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Review

Contribution of chemical toxicity to the overall toxicity of microplastic particles: A review

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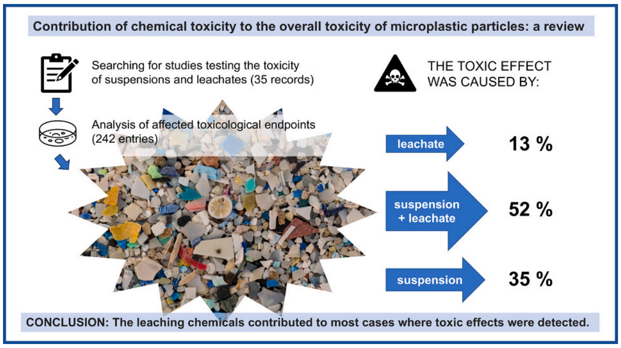
HIGHLIGHTS

- The physical toxicity and chemical toxicity vary widely under different conditions.
- The study revealed that 65 % of toxicity endpoints were due to chemical leaching from microplastics.
- 52 % of the cases showed that both suspension and leachate caused toxic effects.
- Factors such as leaching time and particle size greatly influence toxicity results.

GRAPHICAL ABSTRACT



Contribution of chemical toxicity to the overall toxicity of microplastic particles: a review

Searching for studies testing the toxicity of suspensions and leachates (35 records)

Analysis of affected toxicological endpoints (242 entries)

THE TOXIC EFFECT WAS CAUSED BY:

CONCLUSION: The leaching chemicals contributed to most cases where toxic effects were detected.

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ABSTRACT

Nanoplastics and microplastics are of growing research interest due to their persistence in the environment and potential harm to organisms through physical damage, such as abrasions or blockages, and chemical harm from leached additives and contaminants. Despite extensive research, a clear distinction between the physical and chemical toxicity of plastic particles has been lacking. This study addresses this gap by reviewing studies examining both toxicity types, focusing on environmentally relevant leachates. The chemicals used in plastics manufacturing, which number over 16,000, include additives, processing aids, and monomers, many of which pose potential hazards due to their toxicity, persistence, and bioaccumulation. Studies typically use extraction or leaching methods to assess chemical toxicity, with leaching more closely mimicking environmental conditions. Factors influencing leaching include plastic type, particle size, and environmental conditions. A systematic literature search identified 35 relevant studies that assessed the toxicity of plastic particle suspensions and their leachates. Analysis revealed that, in 52 % of the cases, both the suspension and leachate had toxic effects, while in 35 % of the cases, toxicity was attributed to the suspension alone. At 13 %, only the leachate was toxic. This suggests that leachates contribute significantly to overall toxicity. However, the results vary widely depending on the experimental conditions and plastic type, highlighting the complexity of microplastic toxicity. The preparation methods used for leachates significantly influence toxicity results. Factors such as leaching time, particle size, and separation techniques affect the concentration and presence of toxic chemicals. Additionally, washed particles—those subjected to procedures for removing leachable chemicals—often showed reduced toxicity,

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although the results varied. This underscores the need for standardized methods to compare studies better and understand the relative contributions of physical and chemical toxicity to microplastic pollution.

1. Introduction

In recent decades, microplastic particles have received increased attention, and considerable efforts have been made to elucidate their fate in the environment and their possible impacts on exposed organisms including humans (Tang et al., 2024). The prevailing consensus is that plastic particles can induce toxic effects via different mechanisms. Numerous studies have shown that exposure to these pollutants can physically damage organisms through potentially fatal injuries such as abrasions from sharp plastic fragments (Choi et al., 2021; Wright et al., 2013; Wright and Kelly, 2017) or blockages in the digestive system (Anderson et al., 2015; Fred-Ahmadu et al., 2020; Wright et al., 2013; Li et al., 2024). In addition, the physical presence of plastic particles has been linked to other suggested impacts, such as nutrient dilution, reduced feeding cues, reduced growth rates, inhibition of enzyme production, reduced steroid hormone levels, reproductive failure, or deposition of tiny plastic particles in tissues (Wright et al., 2013). Notably, this issue is always related to the experimental conditions, including organisms, endpoints, and types of microplastics. Numerous studies observed no toxic effects even after ingestion (e.g., Khosrovyan and Kahru, 2022).

Furthermore, plastic particles facilitate the exposure of organisms to various hazardous chemicals (Wang et al., 2020; Wright and Kelly, 2017) and pathogens (Zhong et al., 2023). Plastic materials are known to contain numerous intentionally (Gunaalan et al., 2020; Sridharan et al., 2022) and unintentionally (Muncke, 2009) added chemical compounds. All chemicals present in plastic materials have the potential to migrate from the product to the surrounding environment (Andrady, 2011; Gunaalan et al., 2020; Hahladakis et al., 2018), and many of them have been shown to have adverse effects on living organisms (e.g., metal ions, bisphenols, phthalates or brominated flame retardants) (Gunaalan et al., 2020).

Despite the common categorization of the adverse effects of microplastics and nanoplastics on physical and chemical toxicity, to our knowledge, there is no review summarizing the results of studies that allow us to distinguish between these two mechanisms of toxic behavior, i.e., to simultaneously test the chemical and physical toxicity of identical plastic particles. Therefore, we aimed to identify studies that evaluate the toxicity of microplastics and nanoplastics and their environmentally relevant leachates, compare the methodologies and results, and determine whether physical or chemical properties cause the observed adverse effects. Therefore, only studies with an observed toxic effect were included. This analysis includes a brief introduction to this topic, given the importance of the leaching of chemical compounds from plastics.

2. Chemical substances in plastic and their release and toxicity

A recent report addressed the chemicals used in plastic manufacturing and estimated that >16,000 different chemicals are used in this field. From the functional point of view, the most abundant functional classes are colorants, processing aids, fillers, intermediates, and lubricants (each accounting for >1500 chemicals). The remaining well-known classes account for a much smaller fraction of chemicals (plasticizers 883, antioxidants 478, and flame retardants 389). Regarding safety, only <6 % of these substances are currently subjected to global regulation, and hazard information is not available for 66 % of them. Among the categorized substances, there are >4200 plastic chemicals of concern that are persistent, bioaccumulative, mobile, and/or toxic (Wagner et al., 2024). Among the plastic materials used to manufacture products, base polymers are usually combined with various additives, specifically chemicals, to improve their functional properties (e.g., performance during the manufacturing process, functionality, or aging properties). The most commonly used additives for plastic materials include plasticizers, flame retardants, antioxidants, acid removers, light and heat stabilizers, lubricants, pigments, fillers, antistatic agents, slip agents, and heat stabilizers (Hahladakis et al., 2018). The content of some additives may be a few percent by weight of the material (e.g., odorants, biocides, antistatic agents, antiozonants, or dyes), while others account for a much larger share (stabilizers up to 8 %, flame retardants 10 to 20 %, fillers up to 50 %, plasticizers 10 to 70 %) (Andrady and Rajapakse, 2019; Gunaalan et al., 2020). In addition to other chemicals, plastics also contain starting substances used for initial polymerization (e.g., monomers and catalysts). These are present either as a result of incomplete polymerization during the formation process or due to their release during material degradation. In addition, plastic materials contain unknown amounts of unintentionally added toxic substances, such as impurities of starting materials and additives or intermediate and degradation products generated during processing (Muncke, 2009; Khosrovyan et al., 2022). Due to these diverse sources of plastic chemicals, even additive-free virgin plastics cannot be considered safe in terms of chemical toxicity. All of these chemicals present in the plastic material can potentially leach out of the product (Andrady, 2011; Gunaalan et al., 2020; Hahladakis et al., 2018), as they are not chemically bound to the polymer matrix but can migrate to the surface of the plastic and further into the surrounding environment (Gunaalan et al., 2020).

Although studies testing the chemical toxicity of plastic particles often cite adsorbed contaminants from the environment as one source of toxicity (Bridson et al., 2021; Coffin et al., 2020; Teuten et al., 2009), available modeling has shown that biota exposure to contaminants from this source is negligible compared to exposure from natural prey (Bakir et al., 2016; Bridson et al., 2021; Koelmans et al., 2016). Recent research has focused primarily on the risks posed by additives. The toxic effects of chemicals released from plastics have been documented in many organisms, from bacteria to vertebrates, with endpoints including effects on survival and development, reproductive effects, oxidative stress, and transcriptional changes (Bridson et al., 2021). The chemical toxicity of a wide range of plastic products has been tested by Lithner et al. (2009) and Zimmermann et al. (2019), among others.

Either extracts or leachates are usually used to assess chemical toxicity and potential risks of plastic pollution. Plastic extracts are prepared under extreme conditions, such as organic solvents and elevated temperatures, to ensure the highest recovery of the broadest possible range of substances. Conversely, leaching does not involve extreme conditions, as it aims to simulate realistic environmental exposure conditions (Bridson et al., 2021). Both approaches provide valuable information, as extraction studies identify and quantify chemicals in plastic materials and indicate the highest possible hazard level, while leaching experiments allow the study of physical availability and, subsequently, environmentally relevant doses and exposures (Cummings, 2019). As this study aimed to compare the toxicity of plastic suspensions with solutions containing chemicals released under environmentally relevant conditions, only studies using the leaching method were included.

As leachate preparation is an important process that substantially influences the results of studies using this approach, several reviews have focused on this issue. For example, a review on leachate preparation for toxicology studies has previously been published (Almeda et al., 2023). The kinetics of additive release from plastic particles have been summarized in detail in a review by Do et al. (2022). In addition, Delaeter et al. (2022) reviewed leachate toxicity research with a focus on leachate toxicity to marine microbes and invertebrates. Although the topic of plastic leachate toxicity has recently received considerable attention, analyses of the contribution of leachates to the overall toxicity of suspended plastics are still lacking.

The sorption and desorption of organic compounds between plastic particles and the environment depend on the properties of the specific plastic material, the chemical compound, and various environmental factors (Do et al., 2022). The determining properties of plastic particles are plastic type, morphology, and other physicochemical properties, including molecular weight, crystallinity, glass transition temperature (Poças et al., 2008), and internal pore size. The properties of additives and chemicals that affect their leachability include polymer–additive bond strength, molecular weight, and hydrophobicity (Qiu et al., 2022). Determining environmental factors include pH, salinity, dissolved organic matter, temperature (Do et al., 2022), and flow conditions (Henkel et al., 2023). Leaching is further enhanced by weathering forces and fragmentation of plastic products into smaller particles (Gunaalan et al., 2020; Luo et al., 2019), where weathered plastics have been shown to be more toxic than corresponding new materials (Gewert et al., 2021). Leachates produced under ultraviolet (UV) light have also been described to be more toxic than leachates prepared in the dark (Klein et al., 2021a).

3. Literature search and data processing

The relevant data sources were identified by searching the Web of Science and Scopus databases on 2.5.2024 using the following keywords: (microplastic* OR nanoplastic* OR “micro plastic*” OR “nano plastic*”) AND toxic* AND leachate* and (microplastic* OR nanoplastic* OR “micro plastic*” OR “nano plastic*”) AND toxic* AND migrate*. All abstracts (287 records) were examined, and only original papers assessing the toxicity of micro/nanoplastic suspensions, together with their leachates and full-text documents available online, were retained for further analysis.

After thoroughly reading the articles, several studies were excluded because it was not possible to compare the toxicity of the suspension and

Table 1
Summary of 35 studies analyzed in this review.

Publication	Plastic type	Particle size	Organism	Exposure time [days]	Leaching time [days]
Zhao et al., 2017	PS	108.2 ± 4.5 nm	*Caenorhabditis elegans*	4.5	7
Martínez-Gómez et al., 2017	HDPE, PS	HDPE 0–80 μm, PS 6 μm	*Paracentrotus lividus*	2	30
Kalčíková et al., 2017	PE	71.30 ± 34.29 μm, 96.00 ± 69.99 μm	*Lemna minor*	7	7
Khan et al., 2019	Tire wear	<1 μm	*Hyalella azteca*	2	2
Thomas et al., 2020	PMMA, PS	PS 10, 80, 230 μm; PMMA 10, 50 μm	*Paracentrotus lividus*	0.042; 3	30
Trestrail et al., 2020	Phenolformaldehyde	170.4 ± 147.5 μm	*Physa acuta, Bembicium nanum, Mytilus galloprovincialis, Daphnia magna, Allorchestes compressa, Artemia* sp.	3	1
Piccardo et al., 2020	PET	18 ± 14; 151 ± 126; 928 ± 450 μm	*Vibrio fischeri, Phaeodactylum tricornutum, Paracentrotus lividus*	3	3
Boyle et al., 2020	HDPE, PET, PVC	PVC 152.4 ± 37.6 μm, HDPE 297.9 ± 51.6 μm; PET 257.7 ± 67.4 μm	*Danio rerio*	1	1
Zimmermann et al., 2020	PLA, PUR, PVC	<59 μm	*Allivibrio fischeri, Daphnia magna*	21	23
Zhang et al., 2020	PVC	0.1 mm	Microbial community	15	15
Klein et al., 2021b	PLA	≤150 μm	*Lumbriculus variegatus*	28	1
Koski et al., 2021	Tire wear	8–20 μm	*Acartia tonsa, Temora longicornis*	1	Not stated
Halle et al., 2021	Tire wear	210 ± 116 μm, 176 ± 120 μm	*Hyalella azteca*	2	2
Rozman et al., 2021	Bakelite, PE, PET, tire wear	7.64–652 μm	*Lemna minor*	7	7
Klun et al., 2022	Bakelite	7.64 ± 3.48 μm	*Daphnia magna, Lemna minor, Allivibrio fischeri, Pseudokirchneriella subcapitata*	0.021; 1; 2; 3; 7	0.021; 1; 2; 3; 7
Esterhuizen et al., 2022	HDPE	4 mm ± 1 mm	*Lolium multiflorum*	7	3
Chibwe et al., 2022	Tire wear	1.7 μm - 1.7 mm	*Pimephales promelas*	4,5	1; 3; 10
Song et al., 2022	PE	5–30 μm	*Daphnia magna*	21	0,042
Yang et al., 2022	Tire wear	41 μm	*Tigriopus japonicus*	4	60
Bucci et al., 2022	PE, PP	150–500 μm	*Fathead Minnow*	14	1
Ding et al., 2022a	PA, PE, PP, PS, PVC	30 μm	*Enchytraeus crypticus*	14	10
Ding et al., 2022b	tire wear	225.6 μm	Microbial community	35	10
Zhang et al., 2022	PE, PET, PVC	0.1 mm	Microbial community	Not stated	Not stated
Wang et al., 2022	PS	0.5; 1; 10; 50; 75; 150 μm	Microbial community	13	13
Boháčková et al., 2023	PET, PVC	25; 90 μm	*Oncorhynchus mykiss*	1	0.042; 7; 14
Z. Ni et al., 2023	PS	0.1 μm; 1 μm	*Skeletonema costatum*	4	30
Détrée et al., 2023	Acrylic fibres, nylon fibres (PA), polyester fibres	100 ± 55 μm (average length)	*Crassostrea gigas*	4	9
Roubeau Dumont et al., 2023	Tire wear	<0.2 μm	*Chlorella vulgaris, Lemna minor, Daphnia magna*	2; 7	7
Liu et al., 2023	Face masks	247–450 nm	*Escherichia coli*	0,0833	35
Shao et al., 2023	PLA	2.86 ± 0.46 μm	*Caenorhabditis elegans*	4.5	7
Wang et al., 2023	PE, PET, PLA, PVC	150 μm	Microbial community	38	4
X. Ni et al., 2023	Tire wear	<78 μm	*Eriocheir sinensis*	14	42
Thomsen et al., 2024	Tire wear	<100 μm	*Mytilus edulis*	3; 21	3
Caballero-Carretero et al., 2024	Tire wear	82.3 ± 40 μm	*Chironomus riparius*	1	14
Liu et al., 2024	PE	100 nm	*Caenorhabditis elegans*	4.5	7

leachate due to the different treatments or experimental setups. The most frequent reasons for exclusion were the use of plastic particles of different sizes for suspension and leachate preparation (Beiras et al., 2019; Oliviero et al., 2019; Parlapiano et al., 2022; Schiavo et al., 2020) and the use of resuspended particles after leaching for testing the toxicity of suspensions (these particles contain a decreased amount of leachable chemicals, so the toxicity of a suspension prepared in this way can be decreased) (Cunningham et al., 2022; Ekvall et al., 2019). On the other hand, several studies have tested the toxicity of resuspended/washed particles together with the toxicity of suspensions and leachate, and the results of these experiments are described in Section 7. Studies involving aged particles were included only if the criteria for the comparability of leachates and suspensions were met. After this selection, we obtained 35 articles for inclusion in our analysis (see Table 1). For detailed information about selected articles, see Table S1.

For the analysis of the contribution of leaching chemicals to suspension toxicity, a table summarizing the toxicological endpoints that could be and were tested for both the particle suspension and the leachate was created (i.e., the data regarding particle translocation, particle localization, or defecation cycle length were excluded). Only endpoints significantly affected by at least one exposure scenario were included, which required the respective authors to statistically test the significance of the effects against the respective controls. Data from sequencing or microchip/sensor arrays were excluded due to their complexity. The table was arranged so that every row contained information on one toxicological endpoint analyzed for one type of plastic on one organism/cell type, resulting in a total of 242 entries (see Table S2). Furthermore, every entry contained information on whether the significant effect was recorded following exposure to the suspension, leachate, or both (in yes/no format). If multiple concentration levels of both the leachate and the suspension were tested, the lowest observed effective concentrations were also listed.

All analyses were performed using Microsoft Excel software (Microsoft Corp., USA), and the graphs were generated using OriginPro 2019 software (OriginLab Corp., USA).

4. Differentiating between the chemical and physical toxicity of tiny plastic particles

After analyzing the available data, the percentages of toxicological endpoints significantly affected by plastic suspension, leachate, and both were summarized for each study (Fig. 1). As the toxicity of suspension and leachate is strongly dependent on the specific material and exposure conditions, the percentage of significantly altered endpoints under different scenarios was first calculated for each study separately and then averaged to describe the general trend. As expected, the results substantially differed among the included studies; however, the averaged data showed that in 52 % of the observed endpoints, the effect was caused by both the leachate and suspension; in 35 % of the cases, the adverse effects were attributed to suspension only; and in 13 % of the cases, the toxic effect was caused by leachate only. According to these results, leachate caused toxicity at 65 % of the recorded endpoints.

To our knowledge, we are the first to apply this approach to process the data for suspension and leachate toxicity.

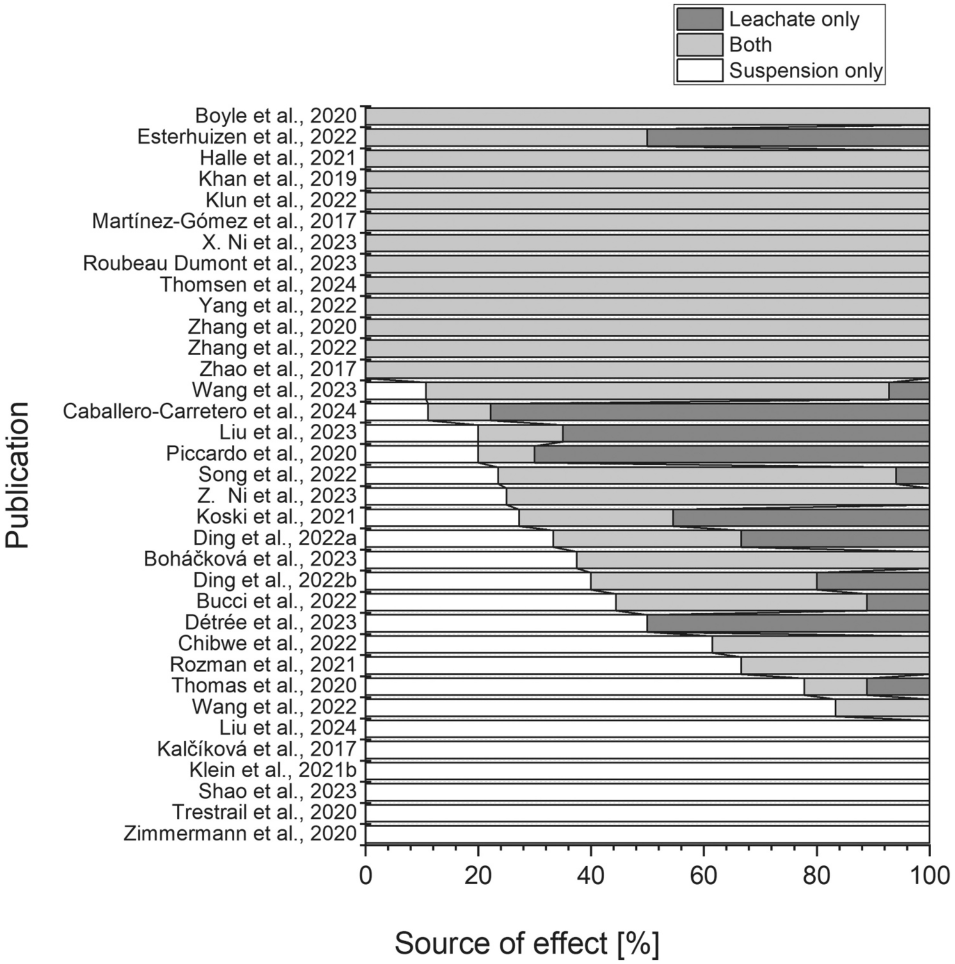

Fig. 1. The contribution of leaching chemicals to the toxicity of suspensions containing plastic micro- and nanoparticles. For each of the listed studies, the percentage of toxicological endpoints that were significantly affected by the suspension only, leachate only, or both was determined.

The toxicity and leaching results strongly depend on many parameters, such as the material used (polymer composition, present additives, age, storage conditions, and particle size) or leachate preparation (leaching time, solvent composition, and temperature). The design of the included studies was also very diverse. Unfortunately, there are not enough data available for reliable statistical evaluation of the contributions of the individual parameters. However, as seen from the broad and diverse list of tested organisms (see Table 1), this effect is not specific to only a few species.

4.1. Suspension and leachate toxicity

In the most common scenario (occurring in 28 studies, 52 % of endpoints), a toxic effect was observed after exposure to the suspension as well as after exposure to the leachate. As the suspension contains the particles together with the leachate, these results suggest that a substantial portion of nano- and microplastic-related toxic effects are related to the leaching of chemical substances.

From studies where both the suspension and the leachate caused toxic effects, ten studies tested more than one concentration of the suspension and the corresponding leachate, enabling a comparison of the lowest effective concentrations measured using the same experimental design. However, the results are highly heterogeneous. In several studies (Caballero-Carretero et al., 2024; X. Ni et al., 2023; Yang et al., 2022; Zhang et al., 2020; Zhao et al., 2017), the leachates exerted lower toxicity than did the corresponding suspensions. In other studies (Boháčková et al., 2023; Martínez-Gómez et al., 2017), the leachate habitually caused an equal level of toxicity. In Bucci et al. (2022), the lowest toxic leachate concentration was the same or lower than the lowest toxic concentration of the particle suspension. Furthermore, according to Halle et al. (2021) and Khan et al. (2019), leachate was more toxic at low concentrations; however, the suspension appeared more toxic at higher concentrations. This finding highlights only the variability of plastic particle toxicity and its underlying mechanisms. It should also be mentioned that the range and exact concentration often differed even when more than one concentration of the suspension and the corresponding leachate were tested in the study. For example, and X. Ni et al. (2023) applied suspension concentrations of 50 and 500 mg/l, while leachate concentrations of 10, 100, 1000, and 3000 mg/l were used.

Moreover, this comparison can be complicated by the fact that several studies have reported that the toxic effects of plastic suspensions or their leachates are not concentration-dependent (Bucci et al., 2022; Caballero-Carretero et al., 2024; Kalčíková et al., 2017; Koski et al., 2021; Martínez-Gómez et al., 2017; Thomas et al., 2020). Martínez-Gómez et al. (2017) attributed this nonconcentration-dependent toxicity of suspensions to the aggregation of particles at higher concentrations.

4.2. Leachate-only toxicity

Occasionally (in 12 studies, 13 % of endpoints), only the leachate treatment caused a toxic effect. As the leachates were not prepared using any type of acceleration (heat, organic solvent, or UV radiation), the leached additives are supposed to also be present in the suspension together with plastic particles, and the leachate was not expected to be more toxic than the suspension. However, in several cases, the explanation for the lower toxicity of the suspension could have been found in the experimental design, specifically in the leachate preparation.

Several studies have reported a long leaching time compared to the exposure time during the experiment (Caballero-Carretero et al., 2024; Détrée et al., 2023; Liu et al., 2023; Thomas et al., 2020). Under such conditions, the concentration of released chemicals in the suspension is substantially lower than that in the leachate, where the compounds have more time to leave the plastic matrix and enter the liquid phase, with lower toxicity as a logical consequence. In the remaining studies, the leaching time was equal to the exposure time (Piccardo et al., 2020), not reported (Koski et al., 2021), or shorter than the exposure time.

Furthermore, even when the leaching time equals the exposure time, the effective concentration of the released additives is not identical because in the leachates, the maximum concentration is present from the beginning of the exposure, while in the suspension (when prepared immediately before the experiment), the concentration gradually increases until equilibrium is established, and for some period, the organism is exposed to lower concentrations or no additives at all. However, it has been reported that a substantial amount of additive is released during the first 24 h of leaching at 25 °C (Luo et al., 2019).

4.3. Suspension-only toxicity

For 35 % of the endpoints (reported in 22 studies), toxic effects were observed only after exposure to microplastic suspensions (containing particles together with the leachate), while leachate treatment caused no toxicity. The absence of leachate treatment toxicity suggests that under the applied experimental conditions, the recorded toxicity was caused by the physical properties of the plastic particles. The lower representation of suspension-only toxicity suggests that in the included studies, physical toxicity is a minor concern compared to the chemical toxicity of leachates.

Furthermore, in some cases, the results can be attributed to the applied methodology rather than exclusively physical toxicity. For example, in Klein et al. (2021b) and Trestrail et al. (2020), the applied leaching time was 24 h, whereas the exposure times in toxicological tests were 24 and 3 days. During this period, more additives may have been released, and the nontoxicity of these samples is insufficient to exclude the leachate contribution.

5. The leachate preparation

As mentioned previously, the preparation procedure greatly affects the toxicity of the tested leachates. Although no studies enhancing the leaching process using heat, organic solvents, or UV radiation were included, many other variables influence toxicity. The most apparent (and certainly the most discussed in this review) is leaching time. Among the studies included in this review, the leaching time ranged from 30 min (Klun et al., 2022) to 60 days (Yang et al., 2022). In 12 studies (34 %), the leaching time corresponded to the exposure time, allowing the best comparability between suspension and leachate toxicity.

Another aspect worth mentioning is that, in most of the studies, even though the leaching time is specified, it is not specified when the particle suspension was prepared with respect to the beginning of the toxicological experiment; therefore, the “leaching time” for the suspension is unknown. The time particles spend in liquids before experiments may substantially affect their toxicity. However, it is difficult to compare the exposure of particles to different solutions and washes during particle preparation or filtering (as is often not documented) and previous exposures of plastic objects used for particle preparation. The same applies to the storage conditions of commercially available suspensions.

In terms of separating the leachate from the particles, the most frequently applied method is filtration using filters with different pore sizes. This method was used in 29 studies, and the pore size was usually smaller than 1 μm. In the remaining cases, centrifugation (5 studies) or sieving (1 study) was used. According to our opinion, this step can also affect the toxicity of leachate, as there can still be particles present in the leachate, either because of imperfect separation using centrifugation or simply because of the presence of particles smaller than the pore size diameter. It is not common practice to inspect the leachate for the presence of nanoplastics.

Other important factors described in the literature that influence leaching are the particle size, solid-to-liquid ratio, and mixing conditions (Almeda et al., 2023).

6. Other trends in the reviewed literature

Alongside the exponentially growing number of published articles concerning plastic particles in the last two decades, it is possible to observe the development of different approaches in this research field as well. For example, testing the toxicity of plastic leachates together with their suspensions appears to be a rather recent trend, as the oldest studies included in this review were published in 2017, and 89 % of the included studies were published in 2020 or later.

Furthermore, the plastic-type distribution in the studies included in this review is substantially different from the plastic-type distribution of microplastic toxicity studies summarized in a review from 2020, wherein >58 % of the reviewed studies used microplastics in the form of a sphere or a bead, which do not frequently appear in environmental samples and are considered less environmentally relevant (De Ruijter et al., 2020). On the other hand, only 26 % of the studies included in this review used spherical particles, and the irregularly shaped particles applied in 60 % of the studies strongly dominated (see Fig. 2). This also relates to the lower representation of PE and PS particles, which are usually commercially available in the form of microparticles. In 1 study (using 5 types of particles), the shape of the particles was not specified.

Another documented change is the increase in publications reporting the concentration in mass units as well as the particle count (37 % of studies). According to a 2020 review, only 17 % of studies at that time used both units to describe concentration (De Ruijter et al., 2020). This is a recommended approach to increase the comparability of results with other toxicological studies, with mass units as the prevailing format for the expression of particle concentrations and simultaneously allowing the comparison of applied particle concentrations with the concentration of plastic particles found in environmental samples that are usually reported in the particle count.

7. The toxicity of the washed particles

In addition to suspension and leachate toxicity, several studies (7 in total) included in our review tested the toxicity of suspensions of particles that had been subjected to some type of washing and were, therefore, expected to contain reduced amounts of leachable additives. Different approaches were applied to create these "additive-depleted" particles (see Table 2). In 3 studies (Boháčková et al., 2023; Klun et al., 2022; Roubeau Dumont et al., 2023), the washed particles were created as a byproduct during leachate preparation (after leaching, the leachate was separated from the particles, and both fractions were used in the toxicological analysis). The study of Boyle et al. (2020) involved different washing scenarios; some were similar to the preparation of leachate (but not the same), and some applied harsh conditions in the form of ethanol, nitric acid, or surfactants. In the three remaining studies (Klein et al., 2021b; Trestrail et al., 2020; Zimmermann et al., 2020), organic solvents were used for the washing procedure.

The procedure used when washing the particles can greatly impact the particle properties. When a more aggressive organic solvent is used, the chance of completely removing free chemicals is more pronounced. However, it can be accompanied by changes in the size and number of washed particles (Zimmermann et al., 2020). These processes further modify the toxicity of the studied particles and complicate the comparison between the toxic effects of the original and washed particles. On the other hand, when only a soft washing method is used, there is a high probability that the leachable chemical compounds have been removed only from the particle surface. After a more extended period of leaching or under different conditions, the remaining amount of chemical compounds starts moving from the center of the particle to its surface and into the media (Gunaalan et al., 2020). The size of the washed particles also greatly impacts the washing efficiency (Boháčková et al., 2023).

When testing the toxicity of the washed particles, the results varied. In summary, 75 % of the monitored endpoints remained affected after the wash. In 4 studies (Boháčková et al., 2023; Boyle et al., 2020; Klun et al., 2022; Roubeau Dumont et al., 2023), the toxicity of washed particles decreased to some extent, while in 3 studies (Klein et al., 2021b; Trestrail et al., 2020; Zimmermann et al., 2020), the suspension of washed particles induced the same toxic effects as the original suspension.

The most substantial decrease in the toxicity of washed particles was described by (Roubeau Dumont et al., 2023), where the number of affected toxicological endpoints decreased by >80 %. Even though this study used the membrane with the smallest pore size (~ 1.5 nm) for the filtration step, ensuring greater efficiency in removing the tiny plastic particles, the most critical factor influencing the toxicity of the washed particles appeared to be either the exposed biological species or some differences in test conditions for these two species. Specifically, the washed particles did cause acute toxicity in *Daphnia magna*; however, they did not influence *Lemna minor* at any of the five endpoints. In this case, the results cannot be attributed to the different leaching times (2 days in the test with *Daphnia magna*, 5 days in the test with *Lemna minor*), as the toxicity toward *Daphnia magna* was recorded after a shorter exposure time. When testing the toxicity of washed bakelite microparticles (Klun et al., 2022), the particles did cause toxic effects at all endpoints except for the *Allivibrio fischeri* test. These results can be either species-specific or caused by the fact that the test with *A. fischeri* has the shortest exposure time (30 min compared to the range from 24 h

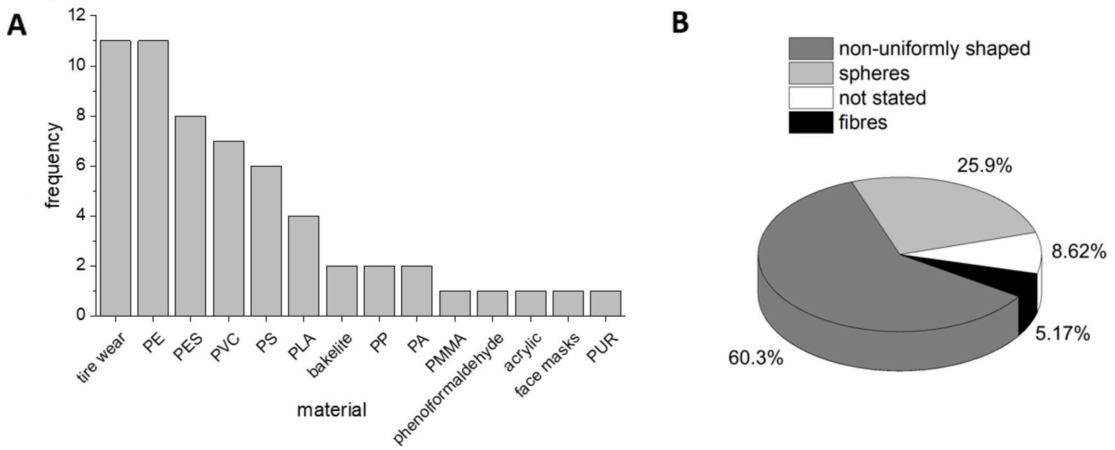

Fig. 2. Material (A) and shape (B) distributions of the tested plastic particles (58 records from 35 publications). PE = polyethylene, PVC = polyvinyl chloride, PS = polystyrene, PES = polyester, PLA = polylactic acid, PP = polypropylene, PA = polyamide, PMMA = polymethylmethacrylate, PUR = polyurethane

Table 2
The preparation procedure of the washed particles and the effect of this wash on the toxicity of their suspension.

Publication	The preparation of washed particles	Washing solvent strength	Number of endpoints where the washed particles were no longer toxic
Boháčková et al., 2023	1 day, 7 days and 14 days in aqueous exposure media at 4 °C, separated by centrifugation	Low (same as during leachate preparation)	4/8
Boyle et al., 2020	Incubation in wash solution (water, 2 % nitric acid, 2 % detergent Neutracon, or 100 % ethanol) at room temperature, filtration through 100 μm and removal of wash solution with water	Different scenarios with low or high wash intensity	1/4
Klein et al., 2021b	Incubation in methanol at room temperature for 24 h on an orbital shaker (100 rpm), vacuum-filtration through 0.2 μm filter, drying at 30 °C in the dark for 3 days	High	0/1
Klun et al., 2022	Incubation under the same conditions as during ecotoxicity tests with different organisms (different aqueous media, 0.5–168 h), filtration through 0.22 μm filter, drying at room temperature for 24 h	Low (same as during leachate preparation)	1/5
Roubeau Dumont et al., 2023	Incubation in aqueous media (hard water, pH 7, stirring) at room temperature in the dark for 1 week, filtration using a 20 kDa filter (~ 1.5 nm)	Low (same as during leachate preparation)	5/6
Trestrail et al., 2020	Soaking in solvent at 250 rpm at 25 °C for 1 h, vacuum filtration through 0.22 μm filter (repeated three times with water, 50 % methanol and water), drying to constant weight at 40 °C	High	0/1
Zimmermann et al., 2020	Sonication in methanol at room temperature for 1 h, vacuum-filtration through 1 μm filter, drying at 30 °C for 24 h	High	0/3

to 168 h in the remaining tests). It is possible that in this particular test, the chemicals did not have enough time to leach from the inside of the particles.

On the other hand, in our previous publication (Boháčková et al., 2023), the toxicity of the washed particles seemed to be substantially dependent on the particle size. Although the 90 μm washed PVC particles did not have any toxic effect on the fish cells after 24 h of exposure, the 25 μm washed PVC particles had the same toxic effect as the suspension of identical particles before washing, probably due to their larger surface area and faster desorption of chemical compounds.

No apparent trend was detected in terms of connecting the strength of the washing solvents to the decrease in toxicity. In the study of Boyle et al. (2020), several washing solvents with different strengths were applied (ultrapure water, 2 % nitric acid, 2 % detergent, and 100 % ethanol), and only washing with HNO_3 mitigated the toxic effects observed on *Danio rerio*. However, all the remaining studies reported a decrease in the toxicity of washed particles when only solvents with low strength (aqueous media) and no organic solvents were used.

8. Conclusions

The concept of differentiating between the chemical and physical toxicity of tiny plastic particles is frequently cited in the literature. However, a comprehensive review of this problem is still lacking. Therefore, relevant publications testing the toxicity of nano- and microplastic suspensions and their environmentally relevant leachates (prepared without elevated temperature or organic solvent) were identified, and the results of significantly changed toxicological endpoints in this study were summarized.

In summary, 65 % of the toxicological endpoints could be attributed (also) to the toxicity of the leaching chemicals, suggesting that chemical toxicity is of greater concern among the reviewed studies. Among studies also testing the toxicity of washed particles, no trend was discovered to connect the strength of the washing solvent with the decrease in toxicity. However, only one study completely removed the toxic effect of the tested particles by applying a wash with 2 % nitric acid.

Regarding the general trends in plastic particle toxicity research, we recorded some improvements in terms of environmental relevance. First, an increasing number of studies have reported both the particle concentrations regarding the particle count and mass units. This approach is recommended because it allows for the comparison between particle concentrations used in toxicological studies and concentrations detected in the environment. Second, a large number of studies have used realistically shaped plastic particles. This is a positive trend; however, it is impossible to determine whether this trend is general or specific for studies dealing with realistic leachates only.

We further conclude that the leaching time and exposure substantially affect the toxicity of plastic particles and their leachates. When the experiment is designed to differentiate between the toxicity of a plastic suspension and leachate, the leaching time is recommended to be equal to the exposure time (under the same conditions) to allow the release of comparable amounts of additives. However, nano- and microplastics are very diverse, and many factors are known to influence the toxicity of their suspensions and leachates. Unfortunately, there are not enough data yet to describe and statistically evaluate the influence of these factors individually.

We would like to emphasize the need for further research in performing comprehensive and detailed chemical analysis to elucidate the causal aspects of the chemical compounds present in plastic particles, especially using non-targeted analytical approaches.

CRediT authorship contribution statement

Jana Boháčková: Writing – original draft, Methodology, Formal analysis, Data curation, Conceptualization. **Tomáš Cajthaml:** Writing – review & editing, Supervision, Funding acquisition, Conceptualization.

Declaration of competing interest

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.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2024.177611.

Data availability

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

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