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Hazard/Risk Assessment

Effects of Polyester Fibers and Car Tire Particles on Freshwater Invertebrates

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Abstract: Microplastic ingestion has been shown for various organisms, but knowledge of the potential adverse effects on freshwater invertebrates remains limited. We assessed the ingestion capacity and the associated effects of polyester fibers (26–5761 μm) and car tire particles (25–75 μm) on freshwater invertebrates under acute and chronic exposure conditions. A range of microplastic concentrations was tested on *Daphnia magna*, *Hyalella azteca*, *Asellus aquaticus*, and *Lumbriculus variegatus* using water only (up to 0.15 g/L) or spiked sediment (up to 2 g/kg dry wt), depending on the habitat of the species. *Daphnia magna* did not ingest any fibers, but low levels of fibers were ingested by all tested benthic invertebrate species. Car tire particle ingestion rose with increasing exposure concentration for all tested invertebrates and was highest in *D. magna* and *L. variegatus*. In most cases, no statistically significant effects on mobility, survival, or reproductive output were observed after acute and chronic exposure at the tested concentrations. However, fibers affected the reproduction and survival of *D. magna* (no-observed-effect concentration [NOEC]: 0.15 mg/L) due to entanglement and limited mobility under chronic conditions. Car tire particles affected the reproduction (NOEC: 1.5 mg/L) and survival (NOEC: 0.15 mg/L) of *D. magna* after chronic exposure at concentrations in the same order of magnitude as modeled river water concentrations, suggesting that refined exposure and effect studies should be performed with these microplastics. Our results confirm that microplastic ingestion by freshwater invertebrates depends on particle shape and size and that ingestion quantity depends on the exposure pathway and the feeding strategy of the test organism. *Environ Toxicol Chem* 2022;41:1555–1567. © 2022 The Authors. *Environmental Toxicology and Chemistry* published by Wiley Periodicals LLC on behalf of SETAC.

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INTRODUCTION

Microplastics are ubiquitous contaminants in freshwater ecosystems (Li et al., 2020; Schell et al., 2020; Yang et al., 2021) and can be ingested by a wide range of organisms (Scherer et al., 2018). This ingestion has been shown to cause adverse effects such as intestinal damage, reduced growth, decreased reproduction, and decreased survival of aquatic organisms (Foley et al., 2018; Kögel et al., 2020). Most studies assessing the effects and ingestion of microplastics under laboratory conditions have been performed with polyethylene and polystyrene fragments or beads, which are not necessarily the most abundant microplastic types in the environment (De Ruijter et al., 2020; Kögel et al., 2020; Kutralam-Muniasamy et al., 2020; Miloloža et al., 2021). Furthermore, data for freshwater benthic organisms remain very limited (Bellasi et al., 2020; Kögel et al., 2020), and most toxicity tests are performed without sediment, thus limiting the applicability of these data for conducting sediment risk assessments.

Fibers are often reported as the dominant microplastics in freshwater ecosystems (Li et al., 2020; Rebelein et al., 2021; Sarijan et al., 2021; Yang et al., 2021). The fragmentation of textiles during laundry and the subsequent environmental discharge of treated and untreated wastewaters constitute one of the most important pathways for these microplastics into the aquatic environment (De Falco et al., 2019; Schell et al., 2020; Ziajahromi et al., 2016). Another important pathway for fibers is atmospheric transport and deposition, which is also responsible

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for the contamination of remote areas (González-Pleiter et al., 2020; Stanton et al., 2020). Most field monitoring studies report the dominance of specific microplastic shapes and polymer types by showing their total percentage instead of providing their actual concentrations (see Rebelein et al., 2021; Yang et al., 2021). Therefore, only a limited number of field studies have reported specific microplastic fiber concentrations in freshwater ecosystems. Measured fiber concentrations in freshwater have been found to range from less than 0.1 to 519 fibers/L (Lahens et al., 2018; Martínez Silva & Nanny, 2020). Several studies have shown that microplastic fibers are retained in freshwater sediments (Deng et al., 2020; Martínez Silva & Nanny, 2020; Tibbetts et al., 2018). Concentrations as high as 1323 microplastics/kg dry weight sediment were measured in a textile industrial area in Shaoxing City, China, with up to 79% being fibers (Deng et al., 2020). Mass fractions of fibers are less frequently reported, but the mass concentration in the water of the Saigon River (Vietnam) has been estimated to vary between 0.05 and 0.22 mg/L (Lahens et al., 2018). In river sediments of central Spain, concentrations up to 1.58 mg fibers/kg dry weight were measured (Schell et al., 2021), and microplastic concentrations (including all shapes) reached 1 g/kg dry weight (equivalent to 4000 microplastics/kg dry wt) in the river shore sediments of the Rhine–Main area in Germany (Klein et al., 2015).

Recently, tire-related particles have been included in the microplastic contaminant group (Hartmann et al., 2019; Kole et al., 2017; Wagner et al., 2018). The terminology used differs between studies, and thus we will use the term "tire particles" for microplastics consisting only of tire material. Tire wear particles, if not specified otherwise, will refer to particles formed by the friction of tires on the road surface and therefore emitted into the environment as particles consisting of polymer tread with pavement encrustations (Kreider et al., 2010; Unice et al., 2013). Global annual tire wear emissions have been estimated to be up to 3.4 million tons (Baensch-Baltruschat et al., 2020). These particles are prone to reach aquatic environments via stormwater runoff and wastewater effluents of combined sewer systems (Kole et al., 2017; Unice et al., 2019; Wagner et al., 2018). The assessment of aquatic exposure concentrations for tire wear particles has been challenging, mainly because of their high black carbon content, which produces a low-quality spectrum that cannot be accurately interpreted by the spectroscopic techniques often used for microplastic identification (Wagner et al., 2018). Nonetheless, 15%–38% of the microplastics (80–260 microplastics/kg dry wt) measured in the sediment of a stormwater treatment wetland were most likely derived from tires (Ziajahromi et al., 2019), and up to 1833 suspected tire wear particles/kg wet weight sediment (corresponding to 0.0023 g/kg wet wt) were observed in the Ashley River (South Carolina, USA; Leads & Weinstein, 2019). Certain studies have relied on chemical markers to assess tire particle exposure (Baensch-Baltruschat et al., 2020; Wik & Dave, 2009). For example, using markers for rubber polymers, Unice et al. (2013) reported a maximum concentration of 5.8 g/kg dry weight of tire particle material in sediments (which amounts to 11.6 g/kg dry weight of tire wear particles, assuming a 50:50 ratio of polymer tread and mineral road encrustations) from the Seine River catchment (France), and in a follow-up modeling study, the same authors reported that water concentrations may be up to 0.12 mg/L (Unice et al., 2019).

The ingestion capacity of fibers and tire particles by freshwater organisms may differ significantly from that described for other microplastics, because not only the polymer type but also the size and morphology are expected to play an important role (Ogonowski et al., 2016; Ziajahromi et al., 2017). For example, longer fibrous materials may have different ingestion rates and gut retention times than spherical particles (Qiao et al., 2019; Wright et al., 2013). Previous studies have also shown that plastic fibers can exert greater adverse effects on benthic and planktonic invertebrates than other microplastic types, for example, due to their longer gut residence time or entanglement capacity (Au et al., 2015; Ziajahromi et al., 2017).

Whereas fibers are expected to mainly cause effects of a physical/mechanical nature, tire particles contain a complex mixture of elastomers and chemical additives (Kreider et al., 2010), which may be taken up by living organisms. Therefore, they may pose a combination of both physical and chemical hazards. Thus far, research on the environmental effects of tire particles has been mainly related to the toxicity of their chemical leachates obtained with different chemical and physical methods that force their desorption (see Hartwell et al., 1998; Marwood et al., 2011; Turner & Rice, 2010; Wik, 2007; Wik & Dave, 2005, 2006). The effects of the leachates may, however, not be the only cause for the tire-related toxicity; the tire particles themselves may cause additional effects and influence the bioavailability of these chemicals (Khan et al., 2019). There is likely to be a complex interplay between physical particle characteristics and the potential chemical hazards that govern the risks posed by this microplastic type (Selonen et al., 2021). Therefore, to reliably assess the impacts of tire particles on living organisms, long-term toxicity studies with entire particles and environmentally relevant exposure and desorption conditions are needed.

The aim of the present study was to determine the ingestion of microplastic fibers and car tire particles by a selection of freshwater invertebrates with different habitat preferences and feeding strategies and to assess their short- and long-term effects. Laboratory experiments were performed with *Daphnia magna* (pelagic; filter feeder), *Hyalella azteca* (epibenthic; shredder), *Asellus aquaticus* (epibenthic; deposit feeder), and *Lumbriculus variegatus* (endobenthic; deposit feeder) using concentrations ranging from present-day environmentally relevant conditions to projected future microplastic pollution scenarios, with water only and water–sediment exposure. Our ultimate goals were to generate toxicity data that can be used to assess the risks of these microplastics for freshwater ecosystems, and to discuss the mechanisms that explain the differences in uptake and toxicity among freshwater organisms.

MATERIALS AND METHODS

Test materials

The microplastics were obtained from the Norwegian Institute for Water Research (NIVA), Oslo, Norway. Fibers were generated by washing polyethylene terephthalate (PET) fleece blankets ("Skogsklocka"; IKEA) in a clean washing machine (model CS 1692D3-S; Candy Smart) on a 15-min cycle at 40 °C and 1200 rpm. During the washing process no detergents or softener were added. To obtain the fibers, the effluent was collected in a stainless-steel pressure vessel (Pope Scientific) and vacuum-filtered through a 10-µm nylon membrane. The fibers were of a cylindrical shape (Supporting Information, Figure S1), and had a density of 1.38 g/cm^3, a mean length of 600 µm (min–max: 26–5761 µm; SD: 554 µm, $n = 618$), and a width of 20 µm. The fiber sizes we used are in the range of those monitored in freshwater ecosystems (Schell et al., 2021; Yang et al., 2021). Fiber stability in water and sediment was not assessed because the fibers were expected to be stable given the relatively short duration of the experiments.

Tire particles were obtained from the Danish tire granulate manufacturer Genan. The particles were a byproduct of granulate production. The granulate was milled from end-of-life passenger tires, which represents the primary source of tire debris found in the environment. Prior to granulate production, the material was separated from the metallic tire scaffolding and textile components. Finally, the material was purified at NIVA (by separating residual metal particles, plastic, and fiber contamination) and sieved into a size range of 25–75 µm. This range was chosen because most tire particles in the environment are expected to be smaller than 100 µm (Järlskog et al., 2020; Kreider et al., 2010). The lower size limit of 25 µm was selected to facilitate the visual assessment step included in the analysis of particle ingestion by organisms. However, during the sieving process some particles stuck together, and thus particles smaller than 25 µm were included in the experiments. Furthermore, particles agglomerated following the sieving process, and as a consequence, a few particles larger than 75 µm were measured. The measured mean size of the long axis of the tire particles ($n = 896$) was 39 µm (min–max: 3–200 µm; SD: 27.7 µm). Tire particles had a density of 1.16 g/cm^3 and were irregularly shaped (Supporting Information, Figure S1). The chemical composition of the tire material, including the content of trace metals and polycyclic aromatic hydrocarbons, is described in Selonen et al. (2021).

Stock solutions were prepared to allow a more precise dosage based on microplastic mass of low concentrations of the test materials in the experiments. The stock solutions of fibers were prepared in ethanol, because it was not possible to achieve a homogeneous solution in water. Even so, a few fiber clumps were observed by visual inspection in the stock solutions and the two highest exposure concentrations. However, fiber clumps have also been observed in environmental samples (see Schell et al., 2021) and should therefore be included as part of environmentally relevant exposure scenarios for freshwater organisms.

The stock solutions of tire particles were prepared in Milli-Q water, and were placed into an ultrasonic bath for 15 min to break up agglomerates before addition to the test beakers. All stock solutions were prepared just before they were added to the test units (i.e., prior to the first spike). The stock solutions used in the chronic *D. magna* tests were prepared directly before the test start and were used for test medium renewal throughout the entire experiment. To estimate the nominal dose of particles added per test system, subsamples of the stock solutions (20–1000 µl depending on the concentration of the stock solution; $n = 6$) were taken, and the microplastics were counted using a stereo microscope (Olympus SZX7 coupled to an Olympus DP21 camera system). The count-based concentration was estimated based on the particle count and the dilution factor in the test medium. In addition, count-based concentrations were calculated based on the nominal mass following Leusch and Ziajahromi (2021), using the particle density and the average measured fiber length or tire particle diameter. Tire particles were assumed to be spherical for the calculation.

Test species

All test organisms came from in-house cultures of the IMDEA Water Institute; they were held at 20 ± 1 °C with a 16:8-h light:dark photoperiod. The *D. magna* were kept in beakers containing synthetic hard water (ASTM International [2007] standard E729-96) or mineral water (AquaBona; Fuenmayor Spring), which was renewed two times a week, and they were fed with the green algae *Chlorella vulgaris*. The *H. azteca* and *A. aquaticus* organisms were cultured in aquaria with water from an artificial pond (filtered through a 20-µm plankton net and autoclaved prior to use) and fed with previously inoculated *Populus* sp. leaves (to obtain microbial and fungal communities that would increase their palatability) and a solution of fish food (TetraMin; Tetra). The *L. variegatus* were kept in aquaria with quartz-sand sediment. The overlying water and the fish food used for their maintenance were the same as for *H. azteca* and *A. aquaticus*.

Experimental design

The characteristics of the different tests performed, such as the exposure route, exposure duration, and endpoints, are summarized in Table 1. For the pelagic organisms (*D. magna*), water-only tests were performed, whereas for the epibenthic organisms (*H. azteca* and *A. aquaticus*) water-only and water–sediment tests were performed. For the endobenthic organisms (*L. variegatus*), water–sediment experiments were performed. The test organisms were exposed to five fiber or tire particle concentrations. In the water-only experiments, the exposure concentrations ranged between 0 and 0.15 g/L, and in the water–sediment tests, the exposure concentrations ranged between 0 and 2 g/kg of sediment dry weight (Table 1). The number of replicates varied depending on the test (Table 1). In the tests performed with fibers, a solvent control was included in addition to the control, to which the same amount of ethanol was added as to the test beakers.

Acute and chronic effects were assessed in the different test organisms. The endpoints evaluated at the end of the experiments were ingestion, immobility, reproduction, or survival, depending on the exposure duration and the test organism

TABLE 1: Summary of the different toxicity tests, including test species, exposure pathway, test duration, exposure concentrations for fibers and tire particles, evaluated endpoints, number of replicates, and the test protocol used as reference

Species	Exposure pathway	Test duration	Exposure concentrations	Endpoints	No. of replicates	Test protocol[a]
Daphnia magna	Water	48 h	0; 0.00015; 0.0015; 0.015; 0.15 g/L	Ingestion; immobility	4	OECD 202
Daphnia magna	Water	21 days	0; 0.00015; 0.0015; 0.015; 0.15 g/L	Ingestion; survival; reproduction	10	OECD 211
Hyalella azteca	Water	4; 14; 28 days	0; 0.00015; 0.0015; 0.015; 0.15 g/L	Ingestion; survival	4	ASTM E1706-05
Hyalella azteca	Water–sediment	4; 14; 28 days	0; 0.002; 0.02; 0.2; 2 g/kg dry wt	Ingestion; survival	4	ASTM E1706-05
Asellus aquaticus	Water	4; 14; 28 days	0; 0.00015; 0.0015; 0.015; 0.15 g/L	Ingestion; survival	4	ASTM E1706-05
Asellus aquaticus	Water–sediment	4; 14; 28 days	0; 0.002; 0.02; 0.2; 2 g/kg dry wt[b]	Ingestion; survival	3	ASTM E1706-05
Lumbriculus variegatus	Water–sediment	4; 14; 28 days	0; 0.002; 0.02; 0.2; 2 g/kg dry wt	Ingestion; survival; reproduction	4	OECD 225

[a]OECD 202, Organisation for Economic Co-operation and Development test guideline 202 (2004); OECD 211, OECD test guideline 211 (2012); OECD 225, OECD test guideline 225 (2007); ASTM E1706-05, ASTM International standard E1706-05 (2005).

[b]Tire particles only: the highest concentration tested for fibers was 0.2 g/kg.

(Table 1). In the chronic tests with macroinvertebrates, ingestion was also assessed on day 14 after the start of the experiment as an intermediate endpoint evaluation. All tests were performed under controlled climate conditions with a photoperiod of 16:8-h (light:dark) and a temperature of 20 ± 1 °C. Water temperature, pH, conductivity, and dissolved oxygen were measured at the start and the end of the tests with a multiparameter meter (Hanna HI91894), as well as after renewal of the exposure medium to ensure they were within the acceptable range of the guidelines.

Tests with D. magna. Standard acute tests were carried out for each microplastic type following Organisation for Economic Co-operation and Development (OECD, 2004) test guideline 202. The fiber stock solution was added to the empty beakers at the respective concentrations, and the ethanol was allowed to evaporate until completely dry in a fume hood. This step was unnecessary for tire particles because the stock solutions were prepared in water. Then 50 ml of test medium (AquaBona; Fuenmayor Spring) and five organisms were added per beaker ($n = 4$). Mobility was checked after 24 and 48 h of exposure. Organisms were considered to be immobile if no movement was recognized after the beaker was gently moved for 10 s.

Chronic toxicity tests were carried out under semistatic conditions according to OECD (2012) test guideline 211. Test units were prepared in the same way as for the acute tests, but the medium was replaced three times a week, and organisms were kept individually in 50 ml of test medium ($n = 10$). Offspring were counted and removed three times a week, prior to medium renewal, to assess reproduction. Reproduction of *D. magna* was calculated as the total number of living offspring/parent animal that did not accidentally or inadvertently die during the test. Adult survival was recorded at the same time that offspring were counted. After each medium renewal, *D. magna* were fed with *C. vulgaris* corresponding to 0.15 mg organic carbon/individual when they were adults, and one-third and one-half this amount when they were neonates and juveniles, respectively.

The chronic fiber experiment was performed once, and the chronic tire particle experiment was carried out twice, once with synthetic hard water (ASTM International, 2007) and *D. magna* strain A and once with mineral water (AquaBona; Fuenmayor Spring) and a new strain of *D. magna* (strain B). Strain B was also used in the acute tests and the fiber experiment. The two different strains were used because a new culture was established at the laboratory.

Tests with H. azteca and A. aquaticus. The tests with *H. azteca* and *A. aquaticus* were performed based on ASTM International (2006) standard E1706-05 with filtered and autoclaved natural water, as described for the culturing of organisms in the previous section, *Test species*. Only adults between 0.5 and 1 mm were used for the experiments. In the water-only tests, 200 ml was added per test beaker. In the water–sediment tests, sediment was prepared following OECD (2007) test guideline 225 except for the addition of clay. The final sediment mixture contained 94.5% sand (less than 2-mm grain size), 5% peat (heated at 100 °C and ground with a ball mill; Retsch MM400), and 0.5% *Urtica* powder. Two days before the start of the experiment, the peat was mixed with Milli-Q water (50% of the sediment dry wt), and the pH was corrected (5.5 ± 0.5 pH) using $CaCO_3$. The mixture was kept under constant stirring for 48 h to allow the pH to adjust to 5.5–6.5 and was then incorporated into the remaining ingredients. Finally, an amount corresponding to 20 g of dry sediment was added to each test beaker.

For fibers, the stock solutions were added to 250-ml beakers containing 5 g dry sand (previously subtracted and not added to sediment mixture). The ethanol added with the stock solutions was evaporated in a fume hood. This process took approximately 2 h. Then the sand–fiber mixture was added to the sediment mixture and blended in properly. For tire particles, the respective amount of the stock solution was added directly into the final sediment mixture. The overlying water used was the same water as for the water-only test and the culturing of organisms. In each test system, 150 ml water and 10 organisms were added. Each test beaker was continuously aerated and covered to avoid contamination from the air and evaporation of the test medium.

Test organisms were fed with preconditioned *Populus* sp. leaf disks (diameter: 2 cm). Each test unit consisted of 10 organisms with three leaf disks. In the water-only tests with *H. azteca*, 300 µl of TetraMin solution (6.66 g ground TetraMin/1 L Milli-Q water) was also added weekly as an additional food source to achieve optimal maintenance of the test organisms (see Besser et al., 2005; Soucek et al., 2016). Each test beaker was continuously aerated and covered to avoid contamination and evaporation of the test medium. Aeration in the water-only test ensured the distribution of microplastics throughout the water phase. Mortality was assessed as the difference between the initial number of organisms added and the number of living organisms after each test period.

Tests with L. variegatus. Each test unit consisted of a 250-ml beaker with 10 individuals. The test organisms used in the experiment were of a similar size (18.4 ± 3.3 cm; mean ± SD). According to OECD (2007) test guideline 225, they were cut in half 14 days before the start of the test to synchronize reproduction. Mortality was assessed after 4 and 14 days. Reproduction was assessed in the chronic test (28 days) as the difference between the initial number of organisms and the number of living organisms after the test period. The preparation of the test units followed the same steps for water–sediment tests as described in the preceding section, *Tests with H. azteca and A. aquaticus*. The *Urtica* powder in the sediment served as a food source.

Microplastic extraction and visual assessment

At the end of each test, all living organisms were transferred into Milli-Q water and subjected to five rinsing steps with Milli-Q water to remove microplastics potentially attached to their body surface. The *D. magna* exposed to fibers were first visually analyzed under a stereo microscope (Olympus SZX7) to assess the external entanglement with fibers. To isolate the ingested particles, the tissue was digested using a potassium hydroxide solution (KOH; 10%) for *L. variegatus*, and a hydrogen peroxide solution (H_2O_2; 15%) for the remaining test organisms for 48 h at 50 °C. Recovery tests with the microplastics from biota used in the present study have been carried out in previously published studies and have shown satisfactory results (Bråte et al., 2020; Kallenbach et al., 2021, 2022). However, in the present study H_2O_2 was used without the addition of chitinase, because pretests showed sufficient digestion of the test organisms' exoskeleton. These solutions were then vacuum-filtered onto filter papers (glass microfiber filters; 0.7 µm; Scharlau), and the number of ingested particles and their size were assessed using a stereo microscope (Olympus SZX7) with a camera attachment (Olympus DP21). Fiber long dimensions and tire particle long and short dimensions were measured with the Olympus DP2-Twain software. In total, 618 fibers and 896 tire particles from the stock solutions were measured. The size of all ingested fibers was analyzed. For tire particles, the size of all particles ingested by benthic invertebrates after chronic exposure (28 days) was analyzed except for those experiments in which organisms ingested a high number of particles (i.e., 70% of particles ingested by *H. azteca* in the water-only test and 8.5% of particles ingested by *A. aquaticus* in the water-only test). For *D. magna*, all ingested tire particles were measured in the acute experiment, as well as 3.7% of particles ingested in the chronic experiment using strain A. For each batch of samples processed, three blanks were included, which were used to assess for potential procedural, container, solution, and air contamination. Blanks were treated with the same sample processing and microscope analysis procedure as the experiment samples. Blanks contained no fibers and a total of three tire particles. Background contamination was therefore considered negligible.

Data analyses

The no-observed-effect concentration (NOEC) and, if possible, the lowest-observed-effect concentration (LOEC) were determined for all evaluated endpoints in the toxicity tests. For this, the normal distribution and homogeneity of variances of the response data were tested using Shapiro's and Levene's tests, respectively. Because parametric assumptions were not met for all data, nonparametric Kruskal–Wallis tests followed by Bonferroni-adjusted Wilcoxon rank-sum tests were carried out. This was done for all assessed endpoints except for *D. magna* survival in chronic toxicity tests, which was analyzed using Fisher's exact tests to identify the NOEC and LOEC values. Wilcoxon rank-sum tests were used to check for differences between the controls and solvent controls. Statistically significant differences between the observed effects in the different treatments and the controls were assumed when the p value was <0.05. All statistical analyses were carried out using the software R Ver 4.1.1 (R Core Team, 2021) in RStudio

(RStudio Team, 2021) and the required extension packages (Alboukadel, 2020; Fox & Weisberg, 2019; Wickham, 2016).

RESULTS AND DISCUSSION

Microplastic ingestion and effects on D. magna

The results of the experiments show that *D. magna* did not ingest fibers, whereas tire particle ingestion increased with increasing exposure concentrations (Figure 1). After 21 days of exposure (assessed only during the first test with strain A), a higher number of tire particles (~20–60 times higher) was observed inside the gut compared with after 48 h. The higher ingestion was probably caused by the longer exposure time and the increase in body size, allowing adults to ingest particles of larger sizes as well as agglomerates. This confirms the importance of life stage and body size for microplastic ingestion, as indicated previously (Scherer et al., 2017). Furthermore, after 21 days of exposure, adults' ingestion was on average higher at the second highest concentration (0.015 g/L) compared with the highest concentration (0.15 g/L). The lower ingestion rate at the highest exposure concentration may have been caused by the increased agglomeration of tire particles in the test beakers, which has also been observed elsewhere (Miloloža et al., 2021). *Daphnia magna* commonly feeds on small, suspended particles in the water (planktonic algae), and their particle selectivity mainly depends on the particle size (Ebert, 2005; Gophen & Geller, 1984). They usually ingest particles between 1 and 50 μm, but larger particles with a diameter of up to 70 μm may also be taken up (Ebert, 2005). Thus, the tire particles were within this ingestible size range (Supporting Information, Figure S2), whereas the length of most of the fibers used in the present study (mean ± SD: 600 ± 554 μm) exceeded this size range, which could have prevented their uptake.

No effects on *D. magna* mobility were observed after acute exposure (48 h) to fibers or tire particles at the tested concentrations. However, chronic exposure to fibers and tire particles negatively affected survival and reproduction. For fibers, the observed NOEC for reproduction and survival was 0.00015 g/L (Figure 2). There was no significant difference between the solvent control and the control. At concentrations equal to or above 0.0015 g/L, fibers were observed to form agglomerates with the green algae provided as food. Adult *D. magna* became entangled in those agglomerates, which impeded their movement (Figure 3), and probably caused the observed reduction in reproduction (up to 85%) and survival (up to 90%) compared with the control (Figure 2). Furthermore, algae–fiber agglomerates may have reduced food availability and food quality. Similarly, Ziajahromi et al. (2017) showed that *Ceriodaphnia dubia* organisms did not ingest PET fibers of a length between 100 and 400 μm but were negatively affected due to external physical damage (i.e., carapace and antenna deformities) caused by the entanglement with fibers.

In contrast to the results of the present study, fiber ingestion by juvenile *D. magna* has been observed in previous studies (Jemec et al., 2016; Kim et al., 2021). For example, Kim et al. (2021) reported that the ingested fiber sizes ranged between 10 and 70 μm and that longer fibers were not ingested.

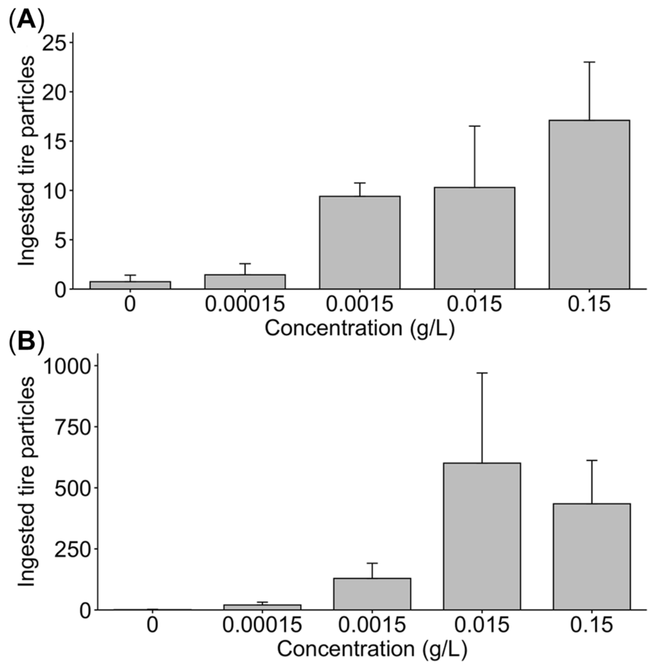

FIGURE 1: Mean number and corresponding standard deviation of ingested tire particles/*Daphnia magna* at (**A**) 48 h (juveniles) and (**B**) 21 days (adults) after the start of the exposure period.

Jemec et al. (2016), however, reported that the size of most ingested fibers was around 300 μm, but also longer ones (up to 1400 μm) were found inside the guts of *Daphnia*, probably because fibers were twisted prior to ingestion. Furthermore, the procedure used to generate the fibers may influence not only the length but also the shape of fibers, which in turn may affect ingestion and entanglement capacity. Similar to our study, Jemec et al. (2016) used PET fleece textile to obtain fibers, but the procedure to generate them differed. Those researchers used a ball mill to grind the textile, whereas in the present study, fibers were obtained by washing the PET fleece blankets. Based on the reported fiber dimension by Jemec

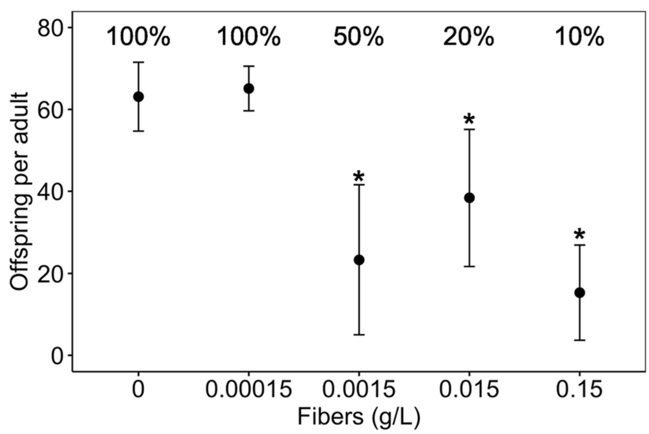

FIGURE 2: Reproduction displayed as mean number of offspring (±95% CI, $n = 10$) per adult *Daphnia magna* after 21 days of exposure to increasing fiber concentrations. Statistically significant differences in reproduction between the control and the different fiber concentrations are displayed by asterisks. The percentage of surviving adults is shown above the respective treatment.

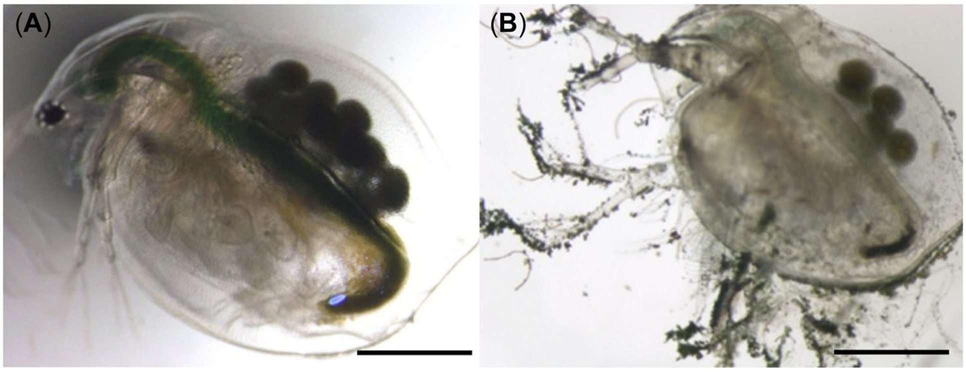

FIGURE 3: *Daphnia magna* after exposure for 21 days to (**A**) the control and (**B**) 0.015 g fibers/L, which resulted in the entanglement of the *D. magna* in algae–fiber agglomerates. The black scale bar represents 1000 μm.

et al. (2016), the fibers used in their experiment were more flattened out compared with the fibers we used, which were more cylindrical. Furthermore, the mortality rate observed in *D. magna* that were not pre-fed prior to the exposure did not increase following a dose–response pattern (i.e., the rate was between 20% and 40% at concentrations from 12.5 to 100 mg/L), whereas the *D. magna* that were fed with algae before the experiment showed no increased mortality at the same test concentrations (Jemec et al., 2016). This is in contrast to our results and the those of Ziajahromi et al. (2017), who saw clear concentration-dependent effects. Ziajahromi et al. (2017) observed a significant reduction in reproductive output with increasing concentration and 40% mortality at the highest test concentration (0.001 g/L) during chronic exposure of *C. dubia*. Moreover, these authors reported a median lethal concentration (LC50) of 0.0015 g/L (1.3×10^4 fibers/L) for acute exposure (Ziajahromi et al., 2017), whereas we found no influence on *D. magna* mobility at this concentration.

For tire particles, the 21-day NOECs for reproduction in the two experiments conducted were 0.015 and 0.0015 g/L (Figure 4). Whereas adult survival was not affected during the first experiment (using synthetic hard water and strain A), the NOEC for survival was 0.00015 g/L in the second experiment (using mineral water and strain B). This might be due to different sensitives of the two *D. magna* strains used (see Toumi et al., 2015). The reduced reproductive output and survival may be caused by a physical effect of the particles themselves and/or incorporated compounds (e.g., metals; organic compounds) that leached out into the test medium or into the organisms' bodies after ingestion of the particles. Tire particle leachates have previously been shown to be toxic to aquatic organisms; however, leachates from different tire material can vary considerably in toxicity (Lu et al., 2021; Wik & Dave 2005, 2006; Wik et al., 2009). Differing compositions depending on the tire type and manufacturer, different wear of tires, and the method used to generate the test material may affect toxicity (Baensch-Baltruschat et al., 2020). Halle et al. (2021) compared the toxicity of tire particles ground from pristine and road-worn tires and observed a greater toxicity of pristine particles for *H. azteca*, which was attributed to a higher abundance of chemical compounds in these particles.

The toxicity of tire leachates to *D. magna* has been previously related to metals, primarily zinc (Zn), and different organic compounds, including benzothiazoles and phthalates (Capolupo et al., 2020; Lu et al., 2021; Marwood et al., 2011; Wik & Dave, 2006; Wik et al., 2009). The tire material used in our study contained a mixture of different metals (with Zn concentrations being by far the highest, at 21.9 g/kg) and

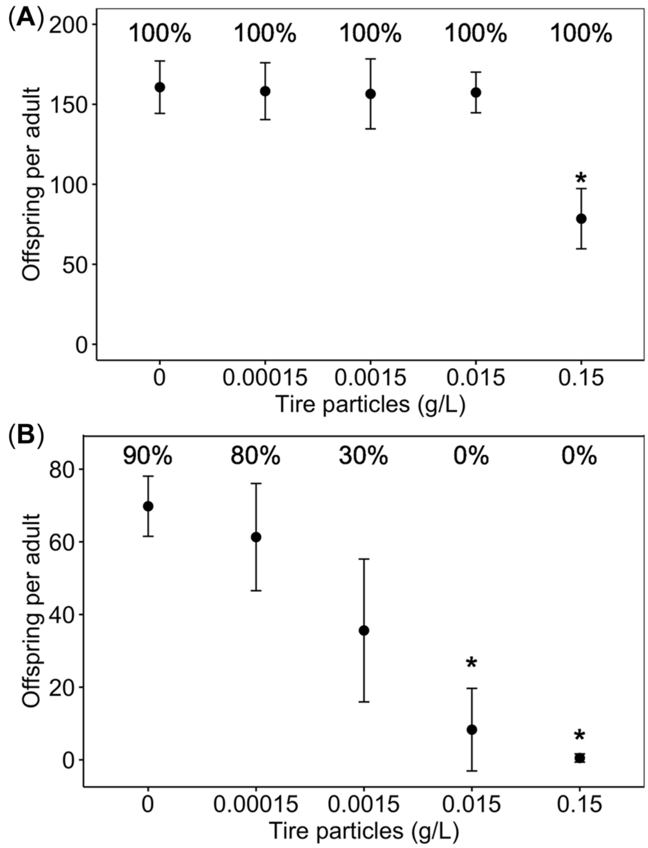

FIGURE 4: Reproduction displayed as mean number of offspring (±95% CI, $n = 10$) per adult *Daphnia magna* after 21 days of exposure to increasing tire particle concentrations in (**A**) the first experiment using synthetic hard water and strain A, and (**B**) the second experiment using mineral water and strain B. Statistically significant differences in reproduction between the control and the different tire particle concentrations are displayed by asterisks. The percentage of surviving adults is shown above the respective treatment.

polycyclic aromatic hydrocarbons (see Selonen et al., 2021 for further details). Roadway particles (collected during outdoor driving) and tire wear particles (collected on a simulated laboratory driving course) have been shown to have lower Zn concentrations than tire particles cryogenically ground from unused tires, but they contained other metals at higher concentrations, probably originating from asphalt (Kreider et al., 2010). Moreover, particles ground from tires, such as those used in the present study, usually have a higher polymer content but lower mineral content (Kreider et al., 2010). Therefore, future studies should be performed with tire wear microplastics recovered from environmental samples to increase the realism of the ecological risk assessment. Also, it remains unclear whether the effects we observed were caused by chemical leaching, the physical effects of the tire particles, or a combination of both, which remains to be investigated in follow-up studies.

Microplastic ingestion and effects on *H. azteca*, *A. aquaticus* and *L. variegatus*

After 4, 14, and 28 days of exposure, all tested macroinvertebrate species showed very low fiber ingestion, suggesting that no accumulation occurred within the organisms' bodies (Figure 5 and Supporting Information, Figure S3). This is supported by previous microplastic accumulation experiments showing that *Gammarus fossarum* egests polyamide fibers within similar time frames as food items (Blarer & Burkhardt-Holm, 2016). In our study, fiber ingestion was higher at the highest test concentration for all organisms and varied slightly depending on the exposure type (i.e., water vs. sediment). For example, ingestion by *H. azteca* was higher after exposure to fibers in the water phase compared with fibers mixed into the sediment (Figure 5 and Supporting Information, Figure S3). The *A. aquaticus* ingested, on average, more fibers than *H. azteca* after exposure to fibers in the water phase at the highest concentration; however, the difference was not statistically significant. In the test systems with sediment exposure, *L. variegatus* ingested more fibers compared with *H. azteca* at the highest test concentration ($p = 0.01$, after 28 days of exposure). The highest fiber concentration tested for *A. aquaticus* was 0.2 g/kg, at which hardly any ingestion was observed for all species. Whereas the average size of fibers in the stock solution was 600 µm, ingested fibers were on average slightly smaller, especially for *A. aquaticus* and *H. azteca* (Figure 6 and Supporting Information, Figure S4). Furthermore, the average size of ingested fibers differed slightly depending on the species. The *L. variegatus* ingested on average the longest and *A. aquaticus* the shortest fibers (Figure 6).

Tire particles were ingested by all three macroinvertebrate species. However, no apparent differences in the number of ingested particles were found from day 4 to 28, indicating that tire particles did not accumulate within the organisms (Figure 5

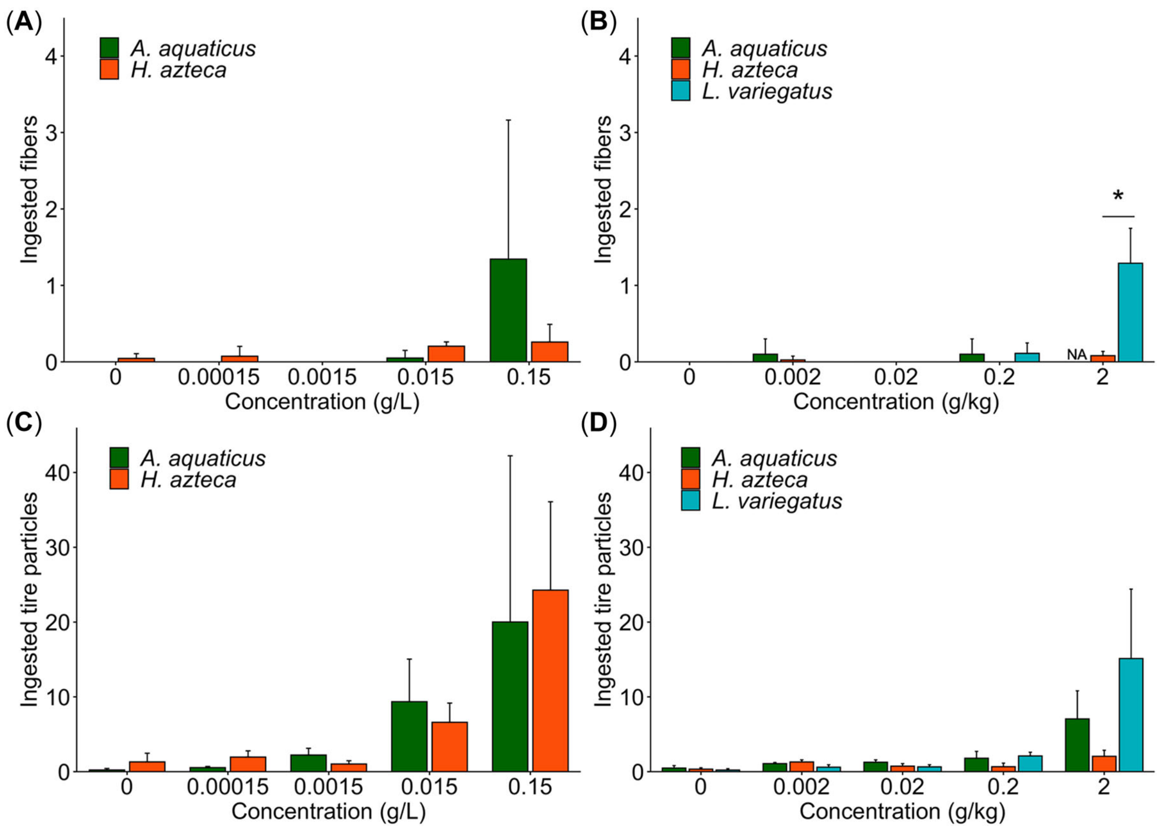

FIGURE 5: Mean number and corresponding standard deviation of ingested microplastics/organism after 28 days of exposure to increasing concentrations of (**A**) fibers in water, (**B**) fibers in sediment, (**C**) tire particles in water, and (**D**) tire particles in sediment. NA means that this concentration was not tested for *Asellus aquaticus*. Statistically significant differences in ingestion between species are indicated by an asterisk.

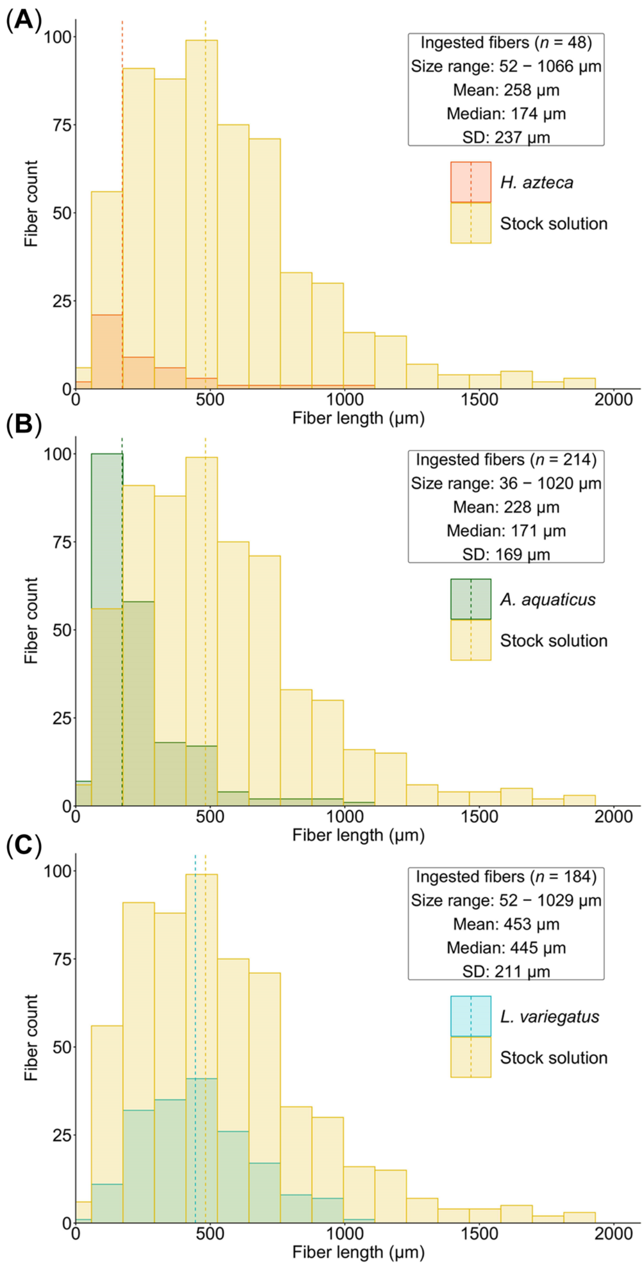

FIGURE 6: Fiber size distribution in the stock solutions and ingested by (**A**) *Hyalella azteca* and (**B**) *Asellus aquaticus* after exposure to fibers dispersed in water, and (**C**) *Lumbriculus variegatus* after exposure to fibers mixed into the sediment. The dashed lines display the respective median of the fiber size distribution. The exposure distribution of the stock solutions was: 26–5761 µm; mean: 600 µm; median: 482 µm; SD: 559 µm ($n = 618$). For the stock solutions the size distribution is only shown up to 2000 µm because only 12 of the 618 measured fibers were outside this range.

and Supporting Information, Figure S3). As has been previously observed, some microplastics can pass through the digestive tract without accumulation, causing little or no observed adverse effects (Gouin, 2020). For instance, Khan et al. (2019) reported a gut clearance time of 24–48 h for *H. azteca* exposed to tire particles. The whole size range of particles present in the tire stock solutions was ingested by the tested macroinvertebrates (Supporting Information, Figure S2).

Tire particle uptake was much higher than fiber uptake by all species (Figure 5), probably due to the difference in particle size and shape. Lower fiber ingestion compared with other particle types has been documented previously (e.g., fragments; Gray & Weinstein, 2017). Furthermore, the exposure pathway influenced microplastic uptake, that is, ingestion by *A. aquaticus* and *H. azteca* was higher after exposure to particles in the water phase compared with particles mixed into the sediment (Figure 5). As for fibers, *L. variegatus* showed the highest ingestion for tire particles of all three benthic species during sediment exposure. Both the epibenthic species *A. aquaticus* and *H. azteca* were probably not in direct contact with the microplastics and thus ingested fewer particles than *L. variegatus*, which is an endobenthic species and feeds directly on sediment particles. The microplastics partly bury and accumulate in the sediment (Scherer et al., 2020; Yao et al., 2019), and thus endobenthic species may encounter microplastics in their natural environment more frequently than epibenthic or pelagic species. Moreover, a higher tire particle ingestion by species following a nonselective feeding strategy (i.e., *L. variegatus* and *D. magna*) was observed. These findings are in agreement with previous studies showing that microplastic ingestion depends not only on microplastic size and shape but also on species characteristics like feeding strategy, habitat, or developmental stage (Fueser et al., 2020; Redondo-Hasselerharm et al., 2018; Scherer et al., 2017).

No significant effects on survival were observed for *H. azteca*, *A. aquaticus*, and *L. variegatus* after acute or chronic exposure to fibers or tire particles (Supporting Information, Figures S5–S7). The solvent controls of the fiber tests showed that the ethanol used in the stock solutions did not influence the survival of *H. azteca* and *A. aquaticus*, or the reproduction of *L. variegatus*. A slight but not significant decrease in reproduction of *L. variegatus* was observed at the highest tire particle concentration after 28 days (Supporting Information, Figure S8). In line with these results, several studies have reported no effects of microplastics (including fibers and tire particles) on freshwater organisms (Redondo-Hasselerharm et al., 2018; Setyorini et al., 2021). For instance, Setyorini et al. (2021) assessed the effects of 50 000 PET fibers/kg with a length of 50 µm on *Chironomus riparius*, showing ingestion but no significant effects. Similarly, Au et al. (2015) observed fiber ingestion by *H. azteca* (polypropylene marine rope; length: 20–75 µm; diameter: 20 µm) but also observed a 10-day LC50 of 71 000 microplastic/L. The present study showed no effects at such a concentration, which may be related to the larger fiber size we used and the lower ingestion. In a chronic experiment, polyamide fibers with a length of 500 µm were ingested and found to decrease food assimilation efficiency of *G. fossarum* at a concentration of 2680 fibers/cm^2, which was possibly caused by physical damage inside the digestive tract (Blarer & Burkhardt-Holm, 2016).

The tire particles NOEC values for benthic invertebrates were greater than 0.15 g/L for water exposure and greater than 2 g/kg dry weight for sediment exposure. This is in line with previous studies showing no effects on benthic freshwater invertebrates exposed to sediments spiked with tire wear

TABLE 2: Maximum measured environmental concentration in different environmental compartments, acute median effect concentration values and chronic no-observable-effect concentrations derived from the present study

		Maximum MEC	48-h EC50	NOEC reproduction		NOEC mortality		
		Fibers/tire particles	*Daphnia magna*	*Daphnia magna*	*Lumbriculus variegatus*	*Daphnia magna*	*Hyalella azteca*	*Asellus aquaticus*
Fibers in water	g/L	0.00022[a]	>0.15	0.00015	NA	0.00015	>0.15	>0.15
	No./L	519[a]	NA	6920	NA	6920	194 550	194 550
Fibers in sediment	g/kg	0.00158[b]	NA	NA	>2	NA	>2	>0.2
	No./kg	1045[c]	NA	NA	$>3.20\times10^{7}$	NA	$>3.20\times10^{7}$	$>4.62\times10^{6}$
Tire particles in water	g/L	0.0008[d] 0.00012[e]	>0.15	0.0015	NA	0.00015	>0.15	>0.15
	No./L	NA	NA	120 000	NA	29 300	$>1.25\times10^{7}$	$>1.25\times10^{7}$
Tires particles in sediment	g/kg	0.0023[g] 5.8[f]	NA	NA	>2	NA	>2	>2
	No./kg	1833[g]	NA	NA	$>7.33\times10^{10}$	NA	$>7.33\times10^{10}$	$>7.33\times10^{10}$

[a]Lahens et al. (2018).
[b]Schell et al. (2021).
[c]Deng et al. (2020); measured 1323 MPs of which 79% were fibers.
[d]Original study by Ni et al. (2008); tire wear concentration estimated by Baensch-Baltruschat et al. (2020).
[e]Unice et al. (2019); concentration modeled.
[f]Unice et al. (2013); concentration measured based on polymer makers.
[g]Leads and Weinstein (2019); concentration shown in particles/kg wet wt.
NA: Endpoint not assessed or concentration not available. Sediment concentrations are reported per kg dry wt sediment if not indicated otherwise.
EC50 = median effective concentration; MEC = measured environmental concentration; MP = microplastic; NOEC = no-observed-effect concentration.

particles up to 10 g/kg (Panko et al., 2013) or with tire particles ground from used tires up to a concentration of 100 g/kg (Redondo-Hasselerharm et al., 2018). However, tire particles ground from worn and pristine tires have been shown to negatively affect *H. azteca* survival, when dispersed in water only, at concentrations slightly higher than the ones we tested (0.2–1 g/L for acute exposure, and 0.6 g/L for chronic exposure; Halle et al., 2021; Khan et al., 2019).

Risk assessment

To compare our test concentrations with measured environmental concentrations, the mass-based concentrations and the estimated count-based concentrations are shown in the Supporting Information, Table S1. For water exposure to fibers, the lowest NOEC value observed (0.00015 g/L) corresponds to approximately 700 fibers/L. The highest measured fiber concentrations in freshwater ecosystems were in the same order of magnitude (Table 2). For tire exposure in the water column, the lowest NOEC observed (0.00015 g/L) corresponds to approximately 30,000 particles/L. Based on mass, the observed NOEC is in the same order of magnitude as modeled maximum tire particle concentrations water column concentrations for the Seine River catchment (France; Table 2).

For sediment exposure to fibers, no effects were observed at the highest test concentration (2 g/kg dry wt), and therefore it can be concluded that the NOEC for all tested species is above this concentration. This corresponds to approximately 3.20×10^{7} fibers/kg, which is much higher than the highest reported environmental concentration (~1000 fibers/kg; Deng et al., 2020). Based on mass, the highest concentration of microplastics in general (not only fibers) was used as a proxy, which is 1 g/kg dry weight sediment (Klein et al., 2015), and thus half of the NOEC concentration observed in our study. Also, for tire particles, the NOEC values for sediments are above 2 g/kg or 7.33×10^{10} particles/kg dry weight sediment, which is significantly larger than the highest monitored concentration of tire particles in freshwater sediments (1833 particles/kg wet wt; Leads & Weinstein, 2019). On the other hand, the maximum sediment concentration estimated by Unice et al. (2013) based on polymer markers (5.8 g/kg) is almost three times as high as the highest tested concentration in our study. However, a previous study observed no adverse effects on benthic freshwater invertebrates exposed to up to 100 g/kg (Redondo-Hasselerharm et al., 2018), suggesting that no risks are expected for this environmental compartment.

CONCLUSIONS

Our results show that microplastic type, size, and exposure pathway determine the ingestion capacity of freshwater invertebrates, as well as the observed effect mechanism. Although adverse effects were only observed for the pelagic species tested (*D. magna*), sediment often contains much higher concentrations than the overlying water. Therefore, epibenthic and endobenthic species are more likely to encounter and ingest microplastics. Based on the comparison between measured environmental concentrations and the NOEC values we determined, it can be concluded that the current risks for benthic and epibenthic macroinvertebrates are generally low or insignificant, whereas for pelagic organisms such as *D. magna*, refined exposure and effect studies with fibers and tire particles are recommended.

Supporting Information—The Supporting Information is available on the Wiley Online Library at https://doi.org/10.1002/etc.5337.

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Data Availability Statement—The raw data used by this study is available upon request to the corresponding author (andreu.rico@uv.es).

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