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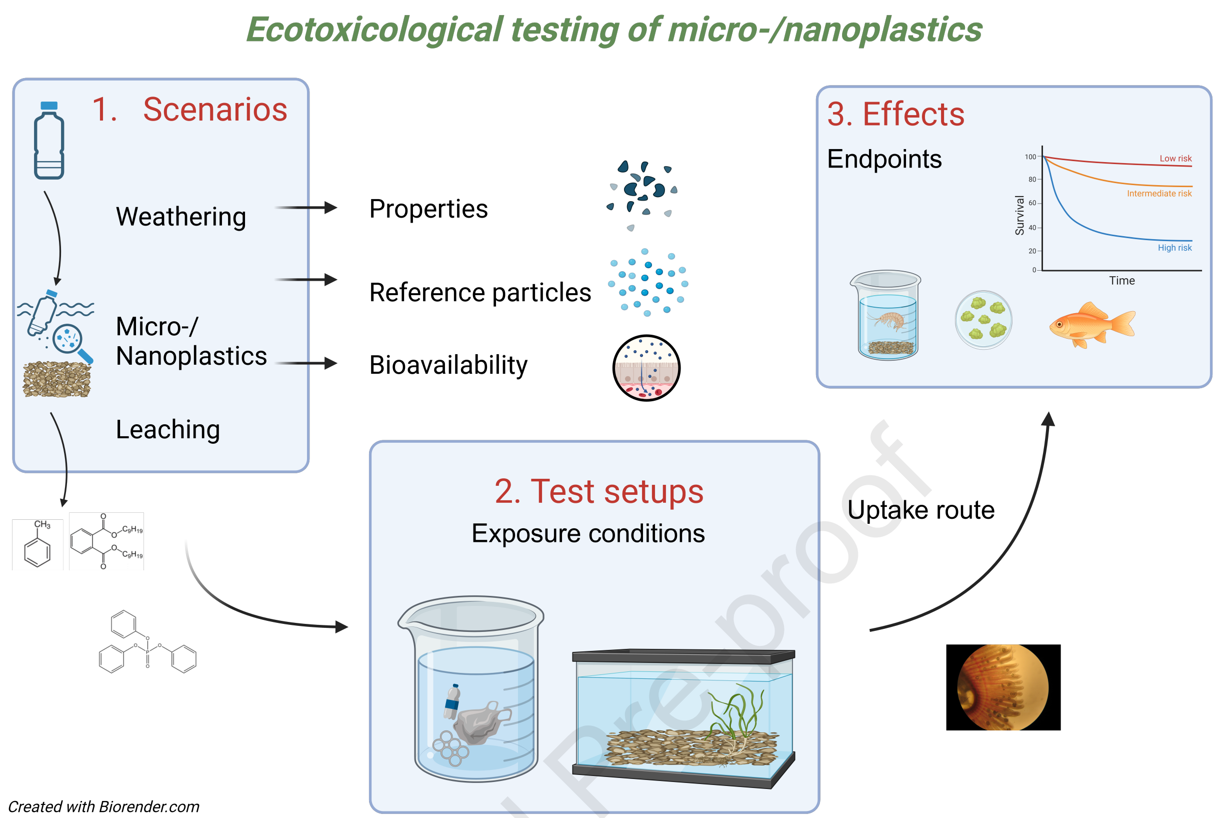
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Review

Towards harmonized ecotoxicological effect assessment of micro- and nanoplastics in aquatic systems

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Abstract

Micro- and nanoplastics are globally important environmental pollutants. Although research in this field is continuously improving, there are a number of uncertainties, inconsistencies and methodological challenges in the effect assessment of micro-nanoparticles in freshwater systems. The current understanding of adverse effects is partly biased by the use of non-relevant particle types, unsuitable test setups and environmentally unrealistic dose metrics, which does not take into account realistic processes in particle uptake and consequent effects. Here we summarize the current state of the art by compiling the most recent research with the aim to highlight research gaps and further necessary steps towards more harmonized testing systems. In particular, ecotoxicological scenarios need to mirror environmentally realistic particle diversity and bioavailability. Harmonized test setups should include different uptake pathways, exposure and comparisons with natural reference particles. Effect assessments need to differentiate direct physical particle effects, such as lesions and toxicity caused by the polymer, from indirect effects, such as alterations of ambient environmental conditions by leaching, change of turbidity, food dilution and organisms' behavior. Implementation of these suggestions can contribute to harmonization and more effective, evidence-based assessments of the ecotoxicological effects of micro- and nanoplastics.

Keywords

Microplastics; Nanoplastics; Test Systems; Exposure Scenario; Effect Assessment; Environmental Pollution

1. Introduction

Plastic pollution in the environment is recognized a major irreversible global threat (Anbumani and Kakkar, 2018; MacLeod et al., 2021; Weis and Alava, 2023). Different types and amounts of plastics are found in all environmental compartments, including the atmosphere, arctic ice, soils, rivers, lakes and oceans as well as in biota, including humans (Allen et al., 2019; Azfaralariff et al., 2023; Bergmann et al., 2022; Koelmans et al., 2019; Kvale et al., 2020; Triebskorn et al., 2019). Reported plastic particle numbers in freshwater systems range from 10^{-2} to 10^{5} particles per m^3 (Triebskorn et al., 2019). Physical and chemical processes in the environment enable the fragmentation and transformation of plastics, which alters the transport and bioavailability (Su et al., 2022). These small-sized micro- and nanoplastics (MNPs) pose a risk to environmental and human health (Azfaralariff et al., 2023; Bucci et al., 2020; Strokal et al., 2023; Zolotova et al., 2022).

The scientific focus on MNPs has increased over the past 20 years (Klingelhöfer et al., 2020), which resulted in a better understanding of MNP emissions, transportation and risk assessment (Thompson et al., 2024). Improved sampling, separation techniques and analytical procedures resulted in a better characterization of dispersal, occurrence and quantification of MNPs. For example, whereas food and food packaging seemed to be the main source of MNPs in human intake, kitchen equipment used to prepare food turned out to be major source of MNPs as well (Snekkevik et al., 2024). However, environmental risk assessment still highly depends on harmonized or standardized procedures in detection and quantification as well as in determining effects, which is not yet achieved (Bao et al. 2024; Ivleva, 2021; Koelmans et al., 2022; SAPEA, 2019).

There is a substantial body of literature reporting effects of MNPs based on laboratory studies, which cover several taxonomic groups, investigate effects on lethal and sublethal endpoints and effects from molecular to food web level. However, it remains questionable if this contributes to a realistic effect and risk assessment, since many studies designs lack comparability, and there are uncertainties in environmental concentrations (Burns and Boxall, 2018; De Ruijter et al., 2020; Gouin et al., 2019; Latchere et al., 2021; Thornton Hampton et al., 2022). Although test systems play a significant role in the evaluation of chemicals, there are currently no established ecotoxicological standard protocols available for non-soluble particulate substances like MNPs. In contrast to soluble substances, the behavior and uptake of particulate substances in water depends on size, density, shape and particle type (Khan et al., 2017). In addition, there are deficits in scientifically based standardization and harmonization of detection procedures within an ecotoxicologically relevant size range in the low µm and nm range (Anger et al., 2018; Dris et al., 2018; Triebskorn et al., 2019; Ivleva et al., 2017; Wang et al., 2023b). A promising first step towards harmonization had been made in a protocol by Monikh et al (2023), who used OECD guidelines as a starting point for ecotoxicological testing. They focus on agglomeration and sedimentation rates, and this information is essential when a future protocol includes sediment layers, co-contamination, aging and biofouling.

MNPs of different polymer composition and aging disperse differently in the environment (Bergmann et al., 2022; Strokal et al., 2023; Su et al., 2022). Therefore, organisms inhabiting the affected habitats are exposed to a complex matrix of natural and plastic particles with diverse physical and chemical characteristics like size, shape, polymer type, additives, adsorbed chemicals or on-growing biofilm (Koelmans et al., 2022; Kooi and Koelmans, 2019; Rochman et al., 2019). Potential negative effects of MNPs and its additives on organisms, populations and biocenoses, as well as fluxes within food chains are not yet sufficiently characterized and understood, which is partly attributed to a lack of standardization and harmonization of testing systems (Weber et al., 2021).

In contrast to previous reviews, that focus on specific aspects within the field of MNP research (analytical procedures, QA/QC criteria, effect data and risk assessment (Anbumani and Kakkar, 2018; Bucci et al., 2020; Jacob et al., 2023; Kotta et al., 2022)), we present a conceptual framework of the key elements in MNP research. We therefore (1) highlight the existing challenges associated with MNP test systems, particularly those related to exposure scenarios, test systems and effect assessment (2) exemplarily discuss steps for harmonization and (3) recommend relevant methodological approaches for a more effective, mechanistic and evidence-based assessments of the ecotoxicological effects of MNP as an outlook.

2. Key elements of MNP testing

Testing strategies for ecotoxicological effect and risk assessment need to consider a range of key variables, as summarized in Figure 1. It is important to consider the interface between the test setup and the reaction of the receptor organism, which is mostly determined by organism specific traits (behavior, feeding type), bioavailability and uptake mechanisms, internal turnover and accumulation.

2.1 Exposure characteristics

2.1.1 Particle characteristics and reference particles

To link MNP properties to toxicity, a detailed characterization of the particles and their behavior in the test system is needed (Brehm et al., 2023). Challenges are mostly related to different polymer types, particle shapes that vary from spheres to fibers, and the use of appropriate reference particles.

The current analytical capacities allow the *a priori* and the *posteriori* determination of particle characteristics (Anger et al., 2018; Ivleva et al., 2023; Primpke et al., 2020). Suggestions on reporting and quality criteria are also available, but not substantially considered, e.g. (Connors et al., 2017; De Ruijter et al., 2020; Koelmans et al., 2019; Kögel et al., 2020; Monikh et al. 2023; Zink and Pyle, 2023). Certain polymer types are under-represented in laboratory studies, e.g. polypropylene (PP), polyester and polyamide (PA) particles, despite their widespread detection in field-based studies (Botterell et al., 2019; De Sá et al., 2018). Consequently, there is a mismatch between MNPs in the environment and those in laboratory experiments (Anbumani and Kakkar, 2018; Burns and Boxall, 2018; Kukkola et al., 2021). This results in a mismatch between the investigated mechanisms of action and ecotoxicological effects (Heinrich et al., 2020; Samadi et al., 2022). Laboratory studies most commonly used polystyrene (PS) and polyethylene (PE) (Haegerbaeumer et al., 2019; Lusher, 2015) (Table 1 for examples), whereas for sediments, high proportions of polyvinylchloride (PVC), PA and polyesters have been reported (Browne et al., 2013; Claessens et al., 2011; Lee et al., 2013). Most common shapes in the environment are beads, fragments and fibers, whereas spherical particles are the most investigated in aqueous studies (Haegerbaeumer et al., 2019; Lusher, 2015). Using commercially manufactured beads in effect studies has the additional disadvantage that often solvents, surfactants or biocides are used as stabilizers and to avoid fouling, which can highly influence the outcome (Heinlaan et al., 2020). Additionally, the majority of ecotoxicological assessments has been conducted with only one type of polymer and only one shape of particle, whereas in nature, the plastic items represent a broad spectrum of polymers and shapes or even heteroaggregates. No ideal reference particle has been agreed upon since the characteristics of inorganic natural particles like kaolin, clay minerals, quartz sand, or glass beads are not in accordance with the various characteristics of MNPs (Heinrich et al., 2020). Reference materials need to reflect the diversity of shapes, and sizes of MNPs found in the environment, as well as changes in porosity during the weathering process (Kefer et al., 2021).

Laboratory-based experiments using plastic particles collected in the environment are scarce (Latchere et al., 2021), presumably due to the high effort in collection and separation of the particles and therefore and therefore the impracticability of this approach (Su et al., 2022). It is furthermore problematic as effects on extraction and separation methods on particle characteristics need to be considered (Enders et al., 2020; Li et al., 2020). Alternatively, custom-made reference particle mixtures could be used, but the availability of suitable material which mimic real MNPs is limited (Ivleva, 2021). However, De Ruijter et al. (2023) developed processed environmentally relevant microplastic (ERMP) standard material that adheres to high-quality requirements (Table 1). Their ERMP was made from plastic items collected from natural sources and cryogenically milled to represent the diversity of microplastics. A stepwise protocol on the technical steps needed to produce MNPs is summarized in Monikh et al. (2023), which includes grinding and milling procedures and summarizes subsequent particle characterization methods. Parolini et al. (2024) describe a method including extrusion at elevated temperatures, which could be a promising approach to generate additivated and non-additivated particles from the same source material. Other approaches using ultrasonication and precipitation also generate a suite of more realistic reference particles (Alimi et al., 2022; Boettcher et al., 2023; De Ruijter et al., 2023; Kefer et al., 2022; Von der Esch et al., 2020). (Kefer et al., 2022) tested different methods to produce microplastics, which have been used in research on the toxicity of phenanthrene combined with MNPs on a freshwater amphipod species (Bartonitz et al., 2020), and in a study examining sample preparation methods for reproducibility and sensitivity in wastewater treatment effluent (Al-Azzawi et al., 2020).

2.1.2 Aging, weathering and biofouling of the MNPs

In the environment, MNPs weather via mechanical action, (photo)oxidative processes, biological degradation, and biological fouling (Browne et al., 2007; Cole et al., 2011; Kaiser et al., 2017; Ventura et al., 2024), which results in the modification of the surface and density (Duan et al., 2021; Lv et al., 2022; Ter Halle et al., 2017). Mechanical weathering will decrease particle size and increase roughness, whereas oxidative processes will make a plastic more brittle, due to changes in functional groups. Enzymes can hydrolyse plastics and biofouling influences their buoyancy. These processes lead to smaller particles, leaching, altered environmental transport and interaction with environmental chemicals (Al Harraq et al., 2022). Changes of surface structure and charge can lead to agglomerations with food, changing both particle and food uptake (Hanna et al., 2018) and influence the adsorption/desorption capacity of contaminants or additives (Bandow et al., 2017) and consequently uptake (Bråte et al., 2018; Fabra et al., 2021) and ecotoxicological effects (Moyal et al., 2023). This hampers the transferability of laboratory data to realistic field situations (Alimi et al., 2022).
During ageing, MNPs can be covered in a biofilm, providing a substrate for food (Figure 2) that attracts shredders (Qi et al., 2021). In that case, MNPs can cause negative effects through food dilution (Al-Azzawi et al., 2020), but can also act positively as a vector of nutritious biofilms. For instance, *Daphnia magna* preferentially ingests biofouled plastic, with consequent higher growth rates (Mazurais et al., 2015), compared to clean plastic (Polhill et al., 2022).

In addition to biological interaction effects, ageing also directly influences particle characteristics in terms of density (Kaiser et al., 2017), surface (Ji et al., 2024) and hydrophobicity (Ji et al., 2024; Kaiser et al., 2017; Kiki et al., 2022; Reineccius et al., 2023). Differences in surface roughness, crystallinity, surface functional groups and biofilm biomass affect the adsorption of organic molecules and heavy metals (Hu et al., 2024; Ji et al., 2024; Town et al., 2023), e.g. by enhancing the adsorption capacity for metal ions on the MNPs' surface (Qi et al., 2021). Oxidative degradation increases the water solubility of metabolites and alters their bioavailability (Lukas et al., 2024). Therefore, ageing and weathering of particles influences particle biotic interaction and toxicity, which needs to be considered in test strategies (Figure 2).

2.1.3 Exposure conditions in the test setup

Although the standard toxicity testing framework used for soluble chemicals enables to assess a large number of effects, it is not fully transferrable to particulate contaminants like MNPs (Khan et al., 2017). Test systems can be static, when the initial test condition is maintained over the test duration, semi-static, when the test solution is renewed partially during the test, or continuous, when the test solution is constantly renewed in a flow through setup. Each of these setups have advantages and disadvantages, mainly with respect to the homogeneity of particle distribution in the exposure medium and therefore bioavailability to the test organisms (Bour et al. 2021; Monikh et al. 2023). Testing of MNP requires adapting testing frameworks, using specific exposure designs and investigating alternative endpoints (Bour et al., 2021). A variety of exposure setups can be used, ranging from cell cultures to mesocosms, but it needs to be considered which parameters in the exposure system can be effectively controlled, such as flotation, settling or mixing of particles (Heinrich et al., 2020). This control depends on whether one wants to study mechanistic effects, by artificially enhancing MNP availability to the test organism, or higher-level community effects, by allowing environmentally realistic particle behavior in the test compartments (Figure 2), and it includes the adjustment of water chemistry, pH, temperature, and particle dosimetry and their respective verification (Figure 3).

The effect of different water matrices on the test substance, such as different media used for different test species, is often neglected. For instance, salinity of the test medium influences degradation rates of plastics (Reineccius et al., 2023) and consequently the physical and chemical properties of MNPs. In addition, ionic strength and pH influence the adsorption of metals (Qi et al., 2021) and the adsorption of organic pollutants (Junaid et al., 2023). As the salinity increases, the adsorption capacity of MNPs for organic molecules, such as norfloxacin (NOR), decreases (He et al., 2023). Therefore, the ionic composition should always be considered and reported for MNP exposure experiments.

The media pH can alter the zeta potential of MNP and the precipitation of metals. It consequently modifies the adsorption of metals (Sizochenko et al., 2021) and the adsorption of organic compounds on biofilms on MNP (Xu et al., 2018).

Exposure duration is particularly important for MNP weathering and MNP fluxes and needs to be adapted to the specific assessment goal. If acute responses are expected, the exposure duration typically ≤96 hours. Chronic exposures cover the life-span or reproductive cycle of a test organism. For MNP effect assessment, single-species tests are commonly used in an acute toxicity setup. Long-term effects on complex biotic communities in more realistic exposure scenarios are less common (Bour et al., 2021), but are of high interest to examine effects with higher ecological relevance (Haegerbaeumer et al., 2019). They bridge between standard laboratory tests and outdoor studies (Haegerbaeumer et al., 2016) and provide essential data for estimates of diversity loss in ecosystems, e.g. by using a Threshold Indicator Taxa Analysis (TITAN) (Li et al., 2023).

To test ecotoxicological effects of MNPs and additives, both short-term and long-term experiments are needed with knowledge on fluxes and retention times of MNPs is crucial determining exposure scenarios. One promising approach to assess the fate of MNPs is by performing mass balances in the water column and the sediment as suggested by Martínez-Pérez et al. (2024). The majority of MNP will cycle through organisms before reaching the sediment, increasing the likelihood of negative ecological effects and transfer in the food web (Gilfedder et al., 2023). MNPs >100 µm are found on the surface of sediment consisting of coarse silt and fine sand, while the smaller particles might infiltrate >10 cm into sediment. Therefore, the texture of sediment should always be reported along with values of MNP concentrations (Waldschläger and Schüttrumpf, 2020). In addition, particle size will decrease in long-term exposure due to weathering, which will increase their bioavailability, and should ideally be monitored.

2.1.4 Dosimetry

It is often difficult to directly compare the reported MNP concentrations/quantities of different studies (Haegerbaeumer et al., 2019; Karami, 2017; Van Cauwenberghe et al., 2015). The methodology and the reporting of effects after MNP exposure are often flawed by presenting only the nominal exposure concentration, without analytical validation (Figure 3). There is a striking discrepancy between high concentrations of smaller particles tested for toxicity and low concentrations of larger particles analyzed in the environment (Triebskorn et al., 2019). Field concentrations of MNPs are influenced by sampling techniques and thus does not accurately represent the actual concentrations in the field (Connors et al., 2017; Su et al., 2022). Bucci et al. (2020) determined that only 17% of the concentrations used in experimental studies have been found in nature, and that 80% of particle sizes used in experiments fall below the size range of the dominant fractions in environmental sampling. Even though the detection limits for small-scale plastic particles (<10 µm) have substantially improved in recent years, there is still a lack of a comprehensive view on the actual global distribution and concentration range (Ivleva et al., 2023). However, MNPs have been tested in concentrations several orders of magnitude higher than current known environmental concentrations, e.g. (Karami et al., 2017; Phuong et al., 2016). With respect to the assessment goals, testing of such high concentrations enables the determination of effect thresholds, but can only aid environmental risk assessment if low concentration ranges are covered as well.

The production of homogeneous aqueous suspensions of MNPs is challenging since material density and polarity of the surface greatly vary (Heinrich et al. 2020). Particles of sparingly soluble substances can form aggregates, resulting in inhomogeneous distribution and unpredictable exposure scenarios (Götz et al., 2021) (Figure 3). Moreover, guidance documents by the OECD on nanomaterial testing mentions particle adhesion to container walls as an additional problem to maintain exposure concentrations and therefore similar processes can be assumed for larger sized particles (OECD, 2022). Surfactants can partially solve this problem (Monikh et al., 2023), but using them will also include an extra toxicity parameter. Even though most often concentrations of MNPs are given (e.g. in mg/L or number of particles per liter), the poorly reported surface-to-volume ratio is at least equally important. Most commonly, particles of beads per liter is used but also g/L or mass % and even volume % is reported (Botterell et al., 2019). If sediment is included, the concentration is often expressed as mg particles per kg sediment (Table 1). Weathering will increase the number of particles per liter, but not change the mass. Adding a sediment layer will lead to a lower concentration of particles in the water phase, depending on their hydrophobicity. Round robins with and without sediment layers can increase the reliability of published concentrations. Further development of analytical methodologies and quality assurance will improve standard laboratory and higher-tier procedures (Gouin et al., 2019).

2.1.5 Bioavailability

The adjusted dose in the testing system is not necessarily the bioavailable fraction (Drago et al., 2020; Redondo-Hasselerharm et al., 2018) (Figure 3). The bioavailability of MNPs depends mainly on the particle behavior in the testing system, the behavior of the test organism, e.g. active or passive feeding, and the barrier function of interface epithelia. Particle shape determines the surface to volume ratio of the particles that influences both its uptake by organisms and the adsorption of chemicals or biofilms at the particle surface. For example, in marine zooplankton, *C. helgolandicus* ingests mostly fragments, *A. tonsa* mostly fibers and *H. gammarus* larvae mostly beads (Kooi and Koelmans, 2019). The feeding mechanism is the main interface between the external particle diversity and the organism, which is further influenced by the feeding strategy (McNeish et al., 2018; Porter et al., 2023; Scherer et al., 2017). Filter feeders, deposit feeders and planktonic suspension organisms are therefore considered the most susceptible to particle ingestion (GESAMP, 2015; Porter et al., 2023). Several key processes are important in influencing the bioavailability of particles in the testing system (Gouin et al., 2019), mostly particle–particle interactions, such as aggregation and agglomeration, biofouling as well as floatation and sedimentation. Due to their hydrophobic nature and often higher densities, more MNPs are associated with the sediment layer compared to a free floatation in the water column (Koelmans et al., 2019).

The current approaches are based on standard procedures to test chemicals that are dissolved in the exposure medium, the so-called test or external concentration. The internal exposure concentration that actually causes toxicity (e.g. by receptor inhibition at the target site) is determined by uptake route, which is mostly driven by species-specific behavior, barrier functions of interface epithelia and internal toxicokinetics of the test substance. Observed effects can only be related to exposure when internal concentrations are correctly estimated. In this context, the quantification of uptake and excretion kinetics becomes mandatory. Since the determination of the relative influence of the various routes of uptake in these multiphase systems is difficult, the approach to estimate bioavailability by measuring organism body burdens seems to be most promising. Consequently, more systematic assessments are necessary to understand the relationship of encounter probability and uptake, as well as the internal kinetics to define the real inner exposure (Koelmans et al., 2016; Rafa et al., 2024).

2.1.9 Leaching additives and interactions with other contaminants

Polymer particles are known to be a source of additives and to interact with environmental chemicals. This increases the complexity in test setups, as mixture effects and particle-chemical-biota interactions need to be considered (Koelmans et al., 2016; Rafa et al., 2024) (Figure 2). Often the chemical speciation in the exposure medium is not characterized and thus the extent to which the organic pollutants are associated with the plastic particles remains unknown. Presumably due to analytical constraints it is often not evident whether the eventual body burden of organic pollutants corresponds to that which has been released from ingested plastic particles or rather represents the sum of the released and remaining particle-bound compounds (Town and Van Leeuwen, 2020; Town et al., 2018). The effects of additives and associated compounds are not always straightforward. Mixture effects depend on the chemical speciation and consequent bioavailability of metals and plastics. For example, a combination of MNPs and metals can cause antagonistic or synergistic toxicity. MNPs promoted metal uptake in the shoot (Chen et al., 2024), which shows they can enhance MNP toxicity. However, in a different study, polystyrene microplastic reduced Cadmium availability to Dandelion plants (Li et al., 2024). MNPs also interact with pesticides through adsorption and desorption processes, which require additional consideration due to the role this plays in changing the environmental transportation, fate, bioavailability, and ecotoxicity of both plastic particles and organic chemicals (Junaid et al., 2023).

Plastics contain a wide variety of additives, like plasticizers, salts, pigments, stabilizers and flame-retardants, which can be toxic for aquatic organisms (Beggel et al., 2024). As aging promotes the internal chain breaking of MNPs and the increase of specific surface area, it stimulates the release of additives that can disrupt a variety of biological processes in organisms (Luo et al., 2022). No studies are known that examine the extent to which plastics additives in sediments are adsorbed to MNPs as opposed to the sediment itself (Onoja et al., 2022), which has potentially implications for the bioavailability of such additives.

The leaching of additives in plastic may induce relevant hazards. These additives may either be associated with the plastic from the production process (e.g. intentionally added compounds, such as UV stabilizers or non-intentionally added substances and byproducts), or sorb to the particles once in the environment (e.g. persistent organic pollutants (POPs), via the vector effect) (Mitrano and Wohlleben, 2020; Gandara e Silva et al., 2016; Schrank et al., 2019). However, De Ruijter et al. (2020) concluded that 73% of published studies did not mention the potential of chemical additives to influence the observed adverse effects, which makes it difficult to distinguish the toxicity of the particles from the toxicity of the released additives (Brehm et al., 2023). To approach this challenge, it would be necessary to identify all compounds in the used plastics or compare the effects between specifically designed particles with and without additives.

2.2 Effect assessment

2.2.1 Uptake

A broad variety of aquatic organisms is used to test for ecotoxicological effects of MNP (Table 1). The main focus is often set on small planktonic crustaceans such as *Daphnia,* whereas key organisms such as aquatic primary producers (Samadi et al., 2022) and riverine species (Feiner et al., 2016) are underrepresented. MNP uptake depends on the type of feeding, so a variety of different species need to be tested, such as filter feeders (mussels), scrapers (insects, snails) and shredders (amphipods) (Figure 3). In aquatic ecosystems, filter feeders such as mussels, are directly exposed to the surrounding medium during food uptake and are therefore particularly vulnerable to MNPs (Kuehr et al., 2022). They typically do not distinguish between natural and MNP particles, and therefore do not cease their filtration during exposure (Ferreira-Rodríguez et al., 2023; Hartmann et al., 2016; Lummer et al., 2016). Since they do not activate their defensive behavior, they ingest particulate contaminants regardless of their chemical composition (Brehm et al., 2022). For other feeding types, oral uptake, dermal adsorption and diffusion uptake can act simultaneously for solutes, whereas the uptake of particulates is more limited to oral uptake only (Kuehr et al., 2022, 2020). Current knowledge about absorption, distribution, metabolism, and excretion of MNPs by organisms is limited by the methods and experimental designs that do not allow distinguish uptakes routes (MacLeod et al., 2021), especially for MNPs that carry other pollutants (Liu et al., 2023). Uptake can take place orally, via contact (dermal, or when water flushes through the gills) or injected into the animal for research purpose. The uptake pathways of particles and sparingly soluble substances must be considered in ecotoxicological research because exposure from the water column is negligible for non-filter-feeding organisms, and guidelines must be updated accordingly (Götz et al., 2021).

Part of the (eco)toxicological effects of MNPs may be anticipated as a direct consequence of the ingestion by filter feeders and predators, thereby competing with food. This likely results in a reduction of the fraction of digestible matter within the gastrointestinal tract and will possibly impair the nutritional status of organisms (Heinrich et al., 2020). In addition, particularly for filter-feeders, metals released from ingested plastic particles may be higher than metal uptake via the water phase (Town et al., 2018). There are indications of non-digested effects as well, such as bioadhesion of MNP to aquatic animals and macrophytes and blockage of gills in fish (Kalčíková, 2023).

Internalized MNPs are able to cross the gut barrier in fish, which has been shown using palladium-labelled NMPs (Clark et al., 2022, 2023), which is a promising methodology to study vector effects. Whereas the MNPs accumulated will possibly be transferred to the predators while feeding, the fate of that transferred MNPs cannot be determined from the available information to date as it is not possible to analyze whether the particle inside the body of an organism occurred by trophic transfer. Therefore, Castro-Castellon et al. (2022) call for more studies on trophic transfers across organisms with differing time scales of life histories and metabolic rate.

Bioaccumulation of MNPs for a possible vehicle effect should be interpreted in relation to ingestion/egestion rates in the animal, as well as the extent of chemicals adsorbed to the MNPs and the possible leaching of chemicals in the plastics. Animals have different feeding habits, so the extent of ingestion can vary greatly. Consequently, the time MNPs are retained inside an animal can influence the extent of desorption/adsorption processes. Especially when uptake rates are higher than elimination rates, assessing these two processes will give more insight into bioaccumulation (Bao et al. 2024). MNP fibers for example, can be retained longer in the digestive tract because they can entangle and get stuck to the walls of the digestive tract more easily than beads (Eder et al., 2021).

2.2.2 Toxicological endpoints & mode of action

Adverse effects of MNP have been reported for a wide variety of species and ecotoxicological endpoints (Table 1), which has been systematically summarized elsewhere (Ahmed et al., 2023; Anbumani and Kakkar, 2018; De Sá et al., 2018; Gaylarde et al., 2021; Haegerbaeumer et al., 2019;

Koelmans et al., 2022; Pelegrini et al., 2023; Pisani et al., 2022; Prokić et al., 2019; Weis and Alava, 2023). The majority of the studies used laboratory-based single-species tests, applying whole organism endpoints such as mortality, feeding rates, behavior and growth as well as toxicological endpoints. Ideally, effect assessment integrates multiple levels of biological organization, from mechanistic studies using "omics" techniques (Beggel et al. 2011; Connon et al. 2012; Eliso et al., 2024) to effects on communities and food webs (De Sá et al., 2018). The four most relevant effect mechanisms are: food dilution, internal physical damage, external physical damage and oxidative stress (Koelmans et al., 2022). MNP can induce both physical effects and chemical toxicity, which needs to be distinguished in terms of mode of action and overall adverse outcome. Physical effects typically occur when particles attach to outer or inner epithelia and cause physical injuries by abrasion or inflammation in the digestive tract (Mbugani et al., 2022a, 2022b). Negative effects can also be associated with a blockage or internalization at adsorptive surfaces such as gills and gut epithelia. PS particles found in the gills, intestines, and livers of fish can promote fatty acid degeneration, alter the composition of the intestinal microbiome, interfere with metabolism, and induce changes in gene expression (Zolotova et al., 2022), which could all be labelled as either direct or indirect effects.

Distinguishing between direct, or chemical, intrinsic particle toxicity (caused by the polymer itself and the respective monomers) and physical effects of the particle and associated yet non-intrinsic toxicity (e.g. by leaching of additives or desorption of surface chemicals) is challenging, as chemical toxicity and physical effects often act simultaneously (Zolotova et al., 2022). They enter in the organism's tissue and can simultaneously have tissue breaking and biochemical effects. Usually, the mode of toxicity of chemicals relates to chemical reaction between the (dissolved) molecule and a sub-organismic receptor in cells or membranes. However, for plastic particles other forms of adverse effects (i.e. physical or mechanical effects) may prevail, that are related to non-chemical properties, such as size, shape, material density and surface quantity and quality (ECETOC, 2018).

Although a direct toxicity of the plastic's polymers is often not proven in exposure studies, the interaction effect with environmental pollutants (vector effect), in which mixture effects depend on the chemical speciation and consequent bioavailability of pollutants (e.g. metals) and plastics, seems to be more prevalent. To study interaction effects of MNPs and environmental chemicals requires an adequate experimental design to resemble realistic underlying ad- and desorption processes in order to avoid over- or underestimation of toxicity caused by the particle-associated chemical (Heinrich et al., 2020). This is reflected in the literature, where reported results are often controversial, ranging from synergistic to antagonistic effects. MNPs can be more toxic with co-contaminants (plasticizers, metals, organic pollutants) (Avazzadeh Samani and Meunier, 2023), and interact with pesticides through adsorption and desorption processes, which require additional consideration due to the role this plays in changing the environmental transportation, fate, bioavailability, and ecotoxicity of both plastic particles and pesticides (Junaid et al., 2023). Especially for hydrophobic, persistent contaminants, like per- and polyfluoroalkyl substances (PFAS), synergistic effects in food webs are expected (Soltanighias et al., 2024). MNPs found in natural conditions absorb large amounts of PFAS (Scott et al., 2021), but the interaction of MNPs with PFAS depends strongly on ionic strength, temperature, pH and functional groups (Salawu et al., 2024).

3. Outlook

Risk assessment related to MNP is improving and promising approaches have been made to deal with the existing effect data situation (Adam et al., 2019; Redondo-Hasselerharm et al., 2023). Testing protocols, validity and reporting quality assessment criteria are continuously improved and implemented. However, there are still key elements that are either understudied, not considered, or technically challenging. To address the existing problems, the following key aspects points are recommended to overcome the existing lack of harmonized test methods applicable to MNP particles (Bour et al., 2021; De Ruijter et al., 2020; Haegerbaeumer et al., 2019; Koelmans et al., 2022; Kotta et al., 2022; Monikh et al. 2023). In particular, particle realism, realistic exposure scenarios, dose-metrics, particle-chemical interaction, choice of test species and mode of action require specific attention. Furthermore, we recommend first defining the assessment goal of the study to adjust the experimental design accordingly (Figure 2). Test setups can thereby differ depending substantially, depending if fundamental mechanistic processes are of interest or complex interaction processes that reflect more natural conditions.

3.1 Particle realism

Analogously to solvent controls in exposures of soluble substances, the use of appropriate and diverse reference materials is also key for MNP particle testing. The following approaches can be recommended. First, the use of different mixes reflecting natural “fingerprints” of particles. Within this scenario, the availability and selectivity is taken into account by providing a standard predefined mix with known descriptor parameters, which can be adjusted to the testing system. This should cover the different shapes, sizes and chemical identities to resemble the variety of natural occurring particle characteristics. Such a reference mix could be combined with non-polymer particles with similar size distribution. Koelmans et al. (2022) introduced the continuum concept, which acknowledges the multidimensional nature of MNP particles, encompassing various sizes, shapes, densities, and chemical compositions which could serve as a basis. Second, a pre-defined ageing and weathering protocol could be implemented to systematically compare the differences between pristine and weathered particles (Table 2). The production of such reference materials has been suggested previously and can technically be implemented (De Ruijter et al., 2023; Kefer et al., 2021; Monikh et al. 2023; Von der Esch et al., 2020), but a harmonized approach needs to be agreed upon. One practical recommendation is to base these reference mixes on known environmental sizes, concentrations and polymer identities to cover a spectrum of different scenarios (Table 2). The use of such scenarios will allow to systematically assess the uptake, internalization and excretion by the test organisms, the internal residual times and the effects based on the descriptor metric of the real internalized particle characteristics suite and duration, which should be one focus in future MNP research.

3.2 Realistic exposure scenarios

The distribution of MNPs in an aqueous suspension is not homogeneous, which impacts their bioavailability. A recommendation for more harmonized testing could include the documentation and control of the processes the particulates undergo. This includes the distribution, aggregation, chemical interaction and weathering (Figure 2, 3). Static systems, overhead stirring, and rotational setups have been compared for maintaining MNPs in suspension, with rotational methods proving most effective (Salaberria et al., 2020; Sun and Wu, 2023). Possible setups for static systems without sediment are outlined in Monikh et al. (2023). However, water-sediment exposure setups are recommended in case of high hydrophobicity and when benthic target species are studied (Table 2).

Test durations need to be adjusted to the assessment goals. Short-term experiments might thereby be suitable if mechanistic relationships are of interest or the study is intended to be a proof of principle. However, this often comes along with the need to apply rather high or environmentally unrealistic concentration ranges. Long-term experiments will become necessary if interaction effects and kinetics are in the focus of interest, and when environmental complexity (e.g. community effects) needs to be included.

A variety of conditions (pH, oxygen content, redox potential, salinity) can be applied and one can choose to use long-term exposure to organisms or communities or to take samples over weathering time and exposure organisms for a short term. Due to their potentially different ageing, both petroleum-based and biodegradable plastics should be considered. By adding a nutrient medium, varying inocula and day/light regime, one can induce biofouling as an extra component in weathering (Table 2). Systematic aging can be incorporated in testing frameworks, for example in procedures that are already under OECD norms. As a standardized weathering one can consistently include 2 or 3 standardized media (from fresh to salt water) during a fixed period (Amariei et al., 2022) (Table 2).

Exposure time should take not only uptake speed of organisms, but also adsorption and desorption of co-contaminants and leachates into account. Hydrophobic leachates, like plasticizers, can leach out of plastic particles for up to hundreds of years (Henkel et al., 2022), and are therefore less relevant in short-term exposures.

Gut retention times are relevant for defining duration of internal exposure, and for digestive fragmentation, which has been shown for a planktonic species (Dawson et al., 2018) but may occur for others as well. To quantify the MNP uptake, biological samples can be enzymatically digested to determine particle body burdens (Silva et al., 2019). Alternatively, one can quantify the concentration in the medium before and after exposure. However, when a sediment layer is used, MNPs must be extracted from the sediment first. More research is needed to improve and validate extraction and purification of MNPs from complex matrices.

3.3 Dose-metrics

The metrics reported in effect assessment are often insufficient to relate the effects to the exposure scenario. Particle size and counts or mass concentrations can lead to wrong interpretations in effect assessment, especially when surface charge is insufficiently taken into account (Kögel et al., 2020). Besides dose in both mass and particle number, surface to volume ratios, densities and hydrophobicity (using log K_{ow}) should be reported in future scientific publications.

3.4 Particle-chemical interaction

For environmental risk assessment, a deeper integration of the potential effects of MNP interactions with co-contaminants, such as metals and organic pollutants needs to be considered. Effects of weathering on adsorption and leaching of chemicals are often neglected when pristine polymers are used. Hence, the number of studies examining effects of co-contaminations is not representing environmental relevant circumstances (De Sá et al., 2018). Partitioning kinetics, including equilibrium of chemical additives in the polymer, the aqueous phase, inorganic particles and biota, need to be determined to enable the design of test scenarios for particle-chemical interaction (Figure 2). Leaching of chemicals from MNPs should be compared to a particle free exposure of the leachate (Table 2).

The development of standard reference materials for MNPs in sediment would significantly increase the understanding of the role of MNPs as conduits of chemical pollutants, partitioning of chemical plastics additives in sediments between sediment particles and MNPs and the impact on bioavailability of additives.

Plastic particle size highly influences the release kinetics of associated organic compounds (Town and Van Leeuwen, 2020). Therefore, detailed descriptions of the physicochemical features of plastic particles are to be provided in experimental studies on MNPs in different types of aquatic environments (Table 2).

Zink and Pyle (2023) proposed a framework of reporting requirements to better understand the interaction between metals and microplastics including their bioavailability. To be reported are environmental parameters, particularly factors known to influence metal behavior (pH, water hardness, organic matter, temperature) and microplastic surface characteristics that affect adsorption capacity. As future research direction, relevant tissue-specific uptake, accumulation, and toxicity should be assessed, to develop an understanding of tissue-specific accumulation and migration across biological membranes.

3.5 Mode of action

The majority of experimental evidence is at the organismal or sub-organismal level and there is limited evidence about how to scale up to higher levels of organization (populations, assemblages). Effects of biochemical biomarkers involved in antioxidant pathways, oxidative damage and neurotoxicity are often evident at high concentrations, generally several fold greater than those found in the environment (Connors et al., 2017). Therefore, these effects only reflect worst-case scenarios and do not take into account more subtle and long-term exposure.

MNP effect studies often do not use adequate particle controls, which would allow to distinguish physical from biochemical effects (based on key parameters shape, size, and porosity). Many properties, such as shape and size, are particle-specific, and influence the interaction with organisms, independent of their composition. It is recommended that MNP or leachate properties that govern adverse effects on organisms are defined. In this context, knowledge obtained on nano-materials or natural sediment and soil-particles can be transferred to MNP effect assessment (Table 2).

4. Conclusions

As identified in this review, the main challenges in ecotoxicological effect assessment of MNPs are (1) providing stable, continuous exposure scenarios (even if the particles settle down in sediment), (2) using test species that represent exposure-relevant organisms with respect to their ecology and physiology, (3) providing a suitable analytical method for external and internal MNP exposure, (4) defining useful endpoints for both physical and biochemical effects, and (5) testing the influence of environmental conditions on speciation and bioavailability of MNPs, leachates and co-contaminants.

In order to address these, we recommend: (1) the use of appropriate and diverse reference materials, (2) the documentation and control of the processes the MNPs undergo before and during exposure,

(3) reporting metrics and hydrophobicity, (4) studying partitioning kinetics, (5) the use of ecologically relevant key species.

These proposed steps in future research will lead towards more effective, mechanistic and evidence-based assessments of the ecotoxicological effects of MNPs.

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Declaration of competing interest

None

Data availability

All data used for the literature review are contained in the manuscript.

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Table 1. Examples of chosen characteristics of micro-nanoplastics, test organisms, exposure time and toxicological endpoints.

MP material	MP Concentrations	MP Sizes	MP shapes	Exposure time	Test organisms	Toxicological endpoints
PE (Mazurais et al., 2015)	10, 25, 50, 100, or 200 mg/L of pristine and biofouled microplastic (Amariei et al., 2022)	10 – 45 µm polyethylene microbeads (Mazurais et al., 2015)	PE Microbeads (Mazurais et al., 2015)	96 hours (Tamayo-Belda et al., 2023)	*Danio rerio* embryo (Bashirova et al., 2023; Tamayo-Belda et al., 2023)	• Mortality • Morphological changes • Regeneration (Tamayo-Belda et al., 2023)
PLA, HDPE and PVC (Green et al., 2016)	500 µg/L polyamide (Bartonitz et al., 2020)	1.4 - 707 µm for PLA, 2.5 - 316 µm for HDPE and 8.7 - 478 µm for PVC (Green et al., 2016)	PET Nanoparticles (Bashirova et al., 2023)	170 days (Parker et al., 2023)	*Chlorella vulgaris* (& *Chlorella reinhardti* (Pencik et al., 2023; Rani-Borges et al., 2023; Wang et al., 2023a)	• Feeding behavior • Regeneration of the head • Locomotion test (Cesarini et al., 2023)
PET (Bashirova et al., 2023)	0, 5, 10, 50, 100 and 200 ppm PET NPs (Bashirova et al., 2023)	9 - 5386 µm ERMP (De Ruijter et al., 2023)	PP powder (Beckingham and Ghosh, 2017)	28 days (Jia et al., 2023)	*Hydro cnidara* & *Hydro viridissima* (Tamayo-Belda et al., 2023)	• Photosynthetic activity • Morphology (Jia et al., 2023)
PET, PA, PS and PLA (Brehm et al., 2022)	0.5, 1, 2.5, 5, 10, 50 mg/L PS NPs) (Bellingeri et al., 2019)	0.5 µm and 15 µm PS microplastics (Hao et al., 2023)	PL fibers (Schell et al., 2022)	10 days (Cesarini et al., 2023)	*Gammarus duebeni* and *Gammarus pulex* (Griffith et al., 2023)	• Feeding rate • Swimming behavior • Mortality • Energy assimilation (Bartonitz et al., 2020; Götz et al., 2022)
PMMA (Cesarini et al., 2023)	8, 40, 200, 1000 µg/mL), corresponding to 70,000 - 620,000 particles/mL (Boháčková et al., 2023)	3 – 7 µm PET microplastics (Pencik et al., 2023)	Fluorescent PS bead (Sussarellu et al., 2016)	12 hours (Weber et al., 2021)	Chironomidae (Prata et al., 2023; Stanković et al., 2022)	• Oxidative stress (enzyme activity) • Particle size reduction • Mortality (Queiroz et al., 2023; Rani-Borges et al., 2023)

PP (Beckingham and Ghosh, 2017)	0.06 g microplastics /L (Zink et al., 2023)	100 nm PS nanoparticles (Jia et al., 2023)	Metal-doped spherical polystyrene nanoplastics (Heinze et al., 2021)	6 hrs (Griffith et al., 2023)	Rainbow trout *Oncorhynchus mykiss* cell lines (Boháčková et al., 2023)	• Growth performance • Antioxidant responses • Changes in the composition of intestinal microbiota communities (Hao et al., 2023)
PS (Rani-Borges et al., 2023)	6.4, 160, 4000, 100,000 particles mL^{-1} PS fragments (Weber et al., 2021)	PL fibers (26–5761 µm) and car tire particles (25–75 µm) (Schell et al., 2022)	Irregular PE fragments (Amariei et al., 2022)	24 hours (Boháčková et al., 2023)	*Daphnia magna* (& *Daphnia pulex* (Hoffschröer et al., 2021; Lee et al., 2023)	• Specific growth rate • Prey consumption (Parker et al., 2023)
PL (Schell et al., 2022)	100,500,1000, 2000 and 4000 mg MP/kg dw sediment (Romero-Blanco et al., 2021)	1 - 10,10 - 100, and 100 - 500 µm PS and PVC (Zhao et al., 2023)	Crystal spherical primary PS (Rani-Borges et al., 2023)	100 days (Stanković et al., 2022)	Aquatic plant *Eichhornia crassipes* (Jia et al., 2023)	• Cell growth • Colony size • Chlorophyll content • Photosynthetic efficiency • Pigment analysis (Pencik et al., 2023; Rani-Borges et al., 2023; Wang et al., 2023a)
LDPE (Muñiz-González et al., 2021)	0, 0.1, 1, 5, 10, 20, 30 and 40% plastic weight in the total sediment mixture (Redondo-Hasselerharm et al., 2018)	5.0–7.0 µm HDPE (Zink et al., 2023)	Fibers, pellets, spheres, and fragments (Waldschläger and Schüttrumpf, 2020)	2 weeks (Bartonitz et al., 2020; Götz et al., 2022)	*Gammarus roeseli* (Bartonitz et al., 2020; Götz et al., 2022)	• MP ingestion • Damage to gut epithelium • Reactive oxygen species • Larval body length (Prata et al., 2023)
ERMP test mixture (De Ruijter et al., 2023)	0.1, 1; and 10 g MPs/ kg sediment (Silva et al., 2022)	~426 ± 175 nm PMMA nanoparticles (Cesarini et al., 2023)	Nylon 6 powder (PA) (Palacio-Cortés et al., 2022)	18 days (Brehm et al., 2022)	Freshwater mussels *Dreissena polymorpha* (Weber et al., 2021) and *Dreissena bugensis* (Brehm et al., 2022)	• Embryo development • Hatching rate • Survival rate • Morphological deformities, e.g. tail detachment

- Heart beat analysis

(Bashirova et al., 2023; Tamayo-Belda et al., 2023)

List of abbreviations:

PE	Polyethylene
PLA	Polylactic acid
HDPE	High density polyethylene
PVC	Polyvinylchloride
PET	Polyethylene terephthalate
PA	Polyamide
PS	Polystyrene
PMMA	polymethyl methacrylate
PP	Polypropylene
PL	Polyester
LDPE	High density polyethylene
ERMP	Environmentally relevant microplastic

Table 2. Representation of challenges and recommendations for different MNP test scenarios.

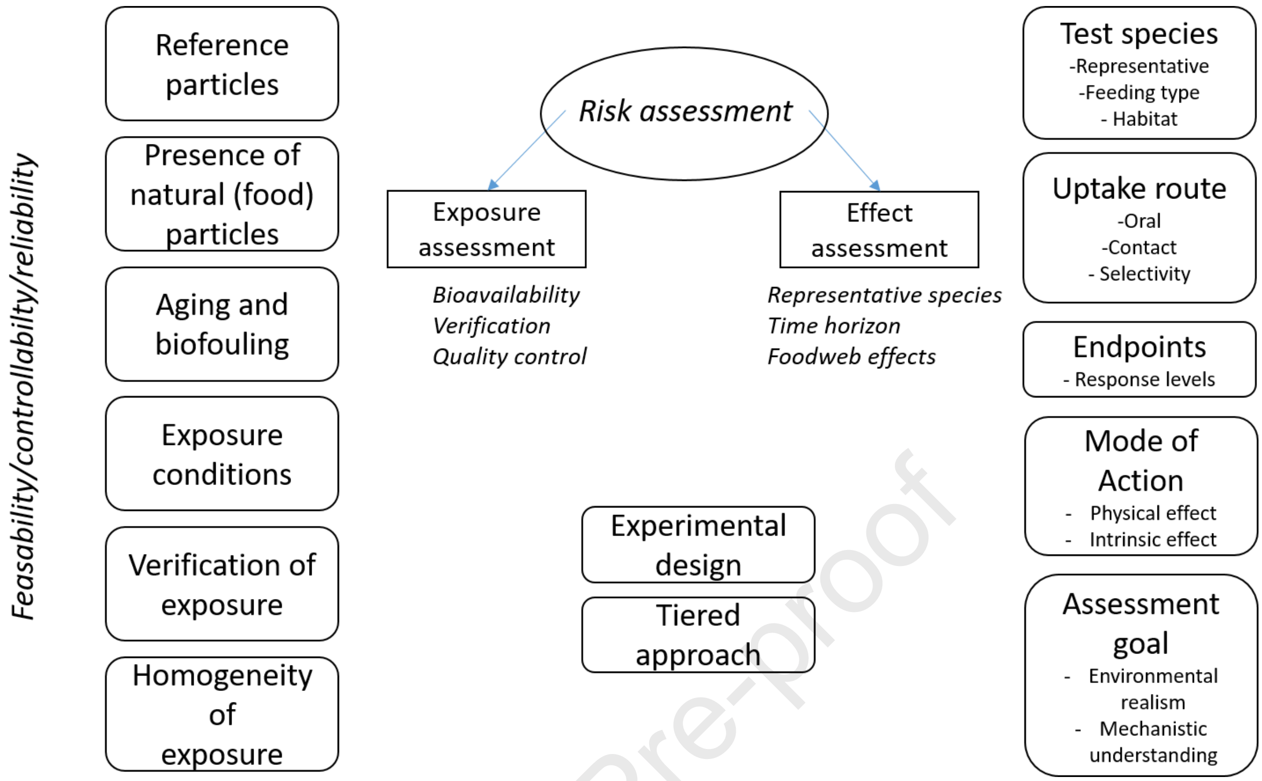
Exposure	Scenario	Challenges	Recommendations
	Weathering tests	Varying weathering conditions	Two standardised media (fresh and salt) at fixed exposure time
	Biofouling tests	Varying algae composition	Standardised medium, light intensity and algae inocculation
	Leaching tests	Leaching time, unknown compositions	Standardised leaching time, full analysis of unknown compounds, determination of partitioning kinetics
	Bioavailability tests	Speciation and kinetics	Flux analyses and modelling
Effects	In vitro test	Particle realism	Standard mix that includes ranges of age, weathering and sizes
	Bioassays	Homogeneity, hydrophobicity	Include sediment layer and/or rotational methods
	Bioassays	Mode of action	Use knowledge on nano-materials and compare
	Bioassays	Dose metrics	Publish mass particle number, surface to volume ratios, densities and hydrophobicity
	Mesocosms	Choice of test organisms	Riverine species
	Mesocosms	Choice of test organisms	Short pelagic food chain

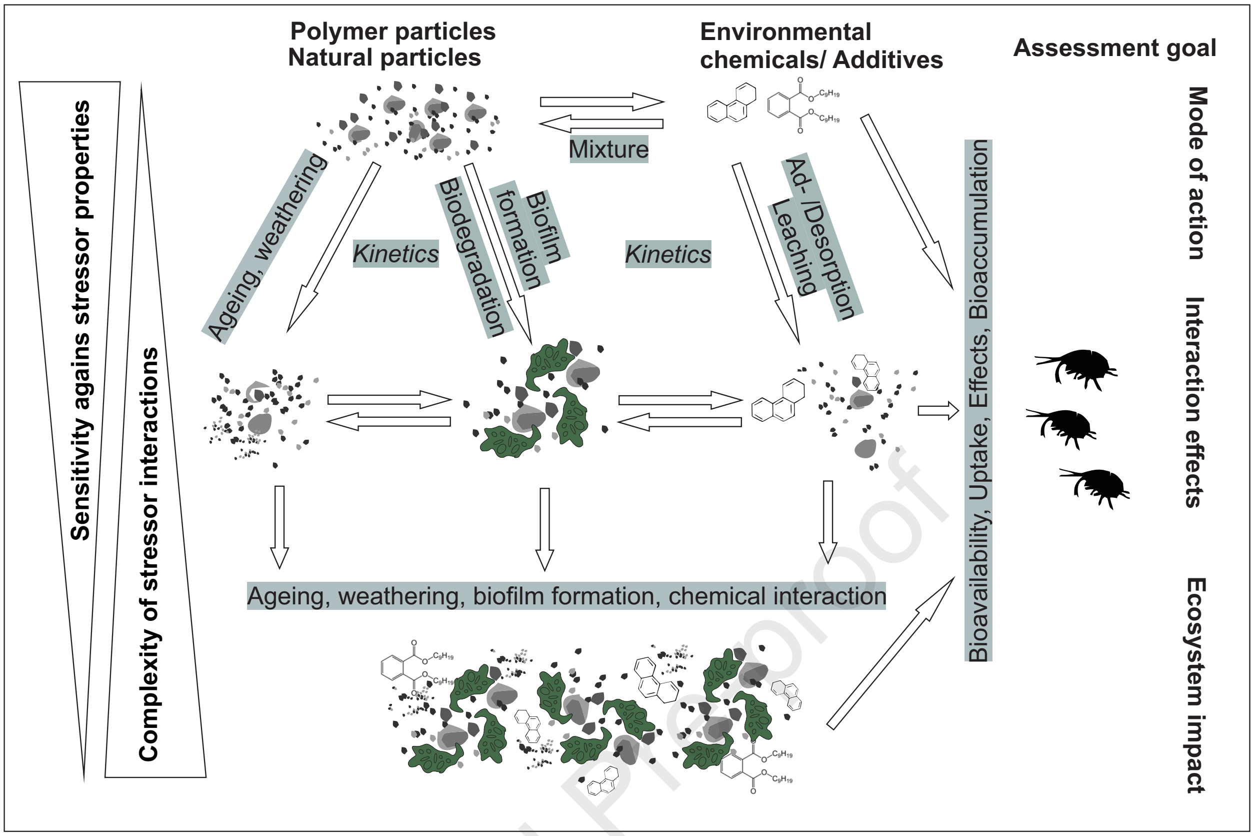
Figure captions:

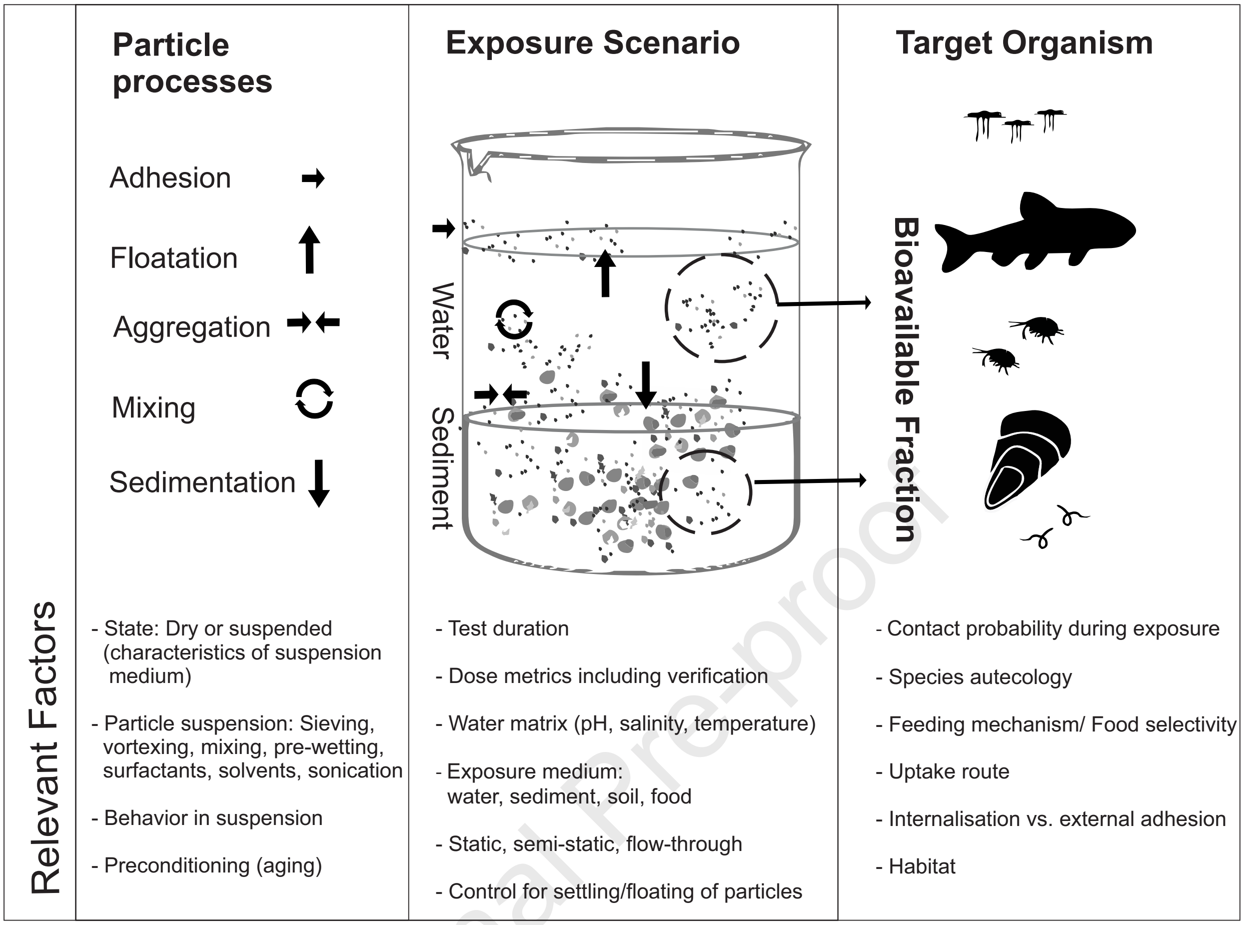
Figure 1: Conceptual framework and key variables in ecotoxicological risk assessment of microparticles.

Figure 2: Illustration of different levels of complexity in test designs, depending on the assessment goal.

Figure 3: Relevant factors and processes to consider during particle exposure to target organisms.


Feasability/controllabilty/reliability
Reference particles
Presence of natural (food) particles
Aging and biofouling
Exposure conditions
Verification of exposure
Homogeneity of exposure
Risk assessment
Exposure assessment
Bioavailability
Verification
Quality control
Effect assessment
Representative species
Time horizon
Foodweb effects
Experimental design
Tiered approach
Test species
-Representative
-Feeding type
- Habitat
Uptake route
-Oral
-Contact
- Selectivity
Endpoints
- Response levels
Mode of Action
- Physical effect
- Intrinsic effect
Assessment goal
- Environmental realism
- Mechanistic understanding


Polymer particles
Natural particles
Environmental chemicals/ Additives
Assessment goal
Mixture
Ageing, weathering
Kinetics
Biodegradation
Biofilm formation
Kinetics
Ad- /Desorption Leaching
Bioavailability, Uptake, Effects, Bioaccumulation
Mode of action
Interaction effects
Ecosystem impact
Sensitivity agains stressor properties
Complexity of stressor interactions
Ageing, weathering, biofilm formation, chemical interaction


Relevant Factors
Particle processes
Adhesion
Floatation
Aggregation
Mixing
Sedimentation
Exposure Scenario
Water
Sediment
Target Organism
Bioavailable Fraction
- State: Dry or suspended (characteristics of suspension medium)
- Particle suspension: Sieving, vortexing, mixing, pre-wetting, surfactants, solvents, sonication
- Behavior in suspension
- Preconditioning (aging)
- Test duration
- Dose metrics including verification
- Water matrix (pH, salinity, temperature)
- Exposure medium: water, sediment, soil, food
- Static, semi-static, flow-through
- Control for settling/floating of particles
- Contact probability during exposure
- Species autecology
- Feeding mechanism/ Food selectivity
- Uptake route
- Internalisation vs. external adhesion
- Habitat

Highlights:

- Ecotoxicological assessment of micro-nanoplastics depends on appropriate methodologies
- Inconsistencies and methodological challenges hamper sound risk assessment
- Realistic particle choice, dose-metrics, test-duration and environmental conditions are key
- Control treatments with reference particles help distinguish physical from toxic effects
- Consideration of this framework contributes to realistic effect assessment and harmonization

Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐ The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: