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Screening study of four environmentally relevant microplastic pollutants: Uptake and effects on *Daphnia magna* and *Artemia franciscana*

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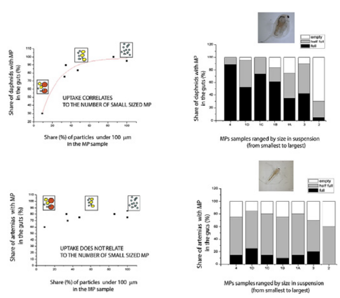
HIGHLIGHTS

- All tested microplastics were found inside the guts of *D. magna* and *A. franciscana*.
- The uptake of microplastics in daphnids is exponentially related to the size of particles.
- A small mass of particles is sufficient to fill the gut of daphnids.
- No acute mortality of daphnids and artemias was observed.
- No delayed acute effects were found and microplastic was depurated from organisms.

GRAPHICAL ABSTRACT



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ABSTRACT

This study investigated four different environmentally relevant microplastic (MP) pollutants which were derived from two facial cleansers, a plastic bag and polyethylene textile fleece. The mean size range of the particles (according to number distribution) was 20–250 µm when measured as a powder and 0.02–200 µm in suspension. In all MP exposures, plastic particles were found inside the guts of *D. magna* and *A. franciscana*, but only in the case of daphnids a clear exponential correlation between MP uptake in the gut and the size of the MP was identified. Exposure tests in which the majority of the MP particles were below 100 µm in size also had higher numbers of daphnids displaying evidence of MP ingestion. As the average MP particle size increased, the percentage of daphnids which had MP in their gut decreased. Using a number distribution value to measure particle size when in a suspension is more experimentally relevant as it provides a more realistic particle size than when samples are measured as a powder. Generally, artemias had fewer MP particles in the gut, than the daphnids, which could be explained by their different food size preferences. No acute effects on *D. magna* were found, but the growth of *A. franciscana* was affected. We conclude that zooplankton crustacean can ingest various MPs but none of the exposures tested were highly acutely hazardous to the test species. In addition, no delayed lethal effects in a 24 h post-exposure period were found.

1. Introduction

Environmental research in recent years has been extensively

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focused towards the potential adverse effects of microplastics (MP) on different organisms (Eerkes-Medrano et al., 2015; Horton et al., 2017). Marine and freshwater zooplankton species are particularly vulnerable to MP exposure since they come into direct contact with suspended MP in the upper water layers (Setälä et al., 2014). Some adverse effects of microplastic on zooplankton crustaceans have already been documented. 50 nm polystyrene particles (above 50 mg/L) affected the motility and moult of marine brine shrimp *Artemia franciscana* larvae after 48 h (Bergami et al., 2016). It was shown that brine shrimp can ingest 40 nm carboxylated polystyrene and 10–20 μm polyethylene particles (Batel et al., 2016). High concentrations of 3–4 μm polyethylene MP (105 particles/mL) caused elevated mortality, increased the inter-brood period and decreased reproduction of freshwater crustacean *Daphnia magna* (Ogonowski et al., 2016). Decreased reproduction, growth and malformation of *D. magna* neonates were also reported upon 21 d exposure to nano-polystyrene (~70 nm; up to 103 mg/L) (Besseling et al., 2014). The following MPs were detected in the gut of *D. magna*: 20 nm and 1 μm fluorescent carboxylated polystyrene (Rosenkranz et al., 2009), 2 μm fluorescent polystyrene (Rist et al., 2017), fluorescent polymethyl methacrylate (29.5 ± 26 μm) (Imhof et al., 2013), 1 μm polyethylene (Rehse et al., 2016), and 3–4 μm polyethylene particles (Ogonowski et al., 2016).

Most of the aforementioned studies have reported on the uptake and effects of MPs in size ranges up to 30 μm. The uptake of particles in this size range by zooplankton is expected as they are of a similar size to their preferred food (Huntley et al., 1983; Setälä et al., 2014). However, very little information is available on the uptake and effects of larger MPs commonly released to environment (Kalčíková et al., 2017a). We therefore performed a screening study using 4 different types of MP of various origins, polymer compositions (polyethylene, polyethylene terephthalate), shapes and sizes. These MPs are environmentally relevant pollutants since they were either derived from products that primarily contain MPs (facial cleansers) or they were produced from plastic waste (bags, and textiles) resulting in so called secondary MPs.

The first aim of this study was to investigate whether these MPs have an acute effect on immobility of *D. magna* and *A. franciscana*, two commonly used zooplankton toxicity test species (ISO 6341:2012; Kos et al., 2016). For this purpose, a very high testing concentration 100 mg/L was used because according to regulatory toxicity testing practise substances above this threshold are classified as non-harmful if no effect is observed (Bondarenko et al., 2016). We also tested if some delayed effects would be evident after additional 24 h incubation in clean medium with food. The second goal was to investigate whether *D. magna* and *A. franciscana* are able to ingest these microplastic particles and if the presence of MP in the gut is related to the size of exposed MP in test medium. A novelty of this study is that the size of MP was measured in powder samples as well as in suspension and the relevance of each mode of measurement in MP ecotoxicity study is discussed.

2. Materials and methods

2.1. Microplastic particles

2.1.1. Preparation of microplastic particles

Microplastic particles were prepared from four commercial products: 2 facial cleanser products, 1 plastic bag and 1 textile fleece. Particles were designated with numbers (MP1A-D; MP2; MP3; MP4) (Table 1). Microplastic particles from commercial facial cleansers (MP1A-D, MP2) were extracted with filtration as described in Jemec Kokalj et al. (2018). Briefly, a small amount of facial cleanser was poured on the filter with the largest mesh size (300 μm) and the soluble ingredients were washed with warm deionised water (40 °C). The washing was repeated until no foam formation was observed in the effluent water. Gradient filtration was used to obtain 3 MP size ranges: three filters were used in a cascade starting with a 300 μm mesh size (MP 1A), followed by 120 μm (MP 1B) and 20 μm (MP 1C). Additionally, MP1A were ground using a planetary ball mill to obtain small size MP 1D. Microplastic particles from a plastic shopping bag (MP3) were prepared according to Jemec Kokalj et al. (2018) by grinding the already fragmented plastic shopping bag using an agate mortar and sodium chloride (NaCl) to enhance the effect of grinding. The sample was then poured on a 120 μm mesh filter and washed with deionised water to remove NaCl. Microplastic particles from fabric (MP4) were prepared using a Retsch PM 100 planetary ball mill (milling balls of diameter 1 cm) according to Jemec et al. (2016). 200 mg of PET fibers were mixed with 1 g of NaCl and poured into 25 mL grinding jar with 5 steel milling balls (diameter 1 cm). Fibers were milled for 2 h at 600 rpm with milling cycle 2 min on and 2 min off. After milling the microplastic particles were washed with deionised water to remove NaCl, then dried and stored in a sealed

Table 1
A list of tested microplastic (MP1-MP4), the source commercial product, the mode of how they were prepared and reference to publication where some characterization data is already available. The mean size of particles in powder (numerical distribution) and number of particles/mg powder are also added.

Name in this paper	Internal Lab. code	Source of MP	Mode of preparation	Chemical composition	Mean diameter ± SD (powder) (μm)	No. of particles/mg
MP 1A	MP4	Facial cleanser 1	extracted with gradient filtration: 1th fraction (the largest)	Polyethylene[a]	183.1 ± 92.46	124.80
MP 1B	MP3	Facial cleanser 1	extracted with gradient filtration: 2nd fraction (middle size)	Polyethylene[b]	102.9 ± 29.1	1051.10
MP 1C	MP2	Facial cleanser 1	extracted with gradient filtration: 3rd fraction (the smallest)	Polyethylene[b]	63.05 ± 24.75	3760.40
MP 1D	MP7	Facial cleanser 1	milled from MP1A	Polyethylene[b]	264 ± 128.3	78.44
MP 2	MP5	Facial cleanser 2	extracted with filtration	Polyethylene[c]	247.9 ± 123.6	/
MP 3	MP8	Plastic bag	grinding fragmented plastic shopping bag using an agate mortar	Polyethylene[a]	136.8 ± 50.89	271.10
MP 4	MP9	Textile fleece	grinding in planetary ball mill	Polyethylene terephthalate[d]	22.8 ± 6.11	50667.03

[a] Jemec et al., 2016.
[b] not published before.
[c] Kalčíková et al., 2017b.
[d] Jemec Kokalj et al., 2018.