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Parental and trophic transfer of nanoscale plastic debris in an assembled aquatic food chain as a function of particle size☆

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ABSTRACT

The existing limitations in analytical techniques for characterization and quantification of nanoscale plastic debris (NPD) in organisms hinder understanding of the parental and trophic transfer of NPD in organisms. Herein, we used iron oxide-doped polystyrene (PS) NPD (Fe-PS-NPD) of 270 nm and Europium (Eu)-doped PS-NPD (Eu-PS-NPD) of 640 nm to circumvent these limitations and to evaluate the influence of particle size on the trophic transfer of NPD along an algae-daphnids food chain and on the reproduction of daphnids fed with NPD-exposed algae. We used Fe and Eu as proxies for the Fe-PS-NPD and Eu-Ps-NPD, respectively. The algae cells (*Pseudokirchinella subcapitata*) were exposed to 4.8×10^{10} particles/L of Fe-PS-NPD or Eu-PS-NPD for 72 h. A high percentage (>60%) of the NPD was associated with algal cells. Only a small fraction (<11%) of the NPD, however, was transferred to daphnids fed for 21 days on the NPD-exposed algae. The uptake and trophic transfer of the 270 nm Fe-PS-NPD were higher than those for the 640 nm Eu-PS-NPD, indicating that smaller NPD are more likely to transfer along food chains. After exposure to Fe-PS-NPD, the time to first brood was prolonged and the number of neonates per adult significantly decreased compared to the control without any exposure and compared to daphnids exposed to the Eu-Ps-NPD. The offspring of daphnids exposed to Eu-PS-NPD through algae, showed a traceable concentration of Eu, suggesting that NPD are transferred from parents to offspring. We conclude that NPD can be transferred in food chains and caused reproductive toxicity as a function of NPD size. Studies with prolonged exposure and weathered NPD are endeavored to increase environmental realism of the impacts determined.

1. Introduction

Nanoscale plastic debris (NPD, size < 1 μm) are released in the environment either because of plastic weathering (Jahnke et al., 2017) or production and application of products containing polymeric nanomaterials (Ekvall et al., 2019). It is estimated that NPD potentially remain in the environment for many years due to their non-biodegradability (Lehner et al., 2019). In the environment, NPD may interact with (micro)organisms and can be taken up by biota (Cole and Galloway, 2015; Pitt et al., 2018a) and, consequently transfer in food chains. For example, it was reported that NPD interact with algae and induce surface damage to the cell (Hazeem et al., 2020) as well as reduce population growth and the chlorophyll content in the cells (Besseling et al., 2014). As a primary producer, microalgae play a vital role in aquatic ecosystems. Since algae have a large surface area to volume ratio, they are more prone to association with NPD (Chae et al., 2018a). With regard to nanomaterials, it was shown that microalgae could accumulate particles on their surface due to the strong association of nanomaterials with algal cells (Abdolahpur Monikh et al., 2019a). It is also likely that

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microalgae accumulate NPD on their surface and transfer NPD into aquatic food chains.

As filter feeders, daphnids consume algae and may be exposed to algae-associated NPD. As a result, our hypothesis is that NPD can transfer from algae to daphnids and then to the organisms of higher trophic levels. In general, understanding the trophic transfer of nanoscale materials is an essential element in assessing their possible risk. Nevertheless, the limitations in analytical techniques for identification and quantification of nanomaterials, particularly polymeric nanomaterials, dramatically hinder gaining new insights on their trophic transfer. Few studies already showed that microplastics (1 μm < size < 5 mm) can accumulate in organisms and may transfer in food chains. For example, trophic transfer of microplastics from mussels to crabs (Farrell and Nelson, 2013a) and from mussels to fish and crabs (Santana et al., 2017) was documented. The trophic transfer of NPD was documented in very few studies, where polystyrene NPD, for example, transferred from algae to daphnids and fish (Chae et al., 2018a). Due to the analytical limitations, to date, trophic transfer of NPD is not quantified and was only determined by using fluorescent-labelled plastic particles (Farrell and Nelson, 2013b; Rist et al., 2017; Setälä et al., 2014). It has been shown that the fluorescence labels can detach from the particles and the detach dyes have completely different uptake kinetics and biodistribution compared to the NPD-bound dyes (Salvati et al., 2011; Tenuta et al., 2011). This can lead to invalid conclusions with regard to the biological fate and trophic transfer of NPD.

Metal-doped polymeric nanomaterials potentially can be used as models of NPD to circumvent the challenges associated with NPD tracking in organisms' bodies by using the metals as proxies (Koelmans, 2019). In this approach, metals are used in the texture of the polymeric nanomaterials while the surface of the particles is polymeric (Mitrano et al., 2019). The polymeric surface of the particle will interact with the surrounding environment and the presence of trace metals inside the particles will, thus, not interfere with the particle interactions with biota. These nanomaterials can be used as models of NPD (Frehland et al., 2020), which allows for the application of well-established mass spectrometry techniques for characterization and quantification of NPD in organisms. Inductively coupled mass spectrometry (ICP-MS), for example, is widely used for measuring elements at trace levels. Performing ICP-MS in a single-particle mode, so-called spICP-MS, allows measuring the size distribution and particle number concentration of metallic nanomaterials (Abdolahpur Monikh et al., 2019b). Single-cell (sc) ICP-MS was recently introduced to measure the concentration of elements in cells on a cell-by-cell basis at a concentration as low as the attogram per liter (Cao et al., 2020; Ho and Chan, 2010; Merrifield et al., 2018). These techniques potentially allow measuring the concentration of metal-doped NPD at trace levels, as expected to be the case in biota.

Exposure to NPD induced mortality and reactive oxygen species production (Liu et al., 2018, 2020) in daphnids. A few studies showed that exposure to waterborne NPD can also cause severe alterations in reproduction (Cui et al., 2017) of daphnids. The parental transfer of NPD was also reported previously, where a dietary exposure of adult zebrafish to polystyrene NPD led to the transfer of the NPD to the offspring (Pitt et al., 2018b). Brun et al. (2017) showed that polystyrene NPD accumulated in or on lipophilic cells in the early stages of embryonic development of *Daphnia magna* while the embryo was still surrounded by a chorion (Brun et al., 2017), which could attribute to brood pouch-mediated NPD uptake by embryos. To the best of our knowledge, it is unknown whether long-term dietary exposure of *D. magna* to NPD can influence their reproduction and lead to the parental transfer of NPD to the offspring. We expect that the dietary uptake of NPD by daphnids may indirectly influence the reproduction rate in the daphnia by affecting the e.g. feeding activity of daphnids. Lesson learned from nanomaterials toxicity showed that physicochemical properties of the particles such as size, shape, and surface chemistry can play a significant role in their uptake, transfer, and toxicity in organisms (Brown et al., 2001; Navarro et al., 2008). It remains, however, largely unknown how the physicochemical properties of NPD influence their adverse effects, particularly, as it is relegalized that weathering of NPD can influence their surface chemistry (Suhrhoff and Scholz-Böttcher, 2016) and thus may have a considerable influence on their behavior in the environment and subsequently their uptake and adverse effect.

Therefore, the aim of this study is to understand whether the particle size influences the trophic transfer of NPD in an aquatic food chain and the reproduction of daphnids fed for long-term on NPD-exposed algae. In this study, we applied spherical metal-doped polymeric nanomaterials being iron oxide-doped polystyrene NPD (Fe-PS-NPD) of 270 nm and Europium-doped polystyrene NPD (Eu-PS-NPD) of 640 nm, as models of NPD. By circumventing the analytical challenges associate with NPD tracking and characterization in a biological matrix, we demonstrated the influence of NPD size on their trophic transfer in an assembled aquatic food chain and their parental transfer in daphnids. Algal cells were exposed to the NPD and the concentration of the Eu and Fe as proxies of Eu-PS-NPD and Fe-PS-NPD, respectively, was measured in the population of the cells on a cell-by-cell basis using scICP-MS. The NPD-exposed algae were used to feed daphnids for 21 days and the mass concentration and particle number of the NPD were measured in the daphnids using ICP-MS and spICP-MS, respectively.

2. Materials and method

2.1. Materials

All chemicals used were of analytical grade and purchased from Sigma-Aldrich (Zwijndrecht, the Netherlands) or Merck (Darmstadt, Germany) unless otherwise mentioned. Spherical Eu-PS-NPD of 640 nm size (PDI = 0.08) doped with Eu^{3+} (<0.2%) and spherical Fe-PS-NPD of 270 nm size (PDI = 0.01) doped with iron oxide (>40%) were purchased from MicroParticles (GmbH, Berlin, Germany) to be used as a model of NPD in this study. The density of the Eu-PS-NPD was ~1.05 g/cm^3 and the density of Fe-PS-NPD was ~2 g/cm^3. Water was deionized by reverse osmosis and purified by a Millipore Milli-Q (MQ) system.

2.2. Characterization of NPD in different media

Polystyrene was used as a model plastic in this study because it is one of the frequently observed plastic in the environment and at the moment it is possible to make metal-doped particle at the nanoscale with this polymer. We used metal-doped PS-NPD of different sizes being 270 and 640 nm with a relatively narrow size distribution. The Eu-PS-NPD and Fe-PS-NPD were dispersed in MQ water to reach a final concentration of 100 mg/L. The dispersions were sonicated using a bath sonicator (35 kHz frequency, DT 255, Bandelin electronic, Sonorex digital, Berlin, Germany) for 1 min. First, we confirmed the stability of the metals in the structure of the NPD by measuring the solubility of the NPD in water over time. Accordingly, dispersions (10 mg/L) of the NPD in MQ water were prepared and left at room temperature. The dispersions were sampled every 12 h for 72 h in total. The collected samples at each time point were centrifuged (using Thermo Scientific Sorvall ST 16R Centrifuge) at 3000×*g* for 10 min and the supernatants were removed. The concentrations of the Eu and Fe in the supernatants were measured using ICP-MS (S1, the Supporting Information).

The hydrodynamic size and the zeta potential were measured using a Zetasizer Nanodevice (Malvern Panalytical, Almelo, the Netherlands). The hydrodynamic size of the NPD was measured over 48 h in MQ water and in the algal exposure medium to determine the aggregation profile of the NPD in different media. Transmission electron microscope (TEM; JEOL 1400) operating at 80 kV accelerating voltage was used to image the NPD and determine their shape and size.

The presence of Eu and Fe in the structure of the PS-NPD increases the density of the NPD and may lead to the sedimentation of the NPD over time. Consequently, the effective exposure concentration in the algal exposure medium could decrease over time. An experiment was performed where the NPD were dispersed in 100 mL algal exposure medium (without algae) and kept shaking continually at 80 rpm using a G10 Gyratory Shaker (Washington, the US), which mimics the conditions at which the algae were exposed to NPD. We expected this condition to maintain the NPD as much as possible in the dispersed phase for 72 h. To evaluate the sedimentation profile of the NPD, aliquots of the samples were taken from the top 1 cm of the flask every 12 h and the concentration of the Eu and Fe in the exposure media was measured in the samples using ICP-MS.

2.3. Exposure of microalgae to NPD

The unicellular microalgae *Pseudokirchinella subcapitata* was cultured in the Woods Hole algal medium according to the OECD guideline 201 (OECD, 2011). The algae density was measured using an AquaFluor fluorometer (Turner Designs, San Jose, CA, USA) and kept at 5×10^3 cells/mL in accordance with the OECD guideline (OECD 201) for initial biomass of algae. In this study, we used particle number as the dose metric to be able to evaluate the influence of the particle size (Abdolahpur Monikh et al., 2019c) on the NPD association with algae and their trophic transfer. The algae were exposed to 1 mg/L of Fe-PS-NPD and 7 mg/L of Eu-PS-NPD, which are equal to 4.8×10^{10} particles/L, during their steady-state phase of growth (6–7 days) (Abdolahpur Monikh et al., 2019a). The estimated particle to cell ratio at the initial concentration was ~9500 particles/cell. Calculation of the NPD particle number is described in S2, Supporting Information. Fifteen replicates were used for each treatment. The flasks containing the exposed algae were placed in a climate chamber (22 °C) on a G10 Gyratory Shaker at a light intensity of 70 mE m^{-2} s^{-1} for 72 h.

The growth inhibition test (72 h) was performed for algae according to the standard guideline (OECD 201) (OECD, 2011) to assure the exposure to NPD did not lead to algal mortality (see S3, Supporting Information). The exposed algae were left at 4 °C for 24 h to sediment. After 24 h, the supernatants, which may contain the free NPD, were removed. Aliquots of the supernatants were digested using *aqua regia* and the total concentrations of the Eu and Fe were measured in the samples using ICP-MS to determine the total removed NPD by algae from the exposure medium (see S1, Supporting Information). The rest of the pellets algae were washed using phosphate-buffered saline (PBS, 10 × , pH 7.5) (see S4, Supporting Information). The washing process removed the unbound and loosely attached NPD from the surface of the algae, while kept the strongly attached NPD on the surface of the algae as explained in the previous study for other nanomaterials (Abdolahpur Monikh et al., 2019a). We removed the loosely attached NPD from the algae to reduce the possibility of the NPD detach from the algal cells when we feed the daphnids with the exposed algae. Subsequently, the possibility of water-borne exposure of daphnids to the detached NPD would decrease. Aliquots of the algal samples were separated for scICP-MS analysis and the rest was used to feed *D. magna*.

2.4. Association of NPD to algal cells

After removing the unbound and loosely attached NPD from the algae, the pellets were dispersed in PBS (10 × , pH 7.5) and diluted to a final volume of 10 mL. The concentration of the algal cells was measured using AquaFluor fluorimeter and 2 mL of the samples were analyzed using scICP-MS. A PerkinElmer NexION 2000 ICP-MS was used to perform scICP-MS. An Asperon spray chamber was used which allowed the introduction of a low volume of algal cell dispersion at a rate of 0.02 mL/min into the plasma. A high-efficiency quartz concentric nebulizer (MEINHARD HEN) was applied. The dwell time and acquisition time was set at 50 μs and 40 s, respectively. Each measured event in scICP-MS represents an algal cell. From the total number of cells measured with the Aquaflour fluorimeter, we determined the number of cells with no NPD. The percentage of NPD containing algae was normalized to the total number of the cells in the population.

2.5. Exposure of Daphnia magna to NPD and reproduction toxicity assay

The *D. magna* specimens were continuously cultured in Elendt M7 medium, at 22 ± 0.5 °C and light: dark (L:D) cycle of 16:8 h. Before the exposure experiments, female *D. magna* specimens were collected and cultured individually. Healthy neonates (<24-h old) from their third brood were separated and cultured for the experiments in Elendt M7 medium. The organisms were kept at 22 C with a 16: 8 h light: dark cycle.

We exposed *D. magna* individually to 0.1 mg of the NPD-exposed algae in a 21-day reproduction bioassay following the OECD guidelines (OECD, 2012). Accordingly, 15 individual *D. magna* were divided into 15 jars (50 mL volume). Three times a week, the *D. magna* were fed with 72 h NPD-exposed algae after washing the algae to remove the unbound and loosely attached NPD from the surface of the algae. Control groups were fed with unexposed algae. The exposure medium of the daphnids was refreshed three times a week with 50 mL of fresh media (pH 8). The total number of surviving individuals was calculated and the dead daphnids were separated on a daily basis. The organisms were considered dead if they did not display any movement for 15 s. During the exposure, some response variables related to reproduction, including the time to the first offspring, the total number of neonates per daphnia, neonates' mortality and the concentration of Fe and Eu in the neonates were measured. The number and the survival of neonates were monitored daily.

After 21 days, the daphnids were harvested and washed three times with MQ water. Depuration experiments were performed to allow the organisms to empty their gut. The fraction of the NPD that could not pass the gut epithelium and internalized into the organisms might be excreted during the depuration period. For the depuration experiment, the daphnids were placed into a clean Elendt M7 medium for 24 h without feeding. Aliquots of the depuration medium were taken at 24 h to measure the total mass of Eu and Fe and the particle number of the excreted NPD from the daphnids using ICP-MS and spICP-MS, respectively. The total concentration of Eu and Fe in the organisms was also measured after acid digestion of the samples using ICP-MS (S1, Supporting information).

2.6. NPD particle number in the organisms

To measure the NPD particle number using spICP-MS, the NPD must be extracted from the organisms. In order to extract NPD from the algae and daphnids, the organisms were digested using 5% Tetramethylammonium hydroxide (TMAH). Accordingly, 0.1 of pellet algae or 3 individuals *D. magna* were put in glass vials and 5 mL of TMAH (5%) was added to the samples. The samples were digested on a water bath at 70 °C for 1 h. After 1 h the algal residual was diluted with MQ water to a final volume of 10 mL. In the case of *D. magna*, the samples were further digested with 30% H_2O_2 to remove the carapace of the organisms, which could not be removed using the TMAH solution alone. It was reported that 5% TMAH does not influence PS-NPD e.g. due to polymer degradation (Cole et al., 2014). The number of NPD extracted from the organisms were quantified using spICP-MS, by using Eu and Fe as proxies of the Eu-PS-NPD and Fe-PS-NPD, respectively. The samples resulting from the depuration experiments were also measured using spICP-MS to determine the number of NPD excreted from the *D. magna* into the depuration medium. The spICP-MS measurements were performed on a PerkinElmer NexION 2000 ICP-MS. The operational parameters for spICP-MS are summarized in Table S2 (Supporting Information). Dispersions of standard gold nanomaterials with sizes of 10, 60, and 100 nm and mass concentration of 50 mg/L were used to determine the transport efficiency. In this experiment, we assumed that the NPD were stable against metal (Fe and Eu) release and against particle agglomeration in the organisms. Therefore, any event measured by spICP-MS represents a NPD particle. The intensity of elements represents the amount of each element in every single particle and was not considered as the number of the particles.

2.7. Data analysis

For all concentrations reported, the respective background concentration of Fe in non-exposed control organisms was subtracted (4.30 ± 0.7 μg Fe/organism, $n = 10$). All statistical analyses were performed using SPSS Statistics 25. Normality was verified using the Shapiro-Wilk test and homogeneity of variances by the Levene test. The one-way ANOVA followed by Dunnett's test analysis was performed to compare the mortality in daphnids between the treatment groups and the control. The significant differences between the treated samples, e.g. with regard to the association of NPD of different size with the algae ($df = 25$), accumulation of NPD of different size in daphnids ($df = 23$), depuration of NPD of different size from daphnids ($df = 26$) and numbers of NPD in daphnids ($df = 25$) compared to algae were analyzed by the *t-test*. The *t-test* was also used to assess the significant differences between the reproduction of treated daphnids with NPD and the controls. The $p < 0.05$ was taken as a significant cut-off. Results are reported as mean and standard deviation of 15 replicates.

3. Results and discussion

3.1. Characterization of NPD in different media

The measured hydrodynamic size and the zeta potential of the NPD in MQ water and in the algae exposure media are reported in Table 1. The TEM image of the Fe-PS-NPD (Fig. 1a) and Eu-PS-NPD (Fig. 1b) showed that the particles are spherical in shape and have a narrow size distribution. No homoaggregation was observed for the NPD dispersion in MQ water (Fig. 1c) as determined by the increase in the hydrodynamic size of the particles over time. However, the hydrodynamic size increased in the algal exposure medium over 48 h (Fig. 1c) as reported for other nanomaterials (Abdolahpur Monikh et al., 2019c). This indicated that both NPD underwent homoaggregation in algal exposure medium without algae. This could be due to the decreases in the absolute value of the zeta potential to −14 mV and −17 mV for Fe-PS-NPD and Eu-PS-NPD in the algal exposure medium, respectively (Table 1). The NPD did not dissolve in water as the concentration of elementary Eu was lower than the detection limit and Fe measured in the supernatant was stable over 72 h of exposure (see S5, Supplementary Information). The Eu concentration in the exposure medium without algae showed (Figure S1) that the particles did not sediment within 72 h. However, we observed a reduction in the concentration of Fe after 24 h of exposure. This indicated that the Fe-PS-NPD have sedimented over time and the concentration of NPD in the exposure medium reached a value lower than 80% of the initial NPD concentration (Figure S1). This decreased the effective exposure concentration of the Fe-PS-NPD to algae over time. Therefore, every 12 h, we gently shook the exposure medium, in which algae were exposed to Fe-PS-NPD, manually for 1 min in a way not to damage the algal cells. By using this approach, we could keep the exposure concertation of the Fe-PS-NPD above 80% of the initial concentration in the exposure medium for 12 h.

3.2. Association of NPD to algal cells

The growth inhibition test showed that 4.8×10^{10} particles/L NPD did not induce toxicity to the algae after 72 h exposure, as tested by measuring the chlorophyll content relative to unexposed controls (Figure S2b, Supporting Information). The NPD were associated with the algae after 72 h of exposure (Fig. 2a–b). Using scICP-MS, we measured the total mass of Eu and Fe associated with the algal cell on a cell-by-cell basis. A high percentage of the cells accumulated both NPD (<80% cells accumulated Fe-PS-NPD and <60% accumulated Eu-PS-NPD) particles on their surface (Fig. 2c–d), with the association of NPD to cells being stochastic as previously reported for other nanomaterials (Abdolahpur Monikh et al., 2019c). This indicated that the number of NPD which were transferred to algal grazing organisms such as daphnids could also differ from organism to organism. The association of the smaller NPD (Fe-PS-NPD) with the cells was significantly (*t-test*, $p < 0.05$) higher than the association of the larger NPD (Eu-PS-NPD). It is likely that smaller NPD, which have a higher volume specific-surface area (2.2×10^5 cm^2/cm^3), attach stronger to the surface of algae compared to the larger NPD, which have a smaller volume specific-surface area (9.3×10^4 cm^2/cm^3) as reported previously for other nanomaterials (Abdolahpur Monikh et al., 2019a). Although a previous study showed that different sized plastic particles can have different toxicity profiles in algal cells (Hazeem et al., 2020), we, for the first time, showed that the size of NPD influences their association with algal cells. This can be of paramount importance for understanding the trophic transfer of NPD in food chains as this

Table 1
Physicochemical properties of the NPD in MQ water and in the algal exposure medium immediately after sonication.

NPD	Hydrodynamic size in MQ water [nm]	TEM measured size	Zeta potential in MQ water [mV]	Hydrodynamic size in algal exposure medium [nm]	Zeta potential in algal exposure medium [mV]
Fe-PS-NPD	360 ± 23	345 ± 7	- 38 ± 3	368 ± 42	- 14 ± 4
Eu-PS-NPD	710 ± 45	680 ± 5	- 37 ± 2	734 ± 58	- 17 ± 2

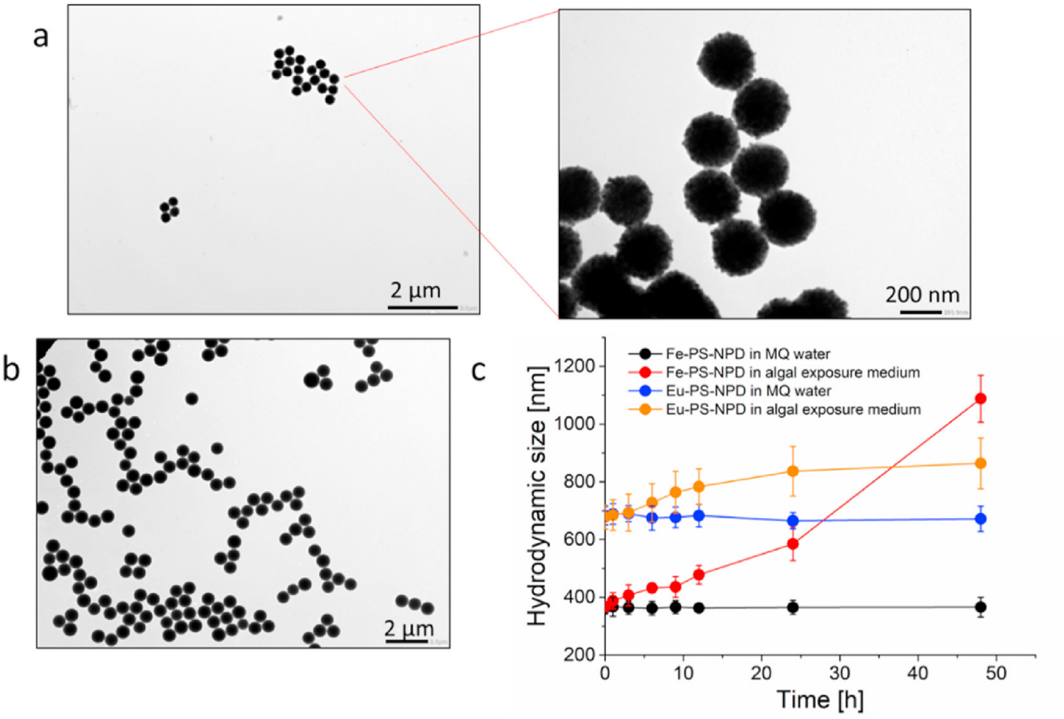

Fig. 1. a) TEM images of the Fe-PS-NPD in MQ water. b) TEM image of the Eu-PS-NPD in MQ water. c) Homoaggregation profile of the NPD in MQ water and in the algal exposure medium as determined by measuring the hydrodynamic size of the particles over 48 h.

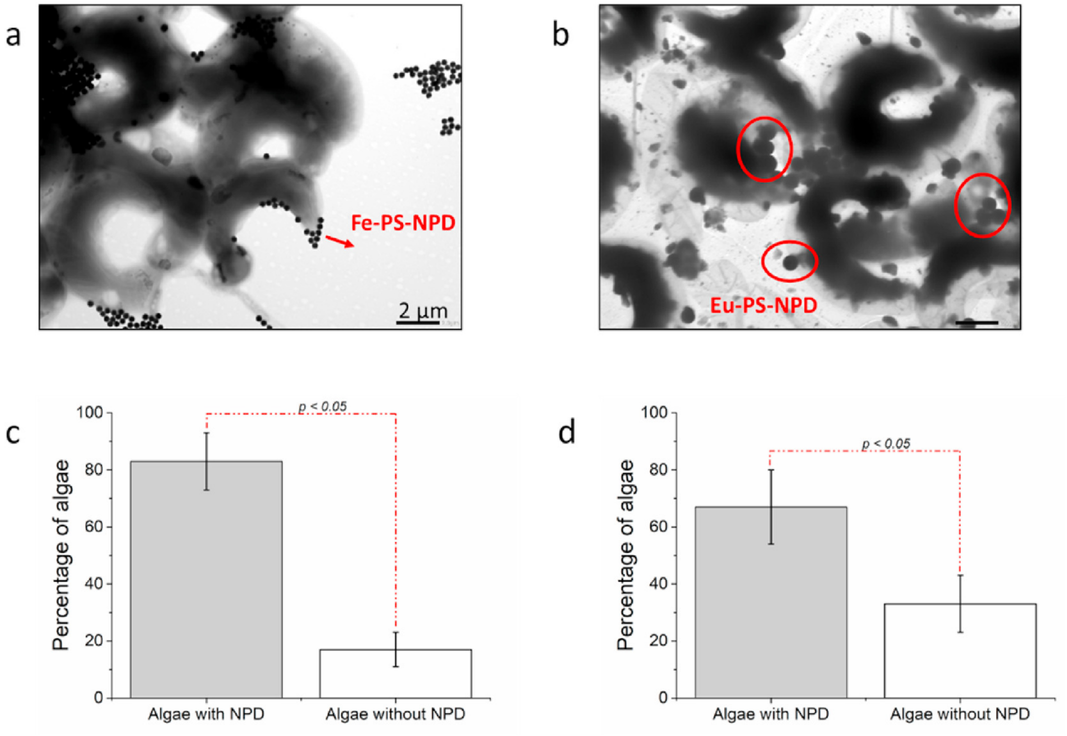

Fig. 2. TEM images showing the association of the Fe-PS-NPD (a) and Eu-PS-NPD (b) with algal cells. Percentage of the algal cell that have Fe-PS-NPD (c) and Eu-PS-NPD (d) associated with their cell surface after 72 h of exposure.

finding implies even when the NPD are not internalized in the algae but accumulated only on the surface of the algae, they may still be transferred to algal grazing organisms.

3.3. *Trophic transfer of NPD from algae to daphnids*

During a 21-day exposure to NPD, the trophic transfer of the NPD from water to daphnids was determined by measuring Fe and

Table 2
Percentage of the NPD in each trophic level, determined by their proxies, normalized to the NPD in the previous level.

NPD	NPD removed by algae from the exposure medium/NPD in the exposure medium [%]	NPD accumulated on algae/ NPD in the exposure medium [%]	NPD in daphnia/NPD in 0.1 mg algae per daphnia [%]	NPD in daphnia after deputation/NPD in 0.1 mg algae per daphnia [%]	NPD depurated from daphnia/NPD in 0.1 mg algae per daphnia [%]
Fe-PS-NPD	72 ± 8.5^b	26 ± 5^b	11 ± 3.5^b	0.8 ± 0.2^b	7.3 ± 3^b
Eu-PS-NPD	58 ± 6^a	14 ± 2.5^a	6.3 ± 0.7^a	0.3 ± 0.01^a	4.2 ± 0.2^a

The letters (a, b) show the significant differences between the NPD size ($p < 0.05$).

Eu as proxies for the Fe-PS-NPD and Eu-PS-NPD, respectively. At the end of the bioassays (21 days), the mortality in the exposed daphnia was not significantly (*ANOVA*, $p < 0.05$) different from the mortality determined in the control (between 1 and 3 organisms out of 15 organisms exposed).

Our findings confirmed that NPD are transferred in the assembled aquatic food chain. Table 2 summarizes the results obtained for the trophic transfer of the NPD. The mass concentration of NPD was measured in each trophic level and normalized to the mass concentration of the NPD in the previous level. A high concentration of NPD (on average 72% for Fe-PS-NPD and 58% for Eu-PS-NPD) was removed from the exposure media by algal cells. After washing the algae, however, only a fraction (on average 26% for Fe-PS-NPD and 14% for Eu-PS-NPD) of the NPD associated with algae remained attached to the cells. The percentage of the removed Fe-PS-NPD by algae from the exposure media was significantly (*t-test*, $p < 0.05$) higher than the percentage of the removed Eu-PS-NPD (58%). The percentage of the remaining NPD on the algae was higher for the Fe-PS-NPD compared to the Eu-PS-NPD. Nevertheless, only 11% of the Fe-PS-NPD and 6.3% of the Eu-PS-NPD in algae were transferred to *D. magna* which fed for 21 days on NPD-exposed algae. The trophic transfer of NPD has already been reported (Chae et al., 2018b). Our study is the first to quantify the amount of NPD transferred in food chains.

Although the passive uptake mechanisms of NPD after attachment to algae were proposed as a pathway for NPD entering daphnids (Rist et al., 2017), this study showed that most of the NPD (on average 7.3% for FE-PS-NPD and 4.2% for Eu-PS-NPD) taken up are depurated from the organisms. It is possible that the NPD accumulated in the gut of *D. magna* and were not taken up into the organisms. After 24 h of depuration, the observed decreases in the body burden of NPD in the daphnids confirmed that only a small percentage (0.8% for Fe-PS-NPD and 0.3% for Eu-PS-NPD) of the NPD remained in the daphnids, whereas the rest were excreted. It was reported previously that 24 h after ingestion, the amount of NPD in daphnids decreases significantly due to the exertion of NPD from the organisms (Rist et al., 2017). The feeding habits of the daphnia could be the reason for the high depuration of NPD. It has been shown, for example, that the addition of algae to water facilitates the release of a significant fraction (~50–85%) of the accumulated carbon nanotubes from *D. magna* (Petersen et al., 2009).

The uptake, body burden and the excreted amount of Fe-PS-NPD were significantly (*t-test*, $p < 0.05$) higher than those of the Eu-PS-NPD. This suggests that NPD with small size are taken up more efficiently than the larger counterparts. This could be due to the stronger attachment to the algae. It was documented for other nanomaterials e.g. gold nanomaterials, that particles of smaller size have a higher uptake and depuration rate in *D. magna* compared to particles of larger size (Skjolding et al., 2014). Our findings showed that NPD with small size are more likely to remain in the gut system of D. magna compared to larger-sized NPD, possibly due to their small size that facilitates their easier penetration through the gut epithelium.

To provide insight into the number-based trophic transfer of NPD, the number of NPD at each trophic level was measured using spICP-MS whilst considering two assumptions. Our first assumption is that there is no homoaggregation between the particles and each measured event represents one NPD regardless of the intensity of the signal. Our second assumption is that there is no Eu and Fe release from the particles in the organism. The number based-trophic transfer of the NPD was similar to their mass-based trophic transfer. Our findings (Fig. 3) showed significantly (*t-test*, $p < 0.05$) lower numbers of NPD in daphnids (ranging on average from 6.3×10^5 for Eu-PS-NPD to 7.2×10^6 for Fe-PS-NPD) compared to algae (ranging on average from 5×10^7 for Eu-PS-NPD to 4.8×10^8 for Fe-PS-NPD). Although the algae were exposed to the same number of NPD of different sizes, the number of NPD which were associated with the algal cells and transferred to daphnids was significantly (*t-test*, $p < 0.05$) different between the two NPD.

Trophic transfer of NPD, as determined based on the particle number, also confirmed the dependency on NPD size. The number of the smaller NPD (Fe-PS-NPD) in all trophic levels was significantly (*t-test*, $p < 0.05$) higher than the number of the larger NPD (Eu-PS-NPD). This is in agreement with a previous study, where the author showed that on average a higher particle number of 100 nm PS-NPD (5.29×10^7 particles/animal of the) compared to 2 μm PS particles (1.24×10^5 particles/animal) were taken up by *D. magna* (Rist et al., 2017). The number of depurated NPD from daphnids was also higher than the number of retained NPD in the daphnids for both NPD after 24 h depuration. Our study for the first time quantified the trophic transfer of NPD on a particle number basis. This approach must be carried out with realistic concentrations and particle properties in terms of shape, size and polymer type when the numbers of environmental concentrations of NPD are available

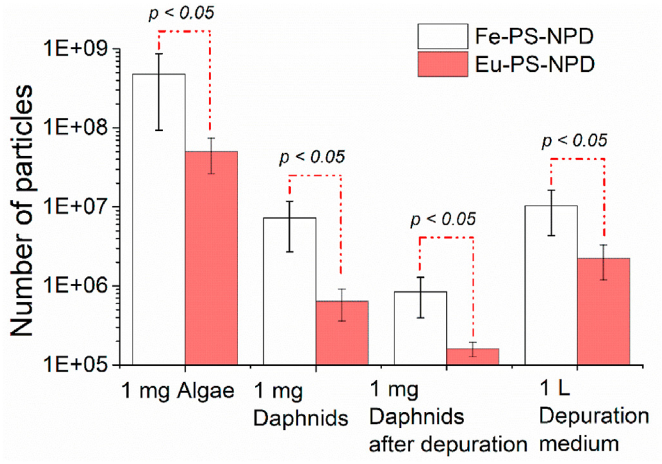

Fig. 3. The number of NPD measured using spICP-MS by measuring the Eu and Fe as the proxies of the NPD in organisms of each trophic level and in daphnids after depuration. The number of NPD in the algae was measured after 72 h of exposure. The number of NPD in daphnids was measured after 21 days of feeding with NPD-exposed algae. After 21 days of exposure, the daphnids were transferred to a clean medium for 24 h, as a depuration period. The number of the particles was also measured in the daphnids after 24 h of depuration and in the depuration medium.

Table 3
Reproduction toxicity of Fe-PS-NPD in the offspring of *D. magna* fed with NPD-exposed algae for 21 days.

Offspring	Time to production [days]		Average number of neonate/organism		Mortality		Fe-PS in offspring/in parent [%]	
	Control	Exposed	Control	Exposed	Control	Exposed	Control	Exposed
First brood	10–11	10–12	13 ± 1.8^{b}	4 ± 0.7^{a}	$0\ \%^{a}$	$5\% \pm 0.4^{b}$	–	1.2 ± 0.6
Second brood	13–14	14–16	11 ± 2.5^{b}	6 ± 2^{a}	$3\% \pm 0.4^{a}$	$7\% \pm 1.3^{b}$	–	0.7 ± 0.3
Third brood	17–18	18–21	12 ± 1.5^{b}	4 ± 1.5^{a}	$1\% \pm 0.01^{a}$	$10\% \pm 2.5^{b}$	–	0.9 ± 0.3
Fourth brood	21–22	NA	10 ± 2	–	0%	–	–	–

a–b indicate significant differences from control ($P < 0.05$). Data are presented as means ± standard deviation (SD). n = 15, NA: not achieved.

in field samples to facilitate the risk assessment of NPD as a function of their physicochemical properties.

3.4. Reproduction toxicity to D. magna

To assess the toxic effects of the NPD on the reproduction of *D. magna* after 21 days of feeding with NPD-exposed algae, we measured the time to the reproduction, the number of offspring per brood, mortality in offspring, and the body burden of NPD in the offspring. The results (Table 3) showed that the Fe-PS-NPD prolonged the reproduction time and significantly (*t-test*, $p < 0.05$) decreased the number of neonates compared to the control. The percentage of the mortality in the offspring of *D. magna* exposed to Fe-PS-NPD was significantly (*t-test*, $p < 0.05$) higher than in the offspring of the control groups. Our finding is in agreement with a previous study that showed exposure to PS-NPD induced the reproduction toxicity in daphnids (Liu et al., 2019; Zhang et al., 2020). The concentration of Fe in the offspring of treated organisms was similar to the concentration of Fe in the offspring of the control and to the Fe in the parents. We could not confirm whether or not there is a parental transfer for Fe-PS-NPD because of the limitation in measuring low concentrations of Fe using ICP-MS and the presence of polyatomic interferences in biological samples which interfered with the Fe of interest (Costo et al., 2019).

During the 21 days exposure, no significant (*t-test*, $p < 0.05$) differences between Eu-PS-NPD treated daphnids and the control were found for the time to first-forth brood (Table 4) and the offspring mortality. The number of neonates per brood in the treated daphnids was significantly lower than the number of neonates per brood in the control groups. Our findings show that the smaller NPD (Fe-PS-NPD) have a higher impact on the reproduction of *D. magna* than the larger NPD (Eu-PS-NPD). Previous results suggested that mortality in offspring and the reproduction of *D. magna* exposed to microplastics were linked to the availability of food rather than particle concentrations. It is also possible that NPD induced reproduction toxicity by influencing the ingestion of NPD-associated algae by *D. magna*. Accumulation of NPD in the gut and retention in the gut epithelia might decrease the feeding activity in the long term and result in further impairment of physiology and fitness of organisms (Cole et al., 2015), thus influencing the reproduction of the organisms (Rist et al., 2017).

The concentration of Eu could be measured in the offspring (0.4%–0.8% of Eu in parent transferred to offspring), suggesting that Eu-PS-NPD might transfer to offspring through parents. We have exposed the mother *D. magna* to the NPD-associated algae after removing the unbound and loosely attached NPD from the algae. This procedure does not fully rule out the possibility that the NPD could be detached from the surface of algae in the exposure media of the daphnids and accumulated in the neonates through brood pouch-mediated uptake as reported previously (Brun et al., 2017). There is also the possibility that when the daphnids ingested the NPD-associated algae, the NPD might be detached from the algal cells in the gut of the daphnids due to e.g. the variation of the composition of the surrounding medium, activities of some enzymes, the activity of gut bacteria and/or the low pH (~4) of the daphnids' gut. After excretion of the detached NPD into the exposure media, they might be taken up again by daphnids through the brood pouch and accumulate in the neonates.

4. Conclusion

Our results show that NPD have the potential to transfer to higher trophic levels. While algae were exposed to similar numbers of each NPD of different sizes (270 nm and 640 nm), a higher amount of the smaller NPD accumulated on algal cells. The association of NPD with algae is an important pathway for NPD transfer in aquatic food webs. Daphnids ingested a considerable amount of algal-associated NPD. However, only a small fraction of the NPD accumulated in daphnids and the rest were depurated from the organisms. The reproduction toxicity in daphnids after feeding with NPD-associated algae also differed for the two NPD sizes. It confirmed that dietary uptake of NPD could also cause reproductive effects in daphnids as a function of NPD size. Parental transfer is occurring for small NPD, either directly through mothers or through water exposure, but we could not differentiate these processes. Taking all findings together it can be concluded that by circumventing the challenges associated with NPD detection, identification, and characterization it was shown that smaller NPD are potentially more hazardous. Future studies can make use of the approach described here to understand the trophic transfer of NPD under environmentally realistic conditions; considering for instance weathering of NPD and long-term exposure of organisms

Table 4
Reproduction toxicity and the concentration of Eu-PS-NPD in the offspring of *D. magna* fed with NPD-exposed algae for 21 days.

Offspring	Time to production [days]		Average number of neonate/organism		Mortality		Eu-PS in offspring/in parent [%]	
	Control	Exposed	Control	Exposed	Control	Exposed	Control	Exposed
First brood	10–11	10–11	14 ± 1^{b}	10 ± 1^{a}	2%	3% ± 0.2	–	0.4 ± 0.06
Second brood	13–14	13–14	13 ± 0.5^{b}	9 ± 0.5^{a}	2% ± 0.4	4% ± 1	–	0.7 ± 0.03
Third brood	17–18	17–18	16 ± 1^{b}	8 ± 0.7^{a}	1% ± 0.01	2% ± 0.6	–	0.8 ± 0.04
Fourth brood	21–22	21–22	13 ± 1.5	11 ± 0.8	4%	$3\% \pm 0.5^{a}$	–	0.7 ± 0.02

a–b indicate significant differences from control ($P < 0.05$). Data are presented as means ± standard deviation (SD). n = 15.

along the food chain to non-biodegradable NPD.

Author statment

Fazel A. Monikh: Conceptualization, methodology, food chain experiment, exposure, measurement, data curation, writing- original draft preparation. Latife Chupani. Methodology, food chain experiment, exposure, writing. Martina G. Vijver: Supervision, editing. Willie J.G.M. Peijnenburg: Supervision, editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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References

Abdolahpur Monikh, F., Arenas-Lago, D., Porcal, P., Grillo, R., Zhang, P., Guo, Z., Vijver, M.G., Peijnenburg W, J.G.M., 2019a. Do the joint effects of size, shape and ecocorona influence the attachment and physical eco(cyto)toxicity of nanoparticles to algae? Nanotoxicology 1–16.

Abdolahpur Monikh, F., Chupani, L., Vijver, M.G., Vancová, M., Peijnenburg, W.J.G.M., 2019b. Analytical approaches for characterizing and quantifying engineered nanoparticles in biological matrices from an (eco)toxicological perspective: old challenges, new methods and techniques. Sci. Total Environ. 660, 1283–1293.

Abdolahpur Monikh, F., Fryer, B., Arenas-Lago, D., Vijver, M.G., Krishna Darbha, G., Valsami-Jones, E., Peijnenburg, W.J.G.M., 2019c. A dose metrics perspective on the association of gold nanomaterials with algal cells. Environ. Sci. Technol. Lett. 6, 732–738.

Besseling, E., Wang, B., Lürling, M., Koelmans, A.A., 2014. Nanoplastic affects growth of S. Obliquus and reproduction of D. Magna. Environ. Sci. Technol. 48, 12336–12343.

Brown, D.M., Wilson, M.R., MacNee, W., Stone, V., Donaldson, K., 2001. Size-dependent proinflammatory effects of ultrafine polystyrene particles: a role for surface area and oxidative stress in the enhanced activity of ultrafines. Toxicol. Appl. Pharmacol. 175, 191–199.

Brun, N.R., Beenakker, M.M.T., Hunting, E.R., Ebert, D., Vijver, M.G., 2017. Brood pouch-mediated polystyrene nanoparticle uptake during Daphnia magna embryogenesis. Nanotoxicology 11, 1059–1069.

Cao, Y., Feng, J., Tang, L., Yu, C., Mo, G., Deng, B., 2020. A highly efficient introduction system for single cell- ICP-MS and its application to detection of copper in single human red blood cells. Talanta 206, 120174.

Chae, Y., Kim, D., Kim, S.W., An, Y.-J., 2018a. Trophic transfer and individual impact of nano-sized polystyrene in a four-species freshwater food chain. Sci. Rep. 8, 284.

Chae, Y., Kim, D., Kim, S.W., An, Y.J., 2018b. Trophic transfer and individual impact of nano-sized polystyrene in a four-species freshwater food chain. Sci. Rep. 8.

Cole, M., Galloway, T.S., 2015. Ingestion of nanoplastics and microplastics by pacific oyster larvae. Environ. Sci. Technol. 49, 14625–14632.

Cole, M., Lindeque, P., Fileman, E., Halsband, C., Galloway, T.S., 2015. The impact of polystyrene microplastics on feeding, function and fecundity in the marine copepod Calanus helgolandicus. Environ. Sci. Technol. 49, 1130–1137.

Cole, M., Webb, H., Lindeque, P.K., Fileman, E.S., Halsband, C., Galloway, T.S., 2014. Isolation of microplastics in biota-rich seawater samples and marine organisms. Sci. Rep. 4, 4528.

Costo, R., Heinke, D., Grüttner, C., Westphal, F., Morales, M.P., Veintemillas-Verdaguer, S., Gehrke, N., 2019. Improving the reliability of the iron concentration quantification for iron oxide nanoparticle suspensions: a two-institutions study. Anal. Bioanal. Chem. 411, 1895–1903.

Cui, R., Kim, S.W., An, Y.-J., 2017. Polystyrene nanoplastics inhibit reproduction and induce abnormal embryonic development in the freshwater crustacean Daphnia galeata. Sci. Rep. 7, 12095.

Ekvall, M.T., Lundqvist, M., Kelpsiene, E., Šileikis, E., Gunnarsson, S.B., Cedervall, T., 2019. Nanoplastics formed during the mechanical breakdown of daily-use polystyrene products. Nanoscale Adv. 1, 1055–1061.

Farrell, P., Nelson, K., 2013a. Trophic level transfer of microplastic: Mytilus edulis (L.) to Carcinus maenas (L.). Environ. Pollut. 177, 1–3.

Farrell, P., Nelson, K., 2013b. Trophic level transfer of microplastic: Mytilus edulis (L.) to Carcinus maenas (L.). Environ. Pollut. 177, 1–3.

Frehland, S., Kaegi, R., Hufenus, R., Mitrano, D.M., 2020. Long-term assessment of nanoplastic particle and microplastic fiber flux through a pilot wastewater treatment plant using metal-doped plastics. Water Res. 182, 115860.

Hazeem, L.J., Yesilay, G., Bououdina, M., Perna, S., Cetin, D., Suludere, Z., Barras, A., Boukherroub, R., 2020. Investigation of the toxic effects of different polystyrene micro-and nanoplastics on microalgae Chlorella vulgaris by analysis of cell viability, pigment content, oxidative stress and ultrastructural changes. Mar. Pollut. Bull. 156, 111278.

Ho, K.-S., Chan, W.-T., 2010. Time-resolved ICP-MS measurement for single-cell analysis and on-line cytometry. J. Anal. Atomic Spectrom. 25, 1114–1122.

Jahnke, A., Arp, H.P.H., Escher, B.I., Gewert, B., Gorokhova, E., Kühnel, D., Ogonowski, M., Potthoff, A., Rummel, C., Schmitt-Jansen, M., Toorman, E., MacLeod, M., 2017. Reducing uncertainty and confronting ignorance about the possible impacts of weathering plastic in the marine environment. Environ. Sci. Technol. Lett. 4, 85–90.

Koelmans, A.A., 2019. Proxies for nanoplastic. Nat. Nanotechnol. 14, 307–308.

Lehner, R., Weder, C., Petri-Fink, A., Rothen-Rutishauser, B., 2019. Emergence of nanoplastic in the environment and possible impact on human health. Environ. Sci. Technol. 53, 1748–1765.

Liu, Z., Cai, M., Yu, P., Chen, M., Wu, D., Zhang, M., Zhao, Y., 2018. Age-dependent survival, stress defense, and AMPK in Daphnia pulex after short-term exposure to a polystyrene nanoplastic. Aquat. Toxicol. 204, 1–8.

Liu, Z., Huang, Y., Jiao, Y., Chen, Q., Wu, D., Yu, P., Li, Y., Cai, M., Zhao, Y., 2020. Polystyrene nanoplastic induces ROS production and affects the MAPK-HIF-1/NFkB-mediated antioxidant system in Daphnia pulex. Aquat. Toxicol. 220, 105420.

Liu, Z., Yu, P., Cai, M., Wu, D., Zhang, M., Huang, Y., Zhao, Y., 2019. Polystyrene nanoplastic exposure induces immobilization, reproduction, and stress defense in the freshwater cladoceran Daphnia pulex. Chemosphere 215, 74–81.

Merrifield, R.C., Stephan, C., Lead, J.R., 2018. Quantification of Au nanoparticle biouptake and distribution to freshwater algae using single cell – ICP-MS. Environ. Sci. Technol. 52, 2271–2277.

Mitrano, D.M., Beltzung, A., Frehland, S., Schmiedgruber, M., Cingolani, A., Schmidt, F., 2019. Synthesis of metal-doped nanoplastics and their utility to investigate fate and behaviour in complex environmental systems. Nat. Nanotechnol. 14, 362–368.

Navarro, E., Baun, A., Behra, R., Hartmann, N.B., Filser, J., Miao, A.J., Quigg, A., Santschi, P.H., Sigg, L., 2008. Environmental behavior and ecotoxicity of engineered nanoparticles to algae, plants, and fungi. Ecotoxicology 17, 372–386.

OECD, 2011. Test No. 201: Freshwater Alga and Cyanobacteria. Growth Inhibition Test.

OECD, 2012. Test No. 211. Daphnia magna Reproduction Test.

Petersen, E.J., Akkanen, J., Kukkonen, J.V.K., Weber, W.J., 2009. Biological uptake and depuration of carbon nanotubes by Daphnia magna. Environ. Sci. Technol. 43, 2969–2975.

Pitt, J.A., Kozal, J.S., Jayasundara, N., Massarsky, A., Trevisan, R., Geitner, N., Wiesner, M., Levin, E.D., Di Giulio, R.T., 2018a. Uptake, tissue distribution, and toxicity of polystyrene nanoparticles in developing zebrafish (Danio rerio). Aquat. Toxicol. 194, 185–194.

Pitt, J.A., Trevisan, R., Massarsky, A., Kozal, J.S., Levin, E.D., Di Giulio, R.T., 2018b. Maternal transfer of nanoplastics to offspring in zebrafish (Danio rerio): a case study with nanopolystyrene. Sci. Total Environ. 643, 324–334.

Rist, S., Baun, A., Hartmann, N.B., 2017. Ingestion of micro- and nanoplastics in Daphnia magna – quantification of body burdens and assessment of feeding rates and reproduction. Environ. Pollut. 228, 398–407.

Salvati, A., Åberg, C., dos Santos, T., Varela, J., Pinto, P., Lynch, I., Dawson, K.A., 2011. Experimental and theoretical comparison of intracellular import of polymeric nanoparticles and small molecules: toward models of uptake kinetics. Nanomed. Nanotechnol. Biol. Med. 7, 818–826.

Santana, M.F.M., Moreira, F.T., Turra, A., 2017. Trophic transference of microplastics under a low exposure scenario: insights on the likelihood of particle cascading along marine food-webs. Mar. Pollut. Bull. 121, 154–159.

Setälä, O., Fleming-Lehtinen, V., Lehtiniemi, M., 2014. Ingestion and transfer of microplastics in the planktonic food web. Environ. Pollut. 185, 77–83.

Skjolding, L.M., Kern, K., Hjorth, R., Hartmann, N., Overgaard, S., Ma, G., Veinot, J.G.C., Baun, A., 2014. Uptake and depuration of gold nanoparticles in Daphnia magna. Ecotoxicology 23, 1172–1183.

Suhrhoff, T.J., Scholz-Böttcher, B.M., 2016. Qualitative impact of salinity, UV radiation and turbulence on leaching of organic plastic additives from four common plastics — a lab experiment. Mar. Pollut. Bull. 102, 84–94.

Tenuta, T., Monopoli, M.P., Kim, J., Salvati, A., Dawson, K.A., Sandin, P., Lynch, I., 2011. Elution of labile fluorescent dye from nanoparticles during biological use. PloS One 6, e25556.

Zhang, W., Liu, Z., Tang, S., Li, D., Jiang, Q., Zhang, T., 2020. Transcriptional response provides insights into the effect of chronic polystyrene nanoplastic exposure on Daphnia pulex. Chemosphere 238, 124563.