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Quantifying nanoplastic-bound chemicals accumulated in *Daphnia magna* with a passive dosing method†

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Because of their large surface area and high hydrophobicity, nanoplastics (NPs) possess a large sorption capacity for and strong affinity to hydrophobic organic chemicals, which may substantially alter the environmental behavior, especially the bioaccumulation of hydrophobic organic compounds. The present study aims to examine the effects of nano-size polystyrene (PS) on the bioaccumulation of polychlorinated biphenyls (PCBs) in *Daphnia magna*. A novel passive dosing method was developed to quantify the accumulation of NPs in *D. magna* in different exposure suspensions with various NP concentrations during 2 h and 24 h exposure. Addition of NPs enhanced the amount of PCBs accumulated in *D. magna* by 1.4–2.6 times compared to no presence of NPs during 24 h exposure. The relative amounts of NP-bound PCBs accumulated in *D. magna* were 18% to 81% for different PCB congeners at various NP concentrations. The significance of different uptake pathways in the accumulation of PCBs in *D. magna* with the presence of NPs was found to relate to the chemical properties of PCBs, the ingestion rate of plastics and the exposure conditions. These results have justified additional research efforts toward investigating the fate and toxic effects of NP-bound contaminants ingested by organisms.

Environmental significance

Nanoplastics (NPs) can interact strongly with organic pollutants and induce adverse effects on aquatic organisms. However, a few studies have examined the contributions of NP-bound chemicals to the total accumulated amounts in biota. This article presents results from examining the effects of nano-sized polystyrene on the bioaccumulation of polychlorinated biphenyls in *Daphnia magna*. A passive dosing method was developed to quantify the accumulation of NP-bound chemicals, and clarify the contributions of different uptake pathways. These results shed light on how nano-sized plastics influence the bioaccumulation of hydrophobic organic chemicals in aquatic organisms and would be beneficial for researchers worldwide who have been, are and will be involved in the investigation of the fate and toxic effects of plastic-bound organic contaminants ingested by organisms.

Introduction

Plastic materials are used in a wide range of applications such as packaging, building and construction due to their unique properties. The annual global production of plastics increased from 1.7 million tons in 1954 to 322 million tons in 2015.[1] Most disposed plastics are not recycled properly[2] and may end up in the marine environment through different pathways such as rainfall, rivers, drainage and wind. Some of the debris is difficult to degrade and hence persists in the environment for a long time, but can be fragmented gradually to micro-particles due to natural weathering. Primary micro-size plastics also come from consumer products such as facial cleaners, which generally pass through sewage systems and are released directly into lakes and oceans. These micro-size plastics may continue to break down,[3–6] and those with at least one dimension less than 1 μm are generally defined as nanoplastics (NPs).[7,8]

Plastics have been reported to cause biological toxicity, and their amounts in an exposure system were found to significantly correlate with the responses to adverse effects, such as signs of stress, mortality, feeding patterns and bodyweights, in biota.[9–11] Recent studies also reported that the toxicity of plastic particles increased with decreasing size,

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† Electronic supplementary information (ESI) available: Four supplemental figures showing: the SEM image of 100 nm PS; the schematic of the passive dosing vial and exposure; the accumulation of PCBs on the fiber after 24 h exposure; and the accumulation of individual PCBs in solution with different concentrations of NPs after 2 h exposure. One supplemental table showing the ingested amount of NPs under different conditions. See DOI: 10.1039/c7en00932a

and nano-sized plastics showed higher toxicity to aquatic orgnisms.[12–14] Because of their large surface area and high hydrophobicity,[15,16] NPs also possess a large sorption capacity for and strong affinity to hydrophobic organic chemicals (HOCs). These attributes may substantially influence the environmental behavior of HOCs affiliated with NPs, *e.g.*, bioaccumulation and toxicity.[17,18] The presence of plastics may enhance or reduce chemical uptake by biota.[10,11,19–21] Such enhancement can be attributed to the uptake of micro-[22–24] and nano-sized[25] plastics by aquatic organisms. However, the mechanisms for accumulation of plastic-bound HOCs in organisms through ingestion remained poorly understood. A few controlled experiments have quantified the relative contribution amounts of plastic-bound and freely dissolved HOCs accumulated in organisms, in a matrix containing NPs.

One main reason may be the limitation of traditional exposure methods. Freshly spiked[19] or field-contaminated plastics[10] have been used to prepare exposure suspensions, which may lead to chemical desorption from plastics to water and then dermal uptake by biota. This would pose challenges for distinguishing bioaccumulation from ingestion or dermal absorption. Zero aqueous phase concentration needs to be demonstrated in this exposure mode.[26] In other studies,[11,20] chemicals at constant concentrations were spiked in exposure suspensions with different amounts of plastics. In this exposure mode, the amount of dermal absorption was difficult to be distinguished from that of ingested plastic particles by only measuring the total bioaccumulation amount in organisms. To confirm the hypothesis that plastics act as a carrier of toxic chemicals, plastics accumulated by organisms should be accurately measured, which however has not been done in some studies, especially for NPs as it is extremely difficult to quantify NPs in organisms.[27]

The present study was conducted to address the abovementioned issue, using a passive dosing vial developed by Mayer *et al.*[28] Selected polychlorinated biphenyls (PCBs), *i.e.*, 2-chlorobiphenyl, 4-chlorobiphenyl, 2,5-dichlorobiphenyl, 3,3′-dichlorobiphenyl and 3,3′,4,4′-tetrachlorobiphenyl (PCB **1**, **3**, **9**, **11** and 77, respectively), were selected as the typical HOCs, nano-sized polystyrene (PS) particles as the representative NPs and *Daphnia magna* as the model organism. The passive dosing vial is able to generate a series of exposure suspensions with constant concentrations of freely dissolved PCBs and different amounts of NPs, beneficial for examining the influences of NPs on the uptake of PCBs in *D. magna*. The objectives of the present study were to (1) develop a passive dosing method to quantify the relative contributions of NP-bound PCBs to the total amount of PCBs accumulated in *D. magna* and (2) clarify the roles of different pathways in the accumulation of PCBs in *D. magna* with the presence of NPs.

Materials and methods

Chemicals and materials

Stock suspensions of nano-sized PS particles were supplied as 2.5% w/v nano-PS dispersed in water : ethanol (1 : 1 in volume) by Aladdin (Shanghai, China). The uniform PS microsphere with a diameter of approximately 100 nm was confirmed by scanning electron microscopy, while the PS composition was confirmed by the FTIR spectra (ESI† Fig. S1). The hydrodynamic diameters of NPs in exposure suspensions were characterized with a particle size analyzer (Brookhaven Instruments Corporation, Holtsville, NY, USA). There was no apparent difference between PS particles incubated for 5 days and spiked with PCBs, and also exposure with *Daphnia* for 24 h (ESI† Table S1). Besides, to confirm if the amounts of interfering materials leached from the PS phase were negligible, 100 μL of the stock suspension was extracted with 1 mL of hexane containing 100 μg L^{-1} deuterated PCB 77 as the surrogate standard. After 1 h of sonication, 2 μL of the upper extractant was analyzed with a gas chromatograph/mass spectrometer in the full-scan mode with m/z in the range of 50–300. The chromatogram shows no PCBs leaching from the PS, and no significant amount of unknown compounds present in the PS stock suspension.

Five solid PCB congeners, *i.e.*, PCB **1**, **3**, **9**, **11** and 77, were obtained from J&K Scientific (Beijing, China). A stock suspension of the PCB mixture at 1000 mg L^{-1} for each congener was prepared by dissolving the solid standards in dichloromethane, and stored at −18 °C. A SYLGARD184 silicone elastomer kit purchased from Dow Corning (Shanghai, China) was used to prepare the passive dosing coating. A home-made polydimethylsiloxane (PDMS) fiber with a length of 0.5 cm, a thickness of 44 μm and a volume of 0.18 μL was used for monitoring the stability of the prepared passive dosing vials.

Preparation of the passive dosing vial and its working principle

A schematic of this device is shown in ESI† Fig. S2, and detailed preparation procedures can be found in the literature.[29] Briefly, a thin layer of PDMS coating (1.00 g) was fixed at the bottom of a 20 mL vial and used for storing the target compounds. The target PCBs were loaded onto the PDMS phase by adding 1 mL of a 25 mg L^{-1} PCB standard solution to the dosing vial and agitated at 250 rpm for 22 h. PCB molecules could accumulate onto the PDMS coating due to chemical potential difference. Then 1, 2, 3, 4 and 5 mL of ultra-pure water were separately added to the vial each time after 22 h of agitation to further decrease the chemical potential in the loading solution and enhance the accumulation factor on the PDMS coating. The loaded solution was discarded and the vials were cleaned with ultra-pure water three times.

Five exposure suspensions with nano-PS concentrations of 0, 2, 5, 10 and 20 mg L^{-1} were prepared, and 10 ml of each suspension was added to each dosing vial and mixed in a rotating agitator for 5 days. Preliminary result indicated that 5 days was sufficient to obtain a stable concentration of PCBs in the exposure suspension (Fig. S3†). In the presence of chemical potential, PCBs will migrate from the PDMS coating

to the exposure suspension and are sorbed by nano-PS (if present) until the chemical potential reaches equilibrium among all interacting phases. Since the solubility of PCB congeners in water is low and the amount of nano-plastics in each exposure suspension was relatively small, the amounts of PCBs decreased in the PDMS coating were insignificant compared to the original loaded amounts and the free concentrations in different exposure suspensions were similar in each vial. The stability of freely dissolved concentrations of PCBs in each exposure suspension was monitored by the PDMS fiber, which only senses the freely dissolved concentrations of analytes. The freely dissolved concentrations of PCBs in different vials were essentially constant (ESI† Fig. S4).

Bioaccumulation of PCBs in *D. magna*

The *D. magna* was obtained from the School of Life Sciences, Sun Yat-sen University, cultured in artificial growth medium (M7 medium) recommended by OECD standards 202 and 211 and fed three times a week with *Scenedesmus obliquus*. The culture and exposure settings for *D. magna* were maintained at 21 ± 1 °C under a photo period of light : dark at 16 : 8 h. Daphnids originating from a single clone with an age of around 10 d were selected for the bioaccumulation exposure experiments.

In accumulation experiments, the daphnids were exposed to suspensions with nano-PS concentrations of 0, 2, 5, 10 and 20 mg L^{-1} for 2 and 24 h. A control group was a culture solution without PCBs and nano-PS particles. Triplicates were conducted at each sampling point with 18 daphnids in each exposure suspension. After exposure, PCBs in daphnids, exposure suspensions and PDMS fibers were measured.

For *D. magna* analysis, 15 of them were transferred from the suspension to a piece of filter paper, rinsed with 100 μL ultra-pure water, dried with another piece of filter paper and transferred to a 2 mL centrifuge tube. Two hundred fifty microliters of 2 mol L^{-1} KOH solution and 500 μL of hexane containing 100 μg L^{-1} deuterated PCB-77 as the internal standard were added to the centrifuge tube and sonicated for 30 min. The upper solution was transferred to a 200 μL inert tube before GC/MS analysis.

For analysis of the exposure suspension, 5 mL of exposure suspension was transferred to a 10 mL vial, which was spiked with 1 mL of hexane containing 100 μg L^{-1} deuterated PCB-77. The vial was sonicated for 30 min and agitated in a rotating agitator at 250 rmp for 24 h. The upper solution layer was removed and transferred to a 2 mL vial for GC/MS analysis.

The PDMS fiber was directly removed from the exposure suspension with a tweezer and rinsed with ultra-pure water for 30 s. The fiber was dried with a piece of Kimwipe and placed in a 200 μL inert tube containing 150 μL of hexane with the internal standard. The inert tube was fixed in a 2 mL vial and agitated for 24 h at 250 rmp before the extraction solvent was analyzed.

Statistical analysis and quality control

The data were processed with both Microsoft Excel and GraphPad Prism (GraphPad Software, La Jolla, CA). Student's *t*-test and one-way ANOVA test were used to compare experimental data, with significant difference attributed where $P \leq 0.05$. The surrogate standard was added into the extracted solution to monitor the extraction recovery. The average recoveries were 101% ± 8%, 90% ± 9% and 96% ± 10% for 2 h exposure, and 101% ± 9%, 102% ± 15% and 100% ± 10% for 24 h exposure in extraction of *D. magna*, exposure suspensions and fibers, respectively. During the instrumental analysis, a standard was injected after the analysis of every 9 samples for calibration of standard curves.

Results and discussion

Uptake amount of PCBs

Polychlorinated biphenyls were not detected in control groups, suggesting that PCBs detected in *Daphnia* were all accumulated from the exposure suspensions. The amounts of PCB congeners accumulated in daphnids varied in exposure suspensions with different concentrations of nano-PS at different sampling time points. The accumulated amounts of individual PCB congeners increased with increasing NP concentration during 24 h exposure for all NP concentrations except for 20 mg L^{-1} (Fig. 1). The presence of NPs enhanced the total uptake amount of PCBs by 1.4–2.6 times compared to the one without NPs during 24 h exposure. Similar enhancement on the accumulation of individual PCB congeners in *Daphnia* was observed for 2 h exposure (ESI† Fig. S5). The accumulated amount of total PCBs was enhanced by 1.9–4.6 times when the NP concentration increased from 2 to 20 mg L^{-1}. As illustrated in the experimental section, the exposure suspensions with different concentrations of NPs maintain a constant concentration of freely dissolved analytes. Therefore, the enhancement effect observed in the suspension with NPs can be accounted as the contribution of NP-bound PCBs.

Contribution of plastic-bound compounds

The plastic-bound compounds can be accumulated on *D. magna* by ingestion or penetration through dermal adsorption. Quantification of the accumulated NPs on *D. magna* was the first step to investigate the contribution of bound compounds, which, however, was challenging especially for NPs

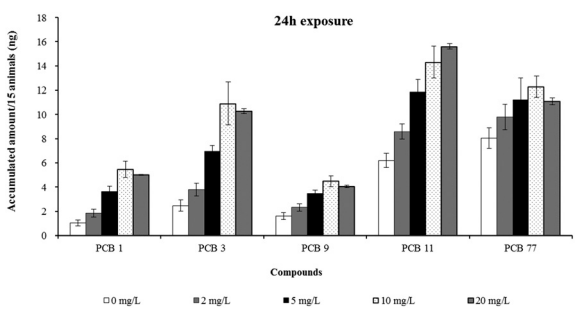

Fig. 1 Accumulation of individual PCBs in daphnids with different concentrations of NPs after 24 h exposure.

due to the lack of robust detection techniques.[27] In the current study, the passive dosing vial provided an opportunity to indirectly quantify the accumulated amount of plastic-bound PCBs. As demonstrated (ESI† Fig. S3), the freely dissolved concentration of PCBs was constant in the suspension with and without NPs ($C_{sol,0}$), while the concentration of PCBs bound to the NPs is equal to the total concentration of PCBs ($C_{sol,i}$) minus $C_{sol,0}$, and further divided by the concentration of NPs in the suspension (C_i). Assuming that the metabolism of PCBs in *D. magna* is negligible or similar under different exposure conditions, the uptake amount of NPs can be calculated by

$$\frac{C_{sol,i} - C_{sol,o}}{C_i} = \frac{n_{dap,i} - n_{dap,o}}{m_{NP}} \quad (1)$$

where C_i is the concentration of NPs in a specific exposure suspension; $C_{sol,i}$ and $C_{sol,0}$ are the total concentrations of PCBs in exposure suspensions with and without NPs, respectively. The left side of the equation is equal to the concentration of chemical accumulated on NPs in the exposure suspension. $n_{dap,i}$ and $n_{dap,o}$ are the total amounts of PCBs accumulated in *Daphnia* from exposure suspensions with and without NPs, respectively, and m_{NP} is the uptake amount of NPs in *D. magna*. Since the free concentration of PCB congeners in each exposure vial was constant, $n_{dap,i} - n_{dap,o}$ can be accounted for the uptake of NP-bound PCBs. Therefore, the right side of the equation is equal to the concentration of chemical accumulated on uptake NPs. The ingestion amount m_{NP} can be quantified using eqn (1). The underlying principle of the current developed method is similar to that of fluorescent labeling which quantifies plastics based on fluorescence intensity. The method developed in the present study labels NPs with chemicals, and quantifies the amount of NPs by measuring the amount of chemicals. Here, the desorption of PCBs from the NP when it passed through the *Daphnia* gut was assumed to be negligible. In the case where the chemicals can be totally desorbed from the plastics and adsorbed by the *D. magna* within the exposure time, the quantified amount of NPs didn't represent the ones accumulated in *D. magna* but the ones that passed through the gut.

The amount of nano-PS (m_{NP}) accumulated in *D. magna* at different exposure times was calculated with eqn (1) for individual PCB congeners (ESI† Table S2). However, only a specific amount of NPs was accumulated in each organism within a fixed exposure period. Therefore, the concentrations of five PCB congeners were used to calculate the average amount of NPs accumulated in *D. magna* in each exposure suspension; the relative standard deviation (RSD) ranged from 11% to 35%. The accumulated amount of NPs in each *D. magna* was 86 ng for 24 h exposure in a NP suspension with a concentration of 2 mg L^{-1} (Fig. 2). This result was in the same order of magnitude of the data published by Rist[30] who reported 23 ng for each *D. magna* during 24 h exposure in a 100 nm PS suspension at 1 mg L^{-1}, demonstrating the accuracy of the method developed in the present study.

The average uptake amount of NPs increased from 75 to 259 ng per animal as the nano-PS concentration increased from 2 to 20 mg L^{-1} during 2 h exposure, indicating a linear relationship between the amounts of NPs accumulated in *D. magna* and the concentrations of NPs in the exposure suspensions. However, only a slight increase of the NP amount in *D. magna* was observed during 24 h exposure with increasing NP concentration, particularly for 20 mg L^{-1} (Fig. 2). A possible reason was the toxic effect of NPs on *D. magna* when the NP concentration was high, resulting in less ingestion of NPs after 24 h exposure. In addition, the amounts of NPs accumulated in *D. magna* exposed in the 2, 5 and 10 mg L^{-1} suspensions were not significantly different between the 2 h and 24 h exposures, suggesting that the accumulation of NPs in *D. magna* reached the steady state within 2 h.

Last but not least, it is worth noting that the accumulation amount of NPs quantified by the developed method cannot distinguish the ingested NP and the one that may accumulate by dermal absorption. The uptake of plastic in *D. magna* was confirmed using the microscope. As shown (Fig. S6†), the gut of the *D. magna* was filled with plastic compared to the control group. In addition, we also observed the presence of blue plastics in the other parts of the *D. magna* body such as the antenna and abdomen, and their uptake pathway should be further clarified. Furthermore, the biomolecules secreted from the *Daphnia magna* may absorb PCBs and increase the concentration of PCBs in the exposure solution ($C_{sol,i}$). However, since the affinity of PCBs to *D. magna* secretion was much lower than that to the NP particles, and the amount of biomolecules secreted from 18 daphnids over 24 h was limited, and the effect should be negligible in the present study.

Contributions from various accumulation pathways

The amounts of PCBs accumulated in *D. magna* can be contributed by both the freely dissolved and plastic-bound forms. The freely dissolved form was mainly attributed to dermal absorption, while the plastic-bound one was mainly accumulated through ingestion. The results presented in the preceding section already demonstrated that the contribution of NP-bound PCBs was similar at two exposure times in the

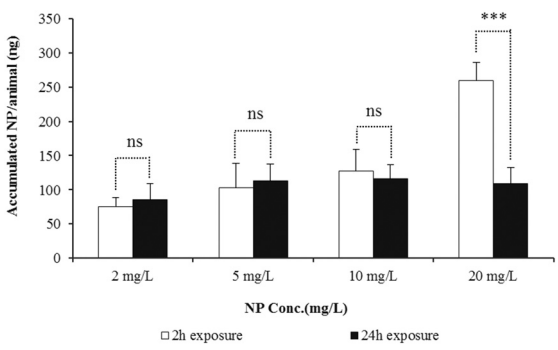

Fig. 2 Average ingestion amount of NPs in *D. magna* at different exposure times.

suspensions with NP concentrations of 2, 5 and 10 mg L^{-1}. Hence the dermal absorption amounts at 2 h and 24 h for different target compounds in these exposure suspensions can be compared. Fig. 3 displays the sum of the accumulated amounts of each PCB congener in daphnids exposed to PS concentrations of 0, 2, 5 and 10 mg L^{-1}. The dermal absorption amounts of low chlorinated PCBs such as PCB **1** and PCB **3** were not significantly different between 2 h and 24 h exposures, *i.e.*, the dermal absorption reached equilibrium within 2 h. On the other hand, the dermal absorption amounts of PCB **9**, PCB **11** and PCB 77 were significantly different between the 2 h and 24 h exposures, and the difference increased with increasing number of chlorine (Fig. 3). This result is consistent with the commonly accepted perception that chemicals with a higher K_{ow} required a longer time to reach equilibrium.

A bioaccumulation model[21] that considered the bioaccumulation of PCBs from an environment containing plastics as the mass balance of uptake and loss processes was used to quantify the ratio of dermal absorption and ingestion for a chemical:

$$\frac{dC_{biota}}{dt} = k_{dermal}C_{free} + UR_t C_{PL} a_{PL} - k_{loss}C_{Biota,t} \quad (2)$$

where the first term in the right side of eqn (2) quantifies the dermal uptake from water by passive partitioning; the second term quantifies the uptake through exchange with plastic particles; the third term quantifies the overall loss due to elimination and egestion, which is negligible for PCBs within a short exposure period; C_{free} is the concentration of a freely dissolved target analyte and k_{dermal} is the first-order rate constant for dermal uptake. In the second term, UR_t represents the particle mass uptake rate and a_{PL} is the absorption efficiency from plastics.

As shown in eqn (2), the total uptake amount of PCBs in *D. magna* was correlated with the physicochemical properties of the target compounds and NPs, as well as the environmental conditions. k_{dermal} correlates with the hydrophobicity of the target chemical, temperature and pH of the system,

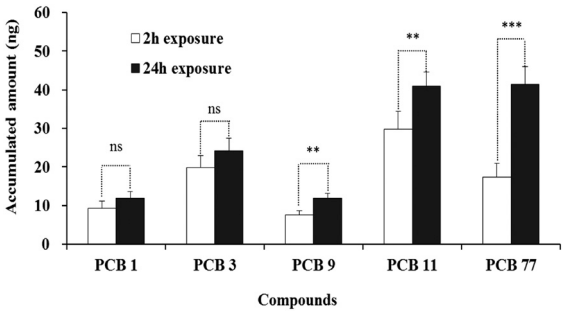

Fig. 3 Sum of the bioaccumulation of PCB congeners in daphnids after 2 h and 24 h exposures in NP suspensions with concentrations of 0, 2, 5 and 10 mg L^{-1}. ** $P < 0.01$, *** $P < 0.001$. ns: no significant difference.

Table 1 Contributions of NP-bound PCBs to the total uptake amount in daphnids at various concentrations of NPs

Compounds	2 mg L^{-1}	5 mg L^{-1}	10 mg L^{-1}	20 mg L^{-1}
PCB **1**	45%	72%	81%	80%
PCB **3**	35%	65%	77%	76%
PCB **9**	31%	54%	64%	61%
PCB **11**	28%	48%	57%	60%
PCB 77	18%	28%	35%	28%

whereas a_{PL} relates to the sorption capacity of NPs to the chemical and $UR_t C_{PL}$ is determined by the uptake amount of NPs. By comparing the total accumulation of PCBs in the exposure suspensions with and without NPs at a specific exposure time, the contributions of freely dissolved compounds and plastic-bound compounds can be distinguished. For example, the total amounts of PCB **1** (1.84 ng) and PCB 77 (9.80 ng) accumulated in *D. magna* after 24 h exposure at 2 mg L^{-1} of NPs were greater than those (1.01 and 8.03 ng) in the control group. The enhanced contribution was 45% for PCB **1** and 18% for PCB 77, which can be attributed to the second term of eqn (2). Because PCB 77 may not reach dermal absorption equilibrium after 24 h exposure, the enhanced contribution of plastic-bound compounds should be lower than 18%. In this scenario, the contribution from uptake of plastic-bound PCBs could be negligible and dermal absorption of freely dissolved compounds was dominant. The enhanced contributions of NP-bound PCBs to the total uptake amounts in *D. magna* at various NP concentrations were in the range of 18–81% (Table 1), which decreased with increasing hydrophobicity of PCBs and decreasing NP concentration. Moreover, the contribution of ingested NPs to the total amounts of PCBs accumulated in *D. magna* could be negligible as reported in the literature if the concentration of NPs was set approximately equal to the actual environmental levels, which is currently ~2 ng L^{-1} by "whole ocean" as estimated by Koelmans.[17]

Conclusions and future perspectives

The significance of NPs as a carrier for bioaccumulation of organic pollutants has been hotly debated.[17] Existing evidence has shown that plastics can either enhance or reduce bioaccumulation of hydrophobic organic chemicals, depending on the experimental conditions but without further explainations.[18] The present study developed a passive dosing method to quantify the uptake amount of NPs and distinguished the contributions of freely dissolved compounds and plastic-bound compounds. The results demonstrated that the relative contributions of plastic-bound compounds were enhanced as the concentration of NPs increased and decreased as the hydrophobicity of the compound increased.

The obtained results provide important references for the explanation of the literature reported results and future investigations of the effects of NPs on bioaccumulation of hydrophobic organic chemicals in aquatic organisms. On the

one hand, the fact that NPs can sorb large amounts of chemicals raises concerns about the potential adverse effects of NP-bound contaminants on aquatic organisms. Ingested NP-bound contaminants can be transported across the food web, posing increased risk to the aquatic ecosystem and eventually humans. On the other hand, because the environmental concentrations or sorption capacity of plastic debris are much lower than those of most other natural media such as dissolved organic matter, black carbon and suspended particulates, the contributions of NP-bound chemicals to the amounts of bioaccumulation are considered to be negligible.[17] One key step to better understand the role of NP-bound chemicals is to quantify the uptake dynamics of NPs in organisms. The present results have shed light on how nano-sized plastics influence the bioaccumulation process of HOCs in aquatic organisms, represented by *Daphnia*. To move forward, future efforts should be directed toward investigating the combined toxic effect of NPs and organic contaminants on aquatic organisms, as well as the fate and trophic level transfer of NP-bound contaminants after ingestion. Also, illustration of the uptake mechanism of NP-bound compounds in biota was the priority research topic. Finally, the environmental conditions for *D. magna* exposure such as food addition and opportunity for gut cleaning should also be investigated in future investigations.

Conflicts of interest

There are no conflicts to declare.

Acknowledgements

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