Toxicity, bioaccumulation and trophic transfer of engineered nanoparticles in the aquatic environment

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Chapter 4

Accumulation kinetics of polystyrene nano- and micro-plastics in the waterflea *Daphnia magna* and trophic transfer to the mysid *Limnomysis benedeni*

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In preparation

Abstract

Trophic transfer of nano-and micro-plastics (NMPs) has attracted the attention of scientists. Here we investigated the accumulation of differently sized polystyrene particles (PSPs) in *Daphnia magna* and their subsequent transfer to *Limnomysis benedeni*, explicitly accounting for the fate of the PSPs in the aquatic system as a function of particle size (26, 500 and 4800 nm). *L. benedeni* were fed with *D. magna* exposed to 8 mg/L PSPs (8.3×10^{11} particles mL^{-1} 26 nm PSPs, 1.2×10^{8} particles mL^{-1} 500 nm PSPs, 1.3×10^{5} particles mL^{-1} 4800 nm PSPs) for 16 h and were subsequently depurated for 48 h (in the absence of food). On the basis of mass of particles accumulated in the organisms, the internal concentration of 4800 nm PSPs was found to be 4-10 times higher than that of 26 and 500 nm PSPs in *D. magna*. Uptake rate constants in daphnids for 26 nm (1.7 ± 0.4 L/g·h) and 4800 nm (1.7 ± 0.4 L/g·h) were significantly ($p < 0.05$) higher than uptake rate constants for the 500 nm (0.7 ± 0.1 L/g·h) PSPs. It was estimated that a small fraction (1 to 5 %) of the PSPs ingested by *D. magna* was transferred to *L. benedeni*. Moreover, the larger the particle size, the higher the extent of transfer in the food chain. Elimination rate constants in *L. benedeni* were found to be 0.03 ± 0.03, 0.1 ± 0.2 and 0.2 ± 0.8 per hour for 26, 500 and 4800 nm PSPs respectively, and were not significantly different from one another. Visual observations of fluorescence showed that PSPs were mainly accumulated in the stomach and intestine of *L. benedeni*. Furthermore, the calculated trophic transfer factor on the basis of mass of particles accumulated in the organisms was < 1 for all PSP treatments, implying that no biomagnification was observed in the predator. These findings indicated that NMPs can be transferred along the daphnia-mysids food

chain, but that biomagnification does not occur. It also highlights that particle size affects accumulation and trophic transfer.

Keywords: Polystyrene particles; Aquatic food chain; Biodistribution; Uptake rate constants, Elimination rate constants

4.1 Introduction

Pollution of aquatic environments by nano- (1 - 100 nm) (Bouwmeester et al., 2015) and microplastics (100 nm - 5 mm) (Waring et al., 2018) originates from the release of primary manufactured particles employed in many industrial and consumer products (Imhof et al., 2016; Mattsson et al., 2018; Napper et al., 2015; van Wezel et al., 2016; Wang et al., 2019), as well as from the degradation of larger plastic items into nano- or micro-sized fragments (Andrady, 2017; Botterell et al., 2019; Carbery et al., 2018; Franzellitti et al., 2019). As a result there are significant quantities of nano- and microplastics (NMPs) present in the aquatic environment (Botterell et al., 2019; Jambeck et al., 2015; Wang et al., 2019). Furthermore, the concentrations of NMPs in the environment are measured and estimated to be between 1 pg/L and 1 g/L, and they are expected to rise exponentially as their particle size decreases (Lenz et al., 2016). These particles can be ingested by organisms and subsequently be transferred across trophic levels (Chae et al., 2019, 2018; Crooks et al., 2019; Elizalde-Velázquez and Gómez-Oliván, 2021).

Particle size is an important factor affecting the accumulation and trophic transfer of NMPs (Markic et al., 2020; Monikh et al., 2021; Provencher et al., 2019). Van Pomeren et al. (2017) found that nanoplastics with particle sizes of 25 and 50 nm migrate through the body of zebrafish embryos and eventually accumulate in specific organs and tissues, whereas nanoplastics larger than 50 nm were predominantly adsorbed onto the intestinal tract and outer epidermis of zebrafish embryos. Similarly, 5 μm microplastics were found to accumulate in the gills, gut and liver of zebrafish (*Danio rerio*), whilst accumulation of 20 μm particles was restricted to the gills and gut (Lu

et al., 2016). In addition, Monikh et al. (2021) found that the uptake and trophic transfer of microplastics with a particle size of 270 nm along an algae-daphnids food chain were higher than the uptake and transfer of 640 nm particles. However, to date the kinetics of the accumulation of NMPs as a function of particle size have been poorly quantified.

In aquatic environments, actual exposure of organisms to NMPs is dependent on the environmental fate of NMPs. One key characteristic which determines the fate of NMPs is considered to be their density (Ding et al., 2021). Plastics with a low density ($\rho < 1.0$ g/cm^3) will primarily remain in the top/surface layers of the water phase and can be ingested by organisms feeding from the water phase (Devriese et al., 2015; Pegado et al., 2018; Zhang et al., 2017). In contrast, plastics where $\rho > 1.0$ g/cm^3 likely settle to the sediment layer, where they can be ingested by zoobenthos (Zhang et al., 2017). When the density of plastics is similar to 1.0 g/cm^3, their spatial distribution is additionally affected by particle size and shape and mediated by turbulent mixing and biofouling rates (Shamskhany et al., 2021).

Within the current study, we hypothesized that trophic transfer increases with decreasing particle size of PSPs, and a smaller particle has a higher likelihood of penetration and translocation in/within a predator. Moreover, the fate of PSPs in the aqueous phase is, apart from their density, hypothesized to be associated with their particle size in the aqueous phase. To test the hypotheses, we selected fluorescently labeled polystyrene particles (PSPs) ($\rho = 1.05$ g/cm^3) with particle sizes of 26, 500 and 4800 nm as model NMPs. A food chain experiment was conducted using the waterflea *Daphnia magna* and the predatory mysid *Limnomysis benedeni*. *D. magna* neonates were exposed to PSPs for 7 hours, and subsequently fed to *L. benedeni* over

the course of 16 hours, followed by 48 hours of depuration. Biota samples were collected at several timepoints to determine uptake and elimination kinetics, and water and sediment samples were collected to explore the spatial distribution of PSPs in the simulated aquatic system.

4.2 Materials and Method

4.2.1 Test materials and medium

Three fluorescent-dye-labeled and spherical PSPs (diameter 26 nm with an excitation/emission at 505/515 nm and diameters 500 and 4800 nm with an excitation/emission at 468/508 nm) were purchased from ThermoFisher Scientific (Waltham, USA). The 500 and 4800 nm PSPs were unmodified, whilst the 26 nm PSPs contained carboxyl groups bound to the particle surface. Stock suspensions of PSPs (100 mg/L) were freshly prepared in ElendtM7 medium according to OECD 202 (OECD, 2004) and subsequently dispersed by sonicating for 10 min at 50 Hz in a water bath sonicator (USC200T, VWR, Amsterdam, The Netherlands).

4.2.2 Quantitative analysis of mass concentration and particle number concentration

The exposure concentrations (mass-based) of PSPs were determined after measuring their fluorescence intensity (FI) using a Sparks Multimode Microplate reader (TECAN, Switzerland). The particle number concentration (particles/mL) converted from the mass concentration (mg/L) was used as a reference dose metric for the PSPs.

This conversion was performed according to following equation (Facts, 2005):

$$\text{Number of PSPs/ mL} = \frac{6C \times 10^{12}}{\rho \times \pi \times \varnothing^{3}} \quad (1)$$

Where, C is the concentration of suspended PSPs in g/mL, ϕ is the diameter of PSPs in μm, and ρ is the density of polystyrene (1.05 g/mL).

4.2.3 Cultures of test organisms

Daphnids were taken from the culture maintained at Leiden University which is kept according to OECD Guideline 202 (OECD, 2004). Elendt M7 medium (OECD, 2004) was used as the culture medium (pH 8.4 ± 0.4). *D. magna* were maintained at a temperature of 22 ± 1 °C with a 16:8 light-dark cycle and fed with the algae *Pseudokirchneriella subcapitata* every two days.

L. benedeni were collected in spring from a pond in the Netherlands. All collected individuals were acclimated for several weeks and fed cultured daphnids. *L. benedeni* was cultured according to the following acclimatization steps in the climate room (22 ± 1 °C, 6:8 light-dark cycle). 1) Day 1: *L. benedeni* were kept in a mixture of water collected from the original pond and Elendt M7 medium, including a small amount of sediment collected from the original pond. 2) Day 2: *L. benedeni* were transferred into ElendtM7 medium with sediment and were fed with cultured daphnia neonates. 3) Day 3: *L. benedeni* were cultured in ElendtM7 medium without sediment and food to clean their guts. *L. benedeni* were cultured under continuously aeration in the same conditions as *D. magna*. Only female *L. benedeni* (1.2 ± 0.1 cm length) were selected for the experiment for reasons of consistency.

4.2.4 Survival tests

To investigate PSPs-induced responses on the selected test organisms, the survival rates of the daphnids and mysids exposed to all PSP treatments for 24 and 72 h were recorded. *D. magna* neonates (< 24 h) were used in the test after cleaning their guts for around 2 h in Elendt M7 medium. Twenty five neonate daphnids (5 replicates) and 10 mysids (single replicate) were used in each treatment. The test organisms were exposed to increasing nominal concentrations of PSPs of 0, 0.5, 1, 2, 4 and 8 mg/L.

4.2.5 Food chain transfer from *D. magna* to *L. benedeni*

The feeding rate of *L. benedeni* was assessed via a pre-feeding test of 16 h (Supplementary data, Figure S4.1). After 2 h of gut cleaning, *D. magna* neonates were non-exposed (control group) and exposed to the PSP suspensions for 7 h accumulation period. Two exposed daphnids were introduced as food to each mysid (previously maintained without food for 24 h); the feeding session lasted for 16 h, and daphnids were washed 3 times with clean ElendtM7 medium to remove potentially adsorbed particles. After the feeding session, the exposed mysids (n = 8) were washed 3 times to remove the adsorbed PSPs. Subsequently, these mysids were transferred to uncontaminated medium without feeding for another 48 h in order to monitor the depuration of PSPs in the predator (*L. benedeni*). The control mysids (n = 8) were fed two non-exposed neonate daphnids during the feeding period. An initial exposure concentration of 8 mg/L of PSPs (i.e., 8.3×10^{11} particles/mL 26 nm PSPs, 1.2×10^{8} particles/mL 500 nm PSPs, 1.3×10^{5} particles/mL 4800 nm PSPs) was chosen in the trophic transfer test.

Daphnid samples were collected at 0, 0.25, 1, 2, 4 and 7 h and mysid samples were collected at 0, 16, 20, 40, 46 and 64 h. All samples were washed once with ElendtM7 medium and twice with MilliQ water (Milipore Milli-Q reference A+ system, Waters-Millipore Corporation, Milford, MA, USA), and afterwards homogenized at 30 rps for 1 minute (TissueLyzer II, QIAGEN, USA). The internal concentrations of PSPs in the test organisms were determined based on fluorescence using a Sparks Multimode Microplate reader. Concentrations of excreted PSPs from mysids into the aqueous medium were measured simultaneously. The aqueous samples were collected from the middle layer of the exposure suspensions. Also, a control group containing non-exposed organisms was included to ensure that the results of the fluorescence analysis of feeding and depuration were associated with exposure to the test materials.

4.2.6 Modelling kinetics of accumulation process

First-order kinetics were used to model the accumulation of PSPs in the test organisms. The uptake rate of PSPs in the organisms was calculated as follows:

$$\frac{dC_{org}}{dt} = k_w C_{external} \quad (2)$$

Where C_{org} is the concentration (mg/g) of PSPs in organisms, k_w is the uptake rate constant (L/g·h), and $C_{external}$ is the exposure concentration (mg/L) of PSPs.

The elimination rate of PSPs in the organisms was calculated with the following formula:

$$\frac{dC_{org}}{dt} = -k_e C_{org} \quad (3)$$

Where k_e is the elimination rate constant (1/h).

The accumulation process can be described as the sum of the rates of uptake and elimination, as presented in Equation 4:

$$\frac{dC_{org}}{dt} = k_w C_{external} - k_e C_{org} \quad (4)$$

4.2.7 Evaluation of the trophic transfer factor

The trophic transfer potential of PSPs from daphnids to the mysid was evaluated by calculating the trophic transfer factor (TTF). The TTF was evaluated by using the ratio of the PSP concentration in the mysid (C_{mysid}, mg/g wet mass) to the PSP concentration in the daphnids (C_{daphnia}, mg/g wet mass):

$$\text{TTF} = \frac{C_{\text{mysid}}}{C_{\text{daphnia}}} \quad (5)$$

If TTF > 1, biomagnification of PSPs occurs in the mysid; if TTF ≤ 1, the extent of transfer of PSPs from the daphnia to the mysid is limited.

4.2.8 Characterization and *in vivo* distribution

The biodistribution of PSPs in the test organisms was visualized using a Leica MZ 16FA fluorescent stereo microscope equipped with a digital camera (DFC 420) and image acquisition software of Leica. A GFP3 filter (excitation at 470/40 nm, barrier at 525/50 nm) was used and exposure was set at 2.5 s, gain at 2.0 and gamma at 0.6. Transmission

settings were set at exposure 146.4 ms, gain 1.0 and gamma 0.6. Daphnia samples were collected at 0 and 7 h. Mysid samples (n = 3, both control and exposure groups) were collected at 0, 16, 20, 40 and 64 h. These samples were rinsed with uncontaminated medium for 1 min before the characterization of the biodistribution of PSPs.

4.2.9 Statistical analyses

Statistically significant differences in the accumulation kinetic rate constants between the PSP treatments and in the mass concentrations between the exposure group and control group were determined by one-way analysis of variance (ANOVA) with the Tukey's HSD post hoc tests, at a significance level of $p < 0.05$ using SPSS 16.0 (IBM SPSS Statistics for Windows, Ver. 19.0, IBM Corp., Armonk, NY). Data were checked for normality and homogeneity before performing the relevant tests. Statistically significant differences in the observed fluorescence between the PSP treatments was analyzed by means of one way ANOVA followed by Tukey's HSD post hoc tests using the SPSS. Prior to analysis, fluorescence data were Log10 transformed to assure that the requirements for residual- and variance distributions were met. Values in all controls and treatments are expressed as mean ± standard deviation.

4.3 Results and discussion

4.3.1 Survival of *D. magna* and *L. benedeni* exposed to PSPs

Survival data for *D. magna* and *L. benedeni* are presented in Table S4.1 and Figure S4.2 (Supplementary data), respectively. As shown in Table S4.1, the survival rate of *D. magna* was close to 100% for all PSP treatments, independent of particle size and exposure concentration of the PSPs. This means that the PSPs showed no acute lethal toxicity to *D. magna* at all concentrations studied. As shown in Figure S4.2, *L. benedeni* exposed to all the PSP treatments showed a time-dependent decrease in survival rates in comparison to controls, implying that the PSPs exhibited time-dependent acute lethal toxicity.

4.3.2 Accumulation of PSPs in *D. magna*

The kinetics of the accumulation of different sizes of PSPs in *D. magna* directly from the aqueous phase over the exposure period of 7 h are depicted in Figure 4.1. The kinetic rate constants of PSPs in the daphnids were calculated as shown in Table 4.1. The background concentrations in daphnia were detected to be around 0.003 ± 0.006 mg PSPs/g daphnia wet mass during the whole experimental period. Moreover, three separate images in Figure S4.3 (Supplementary data) show overlapping curves (Figure 4.1) for the data of background auto-fluorescence. The ingestion of PSPs in *D. magna* was faster in the first hour of exposure and thus the internal concentrations of PSPs subsequently stabilized. During the exposure, the internal mass concentration (based on mg/g dry weight) of 4800 nm PSPs was 4-10 times higher than the mass concentration of 26 and 500 nm PSPs in *D. magna*. The order of the uptake rate constants (on the basis of mass

concentration) of PSPs in *D. magna* decreased as follows: 26 = 4800 > 500 nm (Table 4.1). These findings were not in line with expectations, as we hypothesized that uptake rates as determined on the basis of mass concentration would be inversely related to particle size. The filter apparatus of the *D. magna* acts like a net and thus allows particles to be ingested in certain size ranges related to the individual mesh sizes (Geller and Muller, 1981; Lee and Ranville, 2012; Tervonen et al., 2010). Thereupon, too small particles only will be ingested when they are aggregated, and too large particles will not be taken into the filter apparatus.

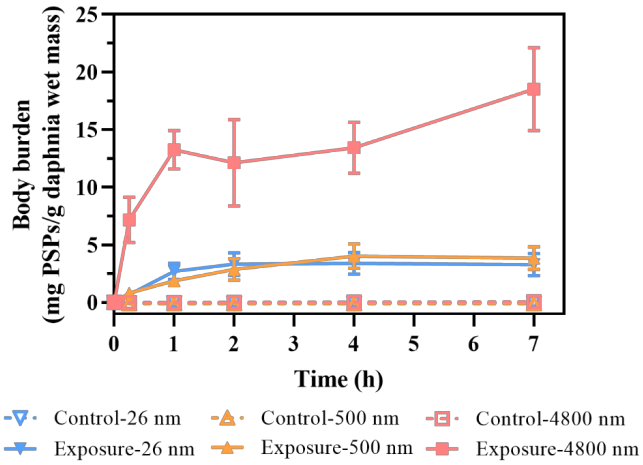

Figure 4.1. Accumulation of PSPs (26, 500 and 4800 nm) in *D. magna* neonates over 7 h exposure period

Table 4.1 Accumulation kinetic rate constants of PSPs in *D. magna* and *L. benedeni* [a] as determined on the basis of mass of particles accumulated in the organisms.

PSPs	k_w (L/g·h, *D. magna*)	k_e (1/h, *L. benedeni*)
26 nm	1.7 ± 0.4a	0.2 ± 0.8A
500 nm	0.7 ± 0.1b	0.1 ± 0.2A
4800 nm	1.7 ± 0.4a	0.03 ± 0.03A

[a] The different letters in a column indicate significant differences among different treatments at $p < 0.05$.

4.3.3 Fate of PSPs introduced by *D. magna* in simulated aquatic system

In the simulated aquatic system used for the food chain transfer, the fate of PSPs introduced by *D. magna* might be divided into three compartments including the predator (*L. benedeni*), the aqueous phase and the depositional phase (excretions of *L. benedeni*). The ratio of PSPs in each compartment (the mass concentration of PSPs in the predator-*L. benedeni*, the aqueous phase and the depositional phase, respectively) compared to the input (the mass concentration of PSPs accumulated in the prey-*D. magna*) as a function of time can be found in Fig 2. As shown in Figure 4.2, the ratios of the PSPs distributed in *L. benedeni* were only in the range of 1 to 5 % over the exposure and depuration periods, which implies that trophic transfer of PSPs was limited. Moreover, for 26 and 500 nm PSPs, the ratios of PSPs in the aqueous phase increased from 31 to 69 % and from 58 to 88 % over time, respectively. This means that 26 and 500 nm PSPs introduced by *D. magna* tended to distribute in the aqueous phase. In contrast, the

ratios of 4800 nm PSPs in the depositional phase reached 93 – 97 %, implying that 4800 nm PSPs introduced by *D. magna* fell sedimented into the depositional phase. It can be concluded that the particle size influenced the fate of PSPs introduced by the prey in the simulated aquatic system.

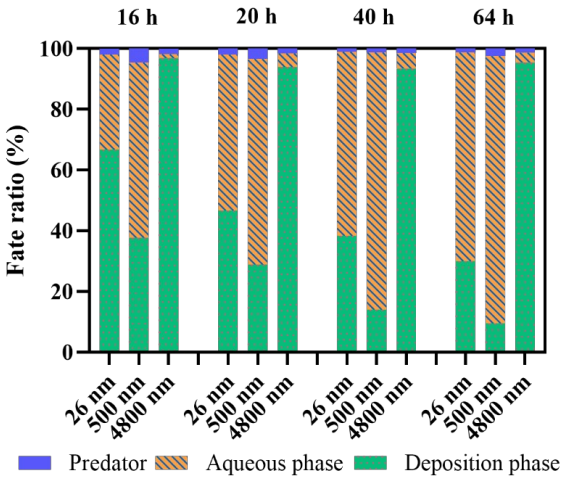

Figure 4.2 Fate ratio (%) of PSPs introduced from *D. magna* to *L. benedeni*, the aqueous phase and the depositional phase.

4.3.4 Trophic transfer of PSPs from *D. magna* to *L. benedeni*

The variation of the mass concentrations of PSPs with different sizes in the predator (*L. benedeni*) transferred from the prey (*D. magna*) over the feeding exposure and depuration periods is presented in Figure 4.3. Generally, the mass concentrations of all PSPs increased in *L. benedeni* after the feeding exposure of 16 h (Figure 4.3) and a significant

difference ($p < 0.05$) from the control group (Table S4.2) was observed, indicating transfer of PSPs from the prey to the predator. The variation in the average mass concentration between the exposure and control groups decreased in order of 4800 nm (4 μg PSPs/g mysid wet mass) > 26 nm (3 μg PSPs/g mysid wet mass) > 500 nm (2 μg PSPs/g mysid wet mass). This also means that the extent of trophic transfer of 4800 nm PSPs to *L. benedeni* was the highest among the studied PSPs based on the mass concentrations, which corresponded to the highest accumulation concentration of 4800 nm PSPs in *D. magna*. Moreover, the extent of trophic transfer of 26 nm PSPs was higher than for the 500 nm PSPs, although their extent of accumulation in *D. magna* was similar. During the 48 h depuration period (Figure 4.3), there was no significant difference in the mass concentration for the 26 nm PSP treatment between the exposure groups and control groups. Note that the average mass concentration of the 26 nm PSPs at 20 h was higher than in the control group. For the 500 nm PSP treatment, although no significant difference in the mass concentration was found between the exposure groups and control groups, the average mass concentrations of the exposure groups at the different time endpoints were slightly higher than the mass concentrations of the control groups. The mass concentration of 4800 nm PSPs in *L. benedeni* did not change significantly ($p > 0.05$) among the exposure groups with time, but was significantly higher than in the control group ($p < 0.05$). This implied that PSPs were retained in the predator. The difference in the average mass concentration between the exposure groups and control groups is supported by the appearance of a fluorescent signal in the PSP-exposed groups observed in the biodistribution (Figure 4.4). Generally, during the 48 h depuration period, the order of magnitude of the average mass concentration was 4800 nm > 500 nm > 26 nm, which

corresponds to the order of mass concentration of PSPs during the feeding exposure period. Furthermore, as shown in Table 4.1, the elimination rate derived from cutting off the background data was found to be relatively low for all treatments. No significant difference in elimination rate constants between PSPs of different sizes was found ($p > 0.05$). This suggests that the particle size of PSPs did not influence the kinetics of depuration process.

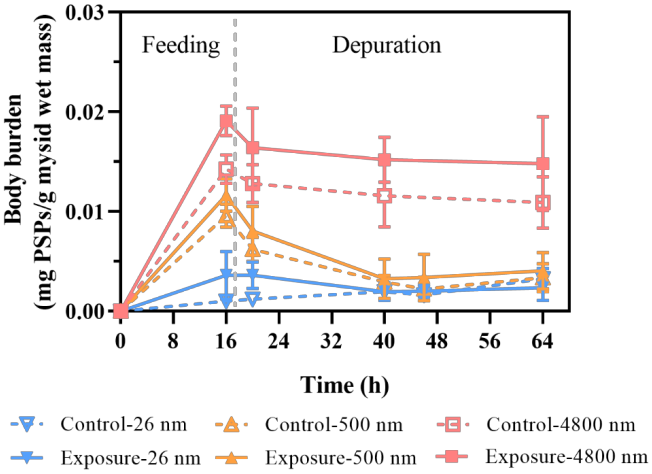

Figure 4.3 Concentration of PSPs (26, 500 and 4800 nm) in *L. benedeni* after a 16 h exposure period and a 48 h depuration period.

The biodistribution of PSPs (26, 500 and 4800 nm) in *L. benedeni* was observed over the 16 h feeding exposure period and 48 h depuration period, as shown in Figure S4.4 (Supplementary data). All controls remained non-fluorescent in the studied areas of *L. benedeni* tissues during all timepoints. After 16 and 20 h of exposure, a strong fluorescence was observed in the organisms exposed to the small sized PSPs (26 nm), while weak fluorescence was detected in the feeding

exposure treatments of 500 and 4800 nm PSPs. The strong intensity of fluorescence of the 26 nm PSPs was in correspondence to the high number concentration of particles in the mysids transferred from daphnids. This is also associated with the initial exposure to a greater particle number of 26 nm PSPs. It is worth mentioning that fluorescent particles were mainly visible in the digestive tract (stomach and intestine) over time, and no translocation of any of the studied PSPs into other tissues was observed in *L. benedeni*. This finding was similar to a previous study on brine shrimp *Artemia franciscana* larvae exposed to 40 and 50 nm PSPs, which were found to be considerably accumulated in the guts of *A. franciscana* (Brun et al., 2017).

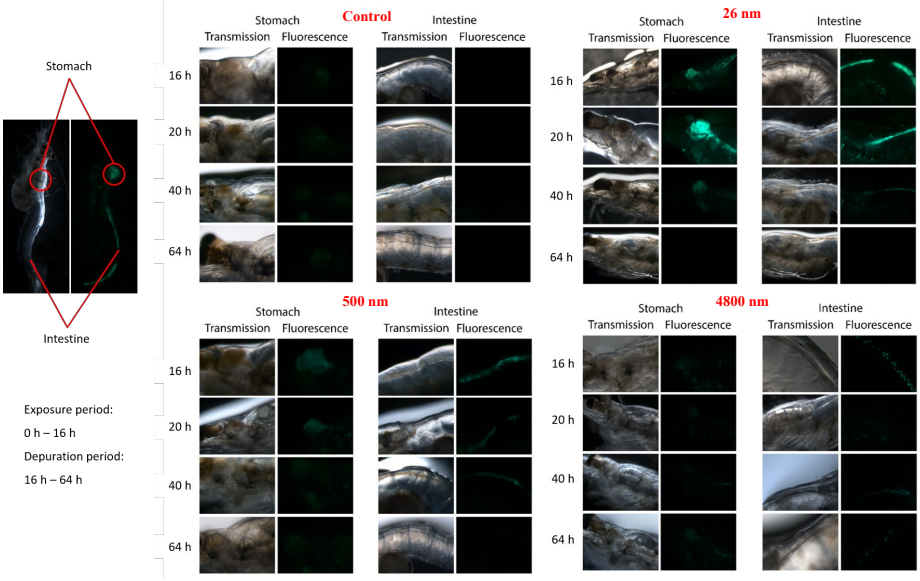

Figure 4.4 Representative images demonstrating the accumulation of PSPs (26, 500 and 4800 nm) in the stomach and intestine of *L. benedeni* over a 16 h feeding exposure period and a 48 h depuration period.

TTFs based on the mass concentration during the feeding and depuration periods were evaluated, as shown in Table 4.2. All TTF values were found to be less than 1, regardless of the size of the PSPs and the exposure time. Hence, the extent of transfer of PSPs from *D. magna* to *L. benedeni* was limited and not associated with their particle sizes. In other kinds of food chains, a low extent of NMPs transfer was also observed. For instance, a TTF value < 1 was determined for 6 μm PSPs as they were transferred from *D. magna* to *Pimephales promelas* (Elizalde-Velázquez et al., 2020a). The biomagnification of 6 μm PSPs from mussels to crabs did not occur as well (Wang et al., 2021).

Table 4.1 Trophic transfer factor (TTF) for the PSPs with sizes of 26, 500 and 4800 nm at the different time intervals [a].

Time (h)	PSPs		
	26 nm	500 nm	4800 nm
16	0.001 ± 0.001	0.000 ± 0.000	0.000 ± 0.000
20	0.001 ± 0.000	0.000 ± 0.001	0.000 ± 0.000
40	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000
64	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000

[a] Data were derived by comparing experimental values to the background auto-fluorescence values.

A challenge in using fluorescently labelled PSPs in uptake and elimination studies is the potential dissociation of dye from the particles (Schür et al., 2019) and Catarino et al. (2019), which can result in false-negative identification of particles in the examined medium, matrix or cell culture when using fluorometric methods for detection. If dye-release contributed to a large extent to the regarding uptake,

elimination and biodistribution, similar kinetic patterns would likely have been observed regardless of particle size (assuming that particle size is not determining the rate of dye-release). In the current experiment, large differences were observed in fluorescent signals in tissues from organisms exposed to different particle sizes, also when accounting for differences in exposure concentrations expressed as fluorescence intensity. In particular, comparisons of fluorescence intensities of PSPs between 26 nm and 4800 nm and between 500 nm and 4800 nm detected after 2 hours exposure to daphnia (uptake reaching to plateau) demonstrated a statistically significant difference ($p < 0.001$), as shown in Table S4.3. There is no a statistically significant difference in the fluorescence intensities of PSPs between 26 nm and 500 nm (Table S4.3), whereas the averaged fluorescence intensities of 26 nm PSPs were higher than the averaged fluorescence intensities of 500 nm PSPs. These evidences indicated that the PSP particles alone considerably contributed to the uptake process rather than the leaching dye, otherwise similar fluorescence intensities should be found between the PSPs with different particle size. Consequently, the labelled PSP particles can be determined in the organisms.

4.4 Conclusions

This work showed that particle size influenced the extent of accumulation and trophic transfer of PSPs from the prey (*D. magna*) to the predator (*L. benedeni*) in the simulated aquatic system. The increase of the mass concentration of PSPs with a large size (4800 nm) was higher than the increase of the mass of internalized PSPs with a small size (26 or 500 nm). Moreover, the uptake rate of 26 and 4800

nm PSPs in *D. magna* was significantly higher than the uptake rate of the 500 nm PSPs. It was estimated that a small fraction of each PSP introduced by *D. magna* was transferred to *L. benedeni*, whereas a large fraction of the PSPs was released in the aqueous phase (26 and 500 nm PSPs) and in the depositional phase (4800 nm PSPs). The extent of trophic transfer from *D. magna* to *L. benedeni* decreased in the order of 4800 nm > 26 nm > 500 nm PSPs. Moreover, there was no significant difference in elimination rate constants between PSPs of different sizes. Furthermore, fluorescence observations in *L. benedeni* revealed that PSPs accumulate mainly in the intestinal tract. In addition, the TTF values of all the studied PSPs were less than 1, implying that trophic transfer was limited in the simulated aquatic food chain. In summary, the study provides evidence that the role of particle size cannot be neglected in regulating the bioaccumulation and fate of PSPs in the aquatic environment.

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Supplementary Information

Table S4.1 Survival (%) of *D. magna* exposed to various concentrations of PSPs with different sizes of 26 nm, 500 nm and 4800 nm after 24 h of exposure [a]

Exposure concentrations (mg/L)	PSPs		
	26 nm	500 nm	4800 nm
0	100 ± 0%	100 ± 0%	100 ± 0%
0.5	100 ± 0%	100 ± 0%	100 ± 0%
1	100 ± 0%	96 ± 9%	100 ± 0%
2	100 ± 0%	100 ± 0%	96 ± 9%
4	100 ± 0%	96 ± 9%	92 ± 18%
8	96 ± 9%	100 ± 0%	100 ± 0%

[a] Values are expressed as mean ± standard deviation (n = 5). Each replicate contained five daphnids.

Table S4.2 Levels for significant (*p*) comparison between the concentrations of PSPs in *L. benedeni* between control groups [a] and exposure groups [b] ($p < 0.05$ indicates a significant difference)

Time (h)	26 nm	500 nm	4800 nm
16	0.00	0.03	0.00
20	0.00	0.06	0.01
40	0.95	0.71	0.00
46	0.12	0.14	--
64	0.07	0.42	0.02

[a] Treatments without exposure.

[b] Treatments fed up with daphnids exposed PSPs with different sizes of 26 nm, 500 nm and 4800 nm.

Table S4.3 Levels for significant (p) comparison among the fluorescence intensities (FI) of PSPs in *D. magna* after 2 hours exposure ($p < 0.05$ indicates a significant difference) [a]

(I) group	log *FI*	(J) group	*p*
4800 nm	4.209 ± 0.165	500 nm	0.000
		26 nm	0.000
500 nm	4.041 ± 0.133	4800 nm	0.000
		26 nm	0.777
26 nm	4.064 ± 0.121	4800 nm	0.000
		500 nm	0.777

[a] Fluorescence data were Log10 transformed to assure that the requirements for residual- and variance distributions were met.

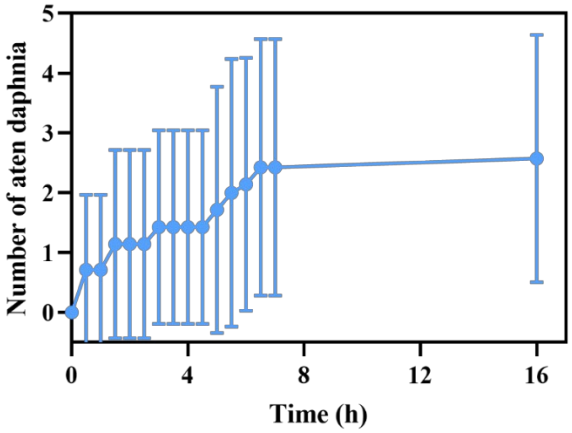

Figure S4.1 The number of the waterflea (*D. magna*) eaten over time by the mysid (*L. benedeni*).

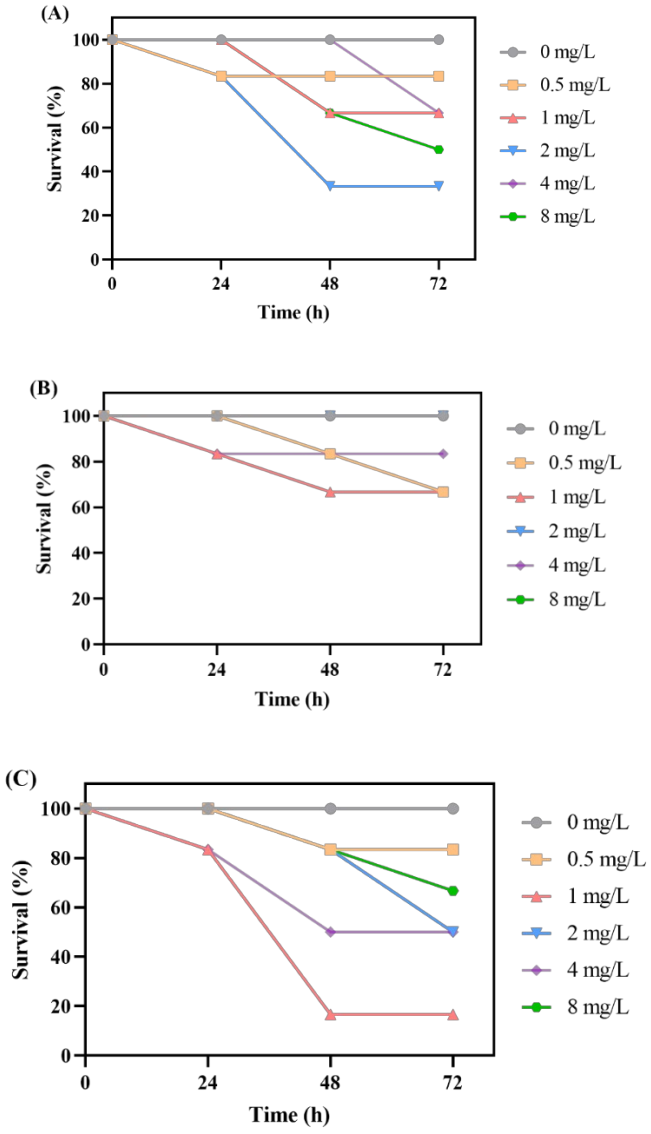

Figure S4.2 Survival (%) of the mysid (*L. benedeni*) exposed to PSPs with sizes of 26 nm (A), 500 nm (B) and 4800 nm (C). The exposure concentrations of PSPs were 0, 0.5, 1, 2, 4 and 8 mg/L. Each treatment contained ten shrimps.

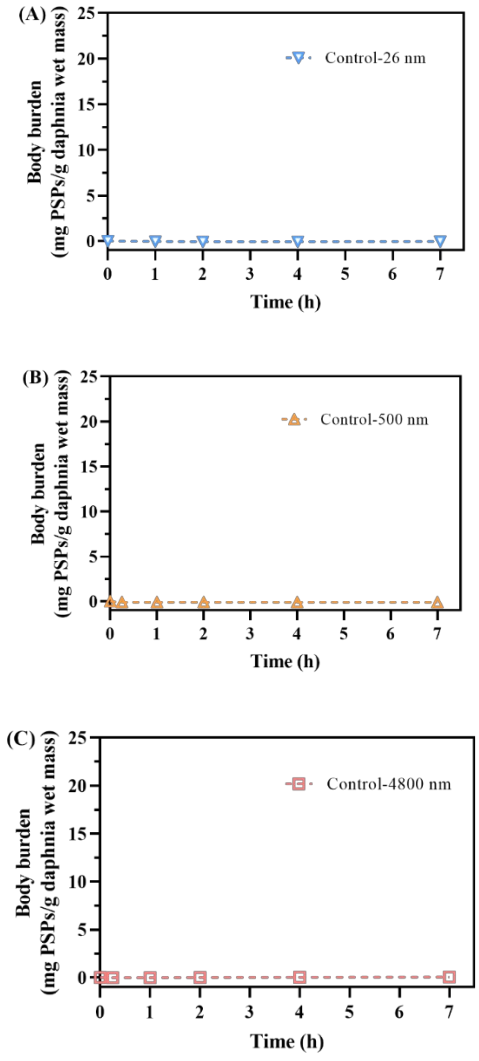

Figure S4.3 Control group data for the accumulation of PSPs (26, 500 and 4800 nm) in *D. magna* neonates over 7 h exposure period.

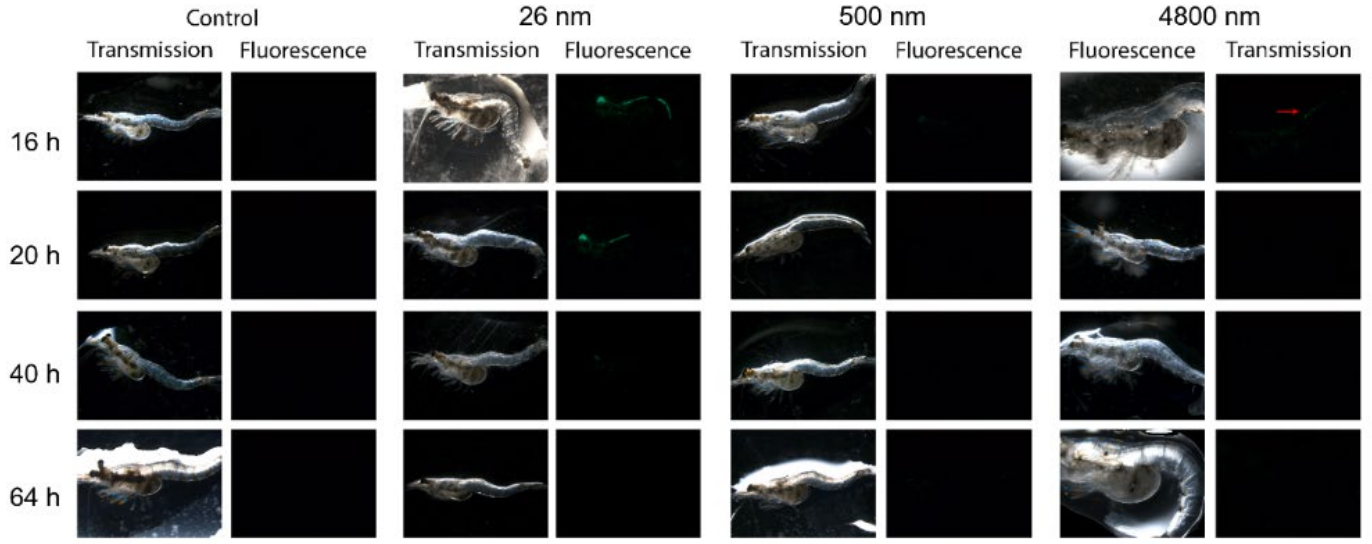

Figure S4.4 Representative images demonstrating the accumulation of PSPs (26, 500 and 4800 nm) in *L. benedeni* over a 16 h exposure period and a 48 h depuration period.