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Review

A brief history of microplastics effect testing: Guidance and prospect☆

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ABSTRACT

Numerous reviews have consistently highlighted the shortcomings of studies evaluating the effects of microplastics (MP), with many of the issues identified in 2016 still relevant in 2024. Here, we summarize the current knowledge on MP effect testing, compare guidelines, and provide an overview of risk assessments conducted at both single species and community levels. We discuss standard test materials, MP characteristics, and mechanisms explaining effects. We have observed that the quality of MP effect studies is gradually improving, and knowledge on enhancing these studies is available. Recommendations include data rescaling and alignment for ecological risk assessment, with preference for using environmentally relevant MPs. A step-by-step protocol for creating polydisperse test materials is provided. Most risk assessments indicate that concentrations observed in ecosystems globally exceed the effect thresholds measured in the laboratory. However, using a higher-tier approach, no risks are expected for freshwater benthic communities at current MP exposure concentrations. Evidence on the mechanisms behind adverse effects is growing; however, more well-designed experiments are needed. A potential solution might involve comparing natural particles with MPs that are as similar in dimensions as possible, providing insight into the mechanisms of food dilution where volume is a critical determinant of toxicity.

1. Introduction

Over the past few decades, concerns have arisen regarding the potentially adverse effects of microplastic particles (MP) in the environment. Understanding these effects is crucial to quantify them within the context of risk assessment (Koelmans et al., 2017). While the testing of more traditional chemical stressors like pesticides, persistent organic pollutants (POPs), and heavy metals has been well-established, resulting in numerous guidance documents, protocols, and standard operating procedures (SOPs), the MP scientific community has undergone a steep learning curve.

MP represent a complex, heterogeneous mixture of particles with varying degrees of aging and weathering (Jahnke et al. (2017). They are associated with biofilms and chemicals, including additives and chemicals sorbed from the environment. These characteristics are highly variable in both time and space, posing significant challenges when evaluating the risks associated with this complex contaminant. Despite the challenges causing the research community to take substantial detours and delays in developing valid testing and assessment strategies, some of these key issues are gradually being addressed in response to landmark papers highlighting the limitations of available data (Fig. 1).

Our brief history of MP effect testing begins as early as 2016 with the work of Lenz et al. (2016), who stressed the importance of using environmentally realistic MP concentrations. In the same year, Phuong et al. (2016) also argued that laboratory experiments often employed MP concentrations significantly higher than those found in the field. Additionally, they noted that MP exposure conditions typically involve a single type of polymer with a precise size and homogeneous shape, which does not accurately represent the diversity of MPs found in the environment. Connors et al. (2017) also emphasized the need to address the environmental relevance of test concentrations and stressed that studies should provide sufficient detail to convert particle concentrations and characterize particles extensively. Moreover, they highlight the need for relevant controls or reference materials. Karami (2017) underscored the importance of quantifying chemicals associated with MP particles, selecting appropriate test organisms, preventing aggregation, and considering the environmental relevance of particle size. Rist and Hartmann (2018) elaborated on these aspects and emphasized

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the need of developing clear definitions for plastic particle categorization, including controls for chemical leaching (e.g., monomers, additives), the development of reference materials for method validation and comparison, and addressing the influence of environmental transformation processes (e.g., 'aging') on MP behaviour and ecotoxicity. De Sá et al. (2018) were among the first to observe the over-representation of certain species in MP effect studies, primarily focusing on fish and small crustaceans. They also highlighted a lack of understanding regarding the mechanisms responsible for MP effects. Burns and Boxall (2018) were the first to provide a systematic comparison of effects across multiple species (Species Sensitivity Distributions) and exposure concentrations, shedding light on low risks in natural settings. However, their analysis still faced challenges due to the incomparability of data, as noted by earlier authors. Advancing further, O'Connor et al. (2020) stressed that testing higher-than-realistic MP concentrations remains essential for assessing dose-dependent effects. Meanwhile, Triebskorn et al. (2019) identified additional imbalances, including an over-representation of certain polymers (e.g., polyethylene and polystyrene), species (e.g., fish), and a notable lack of studies addressing the effects of diverse MP mixtures. Furthermore, Bour et al. (2021) advocated for a more nuanced approach to address the complexity of organism-particle interactions, and Kukkola et al. (2021) reiterated the mismatch between field and laboratory studies regarding plastic types and assessed endpoints. They also emphasized that fibres are more prevalent in field studies, whereas particles dominate laboratory studies. Finally, in 2022, an international group of experts identified data gaps and emphasized the importance of adequate particle characterization and appropriate study design that allow for the derivation of dose-response curves (Thornton Hampton et al., 2022a).

In 2020, de Ruijter et al. (2020) synthesized most of these qualitative observations into a quantitative tool for assessing study Quality Assurance and Control (QA/QC). They retrospectively applied this new tool to all studies that had provided effects data up to that point (n = 105 studies), revealing that none of the studies had obtained non-zero scores for all criteria (de Ruijter et al., 2020). This suggests that, if a strict approach were followed, none of the studies would hold relevance for risk assessment in a regulatory context. In the same year, a group of 23 researchers published a set of reporting guidelines aimed at enhancing the comparability and reproducibility of studies (Cowger et al., 2020). To address disparities in particle testing, Koelmans et al. (2020) proposed a data alignment and rescaling framework, which for the time being would solve the problem of the incomparability of results caused by differences in particles being tested.

In summary, numerous reviews over the years have consistently highlighted the shortcomings of MP effect studies, with many of the same issues identified in 2016 still relevant in 2024 (Fig. 1). Solutions have been proposed by de Ruijter et al. (2020), Cowger et al. (2020), and, to some extent, Koelmans et al. (2020). The question now is to what extent the various guidance documents converge and whether there are still new or unresolved bottlenecks.

The objective of this review and guidance document is to summarize the knowledge accumulated in MP effect testing, compare available guidelines, and provide a comprehensive overview of the knowledge on risk assessment at both the single species and community levels. Additionally, we discuss standard test materials, characteristics of MP, and mechanisms explaining their effects. Finally, we offer recommendations for future research directions.

The article is structured as follows. We first discuss effect testing in the context of risk assessment, introducing the relevant framework and methodologies. Approaches for assessing ecological risks, including techniques such as data rescaling, alignment, and tests that integrate environmentally realistic microplastics, are reviewed. We then address QA/QC in single-species tests, focusing first on the effects of MP particles and subsequently on the assessment of chemicals associated with these particles. Next, we review community-level approaches, emphasizing their role in tiered risk assessments. We provide an overview of community-level testing methods and explore how effect thresholds and ecological risks of MPs can be determined based on community-level data. We then provide guidance on the importance of standardized test materials and how to produce them in a way that maximizes the environmental relevance of the previously discussed single-species or community tests. Subsequently, we discuss key characteristics of MPs

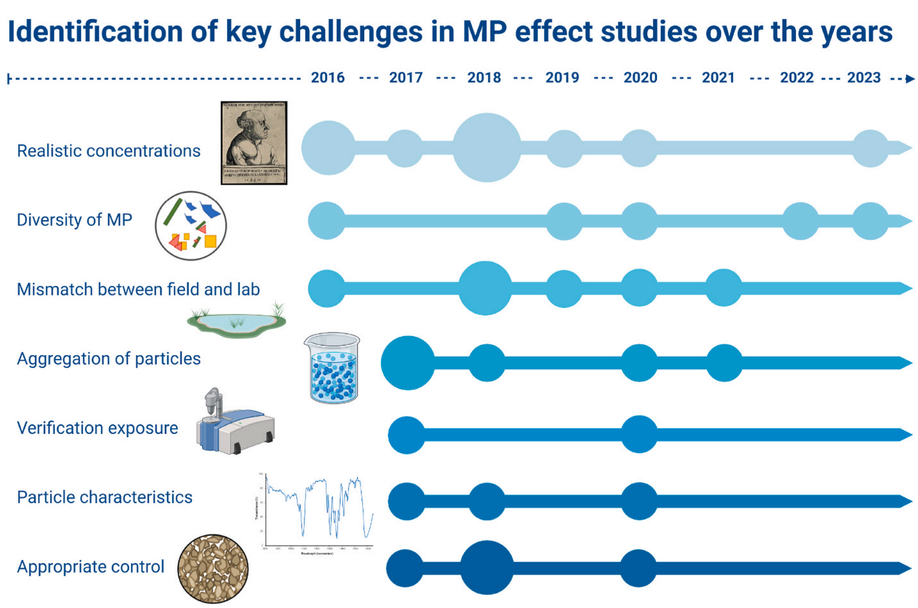

Fig. 1. Identification of key challenges of microplastic (MP) effect studies (n = 16) (Bour et al., 2021; Burns and Boxall, 2018; Coffin, 2023; Connors et al., 2017; de Ruijter et al., 2020; De Sá et al., 2018; Karami, 2017; Kukkola et al., 2021; Lenz et al., 2016; Ogonowski et al., 2018; Phuong et al., 2016; Rist and Hartmann, 2018; Thornton Hampton et al., 2022a; Thornton Hampton et al., 2022b; Triebskorn et al., 2019). Already from 2016, researchers have pointed out the same key challenges and proposed recommendations on how to improve MP effect studies. These key challenges include testing of environmentally realistic concentrations, thoroughly characterizing particles, addressing of aggregation of MP particles during exposure studies, verification of exposure concentrations, testing of MP in their diversity, mismatch plastic type field and lab and natural particle as reference material. Bigger circles indicate that more than one study has pointed out a certain key challenge (Table S1).

that influence their effects and the mechanisms driving these effects. This includes a discussion on the use of natural particles as controls to enhance the ecological relevance of MP effect tests. Finally, we synthesize the findings and provide recommendations for future research directions.

2. Effect testing with relevance for risk assessment: Risk assessment framework

2.1. Assessment of ecological risks using data rescaling and alignment

Similar to more traditional contaminants such as heavy metals and organic micro-pollutants, the evaluation of the risk associated with MP involves comparing exposure to effect thresholds (Koelmans et al., 2017). Exposure is quantified through concentration measurements in environmental samples, while effect thresholds are derived from dose-response relationships measured in the laboratory. One challenge in this regard is that these data are often obtained using different methods, making them incomparable. For instance, exposure to 100 particles/L, ranging in size from 20 to 5000 μm and composed of various polymers, cannot be directly compared to an effect threshold measured in the laboratory for 100 μm-sized MP. These are essentially different stressors, making the comparison like "comparing apples and pears". The only currently available solution is a computational method to correct for these differences. In this approach, all data are converted to an equivalent value as if they were obtained for a standard MP mixture ranging from 1 to 5000 μm (Koelmans et al., 2020; Kooi et al., 2021). We will briefly explain the principle here using an example. Suppose the mechanism causing people to become nauseous is consuming too many apples. In this case, the total volume of those consumed apples is a plausible measure of the relevant dose. We can assume that other factors do not matter in the short term. In this example, it can be presumed that the same effect would occur if too many pears were consumed, as long as the total volume of those pears is equal to that of the apples. The effect thresholds of apples and pears can thus be converted into each other based on volume. In this example, volume is the "effect metric," and food dilution is the mechanism. Interestingly, this example is not coincidental; in the literature, this very mechanism has been identified as the most plausible mechanism for the effects of MP on small organisms such as zooplankton, with the ingested particle volume as the relevant exposure metric (de Ruijter et al., 2020; Thornton Hampton et al., 2022b).

2.2. Past risk assessments

Several previous risk assessments have been carried out with non-harmonized data (Adam et al., 2019; Besseling et al., 2019; Everaert et al., 2020; Everaert et al., 2018; Hataley et al., 2023), which will not be covered in the present review. The data rescaling and alignment techniques, as explained in the previous section, have been applied in a limited number of risk assessments. In 2020, the first assessment for MP in surface waters was conducted, providing an indication of risks for 1.5% of the then-known exposure concentrations worldwide (Koelmans et al., 2020). In 2022, the method was employed by an international working group in the context of California state regulations to set risk management thresholds for coastal waters (Mehinto et al., 2022). A risk assessment was performed for MP in San Francisco Bay (Coffin et al., 2022). Over 75% of the samples exceeded the limit for the most conservative food dilution threshold. Within the Central Bay, 38% of the samples exceeded a higher threshold related to management planning, which was statistically significant within a 95% confidence interval. In 2023, a risk assessment for MP in freshwater sediments worldwide was also conducted (Redondo-Hasselerharm et al., 2023). The exposure concentrations were below or within the uncertainty range of the HC5 values. This implies that the risks of MP for benthic communities at the current freshwater sediment concentrations worldwide could not be ruled out. In 2023, the same methods were used for a risk assessment of MP in the Laurentian Great Lakes (Koelmans et al., 2023b). Due to uncertainties in the parameters used for corrections and conversions, uncertainties in sample volume, and variability in hydrological conditions, the assessment was performed probabilistically (Koelmans et al., 2023b). The probability of a risk occurring due to food dilution was 24% of pelagic exposure in Lake Ontario, 8.3–10.3% of pelagic exposures in Lake Michigan, Lake Huron, Lake Superior, and Lake Erie, and 13–15% of benthic exposures in Lake Erie and Lake Huron. A recent risk assessment for soils also showed that MP concentrations, for a limited number of soils globally, cause a probability of effects occurrence up to 95% (Redondo-Hasselerharm et al., 2024). All these risk assessments indicate that the highest concentrations observed within an ecosystem or globally, for surface water, sediments, and soils, exceed the effect thresholds measured in the laboratory.

2.3. Assessment of ecological risks using tests with environmentally relevant microplastics

The data alignment methods described above aim to correct the differences between particles in the environment and particles used in laboratory effect tests as accurately as possible. However, a significant drawback is the uncertainty introduced by all these computational corrections. It is preferable to conduct laboratory effect tests using environmentally relevant microplastics (ERMP), a mixture of particles that represents the average MP composition of an environmental compartment as closely as possible (de Ruijter et al., 2023). This mixture should thus have a realistic polymer composition, particle size distribution, and composition of shape categories, e.g., fibres, fragments, pellets, and sheets. In addition, tests with such an ERMP mixture should meet crucial QA/QC criteria (de Ruijter et al., 2020; Cowger et al., 2020). de Ruijter et al. (2023) put this into practice by testing 16 invertebrate species while satisfying 20 QA/QC criteria. In a second study, a similarly diverse ERMP mixture was used to test one species and compare the effects with a mixture with a nearly identical particle size distribution, but composed of non-polymer particles (de Ruijter et al., 2025). Threshold effects obtained from dose-response relationships for such realistic mixtures could be more directly compared with exposure data to characterize the risk without computational alignments.

3. Quality assurance in single species tests of microplastic particles

While the previous paragraph addressed the use of single species tests for consistent risk characterization, here we focus on the necessity of using only data that is fit for purpose in risk assessment, as well as the tools to determine this. In de Ruijter et al. (2020), 20 QA/QC criteria were proposed, together forming a standardized protocol to test the effects of MP in aquatic test systems. For each criterion a detailed rationale was provided. The sum of the maximum scores for each individual criterion constitutes the Total Accumulated Score (TAS). These criteria provide guidance to enhance the quality of effect tests in terms of particle characterization, experimental design, applicability to risk assessment, and ecological relevance. An analysis of 105 studies showed that no study scored positively on all criteria, highlighting the need for improved quality assurance (de Ruijter et al., 2020). Specifically, most room for improvement lies in the experimental design, which includes ensuring chemical purity, preventing and measuring contamination in the laboratory, verification of exposure concentrations, and ensuring that tested concentrations are homogeneous and well dispersed within the medium. To enhance applicability to risk assessment, studies should report effect thresholds and include at least 6 doses. Additionally, improving the ecological relevance of studies can be achieved by aging and biofouling MPs rather than testing pristine particles. Furthermore, enhancing the reliability of MP risk assessment can be achieved by diversifying the types of particles tested and extending the exposure time. While acknowledging that some criteria may substantially increase workload, it is essential to recognize that some criteria represent "low-hanging-fruit", or quick wins that can be easily implemented. Given limited resources, a strategic focus on these achievable criteria holds the potential for substantial improvements in informing risks assessment practices.

In parallel with de Ruijter et al. (2020), a diverse group of 23 researchers suggested reporting guidelines to increase the reproducibility of MP research, including considerations for toxicology studies (Cowger et al., 2020). Importantly, their recommendations align with those of de Ruijter et al. (2020) emphasizing the importance of thorough reporting of MP characteristics, such as plastic age, polymer type, size, and shape. Additionally, they emphasize the significance of reporting colour if it is potentially relevant to the organism being tested. Furthermore, they highlight the need for reporting exposure concentrations and their verification, which depend on the exposure media. They also stress the importance of providing detailed descriptions of how test organisms are exposed, and which tissues were analysed.

In line with the recommendations of Cowger et al. (2020) and de Ruijter et al. (2020), Alimi et al. (2022) propose reporting guidelines specifically for studies investigating the effects of weathered MPs. This review once again underlines that the vast majority (90.4%) of MP effect studies only test pristine MP. Alimi et al. (2022) offer highly specific guidance aimed at enhancing comparability and reproducibility when testing weathered MPs. They emphasize the need to clearly report the conditions under which particles have been weathered including exposure time, temperature, humidity, irradiance and also specifying the mimicked weathering pathway.

The body of literature describing the effects of MPs is growing (Granek et al., 2020). Thornton Hampton et al. (2022c) have innovatively designed an open database, complemented by an open source R shiny web application named Toxicity of Microplastics Explorer (ToMEX). This tool enables the compilation and synthesis of existing toxicity data and now includes 160 MP toxicity studies. Moreover, it incorporates the twenty QA/QC criteria proposed by de Ruijter et al. (2020), filtering out poor-quality data for risk assessment. Interestingly, a subset of 14 criteria, referred to as "red criteria", was pragmatically selected; otherwise an insufficient amount of data would have passed the QA/QC screening (Mehinto et al., 2022). Although expert groups may make such decisions in the absence of useful data, the consequence is that the resulting assessment did not meet the full set of twenty critical criteria, and thus, it should not be considered reliable. Nevertheless, while some experts still assigned a value of 4 on a scale of 5 for the reliability of the results, others who gave more weight to the reliability of the data assigned only a score of 1 for the quality of the established thresholds (Mehinto et al., 2022). For instance, the "chemical purity" criterion is excluded, despite its importance in distinguishing particle effects from chemical effects arising from MPs, which is a fundamental component of risk assessment. A potential solution involves thorough particle washing with organic solvents to isolate the particle effect; however, this approach may raise concerns about altering particle characteristics (Cowger et al., 2020; de Ruijter et al., 2020). As an alternative, the distribution of plastic-associated chemicals in the exposure system can often be calculated based on chemical distribution or speciation principles. Subsequently, the calculated concentrations for the various exposure media can be compared to threshold effect concentrations for these chemicals to potentially exclude any contribution of the chemicals to the observed effect (e.g. (Besseling et al., 2014; Redondo-Hasselerharm et al., 2020; Redondo-Hasselerharm et al., 2018; Redondo-Hasselerharm et al., 2021)). Another solution, although laborious, is proposed by Cowger et al. (2020): non-target screening for the presence for additive chemicals. Although the proposed solutions differ, both research groups agree that it is crucial in toxicity testing to know what is being tested. Additionally, several other crucial criteria, despite their importance, have not yet been incorporated as strict prerequisites in ToMEX. These include preventing and measuring contamination in the laboratory, verifying exposure concentrations, ensuring the homogeneity of these exposure concentrations, assessing exposure in tested organisms, and replication. Hopefully, in the future, studies will be able to incorporate these criteria, thereby reducing uncertainties in risk assessment.

In a study conducted by Coffin (2023), an evaluation applying 20 QA/QC criteria from (de Ruijter et al., 2020), assessed whether the quality of MP toxicity tests ($n = 160$) improved from 2012 to 2020. While the TAS has indeed improved, the rate of progress, at 0.002 points per year, suggests that the research community would require many years to achieve a perfect score. Specifically, advancements have been made in the technical aspects, such as particle characterization and experimental design, of MP toxicity tests. However, critical elements, such as including sufficient doses and incorporating environmentally relevant doses withing the tested range, have not shown improvement. Furthermore, a prevalent trend persists wherein many studies choose to test monodisperse pristine MPs, neglecting the environmentally realistic preference for diverse aged and biofouled particles.

To date, only a few studies (Amariei et al., 2022; Verdú et al., 2022) have specifically aimed to incorporate the QA/QC guidelines proposed by de Ruijter et al. (2020). In a follow-up study, de Ruijter et al. (2023) demonstrated the feasibility of meeting all 20 criteria (i.e. 95% of the maximum score while not having 'zero scores') by conducting standardized dose-response tests for 16 benthic invertebrate species (de Ruijter et al., 2023). In another study, they successfully met all criteria while also adhering to standards for control mortality. This study compared the effects of MP with the effects of natural particles (de Ruijter et al., 2025).

All 20 criteria remain to be incorporated for ecotoxicology studies focusing on pelagic species. These existing criteria are applicable to both benthic and pelagic tests. However, the criterion "homogeneity of exposure" may be considered more challenging when working with water as the medium, as particles tend to aggregate and adhere to glass walls. Nevertheless, solutions such as renewing the media, plankton wheels, or applying gentle aeration during exposure are available (Détrée and Gallardo-Escárate, 2017; Gambardella et al., 2019; Gerdes et al., 2019; Tang et al., 2018). Furthermore, the 'verification of exposure concentrations' is not yet a standard procedure when testing MP in water. However, many studies have successfully done so in the past (Long et al., 2017; Peixoto et al., 2019; Reichert et al., 2019; Sussarellu et al., 2016; Wang et al., 2019; Zimmermann et al., 2020). Some studies have utilized slightly more advanced methods such as a flow cytometer (Long et al., 2017; Sussarellu et al., 2016), coulter counter (Zimmermann et al., 2020) or a fluorescence microscope (Peixoto et al., 2019). Alternatively, it is also possible to filter a subsample of the exposure suspension and simply count the numbers under a stereomicroscope (Reichert et al., 2019; Wang et al., 2019). Interestingly, upon screening the ToMEX database, it is notable that, for each individual criterion, there is always at least one study that manages to incorporate that criterion adequately when testing pelagic species. This indicates that the scientific community possesses the knowledge for conducting high-quality MP effect studies. In conclusion, there is no valid reason for ecotoxicological studies testing pelagic species not to adhere to the 20 criteria proposed by (de Ruijter et al., 2020).

Recently, Jemec Kokalj et al. (2021) put forward new quality criteria for nanomaterial studies using the 20 QA/QC criteria (de Ruijter et al., 2020) as a starting point and proposed additional nanoplastic (NP) specific criteria for nanomaterial studies. Moreover, similar QA/QC evaluation tools have been developed for measuring MP in matrices such as water, sediment, air or biota samples (Hermsen et al., 2018; Koelmans et al., 2019; Redondo-Hasselerharm et al., 2023; Wright et al., 2021). Since the measurement of MP in these matrices is also necessary in the context of effect tests, these QA/QC criteria are relevant to such studies. Although the details are beyond the scope of this review, QA/QC criteria for water and biota samples, for instance, have been implemented by the World Health Organization (2019, 2022). While these criteria only apply to testing the effects of particles, there are also specific guidelines for situations where chemicals are associated with the plastics. We will discuss those in the following section.

4. Quality assurance in single species tests of microplastic particle-associated chemicals

MP particles in the natural environment always contain chemical substances, either as a result of the production of the polymers from which the MPs originated, or due to the adsorption of chemicals from the environment. Effects of MPs can, therefore, occur when thresholds for the particles, the chemicals, or both are exceeded, e.g. Tian et al. (2021). When testing these effects in a laboratory test simultaneously, a problem often arises regarding the relevance of exposure to the substances and particles, ideally aiming for exposures that are as environmentally relevant as possible (Koelmans et al., 2016; Koelmans et al., 2022).

The first issue is that when you test chemically contaminated MPs in an uncontaminated test system, the chemical substances will start to desorb immediately. This needs to be measured to understand the exposure, but this is rarely done (Koelmans et al., 2022). The second problem is that some researchers accelerate this desorption by actively extracting the chemicals and determine the toxic effects of the chemical substances after concentrating them (Capolupo et al., 2021; Klein et al., 2021; Zimmermann et al., 2021; Zimmermann et al., 2020), while using unrealistically high plastic-to-water ratios up to 80 g/L. They frame testing of methanolic extracts as "worst case scenario" and testing of water extracts as a "realistic scenario". However, as long as the exposures are not environmentally relevant, this may still be misleading. What is subsequently measured often has little to do with reality. In nature, microplastic-associated chemicals do not desorb as they are in equilibrium with their surroundings, or they desorb very slowly. This means that when chemicals are released, it leaves few effects through dilution. The third problem is that the tests often use relatively clean test organisms, so exposure is caused only by the created thermodynamic concentration gradient. In reality, these gradients are often absent or even reversed, in which case the chemicals adhere to the plastic, and exposure can actually decrease (Koelmans et al., 2016; Koelmans et al., 2013; Mohamed Nor and Koelmans, 2019; Mohamed Nor et al., 2023). The fact that absorption to plastic is typically discussed in the context of the 'vector effect' concerning hazards and risks but is omitted when it occurs in the context of exposure reduction suggests the existence of biases in the literature. The fourth problem is that tests examining the role of MP in the exposure to plastic-associated chemicals usually do not consider parallel exposure routes (Koelmans et al., 2016; Koelmans et al., 2022). The flux of chemical substances through the ingestion of MP is relatively small when compared to the flux through water or food (prey). Because plastic, water, and food are part of the same system, they typically contain the same substances. The fact that the flux through water and food is often greater than that through MP is often overlooked in tests, even though it is a crucial feature of environmentally relevant exposure. Guidance on testing for plastic-associated chemicals is detailed by Koelmans et al. (2022), so we omit it here. The most important point of attention is that the actual chemical exposure must be determined in the test system, for example with passive samplers, that the exposure must be environmentally relevant, and that the data interpretation explicitly states the degree of environmental relevance. So much for the discussion of single-species effects tests; next, we will address community-level approaches.

5. Community level approaches

5.1. Community level approaches as a component of tiered risk assessments

To assess the environmental risks associated with MPs in a cost-effective manner, it is advisable to employ a tiered approach (Koelmans et al., 2017; Posthuma et al., 2008). This framework consists of successive tiers that increase in ecological relevance and complexity, and resource requirements. The lowest tiers encompass the use of databases (Tier 0) and the performance of laboratory single species tests (Tier 1). Toxicity data obtained from these tests can be combined with literature data in Species Sensitivity Distributions (SSDs) (Tier 2). SSDs are cumulative probability distributions that show the sensitivity of a group of species to a stressor. This tool enables the determination of the Hazardous Concentration for 5% of the species (HC5), which is used to estimate the Predicted No Effect Concentration (PNEC). As previously mentioned, several studies have characterized the risks associated with MPs by constructing SSDs using toxicity data for aquatic organisms (Coffin et al., 2022; Koelmans et al., 2023b; Redondo-Hasselerharm et al., 2023). Discrepancies among the PNECs reported in the literature were resolved by aligning exposure and effect data, and by selecting data based on quality criteria and other relevant aspects (*e.g.*, included endpoints). When risks are estimated in Tier 2 through the comparison of the PNEC with measured or predicted environmental concentrations, higher-tier experiments become necessary to assess effects at population and community levels. Higher-tier experiments (Tier 3) are conducted in outdoor artificial or semi-artificial systems, such as mesocosms or enclosures, which mimic the natural environment more effectively than single species tests. Interactions between abiotic and biotic factors play a role in higher tiers, enabling the detection of effects under ecologically relevant and complex scenarios. However, these experiments are more expensive and time-consuming, which consequently limits the amount of toxicity data available for population and communities. Although environmental risks of MPs have been identified for aquatic ecosystems, only a few experiments have been conducted at this level of ecological relevance with marine and freshwater organisms (Foekema et al., 2022; Green, 2016; Marchant et al., 2023; Redondo-Hasselerharm et al., 2020; Yıldız et al., 2022).

5.2. Overview of microplastic community tests

One of these freshwater outdoor mesocosm studies, conducted by Marchant et al. (2023), assessed the effects of two MP polymer types (polyethylene and biodegradable polylactic acid) under two nutrient conditions (enriched and non-enriched) on pelagic community structure and ecosystem functioning over a 12-weeks period. The study demonstrated that environmentally relevant MP concentrations, even at higher levels, did not have an impact on plankton community composition and taxonomic richness, periphyton productivity, or leaf litter decomposition (Marchant et al., 2023). Moreover, Ebbesen et al. (2024) and Klasios et al. (2024) found no effects on marine planktonic or freshwater zooplankton communities after exposure to polyester fibres or weathered MPs, respectively. In another study, a freshwater benthic community in a semi-artificial ditch was exposed to trays containing sediment and either polystyrene NPs or MPs for durations of 3 and 15 months (Redondo-Hasselerharm et al., 2020). While no effects were detected after 3 months of exposure, after 15 months the highest concentration (5% plastic per sediment dry weight) of both NP and MPs led to a reduction in the population of Naididae worms. Furthermore, a freshwater mesocosm study by Yıldız et al. (2022) also showed adverse effects. They reported a significant reduction in the biomass of daphnids, and a significant change in the wing morphology of chironomids following 7 weeks of exposure to MPs through water (polyethylene and polypropylene) and sediments (polystyrene, polyvinyl chloride, polyethylene terephthalate and polyamide) at the highest MP concentration of 20 mg L^{-1} (Yıldız et al., 2022). In a study by Foekema et al. (2022) marine pelagic and benthic communities, including fish, were exposed to 700 μm polystyrene spheres for 2 months using outdoor mesocosms. At the community level, no effects were observed on species richness and diversity. However, reductions in the condition index of fish (*Solea solea*) and the density of barnacles (*Semibalanus balanoides*) were found (Foekema et al., 2022). Lastly, Green (2016) conducted a study that exposed a marine benthic community to MPs. Exposure of polyethylene and biodegradable polylactic acid for 60 days resulted in a significant reduction in species richness and total number of organisms. Furthermore, the abundances of periwinkles (*Littorina* sp.) and isopods (*Idotea balthica*), as well as the biomass of clams (*Scrobicularia plana*), decreased at the highest tested concentrations of 80 μg L^{-1} (Green, 2016).

5.3. Determining effect thresholds and risks of microplastics based on community test data

When comparing the effect thresholds of studies for freshwater and marine populations and communities (Table 1), it becomes apparent that establishing safe limits for the occurrence of MPs in aquatic ecosystems is challenging. Some studies report effect thresholds in mass concentration, while others use number concentrations. Moreover, the characteristics of the tested MPs, the chosen exposure pathways and the selected exposure times greatly differ. Here, we provide an example of how environmental risks of MPs could be calculated using high-tier effect thresholds and compare the measured environmental concentrations of MPs in sediments, as reported by Redondo-Hasselerharm et al. (2023), with the PNEC calculated from the $NOEC_{population}$ for Naididae in Redondo-Hasselerharm et al. (2020). One way to account for the uncertainties and variability in the data is to use assessment factors (AF). This is a conservative approach to ensure that the estimated exposure levels are protective. However, currently, there are no widely accepted AFs specifically tailored for MPs. For this reason, we assessed MPs risks

Table 1
Microplastic effect thresholds collected from the literature for freshwater and marine populations and communities.

	Taxonomic group	Exposure pathway	Polymer type	Size and shape	Effect threshold	Concentration	Exposure time	Reference
FRESHWATER	POPULATIONS							
	Daphnia spp. (Genus)	Surface water	PE, PP	<500 μm (sphere, irregular)	NOEC	0.07 g/m^2	7 weeks	Yıldız et al. (2022) [6]
		Water column	PE	<500 μm (sphere)	NOEC	20 g/m^3		
		Sediment	PS, PVC, PA, PET	<500 μm (irregular)	NOEC	80 g/m^2		
	Chironomidae (Family)	Surface water	PE, PP	<500 μm (sphere, irregular)	NOEC	0.007 g/m_2		
		Water column	PE	<500 μm (sphere)	NOEC	2 g/m^3		
		Sediment	PS, PVC, PA, PET	<500 μm (irregular)	NOEC	8 g/m^2		
	Naididae (Family)	Sediment	PS PS	96 nm (sphere) 20–516 μm (irregular)	NOEC NOEC	5 g/kg 5 g/kg; 1.6 × 10^9 particles/kg	15 months	Redondo-Hasselerharm et al. (2020) [5]
	COMMUNITIES							
	Planktonic communities	Surface water	PE	12.5–500 μm (irregular)	HNOEC	22 × 10^4 particles/L	12 weeks	Marchant et al. (2023) [4]
			PLA	12.5–500 μm (irregular)	HNOEC	23 × 10^4 particles/L		
	Planktonic communities	Surface water	PEST	1–1.5 mm d = 15 μm (fiber)	HNOEC	50 particles/L	12 weeks	Klasios et al. (2024)
	Benthic communities	Sediment	PS PS	96 nm (sphere) 20–516 μm (irregular)	NOEC NOEC	5 g/kgI 5 g/kg; 1.6 × 10^9 particles/kg	15 months	Redondo-Hasselerharm et al. (2020) [5]
MARINE	**POPULATIONS**							
	Solea solea (Species)	Surface water	PS	700 μm (sphere)	NOEC	0.8 g/m^2	2 months	Foekema et al. (2022) [8]
	Semibalanus balanoides (Species)	Surface water	PS	700 μm (sphere)	NOEC	0.8 g/m^{2*}		
	Littorina sp. (Genus)	Surface water (mixed with microalgae)	PE	0.6–363 μm (irregular)	NOEC	80 μg/L	60 days	Green (2016) [7]
			PLA	0.48–316 μm (irregular)	NOEC	80 μg/L		
	Idotea balthica (Species)		PE	0.6–363 μm (irregular)	NOEC	80 μg/L		
			PLA	0.48–316 μm (irregular)	NOEC	80 μg/L		
	Scrobicularia plana (Species)		PE	0.6–363 μm (irregular)	NOEC	80 μg/L		
			PLA	0.48–316 μm (irregular)	NOEC	80 μg/L		
	COMMUNITIES							
	Pelagic and benthic communities	Surface water	PS	700 μm (sphere)	HNOEC	80 g/m^2	2 months	Foekema et al. (2022) [8]
	Planktonic communities	Surface water	PVC, PP, PS, PA	10–120 μm (irregular)	HNOEC	680 particles/ml	5 weeks	Ebbesen et al. (2024)
	Benthic communities	Surface water (mixed with microalgae)	PE	0.6–363 μm (irregular)	NOEC	80 μg/L	60 days	Green (2016) [7]
			PLA	0.48–316 μm (irregular)	NOEC	80 μg/L		

* Non concentration dependent.
Abbreviations: PA: polyamide; PE: polyethylene; PEST: polyester; PET: polyethylene terephthalate; PLA: polylactic acid; PP: polypropylene; PS: polystyrene; PVC: polyvinyl chloride; NOEC: no observed effect concentration; HNOEC: highest no observed effect concentration.

in two ways: 1) without the use of an AF (NOEC = PNEC), and 2) using an AF of 5, as recommended for mesocosm studies by the *Technical Guidance for Deriving Environmental Quality Standards* from the European Union for chemicals (European Commission Directorate-General for Health and Food Safety, 2017). The PNEC values were rescaled to encompass the entire MP size range (1–5000 μm), while also accounting for the polydispersity of environmental MP and the *bioaccessible* MP fraction (Koelmans et al., 2023b; Koelmans et al., 2020; Kooi et al., 2021; Mehinto et al., 2022; Redondo-Hasselerharm et al., 2023; Rico et al., 2023). Following earlier studies (Koelmans et al., 2023b; Redondo-Hasselerharm et al., 2023; Rico et al., 2023), the minimum, mean and maximum measured environmental concentrations of MPs in sediments were compared with the PNECs. These comparisons were repeated for two effect mechanisms: food dilution and toxicity triggered upon translocation, which are caused by the volume of the MPs ingested, and the surface area of the MPs, respectively. The *bioaccessible* MP fraction for volume was set at 1–130 μm based on the maximum ingestible size of particles reported for Tubifex spp., which belongs to the family Naididae (Juget, 1979; Redondo-Hasselerharm et al., 2023). For surface area, the selected *bioaccessible* MP fraction selected was 1–83 μm following previous studies (Koelmans et al., 2023b; Mehinto et al., 2022; Redondo-Hasselerharm et al., 2023; Rico et al., 2023). The rescaled PNECs were 2.68×10^{10} and 7.6×10^{10} particles/kg for volume and surface area when AF = 0, respectively. With AF = 5, the rescaled PNECs for volume and surface area were 5.5×10^{9} and 1.5×10^{10} particles/kg. When plotting the rescaled measured environmental concentrations of MPs in sediments against the rescaled PNECs, we observe that no risks are expected, whether using an AF = 0 or an AF = 5 (Fig. 2). Therefore, we can conclude that no risks are anticipated for freshwater benthic communities at the current environmentally realistic concentrations of MPs.

6. Standard test materials

One of the mentioned QA/QC criteria concerns the use of environmentally relevant plastic particles in tests, preferably standardized particles so that they remain consistent across treatments or when used by different laboratories. To ensure comparability of results across different laboratories, the development of standard reference materials is crucial (ACC, 2022; Dehaut et al., 2023; Martínez-Francés et al., 2023; Teague et al., 2021; Gouin et al., 2024). For instance, standard reference MP particles are essential in calibrating and validating analytical methods, as well as in studies investigating the effects of MPs on organisms. In the latter case, there are valid reasons for using either mono- or polydisperse distributions. While testing monodisperse plastics has provided valuable insights, offering the scientific community a mechanistic understanding of MPs, many researchers have recognized the need to account for the diversity of MPs in study design (Coffin, 2023; de Ruijter et al., 2020; Koelmans et al., 2023a; Lambert et al., 2017; Rochman et al., 2019; Thornton Hampton et al., 2022a). One of the solutions considered to address this key challenge is proposed by Bucci and Rochman (2022). They advocate the separate testing of various components of MPs, taking into account differences in shape, size and polymer type, to assess their potential hazards. However, although such monodisperse toxicity test results are beneficial for mechanistic research, their environmental relevance may be limited. Translating these results to the natural environment, where MP mixtures are polydisperse, is challenging and conducting separate tests for all possible combinations of characteristics would require extensive resources (Koelmans et al., 2023a). A more relevant and efficient approach is to assess the effects of environmentally realistic polydisperse particles. This approach allows for the evaluation of the causal link between exposure and effects by measuring the bioavailable MPs within the continuum of MPs. By using continuous probability distributions (i.e. probability density functions; PDFs) for shape, size and density from empirical data sets, the multidimensionality of MPs can be characterized

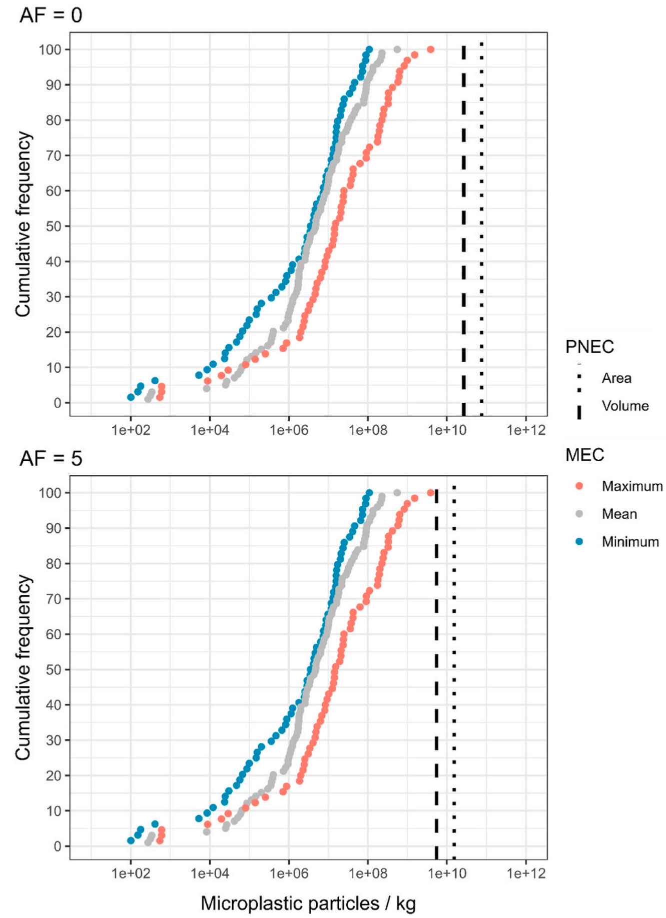

Fig. 2. Cumulative frequency distributions of the rescaled minimum, mean and maximum measured environmental concentrations (MECs) of MPs in freshwater sediments compiled by Redondo-Hasselerharm et al. (2023), plotted together with the predicted no observed effect concentration (PNEC) (dashed lines) derived from $NOEC_{population}$ from Naididae in Redondo-Hasselerharm et al. (2020) for volume (purple) and area (green) as ecologically relevant metrics (ERM) using no assessment factor (AF = 0) (upper panel) or an assessment factor of 5 (AF = 5) (lower panel). This higher tier community level risk assessment shows that there are no MEC values that exceed the PNEC values and thus no risks are expected for freshwater benthic communities at the current environmentally realistic concentrations. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

across various environmental compartments (Kooi and Koelmans, 2019). This new concept of using polydisperse, environmentally relevant microplastics (ERMP) in effect tests has been implemented in recent studies for a wide range of invertebrate species (de Ruijter et al. (2023); de Ruijter et al., 2025). These studies also provide detailed descriptions of the preparation and characterization of the ERMP test mixture (de Ruijter et al., 2025). Additionally, a step by step protocol for creating such a polydisperse ERMP test material is provided as Supporting information.

7. MP characteristics determining effects and mechanisms explaining effects

If single-species or community effect tests are well-executed and use relevant particles, the remaining question is how any observed effects can be explained and which particle properties are important in this context. The most important MP characteristics that determine effects are concentration, particle size, shape and type (Bucci et al., 2020; Kögel et al., 2020). Furthermore, numerous researchers have emphasized the importance of determining the appropriate units for reporting MP concentration in the context of understanding the effects of MPs (Bucci et al., 2020; Burns and Boxall, 2018; Connors et al., 2017; de Ruijter et al., 2020; Karami, 2017; Kögel et al., 2020). In previous studies, the choice between reporting mass or particle count concentration has complicated the comparison of toxicity studies (Thornton Hampton et al., 2022b). Recently, there has been a positive shift towards adopting these recommendations, with an increasing number of studies reporting both metrics (Thornton Hampton et al., 2022b). Consequently, Thornton Hampton et al. (2022b) assert that reporting mass and number concentration should be the minimum requirement. Additionally, metrics such as particle volume and surface area, which may be informative for certain toxicity mechanisms, should also be reported. Already since 2017, researchers noted that mechanisms behind adverse effects are poorly understood (Connors et al., 2017; De Sá et al.; Ogonowski et al., 2018). de Ruijter et al. (2020) found that most evidence supports the food dilution mechanism, but there is also evidence for mechanisms like internal physical damage, external physical damage and oxidative stress (de Ruijter et al., 2020). In a study conducted by Thornton Hampton et al. (2022b), researchers were instructed to explore the ToMEx database using their own expertise, knowledge and statistical tools and determine which MP characteristics are most explanatory for understanding MP effects. In line with the food dilution hypothesis, they found that particle volume was a statistically significant predictor of toxicity. Additionally, they found that surface area was an explanatory factor for toxicity of MPs. While MPs need to be relatively small to translocate from the gut to other tissues (Mehinto et al., 2022), once situated within the tissue, it is likely that the surface area correlates with the extent of inflammation and oxidative stress. However, these findings come with uncertainties as the data in the existing toxicity database was not thoroughly screened for quality before data analysis. Moreover, surface area and total plastic volume in the gut are often not comprehensively reported in toxicological studies (Thornton Hampton et al., 2022b).

While all 20 criteria proposed by de Ruijter et al. (2020) are crucial for testing the effects of MP, three criteria - namely “chemical purity”, “exposure assessment of organism” and “presence of food” - could enhance the strategic testing of mechanisms. For instance, assessing chemical purity helps distinguish the particle effects from the chemical effects. Moreover, studying how organisms are exposed to MP, preferably quantitatively, provides insight into the mechanisms behind adverse effects. Finally, the criteria focusing on the presence of food are equally important. If no food is provided during exposure to MP, it is remains unclear whether the effect is due to starvation of the organisms or the MPs themselves. Only a relatively small number of MP effect studies have incorporated all of these three criteria to a certain extent (Blarer and Burkhardt-Holm, 2016; Kalčíková et al., 2017; Lu et al., 2023; Murphy and Quinn, 2018; Qiao et al., 2019; Redondo-Hasselerharm et al., 2018; Sussarellu et al., 2016; Von Moos et al., 2012; Ziajahromi et al., 2017).

7.1. Natural particles as controls in MP effect tests

Scientists have often emphasized the importance of including natural particles in the experimental design as a control (Gerdes et al., 2019; Ogonowski et al., 2018; Ogonowski et al., 2023). Some argue that, to inform risks assessment, researchers need to demonstrate that the effects of MPs are indeed different from those of natural particles (Ogonowski et al., 2018). While including natural particles provides insight into the specific causes of toxicity to organisms and places the magnitude of toxicity in a broader context, it is not a prerequisite for determining risk. For instance, if negative effects due to food dilution by MP were to increase with rising plastic pollution, while natural inert particles cause similar negative effects, this would not diminish the adverse effects caused by the MPs. Nevertheless, investigating differences in material type, while keeping particle characteristics as similar as possible, enables the testing of mechanisms such as food dilution, where the volume determines toxicity. Until recently, studies have generally indicated that MPs may be slightly more toxic than natural particles (Gerdes et al., 2019; Ogonowski et al., 2018; Ogonowski et al., 2016; Redondo-Hasselerharm et al., 2018; Schür et al., 2020; Yap et al., 2020). However a recent meta-analysis by Ogonowski et al. (2023), comparing the toxic effects of MP and naturally occurring suspended solids (SS), indicated that there was no significant difference between the two types of particles. Researchers however, highlighted that the uncertainties, relating to systematic differences in experimental design such as unreported chemicals in MPs and the predominant use of pristine MPs, were substantial. This underlines the need for studies with a more targeted design (Ogonowski et al., 2023), such as for instance the design provided by (de Ruijter et al., 2025). Here a similarly diverse ERMP mixture was tested on *Lumbriculus variegatus* and compared to the effects of a mixture with a nearly identical particle size distribution, but composed of mineral particles, —specifically, an a priori designed mixture of ten clay, silt, and sand fractions of different particle sizes (de Ruijter et al., 2025). Overall, no differences in growth or reproduction were observed between the two mixtures of particle concentrations of up to 10% (v/v). However, after 14 days of exposure to 5% ERMP, the egestion of faecal pellets was higher compared to exposure to 5% ERMS, suggesting that in order to acquire the same amount of nutrition, *L. variegatus* is spending more energy.

8. Recommendations and prospect

We conclude that the quality of MP effect studies is gradually improving, and QA/QC practices are increasingly being adopted by MP researchers. However, there are specific aspects that require more attention. The most significant advancements in MP effect studies can be achieved through the improvement in the experimental design. Additional focus should be placed on enhancing the applicability to risk assessment and the ecological relevance of MP effect studies. For instance, diversifying the types of particles tested simultaneously could significantly enhance the reliability of MP risk assessment. While data rescaling and alignments are available, it is recommended to use environmentally relevant microplastic (ERMP) mixtures. In this regard, we have provided a step-by-step protocol for designing polydisperse test materials and a targeted approach to test differences between ERMP mixtures and equally diverse mixtures of natural particles. Given the constraints of limited resources, a focused emphasis on these achievable criteria strategically holds the potential for substantial improvements in enhancing risk assessment practices. Nevertheless, in order to produce reliable data for risk assessment studies should adhere to all QA/QC criteria.

The body of literature providing reliable data on the effects thresholds and mechanisms behind adverse effects is still limited, underscoring the need for more research to advance our understanding and accurately inform risk assessments. Additionally, it could provide insights into the specific organisms that are more sensitive to MPs. To achieve this, well-designed experiments focusing on mechanisms are needed, while adhering to strict QA/QC criteria. For instance, a comparative study between natural and MP particles with dimensions as closely aligned as possible, could provide insight into the mechanisms of food dilution, where volume determines toxicity.

Current risk assessments predominantly indicate that concentrations observed globally and within ecosystems, specifically in surface water, sediments, and soils, exceed the effect thresholds measured for MP toxicity in the laboratory. However, a higher-tier approach is presented here as an example, using data from Redondo-Hasselerharm et al. (2020), which reveal no significant risks for freshwater benthic communities at prevailing environmental MP concentrations. Nonetheless, it is important to note that only a limited number of mesocosm studies have been conducted, emphasizing the critical need for broader application of community effect testing and a more comprehensive higher tier risk assessment.

CRediT authorship contribution statement

Vera N. de Ruijter: Writing – review & editing, Writing – original draft, Visualization, Conceptualization. **Paula E. Redondo-Hasselerharm:** Writing – review & editing, Writing – original draft. **Albert A. Koelmans:** Writing – review & editing, Writing – original draft, Supervision, Conceptualization.

Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this work the author(s) used ChatGPT in order to improve grammar. After using this tool/service, the author(s) reviewed and edited the content as needed and take(s) full responsibility for the content of the published article.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Vera N. De Ruijter reports financial support was provided by European Chemical Industry Council. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envpol.2025.125711.

Data availability

No data was used for the research described in the article.

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