried out without cooling or digestion temperature exceeds 50 °C	If no proof is provided that polymers are not affected by the protocol (e.g., heated KOH > 50 °C) or/ insufficient reporting

Activity		Score		
		2	1	0
Microplastic characterization and application for assessing human exposure	**Filter/substrate composition**	Appropriate for subsequent analysis, i.e., inert, flat membrane	Quartz fibre filters (for direct analysis by micro-spectroscopy) or composition that interferes with analysis	Insufficient reporting
	Polymer identification	**Automated, semi-automated or rigorous operator-approach:** Detailed, repeatable method, including whether microparticles are analysed directly in the sample or transferred to new substrate, spread of particles analysed for all samples or per filter ≥ 25% of the surface area analysed. High percentage of suspected microparticles analysed, i.e., all particles for which the numbers of pre-sorted particles are < 100 or ≥ 50% when particle numbers > 100; high hit quality Indices accepted (> 70%); Details of library or/database included or details of software or/programme	Hit quality indices < 70 % when library matches; low percentage of suspected microparticles/ sample area analysed; no indication of whether microplastics are evenly distributed among samples; no indication of whether microplastics were analysed directly in a sample or transferred manually Identification with scanning electron microscopy and energy-dispersive X-ray to distinguish polymer from non-polymer materials	No polymer identification performed or insufficient reporting
	Particle characterization for human exposure	Detailed reporting, including maximum and/minimum particle size and particle size limit of detection Length and diameter of fibres reported Classified as fibres if aspect ratio > 3:1	No mention of minimum size or/ limits of detection Sizes based on suspected (not confirmed) microplastic)	Insufficient reporting

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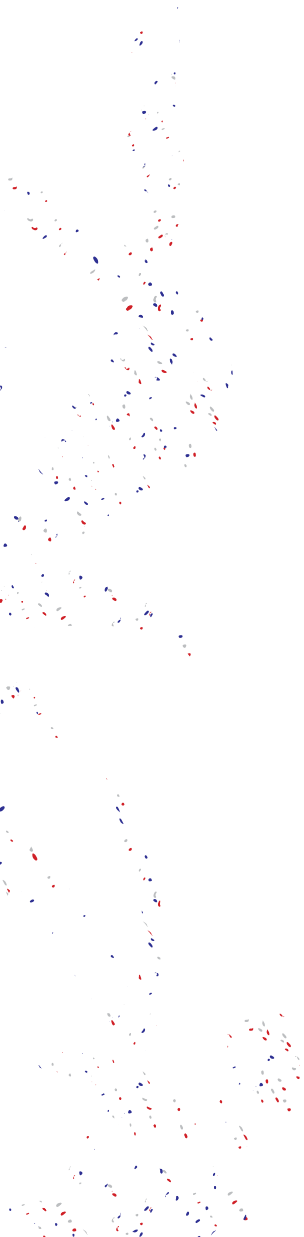

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