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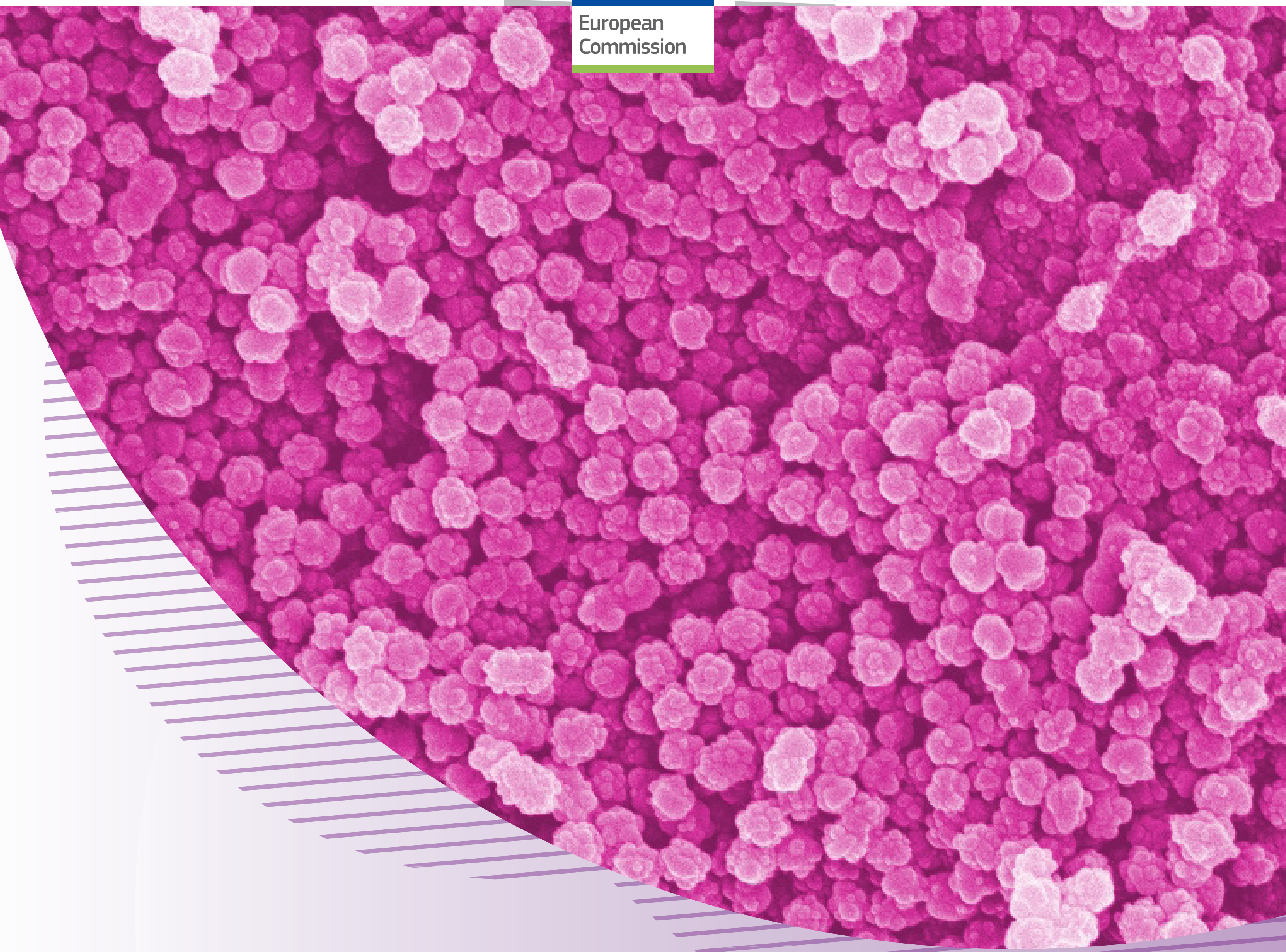

Science for Environment Policy

FUTURE BRIEF:
Nanoplastics: state of knowledge and environmental and human health impacts

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Nanoplastics: state of knowledge and environmental and human health impacts

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Science for Environment Policy Future Brief 27:

Nanoplastics: state of knowledge and environmental and human health impacts

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1. Introduction: nanoplastics in context

Plastics are highly versatile and are ubiquitous in society, fulfilling a wide array of valuable functions in our economy and daily lives. Their adaptability has led to them being used in consumer products such as cosmetics, cooking utensils and food packaging, among others, as well as in medical devices and construction. While there has been a strong push to ban single-use plastics and develop plastic-free and recyclable packaging and products in recent years, notably as part of the EU's [Plastics Strategy1][1] and [Circular Economy Action Plan2][2], plastic disposal remains a key threat to our natural environment, and the material is accumulating in our soil and seas in unprecedented amounts. It has been suggested that an extra 33 billion tonnes of plastic will be added to the planet by 2050 (Galloway, 2015) and some have suggested the current era may even be referred to as the 'Plasticene' (Reed, 2015).

Overcoming the challenge of plastic pollution has been named "the defining challenge of our times" by the United Nations Environment Programme (UNEP, 2018). The impact on our environment is growing to such a scale that plastic litter is considered by some as an emerging threat to how our planet functions and stabilises itself at a global scale (Galloway and Lewis, 2016). Scientists have even proposed marine plastic pollution levels be included as one of the 'planetary boundaries' – a set of nine environmental thresholds that must not be exceeded in order to protect and support the healthy function and development of our planet and its communities (Steffen *et al.* 2015). Plastic pollution does indeed fulfil two of the three boundary criteria in that it is likely irreversible and globally ubiquitous. More knowledge is required to ascertain if it also fulfils the third: disruption to ecosystems or, on a wider scale, the entirety of the Earth system (Villarrubia-Gómez, Cornell and Fabres, 2018). Some researchers suggest that since plastic pollution is occurring faster than it is possible to conduct safety assessments and monitoring, it is exceeding the 'safe operating space' of the planetary boundary for novel entities, demanding prompt action (Persson *et al.*, 2022). The need for such action is especially pressing in light of the potentially synergistic effects of other types of chemical pollution; low capacity of some countries, globally, to abate it through regulation and enforcement; and persistent threat posed by plastics already released.

> **Of the 6.3 billion tonnes of plastic waste generated by 2015, nearly 80% has ended up in either landfill or the natural environment (and only 9% has been recycled)**
>
> Geyer, Jambeck and Law, 2017

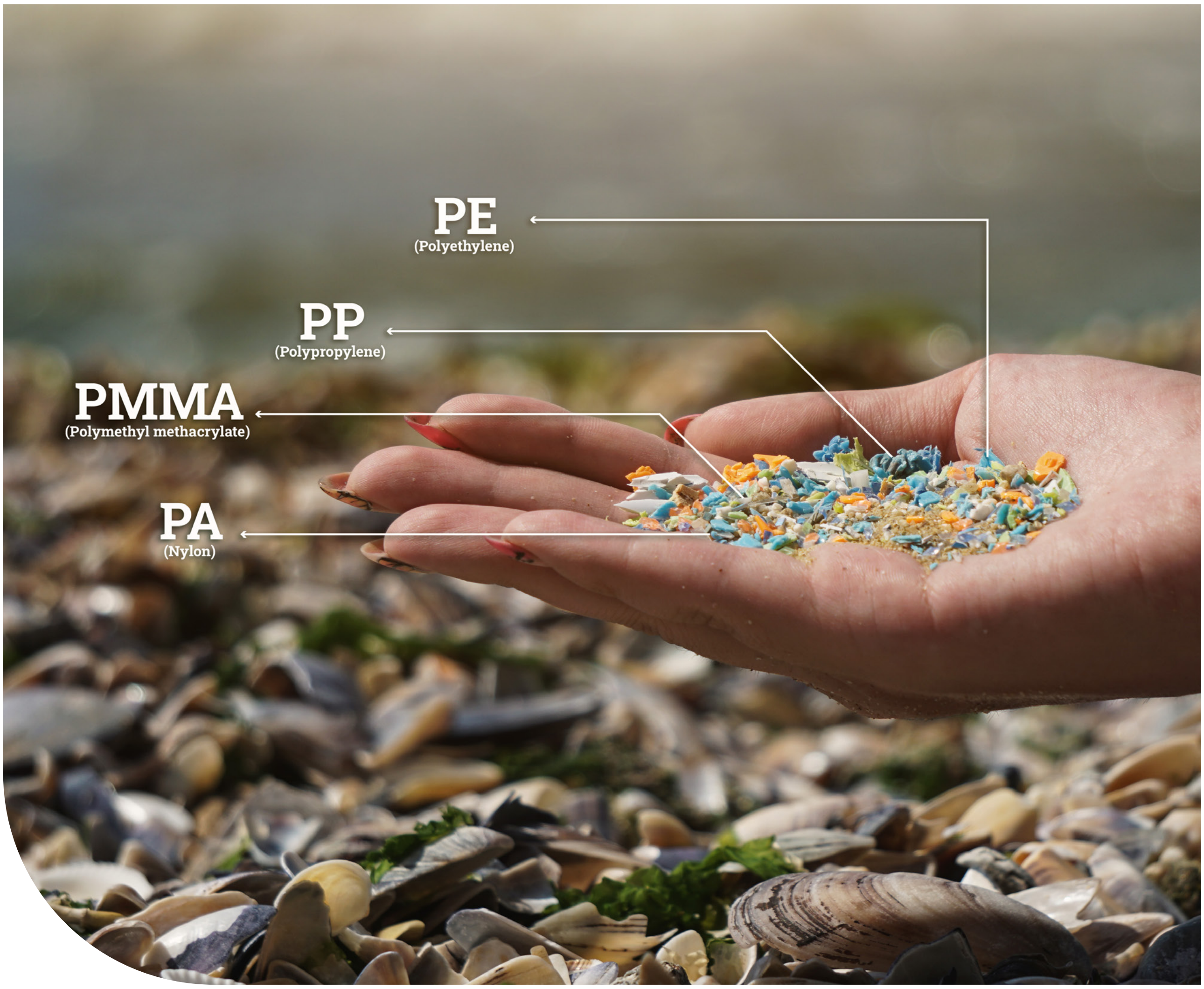
Microplastics may be deliberately manufactured materials – sometimes termed primary microplastics – but there are very few intentionally produced plastic nanomaterials. In the environment, plastic also breaks down to form small fragments known as incidental or secondary microplastics and, at even smaller scales, nanoplastics. Research has focused on microplastics rather than nanoplastics due to their greater visibility and availability of suitable detection methods. There is growing evidence of human and wildlife exposure to microplastics in the environment, and some data on the potential hazard to ecosystem health, but their impacts on human health remain unclear.

For nanoplastics, the situation is even less clear, and data even more sparse. While debate is still ongoing about the size definitions for microplastics (Hartmann *et al.*, 2019), it is an open question at which size a microplastic particle becomes a nanoplastic particle. This discussion is more than a formality. Due to their smaller size, concern is rising over the potential impacts of nanoplastics specifically on the environment, human food chain,

1 https://environment.ec.europa.eu/strategy/plastics-strategy_en
2 https://environment.ec.europa.eu/strategy/circular-economy-action-plan_en

and human health; they are difficult to detect and isolate from environmental samples via standard separation and analytical methods, and there is a risk that these tiny particles may be more efficient at crossing biological membranes than microplastics.

This Future Brief elucidates the current situation regarding nanoplastics and the environment, from their detection and analysis through to their potential health risks, dispersal pathways, and future outlook.



Side view of a woman holding microplastics in her hands with composition infographics (©Shutterstock, photo by SIVStockStudio)

1.1 What we do – and don't – know

According to the European Chemicals Agency (ECHA) and Organisation for Economic Co-operation and Development (ECHA, 2019; OECD, 2021), **microplastics** are small particles of plastic typically no more than 5 millimetres (mm) across. This working definition for a microplastic aligns with that proposed at a National Oceanic and Atmospheric Administration (NOAA) meeting in 2008 and, while not formalised as an international standard, remains widely used (Arthur, Baker and Bamford, 2009; Hartmann *et al.*, 2019).

Generally, **nanoplastics** are considered to be the plastic particles of the smallest size, i.e. smaller than microplastics. Although the EC Recommendation on the Definition of **Nanomaterials**[3] (including plastics) states that they contain particles in the size range 1-100 nm (EC, 2022b), in scientific literature there is currently no agreed distinction on the size of nanoplastics as opposed to microplastics, (Mitrano, 2019; Hartmann *et al.*, 2019). For example, some set the threshold at 1 micrometre (µm; 0.001 mm); others place a nanoplastic's upper size limit 10 times lower, at 100 nanometres (nm; 0.0001 mm) (Ng *et al.*, 2018; EFSA, 2021). Definition of a nanoplastic as **a particle measuring no more than 1µm (0.001mm) across in any one dimension** is widely accepted as a guiding definition (Gigault *et al.* 2018; Dick Vethaak and Legler, 2021; SAPEA, 2019). It is to be noted that this threshold as well as the approach (longest dimension) deviate from the definitions of nano-objects and nanomaterials, which share the prefix but are usually related to features at 1-100nm scale. In addition, the longest dimension of a plastic particle may not be the most appropriate characteristic in assessing risk – the smallest dimension often indicates the likelihood of interaction with biological systems.

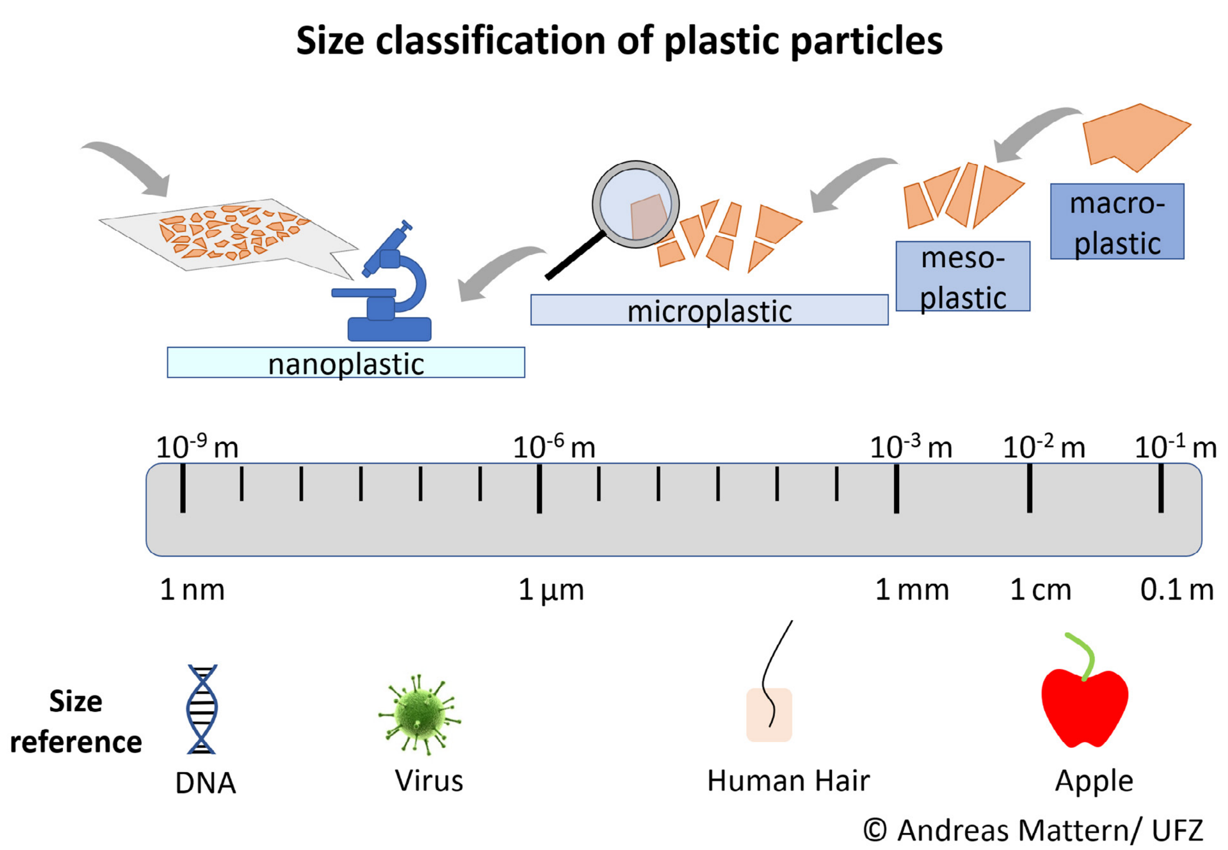


Fig. 1: Classification of plastic particles by their size and size references, according to definition of nanoplastic by Hartmann *et al.* (2019) © Andreas Mattern/ UFZ

3 https://ec.europa.eu/environment/chemicals/nanotech/pdf/C_2022_3689_1_EN_ACT_part1_v6.pdf

Nanoplastics include conventional, bio-degradable and bio-based plastics. Currently, there is no firm evidence that the raw materials chosen to create a plastic product affect the risks resulting from that product entering our environment. Plastic polyethylene terephthalate (PET) bottles derived from petrochemicals exhibit the same chemical and physical properties and behaviour in the environment as plastic bottles created from sugar cane, for example, and so "bio-based alternatives" to conventional plastic particles "are a priori included as microplastics [and nanoplastics] until there is proof for their low potential risk" (EC, 2017).

The terms 'plastic' and 'polymer' are often used interchangeably, but they are not the same thing; not all polymers are plastic. Polymers consist of chains of joined molecules or monomers, and may be found in natural materials such as wool, cotton and wood – and natural polymers are not plastics. Plastic is a material produced from synthetic (e.g. polypropylene) or semi-synthetic polymers (e.g. viscose), or containing a high proportion of these polymers, and often additives. Plastics may be moulded when soft (usually due to heating) and retain a given shape when hardened (either by cooling or chemical processing). Another group of polymeric materials, elastomers (elastic polymers, commonly known as rubber), are often included in the term plastic, although they have different properties. In materials science, elastomers are not plastics because they do not have structural integrity under a load (they stretch), however when we talk of plastics in the environment, it is useful to include elastomers as this encompasses particles from tyre wear, for example.

Fig. 2 All plastics contain polymers, but 'plastic' and 'polymer' are not the same thing.
Source: Aznan, Shutterstock

Box 1: Features of nanoplastics

Nanoplastics:

- are often defined as particles measuring no more than 1 μm (0.001 mm) across in any one dimension (but risk may be related to their smallest dimension)
- include conventional, biodegradable and bio-based plastics
- in the context of environmental science, include elastomers
- include primary, manufactured nanomaterials, and secondary nano-sized particles which are the product of larger plastic particle degradation

1.1.1 How do nanoplastics differ from other plastic particles and nano-sized materials?

Although nanoplastics share many characteristics with microplastics, they are a **behaviourally distinct type of environmental contaminant**. As summarised by Brewer, Dror and Berkowitz (2020), nanoplastics are:

- small enough to penetrate more and different biological barriers, which in turn increases their potential for toxicity and transport of other adsorbed toxins within organisms – including humans
- far **more difficult to quantify** in natural samples, leading to significant knowledge gaps when compared to the body of research tackling microplastics
- able to **adsorb** (hold onto in a thin encasing film) **significant amounts of other pollutants** that are known to be harmful (e.g. heavy metals, pesticides, pharmaceuticals, endocrine disrupting chemicals, microorganisms) due to their high surface area-to-volume ratio, thereby increasing the potential mobility and dispersal of these pollutants. (Microplastics also adsorb substances, however nanoplastics have an even greater capacity to do so **as a result of their higher specific surface area**.)
- **more reactive than larger plastic particles** due to their high surface area-to-volume ratio, therefore more likely to interact with other materials in their environment and potentially more likely to destabilise or aggregate
- small enough to **avoid plastic removal processes** such as physical straining through soil or sediment

As with all plastics, nanoplastics may leach chemical additives and potentially present by-products and monomers, possibly at a faster rate than larger particles due to their greater relative surface area. This raises concerns about the potential toxicological hazards of these substances. Whether coatings that form on nanomaterials as they enter the environment or organisms (eco- or protein coronas) influence their behaviour, is an ongoing topic of research.

For safety assessment of nanomaterials (natural, manufactured or incidental), regulators are concerned with a number of issues – which also apply to nanoplastics (Allan *et al.*, 2021) – such as:

- clear definitions
- ensuring there are appropriate analytical methods for detecting them in a variety of matrices (e.g. in goods and the environment)
- appropriate toxicological methods for hazard assessment

The level of plastic pollution in the environment – indicative of potential levels of nanoplastic pollution – makes such knowledge gaps an urgent focus for investigation and regulatory measures.

> **The extremely large volumes of plastic contaminants entering the environment compared to most engineered nanoparticles further underscore the necessity of singling out nanoplastics as a unique potential hazard**
>
> Brewer, Dror and Berkowitz, 2020

1.1.2 Primary and secondary nanoplastics

Plastic particles enter the environment via multiple pathways, with two key origins: **primary** (nanoplastics that are intentionally manufactured) and **secondary** (those that are formed as larger plastics degrade). Primary nanoplastics are far less prevalent than primary microplastics, and most plastic debris found at the nano-size is secondary (Bianco and Passananti, 2020). The major source of environmental nanoplastics is physical abrasion of larger plastic products across their lifecycles (Gangadoo *et al.*, 2020; Nanotechnology Industries Association, 2020).

Microbeads – small solid plastics that are added to exfoliating face scrubs and other personal care products – are a well-known example of primary plastic particles. **Primary nanoplastics** are also added to a wide range of products for diverse purposes, also being found in personal care and cosmetics products, in products for biomedical or laboratory applications, and textiles (EC, 2017). Table 1 gives a list of micro- and nanoplastic functions. These particles are designed for commercial use, and make their way into the environment via routes such as wastewater (Bianco and Passananti, 2020).

Table 1. Functions of micro- and nano-sized plastic particles in different products. The source table (adapted from EC, 2017) specifically refers to microplastics. However, the upper limit of nanoplastics is currently a matter of debate and many of these functions also apply to nanoplastics as currently defined. Many of the particles referred to here lie in the nanoplastic size regime (below 1 μm (=0.001 mm) in any one dimension). Opacifying nanoplastics, for example, can be as small as 170 nm (=0.0002 mm) in size, while plastics added to improve paint clarity are generally below 1 μm (=0.001 mm) in size.

Function	Products
Abrasive/exfoliating	Cosmetics, detergents, industrial blasting abrasives
Emulsifier, suspending agent	Cosmetics, detergents, paints
Binding	Cosmetics, paints, inks, concrete
Filler	Construction (wall and joint fillers, self levelling compounds/screeds)
Controlled release of ingredients	Pharmaceuticals (nano capsules), cosmetics, fertilisers, crops, detergents (enzymes)
Film forming	Cosmetics, polishing agents
Surface coating	Paper making, polishing agents
Improved chemical and mechanical resistance	Coatings, paints, floor coatings, polymer cement. Coatings can include, for example, house paints with water repellency; glass and façade coatings for high-rise buildings; for transport such as cars, aeroplanes, ships and for structures such as bridges; or coatings used to protect electronic products – both consumer and industrial.

Function	Products
Fluid absorbents	Nappies, water retainer for farming, agriculture, horticulture
Thickening agent	Paints, cosmetics, concrete, oilfield use (drilling fluids)
Aesthetics	Coloured microplastics in make-up, structural effects of paints, enhanced gloss level of paints
Flocculant	Waste water treatment, oilfield use, paper making
Dewatering	Paper making, dewatering of sewage sludge, manure
Dispersing agent	Paints, coatings (pigments)
Opacifying agent	Cosmetics
Anti-static agent	Cosmetics and hair care

Fig. 3. One use of primary nanoplastics is in scratch resistant coatings. Source: Roman Zaiets, Shutterstock

Box 2: Intentionally manufactured nanoplastics

Very small quantities of nanoplastics are produced as test or reference materials in scientific research. For example:

- Germany's Federal Institute for Materials Research and Testing (*Bundesanstalt für Materialforschung und -prüfung* (BAM)) intends to produce small quantities of nanoplastic particles as reference materials to use in its work on the EU's POLYRISK and PlasticsFatE projects, both of which are studying the effects of nanoplastics on human health.
- Research projects for detecting nanoplastics in the environment have used a method to synthesise nanoplastic particles, adding a chemically entrapped metal which acts as a tracer (Mitrano *et al.*, 2019). This provides an effective way to accurately detect nanoplastics in complex media such as sewage sludge.
- Nanoplastics coloured with fluorescent dyes have been used to investigate absorption across biological membranes (Catarino, Frutos and Henry, 2019).
- Polystyrene nanoparticles are used to calibrate scientific instruments.

Secondary nanoplastics, meanwhile, are created via degradation and mechanical abrasion of larger plastic debris, including microplastics, and therefore tend not to have a uniform shape and morphology due to their method of formation. They are largely shaped by their initial composition and the ageing or degradation processes they have been subject to (see section 3.2), exhibiting a wide array of physical and chemical properties (Brewer, Dror and Berkowitz, 2020). Sources of secondary nanoplastics include tyre wear, synthetic textiles, agricultural plastics, fishing equipment and packaging. For example, one study found that on average 1.4 to 2.1 mg of nanoplastic particles was released from a single gram of polyester fleece textile during washing and abrasion experiments, indicating synthetic fabrics may be a significant source of nanoplastics (Yang, Luo and Nowack, 2021). Tyre wear is a well-known source of micro- and nanoplastics in the environment, with 0.3% of the 21,200 t/y of tyre wear particles lost to the environment at the nanoscale, i.e. below 0.1µm (Knight *et al.*, 2020; Prenner *et al.*, 2021). These particles may also derive from mulches made from old tyres, used in playground surfaces and sports pitches.

A further source of nanoplastic pollution is emerging in the form of their unintentional formation and release to the environment as a by-product of other manufacturing processes. One example of this is in the rapidly growing three-dimensional printing sector (Rodríguez-Hernández *et al.*, 2020). Following a printing procedure, equipment is cleaned with an alcohol/resin mixture, which can unintentionally form small nanoplastic particles if it is exposed to UV radiation (e.g. sunlight). If this mixture is subsequently disposed of incorrectly – an emerging risk given the rising domestic use of such printing devices – these nanoplastics could find their way into wastewater and agglomerate in seawater, forming a new and as yet unquantified potential source of nanoplastics.

1.1.3 Knowledge gaps

There are many emerging applications for – and sources of – nanoplastics, but many knowledge gaps remain that must be addressed in order for us to use these materials safely. These require advances in the ability to sample, isolate, detect, quantify, and characterise nanoplastics, as well as standardised methodologies to permit reproducible findings and comparable data. For example, there are challenges in the ability to reliably detect, quantify, and identify target nano-scale particles – particularly in complex mixtures. This may present challenges in enforcement of regulation addressing them (see e.g. ECHA, 2020a; discussed in Chapter 2). In August of 2022, the COM draft proposal on intentionally added micro (and nano) plastics was released, with no lower size limit – thus intentionally added nanoplastics will be covered by the regulation (EC, 2022a). REACH restricts manufacturers from knowingly adding nanoplastics to their products, however it is also acknowledged that at present analytical methods are unable to check products for compliance below 0.1 µm for spherical particles.

Compared to our knowledge of marine plastic pollution, there remains a further lack of research on the **behaviour and fate** of plastic particles in freshwater, atmospheric and terrestrial environments. More work looking into how their morphology affects both behaviour and impacts is needed. It is known that microplastics arise as fragments, pellets, filaments, fibres, broken edges, beads, and irregularly-shaped particles (Rosal, 2021); are some shapes of nanoplastics more problematic than others? How they interact with pharmaceuticals and other emerging contaminants is another important area of research.

Assessing risks of nanoplastics to human health requires **data on exposure**. When considering nanoplastics in the food chain, for example, Toussaint *et al.* (2019) identify the heterogeneity of the methodologies and experimental designs as a key challenge. Exposure to micro- and nanoplastics via other routes such as inhalation is now receiving attention (Prata, 2018; also see sections 3.4 and 5.1), but **standardised methodology** is yet to be implemented.

> **Research into (micro- and) nanoplastics has started only recently and thus, there are many unresolved issues of terminology, definitions, sampling, characterisation and the assessment of hazard and exposure that, in combination, make it difficult, even impossible, to evaluate and regulate the potential risks of nanoplastics**
>
> Allan, Sokull-Kluettgen and Patri, 2020

Box 3: EU projects

A number of European projects are underway that will advance understanding of nanoplastic behaviour, hazards and potential remediation, including:

CE4Plastics[4] (2022-2024) - advancing methods for identification and quantification of nanoplastics released to drinking water from single-use and reusable plastic bottles, and raising awareness in the population about nanoplastic risks.

VORTEX[5] (2018-2023) - investigating microbial transformation of plastic in the ocean.

In-No-Plastic[6] (2020-2023) - developing technology and social initiatives to remove plastic from aquatic ecosystems.

MIGMIPS[7] (2021-2023) - using gut barrier models to study the translocation of microplastics and nanoplastics.

MS4Plastics[8] (2022-2024) - developing innovative pre-treatment and pre-concentration protocols to detect and characterise low µm-range microplastics and nanoplastics (1 nm – 20 µm) in edible fish and shellfish samples.

The EUROqCHARM[9] consortium is evaluating existing methodologies for plastic pollution assessment, with the aim of harmonising them on a European level. The training network MonPlas[10] (Monitoring micro- and nanoplastics), meanwhile, is working with early stage researchers to develop technology for detecting plastic particles in water, in order to provide standardised data.

These initiatives complement the EU's RESPONSE[11] project (2020–2023): Towards a risk-based assessment of microplastic pollution in marine ecosystems. RESPONSE integrates expertise on oceanography, environmental chemistry, ecotoxicology, experimental ecology and modelling to answer key research questions on fate and impact of microplastics (MPs) and nanoplastics (NPs), aiming to provide ecologically relevant strategies for assessing the distribution pathway and biological effects of plastic particles in marine ecosystems.

Other research needs identified in the European Strategy for Plastics in a Circular Economy are being addressed in the European Cluster on Health Impacts of Micro- and Nanoplastics (CUSP)[12]. CUSP research results will contribute to the health-relevant aims of the plastics strategy and the Bioeconomy Strategy, as well as the REACH restrictions on intentionally added MNPs to products, providing new evidence for better preventive policies. CUSP comprises five new projects running from 2021 to 2025:

POLYRISK examines the toxic effects of micro- and nanoplastic particles (MNP) on the immune system. Using advanced methods to chemically detect and quantify MNP, the projects aims to understand the key mechanisms of MNP toxicity *in vitro*, and find biomarkers of toxicity in blood and saliva.

AURORA (Actionable eUropean ROadmap for early-life health Risk Assessment of micro- and nanoplastics) aims to develop a framework for risk assessment to evaluate the impact of microplastic and nanoplastic pollution during pregnancy and early life, using in-depth testing and epidemiological data to reveal impacts on child development and health.

IMPTOX will develop an innovative analytical platform to investigate the effect and toxicity of micro- and nanoplastics combined with environmental contaminants on the risk of allergic disease in preclinical and clinical studies.

PLASTICHEAL aims to develop new methodologies and reliable scientific evidence for regulators to set the knowledge basis for adequate risk assessment of micro- and nanoplastics. It will investigate both short- and long-term potential health impacts.

PlasticsFatE (Plastics Fate and Effects in the human body) will implement a comprehensive measurement and testing programme to improve and validate methods and tools for the identification of micro- and nanoplastics. It will lead to a new risk-assessment strategy supporting the health-relevant aims of the European strategies for plastics.

4 https://cordis.europa.eu/project/id/101059423
5 https://cordis.europa.eu/project/id/772923
6 https://cordis.europa.eu/project/id/101000612
7 https://cordis.europa.eu/project/id/101032657
8 https://cordis.europa.eu/project/id/101023205
9 https://www.euroqcharm.eu/en
10 https://www.monplas.eu/project
11 https://www.response-jpioceans.eu/
12 https://cusp-research.eu/

1.2 Regulatory landscape

The European Chemicals Agency (ECHA) estimate that, each year, around 42 000 tonnes of intentionally added microplastics end up in the environment. The largest single source of microplastic pollution is the granular infill material used on artificial turf pitches, with releases of up to 16 000 tonnes. On the 30th August 2022 the European Commission released a draft proposal regarding the restriction of synthetic polymer microparticles[13].

The restriction in the draft proposal would comprise synthetic polymer microparticles below 5 mm, and fiber-like particles below 15 mm, that are intentionally used in products, and may result in environmental release. It could ban the use of microplastics in cosmetics, cleaning products, pesticides, and sports fields amongst others, and thus covers emissions of microplastics that are preventable. According to the draft, the "Commission considers it appropriate to exclude natural, degradable, and soluble polymers from the definition of synthetic polymer microparticles."

Box 4: Measures in the proposed restriction on microplastics in products placed on the EU/EEA market

On August 30th 2022 the European Commission released a draft proposal for a restriction on microplastics. The proposal does not include a lower limit on the size of microplastics, to incorporate all smaller sized plastic particles – thus including nanoplastics. In addition, the proposal also adjusted the timings for companies to adapt to the law – allowing some cosmetic products up to 12 years to comply. It is worth noting that at present, it is also stated in this draft that enforcement cannot be undertaken on plastic particles below 1µm, due to a lack of suitable detection techniques for particles this small .

The proposed ECHA restriction, which has not yet been adopted by the European Commission, includes several measures detailed below:

- **A restriction on the placing on the market of microplastics (and nanoplastics) on their own, or in mixtures**, where their use will inevitably result in releases to the environment, irrespective of the conditions of use. Examples are cosmetics, cleaning and laundry products, fertilisers, plant protection products and seed coatings. For some of these uses, a transitional period is proposed to allow sufficient time for stakeholders to comply with the restriction. For example, a ban on artificial turf microplastic infill, after six years, is proposed for this material.
- **A labelling requirement to minimise releases to the environment for uses of microplastics (and nanoplastics)** where they are not inevitably released to the environment but where residual releases could occur if they are not used or disposed of appropriately (e.g. paints and inks). These uses are not proposed to be prohibited but would need to be reported to ECHA to ensure that residual releases are monitored and could be controlled in the future, and suppliers would also be obligated to give instructions on how residual releases can be minimised. This provision will also enable information exchange along the supply chain.
- **A reporting requirement to improve the quality of information available to assess the potential for risks for remaining uses of microplastics (and nanoplastics) in the future.** This is considered a cost-effective way to enable the Commission and Member States to consider if, and to what extent, additional action could be needed in 5-10 years.

Source: ECHA, 2019; European Commission draft proposal regarding the restriction of synthetic polymer micropartcles, Comitology register (europa.eu)[14]

13 https://ec.europa.eu/transparency/comitology-register/screen/documents/083921/1/consult?lang=en
14 https://ec.europa.eu/transparency/comitology-register/screen/documents/083921/1/consult?lang=en

The adoption of any lower particle size limit in the scope of restriction, and the level at which this was set, would clearly have large implications for the regulation of nanoplastics.ECHA's initial opinion included such limit of 100nm, which however ECHA's Committee for Risk Assessment (RAC) did not support (RAC/SEAC, 2020a; RAC/SEAC, 2020b) while in contrast, the Committee for Socio-economic Analysis (SEAC) included a lower size limit of 1 nm and recommended a temporary lower size limit of 100 nm to ensure that the restriction can be enforced by detecting microplastics in products (ECHA, 2020a). The 2022 draft Commission proposal does not include a lower limit on microplastic size, thus incorporating nanoplastics within this restriction. However, it includes the text stating that where the concentrations of microparticles cannot be determined by current analytical methods (available at the time of enforcement) only particles of 0.1µm or above shall be considered – for microfibres this is 0.3 µm in length (EC, 2022b).

Specific transitional periods are outlined in the draft proposal released in August 2022 by the EC, to allow stakeholders time to find suitable alternatives. These transitional time periods included 3-12 year compliance periods, depending on the context of the diverse products listed – which ranged from agricultural plant protection pesticides, to cosmetic products such as makeup (EC, 2022b).

Other options for reducing the releases of *unintentionally* formed microplastics in the aquatic environment are being considered by the Commission as part of its Plastics Strategy and the new Circular Economy Action Plan, concerning "microplastics created during the lifecycle of a product through wear and tear or emitted through accidental spills" and provisions to reduce the use of single-use plastic products have been adopted as part of the Single Use Plastic Directive (EU 2019/904) .

All these initiatives include nanoplastics as part of microplastics. They do not include any specific measures on nanoplastics, where knowledge to support such legislation remains lacking.

1.2.2 Other plastics relevant regulation

The ECHA's restriction dossier[15] identifies the **role of plastic additives** (such as fillers, UV stabilisers and plasticisers) in the (eco)toxicity of microplastic (and nanoplastics) as an important data gap. The authors also suggest that the current risk assessment of these substances is unlikely to have considered exposure of organisms via a microplastic (or nanoplastic) vector (ECHA, 2019).

A mapping exercise by ECHA and the chemicals industry identified a list of over 400 functional additives or pigments used in plastics, as well as the polymers in which they are most commonly found, and typical concentration ranges (ECHA, n.d.). Focusing on plasticisers, flame retardants, pigments, antioxidants, antistatic agents, nucleating agents and types of stabilisers, the mapping included substances registered by REACH at production above 100 tonnes per year. This figure is a fraction of the total number of plastic additives that have been used – a more recent review expanded on the ECHA mapping and found more than 6 000 chemicals reported in plastics (Aurisano, Weber and Fantke, 2021). Of these, over 1 500 were identified as 'of concern', meaning they should be prioritized for substitution by safer alternatives.

Since the implementation of REACH in 2007, more than 50 plasticisers have been registered and restrictions placed on some (European Plasticisers, n.d.). For example, from February 2015, DEHP, BBP, DBP and DIBP plasticisers were no longer allowed to be produced in the EU, unless authorisation had been granted for a specific use. Some restrictions precede REACH; e.g. phthalates (which can be used as plasticisers) in children's toys have been restricted in the EU since 1999. (Section 4.7 gives more detail on plastic additive toxicity.) The **Extended Producer Responsibility**

15 Registry of restriction intentions until outcome - ECHA (europa.eu): https://echa.europa.eu/registry-of-restriction-intentions/-/dislist/details/0b0236e18244cd73

(EPR) principle (as contained in Council Directive 2008/98/EC[16] on waste management) aims to transfer the elimination costs of used products from the taxpayer to the consumer, by integrating them in the sales prices of the new products. Each Member State may introduce the EPR concept into its own legal framework, and decide how to encourage manufacturers to participate in the prevention, re-use, recycling and recovery of used plastic products. As one example of implementation, national collecting schemes (NCS) for agricultural waste have been implemented in several Member States, with several countries encouraging users, distributors and producers to recycle agri-plastics (Ireland, Iceland, Sweden, France, Spain, Norway, Germany and also the UK).

The European Commission is also planning to develop a proposal for the **registration of selected polymers** in Europe by 2022. At present, neither registration nor evaluation of polymers has been required by REACH, but pressure is mounting worldwide to limit plastic entry into the environment, and registration of polymers is already required in the USA, Japan, South Korea and China. Citing the generation of micro- and nanoplastics as an "inherent hazardous property of plastic polymers" amongst other risks, some NGOs have called for all synthetic polymers to be included in the pre-registration submissions under REACH (ChemSec, 2020). They also note that plastics excluded from the proposed EU safety checks include polystyrene, which has been linked to lung inflammation in rats, and polyacrylamides, which can degrade to the monomer acrylamide, a neurotoxin (EEB, 2021).

The updated Drinking Water Directive[17], which entered into force in January 2021, addresses microplastics and endocrine disruptors, introducing minimum hygienic requirements for materials in contact with water intended for human consumption in the EU. Any material that is intended to be used in new installations or, in the case of repair works or reconstruction, in existing installations and that comes in contact with drinking water between the abstraction points and the tap will need to be conform with the hygienic requirements.

It is important to recognise that plastic pollution and the risk from nanoplastics is a global problem. At the UN Environment Assembly in 2022, 173 countries agreed to develop a legally binding treaty on plastics. Legislation in North America is beginning to be enacted: California has spearheaded measures to prevent microplastics entering the environment, and Canada is banning single use plastics, however there are currently no regulations specifically addressing nanoplastics.

16 http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=CELEX:32008L0098:EN:NOT
17 https://ec.europa.eu/environment/water/water-drink/review_en.html

2. Detection and assessment

Nanoplastics have now been detected at the North and South Poles, in remote lakes in the Northern Hemisphere, and in snow in the Austrian Alps (e.g. Materić *et al.*, 2020; discussed further in section 3.4). They can be traced within biological cells, and measured in microscopic organisms (see 3.5 and 5.2). However, techniques for detecting nanoplastics in environmental and biological samples are in their infancy, along with ways of assessing their fate and effects on ecosystems, biota and humans.

Suitable methods must be developed, standardised and validated to meet the challenge of assessing the types, amounts, and sizes of fragmented plastic particles in environmental samples – something that remains limited in even comparatively 'clean' samples such as domestic water (Schwaferts *et al.*, 2019; Valsesia *et al.*, 2021). When samples are more complex, for example containing large amounts of biological material, matrix effects and contamination become a greater issue, making an already challenging task even more so.

Isolating nano-sized plastics from the huge numbers of natural particles of similar sizes found in ecosystems is one challenge; assessing their persistence – of utmost importance for evaluation of their risk potential – is another. This is pertinent to assessing biodegradability. European standards exist for assessing the compostability of plastics in industrial composting plants and the biodegradability of mulch films in soil for use in agriculture, for instance, but there is no standard for plastic degradability in water, or within organisms, as yet (EEA, 2020; EC, 2020).

Garbage in the oceans kills live animals (©Shutterstock, photo by HilaryDesign)

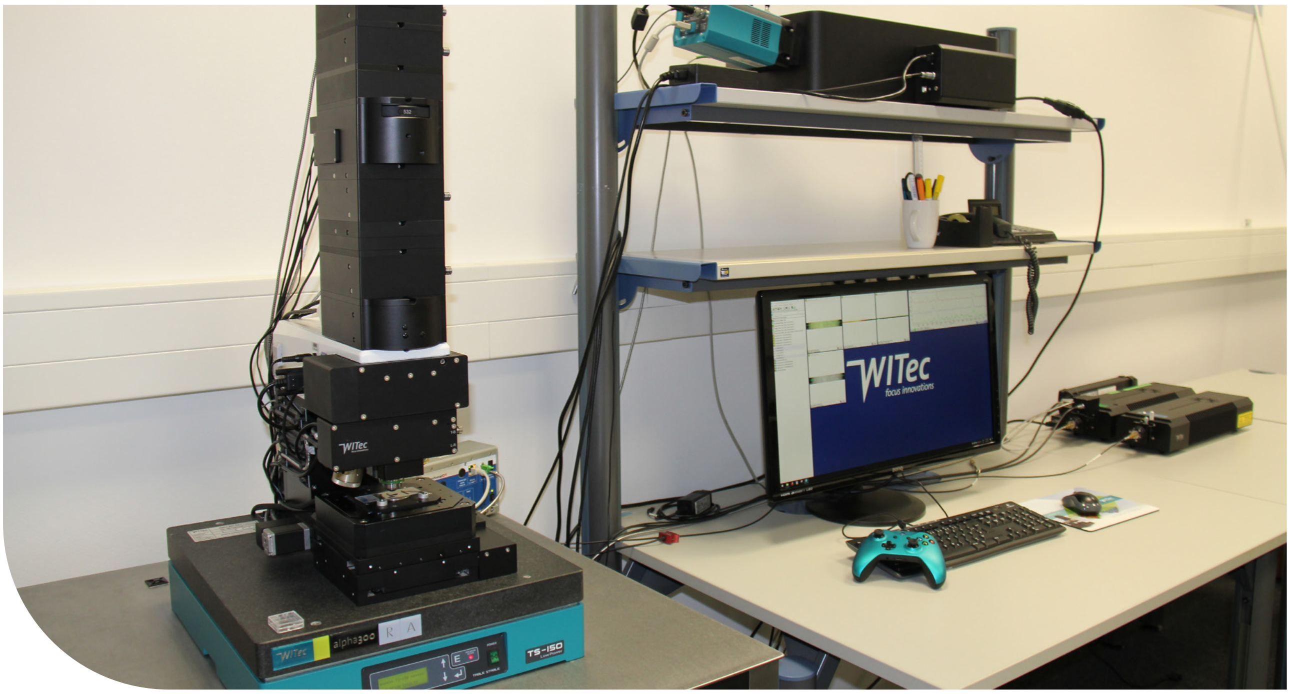
2.1 Methods of characterisation and quantification

Detecting and quantifying nanoplastics in the environment requires methods that can distinguish nanoparticles and separate them from the background matrix. Methods of isolating and characterising nanoplastics utilising dynamic light scattering and Raman microscopy, for example, are under development, but adapting commonly used techniques used to study microplastics can be problematic; conventional filtration, for example, would be excessively time-consuming and costly, as "thousands to tens of thousands of litres of water would have to be filtered through nanoscale-sized pores to acquire statistically relevant quantities of environmental aquatic nanoplastics" (Merzel *et al.*, 2020). Schwaferts *et al.* (2019) provided an overview of methods that are applied to microplastics, that may also be used for nanoplastics (Table 2).

Being composed mainly of carbon, meanwhile – not too different from organic matter – means that nanoplastics evade many available instrumental detection techniques (Jiménez-Lamana *et al.*, 2020; Lehner *et al.*, 2019). Additionally, it is unknown what plastic debris will look or behave like throughout the degradation cycle, introducing further uncertainty into what to search for (EC, 2017).

Many techniques are able to detect and size nanoparticles, but detecting nanoplastics according to size is insufficient – chemical characteristics must be identified to classify particles as plastics. Meanwhile, a technique for detecting one type of nanoplastic may not work for a different type, without adjustment (Correia and Loeschner, 2018).

Some of the methods that show promise include dynamic light scattering (DLS), microscopy, nanoparticle tracking analysis, field flow fractionation (FFF) techniques and mass spectrometry. Techniques are also being developed using doped nanoplastics, whereby particles enriched with detectable metals (e.g. lead, palladium) are added to complex matrices and searched for using robust methods based on mass spectrometry (MS) (Mitrano *et al.*, 2019). Cassano *et al.* (2021) proved able to produce stable, high-throughput, traceable polypropylene nanoplastics by introducing selected markers such as metallic nanoparticles, fluorescent organic and metal-organic dyes or quantum dots – relevant for toxicity and biodistribution studies. At a larger scale, Facchetti *et al.* (2020) successfully identified and characterised fluorescent and metal-doped PVC microplastics (PVC-Platinum octaethylporphyrin (PtOEP) microplastics in the size range of 100 μm) in mussels using *fluorescence microscopy*.



Confocal Raman imaging microscope, ©Wikipedia Commons CC BY 4.0

Table 2: Methods for characterising particle size and morphology Adapted from Schwaferts *et al.* (2019).

Particle characterisation by light scattering

Applying the scattering of laser light on particles to obtain information on physical properties. Generally easy application, and able to gather information including size, particle size distribution, surface charge, stability, aggregation behaviour, and concentration.

Methodology	*Range/Limits*	*Advantages*	*Disadvantages*
Dynamic Light Scattering – DLS	1 nm–3 μm conc. 10^{-6} – 10^{-1}	Fast, cheap, in situ, non-invasive, aggregation, direct coupling	Large particles Polydispersity Complex matrix Non-spherical particles
Multi Angle Light Scattering – MALS	10 nm–1000 nm	Online coupling	Requires clean samples
Laser Diffraction – LD	10 nm–10 mm conc. 10^{-5} – 10^{-1}	Large size range, easy, fast, automated	Only spherical model
Nanoparticle Tracking Analysis – NTA	30 nm–2 μm conc. 10^{-6} – 10^{-5}	Better with polydisperse samples, complex media, particle corona	Complex in operation Spherical model

Particle imaging techniques

Microscopy is the most viable method for obtaining information on a sample's morphology. Three groups of microscope operation modes are most prominent in particle imaging: optical, electron (EM) and scanning probe (SPM).

Methodology	*Range/Limits*	*Advantages*	*Disadvantages*
Transmission Electron Microscopy – TEM	<1 nm	High resolution, precise size information	Quantification difficult Sample preparation Expensive
Scanning Electron Microscopy – SEM	ca. 3 nm	High resolution	Quantification difficult Sample preparation Charging effects

Environmental Scanning Electron Microscopy – ESEM	ca. 30 nm	Wet samples, environmental conditions, non-conductive samples	Reduced resolution
Energy Dispersive Spectroscopy – EDS	nm range	Complementary to EM	Elemental information not sufficient
Atomic Force Microscopy – AFM	ca. 0.1 nm	High resolution, AFM-IR, TERS, In liquid, high resolution	Slow Small area Artefacts due to particle movement
Scanning Tunnelling Microscopy – STM	ca. 1 nm	High resolution	Conductive samples Slow Small area
Confocal Laser Scanning Microscopy – CLSM	>0.2 μm	Fluorescence imaging	Small area Diffraction limit
Near-field Scanning Optical Microscopy – NSOM	ca. 30 nm	Fluorescence	Slow Small area

Chemical identification and characterisation

Chemical identification provides information on type of particle, additives, and ageing. This has usually been achieved by combining vibrational spectroscopy with optical microscopy for imaging. It becomes increasingly difficult with decreasing particle size.

Methodology	*Range/Limits*	*Advantages*	*Disadvantages*
Atomic Force Microscopy Infrared Spectroscopy – AFM-IRIR	>50 nm	High resolution, chemical imaging	Slow, small area
Electrophoretic Light Scattering – ELS	1 nm–3 μm	Fast, cheap, non-invasive, and may be integrated with DLS instrument.	Particle characteristic is sensitive to environment (pH)

Raman Microscopy – RM	>0.5 µm	Non-destructive, easy sample preparation, fast, no interference from water.	Fluorescence
Pyrolysis Gas Chromatography Mass Spectrometry – Py-CG-MS	Limits of detection (LOD): 0. 1µg – 1µg	Little sample preparation.	LOD dependent on polymer type Some polymers difficult Dry sample needed Preconcentration necessary
Thermal Extraction Desorption Gas Chromatography Mass Spectrometry – TED-GC-MS		Measurement with matrix, higher sample masses.	
Other methods of separation and combined techniques			
Flow cytometry (FC)	Down to 200 nm (spatial detectable limit) in freshwater		Early-stage research based on well-characterised samples and controlled plastics – mammalian tissue samples were spiked with nanoplastic beads. Cannot distinguish polymer particles from other particles (ACS, 2020).
Field Flow Fractionation (a family of methods, including Asymmetrical Field Flow Fractionation (AF4), Centrifugal Field Flow Fractionation (CF3) and Thermal FFF	1nm (AF4); ca. 50nm (CF3);	High potential for the processing of environmental samples Particle loss and sample alteration or aggregation are minimized Thermal FFF can separate different polymers of the same molecular weight (Postnova, n.d.)	

Other MS-based techniques, e.g. liquid chromatography coupled to high-resolution mass spectrometry LC-HRMS, thermal fragmentation and matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry MALDI-TOF MS, and Single particle inductively coupled plasma mass spectrometry SP-ICP-MS.	LC-HRMS was recently proposed for the mass quantification of nanoplastics and small microplastics		SP-ICP-MS has been developed based on model studies and synthetic samples of polystyrene plastic debris; "the study of more complex matrices and real environmental samples [would require] further analytical development." (Jiménez-Lamana *et al.*, 2020)

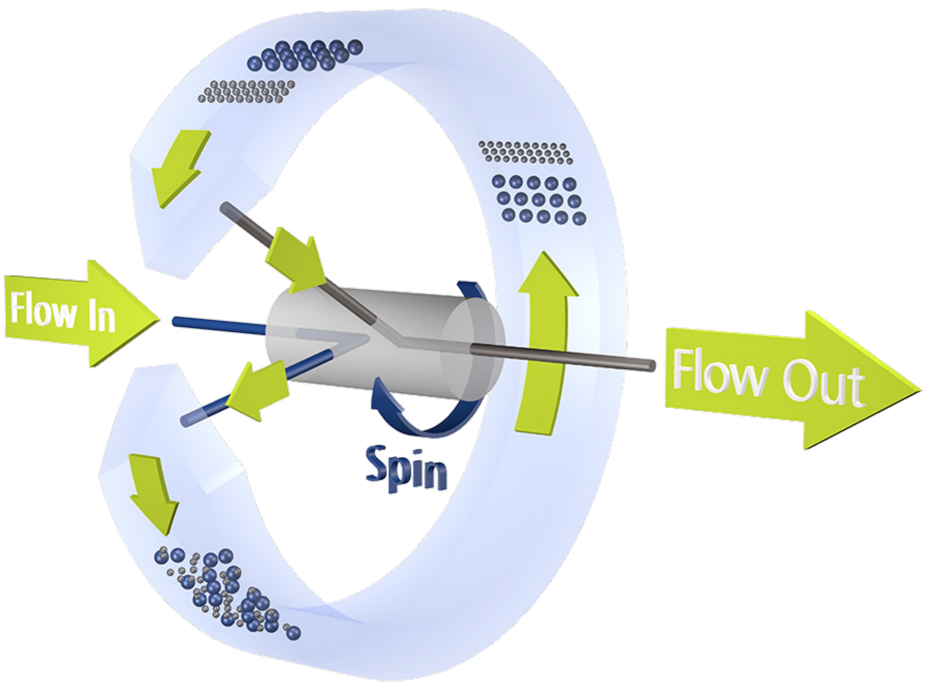


Fig. 4. Centrifugal field flow fractionation allows particles of the least mass to be separated from heavier, larger particles. Source: Postnova.com

Box 5: Examples of methodologies used in detecting and identifying nanoplastics

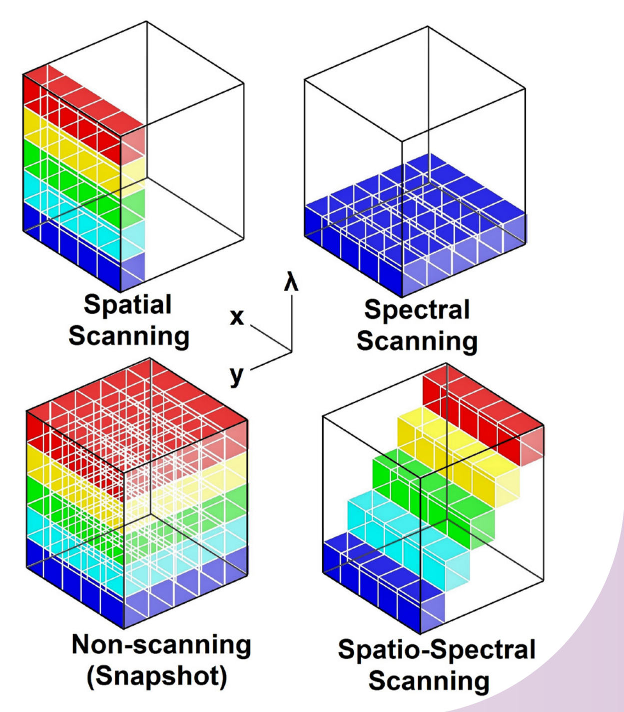
Fourier transform infrared (FTIR) and Raman spectroscopy: These methodologies are widely applied to microplastic detection (e.g. Zhang *et al.*, 2019) and show some promise at the nanoscale, particularly Raman spectroscopy. FTIR obtains an infrared spectrum of absorbed or scattered light/energy of a sample. Molecules can be identified by their characteristic absorption spectrum. Raman spectroscopy also uses scattered light to measure vibrations of molecular bonds. Both techniques measure a different type of molecular fingerprint.

Surface-enhanced Raman spectroscopy has been used to detect nanoplastics. Lv *et al.* (2020) applied this technique to pure water and seawater samples, and successfully detected nanoplastics of 100 nm in size down to concentrations of 40 µg/mL. Valsesia *et al.* (2021) also used Raman spectroscopy to analyse nanoplastics in the tissues of salt-water mussels, a technique that drove nanoplastics to self-assemble and self-isolate. Their process involved eliminating the biological matrix with enzymatic digestion and filtering, followed by collection of nanoplastics on a surface micro-machined with sub-micron-sized cavities. As the solution containing nanoparticles dried, capillary action assembled the suspended particles into the cavities, isolating and trapping them across the surface, enabling analysis.

Ultrafiltration with dynamic light scattering (DLS): DLS measures fluctuations in scattered light to determine the diffusion coefficient – and subsequently size – of particles. Ter Halle *et al.* (2017) used ultrafiltration combined with dynamic light scattering to detect nanoparticles in water samples from the North Atlantic Subtropical Gyre, and subsequently ascertained their chemical composition (plastics) via pyrolysis coupled with gas chromatography-mass spectrometry.

Pyrolysis coupled with gas chromatography-mass spectrometry (GC-MS): In pyrolysis, organic material is heated to high temperatures in the absence of oxygen (to avoid combustion risk) until it decomposes. These decomposition products are then separated via gas chromatography (GC) and characterised by mass spectrometry (MS) at a molecular level, to reveal the quantities and compositions of the substances present. This technique has been used to identify nanoparticles as nanoplastics (Ter Halle *et al.*, 2017).

Hyperspectral imaging: This technique collects many images at different wavelengths for the same spatial area, that can be analysed for individual materials. Mattsson *et al.* (2017) used hyperspectral imaging to detect and characterise polystyrene nanoparticles with a diameter of 52 nm in the brain tissue of freshwater carp, having been passed up the food chain from the small planktonic crustacean *Daphnia magna*.



Acquisition techniques for hyperspectral imaging, visualized as sections of the hyperspectral datacube with its two spatial dimensions (x,y) and one spectral dimension (lambda) - ©Wikipedia Commons CC BY 4.0

Field Flow Fractionation: Some researchers have highlighted the potential of field flow fractionation methods (FFF4) for separating out nano-sized particles in a sample (Gigault *et al.*, 2017; Schwaferts *et al.*, 2020; Loeschner *et al.*, 2022). Asymmetrical FFF (AF4) and Centrifugal FFF (CF3; Fig. 4) use a physical force to separate particles as they flow along a channel, based on their size or mass. Particles can be further characterised by coupling these techniques with other methods such as Raman spectroscopy and multi-angle laser light scattering. In Thermal FFF, a temperature gradient is the driving force of separation, with particles are separated according to their mass and chemical composition.

Loeschner *et al.* (2022) note that sample preparation needs careful consideration when looking for nanoplastics with FFF. Suspensions should not use strong acids or bases, for example, which could affect nanoplastics. Very few studies have attempted to apply FFF for analysing nanoplastics in a complex matrix. Wahl *et al.* (2021) used AF4 in conjunction with pyrGC-MS to detect nanoplastics in soil; Correia and Loeschner (2018) applied AF4 and Multi-angle laser light scattering to fish samples spiked with PS and PE nanoplastics.

Box 6: Core challenges

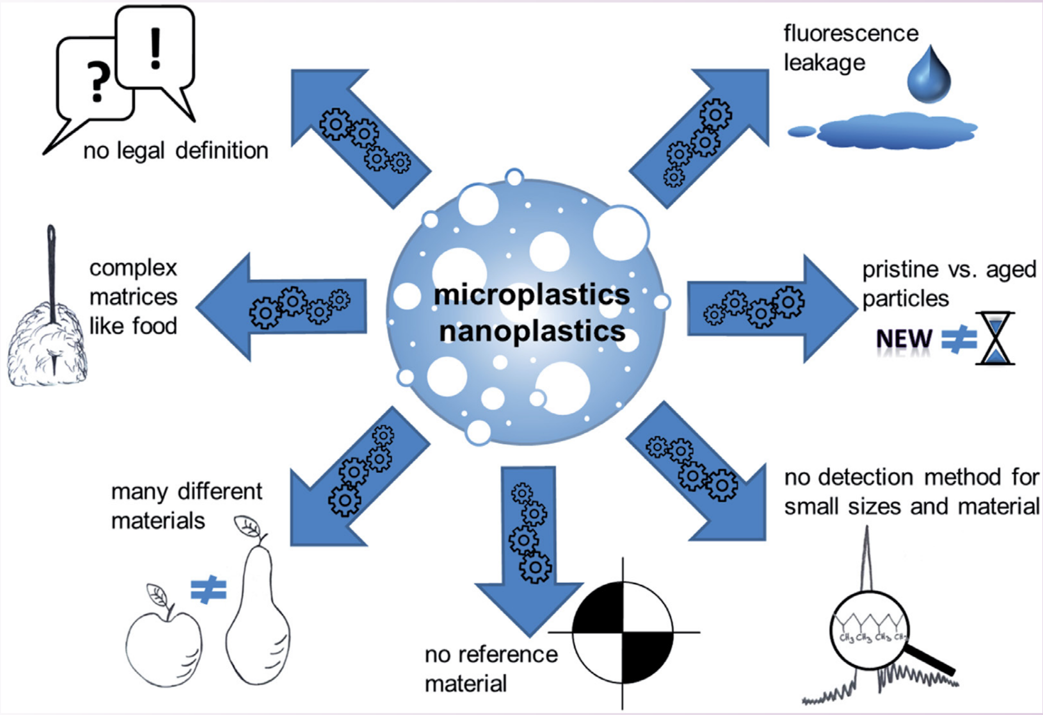


Fig. 5. Challenges and pitfalls in micro- and nanoplastics research. Source: Paul *et al.* (2020)

Some of the challanges to be addressed in developing methodologies for detecting and anlaysing nanoplastics include:

- Complex matrices: samples range from relatively 'clean' such as drinking water, to environmental water (river/sea/lake), wastewater, sediment, soil, alimentary, and biota.
- Many different materials to identify: numerous plastics are used in global markets.
- No reference material, and no clearly defined legal definition or standard metrics.
- Fluorescence/label leakage: markers used on nanoplastics may confound findings (see 3.5.2).
- Pristine vs. aged articles: heterogeneity in target particles increases analytical complexity.
- Avoiding contamination during sample extraction, concentration and handling.
- Improving resolution, sensitivity and specificity in analysis.

2.2 Considerations for policy

It is possible to restrict manufacturing of nanoplastics, addressing the release of primary nanoplastics (as in the restriction on intentionally added microplastics discussed in 1.2.1, for example). However, *secondary* nanoplastics can only really be addressed through policies aimed at macro and microplastics – as in the European Strategy for Plastics in a Circular Economy, for example, or through the European Commission's initiative on reducing un-intentional microplastic pollution into the environment. Increasing recycling rates, regulating single-use conventional plastics (e.g. as in the Plastic Bags Directive[18]), finding safe ways of dealing with end-of-life plastics, and developing biodegradable plastics that pose less risk to health and ecosystems than conventional plastics, are overarching goals that may address the ongoing secondary nanoplastic pollution. In regulation,

18 https://environment.ec.europa.eu/topics/plastics/plastic-bags_en

it may also be useful to target certain types of plastic that are more likely to produce or persist as nanoplastics – or be more hazardous, either due to their inherent characteristics and toxic effects, or their chemical additives. Remediating secondary nanoplastics already in the environment is another challenge altogether.

Meanwhile, the difficulty in detecting nanoplastics presents problems for monitoring the effects of any restrictions on nanoplastic levels in the environment. Currently available methodologies require greater sensitivity, greater ease of use, wider applicability, and often must be combined in order to fully characterise the nanoplastic content of a complex sample.

Additionally, methods must be developed that 'mark' plastics and causally link these to origin, source or manufacturer; and models are needed to assess "the relationships between polymer structural characteristics and the formation of smaller plastic nanoparticles in nature, due to embrittlement, fragmentation or degradation" (SAPEA, 2019). Overall, harmonised protocols for monitoring the concentrations and compositions of marine plastics must be developed and overcome three main issues, say Setälä *et al.* (2019): "how to carry out the field sampling, how to eliminate other particulate matter in the sample without harming the plastic itself, and how to accurately identify the particles", all without introducing contamination to the sample in the process.

To advance the detection, analysis and monitoring of nanoplastic pollution, Horizon 2020 is supporting the European Cluster on Health Impacts of Micro- and Nanoplastics (2021–25), a group of five research initiatives (see Box 3, section 1.1.3). These initiatives complement **the EU's RESPONSE project (2020–2023):** Towards a risk-based assessment of microplastic pollution in marine ecosystems. RESPONSE integrates expertise on oceanography, environmental chemistry, ecotoxicology, experimental ecology and modelling to answer key research questions on fate and impact of microplastics (MPs) and nanoplastics (NPs)", aiming to provide "ecologically relevant strategies for assessing the distribution pathway and biological effects of plastic particles in marine ecosystems".

A key consideration of future policy is safety, with many core research questions aiming to address the level and type of risk that nanoplastics pose to global ecosystems, the marine environment, and, crucially, human health. While nanomaterials and primary nanoplastics can be evaluated to assess if they are "safe by design" across their lifecycle (Allan, Sokull-Kluettgen and Patri, 2020), this is less feasible for secondary nanoplastics – which represent the majority of nano-sized plastic particles found in the environment. As attention has turned to nanoplastics, numerous stakeholders, including plastic producers, have developed strategies to combat plastic pollution, but much work remains to address data gaps pertaining to nanoplastics.

Box 7: Methodological challenges and policy

Robust, accurate, harmonized methodology for detecting and analysing nanoplastics in different media (e.g. biological or environmental samples) is needed to enable various aspects of policy relating to nanoplastics:

- Establish levels in different environments
- Monitor changes to levels in the environment
- Trace origin of nanoplastics in the environment
- Evaluate effects and safety of different nanoplastics, including biodegradable
- Assess hazard posed by nanoplastics in the environment
- Direct restrictions at most hazardous nanoplastics
- Estimate effects of regulations on occurrence of nanoplastics

3. Nanoplastics in the environment

Primary and secondary nanoplastics may arise in terrestrial, and aquatic environments, via a number of key sources – including agriculture and fishing. Wastewater treatment does not remove all nanoplastic particles. Once they enter the environment, their behaviour, transport, and fate, depends on a number of factors – for example, plastics denser than water will sink, while others float. Nanoplastic particles are subject to fragmentation and degradation processes, and there are interesting new findings about biotransformation that occurs during digestion in some organisms.

Ingestion of microplastics by aquatic organisms has been shown in many studies, and the literature on nanoplastic exposure via this route is growing, together with evidence that particles may translocate (move to different organs and tissues) within an organism. In terrestrial habitats, nanoplastics may move downwards through soil and be taken up into plant and fungus tissues, with potential implications for trophic transfer and ecosystem functioning.

3.1 Sources and routes into the environment

Most plastics are produced and used on land, and hence the sources for microplastic and nanoplastics are mainly land-based. However, as these small plastic particles are uniquely light, persistent, and able to remain dispersed in water – they can travel far across the planet. Over 800 million tonnes of plastic pollution in the sea is estimated to come from land-based sources, as well as fishing, other aquaculture activities, and coastal tourism (Yee *et al.*, 2021). There are also some sea-based sources: 'ghost gear' – lost fishing equipment (nets, ropes, lines) makes up 10% of marine litter – with between 500 000 and 1 million tonnes left in the ocean every year (WWF, 2020). This plastic pollution makes up half of the 'Great Pacific Garbage Patch' which is floating in the North Pacific Ocean (and is already deadly to marine life in its macro form, through entanglement). When degraded to micro- and nanoplastic size, these plastics can also be ingested by marine fauna – possibly entering the food chain.

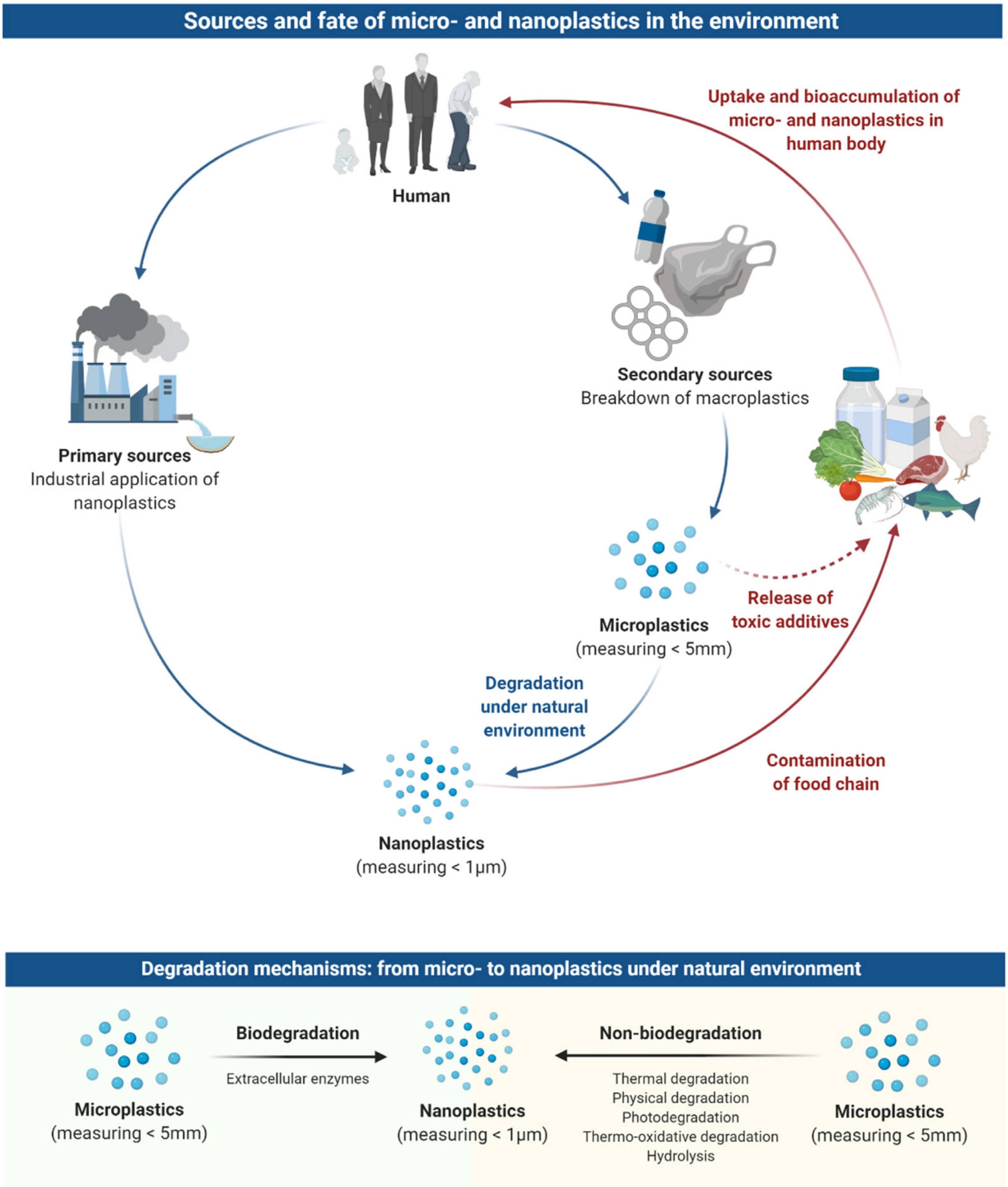


Fig. 6. Sources and fate of micro- and nanoplastics in the environment. Both micro- and nanoplastics can occur in both aquatic and terrestrial environment and undergo a range of abiotic and biologicalbiologicalbiosolid removalbiological degradation processes, and eventually enter the food chain, water supplies, and the air. There is at present only a speculative link between the environment and ingestion and uptake of these plastic particles in the human body. Source: Yee *et al.* (2021)

Wastewater treatment processes may remove more than 95% of microplastics, but there are currently few research findings on levels of nanoplastics remaining in treated water; most studies have only quantified particles greater than 1 μm (Ali *et al.*, 2021). One experiment, using nanoplastics marked with palladium (as well as microplastic particles), found that plastic removal is strongly correlated with suspended solids removal – at least 98% of particulate plastics were associated with solids in the study (Frehland *et al.*, 2020). The researcher acknowledged that it is not currently feasible to measure nanoplastics in a full-scale wastewater treatment plant, but their findings using a simplified model suggest that in real-life treatment – with additional processing steps – even higher levels of solids and plastic particles can be retained.

Any particles that do remain in treated water will be introduced into rivers and oceans, as well as the fresh water supply system – if surface water is used (Frehland *et al.*, 2020; Yee *et al.*, 2021; Browne *et al.*, 2011). Nanoplastics also leach from soil into rivers and oceans via natural erosion. The United Nations Environment Programme estimates that 4.18-12.7 million tonnes of plastic leached into the water systems in 2010 (Yee *et al.*, 2021).

Aerial drone photo of latest technology sewage and sludge processing plant in small island of Psitalia or Psyttaleia, Piraeus, Attica, Greece (©Shutterstock, photo by Aerial-motion)

[N]ovel technologies are highly demanded after tertiary treatment to eradicate small size particles (i.e., NPs, <0.1 μm) and fiber-like MPs (15–0.1 μm) from the final effluent to protect receiving water environment from plastic pollution

Ali *et al.*, 2021

Box 8: Agricultural sources of micro- and nanoplastic pollution

On land, much microplastic pollution is associated with agriculture – and therefore also the product of microplastic degradation, nanoplastic. Sources linked to the agricultural sector include:

- **Microplastics and nanoplastics in sewage sludge** – a large proportion of 'down the drain' plastics, where they are not removed by wastewater treatment, may be released onto agricultural soil via application of biosolids (ECHA, 2020). One study estimated the yearly amount of microplastics transferred to agricultural land from urban sources – including via sewage sludge – at between 125 and 850 tonnes of microplastics per million inhabitants. This is equivalent to an annual input of 63 000 – 430 000 tonnes across Europe (Nizzetto, Futter and Langaas, 2016). Preliminary findings on the fate of PS nanoplastics found that 98% of nanoplastics were present in sewage sludge after wastewater treatment (Frehland *et al.*, 2020).
- **Microplastic in composted domestic and industrial waste** – applied to soil as organic fertilisers or soil conditioners (Weithmann *et al.*, 2018).
- **Residues from controlled-release fertilisers** – plastic pellets in which fertilisers are contained persist in soil, known as 'polymer encapsulation systems'. Anti caking, and anti dust polymer additives, are also a source of plastics added to agricultural fertilisers. However, from 2021 within Europe the EU fertilizing products regulation[19] now restricts the use of non-biodegradable plastics in most of these types of agricultural fertilisers – ¬with a transition period of 5 years to comply. However, controlled release fertilisers are exempt from this restriction. Only polymers meeting biodegradability requirements of the regulation will be allowed on the European market in most fertilisers from 2026.
- **Agricultural plastic mulches** – as mulch film degrades in and on the ground, microplastics and nanoplastics form. Soil contamination is around 467 kt per year in the EU, with 36% of this arriving from mulch film collection – despite mulch being only 12% of the market (by mass)[20]. The removal of plastic mulch contaminated soil from fields, contributes to the loss of soil organic carbon (SOC) – a key component of soil health. Around 7 777 000 tonnes of plastic mulch is used per year in the EU, and only a fraction is collected after use (EIP-AGRI, 2021).

  The 2018 standard EN-17033 states that biodegradable mulch films should have a biodegradation threshold of 90% in 2 years, but since most crops only remain up to 6 months in the field – this is too long to wait before the ground is replanted. Meanwhile, only some European countries, including France and Italy, have national standards for biodegradability of plastic mulch in arable soils.

- **Discarded plastics from greenhouses and polytunnels** – an estimated 117 000 tonnes of plastic film, primarily LDPE, is used in production greenhouses, and 56 300 tonnes in smaller tunnels (EIP-AGRI, 2021). Fragments from degraded film, as well as mismanaged or abandoned plastics, end up in the soil, rivers, and the sea.

19 https://eur-lex.europa.eu/legal-content/EN/ALL/?uri=CELEX:32019R1009
20 EC (2021) Conventional and Biodegradable Plastics in Agriculture: https://environment.ec.europa.eu/system/files/2021-09/Agricultural%20Plastics%20Final%20Report.pdf

Box 9: Nanoplastics from tyre wear

Tyre wear particles (TWP) are produced under normal driving conditions, especially during cornering, braking, accelerating, and other manoeuvres. In western Europe, emissions of TWP have been estimated at 0.5–1.5 kilogrammes of TWP per capita per year (Kole *et al.*, 2017). They may contribute up to 10% of all ocean plastic (*ibid.*), and 40% of microplastics transported from rivers to seas (Siegfried *et al.*, 2017).

Composed primarily of synthetic and natural rubber, tyre material is strictly speaking an elastomer – belongs to the group of elastomers – flexible polymers, distinct from plastic – however, the particles emitted are often classified as micro- and nanoplastics. Emissions are typically at the micro scale, but nano scale TWP have also been detected (Gillibert *et al.*, 2022).

TWP typically enter the environment through rainfall runoff from road surfaces, with around 75% estimated to enter roadside soils – considered a sink that limits the further spread of particles. However, about a fifth of emissions may reach standing water (Baensch-Baltruschat *et al.*, 2021). Where runoff enters urban sewage systems, treatment facilities, such as soil filters that can remove nano-scale particles could be employed (*ibid.*). Using a simulated municipal waste water treatment plant, Mitrano *et al.* (2019) found more than 98% of nanoplastic entering the facility remained in sewage sludge.

3.2 Degradation

Once released into air, soil and water, microplastics and nanoplastics will be subject to transport and fragmentation, and some types to biodegradation processes (Figure 7). Abiotic degradation and transformation processes include physical degradation, photodegradation, thermo-oxidative degradation and hydrolysis (Yee *et al.*, 2021). Physical degradation – where larger plastics fragment into smaller pieces – occurs naturally through weathering. Such processes decompose polymeric structures, altering their mechanical properties and increasing their specific surface area, resulting in enhanced potential for physical-chemical reactions, and interactions with microorganisms (Yee *et al.*, 2021).

Table 3. Types / pathways of plastic particle degradation, from Boyle and Örmeci (2020). Biotic and abiotic processes can occur simultaneously.

<table>
<tr><td colspan="4">Abiotic degradation: mechanical degradation of plastics through weather and climate changes (e.g., freezing, thawing, pressure changes, water turbulence and damage by animal activities) – only morphological changes occur.</td></tr>
<tr><td>Thermal

Breakdown of plastics at high temperatures; while often not hot enough in the environment, plastic production involves thermal treatment</td><td>Oxidation

Thermal or photo-induced; introduces oxygen to plastics and forms degradation-promoting carbonyl and hydroxyl groups</td><td>Hydrolytic

Reaction of plastic compounds with water, resulting in changed molecular weight and reduced strength</td><td>Photo

Most damaging to environmental plastics; ultraviolet and visible light excite polymeric electrons and cause broken bonds</td></tr>
<tr><td colspan="4">Biotic degradation: degradation resulting from organism activity – changes in chemical structure occur over time. Organisms secrete enzymes that break polymer chains and weaken some types of plastics.</td></tr>
</table>

Complete degradation returns nanoplastic back to its constituent elements – largely carbon and hydrogen, among others. Part of this process involves breaking ionic and covalent bonds. Light, for example, can cause a lot of damage to plastic by increasing electron activity within these bonds, resulting in their eventual cleavage (Boyle and Örmeci, 2020). Abiotic processes can, in this way, break the long chain molecules in plastic polymers into shorter chains, and after a long time will eventually convert them into their monomeric forms (Yee *et al.*, 2021).

Usually, biodegradation is used to describe the breakdown of material by micro-organisms, and biotransformation breakdown occurring within organisms, e.g. via enzymatic digestion. When enzymes break up plastic polymer chains, it reduces plastic's molecular mass, promoting further microbial degradation – enhanced by water and oxygen exposure. This gradually breaks down the nanoplastic polymer molecule into shorter chains, and eventually water-soluble oligomers and monomers, that mineralise, and can be used by microorganisms as carbon and hydrogen sources (Boyle and Örmeci, 2020). Some studies have shown that this can happen to biodegradable plastics in composting situations (Jakubowicz, 2003), while a range of fungi have also been found to potentially degrade different petroleum-based plastics – an application that could be further explored in the context of remediation (Sánchez, 2020). It has also been shown that enzymes from environmentally ubiquitous fungi can break down plastic additives, such as phthalates and bisphenol A (Carstens *et al.*, 2020).

"In this study the ability of … fungi to biotransform the PE representatives DBP and DEP, and the plastic precursor chemical BPA was demonstrated. … [M]etabolites in fungal cultures confirmed DBP biotransformation … The results of the present study imply an environmentally ubiquitous fungal potential for the biocatalytic breakdown of plastic additives"

Carstens *et al.*, 2020

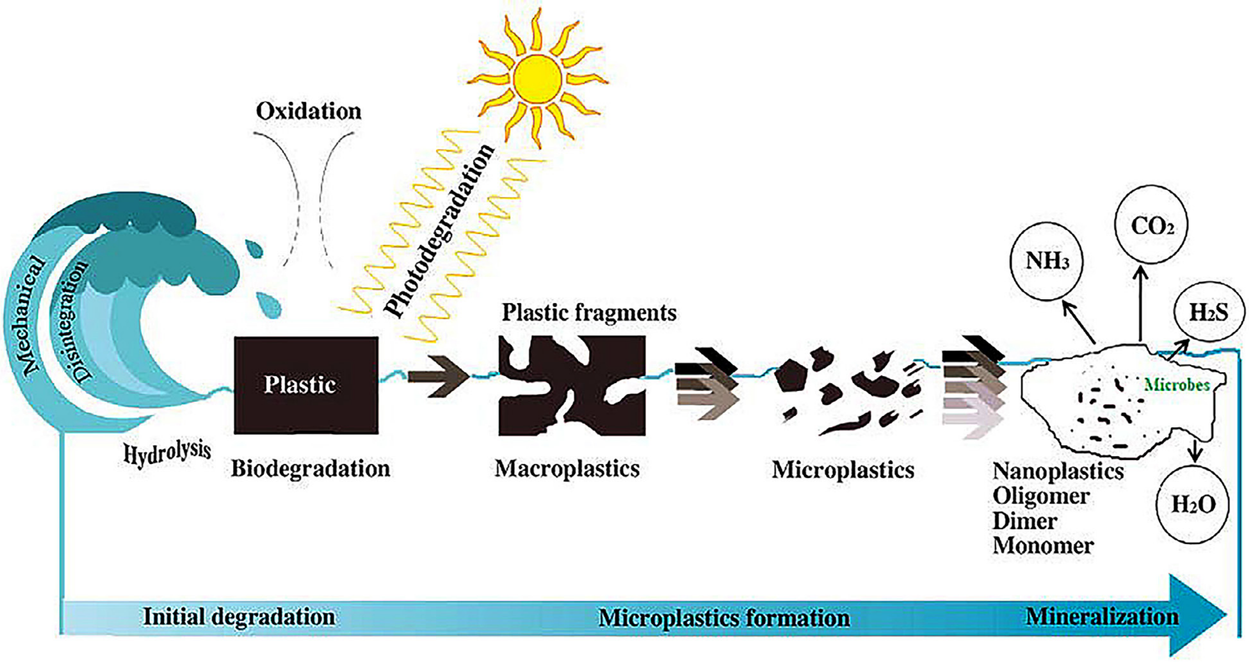


Fig. 7. Degradation pathways of plastic materials in flowing water. Source: Sarkar *et al.* (2020)

Some studies have suggested a degradation pathway within the digestive systems of organisms. Four days after Antarctic krill (*Euphausia superba*) ingested virgin polyethylene (PE) microplastics (particles sized 31.5 µm), measurements showed they had reduced in size by 78% (7.1µm ± 6.2 SD) (Dawson *et al.*, 2018). The authors suggest the bulk of PE MP breakdown in the Krill is due to the stomach and gastric mill mechanically fragmenting the plastic particles, however, they note, they cannot rule out that digestive enzymes also contributed to this breakdown (Dawson *et al.*, 2018). Due to their predominantly herbi- vorous diet, Antarctic krill have complex digestive enzymes with high activity. Another study found the larvae of the greater wax moth, *Galleria melonella*, can also metabolise polyethylene (PE), possibly due to its chemical structure being similar to honeycomb – the larvae's main food (LeMoine *et al.*, 2020). The biotransformation phenomenon demonstrated in these studies could be investigated further, with other types of plastic (Piccardo, Renz and Terlizzi, 2020). Recently, Beale *et al.* (2022) suggested that biotransformation via insects, such as black soldier fly larva (*Hermetia illucens)*, and mealworms (*Tenebrio molitor*), could also be investigated in the context of addressing plastic waste. The bulk of plastic maceration, presumably took place in the stomach and gastric mill, which is responsible for mechanically fragmenting food particles under normal feeding conditions.

3.3 Environmental fate

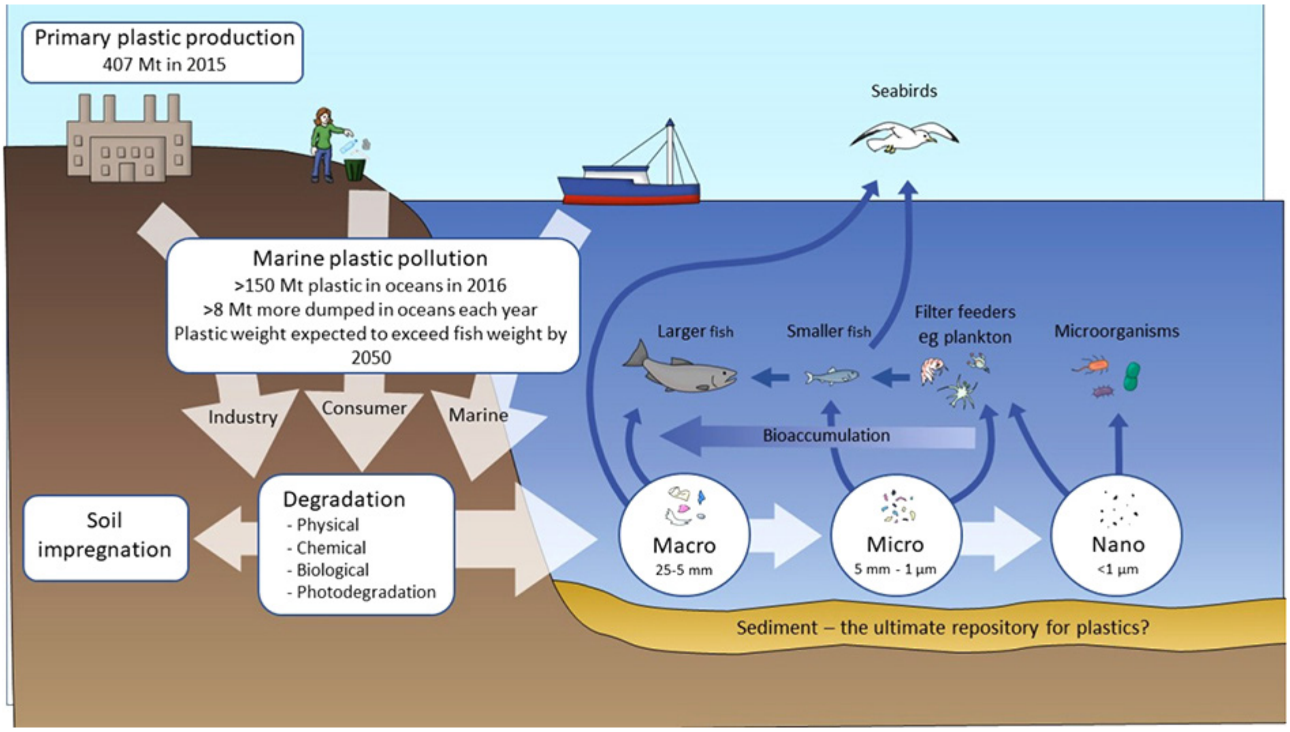


Fig. 8. Schematic diagram showing sources and fate of plastics in the marine environment.
Source: Gangadoo *et al.* (2020)

To understand the potential risks of nanoplastic particles in the environment, it is important to ascertain in what parts of the environment they collect – their environmental fate. The fate of microplastics is better understood than that of nanoplastics – for instance, it is known that they can be washed into rivers during flooding – but there is still insufficient knowledge to reliably model the movement of either across environmental habitats. Existing chemical fate models, such as 'SimpleBox[21]' – an established model already used in the risk assessment of chemicals – may be modified and applied to microplastics and nanoplastics (Kooi, *et al.*, 2017). SimpleBox4Nano and NanoDUFLOW, are

21 https://www.rivm.nl/en/soil-and-water/simplebox

two examples of models adapted for analysis of nanoparticle fate (Meesters *et al.*, 2014; Besseling *et al.*, 2017).

Most research into nanoplastic fate has focused on pollution in the water column of bodies of water, and its effect on open water organisms, for example, pelagic fish or phytoplankton. However, over time, plastic particles tend to aggregate (join together with other particles) and sink. Several plastics are denser than water (PVC, PS, PET) and so sink quite rapidly, while others like low-density polyethylene (LDPE), high-density polyethylene (HDPE) and polypropylene (PP), mainly float in the water column, until being subject to aggregation or biofouling (formation of a biofilm of bacteria and organic matter) (Haegerbaeumer *et al.*, 2019). Relatively high concentrations of nanoplastics in sediment, compared to surrounding water, could pose a risk to benthic (bottom dwelling) fauna. Ingestion of nanoplastics by benthic invertebrates is of particular concern, as they make up 90% of the biomass eaten by fish, with implications for trophic transfer (*ibid.*).

Box 10: Do nanoplastics behave the same in freshwater and seawater?

In order to predict the potential distribution and transport of nanoplastics in real world environments, it is important to investigate their different behaviours in fresh- and seawater. For example, increases in particle sizes have been observed in studies using engineered nanoplastics in seawater, bacterial mediums and biological fluids (Gangadoo *et al.*, 2020; Reynaud *et al.*, 2022). Recently, Lee and Fang (2022) found that particles exhibited less aggregation in freshwater and stronger aggregation and deposition in seawater – due to ionic forces – increasing with temperature. Benthic organisms in warmer waters may be exposed to more nanoplastics than in cooler waters, the researchers suggest. Venel *et al.* (2021), using a 'lab-on-a -chip', showed that larger nanoplastics may aggregate and clump in estuaries.

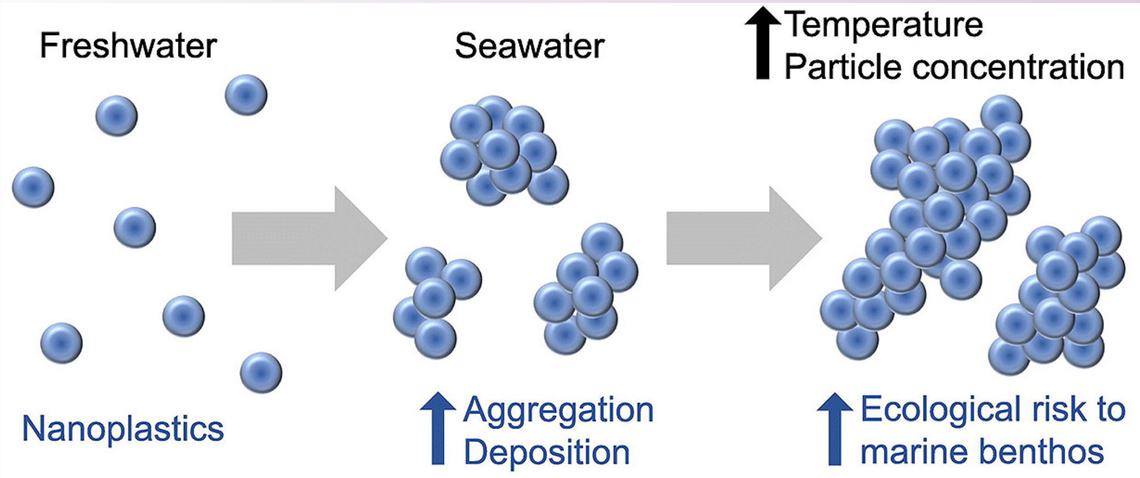


Fig. 9. Laboratory studies suggest nanoplastic particles will remain more dispersed in freshwater than seawater. Higher temperatures may lead to increased homoaggregation and deposition in sediment. Source: Lee and Fang (2022)

Most studies looking at nanoplastic behaviour in water have used engineered study particles, but the behaviour of actual (primary or secondary) nanoplastic particles may be quite different. For example, rough-surfaced and irregularly shaped particles are more likely to aggregate than smooth particles (e.g. Veclin *et al.*, 2022), and coronas may form on particles through interaction with other substances present (see section 4.4).

Meanwhile, Schür *et al.* (2021) note that polystyrene microplastics became less toxic to *Daphnia magna* after adsorbing organic matter in wastewater, inferring that experiments looking at the environmental effects of pristine plastics – micro or nano – may not reflect real-world conditions.

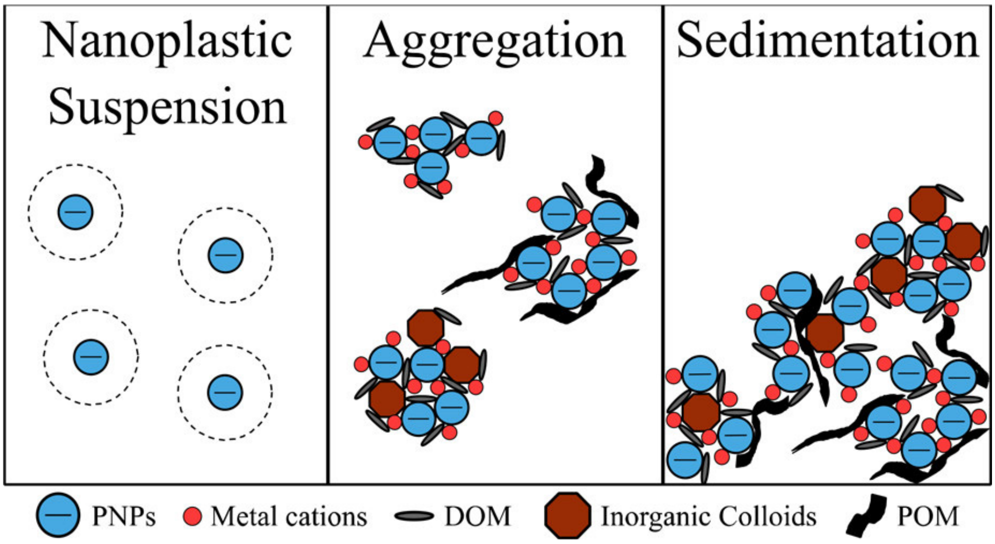


Fig. 10. From Brewer, Dror and Berkowitz (2020), showing the aggregation and sedimentation behaviour of plastic nanoparticles (abbreviated here as PNPs) as they interact with inorganic colloids, metal cations and dissolved and particulate organic matter (DOM, POM)

3.4 Estimating nanoplastics in field samples

Current technological limitations on quantifying environmental nanoplastics mean that most research on nanoplastics as pollutants has focused on laboratory evidence, however some recent studies have used novel techniques to detect particles in field samples (Gillibert *et al.*, 2019; Shen *et al.* 2019; Cai *et al.*, 2021a).

In a recent review, only five studies reported the detection and analysis of nanoplastics in real field samples (Cai *et al.*, 2021b), though new data is being published. Nanoplastic particles are easily destabilised, so many can be lost during sample preparation, making reliable quantification in environmental samples difficult. Despite not being able to estimate the exact concentration of nanoplastics in the environment at present, the expected trend is that they will increase over time: because of increased use in products, as a by-product of industry, and from the degradation of macro and microplastics (Besseling *et al.*, 2019; Koelmans, Besseling and Shim, 2015).

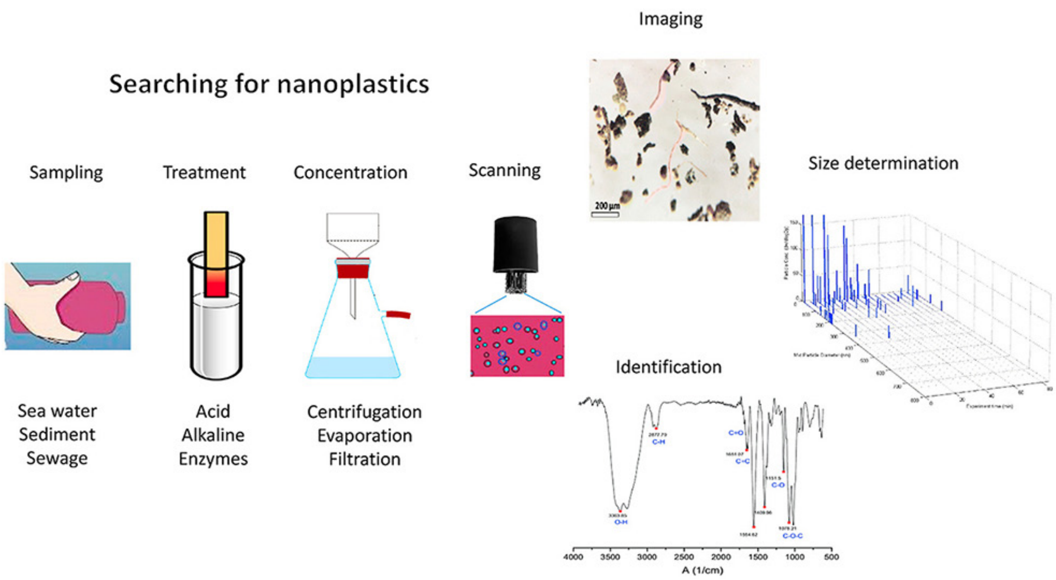


Fig. 11. Methodologies for detecting and quantifying nanoplastics in environmental samples are still being developed. Source: Jakubowicz, Enebro and Yarahmadi (2021)

Nanoplastic occurrence in marine samples has so far only rarely been demonstrated.

One study found nanoplastics present in ocean surface samples from the North Atlantic subtropical gyre (Ter Halle *et al.*, 2017). The researchers detected nanosized polymers in samples taken from the ocean surface using pyrolysis gas chromatography-mass spectrometry. Materić *et al.* (2022) quantified nanoplastics in surface water samples from Sweden and the Siberian Arctic tundra. They found four types of nanoplastic at a mean concentration of 563 μg/L in the Swedish samples, and PVC and polystyrene nanoparticles were detected in Siberian samples, at a mean concentration of 51 μg/L.

Another study detected PET nanoplastic in Alpine snow samples, semi-quantifying its concentration in surface snow as 18.5 ± 1.5 ng/ml of filtered snow (Materić *et al.*, 2020). The authors suggest this observation shows airborne nanoplastic particles, largely PET, are being deposited on the surface of the snow. Another analysis of Alpine snow in Austria found 46.5ng/ml of melted surface snow, including polypropylene as well as PET (Materić *et al.*, 2021). Drawing on air transport modelling, the researchers inferred an average deposition rate of 42 kg of nanoplastic per square kilometre each year in this remote location. The particles mostly originate from urban Europe, they note.

> **The presence of nanoplastics in high-altitude snow indicates airborne transport of plastic pollution with environmental and health consequences yet to be understood**
>
> Materić *et al.*, 2020

Another study demonstrated the use of klarite substrates (gold cavities that can hold nanoplastic particles), in conjunction with surface-enhanced Raman spectroscopy, to collect and analyse nanoplastics in ambient airborne samples (Xu *et al.*, 2020). Klarite may allow detection of particles as small as 360 nm. Airborne particle sampling is important due to the potentially high levels of exposure via inhalation in indoor environments.

In contrast, many studies looking to measure microplastics in the environment have been published. Globally, the highest reported microplastic concentrations in the aquatic environment are 102 particles/L water, and 1529 particles per kg sediment, with a large range dependent on region and type of habitat i.e. freshwater, marine, estuarine (beach is higher than this quoted maximum). Fragmentation of microplastic to nanoplastic has been measured in the laboratory and shown in the environment as well (Gigault *et al.*, 2016; Gillibert *et al.*, 2019). It is not known how fast, or to what extent nanofragmentation of plastics will occur in the environment; however, based on mass conservation principles, if spherical microplastic particles with a size of 0.1 μm to 5 mm degraded into 100 nm nanoplastic particles, this would produce nanoplastic particle concentrations that are more than 1014 times higher, than the current microplastic concentrations (Besseling *et al.*, 2019). This is conservative as it doesn't include the ongoing degradation of macroplastics to microplastics, and ultimately nanoplastics. It is estimated, via model-based studies, that this process of nanofragmentation of plastics would occur over several hundreds of years (Koelmans, Besseling and Shim, 2015).

Given the paucity of data on nanoplastic levels in environmental samples, potential distribution in air, aquatic and terrestrial environments can only be inferred from data on microplastics and modelling studies. For example, the highest reported ranges of microplastics are in near shore or estuarine areas, compared with open ocean or freshwater systems (Besseling *et al.*, 2019). The authors suggest this could be due to accumulation of microplastics (and presumably nanoplastics), due to input from rivers and beaches, as well as backwashing from marine currents, and nearshore hydrodynamics trapping/fouling microplastics. However, as it is easier to sample near shore areas, the higher concentrations reported may also be influenced by the sampling effort.

Kooi *et al.* (2017) used a model to calculate the average, background predicted, environmental steady state concentrations (PECs) of plastic particles of different sizes, in different compartments in the Rhine Catchment in Europe. This calculation assumed a yearly emission of 20 kilotons, with 50% emitted to water and 50% to soil – the results can be seen in Table 4 below. The nanoplastic-sized particles in the first column of the table, can be seen to mirror the trend observed for larger microplastic particles up to 10μm in levels of accumulation – most being found in the soil, fewer in water, and fewer still in sediment.

Table 4. Distribution of plastic particles of different sizes over the soil, water and aquatic sediment compartments, as predicted by the multi-media model SB4N. PECs are based on a yearly emission of 20 Kt, and assumes a fouled plastic density of 1100kg/m3, negligible degradation and fragmentation due to short particle residence time in the system, along with an attachment efficiency for heteroaggregation of 0.01. (Kooi *et al.*, 2017)

Particle size	0.1 μm	1 μm	10 μm	100 μm	1 mm
soil (log μg/m³)	6.43	6.38	6.17	4.57	2.62
water (log μg/m³)	5.45	5.44	5.39	4.89	3.08
sediment (log μg/m³)	1.52	2.41	4.42	6.07	6.26

3.5 Nanoplastics in aquatic organisms

There is a plethora of experimental and field data showing that microplastics can be ingested by an array of species from different taxonomic groups, and occupying various ecological niches, and positions along food chains. Ingestion has currently been documented in around 220 species (Lusher *et al.*, 2017). Although fewer studies exist regarding nanoplastics, interaction with aquatic plants and ingestion by animal species has been demonstrated – including marine birds, fish, mammals, mussels, crustaceans, mussels and zooplankton (Larue *et al.*, 2021; Chae and An, 2018; Barría *et al.*, 2020; Boyle and Örmeci, 2020). Uptake via gills provides an additional exposure route for fish to nanoplastic particles. Nanoplastics may induce adverse effects in aquatic organisms, as their small size means they can be ingested, and also pass through biological membranes – unlike microplastics, which are often too large to pass through (Boyle and Örmeci, 2020). The small size of nanoplastics, means they have the potential to penetrate any part of an organism, causing undesirable effects due to the presence of the particle itself (Boyle and Örmeci, 2020; Hartman *et al.*, 2017). Chapter 4 discusses ecotoxicity in more detail. As noted, the infinite combination of plastic characteristics and potential interactions throughout the marine ecosystem, coupled with a paucity of techniques to quantify amounts of nanoplastics ingested, are challenges, making risk analyses of nanoplastics for marine organisms difficult (Galloway, Cole and Lewis 2017; EASAC, 2020). Meanwhile, there is a risk that bioaccumulation of nanoplastics could occur after ingestion by aquatic organisms, including fish and marine mammals. Organisms at higher trophic levels (higher in the food chain) may also ingest nanoplastics present in organisms they consume.

Studies have shown that microplastics ingested are frequently egested (excreted), and the ECHA (2019) report suggests the accumulation of microplastics in fish, for example, is low as a result. However, invertebrate species such as scallops have been found to ingest microplastics and nanoplastics, with some particle sizes remaining in the organism after a protracted time, and evidence of translocation within the organism has also been highlighted (Al-Sid-Cheikh *et al.*, 2018). Understanding to what extent ingesting small plastic particles differs from ingesting natural particles of sediment, or natural organic material of similar size, is important, but there are few comparative studies. One study on differing responses to microplastics and kaolin clay in *Daphnia magna* found that secondary microplastics have a greater capacity to negatively affect feeding behaviours compared to naturally occurring particles of a similar size (Ogonowski *et al.*, 2016); the same may be true for nanoplastics. Likewise, a study comparing the effects of ingestion of a bio-based microplastic, a petroleum-based microplastic and natural silica particles as a control, found that both types of plastic had detrimental effects on aquatic invertebrate species, *Gammarus fossarum* (Straub, Hirsch and Burkhardt-Holm, 2017). Another study comparing the effects of microplastics PVC and PMMA with natural microparticles on mussels (*Semimytilus algosus*), found that significantly different negative reactions to microplastics only occurred at high concentrations that are unlikely to be found in the environment (Barkhau *et al.*, 2022).

> **… mussels exposed to PVC … microplastics produced over 40% less byssus than those exposed to natural microparticles. This suggests that mussels react differently to natural microparticles and to microplastics, but only at high particle loads that exceed current environmental microplastic concentrations by orders of magnitude**
>
> Barkhau *et al.*, 2022

Because of their ability to cross biological membranes, it can be expected that nano-sized particles might confer worse, or different, toxic effects at lower concentrations.. The very size of nanoparticles – of plastic or any material – may make them more hazardous to aquatic organisms than larger particles. Some organisms will excrete indigestible nanoplastic particles; others may absorb smaller nanoplastic particles through membranes, leading to potentially toxic effects in various organs. Much concern is also focused on the toxic additives carried by plastic particles, which could leach into the bodies of organisms that ingest them (EASAC, 2020). Mechanisms of toxicity are discussed further in Chapter 4.

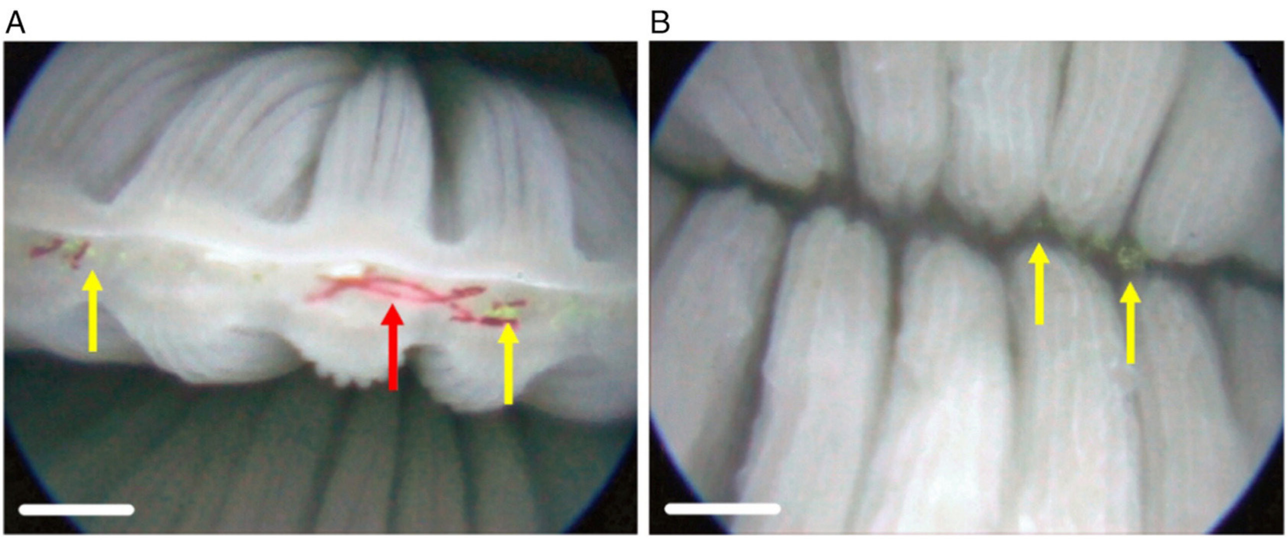


Fig. 12. Oysters appear to select which particles to ingest based partly on their shape. These microscope images show polyester fibres (ca. 65-260 µm long × 16 µm wide, red arrows) and microspheres (10-20 µm diameter, yellow arrows) on the gills of Crassostrea virginica. Only the microspheres are transported to the dorsal tract, indicating their selection for ingestion. Scale bars ≈ 200 µm. Source: Ward, Rosa and Shumway (2019)

3.5.1 Translocation of nanoplastics in aquatic vertebrates

'Translocation' refers to the passage of particles across biological membranes, where they end up in the tissues of organisms. A range of research indicates that nanoplastics do translocate in fish – a finding often correlated with negative health effects (Barría *et al.*, 2020). In the laboratory, for example, researchers have demonstrated translocation of nanoplastics across salmon intestines using palladium-doped polystyrene (Clark *et al.*, 2022); translocation to liver and kidney of fathead minnow (Pimephales promelas) following exposure to fluorescent-labelled polystyrene nanoparticles via diet and injection (Elizalde-Velázquez *et al.*, 2020); and extensive internalisation of nano-sized polystyrene into zebrafish cell cultures (Sendra *et al.*, 2021).

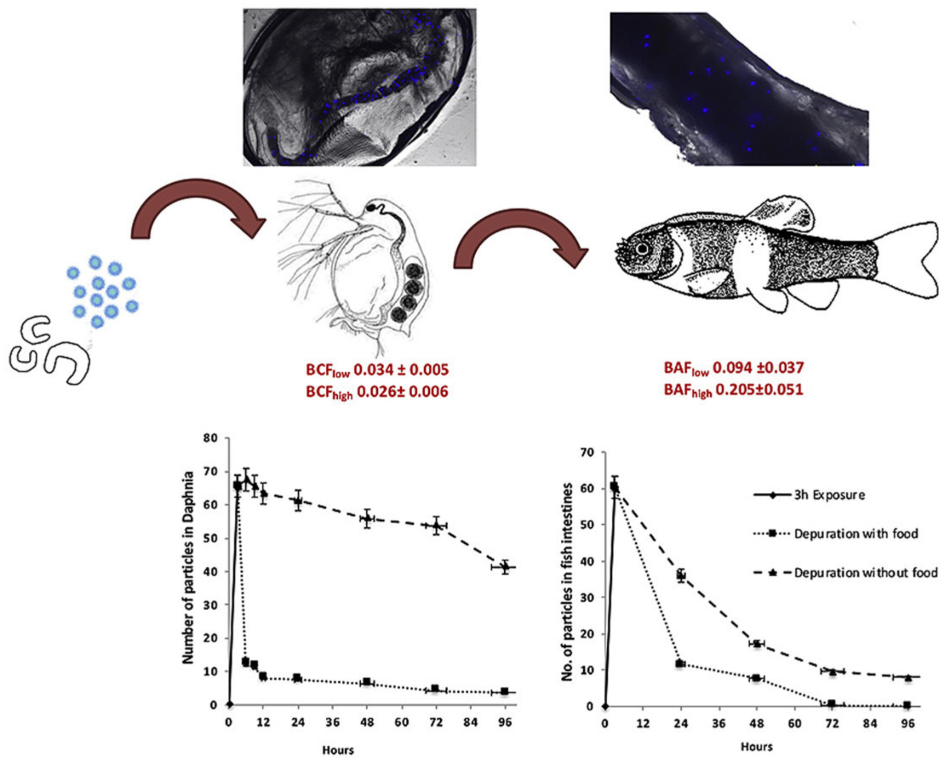


Fig. 13. Experiments with polystyrene nanoparticles have shown that they are able to reach fish organs, with detrimental health effects. Source: Elizalde-Velázquez *et al.* (2020)

Polystyrene nanoparticles were also studied by Sökmen, *et al.* (2020), who found they reached the brains of zebrafish larvae. Mattson et al (2015) noted that brains of fish exposed to nanoplastics were heavier, whiter, and fluffier in appearance than controls, possibly due to the lipophilic nature of polystyrene chains being attracted to the lipid-rich brain tissue. A study using latex particles of <50nm found these particles accumulated throughout the bodies of Japanese rice fish (Oryzias latipes) – in testes, liver, blood and brain – also causing a decrease in survival of affected fish embryos (Boyle and Örmeci, 2020). In another study using diet fed exposure of fish to PS nanoplastics, nanoparticles were identified in the yolk sac, liver and pancreas of larvae and embryos after maternal dietary exposure –suggesting maternal to offspring transfer in fish (Pitt *et al.*, 2018).

The breaching of the blood-brain barrier in zebrafish and Japanese rice fish is particularly concerning for both human and animal health. Importantly, however, many translocation and toxicity studies use fluorescent-marked particles; some research has questioned the validity of this method, suggesting it may lead to inaccuracies in determining translocation of nanoplastic in organisms (Schür *et al.*, 2019; Catarino, Frutos & Henry, 2019). That is, fluorescent compounds themselves may leach from marked particles and accumulate in model organisms, so observations must account for this (Figure 14). Radio-labelled or trace metal doped particles offer an alternative option (e.g. Redondo-Hasselerharm *et al.*, 2021). Additionally, a large proportion of studies use polystyrene particles, which may not well represent the behaviour of other types of nanoplastic within organisms; nor is it the most commony found microplastic found in the environment (and by extension, nanoplastic).

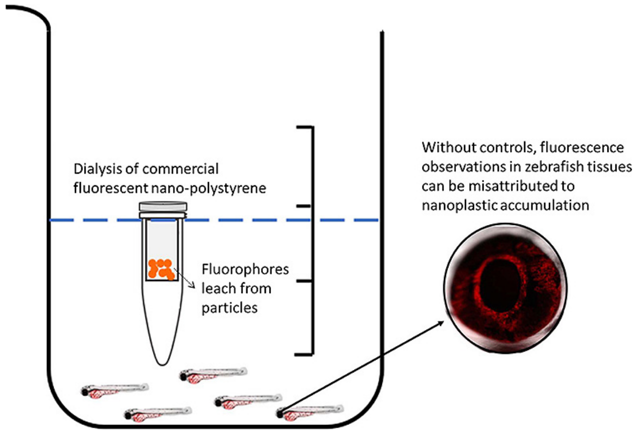


Fig. 14. Recent work has highlighted a methodological problem when using fluorescent-marked nanoparticles to trace nanoplastics in fish tissue. Source: Catarino, Frutos & Henry (2019)

Investigating translocation in fish sampled from the field, meanwhile, faces the challenge of separating and quantifying (unmarked) nanoparticles in biological tissue, while avoiding destruction of these target particles during sample processing. Studies that detect translocation of nanoplastics in fish collected from fresh and seawater environments are lacking. One researcher attempted to retrieve nanoplastics from dissected fish from the Aegean Sea, but was unable to ascertain whether nano-sized particles filtered out were plastics (Gimskog, 2019).

3.5.2 Translocation in aquatic invertebrates

As is the case for the fish studies, translocation in aquatic invertebrates such as shellfish are also mostly carried out with polystyrene (PS) nanoparticles. For example, an experiment with the Mediterranean mussel, *Mytilus galloprovincialis*, demonstrated the translocation of PS nanoplastics into the hemoctyes (immune system cells) (Sendra *et al.*, 2020). The smallest PS nanoplastics tested were detected in the digestive gland and muscle, with swift, size-dependent, translocation to the haemolymph (the invertebrate equivalent of blood), after just 3 hours of exposure. The exposed hemocytes suffered functional losses in motility, cell death, reactive oxygen species (ROS) and loss of phagocytic capacity (ability to surround and destroy micro-organisms and foreign material). However, the hemocytes were resilient when infected with bacteria after this exposure, recovering their phagocytic capacity, despite expression of the mussels' antimicrobial peptide, myticin C, being lowered.

A similar study with a commercially important scallop species eaten by humans, *Pecten maximus* (great or king scallop), found that at environmentally relevant levels (<15 µg per litre of water), nanoplastic PS particles of 24 nm and 250 nm were taken up rapidly into the scallop body (Al-Sid-Cheikh *et al.*, 2018). In this study, Carbon-14 isotopelabelled PS particleswere used, allowing precise tracing of the administered particles within the scallops. The larger 250 nm nanoplastics accumulated in the intestine, whereas the 24 nm particles were distributed throughout the whole body – suggesting translocation across epithelial membranes of the scallop. After 14 days the 24 nm particles were no longer detected, whereas the 250 nm particles were still present after 48 days – indicating differential removal of different particle sizes. Chronic exposure studies would be beneficial to gain a greater understanding of this mechanism. Modelling suggested that after 300 days of environmental exposure to nanoplastics, the scallop body tissues would reach equilibrium – with concentrations below 2.7 mg/g (*ibid.*).

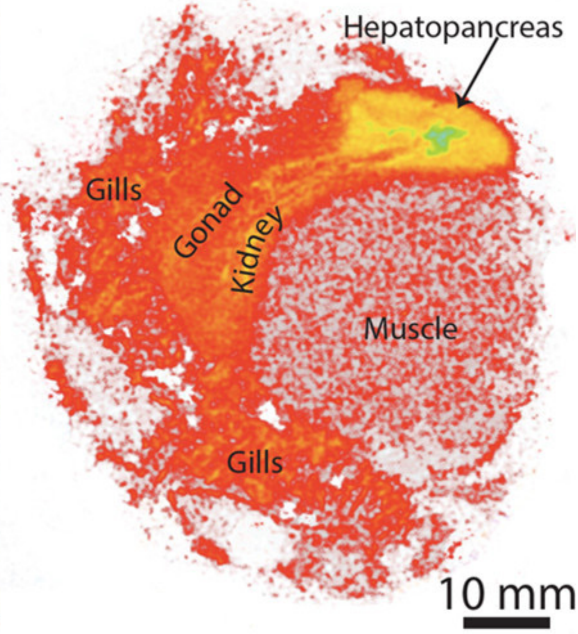


Fig. 15. Pecten maximus, scallop exposed to 14C-radiolabeled nanopolystyrene at predicted environmental concentrations of (<15 µg/L). Uptake was rapid, shown by autoradiograph, and greater for 24 nm than for 250 nm particles. After 6 hours, nanoplastics of 250 nm accumulated in the intestine, while 24 nm particles were dispersed throughout the whole-body, possibly indicating some translocation across epithelial membranes. Red, yellow and green in the right hand x ray image, shows the presence of 24nm radio labelled PS particles, with red indicating lower concentrations, yellow medium, and green higher concentrations. Source: Al–Sid–Cheikh *et al.* (2018)

Some research suggests that nanoplastic particles may have transgenerational effects via translocation to reproductive organs. For example, toxicity was observed in the nematode species Caenorhabditis elegans following prolonged exposure to PS nanoplastics at concentrations higher than 10 µg/L (Zhao *et.al*., 2017). When the concentration was increased to over 100 µg/L, transgenerational effects were observed; the authors suggested this was due to translocation of PS nanoplastics into organs such as the gonad and possible transfer to the next generation. The effect was not found to be due to leachates. In another study, Daphnids fed algae which had been exposed to iron-tagged 270 nm diameter PS were found to contain these nanoplastic particles, suggesting trophic transfer. These daphnids took longer to produce their first offspring, and had lower numbers. In the same study, the next generation of daphnids from parents exposed to europium labelled PS nanoparticles contained a traceable amount of europium – implying it was transferred from parents to offspring (Monikh *et al.*, 2020).

3.5.3 Trophic transfer of nanoplastics

Given the confirmed presence of microplastics (and presumably nanoplastics) in a range of taxa, it has been suggested that trophic transfer of micro- and nano-plastics could occur, through aquatic and terrestrial food chains. Studies have confirmed transfer of microplastics in the laboratory, but it is thought that microplastics may be subject to significant gut clearance in fish (Güven *et al.* 2017; ECHA, 2020) and bivalves, for example, which also may also preferentially capture particles over 1um (Ward, Rosa and Shumway, 2019). However, when nanosized particles are ingested by organisms, they may cross biological membranes, increasing the likelihood of uptake and potential for bioaccumulation, plus subsequent transfer through the food-chain (Besseling *et al.*, 2014; Larue *et al.*, 2021; EASAC, 2020).

Mattsson *et al.* (2015) administered 24 nm and 27 nm PS nanoparticles to carp fish via algae and zooplankton. The researchers observed severe effects on feeding and shoaling of fish, demonstrating the possible uptake of particles through a food chain affecting behaviour of the top consumer. Nano-sized PS particles have been shown experimentally to transfer up to four trophic levels within an aquatic food chain, from algae to end-consumer fish species (Chae *et al.*, 2018). In this experiment, the nanoplastics moved easily through the food chain with negative health impacts for the fish species exposed, which demonstrated lowered levels of activity and tissue changes in their livers. In addition, juveniles were found to have nanoplastics present in their yolk sacs. However, the exposure dosages used in the study were artificially high, and fluorescence as a marker can lead to inaccuracies (Schür *et al.*, 2019), therefore general conclusions cannot be drawn from these findings.

A more recent study found that algae accumulate nanoscale plastic debris (NPD), which is transferred to zooplankton – with greater uptake and trophic transfer of smaller NPD (270 nm), compared with larger NPD (640 nm) (Monikh *et al.*, 2020). In this study, iron and europium were used as a label for the nanoscale PS, rather than fluorescence. The smaller sized NPD were found to detrimentally affect reproduction in the zooplankton and to be passed on to the young.

Further studies using levels of nanoplastics found in the natural environment, i.e. at lower concentrations over a longer period of time, are warranted where there is potential for cumulative build-up in the bodies of aquatic vertebrates. More research taking multiple levels of trophic transfer into account and mixtures of nanoplastics would also improve understanding (Latchere *et al.*, 2021; Zhu et al, 2021). A novel study by He *et al.* (2022), for example, tracked palladium-doped nanoplastics in a constructed freshwater ecosystem with zebrafish, three invertebrates and two aquatic plants, over 49 days. Results showed that chronic exposure led to more uptake than short duration exposures, but no biomagnification effect was identified – consumers higher in the food chain had not absorbed relatively more nanoplastics than organisms further down the chain. Potential bioaccumulation has been noted in studies of environmental microplastics, however: microplastics adhered to seagrass blades in a remote California reserve were found at a higher density per gram in a herbivorous snail present at the site, than on the two species of sea grasses examined (Saley *et al.*, 2019).

3.5.4 Nanoplastics and marine mammals

Nanoplastic presence in the ocean is of concern because of possible toxicological impacts on aquatic organisms. With their potential to bioaccumulate and be passed along food chains, conservationists fear the impact this might have on predators and large filter feeders, such as polar bears and whales (Routti *et al.*, 2021). Plastic additives may add to this risk. Phthalates are a specific group of compounds of concern since these plasticisers are not chemically bound to the polymers of the plastic and may leach out of micro- or nanoplastic particles. These can then enter organisms, or be ingested within plastic particles and then leach out internally (*ibid.*; Hahladakis and Iacovidou, 2018; Paluselli *et al.*, 2018). Detection of phthalates in filter-feeding whales from the Mediterranean Sea and Sea of Cortez has been correlated with high concentrations of microplastics in these areas (Fossi *et al.*, 2016).

At present there is a paucity of data on the presence of phthalates in marine mammals, but also on how nanoplastics might contribute to the presence of these and other plastic additives (Panti *et al.*, 2019). However, as microplastics have been shown to degrade to nanoplastics within the environment, and within organisms, areas with high microplastic concentrations therefore may also contain high concentrations of nanoplastics. Although the source of the plasticisers detected in marine mammals, has not been definitively linked to any particular size of plastic, the presence of plastic pollution of any size in the ocean is a principal source of phthalates.

Box 11: Gathering data on micro- and nanoplastics in cetaceans

A threefold approach to assessing the impact of marine litter on cetaceans has been suggested (Panti *et al.*, 2019). This is to address the multiple potential, physical, and ecotoxicological effects of marine litter interactions, from macro-, micro- and nanoplastics. Data on rate of ingestion, and sublethal short-term and long-term effects of marine plastic litter, can be investigated using the threefold approach – with each method applied independently, or simultaneously, in either stranded or free ranging animals (Panti *et al.*, 2019). The threefold approach consists of:

a) **Analysis of gastro-intestinal content:** Analysis of the gastro-intestinal content in stranded cetaceans, to determine the occurrence and rate of marine plastic litter ingestion and any associated pathology;

b) **Analysis of the levels of plastic additives (biomonitoring), as a proxy for ingestion:** The levels of plastic additives (such as phthalates or PBDEs) and associated Persistent Bioaccumulative and Toxic (PBT) compounds – in free ranging and stranded cetaceans – can aid in quantifying the exposure to marine plastic pollution;

c) **Analysis of biomarker responses:** Biological responses, up to a few hours after death, can be used to detect the potential toxicological effect, related to PBT, and plastic additives from plastic ingestion (Panti *et al.*, 2019).

3.5.5 Nanoplastics, aquatic plants and algae

Nanoplastic pollution also affects aquatic photosynthetic organisms such as seaweed (macroalgae) and phytoplankton (microalgae). This merits investigation, as phytoplankton are not only the foundation of the aquatic food web, but are also responsible for fixing 45% of the carbon dioxide in the air, and producing about half the world's oxygen. Aquatic plants (macrophytes) also offer important ecosystem services.

Larue *et al.* (2021) conducted a review of studies on the impacts of nanoplastics and microplastics on aquatic plants. Most of the articles focused on nanoplastics, with 90% finding that they can have toxic effects. The studies noted that most toxic effects were seen with smaller size particles of plastics, below 1 μm. Leached chemical additives were also implicated in toxicity – and plasticisers such as phthalate esters have been shown to accumulate in some plants, resulting in exposure for people that eat them. The toxicity mechanism for nanoplastic particles acting on phytoplankton is dominated by the chemical effect, i.e. the formation of reactive oxygen species and decreased photosynthesis; the converse is true for bigger microplastic particles where toxicity is driven by physical effects (*ibid.*).

Positively charged plastic particles have been shown to attach to the outside of phytoplankton. As these organisms are the first level in aquatic food chains, adhered plastic particles could be passed up to the next trophic level. Green algae, for example, have been shown to transfer plastic particles to the crustacean *Daphnia magna*, and to two species of fish. Nanoparticles of PS have a higher toxicity to *D. magna* (6x higher mortality), when they are exposed through their diet – ingesting the nanoplastics, rather than encountering them in the water column (Besseling *et al.*, 2014). However, weathering usually induces an overall negative particle surface charge on plastic particles, which has been found to make them less likely to attach to algae, as cellulose in algae also bears a negative charge.

Box 12: Factors influencing toxicity to phytoplankton

After phytoplankton exposure to nanoplastics, expression of genes related to oxidative stress regulation, photosynthesis, fatty acid synthesis, and cell aggregation can be altered. Some microalgae species appear more sensitive than others (Larue et. al., 2021).

Other factors that influence toxicity of nanoplastics to phytoplankton include:

- Concentration

Larue *et al.* (2021) reported that environmental concentrations of PS nanoparticles at which half of a phytoplankton population is affected ranged between 0.5 and 13mg per litre. The highest reported plastic concentration in the aquatic environment so far is 1.56 mg per litre (Lasee *et al.*, 2017), suggesting plastic pollution poses a risk to phytoplankton in these highly contaminated aquatic regions. Few data expressing environmental concentrations in mass per litre are currently available (Larue *et al.*, 2021) – an important knowledge gap to address to assess risk to aquatic life.

- Type of plastic

PVC has often been found to be more toxic than other types of microplastic, such as polystyrene, PP and PET (Zhu *et al.*, 2019; Capolupo *et al.*, 2020). This is likely to be due to the higher amount of toxic chemical additives released from this polymer.

- Aging of particles

Most studies on the toxicity of nanoplastics (and microplastics) to phytoplankton and other species are conducted in a lab, using pristine plastic model particles – yet it has been shown that aged plastic particles taken from the ocean can have different effects. In a study examining the impact of pristine PVC versus environmentally aged PS, it was found that the aged plastic was more toxic to phytoplankton Chlorella vulgaris. A similar effect was noticed using ocean-aged PE versus pristine PE on microalgae *Scenedesmus subspicatus* – perhaps related to molecular-level changes in the structure of the aged PVC, which conferred increased hydroxyl and aromatic groups. In a similar study with PE particles, aged plastic released more additives, meaning it was more toxic to cyanobacteria than the non-aged PE particle leachate (Larue *et al.*, 2021).

- Chemical combinations

A plethora of studies have indicated that microalgae are more susceptible to toxic effects from nanoplastics, and other ecotoxic chemicals, when exposed to them in combination (e.g. Zhu *et al.*, 2019).

The ability of microalgae to absorb micro- and nanoplastics has also been highlighted as a potential remediation and wastewater treatment tool (e.g. Manzi *et al.*, 2022). Uptake of nanoplastics into macrophytes – aquatic plants such as duckweed (Lemna spp.) and water hyacinth (Eichhornia crassipes) – meanwhile, has only recently been documented.

In one study, researchers compared the effects of positively and negatively charged PS nanoplastics on duckweed at different concentrations (Xiao *et al.*, 2022a). Uptake into plant tissues was observed after three days at concentrations of 10-50 μg/ml, together with adverse effects such as reduced growth. However, particles did not appear to be taken up after seven days of exposure at concentrations lower than 0.1 μg/ml, which the researchers say are environmentally relevant. Although there were no obvious effects on the plants at these concentrations, a further experiment exposing plants to particles at 0.015 μg/ml suggested that chronic exposure could induce genetic changes.

Other studies have also detected micro- and nanoplastics on the root surface of duckweed, but no evidence of internalisation (Dovidat *et al.*, 2020). Nevertheless, attached particles may be consumed by organisms that feed on these plants. In the Mediterranean Sea, for instance, microplastic fibres and beads have been found adhered to the sea grass, Posidonia oceanica (Neptune grass), which forms balls of fibrous material that wash up on sandy beaches. Of the detritivorous fauna feeding on these, such as snails, researchers found just under 30% had plastic fibres in their digestive tract (Larue *et al.*, 2021).

Fig. 16. Seagrass ball on a Mediterranean beach. Organisms that feed on this aquatic plant may also be ingesting nanoplastic adhered to it. Credit: Ezu

3.6 Terrestrial habitats

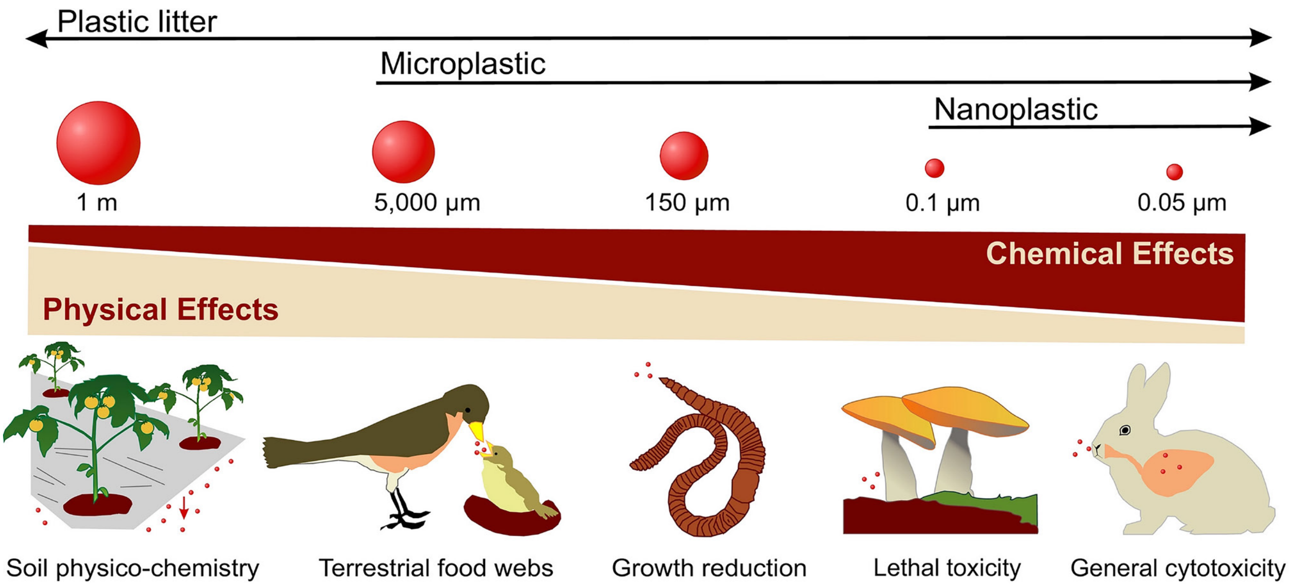


Fig. 17. Potential impacts of plastic contamination: soil biogeochemistry; ingestion by birds; reduction in growth of earthworms; lethal toxicity to fungi; mammal lung inflammation and broad cytotoxicity of nanoplastics. Source: de Souza Machado *et al.* (2018)

Annual plastic production currently exceeds 380 million tons … [and] it is estimated that roughly 32% of plastic waste might find its first receptacle in soils or continental aquatic ecosystems … [A]pproximately 4 977 million tons have accumulated in landfills and the natural environment

Source: de Souza Machado *et al.*, 2018

Public attention on plastics has largely focused on the marine environment, perhaps in part due to the 'Blue Planet effect', referring to the documentary series of the same name (Dunn, Mills and Veríssimo, 2020). However, most marine pollution comes from land; levels of microplastic contamination within terrestrial soil are much higher than in the ocean – some 4 to 23 times larger (de Souza Machado *et al.*, 2018; EASAC, 2020).

There is a growing body of research showing that both micro- and nanoplastics interact with terrestrial organisms from plants, fungi and earthworms to birds, rabbits and rats – many of which provide essential ecosystem services (de Souza Machado *et al.*, 2018). The widespread presence and persistence of micro- and nanoplastics in the global environment, and their interactions with terrestrial and freshwater biota, mean these small plastic particles are viewed as an emerging global threat. Further research is needed on the fate and effects of nanoplastics on land and in freshwater habitats (de Souza Machado *et al.*, 2018; Nizzetto, Futter and Langaas, 2016; EASAC, 2020).

Nanoplastics are anticipated to be introduced to soils as a result of "landfill leachate, agricultural mulches, application of wastewater biosolids to agricultural land, or by direct releases of secondary micro- and nanoplastics from abrasion or maintenance of outdoor plastic goods and coated surfaces", say Alimi *et al.* (2018), and may subsequently "undergo various transformations commonly associated with natural or anthropogenic colloids; namely, homo- and heteroaggregation, interactions with microorganisms and macromolecules (e.g., adsorption of proteins, natural organic matter) and biodegradation".

3.6.1 Soil and earthworms

Studies quantifying the amount of nanoplastics in soil are scarce, probably due to a lack of robust analytical techniques. However, studies addressing microplastics, which degrade to nanoplastics, have found greatly varying concentrations in terrestrial soil at different sites, ranging from under 10 mg/kg of soil to between 55.5 and 67 500 mg/kg (Büks and Kaupenjohann, 2020; Larue *et al.*, 2021). A range of studies have also measured concentrations in terms of numbers of items (of microplastic) in dry soil. Büks and Kaupenjohann (2020) reviewed 23 studies on microplastic contamination in soils and found that median concentrations from studies conducted in Europe amounted to 2 914 items/kg, which is twice as high as in Chinese studies.

Researchers have expressed concern that microplastics could migrate through soil and enter the groundwater, being carried down by rainfall, wet-dry cycles, earthworms and other soil organisms (Boyle and Örmeci, 2021; Blasing and Amelung, 2018). Nanoplastic particles also have the potential to pass through soil pores and enter groundwater aquifers (Rillig *et al.*, 2017; Chae and An, 2018). As PS nanoplastics have been shown to act as carriers for chemical pollutants – pyrene and 'BDE 47' – present in saturated soil, it is possible that these NPs could transport these toxic pollutants into the groundwater of aquifers (Banerjee and Shelver, 2021).

Earthworms suffer detrimental physiological effects due to PE microplastic exposure in soil including damaged immune systems and inhibition of sperm formation in male worms (Kwak and An, 2021), mortality, reduced burrows and reduced growth

(Huerta Lwanga *et al.*, 2016), and gut inflammation (Rodriguez-Seijo *et al.*, 2017). Studies examining the impact of nanoplastics on earthworms, meanwhile, are scarce. Kwak and An (2021) found that they excrete nanoplastic PE particles into the soil, after ingesting/digesting microplastics. This breakdown of microplastics to nanoplastics has been shown to occur due to earthworm gut bacteria (Ng *et al.*, 2018) – a phenomenon also demonstrated in krill (Dawson *et al.*, 2018).

Having established that earthworms can ingest microplastics, and excrete fragmented nanoplastic particles, it is likely that nanoplastics are also being moved further down into the soil depths via similar processes to microplastics. For example, movement of nanoplastic PS particles, of 100 nm size, has been observed in a range of soil types, and one study found that the higher the pH, and lower the iron/aluminium oxide content, the greater the movement of PS nanoplastics in the soil (Boyle and Örmeci, 2021). Different physico-chemical compositions of soil and the array of different nanoplastic particle types are likely to interact in a range of manners together – an area which requires further research.

3.6.2 Terrestrial plants and fungi

Both nanoplastics and small microplastics can be taken up by terrestrial plants (Mateos- Cárdenas *et al.*, 2021; Larue *et al.*, 2021) – raising concerns regarding foodchain contamination. Studies comparing uptake and accumulation of microplastics versus nanoplastics found that nanoplastics accumulate more in root tips of plants. Nanoplastics can be absorbed into the roots of cress (*Lepidium sativum*), broad bean (*Vicia faba*) and onion (*Allium cepa*), for example, and translocate to the shoots of wheat (*Triticum aestivum*). Uptake and translocation of styrene maleic anhydride nanoplastics has also been demonstrated in orange jasmine (*Murraya exotica*) (Larue *et al.*, 2021). A recent study investigated whether three tree species could take up nanoplastics, finding that nanopolystyrene was taken up by roots and into tissues (Murazzi *et al.*, 2022).

Trophic chain transfer of fluorescent PS nanoplastics has been shown to occur from the soil into mung beans, and into African giant snails that feed on these. Studies of commercialised vegetables, grown in greenhouses, show that vegetables and cereals also accumulate high amounts of phthalates – additives in plastics – illustrating a high risk of transfer to consumers (Larue *et al.*, 2021).

Nanoplastics can trigger oxidative stress reactions in plants, affecting germination, photosynthesis and growth. Additives leaching from nanoplastics are toxic to the genes and cells of some plants, (for example, Allium cepa, when exposed to the leachate of a biodegradable nanocomposite of PLA and nanoclays) (Larue *et al.*, 2021). Nanoplastics can also indirectly affect plant growth by changing soil properties and the microbial community (Chen *et al.*, 2022).

Interestingly, nanoplastics in soil may alleviate the toxic effects of other soil pollutants – such as Arsenic (As) and Cadmium (Cd) – on crops, which, when combined with PS nanoplastics were less toxic than when wheat was exposed to them alone. This was due to the nanoplastics attaching the As and Cd to their surface, causing the plants to be less exposed to the toxins. Also, the nanoplastics reduce root activity, which could decrease the uptake of As and Cd (Larue *et al.*, 2021).

Smaller sized and positively charged particles are potentially more toxic to plants. For example, one study found positively charged nanoplastic particles more toxic to Arabidopsis thaliana than negatively charged nanoplastics (Sun *et al.*, 2021). There are a number of other variables that require more investigation, especially to reflect real-world particles and environments in findings. For example, aged particles of plastic have been shown to exert more toxic effects on phytoplankton (Box 12), so lab-based studies using non-aged particles might show less toxic impacts. However, spherical shapes appear to be more easily absorbed by roots than particles of other shapes, while irregular, aged particles may be less bioavailable (Maity *et al.*, 2022). Meanwhile, strong binding of nanoplastic to soil particles may also slow down uptake, but nanoplastics present in water in soil pores appear more readily available to plants (*ibid.*).

Biodegradation of nanoplastics in the rhizosphere – the region of soil around a plant's roots – is yet to receive much research attention. One study found that chemicals exuded from roots in response to nanoplastics may drive their degradation in soil (Yoon *et al.* 2021).

Nanoplastics have been found to be absorbed into fungi cell walls, showing their ability to cross barriers considered impermeable for many other toxic substances (de Souza Machado *et al.*, 2018; EASAC, 2020). Some studies have indicated that nanoplastics inhibit fungal enzyme activity, damage hyphae and affect fungal community structure (Du *et al.*, 2022). This could have knock-on effects in ecosystems, for example leading to lower levels of leaf litter decomposition and nutrient release in freshwater streams. A recent review looking at the effects of micro- and nanoplastics on microbiota in soil and water, also highlighted their complex effects on ecosystems, and individual organism health (Santos *et al.*, 2022).

3.6.3 Other terrestrial organisms

Contamination of terrestrial organisms with microplastics is already widespread, with Zhao *et al.* (2016), for example, finding microplastics in the digestive tract of 94% of dead terrestrial birds in China, with diverse foraging behaviour. Smaller particles of plastic, microplastics and nanoplastics can be ingested or inhaled, blocking the digestive tract, or abrading and irritating mucosa (EASAC, 2020). In one study, nanoplastics inhaled by rats were transported from the lungs to the capillaries, from where the particles could be distributed to the rest of the body (de Souza Machado *et al.*, 2018). Nanoplastics have now been shown to cross highly selective membranes, such as the blood-brain barrier, and the human placenta (Sökmen *et al.*, 2020; de Souza Machado *et al.*, 2018; Campanale *et al.*, 2020).

4. Ecotoxicity of nanoplastics

Studies have demonstrated a number of impacts of nanoplastics on terrestrial, marine and freshwater organisms. Toxicity seems to be chiefly due to damage to cell membranes and oxidative stress (increased free radicals which cause damage to tissues). Findings in this field are mostly laboratory-based, using model organisms and exposure to relatively high concentrations of nanoplastic. At present, technical limitations make quantification of concentrations of environmental levels of nanoplastics challenging, but innovative techniques are emerging that permit detection of nanoplastics in field samples. Establishing evidenced-based environmental concentrations of nanoplastics will further validate lab-based mechanistic studies of ecotoxicity, coupled with determinations of accurate concentrations in both media and tissue samples.

Box 13: Not all nanoplastics are (equally) toxic

Some plastics – and by extension micro- and nanoplastic particles – are more hazardous to health than others. While the surface properties and size of nanoplastics may affect their toxicity, and risk is related to extent of exposure, their chemical constituents are also key.

Constituent monomers

Researchers looked at chemicals used in 55 thermoplastic and thermosetting polymers to develop a hazard ranking of plastic polymers for the EU classification and labelling regulation (CLP) (Lithner *et al.*, 2011). According to the ranking, the most hazardous – polyurethanes, polyacrylonitriles, PVC, epoxy and styrenic copolymers – are made of monomers classified as mutagenic and/or carcinogenic.

Additives

Some plastics are made of monomers considered non-hazardous (e.g. polyethylene and polypropylene), but which contain harmful additives, for instance triclosan, phthalates, bisphenol A and formaldehyde. Leachates from plasticised PVC and epoxy products have been shown to be the most toxic to *Daphnia magna* (Lithner *et al.*, 2009; Lithner *et al.*, 2012), chiefly due to hydrophobic organic compounds. A recent study of eight polymers found that all chemicals leached into water were toxic to some degree, with LDPE, PVC and polyurethane inducing most toxic effects (Zimmerman *et al.*, 2021).

Plastic additives include endocrine disruptors on the Priority List[22]. A recent Expert Consensus Statement outlined their key characteristics as a basis for hazard identification (La Merrill *et al.*, 2020), but no hazard ranking has yet been compiled. Under the EC Chemical Sustainability Strategy, the EU will create a legal definition for endocrine disruptors, splitting them into two categories – known/presumed and suspected, further subdivided into human health and environmental endocrine disruptors – so that they may be addressed in CLP (classification, labelling and packaging) regulation.

Attached contaminants

Persistent organic pollutants (e.g. PCBs and PBDEs) and heavy metals can attach to nanoplastic particles (Davranche *et al.*, 2019), making them more toxic to species that ingest them. For instance, PVC with triclosan caused mortality in lugworms (*Arenicola marina*) in one study (Browne *et al.*, 2013); in another, the toxicity of polyethylene to fish increased when combined with contaminants including metals (Rochman *et al.*, 2013). The degree to which such mixing occurs on the surface of micro- and nanoplastics, under different conditions, is still being studied (Yu *et al.*, 2019).

22 Candidate List of substances of very high concern for Authorisation - ECHA (europa.eu) Available at: https://www.echa.europa.eu/candidate-list-table

Rate of leaching

Physical conditions can enhance the release of toxic additives from plastics. For instance, researchers found that UV radiation enhanced toxicity of low-density polyethylene recyclate (LDPE-R), starch blend (SB), bio-based polybutylene succinate (Bio-PBS) and PVC, but had little or no effect on PET, polystyrene, PP and LDPE (Klein *et al.*, 2021).

Knowledge gaps

Numerous studies reveal toxic effects of plastic leachates. However, there is no comprehensive characterisation of the complex chemical mixtures present in plastics; consumer plastics contain toxic compounds that remain unidentified and unregulated (Zimmerman *et al.*, 2019; Gunaalan *et al.*, 2020). Plastic and nanoplastic toxicity studies also tend to focus on organisms such as plankton (e.g. *Daphnia*), zebrafish (*Danio rerio*) and earthworms (*Annelida*), whose metabolism may not reflect that of mammals (Shen *et al.*, 2019).

Our study demonstrates that consumer plastics contain compounds that are toxic in vitro but remain largely unidentified. Since the risk of unknown compounds cannot be assessed, this poses a challenge to manufacturers, public health authorities, and researchers alike. However, we also demonstrate that products not inducing toxicity are already on the market

Zimmerman *et al.*, 2019

4.1 Potential mechanisms of toxicity

The toxicity of nanoplastic particles (at certain levels of exposure) is suggested to be due to a number of factors: membrane damage, oxidative stress, immune response and genotoxicity – with cytotoxicity (cell destruction) being mostly due to membrane damage and oxidative stress (Bhattacharjee *et al.*, 2014). In one experiment, polyethylene nanoparticles penetrated the plasma membrane bilayer, causing structural changes in these biological membranes (Banerjee and Shelver, 2021). Another study showed that particles of nanoplastics that entered into cells caused internal membranes to become more permeable, and interacted with cellular organelles (Yong *et al.*, 2020). Reactive oxygen species (ROS) can be generated within cells upon processing of the plastic particles, causing cellular stress (Rubio *et al.*, 2020). Smaller nanoplastic particles can also cross the gut/lung barrier – triggering intracellular oxidative stress, followed by cytotoxicity in the organs in which the nanoplastics accumulate. Both particle or ROS translocation into the nucleus of a cell can damage DNA replication and repair machinery, contributing towards particle genotoxicity (*ibid.*).

In mammalian cells nanoplastics can cause nuclear membrane disruption, oxidative stress, release of damage associated molecular patterns and cascading effects of inflammation and cell death pathways. Liver cells of mice have been shown to suffer ROS, due to presence of 50 nm PS particles, as well as cell damage. Human liver cancer cells had a similar reaction with generation of free radicals, and mouse macrophages – immune cells – destroyed themselves in the presence of positively charge PS beads. Most studies suggest oxidative stress is triggered by nanoplastics, however, one type of toxicity mechanism may trigger a cascade of other toxicity mechanisms as they are all interconnected (*ibid.*).

At present a thorough understanding of the effect of nanoplastics on humans and other organisms is incomplete – comprehensive studies using diverse plastic materials at relevant environmental concentrations, modelling for chronic exposure, are needed for a realistic hazard and risk assessment (Lehner *et al.*, 2019). In addition, the terrestrial environment, and freshwater ecosystems are less well studied than marine habitats, and require more extensive research focus in the future. Studies involving terrestrial, and freshwater ecosystems will aid our overall understanding of nanoplastic pollution in the environment, and its possible impact on human health.

Nanoplastic toxicity is likely initiated by a cascade of changes at the subcellular level – such as oxidative stress – that then propagate through the biological hierarchy, impacting for example migratory behaviour, reproduction success and foraging behaviour, or causing death (de Souza Machado *et al.*, 2018). Effects and the extent of toxicity seems to depend on a wide range of variables, including polymer type, presence of additives, size, shape, surface charge of plastics, and dose (Yee *et al.*, 2021; Larue *et al.*, 2021).

Genotoxic and mutagenic effects – changes to DNA – have been demonstrated, with organisms exposed to polystyrene nanoparticles exhibiting altered gene expression, for example, chromosome ruptures and nuclear abnormalities (Guimaraes *et al.*, 2022; Alaraby *et al.*, 2022; Brandts *et al.*, 2022). A study looking at the effects of polystyrene nanoparticles on human white blood cells also found DNA damage, in addition to changes in the expression of cytokines related to inflammation, immune and stress responses (Ballesteros *et al.*, 2020).

Immunotoxicity – effects on cells related to the immune system – has been shown in a number of experiments. Sea urchins (*Paracentrotus lividus*) exposed to polystyrene nanoparticles exhibited reduced phagocytosis (destruction of foreign particles), with a more significant effect from negatively charged particles (Murano *et al.*, 2021; see 4.5); another study suggested that polystyrene and polycarbonate nanoparticles act as stressors on the immune system of fathead minnows (Greven *et al.*, 2016). Brandts *et al.* (2018) found that nanoplastics affect the liver and lipid metabolism of fish, interfering with their ability to use energy reserves, and also affect skin mucus which functions as a barrier against pathogens.

Meanwhile, some researchers highlight the way nanoplastics may act as a 'Trojan Horse' for other potentially toxic substances, for example toxictrace metals attached to their surface(e.g. Hildebrandt *et al.*, 2021; Domenech et al, 2021). Although secondary nanoplastics are different from intentionally manufactured nano-sized polymers, it is worth noting that there has been research into the potential for using the latter to deliver drugs to intercellular compartments, highlighting their ability to act as carriers of other chemical substances.

Studies demonstrating nanoplastic particles crossing cell membranes and translocating – in fish, invertebrates, plants, fungi and humans – are beginning to be published (e.g. Sökmen *et al.*, 2020; de Souza Machado *et al.*, 2018; Campanale *et al.*, 2020) (see section 3.5 and 3.6) The exact translocation mechanisms (for example, between cells of the gut wall epithelium, or through cells) remain unclear.

> **… the question arises whether nanoplastics are able to reach deeper organs besides the intestine due to an altered cellular fate**

Paul *et al.* 2022

4.2 Effect concentrations

To understand whether concentrations of nanoplastics in the environment pose a risk of toxic effects, knowledge on the levels at which they negatively affect organisms is needed. A growing number of laboratory studies are being published, offering data on the effects of exposure to different 'doses' of nano- and microplastics. Besseling *et al.* (2019) reviewed data on exposure via water (mg/ml) and sediment (g/kg/dry weight) across brackish, marine and freshwater ecosystems. Effect concentrations reported in this review are shown in Table 5, which compiles findings for different plastics. The levels vary widely – perhaps because different types of polymer have very different ecotoxic effects, depending on size, particle charge, and presence of chemicals such as plasticisers.

The review highlights the limited data available on effect concentrations of nanoplastics in sediments; most studies to date have focussed on concentrations of nanoplastics in water. There is also a knowledge gap on the potential impacts of nanoplastics on the environment, when contained in food or sediment.

Table 5. Summary of published effect data of nanoplastic on organisms. LC50: lethal dose 50%; EC50: effect concentration 50%; LOEC: lowest observed effect concentration; NOEC: no observed effect concentration (Redrawn from Source: Besseling *et al.*, 2019).

Exposure Medium	Ecosystem	LC50 – level at which 50% of organisms die	EC50 – level at which 50% of organisms are affected	LOEC – lowest level at which effects are noted	NOEC – no effects observed at this level
Water (mg/L)	freshwater	4 - 36	0.5-1.6	4.5-1 x 10^3	0.5 - 1
	brackish	0.2 - 22	-	-	1 - 313
	marine	0.8 - 3.9	13	0.1 - 250	10 - 100
Sediment (g/kg DW)	freshwater	-	-	1	-
	brackish	-	-	-	-
	marine	-	-	-	-

4.3 Species sensitivity distributions and preliminary threshold concentrations for nanoplastics

Toxic effects of chemicals are often reported for tests with single species and such tests inform the determination of predicted no-effect concentrations (PNEC), taking account also of any data available on other species and the level of uncertainty regarding their sensitivity. At concentrations below the PNEC, no adverse effects on the ecosystem are expected. Therefore, PNEC values often form the basis for setting protective thresholds such as the Environmental Quality Standards under the Water Framework Directive.

If test results for several taxonomic groups are available, the results from single species tests can be combined in species sensitivity distributions (SSDs). In Figure 18 below, Besseling *et al.* (2019) have provided information on SSDs for organisms affected by nanoplastics in the water. For nanoplastics, the most sensitive species was found to be the copepod, *Tigriopus japonicus*, (50 nm spherical PS particles) and the least sensitive the algae *Scenedesmus obliquus* (70 nm spherical PS particles). The study finds a hazardous concentration (or HC5, where 5 per cent of species in the SSD exhibit an effect) for nanoplastic of 5.4 mg/L – identical to that of microplastic. Using an assessment factor calculation, the researchers proposed a PNEC of 1.1mg nanoplastic/L water.

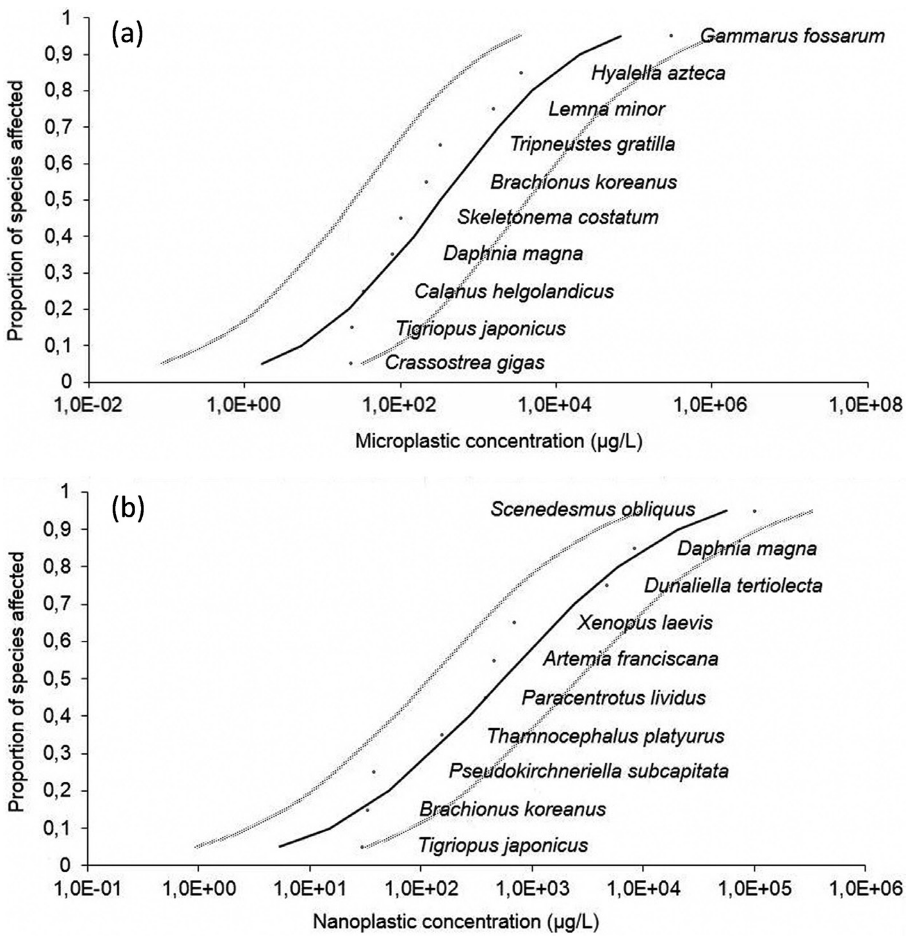


Fig. 18. Species sensitivity distributions of organisms from marine, estuarine and freshwater environments exposed to microplastic (Panel A) or nanoplastic (Panel B) via the water phase. Effect thresholds represent chronic LOECs. Grey curves represent the 95% confidence intervals. Source: Besseling *et al.* (2019)

4.4 Eco-corona

Nanoplastics can adsorb (attach) both environmental pollutants and naturally occurring substances (e.g. natural organic matter or extracellular proteins) onto their surface. This adsorbed material is often referred to as a corona since natural organic matter (NOM) or extracellular proteins may form a layer (a corona) covering the particle surface. The term 'eco-corona' is used to refer to an ecological molecule corona comprised of substances from the environment.

As with other aspects of nanoplastic behaviour, characteristics of the medium influence eco-corona formation (ions, salinity, organic matter, pH), and the eco-corona in turn influences the way nanoplastic particles interact with organisms in that environment. For example, when humic acid was added to a medium containing PS nanoplastics, the nanoplastic became less toxic to algae because it did not adhere to the algae as much as in a control medium (Oberbeckmann and Labrenz, 2020).

When contaminants such as metals, antibiotics, pathogenic micro-organisms (e.g. *Vibrio* spp) and persistent organic pollutants (POPs) attach to nanoplastic particles, those contaminants may become less toxic in the wider environment, through being less bioavailable (*ibid*; Banerjee and Shelver *et al.*, 2021). In some cases, however, nanoplastic may enhance the toxicity of other contaminants, for example if particles are positively charged (Shen *et al.*, 2019), though the reason for increased toxicity in some experiments is not always explained (Campanale *et al.*, 2020). Indeed, there is a lack of knowledge on nanoplastic corona formation, yet the surface structure of nanoplastics in the environment must be taken into account when attempting to predict their interactions with cell membranes – a complex challenge (Kihara *et al.*, 2021).

[T]he particle's "biological identity" should consider the full complexity of the surface structure

Source: Kihara *et al.*, 2021

4.5 Electrostatic charge

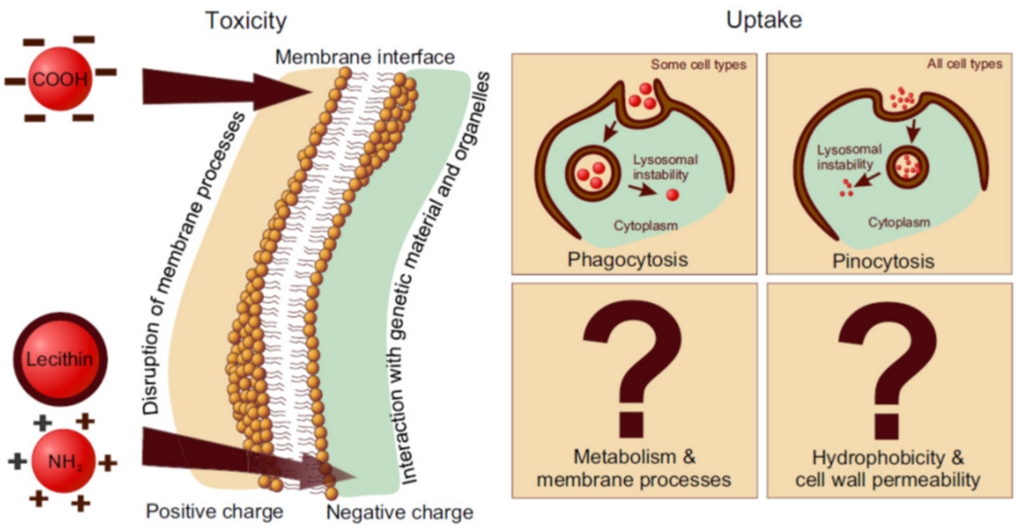


Fig. 19. Potential toxicity and uptake mechanisms of nanoplastics in animal and fungal cells. Carboxyl and amino terminated or lecithin coated polystyrene nanoparticles have diverse cellular fates, that influence the toxicity mechanism. Showing surface properties of nanoparticles are important in determining their level of toxicity. Mechanisms so far reported do not fully explain toxicity and uptake, further mechanisms need to be identified. Source: de Souza Machado *et al.* (2018)

Nanoplastic particles can attach externally to cell membranes and cause toxicity by disrupting essential membrane processes of an organism (de Souza Machado *et al.*, 2018). The surface chemistry and electrostatic charge of nanoplastics determines their loading capacity for chemicals, and ability to cross cell membranes, influencing toxicity (*ibid*; Roach *et al.*, 2006). For example, one experiment showed that positively charged polystyrene beads of 50 nm – because of the positive amino end molecule branch (NH_2) – can stick to cells and cause high toxicity to yeast at concentrations of around 10 mg/L (Miyazaki *et al.*, 2014). Conversely, negatively charged PS nanoplastics – with negative carboxyl end molecules (COOH) – had no effect on the growth rate of cells in this experiment.

The acute toxicity of positively charged PS nanoplastics is due to their electrostatic attraction to negatively charged cell walls. Positively charged particles of amino modified PS nanoplastics – of 60 nm or 400 nm – also triggered greater lung responses, and thrombosis, in hamsters and rabbits (Hamoir *et al.*, 2003; de Souza Machado *et al.*, 2018). However, it has been shown that the surface charge of nanoplastics particles can be neutralised by the occurrence of eco-coronas (see section 4.4.). In their 2017 review, Pulido-Reyes *et al.* found that eco-coronas reduce the aggregation potential and bio-reactivity of some nanoparticles. Natarajan *et al.* (2020) clearly indicated a significant decline in toxic effects of PS nanoplastics on marine microalgae due to formation of an eco-corona, and Saavedra, Stoll and Slaveykova (2019) demonstrated that eco-coronas modulated the surface charge and reduced toxicity to zooplankton. This highlights the need to examine nanoplastic interactions in a natural environment, as well as in laboratory conditions, for a full understanding of their effects. The chemical reactivity and electrostatic interactions of nanoplastics are not the only factors involved in toxicity; nanosized particles of inert substances such as gold can translocate to organs and trigger negative effects in biological systems (Prüst, Meijer and Westerink, 2020).

4.6 Particle and chemical toxicity in plants

Leachates from plastic degradation may contain polymer monomers and/or additives that can cause toxicity to plants. For example, genotoxicity and cytotoxicity were induced in onions, *Allium cepa*, exposed to leachates from the composting of a new biodegradable nanocomposite of PLA and nanoclays (Larue *et al.*, 2021).

PS nanoparticles have been shown to have an effect on the cell division (genetic functioning) of seedlings of two species of plants, with nanoplastics detected inside the cells of one of the species, *A. cepa*, affecting organelles of these seedlings. An impact on lipid mobilisation in exposed seedlings was also detected due to PS nanoplastic exposure.

Oxidative stress was demonstrated in *A. cepa* PS nanoplastic-exposed seedlings; however, the seedlings appeared to be able to prevent this being harmful – up to a certain dose. The authors suggest this implies the mechanism for cell and gene toxicity in A. cepa exposed to PS beads, is not caused by the oxidative stress, but rather a different mechanism (Larue *et al.*, 2021).

In wheat plants, PS nanoparticles affected the metabolic system of these plants –accelerating carbohydrate and amino acid metabolism – a mechanism used to cope with PS toxicity (Larue *et al.*, 2021). PS nanoplastics caused reactive oxygen species formation, and influenced genes related to oxidative stress, when *Arabidopsis thaliana*, (mustard family, includes cabbage and radish) was exposed to them. In addition, changes in root morphology, nutrient imbalances, and alterations in plant root to shoot morphology, have been reported in the literature (*ibid.*).

Micro- and nanoplastics may also carry contaminants that could cause toxicity in plants and other organisms – for example, polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), perfluoroalkyl acids (PFAAs), persistent organic pollutants (POPs), pharmaceuticals and personal care products (PPCPs), and metals (Hartmann *et al.*, 2017).

4.7 Chemical additives in nanoplastics

Several studies have reported that microplastics – and likely nanoplastics – can also cause toxic effects due to chemicals present in the plastic (Chae and *An*, 2018). Plastics can contain additive chemicals such as plasticisers (BPA, DHEP, DiNP), antioxidants, UV stabilizers and flame retardants, which are then released into aquatic environments, causing harm to aquatic organisms. This plastic leachate has been demonstrated for both microplastics and nanoplastics (Zhao *et al.*, 2017; Larue *et al.*, 2021). These contaminants may be transferred through the food chain to predators at upper trophic levels and, as such, their presence could pose a threat to aquatic organisms and ecosystems, and human health.

Plastic additives have been found at moderately high levels in microplastic-rich (and likely nanoplastic-rich) sludge from water treatment plants, used for agricultural purposes (EASAC, 2020; Boyle and Örmeci, 2021), with potential to impact plant health. Researchers have not yet quantified the release of additives into soil or water (Larue *et al.*, 2021).

Capolupo *et al.* (2020) investigated the composition, and effect, of leachates from car tyre rubber (CTR), PP, PET, PS, and PVC. These leachates – which included plasticisers, antioxidants, antimicrobials, lubricants, and vulcanisers – were derived from microplastic particles rather than nanoplastic particles; however, the impact of the leachates themselves is likely unaffected by the particle size. The leachates negatively affected membrane stability, fertilisation and embryo development in Mediterranean mussels (*Mytilus galloprovincialis*) – with 50% of the embryos dying at concentrations ranging from 0.7% (CTR) to 65% (PET) of the total leachate. Notable additives in the different types of polymer included: benzothiazole (CTR), phthalide (PVC), acetophenone (PP), cobalt (CTR, PET), zinc (CTR, PVC), lead (PP) and antimony (PET). This study highlights that chemical composition of plastic leachates is implicated in toxicity to marine biota – with increasing contamination by additives causing increasing toxicity.

5. Impacts on human health

Constant human interaction with plastic items leads to mouth and skin exposure, as well as inhalation. Assessing the risk to human health from nanoplastics specifically, is a complex task. For example, different plastic materials may have different effects on humans and other organisms in the food chain; and potential sources of exposure (food, personal care products, city dust, clothing) may pose different risks. However, some general patterns of impact of nanoplastics (and smaller microplastics) can be noted. Firstly, the potential hazard of nanoplastic particles increases with decreasing size, due to increased bioavailability in the body, and potential to pass through biological membranes (Paul et. al., 2022). The type of hazard posed by larger particles is likely to be mechanical damage, followed by inflammation, whereas smaller particles which could translocate to body organs, may accumulate causing oxidative stress and inflammation more systemically. In addition, quantification and detection techniques for nanoplastics still need to be refined. Considering this, it is understandable that there is no consensus on which aspects of nanoplastics are most harmful to humans and other organisms, however, research is underway to address this knowledge gap.

5.1 Routes into the human body

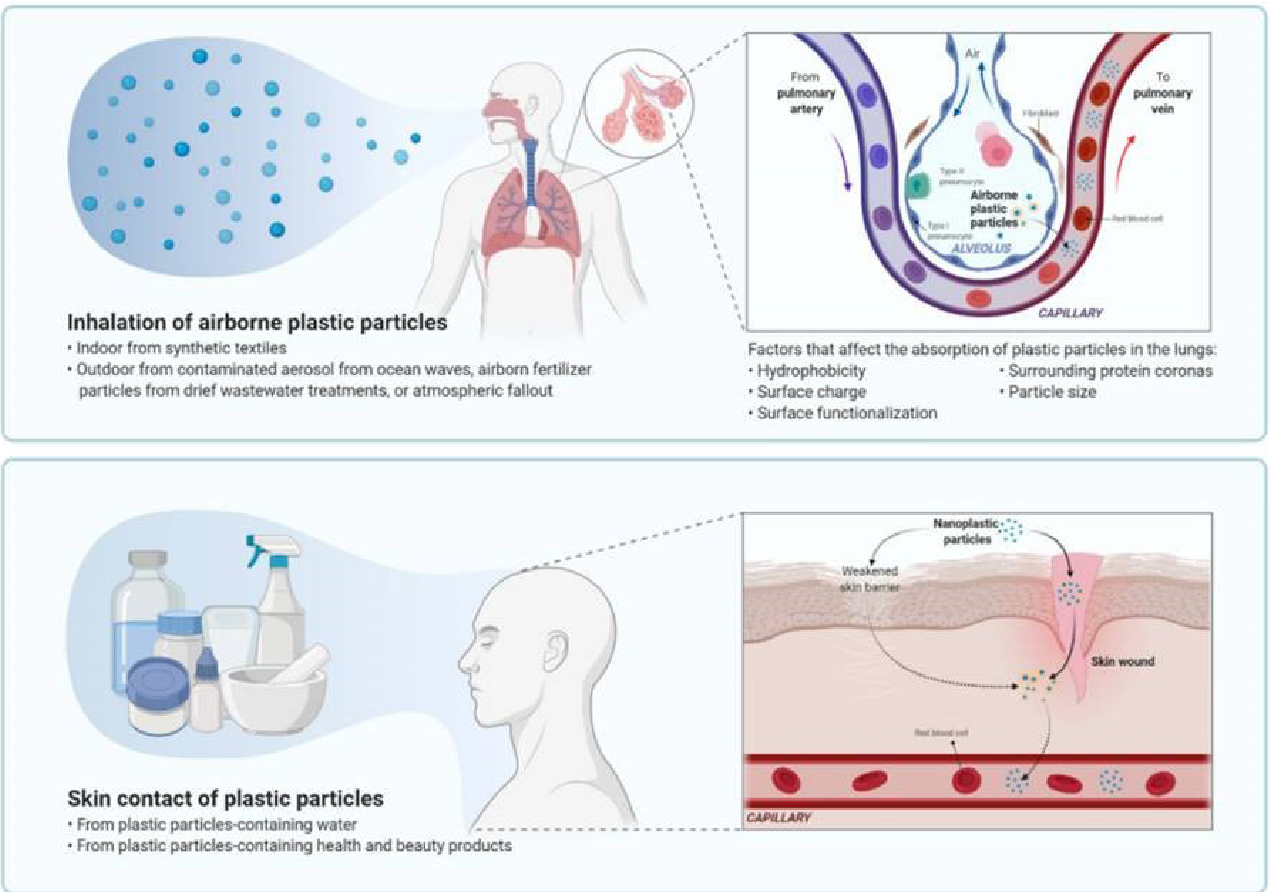


Fig. 20. Key routes by which plastic particles enter the human body. Source: Yee *et al.* (2021)

It is estimated that, on average, an adult person consumes around 39,000–52,000 particles a year or 5 g of plastic every week — the equivalent of a credit card

Schwarzfischer and Rogler, 2022

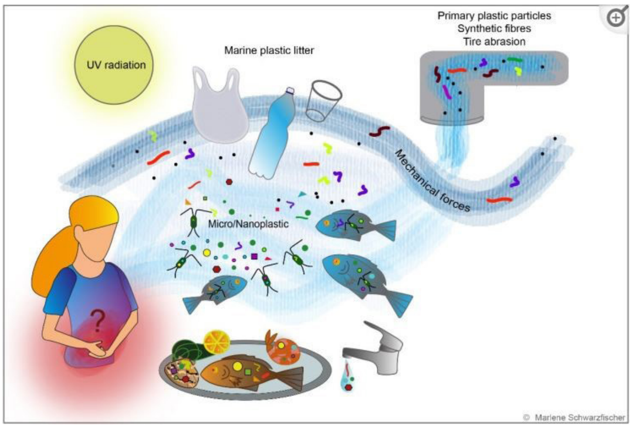


Fig. 21. Nano- and microplastics contamination of fresh and salt water. Secondary microplastic particles emerge with plastic fragmentation whilst in the environment. UV radiation, mechanical forces, biological degradation, and embrittlement result in the formation of nanoplastic particles, which are ingested by marine zooplankton and consequently enter the marine and human food chain. Nanoplastics may be consumed by people in contaminated drinking water, and other foodstuffs. However, the impacts of ingested plastic particles on human health are not yet fully understood. Credit: Marlene Schwarzfischer, Department of Gastroenterology and Hepatology, University Hospital Zurich, University of Zurich, Zurich, Switzerland. Source: Schwarzfischer and Rogler (2022)

Nanoplastics may enter the human body through ingestion of contaminated food and water, inhalation of airborne particles from textiles or polluted outdoor air, or penetration of particles through wounds or weakened skin barriers (Yee *et al.*, 2021; Schwarzfischer and Rogler, 2022). Research has indicated nanoplastics may be consumed via contaminated marine animals, other foods, toothpaste, beer, honey, salt, sugar and drinking water (*ibid*; Koelmans *et al.*, 2019; Al-Sid-Cheikh *et al.*, 2018). Medical implants such as joint replacements may also be a source of internal exposure.

Uptake of nanoplastics and subsequent translocation to organs was reported decades ago in rodents, and more recently in a number of other taxa, including in the brains of fish (Yee *et al.*, 2021; Sökmen *et al.*, 2020). However, at present there is a paucity of systematic studies on nanoplastic translocation and potential health risks in humans. It is important to recognise that it is not possible to extrapolate directly from studies on non-human species, however current assertions of impacts are theoretical assumptions, based on animal model studies and lab-based data (Yee *et al.*, 2021; Prüst, Meijer and Westerink, 2020). Meanwhile, detecting micro- and nanoplastics in human tissues presents its own issues. For example, a study that found microplastics in human placenta and foetal meconium highlighted that contamination of samples – for example from airborne fallout – is a methodological challenge (Braun et al, 2021).

Microplastics have been identified in human stool, but risks from ingestion are not yet fully understood (Schwabl *et al.*, 2019; Zhang *et al.*, 2021). Ingestion of nanoparticles and nanoplastics, is an emerging and complex research field, where the size, shape, charge, and surface modification, as well as the human membrane cell type encountered influence the mode of cellular uptake and entry into the body (Foroozandeh and Aziz, 2022). Nanoplastics may interact with proteins, lipids, carbohydrates, nucleic acids, ions and water in the human body leading to the formation of coronated nanoplastics which increases their absorption and translocation (Yee *et al.*, 2021). It is likely that nanoplastic particles and very small microplastic particles have the potential to be ingested in the human gut, due to their material characteristics conferring higher bioavailability, for example, particles below 2.5 μm can be absorbed by M-cells or Payer's patches in the human gut (Schwarzfischer and Rogler, 2022). Furthermore, tight junction pores between cells in the human gut, have a functional size of 1.5 nm – enabling nanoplastics to potentially enter via this route – whereas microplastics would be too large.

Studies examining the ingestion of nano plastic particles in the gut have noted in models of human digestion, that ingestion and translocation/accumulation of NP and MP occurs in organs distant from the gut (Schwarzfischer and Rogler, 2022). It has been suggested that those suffering with conditions which effect the permeability of the gut lining – such as inflammatory bowel disease or crohns disease – may be more susceptible to nanoplastic particles being ingested in the gut, due to disruption, and increased permeability of the intestinal epithelial barrier. At present, there is insufficient evidence to determine whether nanoplastics cause inflammation in the gut of people suffering with inflammatory bowel disease, or crohns disease, due to conflicting findings in the research literature.

The WHO recently suggested microplastics (they did not include nano-sized plastics in their analysis) in drinking water currently have no discernible overt health risks, though they added a caveat that this may change as concentration levels increase – or as more studies are conducted that elucidate impacts (Mitrano and Wohlleben, 2020). At present in vitro studies and animal models have shown harmful effects of nanoplastics; for example, Lehner *et al.* (2019) found human cell lines showed evidence that nanoplastics are taken up and induce oxidative stress or pro-inflammatory response. However, researchers at Arizona State University, identified monomers - plastic building blocks - in all 47 human tissue samples examined,taken from Alzheimer's patients. Toxic plasticizer, Bisphenol A (BPA), still used in some food containers outside of Europe – where its use is banned – was also present in all 47 human tissue samples. It is unclear how these monomers of plastic came to be present in these human liver and fat tissues, however, these two mammalian organs are likely to be exposed to plastic monomers (possibly resulting from micro or nanoplastic ingestion), as they filter or collect waste products from the human body. The researchers suggested it to be concerning that these non-biodegradable plastic materials, that are present everywhere, can enter and accumulate in human tissues, however, at present the health effects remain unknown (ACS, 2020).

Microplastic fibres have also been found in human lungs, and lung cancer tissue (Jenner *et al.*, 2022; Pauly *et al.*, 1998), and studies have indicated that microplastic inhalation may cause inflammation and increase risk of respiratory diseases (Eschenbacher et al, 2003; Mastrangelo *et al.*, 2003). Prata, estimated that human beings inhale 26–130 MP particles per day, while studies by Vianello *et al.* suggested an average intake of 272 MP particles a day (Prata, 2018; Vianello *et.al*, 2019. Atmospheric microplastics have remained largely unstudied until the last few years (and atmospheric nanoplastics even less so), so there is currently only limited understanding of atmospheric plastic pollution.

In outdoor environments, exposure could happen through breathing in contaminated aerosols from ocean waves or airborne fertiliser particles from dried wastewater treatment plants; indoor environments also contain airborne plastic particles, for example from synthetic textiles (Yee *et al.*, 2021). A recent review on atmospheric microplastics (Zhang *et al.*, 2020) concluded that the effects of atmospheric microplastics, their chemical components and their adsorbed pollutants on human and ecosystem health is unknown, but that the potential of micro- and nanoplastic to influence health is of concern (Lehner *et al.*, 2019; Wright and Kelly, 2017).

Another review asserts that due to their hydrophobicity, nanoplastic should be repelled and eliminated in mucus (Rubio, Marcos and Hernández, 2019). If nanoplastics were able to pass through the physiological tissue barrier in human lungs (which separates air from blood), they could be translocated to other parts of the body – however the risk of this is unknown. Neither is it known whether nanoplastics might pass through the cerebral endothelial membrane (blood-brain barrier). It is thought the main route of exposure that may present a risk to health is via the gastrointestinal tract, in particular via particles that have precipitated onto food from air ((Rubio, Marcos and Hernández, 2019.). Indeed, a study that found microplastics in human breastmilk, suggesting it had translocated into blood via one of the routes of exposure, found no significant relationship to variables including use of personal care products containing plastic compounds, nor consumption of fish/shellfish, beverages, or food in plastic packaging (Ragusa *et al.*, 2022).

Further research is required to identify potential exposure, in conjunction with standardised model systems to evaluate toxicity and long-term effects in humans – prerequisites for human health risk assessments (Brachner *et al.*, 2022; Zarus et al (2021).

5.2 Toxicity in humans

Although toxicity evaluations have been conducted on a range of marine species and rodents (see section 3.5 and 4.1), to date there have been few studies that specifically assess the impacts of nanoplastic particles on human health, or how they move through the gut, lungs and skin epithelia (Yee *et al.*, 2021). Toxic effects, demonstrated in experiments on organisms at lower trophic levels, cannot be directly extrapolated to humans, since the metabolic system of these organisms is quite different, and the ability of clearance and resistance to nanoplastics will likely differ. The release of toxic pollutants from nanoplastics is also affected by many factors, and it is not certain whether such substances would be released in the human body in the same way as in other species (Shen *et al.*, 2019).

"Illustration of human body (©Shutterstock, photo by jijomathaidesigners)"

In addition, most studies to date have used large doses of nanoplastics over short time periods, rather than lower, more environmentally relevant doses, over long time periods. The latter is needed, to provide a more accurate analysis of the potential impacts of nanoplastics on human health from current environmental contamination sources (ECHA, 2020; Shen *et al.*, 2020; Yee *et al.*, 2021). However, as there is some evidence of bioaccumulation of nanoplastics in animal models, and accumulation of plastic monomers in human organs, it has been suggested similarly high concentrations may be achieved in human cells over time (Mukherjee, et. al., 2021; ACS, 2020).

In vitro and in vivo studies have demonstrated that by increasing concentrations of nanoplastics, the genotoxic, inflammatory and cytotoxic responses increase – similar concentrations may be reached with the bioaccumulation process (Mukherjee, et. al., 2021). The risk to human health requires investigation: studies on rats have shown inhaled nanoplastics may cause inflammation in the lungs (Lim *et al.*, 2021); offspring of mice exposed to PS nanoparticles during pregnancy exhibit abnormal brain development (Jeong *et al.*, 2021); and nanoplastics may be able to cross the blood-brain barrier (Kozlovskaya *et al.*, 2014).

5.2.1 Research challenges

Factors that affect toxicity in humans may be indicated by animal studies and human cell studies, but determining the relevance of lab findings to real life environmental exposure is now a research challenge. For example, **surface properties** of nanoplastic particles affect their absorption in biological cells; eco-coronas may reduce their bio-reactivity and potential impacts (Pulido-Reyes *et al.*, 2017; see section 4.4); and negatively charged particles appear to have fewer impacts on cell functioning (Xia *et al.*, 2008; Natarajan *et al.*, 2020; Saavedra, Stoll and Slaveykova, 2019). Aggregation observed with negatively charged nano-PS may also reduce uptake through membranes (Shen *et al.*, 2019). Meanwhile, within organisms, protein coronas appear to promote translocation of nanoplastics (Shen *et al.*, 2019).

Biological systems do not interact with 'bare' nanoplastics, but a complex of nanoparticles and corona. Addressing the knowledge gap on nanoplastic corona formation and cellular interaction is important to corroborate effects observed in vitro (Kihara *et al.* 2021). Researchers using nanoparticles designed for drug delivery often use nanoparticles in the bloodstream 'tagged' with opsonin proteins, allowing macrophages (immune cells) to recognise the nanoparticles and engulf them. This phagocytosis process is critical for eliminating pathogens and dead cells, and leads them to organs such as the liver (see section 5.1), where the human body degrades and clears most toxins. It has been estimated that 30-99% of administered micro and nanoparticles may accumulate in the liver (Zhang *et al.*, 2016).

Another factor affecting movement of nanoplastics within human tissues is size. In one study cited by Shen *et al.* (2019), for example, 44 nm PS entered gastric cancer cells at a higher rate than larger PS particles of 100 nm size. Smaller size may not always lead to higher uptake, though – PS nanoparticles of 100 nm were taken up by human colon cancer cells more easily than 50 nm particles, in one study (Win and Feng, 2005). As well as size, the type of plastic material influences uptake of nanoplasutic particles by intestinal cells, for example, most likely influenced by differing lipophilicity (fat attracting) characteristics of the surface as it interfaces with lipid rich cell mebranes (Paul *et al.*, 2022).

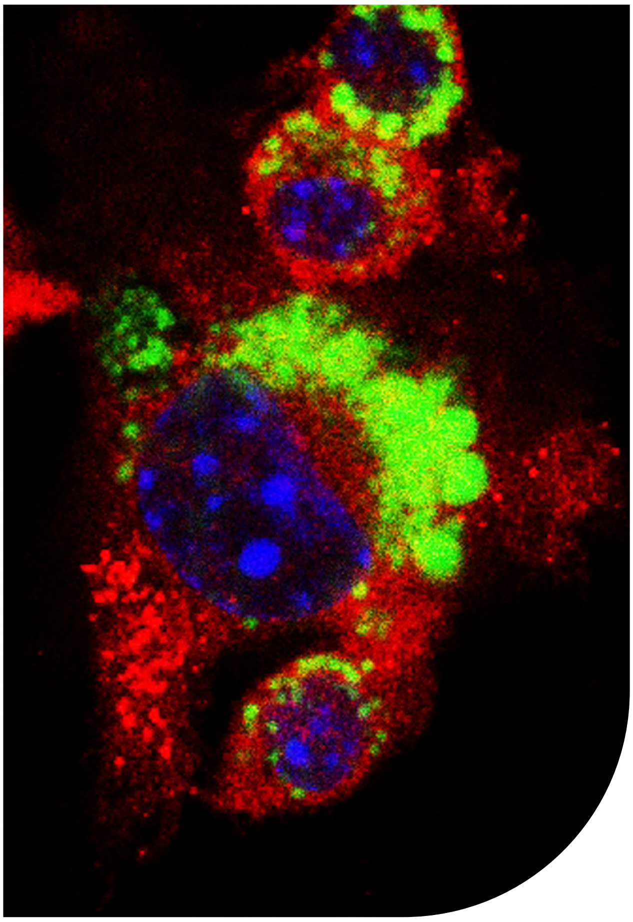


Fig. 22. Human immune system macrophages absorbing nanoplastic. Macrophages are a type of immune cell which find and clear body-foreign particles. In the human body, these "garbage collectors" are absorbing the plastic nanoparticles and recruit further immune cells triggering an immune response. Bone marrow derived macrophages were placed with fluorescent nanoplastic particles (green) in the lab. After 24 hours the cells were fixed and stained – blue = nucleus of macrophage, red = cytoskeleton of macrophage and green = the nanoplastic particles. The NP distorted the shape of the cell. Source: Marlene Schwarzfischer, University Hospital of Zurich. CC -BY-NC-ND

The shape of micro and nanoparticles is also important in cell internalisation; for example, filamentous shapes evade phagocytes better than spheres, and rigid particles are more easily internalised than soft particles (Anselmo *et al.*, 2015). Weber *et al.* (2022) found that irregular polystyrene triggered a higher inflammatory response compared to spherical nanoplastics. Characterising the shapes of nanoplastics to which humans are commonly exposed is probably important for understanding health risk.

Although polystyrene nanoparticles have been much studied, relatively little attention has been paid to how they behave within cells, why cytotoxicity differs between types of cell (Jung *et al.*, 2020), and how cells may expel them on (Han *et al.*, 2021). Studies investigating the behaviour of PS nanoplastics in mouse cells show that they accumulate in cytoplasm (as they cannot be broken down like biodegradable pathogens), causing cellular stress. Indeed, this presents a problem for nanoparticles designed to deliver drugs – they often meet a 'dead end' in the lysosome, the cell's 'waste compartment' (Reinholz et al, 2018). Nanoparticles can also be exported from cells through exocytosis, but there is very little literature on the rate at which this occurs in human cells (Han *et al.*, 2021; Han & Ryu, 2022). One study suggests that smaller polystyrene nanoparticles (50 nm) exit cells more easily than larger particles (500 nm) (Liu *et al.*, 2021). This study also noted that interactions with cells were dominated by forces other than electrostatic charge (the hydrophobic effect and Van der Waals' force).

Some studies suggest plastic composition, and the ability to carry contaminants is the most important factor in nanoplastic toxicity. The inflammation potential of nanoplastic particles themselves compared with nano forms of inorganic metal oxide materials is negligible. The hazard from nanoplastics leaching toxic additives has caused more concern, as as well their ability to act as vectors for attached pollutants (Mitrano and Wohlleben, 2020). All these areas of research require attention to inform risk assessment. With recent research indicating that nanoplastics (Jeong *et al.*, 2022), and plastic monomers may accumulate in human cells (Schwarzfischer and Rogler, 2022), and knowledge that other types of nanoparticles lead to pathological conditions (*ibid.*), the need is pressing.

5.2.1 Nanoplastics and the human body: state of science

While the discovery of microplastics in human lungs, blood and placentas has made news headlines (e.g. Carrington, 2022), evidence of nanoplastics in sampled human tissue is yet to be published. It seems inevitable they will also be detected in the near future. Meanwhile, understanding of their potential effects on human systems, and ability to translocate in the body, draw on in vitro studies with human cells and in vivo studies, chiefly on rodents.

> **… information can be extracted from the fields of nanomedicine and nanotoxicology, where a wide variety of engineered nanoparticles were found to cross the epithelial barrier in both *in vivo* and *in vitro* models**
>
> Rubio, Marcos and Hernández, 2019

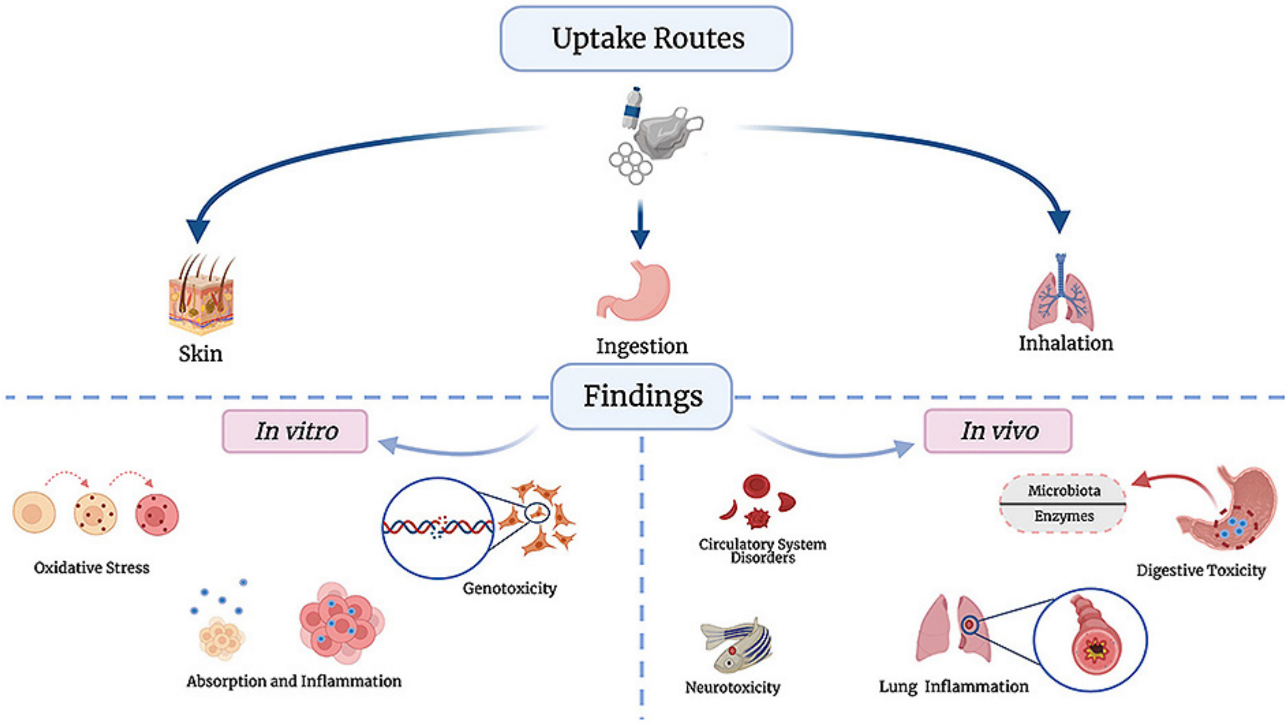


Fig. 23. Studies on nanoplastic interactions with human tissues use human cells and animal models.
Source: González-Acedo *et al.* (2021)

Since ingestion is a likely possible route of human exposure, much research has focused on nanoplastic behaviour in the **intestines**. Ingestion can lead to systemic uptake if nanoplastics are able to pass through the intestinal barrier (epithelium), and be distributed to tissues and organs via the bloodstream or lymphatic system. In a human intestine model, nano-PET was indeed able to cross the gut barrier, reported Magrì *et al.* (2018). Cortés *et al.* (2020) found PS nanoparticles within human intestinal cells exposed at a range of concentrations for 24 and 48 hours, but observed no significant toxic effects – similarly to findings of a different study exposing brain and epithelial cells to PS nanoparticles (Schirinzi *et al.*, 2017).

Some studies have investigated effects of microplastics on the microbiome (Lu *et al.*, 2019), but few have used nanoplastics. Xiao *et al.* (2022) noted changes in intestinal microbiota after exposing mice to 50 nm PS for 30 days, as well as significantly disrupted intestinal mucus secretion, but no adverse effects on the liver, lungs or brain. It is worth exploring a potential link between nanoplastics and bowel problems – one study found higher levels of micro- and nanoplastics in faeces of participants with Inflammatory Bowel Disease (Yan *et al.*, 2022).

Stem cell-based models – in particular organoids including various cells of the epithelium – are emerging that may offer better insights than currently used models based on cancer-derived cell lines (Bredeck *et al.*, 2022). In a recent piece of research using human intestinal organoids, Hou *et al.* (2022) found distinct accumulation of 50 nm PS in cells, causing inflammatory responses and cell death. The degree to which ingested nanoplastics may accumulate in human tissue, over long timeframes, is a key area to be investigated through these new methods. Work by Mohamed Nor *et al.* (2021), for example, suggested microplastics may make up 0.004% of the mass of inorganic particles humans ingest each day, but how this translates to health risk is unknown. Interestingly, Tamargo *et al.* (2022) provided the first evidence that polymers may undergo biotransformation in the human gut, using simulated digestion of PET microplastics.

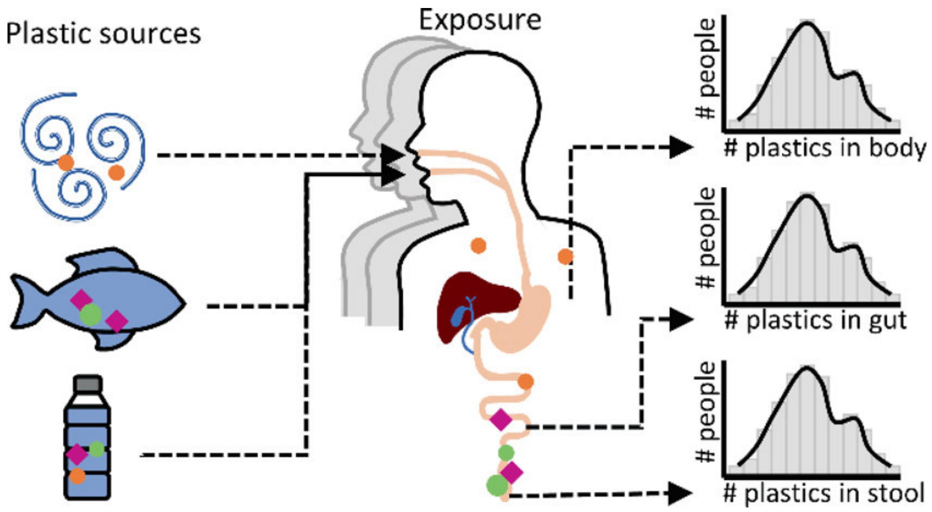


Fig. 24. Mohamed Nor *et al.* (2021) predicted lifetime exposure of children and adults to microplastics. Source: Mohamed Nor *et al.* (2021)

The **liver** is one of the first organs that ingested nanoplastics will encounter. Banerjee *et al.* (2022) studied the interactions of 50-500 nm PS nanoparticles and human liver cells. Smaller particles were more readily internalised and had greater toxic effects, however larger particles also induced cell death and inflammatory response. The researchers emphasise that it is difficult to determine environmentally relevant doses, and clearance mechanisms are not well understood, so the length of time particles remain in systemic circulation – and the length of organ exposure – is not known.

"A comprehensive MP/NP dose response study using more exposure time points, particle sizes, various polymeric composition, and in cells from different organs is necessary to understand the impact of MP/NP exposure more clearly on human health"

Banerjee *et al.*, 2022

Inhalation of aiborne nanoplastics must also occur. In an experiment by Xu *et al.* (2019), human **lung** epithelial cells exposed to 25 nm and 70 nm PS nanoplastics suffered negative effects; however, when Meindl *et al.* (2021) exposed human lung cells to 20 and 200 nm PS nanoparticles for 28 days, they found that healthy respiratory cells adapted to low levels of repeated exposure. Rubio, Marcos and Hernández (2019) assert that nanoplastics should be captured by mucus in the respiratory system; whether scientists will find nanoplastics in human lung samples, as microplastics have been, remains to be seen, however Fournier *et al.* (2020) demonstrated in an animal study that maternal pulmonary exposure to nanoplastics led to nanoplastics in foetal tissues.

Given the expected chronic exposure to nanoplastics in the environment, it is important to investigate whether they may accumulate in human cells. After exposing stem cells to PS nanoplastics for 48 hours, Jeong *et al.* (2022) found "excessive amounts" were internalised, leading to growth inhibition and reduced renewal of cells. They explained that exocytosis may have been hindered by the tightness of cell colonies. In terms of methodology, they emphasise that human induced pluripotent stem cells are an excellent material for investigating long-term toxicity – due to the fact that these cells self-renew – and effects on foetal development. In contrast to many similar studies, they also tested control materials (silica nanoparticles and PE microplastics), concluding that the effect of nanoplastics on human embryonic cells merits further investigation. A recent review of in vitro intestinal models of nanoplastic health effects, suggested future hazard assessments could benefit from co cultures of more than one human cell line – as well as organ on a chip models – as a more reliable insight into doses that trigger biological effects (Bredeck *et al.*, 2022).

the large-scale intracellular accumulation … suggests the possibility of causing unexpected alterations in human embryogenesis …

Jeong *et al.*, 2022

Other areas that currently being investigated include nanoplastic interaction with elements of the immune system – which act as first-line defence against foreign substances in the intestinal tract, lungs and liver, for example – and effects on cancer cells. Nano-PVC and PS have been found to induce inflammatory cytokine release in monocytes (immune cells in blood; Weber *et al.*, 2022); another experiment showed nano-PS impaired lipid metabolism in macrophages (Florance *et al.*, 2022) – but the big picture with regards to nanoplastics and immune function has yet to be seen. Likewise, some studies show that nano-PS may exacerbate cell proliferation in cancers, but evidence is still being gathered (Barguilla *et al.*, 2022).

Box 14: Does research reliably indicate human health risks?

While many studies are now being published that find negative impacts on human cells and animal models (Xu *et al.* 2022), it must also be noted that there is still much work to be done on reproducible findings and robust methodology. For example, concentrations and exposure times used in experiments vary greatly.

Leakage of fluorescent stains can distort results, as can solvents in which nanoparticles are dissolved (Stock *et al.*, 2022). Bulky, hydrophobic flurophores themselves may influence in vivo fate of particles. Keinänen *et al.* (2022) have proposed radiolabelling of nanoplastics and positron emission tomography as an alternative way of tracking particles in mammals. Their initial work found that the majority of PS nanoparticles ingested by mice were not absorbed, but were eliminated within 48 hours. However, they only administered a single dose, which does not reflect real world, continuous exposure.

[N]anoplastics cannot be considered one homogenous entity when assessing their health implications and the use of spherical polystyrene nanoplastics may underestimate their inflammatory effects

Weber *et al.*, 2022

Yan et al (2022) found 15 types of plastic in faeces, dominated by PET and polyacrylamide, yet the majority of toxicological studies still use polystyrene – which accounts for only about 7% of plastic production. Busch *et al.* (2021) found that PVC nanoplastic was more toxic to inflamed liver cells than PS and other work indicates that PET, nylon and polyacrylonitrile had different inflammatory effects compared to PS (Busch *et al.*, 2022). More relevant sample material must be made available; for example, a new way to obtain PET nanoplastics from plastic bottles has recently been presented (Villacorta *et al.*, 2022). Non-spherical nanoplastics should also be studied.

In their review of studies on the human health impacts of micro- and nanoplastics, Xu *et al.* (2022) found that only 16 of 133 studies stated the health impacts were not of concern. The consensus is that nanoplastics must be treated with caution, even if we are still unsure of their effects on human health.

Box 15: Nanoplastics and the brain

An ongoing EU funded study called 'Nanoglia' is examining the impact that nanoplastic particles in the human brain may have on neurological functioning. The researchers note that nanoplastics can translocate from the gut to the lymph and circulatory systems in mammals, also crossing the blood-brain barrier (EC, 2021). The Nanoglia project seeks to understand the impact of the nanoplastic particles on the brain's immune cells – microglia – which engulf these plastic particles. These brain cells are important for neuronal homeostasis, and may be activated by nanoplastic reaching the brain. The team will use an animal brain model, to understand how this activation of microglial cells by nanoplastics, could affect foetal brain development –with respect to potential cellular, molecular and behavioural changes.

It has been shown that nanoplastics cause behavioural disorders in fish (Sökmen *et al.*, 2020), and thus may also represent a risk for human health, in particular for brain function. However, the long-term bioavailability and toxicity of nanoplastics in the brain are unknown. Microglia, as the main neuroimmune cells of the brain, have a defence function – needed during inflammatory conditions – but also constantly sense, and respond to environmental changes, to maintain neuronal homeostasis. This places microglia at the interface between normal and abnormal brain development, and function – shown by a recent discovery that chronic microglial activation causes neurodegeneration (EC, 2021). When microglia internalise nanoplastics in the brain, this might lead to their acute or chronic activation, triggering neurological disorders (*ibid*.).

The Nanoglia project team hope to understand how nanoplastics trigger microglial activation during embryogenesis and postnatal stages – alongside establishing whether this immune activation leads to permanent changes in brain development and function. These mechanistic insights into microglia and nanoplastics in rats may elucidate environmentally triggered pathogenesis of neurological disorders in humans.

"Human brain digital illustration (©Shutterstock, photo by Yurchanka Siarhei)"

5.3 Risk assessment

As outlined above, research into potential human health impacts of nanoplastics is underway. Despite inconclusive evidence of impacts in relation to real world exposure, and in light of concerns regarding toxic chemical additives leaching from ingested nanoplastics, many believe immediate action should be taken to ensure humans are not exposed to levels of nanoplastics that may be hazardous to health. The extent to which micro- and nanoplastics are present in the environment, drinking water and seafood, however, poses challenges that are not easily addressed by standard risk analysis (EASAC, 2020).

In nanoplastic risk assessment, it is key to consider:

- Should we apply the Precautionary Principle?

An important issue is the 'precautionary principle', in relation to both negative effects of nanoplastics on the environment and potential human health impacts (Table 6). Under the precautionary approach, risk management is based on fragmentary evidence but strong indicators of negative impacts. Many highlight the co-benefits of taking this approach, as summarised by Martin Wagner in his debate over the risks of microplastics with Thomas Backhaus: "Based on my values, I favor a precautionary approach to microplastics, not because I consider them doomsday devices but because I believe in positive change" (Wagner & Backhaus, 2019).

- Early action can be most cost effective

ECHA (2020) notes that as microplastics (and nanoplastics) are extremely persistent, based on the 'option value theory of resource economics', it is appropriate to take cost effective action now – despite uncertainties. The costs of inaction on nanoplastics require further exploration.

- Heed early warning signs

The European Environment Agency proposed early warning signs, to be taken into account by regulators when considering materials and substances; these are highly relevant for the design and regulation of polymeric materials (Paulsen *et al.*, 2021).

Table 6. Comparing an evidence based and precautionary principle approach to microplastics, also applicable to nanoplastics. (Source: Backhaus and Wagner, 2018).

	Strictly evidence-based approach	Precautionary approach
Arguments in favour	Insufficient knowledge • Low exposure (on current estimates) • Low toxicity (on current knowledge) • Presence of natural particles at higher levels • Likelihood of negative impacts low	Sufficient knowledge • Ubiquity • Persistence • Mobility in food web • Increasing emissions • Part of microplastics problem where sufficient knowledge on impacts exists • Existence of unknown, negative impacts
Actions in favour	• Identify knowledge gaps • Perform more research filling these gaps • Take risk decision • Depending on outcome: develop and implement risk management measures	• Take risk decision • Develop and implement risk management measures based on fragmentary knowledge • Perform research into the effectiveness and efficiency of these measures • Refine measures
Advantages	• Avoids inefficient risk management measures • Avoids unnecessary opportunity and unintended externality costs • Avoids regrettable substitutions • **Reduces cost of action**	• Early action avoids negative impacts later • Motivates positive and economic change (vision of better society) • Fosters technological and societal innovation • **Reduces cost of inaction, induces change**

Risks from plastic, including nanoplastic, are not only related to health. Marine litter also impacts negatively on human welfare, affecting fisheries, tourism and aquaculture, and often brings losses to communities. Smaller-scale marine plastic litter causes chemical and microbial transfer when ingested, acts as a transporter of biota, and can change species assemblages (JRC, 2016; EASAC, 2020).

Nanoplastics could carry chemicals into the marine environment, as well as adsorbing trace contaminants present in the environment onto their surface – concentrating them – so they may have a toxicological impact when ingested. In a lab-based study, unidentified components of common consumer plastics (PP, LPDE, PS and PLA) had a number of toxic effects (EASAC, 2020). The EASAC report (2020) lists 906 chemicals 'probably' associated with plastics, including 63 associated with human health hazards, 68 with environmental hazards, and seven classified in the EU as persistent, bio-accumulative and toxic. Frond *et al.* (2018) calculated that 20 of these chemicals would amount to 190 tonnes in the environment, and that microplastics in coastal areas (which degrade to nanoplastics) were associated with high level of PCBs in the marine environment – showing how small plastic particles can be a route of toxic chemicals into the environment. A more recent review reportedmore than 6 000 chemicals in plastics, including 1 518 plastic-related chemicals of concern, which the authors suggest should be substituted for safer alternatives (Aurisano, Weber and Fantke, 2021). The authors highlight the need for a global and overarching regulatory framework for plastics and related chemicals, in support of a circular economy for plastics and of target 12.4 of the UN Sustainable Development Goals.

The research literature has established the presence of microplastic particles in food destined for human consumption, however, a similar level of studies investigating nanoplastics in food items is not yet available. Nanoplastic particles have been found in some crop plants grown in polytunnels, as well as in fish and shellfish such as scallops (Larue *et al.*, 2021; Al-Sid-Cheikh *et al.*, 2018). Additionally, as microplastic particles are known to degrade to nanoplastics when ingested by earthworms, krill and other organisms (see section 3.2), it is not unreasonable to conclude this might also occur in humans. Indeed, potential biotransformation of plastics in human digestion has recently been demonstrated (Tamargo *et al.*, 2022). Evidence on which to base a risk assessment of human health impacts of nanoplastics per se is currently insufficient, but the potential impacts, combined with problems related to macro- and microplastic pollution, strongly suggest formal risk assessment for nanoplastic exposure needs to be developed. As the body of literature on nanoplastic toxicity grows, such assessment will in future be able to draw on frameworks such as the ‘adverse outcome pathways’ (AOP) approach to classifying evidence on the hazards of substances including nanoplastics (Jeong & Choi, 2019; Hu & Palić, 2020).

To date, only four papers have been published about the estimation of human intake of microplastics, consistently concluding that the data available in the literature is hard to compare, incomplete and insufficient for a reliable assessment of MP ingestion. The assessment of the food safety risk related to MP/NP contamination is, consequently, impeded

Vitali *et al.*, 2022

5.4 Biomonitoring of plastic-associated chemicals and metabolites

Although there is a lack of evidence upon which to formulate a risk assessment of nanoplastics for human health, research has found chemicals associated with plastics (including nanoplastics) in the human body. Human biomonitoring involves measuring concentrations of environmental contaminants, and/or their metabolites, in human tissues or body fluids, such as blood, amniotic fluid, breast milk, saliva, hair or urine sources. Biomonitoring is the gold standard in assessing the health risks of environmental exposures, because it provides an integrated measure of an individual’s exposure to contaminants from many sources (Royal Society of Chemistry, 2019).

For some chemicals, their widespread presence in the general population at concentrations capable of causing harm – in animal models – has raised public health concerns. However, it should be noted that although biomonitoring is useful in identifying plastic-related chemicals and their metabolites in the human body, it cannot be inferred that the source of these is nanoplastics. Additionally, any data for regulatory risk assessment must meet quality standards.

Biomonitoring has proved some chemicals used in the manufacture of plastics are present in the human population. For example, the American, ‘National Health and Nutrition Examination Survey’

(NHANES)[23] is one of the most comprehensive human biomonitoring programs yet undertaken. NHANES reports on several chemicals associated with the use or production of plastics, including bisphenol A (BPA), phthalates, styrene, acrylamide, triclosan and brominated flame retardants, and their concentrations in the general population. In the EU, the H2020 European Human Biomonitoring Initiative (HBM4EU)[24] coordinates work in this field to provide evidence for chemical policy making, with bisphenols and phthalates among its list of priority substances. Studies under this initiative are ongoing, but results so far have reported average BPA levels of 1.78-1.97 µg/L in human urine, for example (HBM4EU, 2020) – well under guidance values of 230 µg/L (adults) and 135 µg/L (children) urinary total BPA derived from toxicological data (Ougier *et al.*, 2021). European populations are estimated to have average daily intakes of BPA of 0.05 µg per kg of bodyweight – a value much lower that the tolerable daily intake set by EFSA, 4 µg per kg of bodyweight. The results do not indicate how much BPA exposure may derive from nanoplastics.

5.5 Nanoplastics in the food chain

Microplastics have been detected in beer, honey, salt, sugar, fish, shrimps and bivalves, but there is currently little data regarding the presence of nanoplastics in samples of commercially available foods on the market. However, nanoplastics have been detected in lab-based studies of organisms, some of which are species which can be produced commercially as foods, such as crop plants, shellfish and fish (Larue *et al.*, 2020; Shen *et al.*, 2019; Al-Sid-Cheikh *et al.*, 2018). Meanwhile, data is just emerging on the volume of nanoplastics that may be released from products that come into contact with food and drink: Hernandez *et al.* (2019) found that plastic teabags release billions of nanoplastic particles into the drink; Zangmeister *et al.*, (2022) report the release of trillions of sub-100 nm particles from LDPE-lined cups.

A more exhaustive and trustworthy insight on MP and NP pollution along the food chain is fundamental to inform decision-makers towards the institution of strategies and infrastructures for the monitoring of those contaminants and for the setting of legal safety limits

Vitali *et al.*, 2022

In the study looking at microplastics in beer, granular material aside from fibres of plastic and microplastic were present, but couldn't be distinguished as either sand or nanoplastics, as spectroscopic analysis was not used in the study. It is likely – as microplastics degrade to nanoplastics – that both types of particle are present in food that are consumed by humans (Yee *et al.*, 2021; Liebezeit and Liebezeit, 2014).

In a study using Fourier transform infrared spectroscopy on tap, bottled and spring water – all these sources were found to contain microplastic particles (Kosuth *et al.*, 2018). Over 80% of samples of tap water collected from 159 worldwide sources contained microplastics, whilst 93% of samples, from 11 different brands of bottled water contained microplastics. From figures of average microplastic particles in different food and drink types, it has been estimated that humans consume around 39 000 to 52 000 microplastic particles per year – more including inhalation of microplastic particles: 74 000 to 121 000 particles per year (Yee, *et al.*, 2021). Data concerning nanoplastics are not yet available mainly due to a lack of reliable quantification techniques (Yee, *et al.*, 2021; EASAC, 2020). Yet, it seems probable that they will be present at higher concentrations than microplastic particles in water and the air (Frehland *et al.*, 2020).

23 http://www.cdc.gov/nchs/nhanes.htm
24 https://www.hbm4eu.eu/

Vitali *et al.* (2022) examined 136 studies on micro and nanoplastics in food, including marine organisms considered as food which were sampled in the field (Figure 25). Microplastic contamination of seafood was widely found, however they noted that about half of the studies examined mussels. Mussels for human consumption are usually produced in dedicated farms and depurated before going to market, therefore wild mussels are not relevant for food safety assessment. Although one study identified microplastic in chicken, it did not prove presence in chicken muscles. The reviewers note that the assessment of micro and nanoplastic contamination in animal products calls for methods able to digest protein and fat rich animal tissues, and techniques for accurately quantifying particles small enough to pass the gut barrier into the animal's edible tissues.

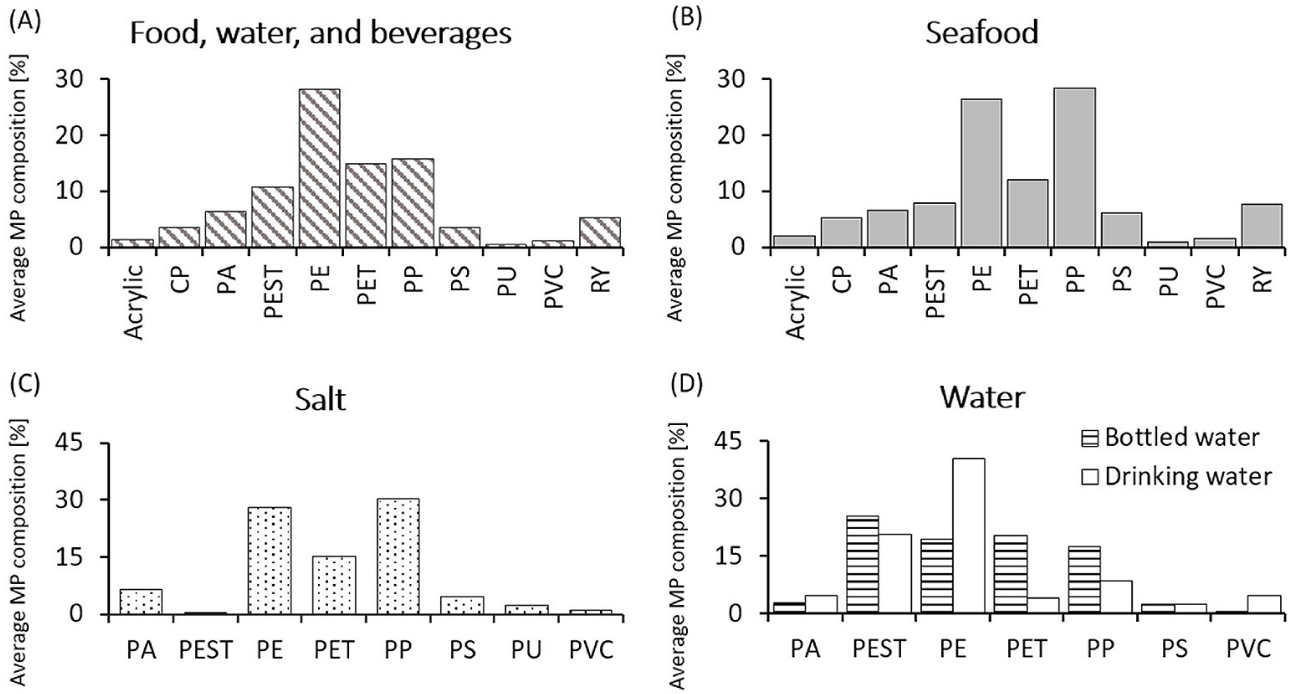


Fig. 25. Average chemical composition of microplastics isolated from (A) food, water and beverages; (B) seafood; (C) salt; and (D) water. CP, Cellophane; PA, polyamide; PEST, Polyester; PE, Polyethylene; PET, Polyethylene terephthalate; PP, Polypropylene; PS, Polystyrene; PU, Polyurethane; PVC, Polyvinylchloride; RY, Rayon. Source: Vitali *et al.* (2022)

Micro and nanoplastics in crops have received very little attention, though nanoplastics can penetrate roots, stems and leaves (see section 3.6). Of carrots, lettuce, broccoli, potato, apples and pears, sampled from a market in Catania, Italy, peeled apples were the most contaminated with microplastics, according to Conti et al (2020) – however there are no other studies with comparable data, nor data on nanoplastics in crops. Vitali *et al.* (2022) therefore recommend a thorough investigation of the nanoplastic content in fruit and vegetables, to assess the food safety risk linked to crops and agricultural soil contamination. However, until reliable, high-throughput methods for analysing plastic nanoparticles are available – along with standardised, comparable reporting – such assessment remains a challenge.

6. Summary

This Future Brief presents the current science on nanoplastics: their detection, assessment and monitoring; their impacts in the environment, ecotoxicity, and environmental fate; and their potential impacts on human health. In 2022, nanoplastics research is a very fast-moving area. New techniques and methods are currently in development to detect, identify and analyse nanoplastics and their impacts, in the environment and in organisms. Methods of interest currently include various advanced techniques in light scattering and chemical identification. Harmonised protocols and reference materials are needed as well as advances in isolation, identification and field sampling.

However, there is much we do know already. Despite the challenges of measurement at a nano-scale, we know that microplastics degrade into nanoplastics, so we know they are there, even if we cannot yet detect them. There is abundant evidence of significant detrimental effects of microplastics – and of plastics at any scale, with plastics and their additives being found frequently in the environment and in organisms.

Plastic is now ubiquitous in the environment, and we, and Earth's other lifeforms, are currently ingesting and inhaling nanoplastics at unknown concentrations. Evidence has emerged that nanoplastics are small enough to cross biological membranes, and to cross into the blood and brain, which may have implications for human health. The effects of nanoplastics once they enter organisms is less well studied – although there is some evidence of lethal and sub-lethal effects in various species, of various plastic types, at both high concentrations and levels that may be found in the environment. To help advance research in this area, projects under the European Cluster on Health Impacts of Micro- and Nanoplastics will be looking at nanoplastics' impacts on health over the next few years.

It is also understood that interaction with other organic matter in the environment creates 'eco-coronas' – organic coatings, which can modulate nanoplastics' charge, chemistry, behaviour and toxicity. Since there are still challenges when studying nanoplastics in the environment (most research has been conducted in the laboratory), understanding of the implications of eco-coronas for nanoplastics risk assessment in naturalistic scenarios is as yet embryonic. Science is only beginning to understand where nanoplastics are, and what their characteristics are – and is only scratching the surface of understanding of interactions at the nano-scale.

We also know that deliberate regulatory response is lagging behind the pace of nanoplastic release and abundance in the environment. Recent and ongoing efforts to regulate intentionally added microplastics (i.e. ECHA opinion developed in 2019) have run into debate about the most appropriate lower size limit for classifying microplastics. This debate has been mainly inspired by the difficulty of enforcement when detection is so challenging at smaller sizes. **However, scientific evidence does not support a cut-off point for the environmental and human *effects* of plastics at a particular size limit; indeed, while the relation between physico-chemical properties and (adverse) biological effects appears complex and evades simple threshold descriptions, the smaller the plastic particles, the more likely they can cross biological membranes and the more thoroughly they can permeate organisms.** It follows, perhaps, that a combination of inclusive categorisation (where 'microplastics' also include 'nanoplastics'), as now stands in the draft Commission proposal of 30 August 2022, placing the burden of responsibility on the producer, would avoid leaving a gaping hole in the regulatory regime for intentionally added nanoplastics, and would reflect what we do know so far.

However, the release of secondary ('unintentional', degraded plastics) represents by far the greater volume of plastic in the environment. Most of the research on environmental fate of micro- and nanoplastics has focused on water pollution. Pollution of soil and of the air has received less attention, and so these are areas where more research efforts could usefully be focused in coming years. The aggregation, concentrations, movement, bioavailability and bioaccumulation of nanoplastics, as well as their eventual endpoints and lifecycle durations, most likely differ significantly according to the particular plastic material and its characteristics and additives. We also know that some plastics and plastic additives are more harmful than others. This gives one clear route for regulation: regulating on the basis of particular plastics and materials, whatever their size.

The plastics industry is a valuable part of the European economy with a complex, international value chain, employing 1.5 million people and generating a turnover of €340 billion in 2015 (EC, 2018). However, it is both inevitable and urgent that the value and disposal chains of plastic materials are significantly rewritten by plastic producers and other decision-makers as Europe transitions to a more circular and environmentally conscious economy. It is clear from the research presented here that the full lifecycle of plastics – from 'cradle' to 'grave' – is not complete when we can no longer see the plastic: plastics continue to have environmental effects far past the point they become invisible. Failure to control these invisible pollutants, which are already permeating the terrestrial, atmospheric, aquatic and biological environments, contributes to an escalating hazard, the full proportions of which we may not fully understand until it is too late.

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