



## Quantification of the combined toxic effect of polychlorinated biphenyls and nano-sized polystyrene on *Daphnia magna*

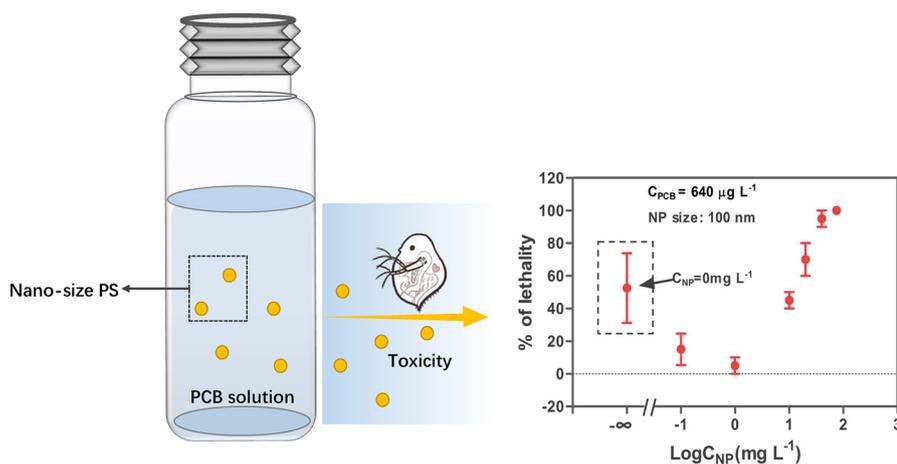
Wei Lin<sup>a</sup>, Ruifen Jiang<sup>b,\*</sup>, Yaxin Xiong<sup>a</sup>, Jiayi Wu<sup>a</sup>, Jianqiao Xu<sup>a</sup>, Juan Zheng<sup>a</sup>, Fang Zhu<sup>a</sup>, Gangfeng Ouyang<sup>a,\*</sup>

<sup>a</sup> MOE Key Laboratory of Aquatic Product Safety/KLGHEI of Environment and Energy Chemistry, School of Chemistry, Sun Yat-sen University, Guangzhou 510275, China

<sup>b</sup> Guangzhou Key Laboratory of Environmental Exposure and Health, and Guangdong Key Laboratory of Environmental Pollution and Health, School of Environment, Jinan University, Guangzhou 510632, China



### GRAPHICAL ABSTRACT



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### ABSTRACT

It has been reported that nanoplastics (NP) could cause serious toxicity and accumulative effects on aquatic organisms as well as interact with organic pollutants and influence potential hazards when exposed to biota. The current study aimed to quantitatively investigate the combined acute toxic effect of polychlorinated biphenyls (PCBs) and nano-sized polystyrene (PS) plastic on aquatic organisms based on analyte speciation. First, the combined acute toxicity of PCB-18 and 100 nm PS to *Daphnia magna* (*D. magna*) in water was evaluated. Then, speciation analysis of the exposure system was conducted by measuring the sorption coefficients ( $\log K_{NP}$ ) of PCBs to nano-sized PS (ranging from 5.28 to 6.56), which demonstrated the PS could substantially decrease the free concentrations of PCBs. The results showed that a low concentration of the PS could decrease the toxicity to *D. magna*, which might be originated from the decreased free concentration of PCB-18. However, when the PS concentration was high enough, an opposite effect was observed because the PS dominated the lethality instead of PCB-18. The current study is helpful to clarify the PCB occurrence in ecosystems and provide an in-depth understanding of the eco-toxicological effects of nanoscale plastics.

\* Corresponding authors.

E-mail addresses: [jiangrf5@jnu.edu.cn](mailto:jiangrf5@jnu.edu.cn) (R. Jiang), [cesoygf@mail.sysu.edu.cn](mailto:cesoygf@mail.sysu.edu.cn) (G. Ouyang).

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## 1. Introduction

With the large volumes of plastics produced in recent years, much plastic debris of a multitude of shapes and sizes has accumulated in the environment [1–7]. These plastics can break down into smaller sizes under microbiological activities [8] and natural weather conditions such as ultraviolet light [9,10], wind and rainfall [11]. Nanoplastics (NP), with at least one dimension less than 1  $\mu\text{m}$  [12,13], were claimed to be much more harmful because they can spread widely with water and air currents or be ingested by organisms causing potential toxicity to biota [14–21]. Some reports have claimed that the NP themselves could cause toxicity to aquatic organisms [11,15,18,22–27]. Ma et al. reported a relatively low LC<sub>50</sub> value ( $15 \pm 3 \text{ mg L}^{-1}$ ) for 50 nm polystyrene (PS) to *D. magna* after 48 h exposure [28]. Besseling et al. also claimed that 70 nm PS with  $32 \text{ mg L}^{-1}$  could cause malformation of *D. magna* after two generations (21 days exposure) [29]. Besides these, due to the large surface area, NP have the potential for adsorbing large amounts of organic pollutants, especially hydrophobic organic chemicals (HOCs) [30–32]. The presence of NP can decrease the free-dissolved concentration of HOCs and the toxicity of HOCs to the biota, and affect the environmental distribution, fate and ecotoxicity of these chemicals [33–35]. Therefore, the combined toxic effect should be investigated in the complex matrix that contains both NP and HOCs.

Several studies have examined the influence of plastic addition on the toxicity of chemicals to different types of aquatic organisms. Significant correlation between the addition of plastic and the toxicity endpoints including chemical uptake amount [33,36–39], larval development [40–42], biomarker responses [43–45] and mortality [46] were observed. For example, Silva et al. [42] studied the embryo development of the brown mussel and found that the toxicity of the leachate from microplastic-contaminated pellets was higher than that from the virgin ones. Another study [44] compared several biomarker responses such as the degree of tissue change (DTC) in the liver, the transcription levels of forkhead box L2 (*foxL2*) and tryptophan hydroxylase 2 (*tph2*) in the brain of African catfish (*Clarias gariepinus*) and found that the addition of the microplastic significantly caused toxicity and modulated the negative impacts of phenanthrene to the organism. However, among all the previous studies, few attempted to quantify the species of chemicals in the exposure system and correlated the toxicity endpoints with the concentrations of different species, which were the most essential facets of the toxic effect.

One of the key parameters of HOC speciation in an aqueous solution containing a complex matrix was the sorption coefficient. To date, although several studies have been performed to determine the sorption coefficients of polychlorinated biphenyls (PCBs) and polycyclic aromatic hydrocarbons (PAHs) to millimeter-sized polymer particles and aged particles in seawater [47,48], limited data, especially for NP, can be obtained in the literature. The data may be limited because of the difficulty of phase separation for NP. Traditional methods have been applied to the determination of the sorption coefficients of chemicals

and particulates usually require phase separation [49–51], which is not easy to achieve for nano-sized plastic particles. Among the emerging methods without phase separation, passive dosing introduced by Mayer et al. [52] has been successfully applied to determining the sorption coefficients of HOCs between dissolved organic matter and water [53]. The results showed that the passive dosing method could provide precise binding and speciation measurements without phase separation, as it allows the concentration of freely dissolved chemicals to be controlled at a pre-determined level during the process.

The present study aimed to quantitatively interpret the combined toxicity of nano-sized PS (100 nm) and PCB to the *D. magna* based on chemicals speciation. A passive dosing method was applied to determine the sorption coefficients of 8 chemicals between water and the 100 nm PS particle. The speciation results were correlated with the observed toxic endpoints and well clarified the combined toxicity of PCB-18 and nano-sized PS to *D. magna*.

## 2. Materials and methods

### 2.1. Materials

Eight solid chemicals, PCB-1, 3, 9, 11, 18, 77, pentachlorobenzene (Penta-CB), hexachlorobenzene (Hexa-CB) were purchased from J&K Scientific Ltd. (Shanghai, China), while nano-sized PS with dimensions of 100 nm were purchased from Aladdin (Shanghai, China). A SYLGARD 184 silicone elastomer kit purchased from Dow Corning (Shanghai, China) was used to prepare a passive dosing vial. The polydimethylsiloxane (PDMS) tubing (i.d. 212  $\mu\text{m}$ , o.d. 300  $\mu\text{m}$ ) was purchased from PermSelect (Ann Arbor, MI, USA), and the stainless-steel wire (diameter of 250  $\mu\text{m}$ ) was purchased from Vita Needle Co. (Needham, MA, USA). An Agilent 7890B GC coupled with 5977 A MS (Santa Clara, CA, USA) was used for separation and quantification purposes. A GERSTEL Multi-Purpose System (MPS) was applied to the automation process (Mülheim an der Ruhr, Germany).

### 2.2. *D. magna* exposure experiment

Acute toxicity assessments of PCB-18 and 100 nm NP were conducted based on the OECD test guidelines 202. The tests were conducted in 20 mL glass vials (Fig. 1a). First, the concentrations of PCB-18 in exposure solutions were set at concentrations of 0, 100, 200, 300, 350, 500, 600, 700, 900, 1500  $\mu\text{g L}^{-1}$ . The original solvent of PCB-18 was volatilized, and 100- $\mu\text{L}$  methanol was added to re-dissolve the PCB as co-solvent, after which a 10 mL culture solution was added in each vial. Second, five *D. magna* not older than 24 h at the start of the test or the first brood progeny were placed into each vial. Then, they were maintained in a temperature-monitored illumination incubator (A1000, Conviron Corporation, Canada) with a 16:8 (light:dark) photoperiod at  $20 \pm 1 \text{ }^\circ\text{C}$  for 48 h, which were exactly the same condition as that the *D. magna* cultivated before the experiments. The immobilization was

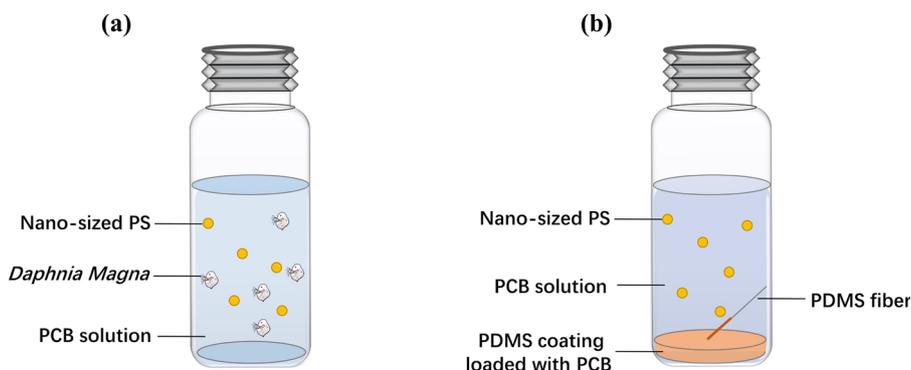


Fig. 1. Experimental devices for the (a) exposure experiment and the (b) sorption experiment.

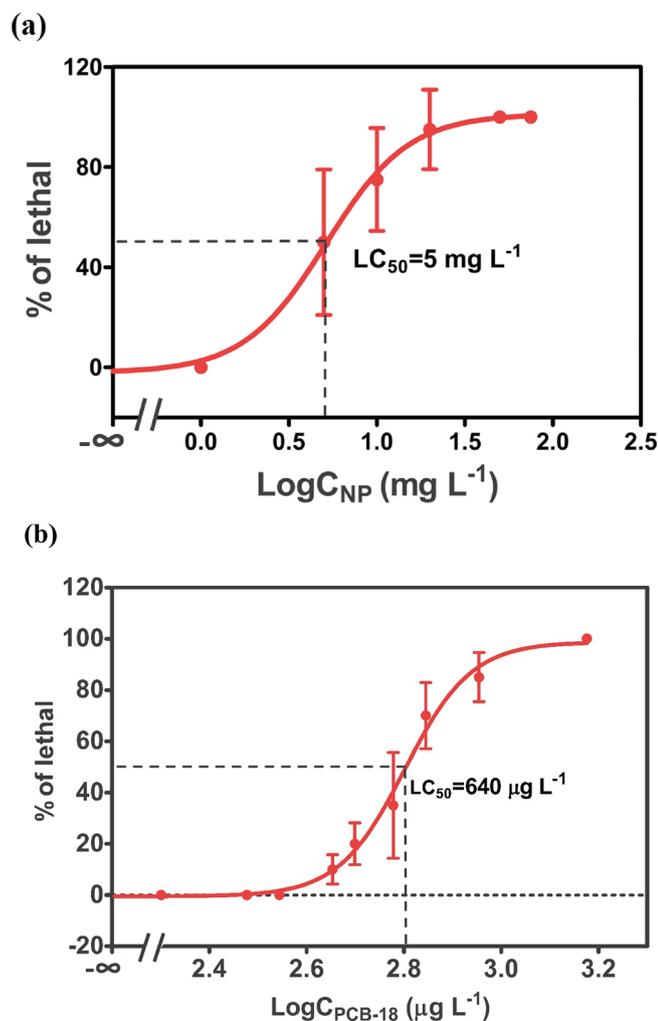


Fig. 2. Concentration-effect curve of (a) PCB-18 and (b) 100 nm PS on *D. magna* after 48 h exposure. Data are the mean  $\pm$  95% confidence intervals ( $n = 4$ ). The curve in Fig. 2 was fitted by the following Boltzmann function:

$$y = y_2 + \frac{y_1 - y_2}{1 + 10^{(\log LC_{50} - x) \cdot \text{Hill slope}}}$$

recorded at 0, 24 and 48 h with a phenomenon that *D. magna* was unable to move within 15 s of gentle agitation of the test vessel. A similar experimental procedure was applied to obtain the dose-effect curve of 100 nm PS on *D. magna*, and the concentrations of NP were set at 0, 1, 5, 10, 20, 50 and 75 mg L<sup>-1</sup>. For the combined toxicity, the exposure solutions with constant PCB-18 concentration (the median lethal concentration (LC<sub>50</sub>) from above experiment) and different concentrations (0, 0.01, 0.1, 1, 5, 20, 40, 50, 75 mg L<sup>-1</sup>) of 100 nm PS were prepared then agitated for 3 days to be better combined. Afterwards, the lethality of *D. magna* was monitored after 48 h of exposure. LC<sub>50</sub> value of *D. magna* exposure experiment was obtained by fitting the percentage of lethality to Boltzmann function, and the associated 95% confidence intervals (95% CI) was calculated. All statistical analysis was performed using Graphpad Prism5. Each experimental treatment was conducted in quadruplicate.

### 2.3. PCBs and PS sorption experiments

A passive dosing method (Fig. 1b) introduced by Mayer [54] was applied to determine the sorption coefficients of PCBs between NP and water. Briefly, a layer of PDMS weighing approximately 0.5 g was first coated on the bottom of a 20 mL vial. Then, the vial was loaded with PCBs by adding 1 mL standard solution with a concentration of 25 mg L<sup>-1</sup> in methanol and incubating for 48 h at a constant agitation

rate (200 rpm). Next, 1, 2, 3, 4 and 5 mL of ultra-pure water were separately added to the dosing vials each time after 22 h agitation. The purpose of adding water was to decrease the solubility of PCBs in the solution and increase the loaded amount of PCBs in the PDMS coating. After loading, the solution was disposed, and the vials were cleaned by methanol and ultra-pure water twice.

The PCB-loaded vials were then filled with 15 mL solutions with different concentrations of PS (ranging from 0 to 10 mg L<sup>-1</sup>), and a homemade PDMS fiber [55] used to monitor the stability of the free-dissolved concentration of PCBs was added to each vial, where 0.05% NaN<sub>3</sub> was added as a microorganism inhibitor. Then, the passive dosing vials were placed on an orbital shaker at 200 rpm at room temperature (25  $\pm$  1 °C) for 3 days. After exposure, both solutions and fibers were extracted by hexane and analyzed by GC-MS. Here, deuterated PCB-77 was used as the surrogate standard to monitor the extraction recoveries.

### 2.4. Quality control

All analytical data in the current study were subject to strict quality control procedures. The correlation coefficients of calibration curves for all the compounds with GC-MS were higher than 0.99, and calibration solutions were injected periodically to ascertain the stability of the instrument. The average recoveries of the surrogate standard were 95%  $\pm$  8.2% and 78%  $\pm$  5.9% for the solution and fiber extraction, respectively. The stability of the passive dosing vials was monitored by the PDMS fiber added to the exposure solution. Since PDMS fibers only sense the free-dissolved concentration of analytes in the solution, the stability of the passive dosing vial could be monitored by the extraction variation of the PDMS fibers in each vial. The relative standard deviation (RSD%) of the fiber extracted amount in all vials were 7.9%, 8.4%, 6.8%, 9.1%, 33%, 7.5%, 11% for PCB-1, 3, 9, 11, 18, 77, penta-CB, and hexa-CB, respectively.

In the toxicity experiment, the concentration of added NP was pre-determined based on the results of the preliminary test. The highest concentration pre-set should result in 100% immobilization while the lowest concentration (only containing 100  $\mu$ L methanol as co-solvent) exhibited no observable effect during the experiment, indicating that all the substances (the culture solution and co-solvent) added in the control group were biocompatible.

## 3. Results and discussion

### 3.1. Combined toxicity of nano-sized PS and PCB-18 to *D. magna*

Acute toxicity of 100 nm PS particles to *D. magna* was first tested in terms of lethality percentage (LC<sub>50</sub>) of *D. magna*. The LC<sub>50</sub> of 100 nm PS from the dose-effect curve (Fig. 2a) was 5  $\pm$  1 mg L<sup>-1</sup>. The acute toxicity of plastic to *D. magna* has been investigated in several studies, and the results demonstrated that the plastic itself did show toxicity, especially for nano-sized plastic [28,29]. Plastic usually caused physical damage to organisms, and the damage was enhanced as the size of plastic decreased [24]. Compared to the reported results, the obtained LC<sub>50</sub> value in the current study was in the same order of magnitude reported by Ma et al. (15  $\pm$  3 mg L<sup>-1</sup> for 50 nm PS at 48 h exposure) [28]. However, the currently used nano-PS was more toxic than other manufactured nanomaterials such as 20 nm TiO<sub>2</sub> and 80 nm Al<sub>2</sub>O<sub>3</sub>, whose LC<sub>50</sub> values were 35 mg L<sup>-1</sup> and 114 mg L<sup>-1</sup>, respectively, to *D. magna* after 48 h exposure [26]. The severe toxicity of nano-sized PS may result from remaining styrene monomers or the addition of surfactant during the synthesis process [56]. Another possibility was that physical wrapping killed the *D. magna*. In the experiment, we observed that the NP could be ingested by *D. magna*, excreted with intestinal secretion, and formed floccule that wrapped the body of *D. magna*, limited its activities, and presumably caused death. In the absence of nano-sized PS, the LC<sub>50</sub> of PCB-18 was 640  $\pm$  1  $\mu$ g L<sup>-1</sup> calculated from the dose-effect curve using the fitting model (Fig. 2b).

The combined toxic effect was tested by monitoring the 48 h lethality of *D. magna* in exposure solutions with constant concentration of PCB-18 ( $640 \mu\text{g L}^{-1}$ ) and various NP concentrations ranging from 0 to  $75 \text{ mg L}^{-1}$ . As shown in Fig. 3, when the concentration of PS in the exposure solution was lower than  $1 \text{ mg L}^{-1}$ , the lethality of *D. magna* decreased as the PS concentration increased. In contrast, when the concentration of PS was higher than  $1 \text{ mg L}^{-1}$ , the addition of PS increased the lethality of *D. magna*. A similar trend was observed by M. Oliverira et al. [57] when studying the combined toxicity of polyethylene microsphere and pyrene to *Pomatoschistus microps* juveniles. However, in the report by Ma, the addition of nano-sized PS enhanced the toxicity of phenanthrene for the selected concentrations [28].

A simple pile up effect of PCB-18 and PS did not cause the combined toxicity, so it was necessary to further investigate the interesting observation above, particularly the combined effect with nano-sized PS concentrations below  $1 \text{ mg L}^{-1}$ . Hereon, the following hypotheses were proposed: (1) the nano-sized PS poses competition to PCB-18 and changes the substance uptake, which are deadly to *D. magna*; (2) the nano-sized PS decreases the total amount of PCB-18 in the aqueous solution by way of combination and coagulation, and then the cluster drops to the bottom or adsorbs to the wall of the vials, becoming unavailable to *D. magna*; (3) the combination of nano-sized PS and PCB-18 reduces the free concentration of PCB-18, so does the toxic effect towards *D. magna*, while the combined PCB has no more toxicity.

### 3.2. Speciation analysis of solution containing NP

To test the hypotheses and further explain the combined toxic effect of PCB and nano-sized plastic on *D. magna*, the sorption coefficient of PCB on 100 nm PS plastic was studied. In accordance with the fundamentals of passive dosing, the following equation can be obtained [53].

$$\frac{C_{\text{solution}}}{C_{\text{water}}} = \frac{C_{\text{bound}} + C_{\text{water}}}{C_{\text{water}}} = K_{\text{NP}} \times C_{\text{NP}} + 1 \quad (1)$$

where  $C_{\text{solution}}$  and  $C_{\text{water}}$  are the concentrations of PCBs in the solution with or without NP, respectively.  $C_{\text{bound}}$  is the concentration of bound PCBs in water.  $C_{\text{NP}}$  is the concentration of NP in the sample.  $K_{\text{NP}}$  is defined as the ratio of chemical concentration on NP to that in water, and it is called the sorption coefficient when the exposure time is long enough and the sorption process reached equilibrium. According to Eq. 1,  $K_{\text{NP}}$  values can be obtained from the slope of the linear curves by fitting the  $\frac{C_{\text{solution}}}{C_{\text{water}}}$  with  $C_{\text{NP}}$ . A high fitting degree of the linear regressions was obtained ( $R^2 > 0.94$ ) for all PCBs in the current experiment (Fig. 4).

Since the exposure solution for the combined toxic test was prepared for 3 days before *D. magna* exposure, the same exposure time was chosen to determine the sorption coefficient. The results (Fig. 4) showed that the  $\log K_{\text{NP}}$  value for PCB-18 was 5.38. The free concentration ( $C_{\text{water}}$ ) could be quantified by Eq. 1 when the total PCB-18 concentration ( $C_{\text{solution}}$ ) and NP concentration ( $C_{\text{NP}}$ ) in the exposure solution were known.

### 3.3. Interpretation of the combined toxic effect of PCB-18 and 100 nm PS

Based on the obtained sorption coefficient, the free-dissolved concentration of PCB-18 in the exposure solution of the acute toxicity test with different concentrations of PS could be quantified. For example, in the exposure solution with a PCB-18 total concentration of  $640 \mu\text{g L}^{-1}$  and a 100 nm PS concentration of  $1 \text{ mg L}^{-1}$  ( $\log C_{\text{NP}} = 0$ ), the free-dissolved concentration of PCB-18 was  $516 \mu\text{g L}^{-1}$ . From Figs. 2b and 3, the corresponding lethality of *D. magna* was approximately 16% (only PCB-18) and  $5\% \pm 12\%$  (PCB-18 with  $1 \text{ mg L}^{-1}$  100 nm PS), respectively. The similar lethality indicated that the combined toxicity to *D. magna* was mainly determined by the free-dissolved concentration of PCBs when the concentration of added PS was low. To specify, the lethality induced by PCB-18 alone had no significant difference with

that induced by a coexistent system that contained sufficiently less PS and the same free concentration of PCB-18 as above. As the  $\text{LC}_{50}$  of PCB-18 and 100 nm PS were on different scales, they should not replace each other with regard to the toxic effect, so hypothesis (1) was rejected.

On the other hand, the results also indicated that when the PS concentration was higher, the toxicity was mainly caused by the 100 nm PS itself. Taking the exposure solution with  $C_{\text{NP}} = 5 \text{ mg L}^{-1}$  as an example, the calculated free-dissolved concentration of PCB-18 was  $290 \text{ mg L}^{-1}$ , which showed no acute toxicity to *D. magna*, as illustrated by Fig. 2b. However, the lethality in this exposure solution was  $50\% \pm 8\%$ , which agreed with the lethality in the solution with  $5 \text{ mg L}^{-1}$  PS (Fig. 2a). The nano-sized PS dominated the lethality when its concentration was high enough, but hypothesis (2) should not be valid in this case. Otherwise, the combination and sedimentation of PS and PCB-18 would reduce the concentration of nano-sized PS in the aqueous solution and the lethality of *D. magna*, as shown in Fig. 3.

Therefore, the calculated results well supported hypothesis (3), indicating that the addition of plastic could decrease the free-dissolved concentration of organic pollutants and the toxicity. Meanwhile, the excessive amounts of plastic could also cause toxic effect towards *D. magna*. More examples at different concentrations of the plastic are presented in Table 1.

## 4. Conclusions

This study revealed that the combined toxicity to *D. magna* depended on the relative concentration of nano-sized PS and PCB. To be specific, PCB was less toxic towards *D. magna* when it was combined with a certain amount of nano-sized PS, while excessive amounts of nano-sized PS enhanced the lethality of *D. magna*. The toxicity effect of PCB and nano-sized PS could be verified from the point of quantitative speciation, which was conducted by measuring the sorption coefficients. Through the exploration of the combined toxic effect of NP and PCBs in this study, we linked the toxicity endpoints of organisms with the speciation of chemicals preliminarily. The current study provides an efficient method and applicable data for studying the effect of plastics on the environmental fate of HOCs for the future.

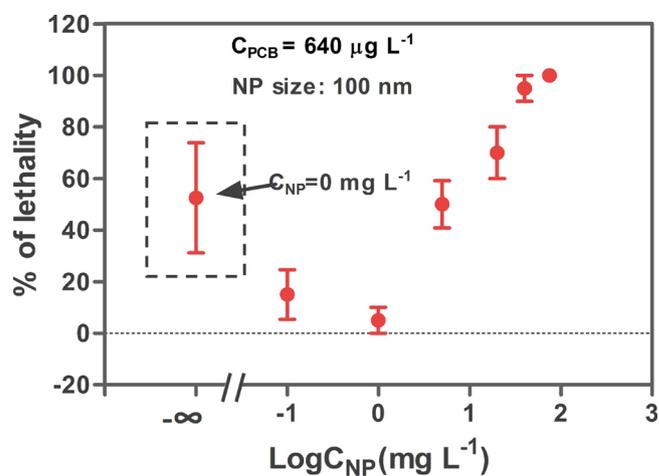
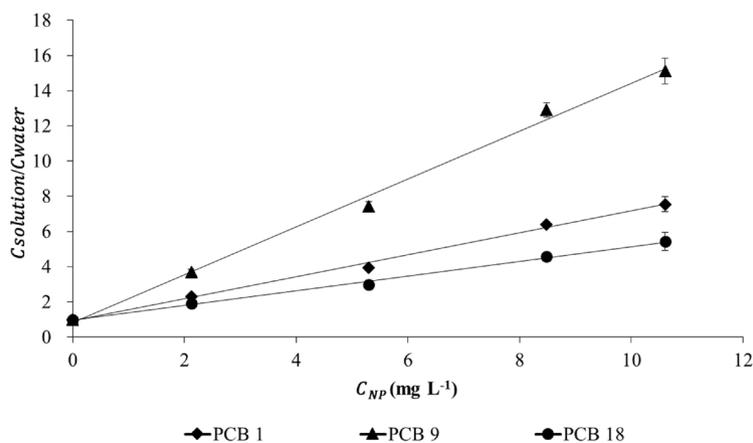


Fig. 3. Lethality of *D. magna* in the exposure solution with constant concentration of PCB-18 ( $640 \mu\text{g L}^{-1}$ ) and different concentrations of 100 nm PS particles after 48 h of exposure. Five *D. magna* samples are placed into each vial, and each experimental treatment was conducted in quadruplicate. The values are the mean of twenty *D. magna* with 95% confidence intervals. The lethality of  $640 \mu\text{g L}^{-1}$  PCB-18 without 100 nm PS is denoted in the dashed box, which is according to that (50% of the lethality in Fig. 2b) found in the experiment studying toxic effect of PCB-18 alone.



Chemicals	PCB-1	Penta-CB	PCB-3	PCB-9	Hexa-CB	PCB-11	PCB-18	PCB-77
$\log K_{NP}$	5.28	5.44	5.84	5.50	5.31	5.63	5.38	6.07

**Fig. 4.** Speciation analysis of  $K_{NP}$  values by fitting curves of  $\frac{C_{solution}}{C_{water}}$  and  $C_{NP}$  based on Eq. 1 for selected PCBs (take PCB-1, 9, 18 as examples) and the  $\log K_{NP}$  ( $L\ kg^{-1}$ ) of PCBs on 100 nm PS after different exposure times. Each value represents the mean  $\pm$  standard deviation. This sorption experiment is conducted under the condition where the exposure time is 3 days, exactly the same as that in the toxicity experiment.

**Table 1**

Comparison of the lethality in the solution containing 100 nm PS only (Fig. 2a), PCB-18 only (Fig. 2b), and both PCB-18 and 100 nm PS (Fig. 3) for *D. magna*. The total concentration of PCB-18 is fixed at  $640\ \mu\text{g}\ L^{-1}$ . Eq. 1 is used to calculate the free concentration of PCB-18 ( $C_{water}$ ). The percentage of lethal for PCB-18 only was determined according to Fig. 2b based on the calculated  $C_{water}$  value.

$C_{NP}$ ( $\text{mg}\ L^{-1}$ )	$C_{water}$ ( $\mu\text{g}\ L^{-1}$ )	% of lethal		
		NP only (Fig. 2a)	PCB-18 only (Fig. 2b)	Combined (Fig. 3)
0.01	638	0	48	$50 \pm 12$
1	516	0	16	$5 \pm 12$
5	290	$50 \pm 26$	0	$50 \pm 8$
20	110	$91 \pm 15$	0	$70 \pm 10$
40	60	$99 \pm 0$	0	$95 \pm 5$
75	34	$100 \pm 0$	0	$100 \pm 0$

## Conflicts of interest

There are no conflicts to declare.

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