Toxicity of Microplastics and Nanoplastics to *Daphnia magna*: Current Status, Knowledge Gaps and Future Directions

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Abstract

We conducted a systematic review of 124 published articles that investigated the toxicity of microplastics and nanoplastics to *Daphnia magna*. This review summarizes studies assessing acute, chronic, and multigenerational impacts, as well as the effects observed via leached chemicals from plastics and the role of plastics as contaminant vectors. Overall, observed toxicity varies across different polymer types, and shapes. One of the most visible findings is that targeted research synthesis of the acute toxicity tests found more toxicity in smaller-sized particles. Most studies use spherical plastics that are commercially available, especially polystyrene, while the use of irregular-shaped and/or secondary plastics is still emerging. Also, there are still various confounding factors that make the comparison of the observed results difficult. Future studies should focus on irregular-shaped particles, and other polymer types, besides polystyrene. More research efforts are needed to understand the impacts of environmental factors and complex matrices.

List of Abbreviations

ABS	Acrylonitrile butadiene styrene
EAA	Ethylene acrylic acid
EPA	Environmental Protection Agency, USA
HDPE	High density polyethylene
LDPE	Low-density polyethylene
OECD	Organization for Economic Co-operation and Development
PE	Polyethylene
PET	Polyethylene terephthalate
PLA	Polylactic acid
PMAA	Polymethacrylic acid
PMMA	Polymethyl methacrylate
PP	Polypropylene
PPDO	Polydioxanone
PS	Polystyrene
PVA	Polyvinyl alcohol
PVC	Polyvinyl chloride

1. Introduction

Since the discovery of plastics in the 1900s[1], their durability and low cost have led to excessive usage and disposal of plastic products resulting in about 400 million metric tons of plastic waste annually.[2] Only ~9% of the waste is recycled while most of the remaining unrecycled plastics end up in landfills or in the ocean.[3] Plastic debris was first observed in the oceans in the 1960s. At that time, they were considered biologically inert. Their effects in the marine environment were merely reported as reduced aesthetic values by the tourism industry, and seen as obstruction hazards for shipping and energy production processes.[4] Since then, plastic waste have been found in water bodies and published literature has consistently classified plastics to be the most abundant anthropogenic material in marine bodies.[5-9] The task of quantifying plastic debris in the ocean is often complicated and unrealistic. Eriksen *et al.*[10] used an oceanographic model analysis which predicts that at least 5.25 trillion plastic particles weighing over 250,000 tons are floating over the sea. In fact, it is estimated that roughly 10% of produced plastics end up in the ocean[11,12] and other reports suggest that there may be more plastic than fish in the ocean by 2050[13].

Marine plastic pollution stems from direct routes (e.g., release from wastewater treatment plants, run off from tourism, recreational and commercial fishing activities, waste from plastic production facilities) or indirect routes (e.g., infiltration and runoff from landfills).[14] Based on their size, plastics debris has been commonly categorized as macroplastics, microplastics and nanoplastics. For the purpose of this review and in agreement with previous reports, we define microplastics as particles greater than 1000 nm and less than 5 mm, and nanoplastics as particles smaller than or equal to ~1000 nm in size.[15-17] The exact quantity of microplastics and nanoplastics in the aquatic environment cannot be ascertained because they are too small and difficult to detect in complex natural environments. However, Gregory and Andrady[18] and Thompson *et al.*[19,20] are of the opinion that microplastics and nanoplastics have been accumulating in the oceans for at least four decades. Substantial evidence suggests the potential for higher particle concentrations of the microplastics and nanoplastics as the larger debris disintegrates into smaller micrometre and sub-micrometre sized particles.[21-24]

Microplastics and nanoplastics are present in different types of water bodies including freshwaters, seawater, estuarine surface water, ocean surface and even marine sanctuaries,[25-27] and are bioavailable to vertebrate and invertebrate aquatic organisms.[28-31] Also, the observed increase in both the amount and occurrence of microplastics and nanoplastics in aquatic organisms supports the arguments that there is an increase in the presence of microplastics and nanoplastics in the aquatic environment.[32,33] Aquatic invertebrates such as water fleas (*Daphnia*) are vulnerable primary consumers of small microplastics and nanoplastics in the aquatic ecosystem.[34,35] These invertebrates are an important component of the ecosystem, and a disruption of their population due to stressors such as microplastic and nanoplastic pollution may have significant impact on other key species.[36-38] Furthermore, the particles ingested by these invertebrates may result in bioaccumulation or biomagnification up the aquatic food chain, and even to humans.[39,40] For example, Hairui *et al*.[41] found that zebrafish contain PE microplastics after feeding on water fleas which have been earlier exposed to PE microplastics (1 mg/L). Hence, there is a need for comprehensive knowledge on the potential health impacts of microplastics and nanoplastics to aquatic invertebrates.

1.1. ***Daphnia magna* as a model organism for microplastic and nanoplastic toxicity studies**

Daphnia magna (D. magna) is one of the most commonly used organisms to study the toxicity of contaminants to aquatic invertebrates. The first application of *D. magna* in ecotoxicology began in the 1930s through the works of Naumann (1934)[42] and Anderson (1944)[43] who used the organism to assess the toxicity of contaminants found in industrial wastewater. This was closely followed by other similar works to investigate the toxicity of various compounds and contaminants.[44,45] Since then, *D. magna* has been a popular model organism in examining the toxicological effects of environmental contaminants.[46-48] *D. magna* has various desirable advantages including high sensitivity to environmental contaminants, ease of culturing in the laboratory, parthenogenetic reproduction, short generation time, large brood size, as well as their extensive range of endpoints in toxicology studies.[49-51] In fact, highly reputable organizations such as the Organization for Economic Co-operation and Development (OECD), Environmental Protection Agency, USA (EPA) and Environment and Climate Change Canada have standardized guidelines for investigating the acute (short-term) and chronic (long-term) toxicity of contaminants on *D. magna*, (e.g. OECD 202,[46] OECD 211,[52] EPA-821-R-02-012,[53] EPA-821-R-02-013,[54] EC Biological test method,[55] etc.). The acute tests (typically range from 48 h to 96 h) examine the mortality or immobilization of the organisms while the chronic tests (typically 21 days) include sub-lethal effects such as survival, growth, and reproduction.

The application of *D. magna* also allows extended examination of physiological or behavioral endpoints to understand contaminant effects at the subcellular, cellular, organ, and ecosystem levels.[56,57] Examples of emerging indicators used in understanding these sublethal effects include swimming behavior parameters (e.g., swimming speed, swimming time, distance traveled, hopping frequency, vertical migration, and resting time[58-61]), feeding rate, thoracic limb movement, filtration rate, heartbeat rate, and food ingestion rate.[62-64] The transparent body of *D. magna* is another advantage of the organism, which allows to measure different physiological endpoints simultaneously using optical methods such as imaging, and video recording, as well as quantitative measurement of particle uptake using laser particle analysis, especially for studies using fluorescent-labelled microplastics.[63,65-67] Recent chronic toxicity experiments have also been extended to include multigenerational studies using *D. magna* as the model organism.[68-71] This characteristic is of particular interest for ecotoxicological studies because it allows the investigation of long-term effects of contaminants on organisms. Notably, it also sheds light on how they adapt to the contaminants' effects or other environmental stressors over extended periods.

1.2. Research objective and methodology

Several studies have investigated the effects of microplastics and nanoplastics on aquatic invertebrates, including *D. magna*. Yet, these existing studies target different types of toxicity tests using different toxicity indicators. Furthermore, the toxicity results are complicated due to the heterogeneity in plastics with researchers examining different polymer types, particle sizes, particle shapes, concentrations, and metrics. In addition, there are other confounding factors such as the presence of preservatives/additives in the tested particles and leaching of chemicals from the particles, which may further impact the toxicity results.[72] Reviews have been conducted to investigate the toxicity of microplastics and nanoplastics on aquatic invertebrates.[73-76] These reviews, however, combine the toxicity results across multiple species; hence they are limited in the extent of comparison that can be carried out to understand the broad impacts of microplastics and nanoplastics to the tested organisms.

The scope of this study has been limited to focus on *D. magna* due to its widespread use as a model organism for toxicity studies on microplastics and nanoplastics, and to allow for easier comparison of the toxicity data. This paper provides a comprehensive review of research publications that have used *D. magna* as a model organism to examine the acute, chronic, and multigenerational toxicity of microplastics and nanoplastics as well as the impacts of microplastics and nanoplastics as contaminant vectors.

We conducted an electronic search for research papers using the terms (Microplastic OR Nanoplastic) AND (Toxicity OR Exposure OR Effects) AND (*Daphnia magna* OR *D. magna*) on the following databases: Google Scholar (scholar.google.com), Science Direct (sciencedirect.com), Toxicity of

Microplastic Explorer (sccwrp.shinyapps.io/aq_mp_tox_shiny), and PubMed (pubmed.ncbi.nlm.nih.gov). The search strategies identified 110 publications. The reference lists of these 110 papers were consulted to identify any relevant papers that may have been omitted. This increased the total number of papers to 138, after the removal of duplicates.

Next, we established exclusion criteria to ensure that we focused on relevant papers within the scope of the review. Based on a preliminary review of the titles and abstract, we excluded studies that (1) were not written in English language; (2) investigated other *Daphnia* species (other than *D. magna*); (3) investigated other toxicity topics outside the scope of this paper (e.g., phototoxicity, feeding behavior, uptake and depuration, biochemical responses, and gene expression). No exclusion criteria were specified regarding the microplastics and nanoplastics due to the complexities in the properties of the tested plastics (e.g., polymer type, particle size, and concentration). However, given that *D. magna* are generally small sized organisms, none of the research papers found in the search results used particles larger than 5 mm in size. Also, no publication date criteria were set, however, the dates of the publications produced by the search were between 2011 and 2023. The search deadline was February 2023, and the reviewed papers included all published papers, including both indexed and non-indexed papers. The screening and selection process was conducted in accordance with the guidelines of Preferred Reporting Items for Systematic reviews and Meta Analyses (PRISMA) and the PRISMA flow diagram is shown in the Supporting Information (**Figure S1)**.

After applying the exclusion criteria stated above, the total number of research papers was reduced to 124 papers. The full text of these 124 papers were read completely and summarized. No authors were contacted for additional information on their study as part of this review. Based on the summaries, the papers were categorized according to the type of toxicity investigated. In certain cases, single papers covered more than one type of toxicity, and were added to multiple categories. We reviewed the papers assigned to the different categories, summarized the results, and derived conclusions where possible. Additional exclusion criteria were applied during a targeted research synthesis based on the types of toxicity being investigated and are discussed under the toxicity result sections, and the reasons for their exclusion were provided in the SI (where applicable). Finally, we derived conclusions, identified gaps, and proposed future directions towards understanding the impact of microplastics and nanoplastics to aquatic invertebrates.

2. Microplastics and Nanoplastics used in Toxicity Studies with *D. magna*

The physical and chemical properties of the particles used in investigating the impact of microplastics and nanoplastics to *D. magna* are very diverse. The shapes range from spherical beads to fibres, irregular shapes (fragments), films, and pellets, while the polymer types include polystyrene (PS), polyethylene (PE), polypropylene (PP), polyvinyl chloride (PVC), polyethylene terephthalate (PET), polymethyl methacrylate (PMMA), ethylene acrylic acid (EAA), polyvinyl alcohol (PVA), polymethacrylic acid (PMAA), acrylonitrile butadiene styrene (ABS), polylactic acid (PLA), polydioxanone (PPDO), epoxy, rubber and Bakelite. For the purpose of this review, microplastics and nanoplastics are broadly categorized as **spherical** micro- and nanoplastics and **non-spherical** micro- and nanoplastics.

2.1. Spherical micro- and nanoplastics

Spherical micro- and nanoplastics are the most common type of microplastics and nanoplastics used for toxicity studies with *D. magna*, accounting for almost 70% of the studies included in this review **(Figure 1**). They are obtained as dry powder or suspensions from different sources including commercial vendors and laboratory synthesis. In some cases, the suspensions were in deionized water or included additional preservatives or surfactants such as Tween 20 and sodium azide. While some studies washed the particles using centrifugation or dialysis to remove the added chemicals,[77-79] others simply used the particles as purchased or did not provide any information about whether the particles were washed or not.[80-83]

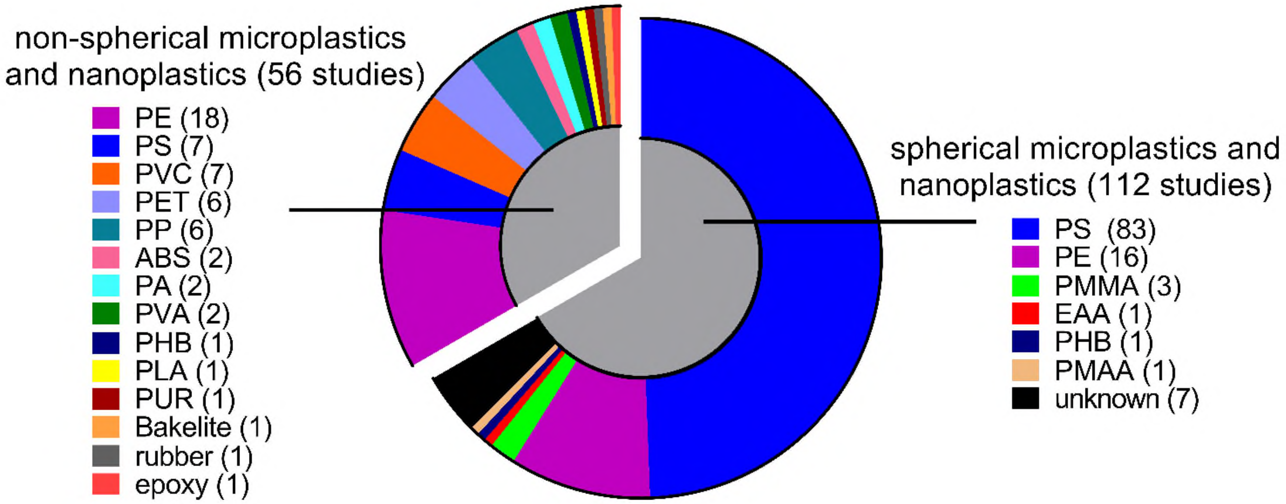


Figure 1. Summary of the type of plastic and polymers used in toxicity studies based on 168 studies reported in 124 papers (note that some papers investigated multiple plastic polymers and each polymer type was considered as a separate study, hence, the total number of studies = 168). Studies using different sizes, preparation methods, or test concentrations of the same polymers were counted as 1.

As shown in **Figures 1 and 2**, commercial PS spherical micro- and nanoplastics are the most popular type of spherical micro- and nanoplastics used in the toxicity studies. Other common types of spherical micro- and nanoplastics used in *D. magna* toxicity studies include PE and PMMA. However, there are fewer tests using these polymer types compared to PS. This is not surprising as PS is one of the most widely used plastic type due to its strength, stability, inexpensive cost, as well as high detection rate in natural waters.[84] Also, the commercial formulations of PS microplastics and nanoplastics are one of the only types of commercial particles that provide options of surface modification through the addition of functional groups (e.g., carboxyl, sulfate, amino). This is particularly important to understand the role of different functional groups in toxicity as these functional groups may be introduced to the surfaces of plastic particles upon exposure to environmental conditions such as UV irradiation and biological degradation.[22] Hence, the use of functionalized particles can contribute to better understand the role of functional groups in the mechanism for toxicity. Another desirable property of the PS particles is the fluorescent-labelling option which allows visualization and tracking of particle uptake by the exposed organisms. Also, PS spherical micro- and nanoplastics are available in uniform sizes compared to particles of other polymer types. Hence, it is a convenient polymer type to compare the impacts of different sizes of particles although it may be argued that the uniform sizes of these particles do not mimic the shapes or sizes of particles found in the environment.

Overall, the tested sizes of spherical micro- and nanoplastics exposed to *D. magna* ranged from 20 nm to 150 µm and the concentrations ranged from 0.01 mg/L to 5000 mg/L. The most common test conditions include PS (20 nm - 150 µm; 0.01 mg/L - 1000 mg/L), PE (1 µm - 100 um, 100 mg/L - 5000 mg/L), and PMMA (0.1 µm, 0.01 mg/L - 1000 mg/L). In our review, we did not find any study that has investigated the toxicity impacts of PP or PVC spherical microplastics and nanoplastics on *D. magna*. A summary of the polymer types, particle sizes and tested concentrations of the spherical micro- and nanoplastics that have been used in toxicity studies with *D. magna* is reported in **Figure 2**. The unit of concentrations used in **Figure 2** were reported as mg/L. Studies whose concentrations were reported in other units were converted to mg/L based on the calculation method described by Leusch *et al.*[85] and using the appropriate polymer densities, i.e., PS (1.055 g/cm^3)[86], PE (0.93 g/cm^3)[87], EAA (0.92 g/cm^3)[88], and unknown polymer (1.30 g/cm^3)[89,90].

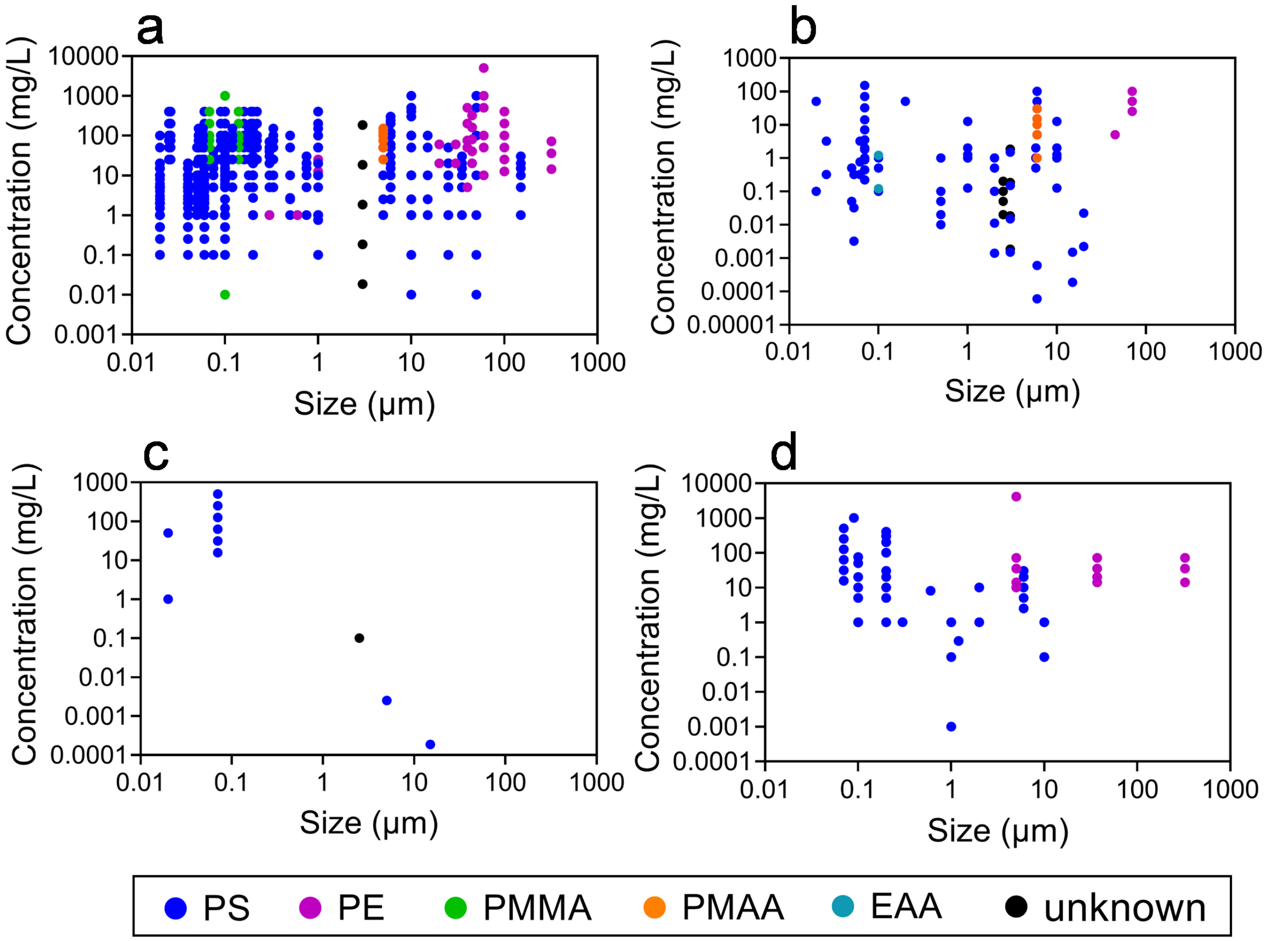


Figure 2. Polymer type, size (diameter), and concentration of spherical microplastics and nanoplastics used in (a) acute toxicity tests (n = 605); (b) chronic toxicity tests (n = 91); (c) multigenerational tests (n = 11); and (d) co-contaminant tests (n = 54). Each dot represents a test conducted on one particle type of a given size and concentration. The colour of the dots represents the polymer type. (Data provided by studies using mass concentration were used as is. For studies whose data were presented as particle number per volume, necessary conversions were carried out to derive the equivalent mass concentration).

2.2. Non-spherical microplastics and nanoplastics

Non-spherical micro- and nanoplastics used in the reviewed studies include fibres and fragments obtained directly from commercial vendors or by grinding larger particles. To mimic environmental weathering, some researchers exposed the spherical microplastics and nanoplastics to weathering conditions such as UV irradiation and stirring[91,92] while others obtained microbeads from the filtration of personal cosmetic products such as facial scrubs.[93,94] The rationale for the toxicity assays using such particles was either to assess (1) an increase of biological interaction and toxicity for weathered particles due to surface changes, or (2) an increased toxicity for non-spherical particles due to mechanical damages. The resulting particles obtained from these weathering processes or cosmetic products will be considered as non-spherical because, as shown in **Figures S2** and **S3**, the particle images from their characterization show that they appear as irregularly shaped fragments, not spheres.

These types of particles account for ~30% of the reviewed studies as shown in **Figure 1** and are mainly PE, PS, or PVC polymers. They typically are polydisperse in size within a given toxicity test, and therefore, are not suitable for investigating the impacts of specific micro- or nanoparticle sizes. However, plastic fragments mimic the microplastics and nanoplastics generated from mechanical abrasion or UV-irradiation of larger debris in the environment, or waste particles released from plastic production factories.[24] For example, PE is commonly used for different types of plastic products and accounts for more than 30% of total plastic production annually.[95] Hence, testing the toxicity of irregular PE microplastics and nanoplastics is necessary for the assessment of this ubiquitous polymer type. Likewise, fibres are important to consider because they allow the assessment of the toxicity of fibres released from washing clothes and not removed by wastewater treatment.[23,96] Overall, the most common types and concentrations of the non-spherical micro- and nanoplastics used for toxicity tests on *D. magna* include PS (0.1 mg/L - 50 mg/L), PE (10 mg/L - 100 mg/L), PET (12.5 mg/L - 100 mg/L), and PP (1 mg/L - 100 mg/L). Although some studies have used microplastics obtained from the natural environment[97] or exposed organisms to the natural marine environment,[98] no such studies have been conducted on *D. magna*.

3. Toxicity of Microplastics and Nanoplastics to *D. magna*

3.1. Acute toxicity

Acute toxicity is one of the most common methods of assessing the toxicity of plastics to *D. magna*. The experiments are typically conducted on neonates (≤ 24 h) using standardized laboratory protocols such as the OECD 202 with durations ranging from 48 h to 96 h. A few studies have modified the acute tests by conducting them on older organisms,[99-101] in the presence of food,[102] or reducing the test duration.[103-105] While the most common endpoints are mortality or immobility, some studies also explored other endpoints such as swimming behavior[77], food ingestion rate[106], and particle uptake[107].

We carried out a targeted research synthesis using the available data on the acute toxicity tests that have been conducted on *D. magna* using microplastics and nanoplastics. To ensure that similar test conditions were compared, the targeted research synthesis included results of studies that aligned with the minimum OECD 202 requirements for immobilization tests on *D. magna*. This includes tests with a minimum of 20 animals per condition and a valid control result (i.e., maximum of 10% immobility). Also, the targeted research synthesis only included studies which involved neonates (≤ 24 h) and were not fed during the duration of the tests. For studies extended to 72 h or 96 h, only the results at the end of 48 h were considered. In studies that included additional objectives such as co-exposure with other compounds, only the results of the acute toxicity of plastic particles alone were selected and used in the analysis provided in this section. The nominal size of the particles was used if it is provided for the study. Otherwise, the size obtained by the authors from their characterization tests were used. In cases where there is a size range of the particles, an average size was selected and used. When the same study investigated multiple polymer types, sizes and/or concentrations, each of these conditions were treated as separate tests in the analysis. Most studies reported the results of acute toxicity tests in terms of immobilization or mortality.

In instances where the authors have reported both mortality and immobility, the latter findings were used. However, when no information on immobility was provided, the mortality results were considered. The selected studies including the polymer types, particle size, and tested concentration are shown in **Table 1**. This table also indicates whether the studies have used some washing methods such as dialysis or centrifugation to remove preservatives/surfactants from the plastic suspensions and labelled “unknown” if the information was not provided. A summary table for the excluded studies and the reason for the exclusion are provided in the Supporting Information (**Table S1**).

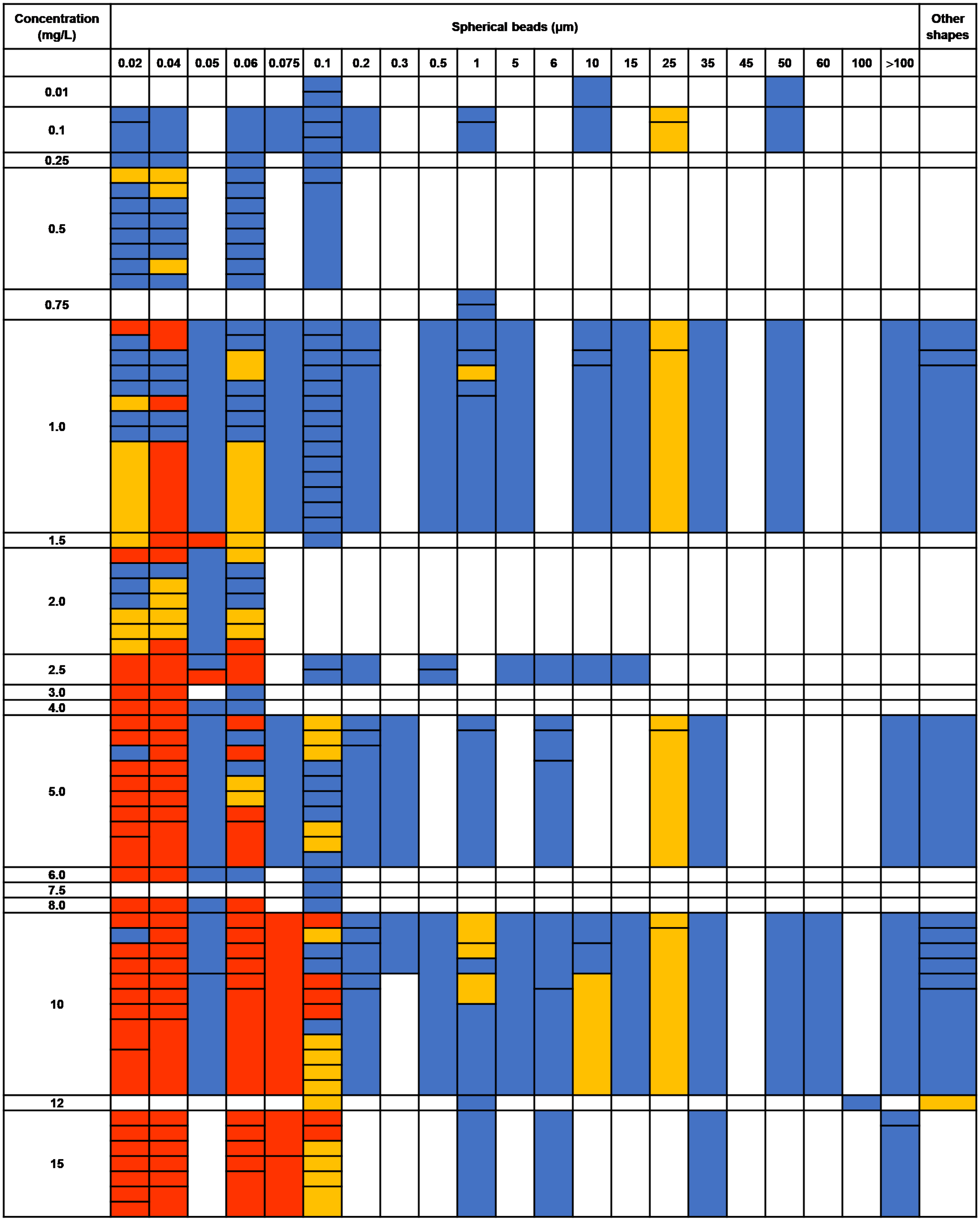
Table 1. Studies investigating the acute toxicity of microplastics and nanoplastics to *D. magna* (included in the targeted research synthesis). -COOH and -NH_2 means carboxylated and aminated functional groups, respectively. (f) means fluorescently labelled. Where applicable, circled numbers were used to distinguish particles that possess different properties within the same study.

Type of plastic	Shape	Sizes	Concentration	Source of plastic	Was the plastic washed?	Refs.
PS	spheres	5.8 µm	5, 10, 20, 50, 100, and 200 mg/L	BaseLine ChromTech Research Center, China	unknown	[80]
PS-COOH; PS-NH_2	spheres	1 µm	0.1, 0.75, 1, 10, 20, and 50 mg/L	Nanjing Xfnano Materials Tech Co., China	unknown	[108]
PS-NH_2	spheres	~100 nm	1, 10, 50, 100, 200, and 400 mg/L	Sigma Aldrich, US	yes (ultra-centrifugation)	[79]
①PS; ②PS-COOH; ③PS-n-NH_2; ④PS-p-NH_2	spheres	① 100 nm; ② 300 nm; ③ 50-100 nm; ④ 110 nm	① 1, 5, 10, 20, 50, and 75 mg/L; ② 5, 10, 20, 30, 40, 50, and 70 mg/L); ③ 0.1, 1, 5, 10, 15, 20, 30, and 40 mg/L; ④ 0 to 100 mg/L	①②③ Aladdin, China; ④ Thermo Scientific, China	unknown	[109]
PS	spheres	① 50 nm; ② 500 nm; ③ 5 µm; ④ 10 µm; ⑤ 15 µm	① 1, 2.5, 5, 10, 20, and 50 mg/L; ②③④⑤ 1, 2.5, 10, 50, and 100 mg/L	BaseLine ChromTech Research Center, China	unknown	[110]
PS (f)	spheres	6 µm	5, 15, 30, 60, 120, 240, and 300 mg/L	Polysciences, Germany	unknown	[111]
PS	spheres	10 µm and 50 µm	0.01, 0.1, 1, 10, 100, 500, and 1000 mg/L	BaseLine Chromtech Research Centre, China	unknown	[112]
PE	spheres	1-4 µm and 90-106 µm	12.5, 25, 50, 100, 200, and 400 mg/L	Cospheric, USA	clean, (vendor's declaration)	[113]
PS	spheres	① 1 µm; ②10 µm	① 1, 5, 10, 50, 100, and 150 mg/L; ② 1, 10, 100, 300, 400, and 500 mg/L	Tianjin Goose Technology Co., China	clean, (vendor's declaration)	[114]
PS-NH_2	spheres	100 nm and 1 µm	1, 10, 50, 100, 200, and 400 mg/L	Sigma-Aldrich, USA	yes (ultra-centrifugation)	[115]
PS; PS-COOH	spheres	200 nm	1, 5, 10, 20, and 30 mg/L	Bangs Laboratories Inc., USA	unknown	[116]
PS	spheres	100 nm	1, 5, 10, 20, 50, and 75 mg/L	Aladdin, China	unknown	[117]
PS-NH_2	spheres	① 20 nm; ② 40 nm; ③ 60nm; ④100 nm	①②③ 0.5, 1, 2, 5, 10, and 15 mg/L; ④ 1, 5, 10, 15, 20, and 30 mg/L	Thermofisher Scientific, USA	clean, (vendor's declaration)	[118]

PET	fibres	length: 60-1400 µm; width: 31-528 µm; diameter: 2-21.5 µm	12.5, 25, 50, and 100 mg/L	Milling of a PET fabric	yes (washed in deionized water)	107
PP	fibres	diameter: 21.2 ± 1.5 µm (inner layer), 22.1 ± 1.6 µm (outer layer), and 4.3 ± 2.2 µm middle layer). Length not provided	1, 10, and 100 mg/L	Cryo-milling of medical single-use face masks	unknown	119
PS-NH$_2$	spheres	50 nm, 200 nm, and 500 nm	2.7 mg/L	Bangs Laboratories Inc., USA	yes (dialysis)	120
PET	fibres	diameter: 14 ± 3 µm; length: 366 ± 275 µm	20, 100, and 500 mg/L	Milling continuous PET filament (Goodfellow)	unknown	121
PE	fragments	1A, 1B, 1C, 1D, 2, 3 and 4 are ~200 µm, ~100 µm, ~50 µm, ~250 µm, ~250 µm, ~150 µm, and ~20 µm respectively.	100 mg/L	2 facial cleanser products, 1 plastic bag and 1 textile fleece	yes (washed in deionized water)	93
PE	spheres	40-48 µm	20, 40, 80, 160, and 320 mg/L	Sigma Aldrich, USA	unknown	83
PE	① spheres ② fragments	① 10-106 µm; ② 10-75 µm	10, 50, 100, 500, 1000, and 5000 mg/L	① Cospheric, USA; ② a plastic recycling company, Denmark	unknown	106
PS-COOH (f)	spheres	20 nm and 200 nm	0.1, 1, 5, 10, 20, 50, and 100 mg/L	ThermoFisher	yes (dialysis)	77
PE	fragments	180.5 ± 118.7 µm	10 and 100 mg/L	Commercial body scrub	yes (washed in deionized water)	122
PP; PE; PVC; PVC/PE	fragments	10-100 µm	50 mg/L	Grinding bulk plastic pieces	yes (washed in ethanol)	123
PS (aged)	spheres	0-1.5 µm; 10-60 µm; and 60-230 µm	1, 5, 10, 15, 20, and 30 mg/L	Guanbu Electromechanical Technology Co., Ltd., China, (aged under natural light for 180 days)	unknown	92
① PS-COOH; ②PS-NH$_2$	spheres	200 nm	① 10, 50, 100, 125, and 150 mg/L; ② 10, 25, 50, 75, and 100 mg/L	Invitrogen (Thermofisher Scientific), USA	clean, (vendor's declaration)	124
pristine PS; aged PS	spheres	0.1-50 µm	0.1, 1, 5, 10, 20, and 50 mg/L	Dongguan Huachuang Plastic Chemical Co., Ltd, China. Aged samples were obtained through continuous stirring and UV light for 6 months.	unknown	91
PE	fragments	140.6 ± 80.0 µm	100 mg/L	facial scrub product	yes (washed in deionized water)	94
PS (f)	spheres	51 nm	2, 4, 6, 8, and 10 mg/L	Bangs Laboratories Inc., USA	unknown	81
PMMA (f)	spheres	86-125 nm	0.01, 0.1, 1, and 1000 mg/L	Synthesized in the laboratory (fluorescent and non-fluorescent)	unknown	125

PE	fragments	44.39 ± 11.16 µm	5, 10, 25, 50, 100, 125, and 250 mg/L	Cryo-milling of PE pellets (Sigma Aldrich, USA)	yes (rinsed with hexane, methanol, and deionized water)	[126]
① PMAA; ② PVA	① spheres; ② films and fibres	① 1.5-10 µm ② size not provided	① 25, 50, 75, 100, 125, and 150 mg/L; ② 25, 50, 75, 100, and 125 mg/L	Synthesized in the laboratory	unknown	[127]
PS (f)	spheres	6 µm	2.5, 5, 10, 20, and 30 mg/L	Polysciences, USA	unknown	[128]
PE	spheres	300-354 µm	14.3, 35.7, and 71.4 mg/L	Cospheric, USA	unknown	[129]
PS-NH_2	spheres	① 20 nm; ② 40 nm; ③ 60 nm; ④ 100 nm	Media 1: ①②③ 0.5, 1, 2, 3, 4, 5, 6, 8, 10, 15, and 20 mg/L; ④ 0.5, 1, 5, 6, 7.5, 8, 10, 12.5, 15, and 20 mg/L. Media 2: ①②③④ 0.5, 1, 1.5, 2, 2.5, 5, 10, and 20 mg/L	Invitrogen (Thermofisher Science), USA	unknown	[130]

A summary of the targeted research synthesis results is presented in **Figure 3** while the detailed results (including polymer types and references) are presented in the Supporting Information (**Table S2)**. In both, all spherical micro- and nanoplastics were presented based on their size (diameter) and concentration, while non-spherical micro- and nanoplastics are presented in the last column of the table based on their concentration alone, as information on size range could not be easily presented due to the different shapes and size ranges. Details on the particle sizes for each test using non-spherical micro- and nanoplastics are presented in **Table S2**. Also, when necessary, the concentration of the particles and the diameter of the spherical particles were rounded up for a more concise number. For example, 5.8 µm was approximated to 6 µm while 1.4 mg/L concentration was approximated to 1.5 mg/L. There was a total of 505 tests including spherical micro- and nanoplastics and non-spherical micro- and nanoplastics across five major polymer types (**Figure 3** and **Table S2**). As shown in **Figure 3**, the toxicity data are categorized as insignificant, mild, or severe when the percentage of mortality or immobility are ≤ 20%; >20% and ≤50%; and > 50%, respectively.



Concentration (mg/L)	**Spherical beads (µm)**																					**Other shapes**
	0.02	**0.04**	**0.05**	**0.06**	**0.075**	**0.1**	**0.2**	**0.3**	**0.5**	**1**	**5**	**6**	**10**	**15**	**25**	**35**	**45**	**50**	**60**	**100**	**>100**	
0.01						🟦 🟦							🟦					🟦				
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0.25	🟦	🟦		🟦		🟦																
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1.5	🟨	🟥	🟥	🟨		🟦																
2.0	🟥 🟦 🟦 🟦 🟨 🟨 🟨	🟥 🟦 🟨 🟨 🟨 🟨 🟥	🟦	🟨 🟦 🟦 🟦 🟨 🟨 🟥																		
2.5	🟥	🟥	🟦 🟥	🟥		🟦 🟦	🟦		🟦 🟦		🟦	🟦	🟦	🟦								
3.0	🟥	🟥		🟦																		
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5.0	🟥 🟥 🟦 🟥 🟥 🟥 🟥 🟥 🟥	🟥 🟥 🟥 🟥 🟥 🟥 🟥 🟥	🟦	🟥 🟦 🟥 🟦 🟨 🟨 🟥 🟥	🟦	🟨 🟨 🟨 🟦 🟦 🟦 🟦 🟨 🟨 🟦	🟦 🟦 🟦	🟦		🟦 🟦		🟦 🟦 🟦			🟨 🟨	🟦					🟦	🟦
6.0	🟥	🟥	🟦	🟦		🟦																
7.5						🟦																
8.0	🟥	🟥	🟦	🟥		🟦																
10	🟥 🟦 🟥 🟥 🟥 🟥 🟥 🟥 🟥	🟥 🟥 🟥 🟥 🟥 🟥 🟥 🟥	🟦 🟦	🟥 🟥 🟥 🟥 🟥 🟥	🟥	🟥 🟨 🟦 🟦 🟥 🟥 🟥 🟦 🟨 🟨 🟨 🟨	🟦 🟦 🟦 🟦 🟦	🟦	🟦	🟨 🟨 🟦 🟨 🟦	🟦	🟦 🟦	🟦 🟦 🟨	🟦	🟨 🟨	🟦		🟦	🟦		🟦	🟦 🟦 🟦 🟦 🟦 🟦
12						🟨				🟦										🟦		🟨
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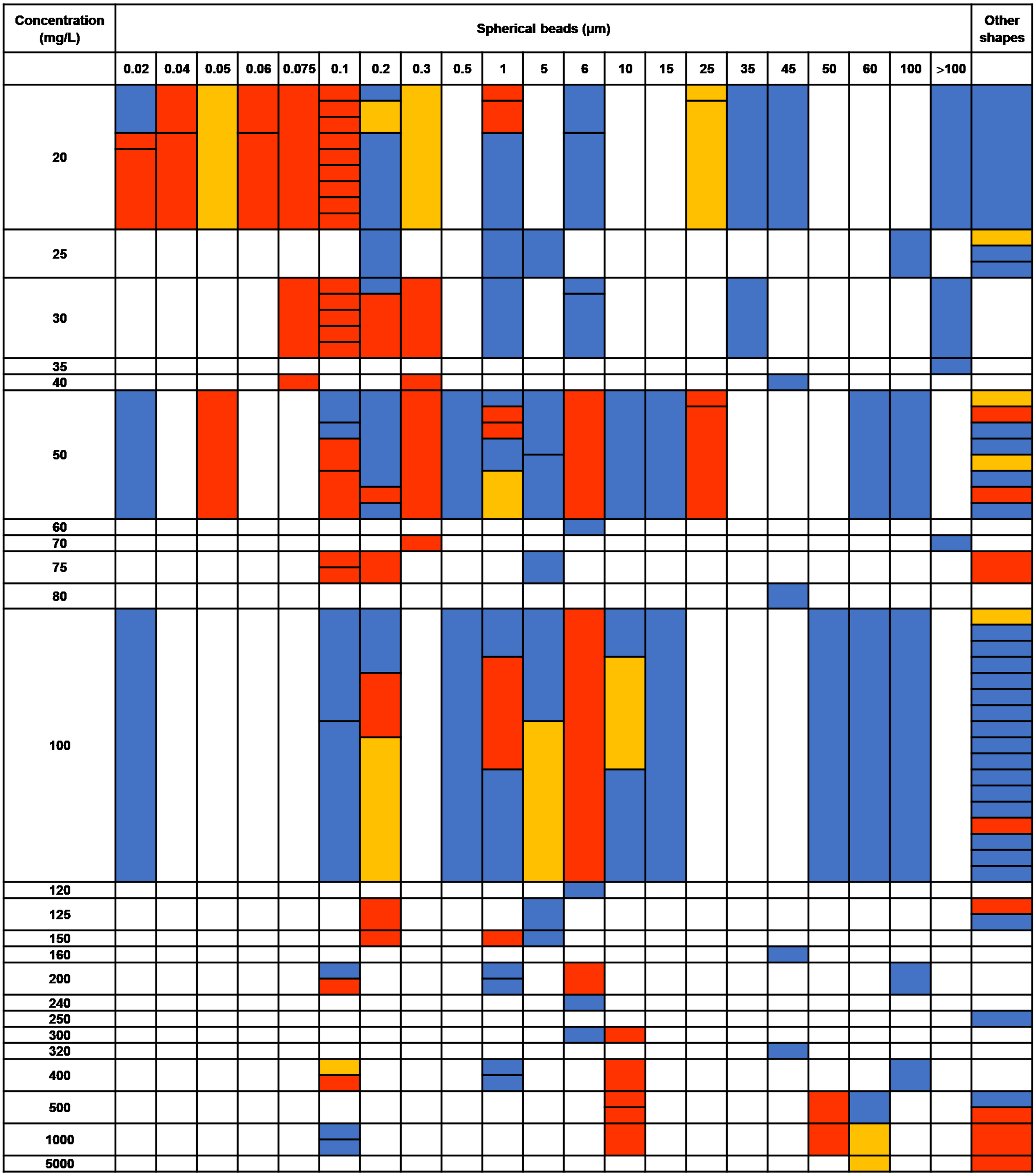

Figure 3. Targeted research synthesis of immobility or mortality observed on *D. magna* neonates (⩽ 24 h) after 48 h exposure to microplastics and nanoplastics. Each colored cell represents a toxicity test conducted on one particle type of a given size and concentration. The spherical beads columns provide the particle diameter and the corresponding concentration of each toxicity test. For the non-spherical or irregular-shaped plastics, the cells under the "other shapes" column present the concentration of each toxicity test. The percentage of

dead or immobile neonates are represented by the cell colors. Blue cell color represents no significant acute toxicity (⩽ 20%), yellow cells represent mild acute toxicity (> 20% and ⩽ 50%) and red cells represent severe acute toxicity (> 50%), N = 505. Detailed information including the polymer types, functional groups (where available), presence of fluorescent dye, whether the particles were washed or not, references, as well as the sizes of the non-spherical particles are presented in the Supporting Information (**Table S2)**.

3.1.1. Acute toxicity of spherical plastic particles

The size and concentration of the tested spherical micro- and nanoplastics for acute toxicity ranged from 20 nm - 150 µm and 0.01 mg/L to 5000 mg/L, respectively. However, the most commonly used concentrations and sizes were 10 mg/L, 50 mg/L and 100 mg/L; and 0.1 µm, 0.2 µm and 1 µm, respectively. The observed acute toxicity from the tested spherical micro- and nanoplastics varied due to various factors including their polymer type, size, and concentration. The results are summarized below according to the different plastic polymer types.

Polystyrene (PS): There are a total of 417 tests conducted on PS spherical micro- and nanoplastics to investigate acute toxicity to *D. magna* (**Figure 3** and **Table S2**).[77,79-81,91,92,108-111,114-118,120,124] The tests varied largely in the size of particles, functional groups and test concentrations. Due to the high number of tests in the category and for easier analysis and discussion, the tests are divided into the following size categories: (i) ≤ 100 nm; (ii) >100 nm and < 35 µm; and (iii) ≥ 35 µm). Spherical PS (≤ 100 nm) were tested on *D. magna* between 0.1 mg/L and 400 mg/L in 269 tests. Out of the 75 tests conducted at ⩽ 1 mg/L in this size range, 84% did not observe significant acute toxicity while 11% and 5% showed mild and severe acute toxicity, respectively. The results were quite opposite for the 194 tests conducted > 1 mg/L. In those tests, 60% found severe acute toxicity while 17% found mild toxicity. Only 23% of the tests did not find significant toxicity at the end of the acute toxicity tests. At increased size range of the spherical micro- and nanoplastics (>100 nm and < 35 µm), 129 acute tests have been conducted at concentrations ranging from 0.01 mg/L to 1000 mg/L. This size range accounts for ~30% of all spherical PS acute toxicity tests on *D. magna*. It includes 66 tests (<

20 mg/L), 49 tests (≥ 20 mg/L and ≤ 100 mg/L) and 14 tests (> 100 mg/L). 80% of tests conducted at < 20 mg/L found no toxicity while the remaining 20% reported mild acute toxicity to *D. magna*. When the test concentrations increased between 20 mg/L and 100 mg/L, 51% showed no significant toxicity while 14% and 35% observed mild and severe acute toxicity, respectively. At above 100 mg/L, the proportion of tests in this size category that showed severe acute toxicity increased, accounting for 64% while the remaining 36% showed no acute toxicity. 89% of 19 tests exposed to spherical PS (≥ 35 μm) consistently did not show acute toxicity, with the 11% remaining which represents 2 tests exhibiting severe acute toxicity at 500 mg/L and 1000 mg/L[92,112] (**Figure 3** and **Table S2**).

Polyethylene (PE): Twenty-six (26) tests were reported in four different studies to assess the acute toxicity of spherical PE to *D. magna* (**Figure 3** and **Table S2**).[83,106,113,129] Two tests on spherical PE (10-100 μm) conducted at 1000 mg/L and 5000 mg/L showed mild acute toxicity while the remaining 24 tests (92%) did not show significant acute toxicity to *D. magna*. The size and concentration ranges of the spherical PE tests which showed no acute toxicity are 10-100 μm (10-500 mg/L)[106], 40-48 μm (20-320 mg/L)[83], 1 μm (12.5-400 mg/L)[113], 100 μm (12.5-400 mg/L)[113], and 300-354 μm (15-70 mg/L)[129].

Polymethyl methacrylate (PMMA): Eight acute toxicity tests were conducted on plain and functionalized PMMA plastic beads (85-125 nm).[125] The particles were synthesized in the laboratory and the tests were conducted at 0.01 mg/L, 0.1 mg/L, 1 mg/L, and 1000 mg/L.[125] All 8 tests showed no acute toxicity to *D. magna* (**Figure 3** and **Table S2**).

Polymethacrylic acid (PMAA): Six acute toxicity tests were conducted using PMAA particles (1.5 -10 μm) at concentrations ranging from 25 mg/L to 125 mg/L. Only one test, at 100 mg/L, showed mild toxicity while other concentrations do not cause any significant mortality after 48 h exposure.[127]

Overall, **Figure 3** clearly shows more severe toxicity at lower particle sizes, at concentrations above 1 mg/L. This aligns with other reports that have shown that when the same concentration of particles was tested, nanoplastics showed higher acute toxicity than larger sized particles.[82,110] Previous studies also suggested that nano-sized particles (≤ 100 nm) may have easier diffusion abilities into the cells and can more readily localize in small structures of organs and tissues through the circulation system of the organism while larger sized particles are egested without being retained in the intestinal tract.[131] Hence, the impacts of microplastics may be limited to physical injuries in the intestinal tracts of animals while nanoplastics, just like other nanoparticles, may be more harmful due to their higher penetration ability.[109] This may explain the increased toxicity observed in the spherical nano-sized PS compared to larger sized particles of the same polymer type and concentration.[82] In addition, Ma *et al.*[110] showed no impact of the larger sized PS particles (500 nm - 15 μm) but observed severe immobility as well as visible physical damage with 50 nm PS particles at the same concentration. Specifically, there was damage to the thoracopods which play an important role in the mobility and filter-feeding of daphnids. Interestingly, Frankel *et al.*[120] reported that the aggregation of 50 nm PS-NH_2 nanoplastics does not diminish their toxicity towards daphnids. It is suggested that when entering the organism, the aggregates rapidly break down into free 50 nm NH_2-functionalized PS beads that are inherently toxic.

The surface chemistry of the particle can also play a pivotal role in toxicity. Lin *et al.*[109] showed this connection by studying different types of functionalized PS particles. Interestingly, they noted that plain PS nanoplastics are more toxic than modified PS nanoplastics such as negatively charged carboxylated PS nanoplastics (PS-COOH), and positively charged aminated PS nanoplastics (PS-NH_2). Among functionalized PS particles, the NH_2-functionalized PS particles show higher toxicity than their COOH-functionalized counterpart.[108] Particularly on the acute exposure of *D. magna* resulting in the immobilization of the organisms, PS-NH_2 nanoplastics were found to be three times more toxic than PS-COOH nanoplastics.[124] This observation is due to the positively charged surface of PS-NH_2 nanoplastics that can readily interact with the overall negatively charged microorganisms. Nasser *et al.*[132] also explored the role of secreted proteins that can coat the surface of PS nanoplastics, forming an eco-corona, and alter the toxicity of the particles. Eco-corona-coated nanoplastics had a lower EC_{50} than their uncoated counterparts. However, it was noted that there was a greater retention for corona-coated nanoplastics in *D. magna* gut, affecting its ability to feed on algae.[132] Subsequent studies could examine the long term effects of eco-corona-coated nanoplastics and how such bioaccumulation can impact toxicity assessments.

Minimal acute toxicity was observed during exposures to spherical PE. However, it is noteworthy that most of the tests used larger particles (e.g., 100 µm). At such sizes, it is uncertain whether the particles were bioavailable to be ingested by the daphnids.[113] Also, it can be hypothesized that the observed severe toxicity at 1000 mg/L and 5000 mg/L may be due to hindrance in their movement followed by exhaustion and drowning, rather than the ingestion of the particles. The only study that investigated PMMA nanoplastics (86-125 nm) found no toxicity at up to 1000 mg/L.[125] The results of the toxicity data from the different polymer types are incomparable due to the different size range of the exposed particles. Hence, there is need for more studies on other polymer types, particularly using smaller sized particles. Also, most of the studies did not provide information on whether they washed the commercial formulations to remove the chemicals that may be present. Hence, the extent of effects from these chemicals, if present, could not be confirmed.

3.1.2. Acute toxicity of non-spherical plastic particles

There was a total of 48 acute toxicity tests on non-spherical micro- and nanoplastics accounting for less than 10% of the total acute toxicity tests covered in this study (**Figure 3** and **Table S2**). The tests include fragments and fibres from PE, PP, PET, PVA, and PVC and the results of the acute toxicity upon exposure of daphnids to the particles from each polymer type is summarized below:

Polyethylene (PE): Six studies conducted a total of 24 tests on PE non-spherical micro- and nanoplastics at concentrations ranging from 10 mg/L to 5000 mg/L (**Figure 3** and **Table S2**).[93,94,106,122,123,126] Different sizes of washed PE fragments ranging from 50 to 250 µm obtained from facial cleansers, as well as 150 µm and 20 µm sized fragments obtained from fleece textile and plastic bag, respectively were tested at 100 mg/L.[75,76] No significant acute toxicity to *D. magna* was observed across all types of PE fragments and sizes. Also, Song *et al.*[126] did not observe significant toxicity upon exposing PE fragments (44.39 ± 11.16 µm) at up to 250 mg/L while Renzi *et al.*[123] showed mild acute toxicity upon exposure of PE fragments (10-100 µm) obtained by grinding bulk plastic pieces at 50 mg/L. Also, at 10 mg/L, acute toxicity tests on PE fragments, 10-75 µm[106] and 180.5 ± 118.7 µm[122] did not show any significant acute toxicity[106,122] while all three tests conducted on PE fragments (10-75 µm) at ⩾ 500 mg/L showed severe acute toxicity to *D. magna*[106].

Polypropylene (PP): There were a total of 10 tests on non-spherical PP to assess acute toxicity to *D. magna*.[119,123] Kokalj *et al.*[119] obtained PP fibres (diameter = 4-24 µm, length not provided) by milling medical masks. The tests were conducted on the three layers of the masks. Nine tests were conducted, at 1 mg/L, 10 mg/L, and 100 mg/L on the three layers of single-use face masks. All nine tests showed no acute toxicity to *D. magna*.[119] Also, Renzi *et al.*[123] tested PP fragments (10-100 µm) obtained by grinding bulk particles. Although the source of the bulk particles was unspecified, exposures at 50 mg/L showed no significant acute toxicity to *D. magna*.

Polyethylene terephthalate (PET): Jemec *et al.*[107] and Tourinhoa *et al.*[121] carried out a total of 7 tests to examine the acute toxicity of PET particles on *D. magna*. Jemec *et al.*[107] tested washed fibres obtained from a PET fleece material (length = 60-1400 µm, width = 30-530 µm, diameter = 2-21.5 µm) at 12.5 mg/L, 25 mg/L, 50 mg/L and 100 mg/L. In all 4 tests, the authors reported mild acute toxicity. In contrast, Tourinhoa *et al.*[121] did not observe any immobility when fibres obtained from a continuous PET filament (length = 366 ± 275 µm, diameter = 14 ± 3 µm) were tested at 20 mg/L, 100 mg/L, and 500 mg/L.

Other polymer types: Only two studies were found in this review that tested other types of plastic for acute toxicity to *D. magna*. Renzi *et al.*[123] tested 50 mg/L of washed PVC particles (10-100 µm) and a mixture of

PVC/PE particles (10-100 µm) for acute toxicity and found no significant immobility to *D. magna* at both conditions. Gökçe *et al*.[127] tested a mixture of PVA films and fibres (unknown size) at five different concentrations up to 125 mg/L. The study found severe toxicity at 50 mg/L, 75 mg/L, and 125 mg/L but no toxicity at 25 mg/L and 100 mg/L.[127]

Only the PE studies that used unwashed particles and high concentrations (50-5000 mg/L) found acute toxicity to *D. magna*[106] while other studies using washed particles at various concentrations did not observe similar results. While some particles of the tested size ranges may not be ingested by *D. magna*, the chemicals present in the particle suspension may be responsible for the observed toxicity. Also, PET fibres (14 ± 3 µm in diameter, 20-500 mg/L) from filament showed no acute toxicity while those from a PET fleece material showed mild acute toxicity within similar particle size and concentration range.[107,121] Tests on PP fibres showed no acute toxicity as well.[119,123] It is important to note that there are only a few studies that investigated these particle shapes; hence, it is difficult to assess the role of the shape or polymer type. While our understanding of the impact of particle shape on toxicity is still in the early stages of development, some studies demonstrated that the shape of the microplastics may influence the impact on *D. magna.*[101,133] For instance, Frydkjær *et al*.[106] using 10-75 µm PE fragments and 10-106 µm PE spheres demonstrated that irregularly shaped microplastics were far more likely to be retained in the gut of daphnids after 90 minutes of exposure. It is implied that the regular spherical shaped plastics possessed a smoother surface resulting in depuration. This is concerning as most plastics present in the aquatic ecosystem are irregularly shaped.

3.2. Chronic toxicity

Chronic toxicity studies target sublethal endpoints such as growth, development, behavior, reproduction, as well as survival to better understand effects of contaminants on population dynamics in the ecosystem. We reviewed 36 chronic studies of microplastics and nanoplastics on *D. magna*. Eighteen out of the 36 were focused solely on PS while an additional 2 studied PS along with other polymer types. The number of studies on other polymers are as follows: PE (9 studies), PVC (4 studies), PET (2 studies), PA (1 study), PLA (1 study), EAA (1 study), PMAA (1 study) PVA (1 study), and PUR (1 study). Some studies only assessed a single polymer type while others investigated multiple polymers. Four studies used commercial particles with no information on the polymer type. In some cases, metrics varied from mass concentration (mg/L) to particle concentration (particles/mL); hence comparison of the results and synthesis of effect across studies was difficult for similar polymer type. Like the acute toxicity section, some studies assessed microplastic and nanoplastic toxicity in the presence of co-contaminants and/or other factors, such as temperature changes or UV, and in these situations only the results obtained from microplastic and nanoplastic exposure alone were reported in this section. A summary of the studies is presented in **Table 2**.

Table 2. Studies investigating the chronic toxicity of microplastics and nanoplastics to *D. magna*. -COOH and -NH$_2$ means carboxylated and aminated functional groups, respectively. (f) means fluorescently labelled. Where applicable, circled numbers were used to distinguish particles that possess different properties within the same study.

Type of plastic	Shape	Size	Concentration	Source of plastic	Was the plastic washed?	Duration of test	Target endpoints	Refs.
① unspecified (f), (1.30 g/cm^3); ② PE	① spheres; ② fragments	① 1-5 µm; ② 1-10 µm	10^2, 10^3, 10^4, and 10^5 particles/mL	① Cospheric, USA; ② Grinding PE spheres (850-1000 µm, Cospheric, USA)	unknown	21 days	**reproduction** (time to first brood, size of first brood, number of broods, size of first three broods, number of neonates); **growth** (terminal body length).	89
① unspecified (f), (1.30 g/cm^3); ② PE	① spheres; ② fragments	① 1-5 µm; ② 2.6 ± 1.8 µm	10^2, 10^3, 10^4, and 10^5 particles/mL	① Cospheric, USA; ② Grinding PE spheres (1 mm, Cospheric, USA)	unknown	21 days	**survival**; **growth**; **reproduction** (time to first brood, time between broods, number of broods, number of neonates).	90
PVC	fragments	① 2 ± 1 µm; ② 50 ± 10 µm	① 2.05 mg/L ② 4.97 mg/L	Guangzhou Huayu Trade Co., Ltd., China	clean, (vendor's declaration)	21 days	**reproduction** (time to first brood, size of first brood, number of broods, total number of neonates); **molting** (frequency of molting)	134
①rigid PVC; ②flexible PVC	fragments	① 4-141 µm; ② 12-276 µm	4342 particles/L	① Nexans Research Center, Germany; ② modification of ① using diisononylphthalate, chalk, and stabilizer	unknown	25-31 days	**survival**; **growth** (body length, body width, tail spine length); **reproduction** (number of neonates in first six broods, time to first brood)	135
unspecified (f), (1.30 g/cm^3)	spheres	1-5 µm	0.02 and 0.2 mg/L	Cospheric, USA	unknown	21 days	**survival**; **growth** (somatic growth); **reproduction** (time to first brood, number of broods, number of immobile neonates, number of aborted eggs)	136
① virgin PA; ② weathered PA	fragments	0-180 µm	100 and 300 mg/L	① Abifor AG, Germany; ② UV-weathering of ① for 26 days	no	21 days	**survival**; **reproduction** (number of neonates)	137
① PS; ② PET; ③ ABS	fragments	3.2-3.7 µm	① 7.3×10^4 particles/mL; ② 1.15×10^5 particles/mL; ③ 1.19×10^5 particles/mL	Grinding plastic products made of the corresponding materials	yes (centrifugation)	14 days	**survival**; **growth** (body length); **reproduction** (number of neonates)	78
① PE; ② PP; ③ PS; ④ PVC	unclear	①② 50 nm; ③ 200 and 600 nm; ④ 200 nm	3×10^{10} particles/mL	CD-bioparticles	no	21 days	**survival**; **reproduction** (number of broods, number of neonates, number of neonates/brood)	82
① PVC; ② PUR; ③ PLA	fragments	① ≤ 20 µm; ②③ ≤ 40 µm	10, 50, 100, and 500 mg/L	Grinding plastic products made of the corresponding materials	unknown	21 days	**survival**; **growth** (body length); **reproduction** (time to first brood, number of neonates)	138

① virgin LDPE ② recycled LDPE	fragments	① 39.8 ± 8.82 µm; ② 205 ± 144 µm	1, 10, and 100 mg/L	① ICO Polymers, USA; ② cryomilling recycled LDPE granules/pellets (Estonia)	unknown	21 days	**survival**; **growth** (body length); **reproduction** (time to first brood, number of broods, number of neonates)	139
PE (f)	spheres	63-75 µm	25, 50, and 100 mg/L	Cospheric, USA	unknown	21 days	**survival**; **reproduction** (number of neonates, average number of neonates/day)	64
PE	① spheres; ② fragments	① 40-48µm; ② 17 ± 3 µm and 34 ± 13 µm	5 mg/L	① Sigma Aldrich, USA; ② Grinding and cryomilling of PE pellets	no	21 days	**survival**; **growth** (body length of adults and neonates); **reproduction** (time to first brood, number of broods, number of neonates, number of neonates/brood)	140
HDPE	fragments	90-200 nm	unspecified	Grinding bulk pieces	yes (filtration)	134 and 98 days	**survival**; **reproduction** (number of neonates)	141
PS (f)	spheres	100 nm and 2 µm	0.1, 0.5, and 1 mg/L	Phosphorex	unknown	21 days	**survival**; **growth** (body length); **reproduction** (time to first brood, number of broods, total number of neonates, number of neonates/brood); **molting** (number of molts)	142
PS	fragments	13.03 ± 7.75 µm	101.6 mg/L	Cryomilling larger particles	unknown	19 days	**growth** (body length, tail length, body width); **reproduction** (time to first brood, number of neonates per brood, sum of neonates from first two broods); **others** (proteomic analysis)	99
PS	spheres	5.8 µm	0.5, 1, and 2 mg/L	BaseLine ChromTech Research Center, China	unknown	21 days	**growth** (body length); **reproduction** (time to first brood, number of neonates in first brood, number of broods, total number of neonates)	80
PS	① spheres; ② fragments; ③ fibres	① 6 µm, 20 µm; ② 5.7 µm, 17.7 µm; ③ diameter = 3 µm, length = 75.5 µm	500 and 5000 particles/L	① Polysciences, USA; ② wet milling; ③ electrospinning	unknown	21 days	**survival**; **growth** (body length); **reproduction** (number of neonates per brood, total number of neonates)	143
PS (f)	spheres	2 µm	1.39×10^{-3} and 1.11×10^{-2} mg/L	Sigma Aldrich, UK	unknown	21 days	**survival**; **growth** (body length); **reproduction** (number of neonates)	102
PS (f)	spheres	1-5 µm	10^2, 10^3, 10^4, and 10^5 particles/mL	Cospheric, USA	unknown	21 days	**growth** (body length); **others** (total biomass, number of ephippia)	37
PS	spheres	0.07, 1, and 10 µm	1 and 2 mg/L	BaseLine ChromTech Research Centre, China	clean, (vendor's declaration)	27 days	**survival**; **reproduction** (net reproductive rate, generational time and intrinsic rate of population increase)	144
① PS-COOH; ② $PS-NH_2$	spheres	① 26 and 62 nm; ② 53 nm	① (26 nm): 0.32 and 3.2 mg/L; ① (62 nm): 0.32, 0.76, 3.2, and 7.6 mg/L; ② 0.0032, 0.032, and 0.32 mg/L	Bangs Laboratories Inc., USA	yes (dialysis)	103 days	**survival**; **reproduction** (total number of neonates)	145
① PS; ② PS (f);	spheres	① 90 ± 5 nm; ② 95 ± 4 nm; ③ 100 ± 4 nm	① 1.6-26.6 mg/L; ② 1.7-28.6 mg/L; ③ 0.4-7.7 mg/L	Synthesized in the laboratory	unknown	21 days	**survival**; **growth** (body length); **reproduction** (time to first brood, number of neonates/brood)	146

③ PS (f) and palmitic acid (PS(f)-PA))								
PS (f)	spheres	50 nm	0.05 and 0.5 mg/L	Fisher Scientific, USA	unknown	21 days	**other** (gene expression analysis, oxidative stress and energetic biomarkers, swimming activity)	147
PS (f)	spheres	1 µm and 10 µm	0.125, 1.25, and 12.5 mg/L	Sigma Aldrich, Italy	unknown	21 days	**growth** (body length); **other** (swimming activity, phototactic behaviour)	148
PS (f)	spheres	70 nm	0.22, 0.44, 0.88, 1.8, 3.5, 7, 14, 32, 70, and 150 mg/L	AVT-PCC, Wageningen UR.	unknown	21 days	**survival**; **growth** (body length of adults and neonates); **reproduction** (total number of neonates in first three broods, number of neonates/brood); other (malformations in neonates)	149
unspecified (f), (1.30 g/cm^3)	spheres	1-5 µm	0.05, 0.1, and 0.2 mg/L	Cospheric, USA	unknown	21 days	**survival**; **growth** (somatic growth); **reproduction** (time to first brood, size of first brood, number of broods, number of neonates, number of dead/immobile neonates, number of aborted eggs)	68
PS-COOH (f)	spheres	500 nm	1 mg/L	Sigma Aldrich, UK	unknown	until second brood	**survival**; **growth** (body length, growth rate); **reproduction** (size of brood, number of neonates); **other** (haemocyte counts)	150
PS (f)	spheres	6 µm	5, 15, 30, 50, and 100 mg/L	Polysciences, Germany	unknown	21 days	**survival**; **growth** (body length of adults and neonates); **reproduction** (time to first brood, number of neonates)	111
PS-COOH (f)	spheres	① 20 nm; ② 200 nm	① 0.1 and 50 mg/L; ② 50 mg/L	ThermoFisher	yes (dialysis)	21 days	**survival**, **growth** (body length); **reproduction** (time to first brood, number of neonates); o**ther** (time to first molt, number of molts, swimming activity, hopping frequency)	151
PS (f)	spheres	6 µm	5 mg/L	Polysciences, USA	unknown	21 days	**survival**; **growth** (adult body length, first neonate body length, neonate body length); **reproduction** (time to first brood, number of neonates)	128
PE	fragments	≤50 µm	10, 25, 50, 100, and 200 mg/L	Sigma Aldrich	yes (washed with methanol and deionized water)	10 days	**survival**	152
① PMAA; ② PVA	① spheres; ② films and fibres	① 1.5-10 µm ② size not provided	① 1, 5, 10, 15, and 30 mg/L; ② 2.5, 5, 10, 25, and 50 mg/L	Synthesized in the laboratory	unknown	21 days	**survival**; **growth** (body length, width length, spine length, specific growth rate); **reproduction** (number of neonates); **other** (morphologic deformations)	127
PS-COOH (f)	spheres	0.5 µm	0.01, 0.02, 0.05, and 0.1 mg/L	Sigma Aldrich	unknown	7 days	**survival**; **reproduction** (occurrence of egg clutches)	153
PE	fragments	44.39 ± 11.16 µm	4.5 mg/L	Cryo-milling of PE pellets (Sigma Aldrich, USA)	yes (rinsed with hexane, methanol, and deionized water)	17 days	**survival**; **growth** (adult body length, neonate body length); **reproduction** (number of neonates, embryonic development rate); **other** (phototactic behavior, oxidative stress analysis)	126

EAA	spheres	103 nm	2.3×10^{11} and 2.3×10^{12} particles/L	Dow Chemical Co., USA	no	21 days	**survival**; **growth** (body length); **reproduction** (number of neonates); **other** (transcriptomic responses)	[88]
PS-COOH	spheres	15 µm	100 and 800 particles/L	Sigma Aldrich, UK	unknown	21 days	**survival**; **growth** (body length); **reproduction** (number of neonates)	[154]

Exposure to microplastics and nanoplastics has been documented to affect population and individual size, trigger malformation and mortality at high concentrations, but those effects are commonly dependent on (1) polymer type[138], (2) particle size[140], (3) duration of exposure[145], and (4) concentration[127], with a broad range of variation across studies. We list and compare below the effects described in the reviewed studies by particle type, followed by the size of the particles. Most research articles using commercial particles did not wash the particles prior to exposures, hence the absence of washing was mentioned when it could introduce confounding effects regarding toxicity results.

3.2.1. Chronic toxicity of spherical plastic particles

Polystyrene (PS): Many studies have highlighted PS microplastic and nanoplastic toxicity to *D. magna* across a range of different endpoints, from standard apical endpoints such as survival, to molecular stress markers. For instance, Yin *et al*.[80] reported that 5.8 µm PS showed effects on reproduction after 21 days at concentrations as low as 2 mg/L, with a decrease in the number of broods and neonates. Jeong *et al*.[128] found a decrease in survival, reproduction, and adult body length in *D. magna* exposed to 5 mg/L PS (6 µm) for 21 days. Growth rate was decreased by accumulation of PS particles, in response to which mitochondrial biogenesis was enhanced through *PGC-1a* gene upregulation. They also observed decreased cellular reactive oxygen species levels compared to controls after 24 h, and decreased superoxide dismutase and catalase activities. Likewise, Schwarzer *et al*.[143] found that 6 µm PS inhibited growth and reproduction when the study tested 6 and 20 µm PS at two concentrations, 500 and 5000 particles/L. No significant effects were observed in the *D. magna* exposed to 20 µm particles and the effects at both concentrations were comparable for both particle sizes. Another study from Bosker *et al*.[37] found a decrease of 26% in the population of adults after 21 days of exposure to 1 µm and 5 µm PS and decrease of total biomass of 21%. This study reported particle concentration rather than mass concentration (from 10^2 to 10^5 particles/mL). Similarly, Pikuda *et al*.[151] exposed *D. magna* for 21 days to washed 200 nm and 20 nm PS for 21 days to assess whether different metrics (particle counts versus mass) impacted the observations. No mortality was observed, however exposures impacted growth, reproduction through the number of neonates, and molting, at 50 mg/L in both tested sizes, and concentrations as low as 0.1 mg/L in the 20 nm PS. Another study did not observe mortality upon exposure of 70 nm PS particles, however, the particles caused a significant decrease in the total number of neonates and brood from the first three broods, starting from 0.2 mg/L.[149] Behavioral effects have also been observed - exposure to 1 and 10 µm PS from concentrations ranging from 0.125 to 12.5 mg/L increased swimming activity and overall body size of *D. magna* adults after 21 days of exposure.[148] The phototactic behavior was impacted as well, suggesting potential long-term consequences upon exposure. A study from Aljaibachi *et al.*[102] highlighted selective behavior from *D. magna* during a 21-day exposure to 2 µm PS, with a decrease of uptake in the presence of algae. De Felice *et al.*[147] went further into physiological and molecular endpoints, and exposed *D. magna* to very low concentrations of 50 nm PS beads (0.05 and 0.5 mg/L) to observe changes in genes involved in stress response. A slight modulation in the transcription of those genes was observed, notably genes involved in energy reserves, but no increase in oxidative stress markers was noted. Sadler *et al.*[150] observed an increase of hemocyte number after 21 days of exposure to 500 nm PS beads for 8 different clones, although the amplitude of the response was clone-dependent. The surface chemistry of PS particles also plays an important role in uptake and toxicity. For instance, Vicentini *et al.*[146] showed that ~90 nm particles functionalized with palmitic acid were more taken up than non-functionalized particles after 21 days, with a lowest observed effect concentration of 3.8 ng/L, and were 6.9 times more toxic, with a decrease in growth and reproduction at concentrations below 30 mg/L. Similarly, Huang *et al.*[153] studied the impact of carboxylated PS (500 nm) at low concentrations (as low as 0.1 mg/L) and found no mortality despite a high body burden. Due to fluorescent labelling, they were able to positively correlate uptake with duration of exposure (7 days). They also observed a delayed sexual maturation in exposed individuals at concentrations as low as 0.01 mg/L, correlated with a strong decrease in offspring numbers starting at 0.02 mg/L. Some studies noted fewer effects with no impact on survival nor reproduction. Rist *et al.*[142] observed uptake and retention times of 100 nm and 2 µm PS beads at concentrations ranging from 0.1 to 1 mg/L and observed a higher ingestion of the bigger particles at high concentration compared to the 100 nm sized ones, with no toxicological effects. They did observe a 21% decrease of the feeding rates with the smaller particles but no effects on reproduction. Prolonged exposures (103 days) showed that both carboxylated and aminated PS nanoplastics (washed) shortened the life-time of *D. magna*, with a decrease of almost three times in the aminated particles.[145] It is noteworthy that the studies that washed PS-COOH nanoplastics at 0.01 mg/L and 50 mg/L did not impact survival after 21 days chronic toxicity test[151], while washed PS-COOH with comparable size range at concentrations ranging from 0.32-7.6 mg/L significantly reduce survival in longer term exposures (103 days) with the decrease in survival observable after about 30 days[145]. This suggests that washed PS particles even at low concentrations may impact survival in daphnids after prolonged exposure.

Polyethylene (PE): Cannif *et al.*[64] studied PE beads with a size ranging from 63 to 75 µm and concentrations ranging from 25 to 100 mg/L. They did not observe toxic effects, despite a high uptake and body burden. An *et al.*[140] also investigated 5 mg/L of 40-48 µm PE beads and found no significant effects on survival, growth and reproduction.

Other polymer types: Gökçe *et al.*[127] studied the effects of lab-generated 1.5 to 10 µm PMAA on *D. magna* for 21 days and found toxicity, with a LC_{50} of 14.8 mg/L, and a decrease in population was observed throughout exposure. These particles also influenced body length and width, but the effect did not follow a dose-response relationship. According to Coady *et al.*[88], no effects on survival, growth and reproduction were observed in *D. magna* after 21 days of exposure to 103 nm EAA (2.3×10^{11} and 2.3×10^{12} particles/L). However, genes involved in metabolism, reproduction and oxidative stress were upregulated at the highest concentration of ethylene acrylic acid copolymer, suggesting potential effects for concentrations above 2.3×10^{12} particles/L.

Unknown polymer type: Several researchers investigated the toxicity of spherical microplastics with no specific polymer identification (*i.e.*, absence of FTIR/Raman to identify polymer type, and no information from the provider). Although these studies do provide information on potential toxicity, the impossibility to link them to a polymer type decreases the relevance of such studies for mechanistic comprehension of microplastic and nanoplastic toxicity. These studies highlighted the need to include characterization of polymer type for comparison across studies. For instance, Guilhermino *et al*.[68] found that microplastics from 1 to 5 µm caused parental and juvenile mortality at 0.2 mg/L after 21 days of exposure, with a strong decrease in reproduction and offspring EC_{50} ranging from 0.101 to 0.146 mg/L particles. Another study found effects on reproduction with similar particle size and concentration after 14 days,[136] with the production of immobile juveniles, but no effects on mortality nor individual growth. Ogonowski *et al*.[90] used similar sized pristine particles (1-5 µm) from the same supplier to compare effects of primary and secondary microplastics. No effects on life-history traits were found after 21 days exposure on adults and juveniles. Decrease of growth, fecundity, and survival in F_1 females were attributed to limited food availability due to the experimental design and the addition of plastic particles. A similar study by Jaikumar *et al*.[89] using the same particles from the same supplier found a decrease in neonates after 21 days of exposure to these microplastics, as well as a decrease in individuals' growth, but survival was not monitored.

3.2.2. Chronic toxicity of non-spherical plastic particles

Polystyrene (PS): Hiltunen *et al*.[78] generated 3.2 to 3.7 µm PS microplastic particles from a PS tray, and introduced the particles as 1% of *D. magna* diet. They did not find effects on survival, size, and reproduction after 14 days of exposure. Schwarzer *et al*.[143] generated two sizes of PS fragments (5.7 µm and 17.7 µm) and one size of PS fibres (diameter = 3 µm, length = 75.5 µm). The fibres did not show any significant effects to *D. magna* while the fragments impacted reproduction. Similarly, Trotter *et al*.[99] observed reduction in growth and reproduction when *D. magna* were exposed to ~100 mg/L of 13.03 ± 7.75 µm PS fragments.

Polyethylene (PE): Ogonowski *et al*.[90] found significant impacts on survival but slight effects on growth and reproduction upon 21-day chronic exposure to 2.6 ± 1.8 µm irregularly shaped PE particles. Another study exposing *D. magna* to 1-10 µm irregularly shaped PE particles observed reduced size of the first brood and the total number of neonates produced within the first three broods but not any significant impact on growth.[89] An *et al*.[140] demonstrated that PE fragments (~17 and ~34 µm) were internalized (*i.e*. ingested) to a greater extent than commercial microbeads of comparable size (~40 µm). Survival after 21 days was heavily impacted in a size-dependent manner, with a decrease of 20% and 60% for particle sizes of 17 and 34 µm, versus 90% for the spherical particles. They also observed that the smaller fragment size reduced the feeding rate, growth, and reproduction of *D. magna*, likely due to a higher retention time. In contrast, studies investigating larger sized fragments observed less significant toxicity. Song *et al*.[126] assessed the influence of PE fragments (48 µm) on *D. magna* survival, reproduction and development. Interestingly, after 17 days of exposure to PE, they observed no change in growth, reproduction, and phototactic behavior. Concurring to this finding, Kokalj *et al*.[139] did not find any effects on growth, reproduction, and lipid content after 21 days of exposure to ~40 and ~200 µm low density PE (LDPE) fragments, with concentrations from 1 to 100 mg/L. Using two prolonged exposures (134 and 98 days), Ekvall *et al*.[141] showed that high density PE (HDPE) 90 and 200 nm did not cause any toxicity to *D. magna*. However, a smaller fraction containing nanoparticles and oligomers with a size < 3 nm resulting from the breakdown of PE chains, and associated leached chemicals, decreased reproduction over time. To investigate further, they separated the small particles from the leached chemicals with sequential filtration, concentration, and dilution, and determined that toxicity was substantially reduced with only the small particles. They therefore showed that chemicals leaching from the plastic particles were highly toxic to *D. magna*, highlighting the potential long-term risk of additive leaching from plastics into the environment.

Polyvinyl chloride (PVC): A study aimed at comparing rigid PVC microplastics (4-141 µm) to flexible PVC microplastic (12-276 µm) containing 30% diisononylphthalate plasticizer.[135] Both PVC types did not impact survival, although the flexible microplastics reduced offspring number and increased body length. Similarly, in another study by Zimmerman *et al*.[138], flexible PVC particles reduced reproduction and the effect was mostly attributed to the same additive, diisononylphthalate, leaching from the particles. Indeed, separation of leached chemicals from the particles showed that toxicity could mostly be attributed to these chemicals because when separated and used alone, they had similar effects on reproduction compared to the results in the presence of the combination of the particles and leached chemicals. Effects of PVC were studied further by Liu *et al.*[134], who observed disruption of detoxification enzymes after 21 days exposure to 2 µm PVC and a change in transcription activities of superoxide dismutase and catalase enzymes after 50 µm PVC particle exposure. Both sizes of PVC particles were shown to decrease the number of neonates, but the effect of potential additives was not investigated.

Other polymer types: Khosrovyan *et al.*[137] showed no effects on survival nor on reproduction of 180 µm PA, pristine or UV-weathered, after 21 days, despite proof of ingestion of particles by *D. magna* with a high body burden. Hiltunen *et al.*[78] generated PET and ABS microplastics with a size ranging between 3.2 to 3.7 µm from a shredded bottle and a toy brick, respectively. They did not find effects on survival, size, and reproduction after 14 days of exposure to ~10^5 particles/mL of both polymer types, but microplastic represented only 1% of *D. magna* diet, and concentrations may have been too low compared to other studies to observe an effect. Zimmerman *et al.*[138] reported slight effects on survival from exposure to irregular microparticles of 10-500 mg/L of PLA and PUR with sizes up to 40 µm after 21 days exposure. They showed that effects of PUR and PLA particles were due to the particles themselves, but not their associated chemicals, highlighting that polymer composition is also an important driver of potential toxicity. Furthermore, PLA had an overall higher effect on survival, highlighting the concern for bio-based and biodegradable plastics that can be as harmful as non-biodegradable polymers.[138] Exposure to a mixture of PVA films and fibres impacted survival, growth and reproduction.[127] The LC_{50} for the 21-day study was found to be 24.4 mg/L and no neonates were born at concentrations higher than 5 mg/L, hence total progeny could not be assessed. However, they observed an increase in organisms' growth until the end of exposure, and malformations after the 10th day. The limited availability of research articles on potential toxicity of these polymer types highlights a knowledge gap that needs to be addressed in future studies.

3.3. Multigenerational effects of microplastics and nanoplastics

Multigenerational effects of microplastics and nanoplastics have been examined in 9 studies compared to the 36 studies that assessed chronic toxicity.[69-71,133,149,154-157] The details of the studies are summarized in **Table 3**. Similar to the single-generation chronic studies, 7 out of those 9 studies investigated effects of PS. Reported effects varied depending on multiple factors such as polymer type, particle size and exposure time, and ranged from no sensitivity[155] or a decrease in population or reproduction over time[156] to complete population extinction.[69] Milder effects such as malformation[149] and overall fitness decrease[133], as well as subsequent effects on non-exposed offspring have also been observed, which could indicate potential long-term effects, such as population weakening.

Besseling *et al.*[149] found that aged PS increased malformation rates up to 68% starting from 30 mg/L in the F_1 generation. Another study using the same duration of exposure with a similar particle size of ~73 nm observed impacts on the reproduction and age at first brood of F_1 and F_2 generations of *D. magna* after exposure to 6.62 mg/L and 13.24 mg/L PS.[156] Similar results were found for PS[71] and PE microplastics[70]. One study using commercial particles of unknown polymer type found a complete extinction of *D. magna* population after 2 generations upon 21 days exposure to 0.1 mg/L pristine microplastics ranging from 1 to 5 µm in size, however there are no information regarding additives potentially present in the commercial suspension that may influence toxicity results.[69] Schur *et al.*[133] showed that irregular ≤ 63 µm PS particles generated from coffee lids decreased size and growth of *D. magna* across 4 generations at a concentration of 2000 particles/mL, and resulted in extinction within one generation at 10000 particles/mL. They also tested the fitness of neonates from each generation by exposing them to potassium dichromate but saw no significant effects - hence maternal exposure to PS particles had no impact on their offspring's fitness. Using the same particles, the authors conducted a second multigenerational study over 21 days investigating (1) effect of aged versus pristine particles and (2) potential toxicity mitigation using wastewater for PS incubation, with a broader range of concentrations (from 80 to 10000 particles/mL, Schur *et al.*[157]). They observed that both particle types affected survival, reproduction, and organisms' length - with a concentration-dependent impact on survival in F_0-F_2, but not F_3. The main effect observed at the lowest concentration (80 particles/mL) was 25% mortality in the fourth generation. Similar to the earlier study, only 5% of the organisms survived when exposed to 10000 particles/mL. Interestingly, PS incubation in wastewater decreased overall mortality despite no observed changes in particle size, surface, and structure.

Other research on 20 nm PS particles reported opposite results, with no effects on offspring growth and reproduction despite proof of particle transmission to the next two generations at 50 mg/L.[155] Similarly, a mesocosm study over 12 weeks using 15 µm unwashed PS particles at 100 particles/mL saw weekly changes in *D. magna* abundance in the first half of the mesocosm experiment, but the population recovered and no effects were observed after 12 weeks, suggesting an acclimatization over time.[154]

Most studies focused on apical and population endpoints, such as survival, number of broods and growth, as they are considered the most relevant to evaluate long-term effects. Only one study aimed at investigating epigenetics molecular biomarkers as a potential mechanism for multigenerational stress response upon microplastic exposure in *D. magna.*[70] The toxicity of 16.7 µm PE (4.35 mg/L) was studied for 21 days across 4 generations - direct exposure induced mortality, but the decrease in population was recovered after 3 generations. Growth and reproduction remained lower than the controls despite no DNA methylation observed across generations. This study highlights that mechanisms other than epigenetics may play key roles in long-term toxicity and response to microplastic exposure.[69,70]

Table 3. Studies investigating the multigenerational toxicity of microplastics and nanoplastics to *D. magna*. -COOH means carboxylated functional group. (f) means fluorescently labelled.

Type of plastic	Shape	Sizes	Concentration	Source of plastic	Was the plastic washed	Duration of test	Number of generations	Targeted endpoints	Refs.
unspecified (f), (1.30 g/cm^3)	spheres	1-5 µm	0.1 mg/L	Cospheric, USA	unknown	21 days	F_0, F_1, F_2, F_3	**survival**; **growth** (body length); **reproduction** (time to first brood, number of brood, number of neonates, number of dead/immobile neonates)	69
PE	fragments	16.68 ± 7.04 µm	4.35 mg/L	Cryomilling of PE pellets	yes (rinsed with hexane, methanol, and deionized water)	21 days	F_0, F_1, F_2, F_3	**survival**; **growth** (somatic growth); **reproduction** (number of neonates); **other** (gut damage, global DNA methylation)	70
PS-COOH (f)	spheres	15 µm	100 particles/L	Sigma Aldrich, UK	unknown	12 weeks	unspecified	**survival**; **growth** (body length); **reproduction** (number of neonates)	154
PS	fragments	≤ 63 µm	80, 400, 2000, and 10000 particles/mL	PS lids of coffee-to-go cups	unknown	21 days	F_0, F_1, F_2, F_3	**survival**; **growth** (body length of adults and neonates); **reproduction** (time to first brood, number of neonates)	157
PS (f)	spheres	5 µm	2.5 µg/L	Da'e Scientific Co., Ltd, China	unknown	21 days	F_0, F_1	**survival**; **growth** (body length); **reproduction** (time to first brood, number of neonates); **other** (population growth rate, swimming and feeding behavior)	71
PS (f)	spheres	20 nm	1 and 50 mg/L	ThermoFisher Scientific, Canada	yes (dialysis)	Until the production of first neonates (F_1)	F_0, F_1, F_2	**survival**; **growth** (body length); **reproduction** (number of neonates); **others** (swimming distance, heartbeat rate, appendage beat rate, tentacles beat rate, post-abdominal curling rate)	155
PS	fragments	≤ 63 µm	400, 2000, and 10000 particles/mL	PS lids of coffee-to-go cups	unknown	21 days	F_0, F_1, F_2, F_3	**survival**; **growth** (body length of adults and neonates); **reproduction** (time to first brood, number of neonates)	133
PS (f)	spheres	70 nm	0.22, 0.44, 0.88, 1.8, 3.5, 7, 14, 32, 70, and 150 mg/L	AVT-PCC, Wageningen UR.	unknown	21 days	F_0, F_1	**survival**; **growth** (body length of adults and neonates); **reproduction** (total number of neonates in first three broods, number of neonates/brood); other (malformations in neonates)	149
PS (f)	spheres	73 ± 7 nm	1.65, 3.31, 6.62, and 13.24 mg/L	Synthesized in the laboratory	unknown	21 days	F_0, F_1, F_2	**survival**; **growth** (body length of neonates); **reproduction** (time to first brood, number of neonates, number of neonates/brood)	156

3.4. Impacts of co-contaminants on toxicity results

In polluted environments, *D. magna* is not only exposed to plastics, but also to co-existing contaminants when they accidentally ingest or encounter microplastics. The vector role of microplastics and the combined pollution exposure complicate their toxic effects on *D. magna.* Commonly found co-existing contaminants mainly include two categories: plastic additives released from the particles themselves and environmental pollutants sorbed onto the particles from the environment.[158]

3.4.1. Impacts of plastic additives

Plastic additives primarily refer to chemicals that are incorporated into plastics during the manufacturing process to meet different product performance requirements. These chemicals include plasticizers, antioxidants, adhesives, antimicrobials, stabilizers, and flame retardants. Other chemicals, such as certain catalysts used during manufacturing and remaining oligomers, alongside non-intentionally added substances, can also be present at high concentrations and behave similarly to additives. **Table 4** presents a summary of studies which have investigated the impacts of plastic additives in the observed toxicity of *D. magna*.

Table 4. Studies investigating plastic additives and microplastics co-exposure to *Daphnia magna*. Where applicable, circled numbers were used to distinguish particles that possess different properties within the same study.

Plastic Type	Size and Shape	Exposure concentration	Type of exposure	Observed effects from leachates	Additives	Concentration of additive in leachate	Common percentage in plastic	Plastic concentrations in medium (solid to liquid ratio)	Additives leaching %	Mixture toxicity	Refs.
① rigid PVC ② flexible PVC	① 4-141 µm (fragments) ② 12-276 µm (fragments)	N/A	chronic (25-31 d)	Rigid PVC caused delay in time to first brood Flexible PVC increased body length and reduced reproduction	diisononyl phthalate (endocrine-disrupting chemical)	2.67 µg/L	/ 30%	N/A	N/A	synergistic	135
① PVC ② PUR ③ PLA	① ≤ 20 µm; ②③ ≤ 40 µm (fragments)	① 500 mg/L ②③ 0.2-500 mg/L	chronic (21 d)	Delayed reproduction, reduced body length Reduced the reproduction, reduced the somatic growth of daphnids	N/A	N/A	N/A	1:2000 1:5000000 1:500000, 1:50000 3:5000, 1:10000, 1:2000	N/A	synergistic	138
PET	length: 60-1400 µm; width: 31-528 µm; diameter: 2-21.5 µm (fibres)	12.5, 25, 50, and 100 mg/L	acute (48 h)	Mortality increased	N/A	N/A	N/A	12.5, 25, 50, and 100 mg/L	N/A		107
PE	39.72 ± 14.45 µm (fragments)	20 mg/L	acute (48 h)	Mortality increased	benzophenone-3	10.27 ± 0.40%	5-10%	1:2000, 1:50000	41.1 ± 2.7%	synergistic	101
Mixture (HDPE, PET, PP, and nylon)	≤ 17 µm	10% and 50% of the plastic leachate.	acute (48 h)	No mortality	Mixture: BPA confirmed	N/A	0.20%	1:50	N/A	independent	155
		100% of the plastic leachate.	chronic (10 d)	Plastic leachate reduced the appendage curling rate but increased growth and reproduction. No effect on the heartbeat rate and swimming distance was observed.							

PA	15-20 µm (fragments)	200 mg/L	acute (48 h)	No leachate effect on acute exposure.	N/A	N/A	N/A	1:500	N/A	N/A	159
rubber	155.72 ±143.68 µm (fragments)	1000 mg/L 3.125%, 6.25%, 12.5%, and 25% of the sneaker leachate.	acute (24 h and 48 h)	sneakers (shoes) leachate showed immobility and mortality	benzothiazole, carbon disulfide, ethyl acetate, p-xylene, Zn, As, etc.	N/A	0.1%, 0.5%	1:1000; 1:500	N/A	synergistic	160
Bakelite	7.6 ± 3.5 µm (fragments)	15, 25, 30, 40, 50, 60, 75, and 100 mg/L	acute (24 h and 48 h)	Leachates had more severe adverse effects than microplastics alone	phenol and phenol- like compounds	N/A	0.1%, 1.0%	1:10000	N/A	synergistic	161
PE	17.35 ± 5.50 µm (fragments)	5 mg/L	multi-generational (F_0-F_3)	Survival rate decreased, number of offspring decreased (F_3), population growth rate decreased.	benzophenone-3	N/A	2.85 ± 0.16%	1:200000	N/A	synergistic	70
PP	diameter: 21.2 ± 1.5 µm (inner layer), 22.1 ± 1.6 µm (outer layer), and 4.3 ± 2.2 µm middle layer). Length not provided (fibres)	1, 10, and 100 mg/L	acute (24 h and 48 h)	No effects on mobility and survival	mixture	N/A	N/A	1, 10, and 100 mg/L	N/A	N/A	119
PPDO, PE, PS	5 mm (pellets, films)	0.0001, 0.001, 0.01, 0.1, and 1 g plastic/L leachate	acute (48 h)	Elevated mortality occurred in biodegradable plastics (PPDO) compared to conventional plastics (PE and PS)	mixture	N/A	N/A	0.0001, 0.001, 0.01, 0.1, and 1 g plastic/L leachate	N/A	N/A	162
PVC	2-3 mm (fragments)	10, 100, and 500 mg plastic/L leachate	acute (48 h); chronic (F_0-F_1)	Low mortality occurred, decrease in population development	mixture	N/A	N/A	10, 100, and 500 mg plastic/L leachate	N/A	N/A	163
PE	0.2-9.9 µm, 30-45 µm, 300-354 µm (spheres)	1, 2.5, and 5 mg in 70 mL of assay media with and without BACs.	acute (48 h)	Survival rate increased.	benzalkonium chlorides: (BAC_{12} ($C_{21}H_{38}NCl$), BAC_{14} ($C_{23}H_{42}NCl$),	N/A	N/A	1:9	N/A	antagonistic	129

					BAC_{16} ($C_{25}H_{46}NCl$))						
PE	44.39 ± 11.16 µm (fragments)	4.5 mg/L	chronic (17 d)	Embryonic development inhibited, and offspring growth decreased.	benzophenone-3 (BP-3)	(10.82 ± 1.20% w/w)	2.85 ± 0.16%	1:200	N/A	synergistic	[126]
PP, HDPE, PVC, ABS, and epoxy	4 cm^2 (square pieces)	250 g particle/L	acute (24 h and 48 h)	Leachates from plasticized PVC and epoxy had more severe effects compared to HDPE. No effects observed in leachates from PP, ABS, and rigid PVC.	N/A	N/A	N/A	1:4	N/A	N/A	[164]

According to the studies reported in **Table 4**, there are already 104 kinds of additives or non-intentionally added substance that have been reported in the leachates of microplastics[119,162,163] (**Figure 4**), which may represent only a fraction of the range of chemicals incorporated into plastics. When plastics are degraded and fragmented into smaller microplastics and nanoplastics, the higher specific surface area facilitates migration of plastic additives into the environment.[17,158,165]

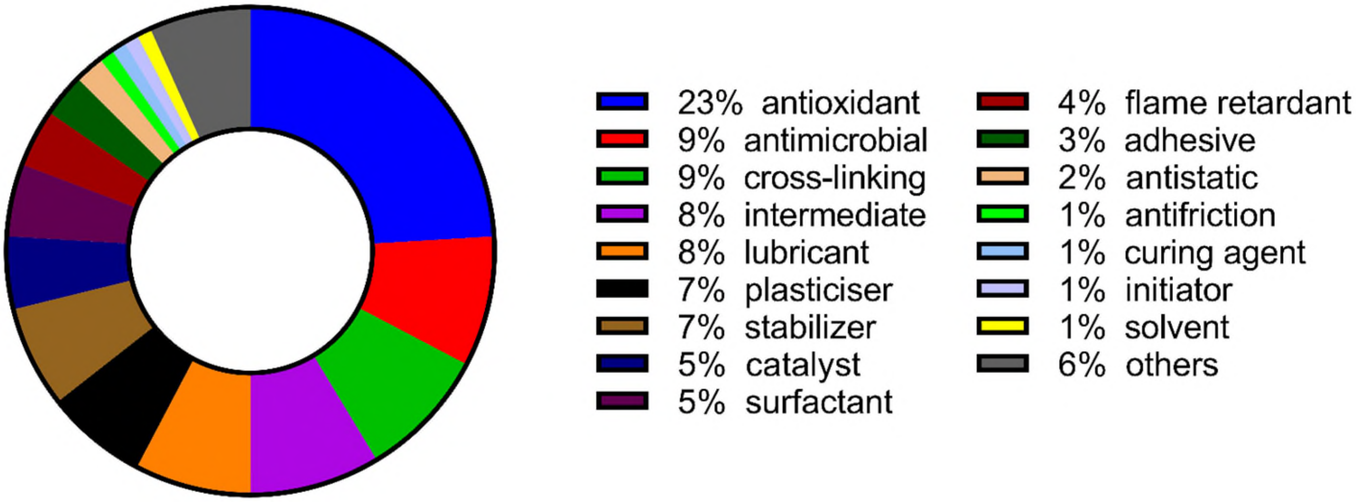

Figure 4. Compositional ratios of the various types of additives in plastic leachate mixtures. The analyzed data were obtained from the *D. magna* toxicity studies listed in **Table 4** which have (tentatively) quantified the additive compositions in the plastic leachates.

The leaching of additives makes the environmental behavior of microplastics very complex and its toxicological effects difficult to predict especially when a mixture of compounds is released. The composition of leached additives and their toxic effects have been studied extensively. The proportion of additives incorporated in plastics can be quite high and thus their leaching is generally unavoidable. As shown in **Table 4**, the content of some commonly used additives in plastics ranged from 0.1-30% w/w while sometimes this percentage can be even higher, reaching 180% for plasticizers (more than the polymer itself) in soft rubber-like plastics, according to some manufacturers.[70,101,126,135,160,161] Moreover, many plastic additives, such as phthalates, are in the form of non-covalent bonds, and therefore they can be easily released upon entering the environment.[166]

Aquatic organisms in the natural environment, such as *D. magna*, can be exposed to plastic additives during both aqueous and dietary exposure to microplastics which subsequently may lead to toxicological effects. *D. magna* has been exposed to additives such as antioxidants, plasticizers, and stabilizers to evaluate their toxicological effects. In most cases, co-exposure of microplastics and additives usually leads to synergistic effects.[72,135,155,160,161] For instance, in comparison to the additive benzophenone-3 alone, the presence of microplastics further enhanced the toxicity of benzophenone-3 by increasing the generation of reactive oxygen species and lipid peroxidation levels. This is partly due to the increased bioconcentration of benzophenone-3 in *D. magna* in the presence of microplastic, and because irregular microplastic fragments (20 mg/L, 39.72 ± 14.45 μm) caused physical damage which subsequently caused severe intestinal injury.[101]

The solid-to-liquid ratio (SLR) varies widely among different studies and can directly affect the concentrations of released additives (**Figure 5a**). Although there is no significant difference between the laboratory-simulated solid-to-liquid ratio (10^{-7}~10^{0}) and the real environmental solid-to-liquid ratio (10^{-7}~10^{-3}), the average solid-to-liquid ratio value in exposure studies is two orders of magnitude higher than that in the real environment. Hence, most results mainly reflect the potential toxicity of combined pollution of microplastics and leached additives in pollution hotspots. Also, we observed that, with the exception of bisphenol A (BPA), which was much higher than those in the field, the co-exposed pollutant concentrations were roughly in the same range as those in the actual environment (**Figure 5b**).

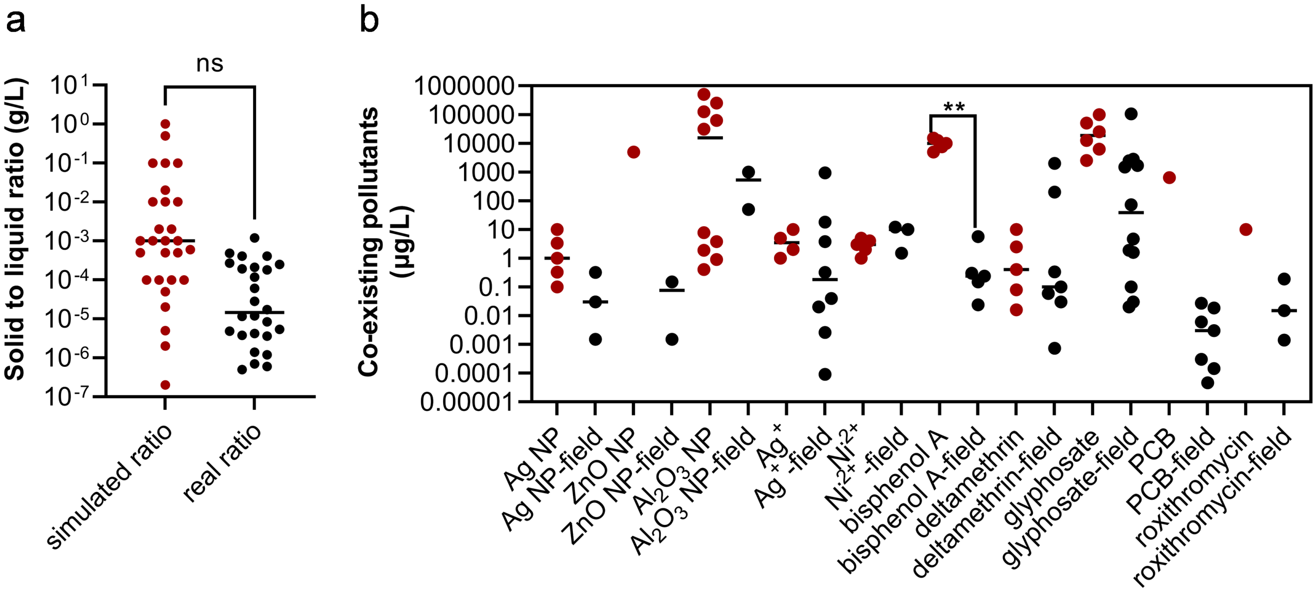

Figure 5. (a) Co-exposure with additives: the plastic weight to liquid volume ratio; Simulated ratios are used in laboratory simulation experiments, while real ratios are plastic mass to liquid volume ratios found in nature. (b) Co-exposure with environmental pollutants: the concentrations used in experiments (red dots) versus those measured in the field (black dots).

It should be noted that the reporting of the final aqueous concentrations and/or released percentages of additives is largely missing. Only very limited studies quantified the freely dissolved additive concentrations. Schrank *et al.*[135] reported the final aqueous freely dissolved concentration (2.67 μg/L) of diisononyl phthalate from PVC microplastics, which is important for the understanding of the joint toxicity of microplastics and additives. In addition to the fact that the quantification of additive concentrations was largely unknown in most studies, there are also two other issues that require consideration. First, plastics often contain a variety of additives, and their composition is predicted by non-target screening, and identification of each compound is time consuming and sometimes very difficult due to a lack of standards. Second, as most researchers are unable to obtain the actual amounts of additives incorporated in plastics, the percentage of additives released is difficult to determine. Also, we noted that little attention has yet been paid to emerging flame retardants, such as organophosphates, and novel brominated flame retardants, which are used as substitutes for conventional flame retardants, *i.e.*, polychlorinated biphenyls and polybrominated diphenyl ethers. Moreover, some plasticizers can reach an extremely high incorporation rate (i.e., 70%)[167], which also deserves more attention in the future.

3.4.2. Impacts of environmental pollutants.

In this review, the environmental pollutants on microplastics refer to the associated pollutants coming from the surrounding environment, which can sorb onto microplastics, and most have reached sorption-desorption equilibrium and are at relatively lower concentrations in comparison to plastic additives.

Several studies have employed laboratory simulation approaches to explore the impacts of co-exposure of environmental pollutants and microplastics or nanoplastics to *D. magna* **(Table 5)**. However, most of these works utilized similar research methods and experimental designs to those for microplastic additives, and thus the results cannot well reflect the real accumulation and/or toxicity of these associated environmental pollutants. First, the tested concentrations of the environmental pollutants are considerably higher compared to those detected in the natural environment and no background trace contamination was taken into account.[159] Second, various joint toxic action modes, such as synergistic,[100,114,116,121,156,168] antagonistic,[159,169] and independent,[170] have been reported for the co-exposure of microplastics and environmental pollutants; however, most of the studies do not reflect the actual scenario in the natural environment. Specifically, most laboratory-based studies adopted the non-equilibrium experimental design, only using pollutant-contaminated microplastics for exposure, without considering other ubiquitous environmental matrices (e.g., natural organic matter, food particles, and sediments), and only clean organisms were used for testing, which promotes the migration of pollutants from microplastics to *D. magna*.

Table 5. Studies investigating environmental pollutants and microplastics co-exposure to *Daphnia magna*. -COOH and -NH_2 means carboxylated and aminated functional group, respectively. (f) means fluorescently labelled. Where applicable, circled numbers were used to distinguish particles that possess different properties within the same study.

Type of test	**Plastic Type**	**Size and shape**	**Exposure concentration**	**Co-exposed compound name and CAS number (if available)**	**Compound concentration**	**Mixture toxicity**	**Effects on *D. magna***	**Refs.**
acute (48 h)	PS; PS-COOH	~200 nm (spheres)	1, 5, 10, 20, and 30 mg/L	nickel	1, 2, 3, 4, and 5 mg/L	when combined with Ni, PS had a slight antagonistic effect on toxicity, whereas PS-COOH had a slight synergistic effect.	Higher immobilization toxicity observed in combined exposure group in comparison to microplastics alone	116
chronic (7 days)	PS	1 µm and 10 µm (spheres)	0.1 mg/L	roxithromycin	0.01 mg/L	synergistic effect	glutathione peroxidase and glutathione S-transferase enzyme activities and malondialdehyde levels decreased in combined exposure group in comparison to microplastics alone	114
chronic (7 days)	① PE ② PET/PA	① 1-10 µm, average diameter of 2.09 µm (spheres); ② length = 10 µm; width = 2 µm (fibres)	① 2.2×10^6 particles/mL (0.01 mg/mL) ② 10-30 particles/mL	① glyphosate acid; ② a commercial formulation of Roundup: 47.5% n-(phosphonomethyl)-glycine-monosodium salt powder; ③n-(phosphonomethyl)-glycine, monoisopropylamine salt solution	2.5 mg/L	① glyphosate acid, but also with Roundup Gran: synergistic effect；② glyphosate-IPA salt: antagonistic effect	Higher mortality was observed in combination with glyphosate acid and Roundup Gran, but decreased when in combination with glyphosate-IPA salt	168
acute (48 h)	PS	100 nm (spheres)	1-75 mg/L	polychlorinated biphenyl-18	640 µg/L	antagonistic effect (the addition of plastic could decrease the free-dissolved concentration of organic pollutants and the toxicity. Meanwhile, the excessive amounts of plastic could also cause toxic effect towards *D. magna*)	Lethality decreased when polychlorinated biphenyl combined with low amounts of PS nanoplastics, whereas the lethality was enhanced when combined with excessive amounts of PS nanoplastics.	117

acute (48 h)	PA	15-20 µm (fragments)	200 mg/L	bisphenol A	5, 7.5, 10, 12.5, and 15mg/L	antagonistic effect	The combination of BPA and PA led to decreased immobilization.	159
acute (72 h)	PS (f)	1.2 ± 0.2 µm (spheres)	0.29 µgmL^{-1}	dimethoate, deltamethrin	0.156-5 mg/L for dimethoate, 0.016-10 µg/L for deltamethrin	no effects (no alteration in the acute toxicity of either deltamethrin or dimethoate to *D. magna*, regardless of the chemical binding capacity (log K_{ow}). Mortality and mobility impairment increased with concentration and time for both pesticides, however the concentrations at which detrimental effects occurred were not influenced by the presence of microplastics.	Pesticide toxicity was not affected by the presence of microplastics	170
acute (48 h)	PS (f)	~73 ± 7 nm (spheres)	15.6, 31.2, 62.5, 125, 250, and 500 mg/L	glyphosate	6.2, 12.5, 25, 50, 100, and 200 mg/L	synergistic effect	Increase in immobility and reactive oxygen species production and decrease in swimming activity for combined exposure.	156
acute (72 h)	① PS ② PE	① 300 and 600 nm (spheres) ② 300-9000 nm (spheres)	6.74×10^{10} particles/L	① Ag^{+}	① Ag^{+}: 1, 2, 5 and 10 µg/L	① Ag-PS-NPD: synergistic effect; ② Ag-PE-NPD: no effects. PE-NPD did not change the toxicity profile of 1 µg/L Ag^{+} compared to the PS-NPD and the control. The presence of PS-NPD led to an increased toxicity of Ag^{+}	When the Ag^{+} concentration in the exposure media increased the survival decreased.	100
acute (48 h)	PS (f)	~90 nm (spheres)	31.25, 62.5, 125, 250, and 500 mg/L	palmitic acid	N/A	synergistic effect	Increased immobility; reduced reproduction	146
chronic (21 days)			0.4, 0.9, 1.9, 3.8, and 7.7mg/L				PSNP/F-PA was almost 3 times more toxic than PSNP, led to reproduction inhibition and mortality following long-term exposure at lower concentrations. Palmitic acid induced increased immobility and reduced reproduction	
acute (48 h - 96 h)	PET	diameter: 14 ± 3 µm; length: 366 ± 275 µm (fibres)	100 mg/L	① AgNP ② $AgNO_3$	0.33 µg Ag/L	synergistic effect	Sublethal effects of cellular energy allocation was decreased	121
acute (8 h)	PS	0.1 µm (spheres)	1 mg/L	nanoparticles	5 mg/L	synergistic effect	Increased the ion-related toxicity (mortality) in the combined exposure group	171
acute (48 h)	PS-COOH, PS-NH_2	200 nm (spheres)	10-400 mg/L	① alginate ② SRHA/Humic acid	2 mg/L	antagonistic effect	Significantly reduced the immobilization of both amidine and carboxyl PS nanoplastics	124

acute (up to 72 h)	PS (f)	1 µm (spheres)	1820 particles/mL (10^{-9} g/ mL)	chromium	2 and 5 mg/L	synergistic effect	Mortality increased with the presence of MPs.	[172]
acute (24 h and 48 h) chronic (21 days)	PS (f)	6 µm (spheres)	10 mg/L 5 mg/L	chromium	0.1-1.0 mg/L 10 and 100 µg/L	synergistic effect	Acute mortality increased with the presence of MPs. Survival, growth, and reproduction decreased in co-exposure to 100 µg/L chromium	[128]
chronic (21 days)	PS-COOH (f)	2 µm (spheres)	1 and 10 mg/L	zinc	N/A	synergistic effect	Reproduction reduced, body length decreased, survival rate decreased.	[173]
chronic (21 days)	PE	1-4 µm (spheres)	1 and 10 mg/L	pyrethroid insecticide (deltamethrin)	40 ng/L	synergistic effect	Increase in mortality and a reduction in longevity, number of neonates per surviving adult, number of broods, body length and cumulative number of molts	[174]
acute (48 h)	PS	30 nm (spheres)	12.5, 25, 50, 100, and 200 mg/L	Wastewater effluent containing chemicals such as antibiotics, anti-inflammatories, contrast agents, antidepressants, beta-blockers, and several intermediate metabolites, etc.	N/A	antagonistic effect (the formation of PS-wastewater heteroaggregates was observed which could result in lower bioavailability of PS NPs and sorbed micropollutants, thus lowering toxicity.)	A decrease of toxicity when *D. magna* was exposed to PS-wastewater mixtures	[175]
chronic (21 days)	unspecified (f), (1.30 g/cm^3)	1-5 µm (spheres)	0.05, 0.10, and 0.20 mg/L	lithium	0.02, 0.04, and 0.08 mg/L	The toxicological interaction between lithium and microplastic was antagonism at the lowest and the highest concentrations, and synergism at the medium concentration.	Significantly reduced the population fitness. Growth and reproduction decreased	[176]

In a real co-exposure scenario of microplastics and environmental pollutants, the pollutants have (nearly) reached equilibrium, and other particle/matter migration routes for environmental pollutants should be considered besides the one mediated via microplastics. Koelmans *et al*.[177] verified that the contribution of environmental pollutants to bioaccumulation through microplastics is negligible and far below that of other natural matrix carriers in a quantitative modeling study. Recently, we also demonstrated that microplastic has negligible contribution to the bioaccumulation and toxicity of the co-existing environmental pollutants at environmentally relevant microplastic concentrations when compared with other natural particles.[158]

Research on the toxic effects of microplastics and co-existing pollutants on *D. magna* is newly emerging. There is still a dearth of information on the conditions in which the presence of multiple pollutants can have synergistic adverse effects. We also need to pay more attention to additives and investigate their toxicity in microplastics independently of the coexisting environmental pollutants.

General toxicity endpoints (*i.e.*, immobilization, mortality, and reproduction) are commonly monitored in combined pollution exposure studies (**Figure 6)**. Less attention has been paid to the specific effects of additives or environmental pollutants. For instance, the baseline toxicities of Ni, bisphenol A, and deltamethrin have been studied in the presence of microplastics, mainly focusing on the mortality, immobilization endpoints.[100,170,178] However, some unique sublethal toxicities of these chemicals, such as the respiration function impairment caused by Ni,[179] the affected energy metabolism and neurotoxicity caused by bisphenol A and pesticides,[178,180] can also be affected by the presence of microplastics but have not been emphasized yet.

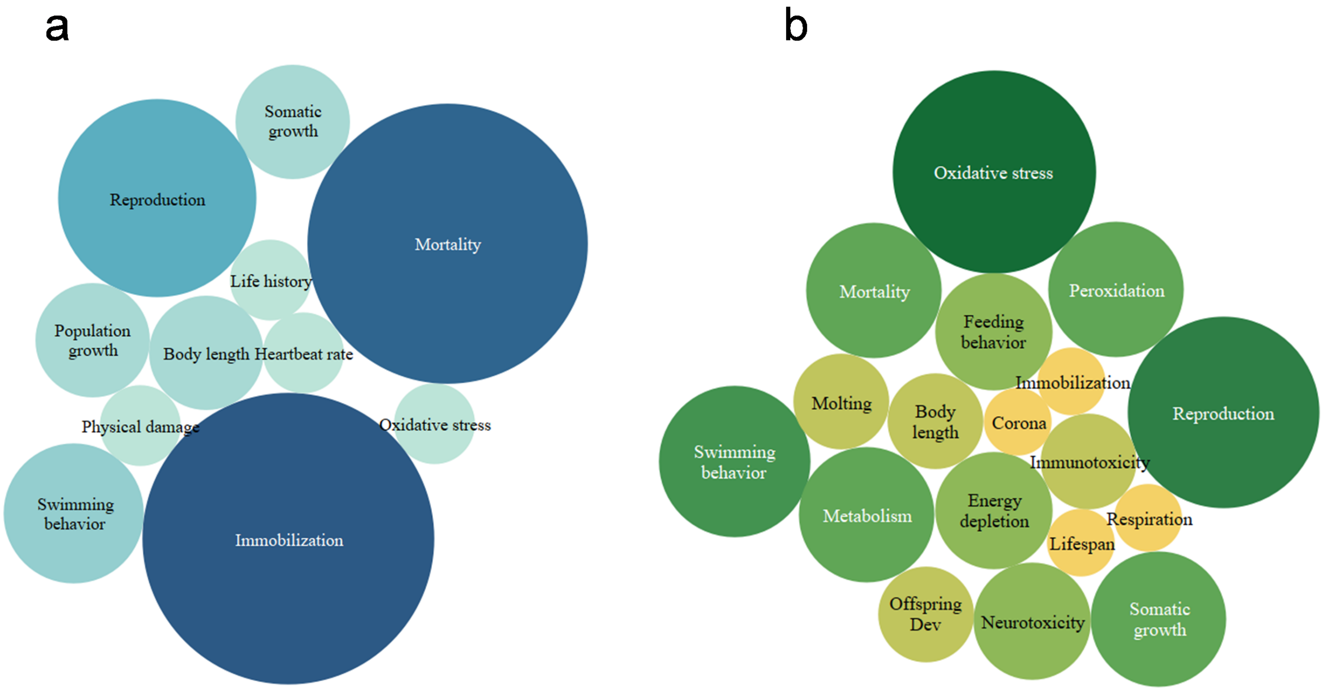

Figure 6. Different bioassay endpoints in exposure experiments of: (a) microplastics and co-existing pollutants (including both additives and environmental pollutants) and (b) co-existing pollutants alone.[119,155,162] The size of each circle represents the relative number of studies incorporating the bioassay endpoints.

Additives and environmental pollutants have different sources, exposure concentrations and distinct environmental behaviors. For the co-exposure of microplastics and environmental pollutants, it is necessary to carry out studies under relative equilibrium scenarios at concentrations closer to environmentally relevant ones, and the influence of natural substances (e.g., food particles, natural organic matter, sediments) should be considered for reliable conclusions to be made.

Currently, around 75% of additive co-exposure studies focused on only a single additive. But a cocktail of various additives is present in microplastics, and thus the mixture toxicity becomes extremely complex. Only a few studies have explored the mixture toxicity of additives. For instance, Kokalj *et al.*[119] and Dao *et al.*[163] analyzed the toxic effects of additive mixtures released from PP and PVC microplastics on the mortality and population development of *D. magna*. Recently, Gao *et al.*[162] also developed the *D. magna* toxicity test into a rapid screening method for microplastic additive mixture toxicity analysis. More studies are needed in this area to explore the effects of additive mixtures.

4. Methodological Innovations and Limitations of the Study

This study employed an innovative approach to conduct a comprehensive review of the state of knowledge and available data for the toxicity of micro- and nanoplastics to *Daphnia magna*. First, the study conducted a rigorous screening using detailed inclusion and exclusion criteria to ensure unbiased selection of relevant papers and increase the degree of comparability of the toxicity results reviewed. Another methodological innovation is the analysis and discussion of different types of toxicity (i.e., acute, chronic, multigenerational, and co-contaminant studies) on a single organism, *Daphnia magna*. This approach allowed for a more comprehensive evaluation of the toxicity of the particles, capturing the different endpoints associated with each toxicity type. Also, the synthesized data from each type of toxicity enabled the identification of potential patterns or trends in the observed toxicities for the different plastic polymer types. Furthermore, for the first time, this study used a targeted research synthesis method to present a graphical representation of the acute toxicity of micro- and nanoplastics and the potential effects of the relationship between particle size and exposure concentration which will improve our understanding of existing data in this area.

Despite these innovative methods, we recognize that the study may have a few limitations which are highlighted below:

Publication Bias: This review paper only assessed the results of published scientific papers. Hence, it may be biased by the fact that some studies showing insignificant toxicity (i.e., no effects studies) or positive effects to *Daphnia* may not have been submitted to peer-reviewed journals for publication.

Limited Long-Term Studies: Only very few studies investigated the multigenerational effects and even fewer studies investigated longer term effects beyond the 21-day duration that is typical for chronic studies.

Considering the potential for prolonged exposure of micro- and nanoplastics in the natural environment, these short-term studies may not fully cover the environmental impacts of micro- and nanoplastics to the model organism, *Daphnia magna*.

Focus on a Single Specie: While *Daphnia magna* is commonly used as a model organism for micro- and nanoplastic toxicity studies and toxicity studies in general, it is essential to acknowledge that the toxicity results observed upon exposure to this organism may not fully represent the effects of the same particles on other aquatic invertebrates. Hence, readers are encouraged to exercise caution when extrapolating findings from this review to other species or organisms.

Lack of Real-World Complexity: The acute, chronic, and multigenerational experiments conducted in the reviewed papers employed simplified laboratory conditions in which the organisms were exposed to the particles, rearing media, and food for longer term studies. Also, the co-contaminant studies limited co-exposure to single pollutants being investigated. Hence, the absence of other factors or materials that are present in the real environment such as fluctuating temperature, interaction with mixture of pollutants or contaminants, interaction with natural organic matter, etc. may limit the environmental relevance of the studies or the findings of this review.

5. Conclusions and Future Directions

Research findings show that there is no one factor that governs the toxicity of the particle to an organism. Larger microplastics (e.g., > 35 µm) do not have impacts towards *D. magna* at environmentally relevant concentrations - both in the short term and the long term while nanoplastics exhibit high toxicity levels not only on short term exposure but also in the long term with impacts on reproduction and gut integrity of the exposed organism. Existing data suggests that smaller sized particles may be more bioavailable to *D. magna*. It is unclear whether fragments are more toxic than their spherical counterparts as only a limited number of studies have explored this topic. Moreover, most of the studies using fragments either focused on short-term exposures or used large-sized particles that may not be ingested by the organisms.

Polymer type and tested concentrations may also play important roles. However, in this review, most of the studies used PS particles with a limited number of studies exploring the impact of other polymer types. Also, it is difficult to compare concentrations across different plastic types owing to differences in shapes, sizes, and densities and that several studies use different concentration metrics such as mass of particles or number of particles.

One of the common reasons for using spherical microplastics and nanoplastics was because of their potential release from personal cosmetic products. However, the studies which have obtained microplastics from these products found that the particles appear as fragments rather than spheres. Furthermore, most of these products are gradually being banned by various countries. Hence, although use of these "simple-shaped" particles is an important first step in understanding the isolated impacts of plastics before advancing into more complex parameters or environmental interactions, it is equally important to increase the focus on other shapes of microplastics and nanoplastics.

Finally, observed toxicity is not limited to isolated plastic; it can also arise from the presence of leached chemicals or additives. Based on our review, we have identified several gaps and suggested the following recommendations for future research to improve our understanding of the toxicity of microplastics and nanoplastics:

1. A vast majority of studies have focused on spherical particles, particularly PS. Future research should aim to explore the impacts of other polymer types. Also, there is a need for further studies to assess the impacts of particle shape, particularly the toxicity of irregular-shaped particles which may be more representative of the particles in the real environment.

2. Only limited studies carry out characterization to understand the properties of the tested particles. In fact, there are studies in which the polymer types were neither identified by the vendors nor verified by the researchers. Future toxicity assessments should include comprehensive characterization of the particles used.
3. Future studies are encouraged to use standard protocols and parameters including number of replicates, duration or exposure, and validity of controls to ensure that the results are comparable, for future meta-analysis to further improve our understanding of the toxicity of microplastics and nanoplastics.
4. While an increasing number of studies are washing tested particles before toxicity exposure, most studies still use the particles or suspensions as purchased. Commercial preparations of microplastics and nanoplastics often contain additives which may confound toxicity results. To aid the comparability of toxicity data and to be able to separate the effects of solution additives from the particles and their leached chemicals, there is a need to wash commercial suspensions of particles used for toxicity exposures.
5. Although it could be argued that there is still limited data and technological methods to inform the actual concentration of micro- and nanoplastics in the aquatic environment, several studies have tested considerably high concentrations, up to thousands of mg/L. Hence, there is a need for more studies using advanced techniques to understand the existing range of concentrations of plastic particles in the environment to better inform the tested concentrations in future toxicity studies.
6. More research efforts are needed to understand the impacts of leached chemicals, additives, and co-contaminants, as well as other environmental factors such as natural organic matter, temperature, and pH.

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Conflict of Interest

The authors declare no conflict of interest.

Associated Content

Supporting Information Available: PRISMA flowchart was shown **Figure S1.** Images of aged PS and microplastics obtained from facial cleansers are provided in **Figures S2** and **S3**, respectively (reproduced with permission). Studies investigating the acute toxicity of microplastics and nanoplastics to *D. magna* which were excluded from the targeted research synthesis (**Table S1**), targeted research synthesis for acute toxicity tests including polymer types and references (**Table S2**).

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Toxicity of Microplastics and Nanoplastics to *Daphnia magna*: Current Status, Knowledge Gaps and Future Directions

Submitted to:

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Supporting Information

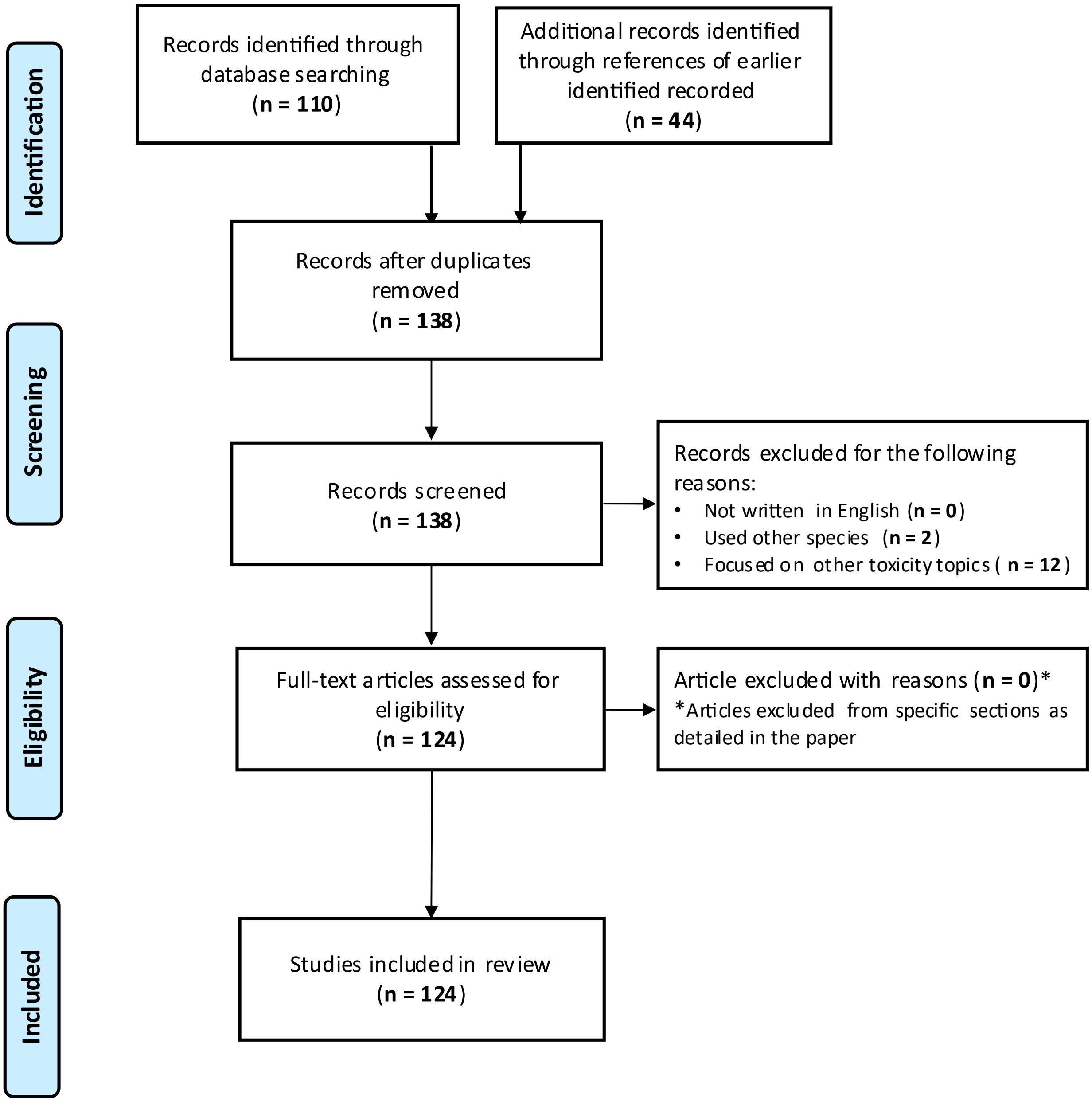


Figure S1. PRISMA flow diagram showing the screening and selection process. Initial electronic search was conducted using four different databases namely, Google Scholar (scholar.google.com), Science Direct (sciencedirect.com), Toxicity of Microplastic Explorer (sccwrp.shinyapps.io/aq_mp_tox_shiny), and PubMed (pubmed.ncbi.nlm.nih.gov). A total of 154 articles were first identified. 16 of them were duplicates while 14 were excluded for other reasons. A total of 124 articles were eligible and included in review.

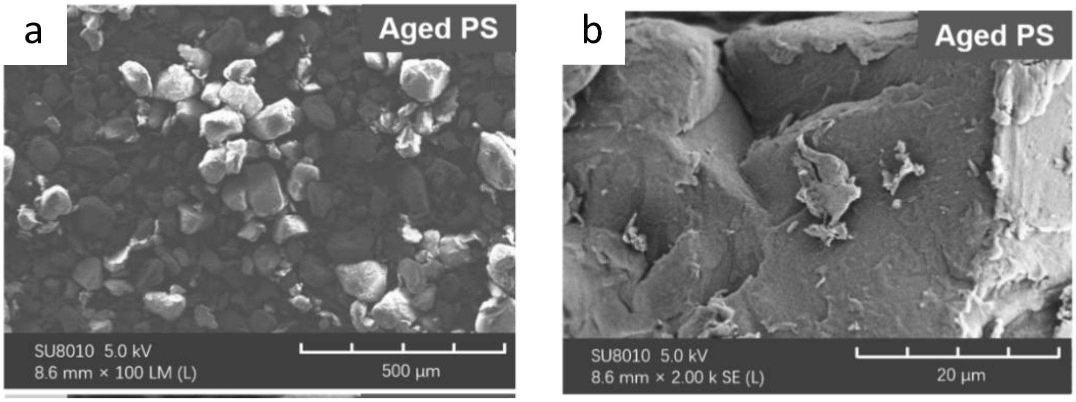


Figure S2. Scanning electron microscopy images of aged PS under (a) lower and (b) higher magnifications. Reproduced from Chen *et al*.[1] (with permission from Elsevier).

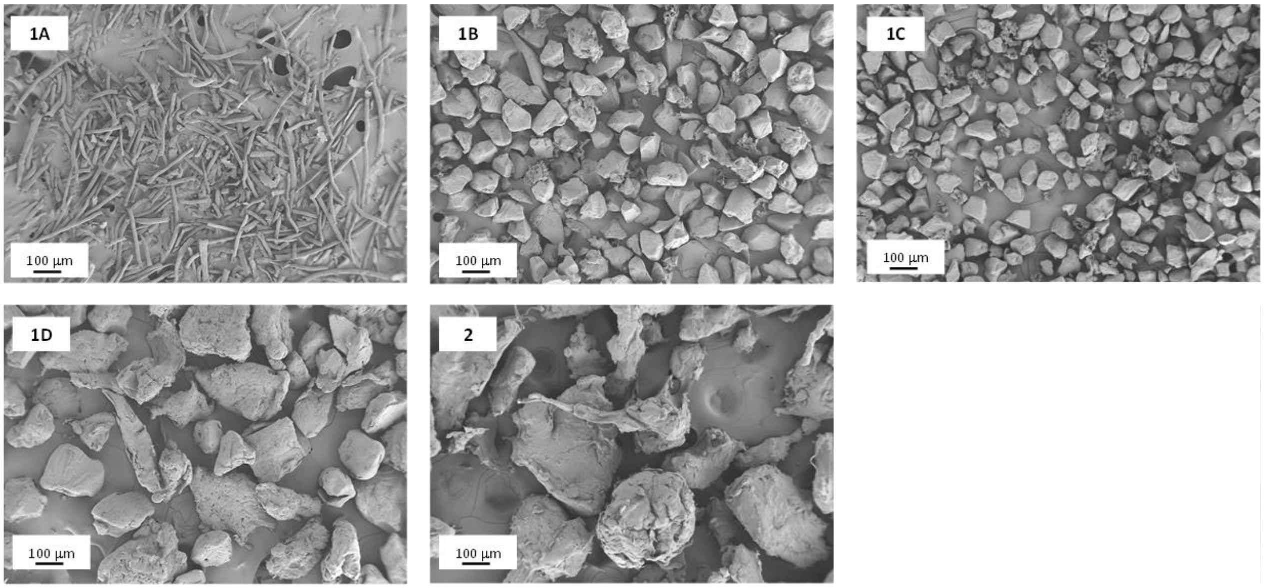


Figure S3. Scanning electron microscopy images of microplastics obtained from different facial cleansers. Reproduced from Kokalj *et al*.[2] (with permission from Elsevier).

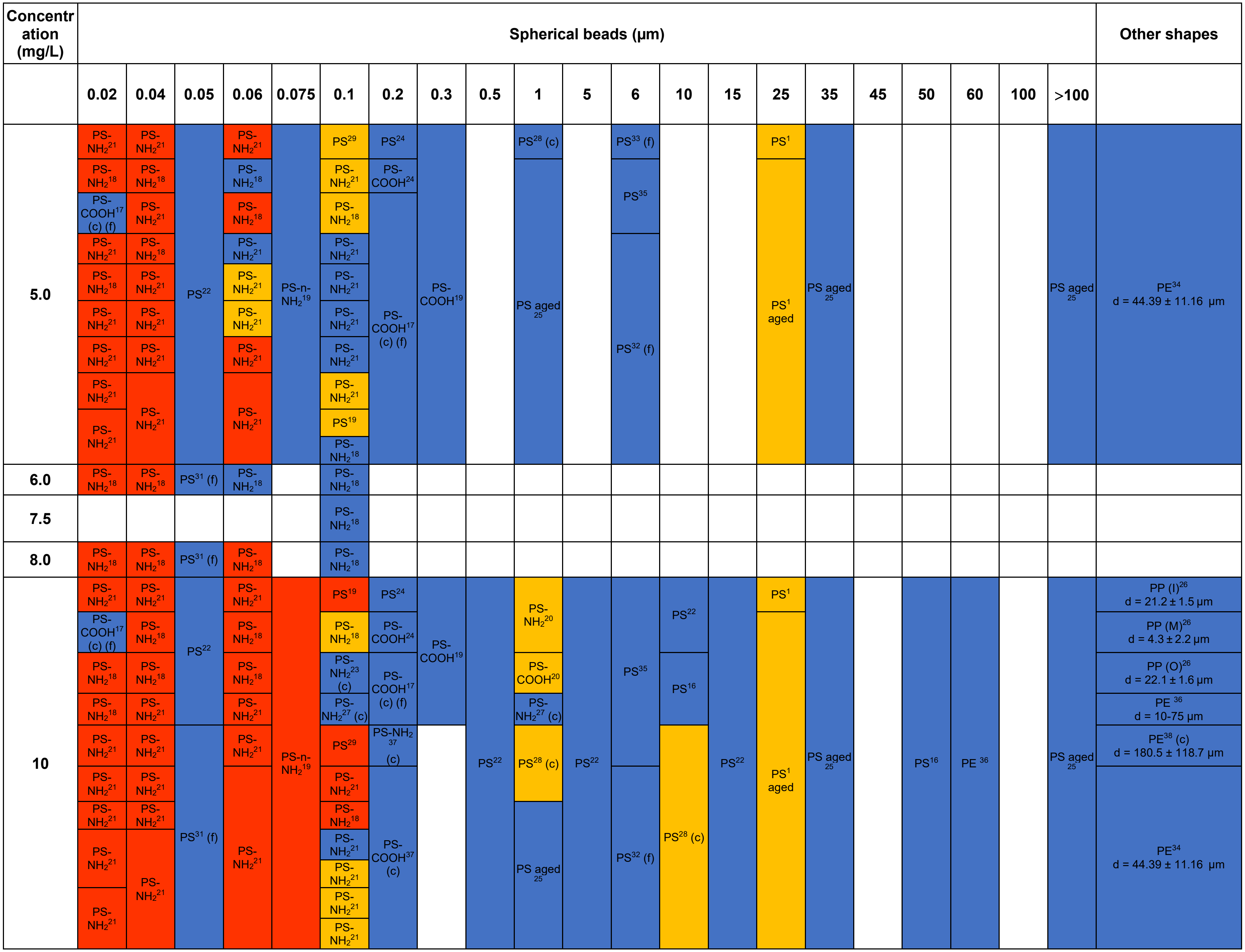
Table S1. Studies investigating the acute toxicity of microplastics and nanoplastics to *D. magna (excluded from the targeted research synthesis)*. -COOH, -HSO_4, and -NH_2 means carboxylated, sulphated, and aminated functional groups, respectively. (f) means fluorescently labelled. Where applicable, circled numbers were used to distinguish particles that possess different properties within the same study.

Type of plastic	Shape	Sizes	Concentration	Source of plastic	Was the plastic washed?	Reason for exclusion	Refs.
① PS-COOH; ② PS-HSO_4; ③ PS-NH_2 and ④ PMMA	spheres	① 26 nm, 60 nm, 90 nm, 160 nm, 190 nm, and 220 nm; ② 25 nm, 200 nm; ③ 52 nm, 53 nm, 57 nm, 58 nm, 120 nm, 180 nm, 330 nm; ④ 68 nm, and 140 nm	①②④ 25, 50, 75, 100, 200, and 400 mg/L; ③ 5, 10, 25, 50, 75, 100, and 150 mg/L	Bang laboratories, USA	yes (dialysis)	24 h exposure	[3]
①unspecified (f); ② PE	① spheres; ② fragments	① 1-5 µm; ②1-10 µm	10^3, 10^4, 10^5, 10^6, and 10^7 particles/mL	Cospheric LLC, USA	① unknown; (②yes (centrifugation)	Concentration in particles/mL	[4]
PET	fragments	3-10 µm	0.1, 1, 10, 100, 1000, and 10000 mg/L	Goodfellow	unknown	96 h test, no data available for end of 48 h period	[5]
① PS; ② PS-COOH; ③ PS-NH_2; ④ PS (f)	spheres	① 0.1 µm, 1 µm, and 10 µm; ②③④ 0.1 µm	1 mg/L	Tianjin Baseline Chromtech Research Centre, China	unknown	Adult organisms were used, and toxicity tests lasted for 8 h	[6]
① PS; ② PE	spheres	① 300 nm, 600 nm; ② 300-9000 nm	1 mg/L	① 300 nm (Thermo Fisher Scientific, the Netherlands); ① 600 nm (GmbH Forschungs, Germany); ② Cospheric LLC, USA	yes (PE particles washed in ethanol)	9-day old daphnia and 72 h tests	[7]
PS, PS-COOH and PS-NH_2	spheres	50-100 nm	10 mg/L	Aladdin Industrial Co., China	unknown	Invalid control, 35% (at pH of 7.8)	[8]
PS-COOH (f) and PS-NH_2	spheres	100 nm	10 mg/L	Obtained in the laboratory from a previous project	unknown	1–3-day old neonates and 24 h exposure	[9]
PS (f)	spheres	100 nm	10, 20, 30, 40, 50, 60, 70, 80, 90, and 100 mg/L	unavailable	unknown	24 h	[10]
PE	①spheres; ②fragments	① 25-50 µm; ② 40-48 µm	① 5, 50, 75, 200, and 500 mg/L; ② 0.05, 0.1, 0.5, 1, 5, 10, and 20 mg/L	① Sigma Aldrich, USA; ② prepared from PE pellets (Sigma Aldrich, USA).	yes (washed with hexane, methanol, and deionized water)	4-day old neonates	[11]
PE (f)	spheres	20 µm and 30 µm	20 and 60 mg/L	Cospheric	no	24 h	[12]
PS	spheres	26 nm and 100 nm	unavailable	Bang Laboratories, USA	yes (dialysis)	unavailable data for test concentrations	[13]
① PET; ② PP	fibres	① 120.49 ± 82.46 µm; ② 134.83 ± 88.70 µm	1000 and 2000 mg/L	Korea Institute of Industrial Technology, Korea	clean, (author's declaration)	3-day old neonates	[14]

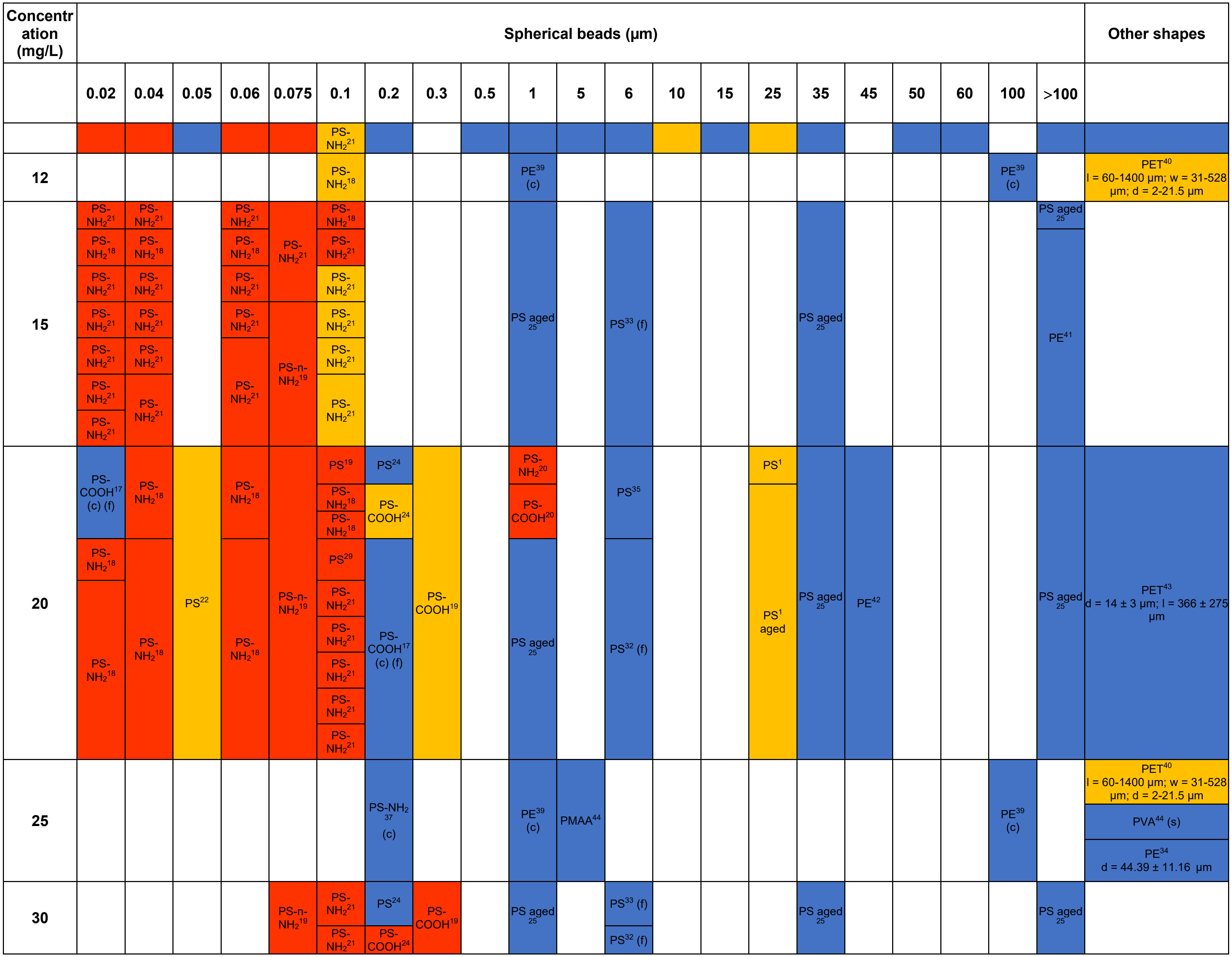
Table S2. Targeted research synthesis of immobility or mortality observed on *D. magna* neonates (≤ 24 h) after 48 h exposure to MPs and NPs. Each colored cell represents a toxicity test conducted on one particle type of a given size and concentration. The "other shapes" column represents the non-spherical plastics. The spherical beads columns provide the particle diameter and concentration of each toxicity tests while the sizes of the non-spherical particles are provided within each cell (l = length; w = width; d = diameter; (s) means size not provided). The percentage of dead or immobile neonates are represented by the cell colors. Blue cell color means no significant acute toxicity (≤ 20%), yellow cell means mild acute toxicity (> 20% and ≤ 50%) and red cell means severe acute toxicity (> 50%). N = 505. (c) means that the particles were washed. (f) means fluorescently labelled. -COOH and -NH_2 means carboxylated and aminated functional groups, respectively.

Concentration (mg/L)	**Spherical beads (µm)**																					**Other shapes**
	0.02	**0.04**	**0.05**	**0.06**	**0.075**	**0.1**	**0.2**	**0.3**	**0.5**	**1**	**5**	**6**	**10**	**15**	**25**	**35**	**45**	**50**	**60**	**100**	**>100**	
0.01						🟦 PMMA[15] (f) 🟦 PMMA-F[15] (f)							🟦 PS[16]					🟦 PS[16]				
0.1	🟦 PS-COOH[17] (c) (f) 🟦 PS-NH_2[18]	🟦 PS-NH_2[18]		🟦 PS-NH_2[18]	🟦 PS-n-NH_2[19]	🟦 PMMA[15] (f) 🟦 PMMA-F[15] (f) 🟦 PS-NH_2[18]	🟦 PS-COOH[17] (c) (f)			🟦 PS-NH_2[20] 🟦 PS-COOH[20]			🟦 PS[16]		🟨 PS[1] 🟨 PS[1] aged			🟦 PS[16]				
0.25	🟦 PS-NH_2[18]	🟦 PS-NH_2[18]		🟦 PS-NH_2[18]		🟦 PS-NH_2[18]																
0.5	🟨 PS-NH_2[21] 🟦 PS-NH_2[21] 🟦 PS-NH_2[21] 🟦 PS-NH_2[21] 🟦 PS-NH_2[21] 🟦 PS-NH_2[21] 🟦 PS-NH_2[18] 🟦 PS-NH_2[18]	🟨 PS-NH_2[21] 🟨 PS-NH_2[21] 🟦 PS-NH_2[21] 🟦 PS-NH_2[21] 🟦 PS-NH_2[21] 🟦 PS-NH_2[21] 🟨 PS-NH_2[18] 🟦 PS-NH_2[18]		🟦 PS-NH_2[21] 🟦 PS-NH_2[21] 🟦 PS-NH_2[21] 🟦 PS-NH_2[21] 🟦 PS-NH_2[21] 🟦 PS-NH_2[21] 🟦 PS-NH_2[18] 🟦 PS-NH_2[18]		🟦 PS-NH_2[18] 🟦 PS-NH_2[18]																
0.75										🟦 PS-NH_2[20] 🟦 PS-COOH[20]												
1.0	🟥 PS-NH_2[21] 🟦 PS-NH_2[21]	🟥 PS-NH_2[21]	🟦 PS[22]	🟦 PS-NH_2[21] 🟦 PS-NH_2[18]	🟦 PS-n-NH_2[19]	🟦 PS-NH_2[23] (c) 🟦 PS[19]	🟦 PS[24]		🟦 PS[22]	🟦 PS-NH_2[20]	🟦 PS[22]		🟦 PS[22]	🟦 PS[22]	🟨 PS[1]	🟦 PS aged[25]		🟦 PS[16]			🟦 PS aged[25]	🟦 PP (l)[26] d = 21.2 ± 1.5 µm

Concentration (mg/L)	Spherical beads (µm)																					Other shapes
	0.02	0.04	0.05	0.06	0.075	0.1	0.2	0.3	0.5	1	5	6	10	15	25	35	45	50	60	100	>100	
	🟦 PS-COOH[17] (c) (f) 🟦 PS-NH_2[18] 🟦 PS-NH_2[21] 🟨 PS-NH_2[18] 🟦 PS-NH_2[21] 🟦 PS-NH_2[21] 🟨 PS-NH_2[21]	🟦 PS-NH_2[18] 🟦 PS-NH_2[21] 🟦 PS-NH_2[21] 🟥 PS-NH_2[18] 🟦 PS-NH_2[21] 🟦 PS-NH_2[21] 🟥 PS-NH_2[21]	🟦	🟨 PS-NH_2[18] 🟦 PS-NH_2[21] 🟦 PS-NH_2[21] 🟦 PS-NH_2[21] 🟦 PS-NH_2[21] 🟨 PS-NH_2[21]	🟦	🟦 PS-NH_2[27] (c) 🟦 PS[29] 🟦 PS-NH_2[21] 🟦 PMMA[15] (f) 🟦 PMMA-F[15] (f) 🟦 PS-NH_2[18] 🟦 PS-NH_2[18] 🟦 PS-NH_2[21] 🟦 PS-NH_2[21] 🟦 PS-NH_2[21] 🟦 PS-NH_2[21] 🟦 PS-NH_2[21]	🟦 PS-COOH[24] 🟦 PS-COOH[17] (c) (f)		🟦	🟦 PS-NH_2[27] (c) 🟨 PS-COOH[20] 🟦 PS[28] (c) 🟦 PS aged[25]	🟦		🟦 PS[28] (c) 🟦 PS[16]	🟦	🟨 PS[1] aged	🟦		🟦			🟦	🟦 PP (O)[26] d = 22.1 ± 1.6 µm 🟦 PP (M)[26] d = 4.3 ± 2.2 µm
1.5	🟨 PS-NH_2[18]	🟥 PS-NH_2[18]	🟥 PS[30] (c)	🟨 PS-NH_2[18]		🟦 PS-NH_2[18]																
2.0	🟥 PS-NH_2[21] 🟦 PS-NH_2[18] 🟦 PS-NH_2[21] 🟦 PS-NH_2[21] 🟨 PS-NH_2[21] 🟨 PS-NH_2[21] 🟨 PS-NH_2[21]	🟥 PS-NH_2[21] 🟦 PS-NH_2[18] 🟨 PS-NH_2[21] 🟨 PS-NH_2[21] 🟨 PS-NH_2[21] 🟨 PS-NH_2[21] 🟥 PS-NH_2[21]	🟦 PS[31] (f)	🟨 PS-NH_2[21] 🟦 PS-NH_2[18] 🟦 PS-NH_2[21] 🟦 PS-NH_2[21] 🟨 PS-NH_2[21] 🟨 PS-NH_2[21] 🟥 PS-NH_2[21]																		
2.5	🟥 PS-NH_2[18]	🟥 PS-NH_2[18]	🟦 PS[22] 🟥 PS[30] (c)	🟥 PS-NH_2[18]		🟦 PS-NH_2[18] 🟦 PS-NH_2[21]	🟦 PS[30] (c)		🟦 PS[22] 🟦 PS[30] (c)		🟦 PS[22]	🟦 PS[32] (f)	🟦 PS[22]	🟦 PS[22]								
3.0	🟥 PS-NH_2[18]	🟥 PS-NH_2[18]		🟦 PS-NH_2[18]																		
4.0	🟥 PS-NH_2[18]	🟥 PS-NH_2[18]	🟦 PS[31] (f)	🟦 PS-NH_2[18]																		

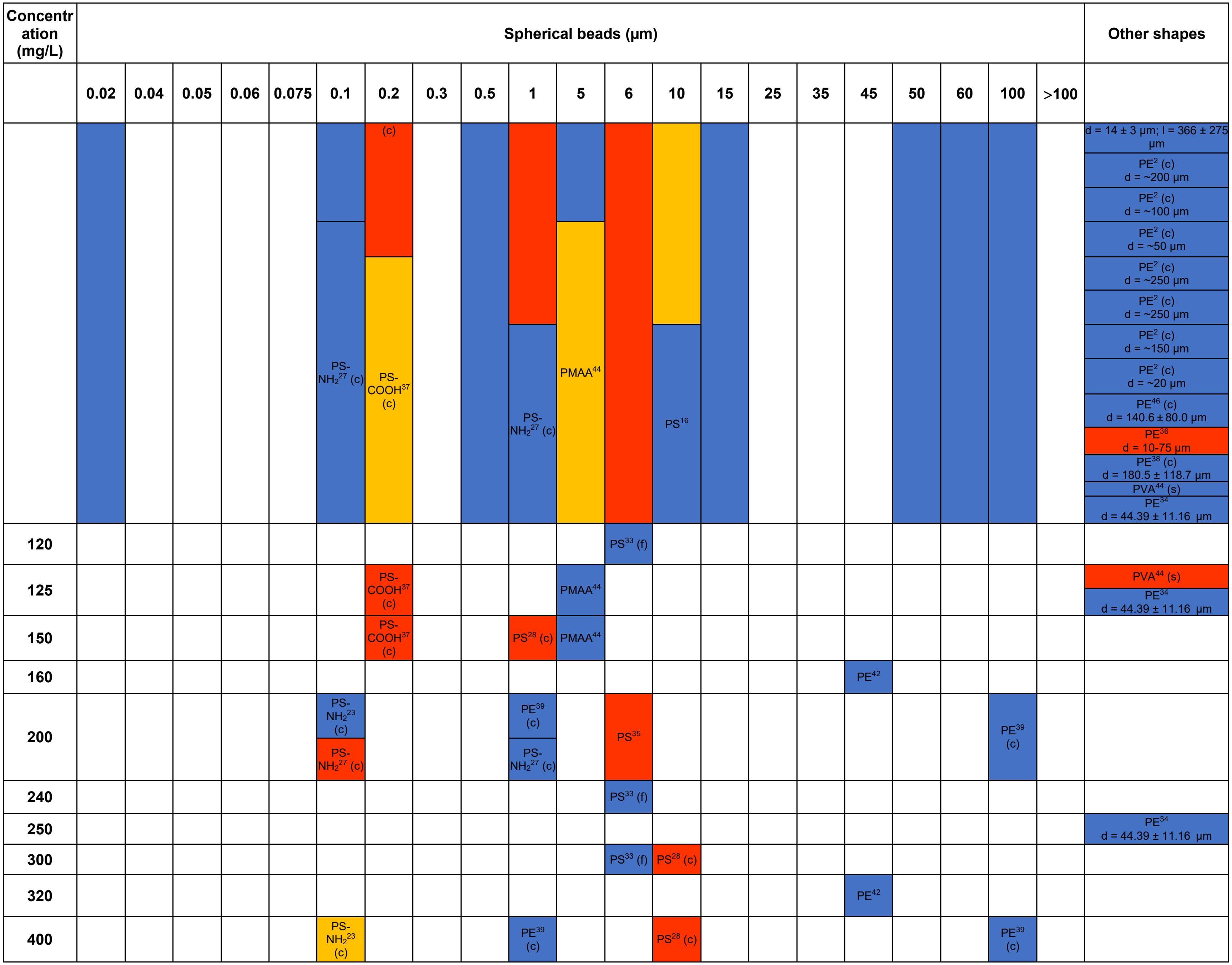


Concentration (mg/L)	Spherical beads (µm)																					Other shapes
	0.02	0.04	0.05	0.06	0.075	0.1	0.2	0.3	0.5	1	5	6	10	15	25	35	45	50	60	100	>100	
5.0	PS-NH$_2$[21] PS-NH$_2$[18] PS-COOH[17] (c) (f) PS-NH$_2$[21] PS-NH$_2$[18] PS-NH$_2$[21] PS-NH$_2$[21] PS-NH$_2$[21] PS-NH$_2$[21]	PS-NH$_2$[21] PS-NH$_2$[18] PS-NH$_2$[21] PS-NH$_2$[18] PS-NH$_2$[21] PS-NH$_2$[21] PS-NH$_2$[21] PS-NH$_2$[21]	PS[22]	PS-NH$_2$[21] PS-NH$_2$[18] PS-NH$_2$[18] PS-NH$_2$[21] PS-NH$_2$[21] PS-NH$_2$[21] PS-NH$_2$[21] PS-NH$_2$[21]	PS-n-NH$_2$[19]	PS[29] PS-NH$_2$[21] PS-NH$_2$[18] PS-NH$_2$[21] PS-NH$_2$[21] PS-NH$_2$[21] PS-NH$_2$[21] PS-NH$_2$[21] PS[19] PS-NH$_2$[18]	PS[24] PS-COOH[24] PS-COOH[17] (c) (f)	PS-COOH[19]		PS[28] (c) PS aged[25]		PS[33] (f) PS[35] PS[32] (f)			PS[1] PS[1] aged	PS aged[25]					PS aged[25]	PE[34] d = 44.39 ± 11.16 µm
6.0	PS-NH$_2$[18]	PS-NH$_2$[18]	PS[31] (f)	PS-NH$_2$[18]		PS-NH$_2$[18]																
7.5						PS-NH$_2$[18]																
8.0	PS-NH$_2$[18]	PS-NH$_2$[18]	PS[31] (f)	PS-NH$_2$[18]		PS-NH$_2$[18]																
10	PS-NH$_2$[21] PS-COOH[17] (c) (f) PS-NH$_2$[18] PS-NH$_2$[18] PS-NH$_2$[21] PS-NH$_2$[21] PS-NH$_2$[21] PS-NH$_2$[21] PS-NH$_2$[21]	PS-NH$_2$[21] PS-NH$_2$[18] PS-NH$_2$[18] PS-NH$_2$[21] PS-NH$_2$[21] PS-NH$_2$[21] PS-NH$_2$[21] PS-NH$_2$[21]	PS[22] PS[31] (f)	PS-NH$_2$[21] PS-NH$_2$[18] PS-NH$_2$[18] PS-NH$_2$[21] PS-NH$_2$[21] PS-NH$_2$[21]	PS-n-NH$_2$[19]	PS[19] PS-NH$_2$[18] PS-NH$_2$[23] (c) PS-NH$_2$[27] (c) PS[29] PS-NH$_2$[21] PS-NH$_2$[18] PS-NH$_2$[21] PS-NH$_2$[21] PS-NH$_2$[21] PS-NH$_2$[21]	PS[24] PS-COOH[24] PS-COOH[17] (c) (f) PS-NH$_2$[37] (c) PS-COOH[37] (c)	PS-COOH[19]	PS[22]	PS-NH$_2$[20] PS-COOH[20] PS-NH$_2$[27] (c) PS[28] (c) PS aged[25]	PS[22]	PS[35] PS[32] (f)	PS[22] PS[16] PS[28] (c)	PS[22]	PS[1] PS[1] aged	PS aged[25]		PS[16]	PE[36]		PS aged[25]	PP (I)[26] d = 21.2 ± 1.5 µm PP (M)[26] d = 4.3 ± 2.2 µm PP (O)[26] d = 22.1 ± 1.6 µm PE[36] d = 10-75 µm PE[38] (c) d = 180.5 ± 118.7 µm PE[34] d = 44.39 ± 11.16 µm



Concentration (mg/L)	Spherical beads (µm)																					Other shapes
	0.02	0.04	0.05	0.06	0.075	0.1	0.2	0.3	0.5	1	5	6	10	15	25	35	45	50	60	100	>100	
	🟥	🟥	🟦	🟥	🟥	🟨 PS-NH$_2^{21}$	🟦		🟦	🟦	🟦	🟦	🟨	🟦	🟨	🟦		🟦	🟦		🟦	🟦
12						PS-NH$_2^{18}$				PE^{39} (c)										PE^{39} (c)		PET^{40} l = 60-1400 µm; w = 31-528 µm; d = 2-21.5 µm
15	PS-NH$_2^{21}$ PS-NH$_2^{18}$ PS-NH$_2^{21}$ PS-NH$_2^{21}$ PS-NH$_2^{21}$ PS-NH$_2^{21}$ PS-NH$_2^{21}$	PS-NH$_2^{21}$ PS-NH$_2^{18}$ PS-NH$_2^{21}$ PS-NH$_2^{21}$ PS-NH$_2^{21}$ PS-NH$_2^{21}$		PS-NH$_2^{21}$ PS-NH$_2^{18}$ PS-NH$_2^{21}$ PS-NH$_2^{21}$ PS-NH$_2^{21}$	PS-NH$_2^{21}$ PS-n-NH$_2^{19}$	PS-NH$_2^{18}$ PS-NH$_2^{21}$ PS-NH$_2^{21}$ PS-NH$_2^{21}$ PS-NH$_2^{21}$ PS-NH$_2^{21}$				PS aged^{25}		PS^{33} (f)				PS aged^{25}					PS aged^{25} PE^{41}	
20	PS-COOH^{17} (c) (f) PS-NH$_2^{18}$ PS-NH$_2^{18}$	PS-NH$_2^{18}$ PS-NH$_2^{18}$	PS^{22}	PS-NH$_2^{18}$ PS-NH$_2^{18}$	PS-n-NH$_2^{19}$	PS^{19} PS-NH$_2^{18}$ PS-NH$_2^{18}$ PS^{29} PS-NH$_2^{21}$ PS-NH$_2^{21}$ PS-NH$_2^{21}$ PS-NH$_2^{21}$ PS-NH$_2^{21}$	PS^{24} PS-COOH^{24} PS-COOH^{17} (c) (f)	PS-COOH^{19}		PS-NH$_2^{20}$ PS-COOH^{20} PS aged^{25}		PS^{35} PS^{32} (f)			PS^{1} PS^{1} aged	PS aged^{25}	PE^{42}				PS aged^{25}	PET^{43} d = 14 ± 3 µm; l = 366 ± 275 µm
25							PS-NH$_2^{37}$ (c)			PE^{39} (c)	PMAA^{44}									PE^{39} (c)		PET^{40} l = 60-1400 µm; w = 31-528 µm; d = 2-21.5 µm PVA^{44} (s) PE^{34} d = 44.39 ± 11.16 µm
30					PS-n-NH$_2^{19}$	PS-NH$_2^{21}$ PS-NH$_2^{21}$	PS^{24} PS-COOH^{24}	PS-COOH^{19}		PS aged^{25}		PS^{33} (f) PS^{32} (f)				PS aged^{25}					PS aged^{25}	

Concentration (mg/L)	Spherical beads (µm)																					Other shapes
	0.02	0.04	0.05	0.06	0.075	0.1	0.2	0.3	0.5	1	5	6	10	15	25	35	45	50	60	100	>100	
					🟥	🟥 PS-NH$_2$[21] 🟥 PS-NH$_2$[21] 🟥 PS-NH$_2$[21]	🟥	🟥		🟦		🟦				🟦					🟦	
35																					🟦 PE[41]	
40					🟥 PS-n-NH$_2$[19]			🟥 PS-COOH[19]									🟦 PE [42]					
50	🟦 PS-COOH[17] (c) (f)		🟥 PS[22]			🟦 PS-NH$_2$[23] (c) 🟦 PS-NH$_2$[27] (c) 🟥 PS[29] 🟥 PS[19]	🟦 PS-COOH[17] (c) (f) 🟥 PS-NH$_2$[37] (c) 🟦 PS-COOH[37] (c)	🟥 PS-COOH[19]	🟦 PS[22]	🟦 PE[39] (c) 🟥 PS-COOH[20] 🟥 PS-NH$_2$[20] 🟦 PS-NH$_2$[27] (c) 🟨 PS[28] (c)	🟦 PS[22] 🟦 PMAA[44]	🟥 PS[35]	🟦 PS[22]	🟦 PS[22]	🟥 PS[1] 🟥 PS[1] aged				🟦 PE[36]	🟦 PE[39] (c)		🟨 PET[40] l = 60-1400 µm; w = 31-528 µm; d = 2-21.5 µm 🟥 PE[36] d = 10-75 µm 🟦 PP[45] (c) d = 10-100 µm 🟦 PVC[45] (c) d = 10-100 µm 🟨 PE[45] (c) d = 10-100 µm 🟦 PE[34] d = 44.39 ± 11.16 µm 🟥 PVA[44] (s) 🟦 PVC/PE[45] (c) d = 10-100 µm
60												🟦 PS[33] (f)										
70								🟥 PS-COOH[19]													🟦 PE[41]	
75						🟥 PS[19] 🟥 PS[29]	🟥 PS-NH$_2$[37] (c)				🟦 PMAA[44]											🟥 PVA[44] (s)
80																	🟦 PE [42]					
100	🟦 PS-COOH[17] (c) (f)					🟦 PS-NH$_2$[23] (c)	🟦 PS-COOH[17] (c) (f) 🟥 PS-NH$_2$[37]		🟦 PS[22]	🟦 PE[39] (c) 🟥 PS[28] (c)	🟦 PS[22]	🟥 PS[35]	🟦 PS[22] 🟨 PS[28] (c)	🟦 PS[22]				🟦 PS[16]	🟦 PE[36]	🟦 PE[39] (c)		🟨 PET[40] l = 60-1400 µm; w = 31-528 µm; d = 2-21.5 µm 🟦 PP (I)[26] d = 21.2 ± 1.5 µm 🟦 PP (M)[26] d = 4.3 ± 2.2 µm 🟦 PP (O)[26] d = 22.1 ± 1.6 µm 🟦 PET[43]



Concentration (mg/L)	Spherical beads (μm)																				Other shapes
	0.02	0.04	0.05	0.06	0.075	0.1	0.2	0.3	0.5	1	5	6	10	15	25	35	45	50	60	100	>100	
	🟦					🟦 🟦 PS-NH_2[27] (c)	🟥 (c) 🟨 PS-COOH[37] (c)		🟦	🟥 🟦 PS-NH_2[27] (c)	🟦 🟨 PMAA[44]	🟥	🟨 🟦 PS[16]	🟦				🟦	🟦	🟦		🟦 d = 14 ± 3 μm; l = 366 ± 275 μm 🟦 PE[2] (c) d = ~200 μm 🟦 PE[2] (c) d = ~100 μm 🟦 PE[2] (c) d = ~50 μm 🟦 PE[2] (c) d = ~250 μm 🟦 PE[2] (c) d = ~250 μm 🟦 PE[2] (c) d = ~150 μm 🟦 PE[2] (c) d = ~20 μm 🟦 PE[46] (c) d = 140.6 ± 80.0 μm 🟥 PE[36] d = 10-75 μm 🟦 PE[38] (c) d = 180.5 ± 118.7 μm 🟦 PVA[44] (s) 🟦 PE[34] d = 44.39 ± 11.16 μm
120												🟦 PS[33] (f)										
125							🟥 PS-COOH[37] (c)				🟦 PMAA[44]											🟥 PVA[44] (s) 🟦 PE[34] d = 44.39 ± 11.16 μm
150							🟥 PS-COOH[37] (c)			🟥 PS[28] (c)	🟦 PMAA[44]											
160																	🟦 PE[42]					
200						🟦 PS-NH_2[23] (c) 🟥 PS-NH_2[27] (c)				🟦 PE[39] (c) 🟦 PS-NH_2[27] (c)		🟥 PS[35]								🟦 PE[39] (c)		
240												🟦 PS[33] (f)										
250																						🟦 PE[34] d = 44.39 ± 11.16 μm
300												🟦 PS[33] (f)	🟥 PS[28] (c)									
320																	🟦 PE[42]					
400						🟨 PS-NH_2[23] (c)				🟦 PE[39] (c)			🟥 PS[28] (c)							🟦 PE[39] (c)		

Concentration (mg/L)	Spherical beads (µm)																					Other shapes
	0.02	0.04	0.05	0.06	0.075	0.1	0.2	0.3	0.5	1	5	6	10	15	25	35	45	50	60	100	>100	
						🟥 PS-NH$_2$[27] (c)				🟦 PS-NH$_2$[27] (c)			🟥							🟦		
500													🟥 PS[16] 🟥 PS[28] (c)					🟥 PS[16]	🟦 PE[36]			🟦 PET[43] d = 14 ± 3 µm; l = 366 ± 275 µm 🟥 PE[36] d = 10-75 µm
1000						🟦 PMMA[15] (f) 🟦 PMMA-F[15] (f)							🟥 PS[16]					🟥 PS[16]	🟨 PE[36]			🟥 PE[36] d = 10-75 µm
5000																			🟨 PE[36]			🟥 PE[36] d = 10-75 µm

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