Bioconcentration of Perfluoroalkyl Substances by *Chironomus Plumosus* Larvae in Water with Different Types of Dissolved Organic Matters

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Abstract

The effects of four types of dissolved organic matters (DOM) on the bioconcentration of perfluoroalkyl substances (PFASs) in *Chironomus plumosus* larvae have been studied. The PFASs included perfluorooctane sulfonate (PFOS), perfluorooctanoic acid (PFOA), perfluorononanoic acid (PFNA), perfluorodecanoic acid (PFDA), perfluoroundecanoic acid (PFUnA), and perfluorododecanoic acid (PFDoA). The DOM included humic acid (HA), fulvic acid (FA), tannic acid (TA), and a protein, peptone (PEP), and their concentrations ranged from 0 to 50 mg·L⁻¹. The results showed that, upon bioconcentration equilibrium, the body burdens of longer perfluoroalkyl chain PFASs (PFOS, PFDA, PFUnA and PFDoA) decreased with PEP and HA concentrations while increased with FA and TA concentrations. When FA and TA concentrations increased from 0 to 50 mg·L⁻¹, body burdens of these PFASs increased by 7.5%-148.8% and 5.7%-37.1%, respectively. However, the DOM had no significant impact on the body burdens of shorter perfluoroalkyl chain PFASs (PFOA and PFNA). All of the four types of DOM lowered not only the uptake rate constants (k_u) of PFASs due to the decrease of freely dissolved PFAS concentrations, but also the elimination rate constants (k_e) due to the inhibition effect of DOM on the PFAS elimination from the larvae. The reduction in the two constants varied with both DOM and PFAS types. In the presence of PEP and HA with larger molecular weights, the k_u values decreased more than k_e , leading to the decreased body burdens of longer perfluoroalkyl chain PFASs. As for FA and TA with smaller molecular weights, the k_e values decreased more than k_u , resulting in increased body burdens of longer perfluoroalkyl chain PFASs. This study suggests that the effects of DOM on PFAS bioconcentration depend not only on the concentration but also on the molecule weight of DOM, which should be considered in the bioavailability assessment of PFASs.

Keywords: Perfluoroalkyl substances (PFASs); Bioconcentration; Dissolved organic matters (DOM); Proteins; Bioavailability

Capsule: DOM has effects not only on uptake rate constants of PFASs in the larvae but also on their elimination rate constants.

1 Introduction

Perfluoroalkyl substances (PFASs) are a class of anionic fluorinated surfactants characterized by a hydrophobic perfluoroalkyl chain and a hydrophilic carboxylate or sulfonate functional group. Because of their oil, stain, and water repellant properties, PFASs are widely used in commercial and industrial products, such as textiles, paper products, carpets (Renner, 2001). As a result, PFASs have been detected in water, such as river, lake, groundwater, and drinking water (Eschauzier et al., 2013; Lam et al., 2014; Zhou et al., 2014), and concentrations of some PFASs in groundwater and surface water were up to $\text{mg}\cdot\text{L}^{-1}$ level (Moody et al., 2002; Schultz et al., 2004). In addition, PFASs have also been detected in plankton, fish, mammals, and even humans (Lam et al., 2014; Zhou et al., 2014). Studies showed that some PFASs have toxicological risks to organisms, such as developmental toxicity and endocrine disrupting effects (Kim et al., 2013; Wang et al., 2011a). It is widely recognized that PFASs are persistent in the environment (Conder et al., 2008), and can be accumulated in many kinds of organisms (Martin et al., 2003; Yeung et al., 2009). Some studies found that the bioconcentration of PFASs in aquatic organisms can be influenced by many factors, including PFAS properties, organism species, and environmental conditions (e.g. dissolved organic matters (DOM) and concentrations of Ca^{2+} and Mg^{2+}) (Loi et al., 2011; Naile et al., 2013b; Xia et al., 2015a). For example, the bioconcentration of PFASs in *Daphnia magna* decreased with the increase of Ca^{2+} and Mg^{2+} concentrations in water with the presence of DOM (Xia et al., 2015b).

DOM, which is ubiquitous in aquatic environment, is a complex and heterogeneous mixture of organic compounds, composed of various molecular weight aromatic and aliphatic organic compounds, attached with kinds of groups, such as carboxyl and hydroxyl (Lam et al., 2007; Leenheer and Croue, 2003). It is generally accepted that DOM significantly affect the bioavailability of organic contaminants (Haitzer et al., 1998; Xia et al., 2015a; Xia et al., 2013). Many studies have shown that the presence of DOM will lower the freely dissolved concentrations of hydrophobic organic compounds (HOCs); the origin, quantity, quality and composition of DOM

will affect the uptake and bioconcentration of HOCs in aquatic organisms (Chen et al., 2010; Gouliarmou et al., 2012; Haitzer et al., 1999). For example, the presence of natural DOM decreased the permethrin uptake by *Daphnia*, and statistical analyses suggested that permethrin associated with DOM was completely not bioavailable (Yang et al., 2007). Bioconcentration of benzo[k]fluoranthene in Japanese medaka (*Oryzias latipes*) was reduced with the increase of humic acid (HA) concentration in the exposure medium (Chen et al., 2008). However, previous studies mainly focused on traditional HOCs, and limited information is available about the influence of DOM on the bioconcentration of PFASs. Only some researchers have reported the binding of PFASs with DOM and the influence of DOM on PFAS bioaccumulation (Jeon et al., 2011; Li et al., 2009; Xia et al., 2013). As a type of proteinophilic compounds with resistance to be degraded by organisms, the bioconcentration of PFASs by aquatic organisms in the presence of DOM may be different from other HOCs which are lipophilic compounds and some of which can be metabolized in biota, such as polybrominated diphenyl ether (Zeng et al., 2013). Therefore, it is worth to study the influence of DOM on the bioconcentration of PFASs in aquatic organisms.

Protein is one of the important components of DOM. PEP, which is ubiquitous in aquatic environments and contains dissolved protein derivatives produced from proteolysis, is widely used in biological and environmental experiments (Banihashemi and Droste, 2014; Garcia et al., 2010; Xia et al., 2015a). HA and fulvic acid (FA), which are the main components of humic substances, are also widely used in biological and environmental experiments (Al-Reasi et al., 2013; Xia et al., 2015a). Tannic acid (TA) is extensively studied as a typical organic acid with a smaller molecular weight (Laue et al., 2014; Liu and Li, 2010). Studies found that PFASs could bind with proteins, such as serum albumin and liver fatty acid-binding protein, as well as HA (Bischel et al., 2010; Luebker et al., 2002).

The objectives of this study were to investigate the impact of four types of DOM, including PEP, HA, FA, and TA, on the bioconcentration of PFASs in *Chironomus plumosus* larvae, a part of

the aquatic food web and one of the food sources to fish which is a major exposure source of PFASs for the general public (Xiao et al., 2013; Ye et al., 2008). The studied PFASs included perfluorooctane sulfonate (PFOS), perfluorooctanoic acid (PFOA), perfluorononanoic acid (PFNA), perfluorodecanoic acid (PFDA), perfluoroundecanoic acid (PFUnA), and perfluorododecanoic acid (PFDoA). These six types of PFASs widely exist in the aquatic environment, and many researchers have reported that PFOS, with a sulfonate group, is one of the main PFASs found in the environment media (Pan et al., 2014; Ye et al., 2008). The bioconcentration kinetics of PFASs in the presence of DOM was studied, and the effect of DOM concentrations and molecular weights on the PFAS bioconcentration was investigated. In addition, the influence of DOM on the bioconcentration of different PFASs was also compared, and the influencing mechanisms of different types of DOM on the bioconcentration were discussed.

2 Materials and Methods

2.1 Chemicals

PFOA (96%), PFNA (97%), PFDA (98%), PFUnA (95%), and PFDoA (95%) were purchased from Acros Organics (New Jersey, US). PFOS (98%) was obtained from Tokyo Chemical Industries (Tokyo, Japan). The stock solution of PFASs was prepared by dissolving the standard substances in methanol at 500 mg·L⁻¹ for each PFAS. Recovery indicators, [1,2,3,4-¹³C₄] perfluorooctane sulfonate (MPFOS) (≥ 99%) and [1,2,3,4-¹³C₄] perfluorooctanoic acid (MPFOA) (≥ 99%), were supplied by Wellington Laboratories (Guelph, Canada). Ammonium acetate (98%), Methyl-tert-butyl ether (MTBE, 99.5%), and tetrabutyl-ammonium hydrogen sulfate (TBA), which were used for extracting PFASs from the larvae, were obtained from Sigma-Aldrich Chemical Co. (St. Louis, MI, USA). Methanol (chromatography grade) was purchased from J.T. Baker (Phillipsburg, NJ, USA). PEP, HA, FA, and TA were purchased from Beijing Aoboxing Co., Ltd (Beijing, China), Aldrich (Steinheim, Germany), Henan Changsheng Co., Ltd (Henan, China), and

Acros Organics (New Jersey, US), respectively. Stock solutions of 200 mg·L⁻¹ PEP, TA, and FA were prepared in deionized water. The HA stock solution was prepared by dissolving HA in sodium hydroxide solution (pH = 12) (Laor et al., 1999). The solution was stirred and filtered with 0.45 μm nylon membrane to remove undissolved particles. After that the TOC of HA solution was measured by TOC-L CPH/CPN (Shimadzu, Japan) and then diluted with deionized water to 200 mg·L⁻¹. Then, the pH of the solution was adjusted to 7.0 with dilute HCl. All of the stock solutions were stored in the dark at 4 °C before use.

2.2 Kinetic and steady state experiments of PFAS bioconcentration in the larvae

Chironomus plumosus larvae, collected from uncontaminated Yangliuqing River in the outer suburbs of Tianjin city, were used for the PFAS bioconcentration experiments. The larvae were acclimated to laboratory conditions in artificial freshwater (AFW) for 2 d to purge their guts. The AFW was prepared according to our previous study (Dai et al., 2013). The acclimation and following exposure experiments were conducted with a 16:8 photoperiod at 25±0.5 °C. Dissolved oxygen was maintained at 7.1-7.2 mg·L⁻¹ and pH at 7.4-7.5, and no food was provided to the larvae.

All exposure experiments were conducted in 500 mL polypropylene (PP) beakers. The stock solution of each DOM was diluted to 1, 5, 10, 30, and 50 mg·L⁻¹ with AFW, respectively. Each beaker was added with 200 mL DOM solution and 200 μL PFAS solution (500 mg·L⁻¹), obtaining a nominal concentration of 0.5 mg·L⁻¹ for each PFAS. The beakers without DOM were used as the control, and the beakers with AFW only were used as the blank. All the beakers were shaken at 95 rpm in darkness at 25±0.5 °C for 72 h to equilibrate the PFASs between DOM and water before exposure experiments. The male larvae, which were less sensitive to PFASs than females, in the fourth instar based on uniformity in size were selected for exposure, and the details are shown in Supplementary Information.

As for the bioconcentration kinetics of PFASs, the beakers contained 10 mg·L⁻¹ DOM were used, and the control and blank beakers were used as well. A total of 25 male larvae were placed

in each beaker and cultivated under the conditions as mentioned above. Fifty percent of the exposure solutions were renewed every three days. Two larvae were collected from each beaker at each predetermined interval (1, 2, 3, 5, 7, 9, and 11 d). The collected larvae were rinsed with AFW and dried with filter paper, and then transferred to PP centrifuge tube and the wet weight was obtained. The larvae were freeze-dried for 48 h and the dry weight was obtained prior to subsequent extraction. As for the steady state bioconcentration, a total of 15 male larvae were placed in each beaker containing 0, 1, 5, 10, 30, or 50 mg·L⁻¹ DOM. After 10 d exposure, four larvae from each beaker were collected for lipid analysis and the others were collected for PFAS analysis. Each experiment set, including the kinetics and steady state batch, were conducted in three replicates for each exposure concentration.

2.3 Sample preparation and PFAS extraction

Lipid was measured using the method reported by Millward et al (Millward et al., 2005). According to the principle of the ecotoxicological experiment (Harris et al., 2014), the PFAS concentrations of the exposure solution were measured at the beginning and end of the experiment, as well as before and after each renewal of the exposure solution. For the PFAS analysis in larvae tissue, the dried larvae of each set were ground into fine powder and PFASs were extracted using the iron-pairing method reported by Kannan et al (Kannan et al., 2006) and Liu et al (Liu et al., 2011). In brief, the powder of the larvae was added to 10 mL PP centrifuge tube, and 10 ng ¹³C₄-PFOA and ¹³C₄-PFOS (100 μL, 100 μg·L⁻¹) were added as recovery indicators. Then 0.25 mL of 0.5 M TBA (pH=10), 1 mL of 0.25 M sodium carbonate buffer (pH=10), and 2.5 mL MTBE were added into each tube. The mixture was vortexed, and then shaken (250 rpm, 30 min, 25±0.5 °C). The organic layer was separated by centrifuging (4000 rpm, 15 min). The extraction was repeated with 2.5 mL of MTBE twice. All the organic layer were combined in 15 mL PP centrifuge tube and vortexed. Then the extracts were evaporated to dryness under a gentle nitrogen stream and reconstituted with 1 mL methanol. After that, they were vortex mixed and filtered with 0.2-μm

nylon membranes into PP autosampler vials with PP caps.

2.4 Instrumental analysis

PFASs were analyzed by liquid chromatography-tandem mass spectrometry (LC-MS/MS; Dionex Ultimate 3000 and Applied Biosystems API 3200) in electrospray negative ionization mode. Chromatographic separation was in combination with a binary pump, an autosampler and a column oven equipped with a Waters Sunfire C18 Column (5 μm pore size, 4.6 $\times$ 150 mm). The injection volume was 10 μL . 50 mM ammonium acetate and methanol were used as mobile phase. At a flow rate of 1 $\text{mL}\cdot\text{min}^{-1}$, the mobile phase gradient was ramped from 70% to 95% methanol in 4 min, held at 95% methanol for 3 min, and then ramped down to 70% methanol. For quantitative analysis, analyte ions were monitored using multiple reaction monitoring mode. The details of primary and production ions are listed in Table S1. Concentrations of all target analytes were quantified from calibration curves drawn using external standards.

2.5 Data analysis

The kinetic model expressed in equation (1) (Bruggeman et al., 1981) was used to fit the uptake and elimination rate constants of PFASs in the larvae,

$$C_b = k_u C_w \left(\frac{1 - e^{-k_e t}}{k_e} \right) \quad (1)$$

where C_b is the PFAS concentration in the larvae ($\mu\text{g}\cdot\text{kg}_{\text{ww}}^{-1}$) at time t (d); C_w is the PFAS concentration in the water ($\mu\text{g}\cdot\text{L}^{-1}$), and here is the spiking concentration (500 $\mu\text{g}\cdot\text{L}^{-1}$); k_u is the uptake rate constant of PFASs from the water ($\text{L}\cdot\text{kg}_{\text{ww}}^{-1}\cdot\text{d}^{-1}$) and k_e is the elimination rate constant of PFASs from the larvae (d^{-1}).

Bioconcentration factor (BCF , $\text{L}\cdot\text{kg}^{-1}$) expresses the degree to which chemicals can be accumulated in biota only through its respiratory and dermal surfaces in the environment (Arnot and Gobas, 2006). The BCF values of PFASs in the larvae based on the steady state (BCF_{ss}) and kinetic (BCF_{kinetic}) methods were calculated with equation (2) and (3), respectively:

$$BCF_{ss} = \frac{c_b}{c_w} \quad (2)$$

$$BCF_{kinetic} = \frac{k_u}{k_e} \quad (3)$$

All statistical analyses were performed using SPSS 18.0 for windows (SPSS Inc., Chicago (IL), USA) and Microsoft Excel 2013. Analysis of variance (ANOVA, one factor) was carried out to test differences between each two compared groups. Difference was considered significant when the significance level was smaller than 0.05.

2.6 Quality assurance and quality control (QA/QC)

Throughout the experiments, disposable PP beakers, bottles, tubes, and pipettes were used to prevent target compounds adsorption and contamination from glassware and other vessels containing fluorochemicals. All vessels were cleaned by methanol three times before use.

The limits of quantification (LOQs) for each target analyte were defined as 10 times of the signal to noise ratio (S/N=10), which were in the range of 0.01-0.058 $\mu\text{g}\cdot\text{L}^{-1}$ and 0.25-0.75 $\text{ng}\cdot\text{g}^{-1}$ dry weight for the water and the larvae tissue, respectively. The concentrations of PFASs in *Chironomus plumosus* larvae of the blank experiments without PFASs were lower than LOQs. Here the PFAS concentrations were regarded as zero. All the target chemicals in each sample were quantified by extra-calibration curve. The correlation coefficients of the standard calibration curves were higher than 0.99, and the repeatability of these calibration curves was confirmed prior to each set of determinations. To ensure the extraction efficiency and accuracy of determination, a recovery experiment for PFASs and recovery indicators in the larvae was conducted. The detailed procedure of the recovery experiment is shown in Supplementary Information. Recoveries for the target analytes in the larvae were between 73% and 115%, and the recoveries for $^{13}\text{C}_4$ -PFOS and $^{13}\text{C}_4$ -PFOA were 85% and 113%, respectively. The recoveries were consistent with other references (Zhou et al., 2013). Procedural blanks and calibration verification standards

were run after every 10 samples to check the background contamination and the validity of the calibration. A new calibration curve was run if the quality-control standard was not measured within $\pm 20\%$ of its theoretical value. The sample concentrations of target chemicals were corrected according to the recovery indicators. In addition, the measured PFAS concentration of the exposure solution ranged from 496 to 520 $\mu\text{g}\cdot\text{L}^{-1}$; the variation was within 4% compared to the spiking concentration (500 $\mu\text{g}\cdot\text{L}^{-1}$). Therefore, the spiking concentration was used to calculate the *BCF* values of PFASs.

3 Results and discussion

3.1 PFAS bioconcentration kinetics in the larvae in the presence of DOM

Survival rates of the larvae were $87 \pm 5.1\%$ and $85 \pm 3.2\%$ in the exposure systems with and without presence of DOM, respectively. Previous study (MacDonald et al., 2004) showed that *Chironomus tentans* were uniquely sensitive to PFOS, but the similar results were not obtained in our study. This may be due to the different species of chironomid larvae and their sex/instar. The average lipid contents were $6.9 \pm 0.3\%$ and $6.9 \pm 0.6\%$ in the larvae in the presence and absence of DOM, respectively. No significant differences in survival rate and lipid content were observed among all the bioconcentration systems ($P > 0.05$).

As shown in Fig.1, whether with or without the presence of DOM, the PFAS levels in the larvae increased with exposure time till a steady state was reached, and the equilibrium time increased with the perfluoroalkyl chain length of PFASs. For example, in the absence of DOM, PFOA reached steady state condition at 3 day's exposure while PFDoA at 9 day's exposure. This was consistent with the results reported by Liu et al (Liu et al., 2011) that the PFAS perfluoroalkyl chain length had obvious influence on the equilibrium of PFASs between the green mussels and the environmental media. In the presence of DOM, FA and TA changed the equilibrium time while PEP and HA did not. Compared with control group without DOM, the equilibrium time of PFASs except

PFDoA increased by 2-6 days in the presence of 10 mg·L⁻¹ FA or TA, and the increase for PFASs with shorter perfluoroalkyl chain was more than those with the longer ones. For example, relative to the control group, the equilibrium time of PFOA and PFDA increased by 6 and 2 days, respectively in the presence of 10 mg·L⁻¹ FA.

The first-order accumulation rate model was used to fit bioconcentration kinetics of PFASs and the results are shown in Table 1. In the presence or absence of DOM, the k_u values increased with the perfluoroalkyl chain length of PFASs. For example, when the fluorinated carbon number increased from 7 to 11, the k_u value rose from 1.47 to 3.36 L·kg_{ww}⁻¹·d⁻¹ in the absence of DOM. This was consistent with the previous reports (Liu et al., 2011; Xia et al., 2015a; Xia et al., 2013). Compared with the control group without DOM, both k_u and k_e values of each PFAS decreased in the presence of DOM. For example, the k_u and k_e values of PFDoA in the larvae decreased from 3.36 to 2.27 L·kg_{ww}⁻¹·d⁻¹ and from 0.70 to 0.62 d⁻¹, respectively when HA concentration increased from 0 to 10 mg·L⁻¹ HA. Therefore, DOM had influence not only on the uptake rate constants of PFASs to the larvae but also on the elimination rate constants of PFASs from the larvae. The decrease of the two bioconcentration kinetic constants caused by the presence of DOM may be responsible for the increase of equilibrium time for PFAS bioconcentration in the presence of FA and TA as mentioned above.

Fig. 1 Bioconcentration curves of PFASs by *Chironomus plumosus* larvae in water with 10 mg·L⁻¹ DOM

Table 1 Bioconcentration kinetic parameters and *BCF* values of PFASs in *Chironomus plumosus* larvae with the presence of 10 mg·L⁻¹ DOM (average ± standard deviation, *n*=3)

	PFOA	PFNA	PFOS	PFDA	PFUnA	PFDoA
<i>Uptake rate constant, k_u (L·kg_{ww}⁻¹·d⁻¹)</i>						
Control	1.47 ± 0.12	1.42 ± 0.21	1.94 ± 0.18	2.20 ± 0.06	2.10 ± 0.08	3.36 ± 0.16
PEP	0.78 ± 0.16	0.81 ± 0.19	0.95 ± 0.11	1.29 ± 0.16	1.44 ± 0.30	2.26 ± 0.37
HA	0.68 ± 0.11	0.73 ± 0.13	0.87 ± 0.07	1.03 ± 0.14	1.59 ± 0.17	2.27 ± 0.21
TA	0.90 ± 0.16	0.97 ± 0.15	1.10 ± 0.08	1.36 ± 0.15	1.65 ± 0.20	2.44 ± 0.55
FA	0.68 ± 0.15	0.73 ± 0.15	0.89 ± 0.11	1.16 ± 0.18	1.58 ± 0.24	3.12 ± 0.27
<i>Elimination rate constant, k_e (d⁻¹)</i>						
Control	1.11 ± 0.15	0.86 ± 0.15	0.99 ± 0.10	1.06 ± 0.03	0.67 ± 0.02	0.70 ± 0.03
PEP	0.53 ± 0.08	0.49 ± 0.09	0.54 ± 0.05	0.67 ± 0.08	0.48 ± 0.07	0.57 ± 0.08
HA	0.50 ± 0.06	0.52 ± 0.07	0.56 ± 0.04	0.60 ± 0.07	0.62 ± 0.05	0.62 ± 0.05
TA	0.61 ± 0.09	0.57 ± 0.07	0.60 ± 0.04	0.61 ± 0.06	0.52 ± 0.05	0.51 ± 0.09
FA	0.39 ± 0.05	0.39 ± 0.05	0.43 ± 0.03	0.49 ± 0.06	0.45 ± 0.05	0.51 ± 0.05
<i>BCF_{kinetic} (L·kg_{ww}⁻¹)</i>						
Control	1.33 ± 0.18	1.66 ± 0.29	1.97 ± 0.20	2.08 ± 0.06	3.12 ± 0.10	4.77 ± 0.19
PEP	1.47 ± 0.22	1.64 ± 0.28	1.74 ± 0.15	1.92 ± 0.22	3.01 ± 0.44	3.98 ± 0.51
HA	1.36 ± 0.16	1.40 ± 0.18	1.55 ± 0.10	1.73 ± 0.19	2.57 ± 0.20	3.68 ± 0.29
TA	1.48 ± 0.22	1.70 ± 0.21	1.84 ± 0.11	2.23 ± 0.21	3.18 ± 0.30	4.82 ± 0.82
FA	1.76 ± 0.23	1.87 ± 0.22	2.08 ± 0.16	2.39 ± 0.26	3.53 ± 0.35	6.06 ± 0.52
<i>BCF_{ss} (L·kg_{ww}⁻¹)</i>						
Control	1.58 ± 0.03	1.77 ± 0.03	1.87 ± 0.02	2.11 ± 0.15	3.09 ± 0.27	4.83 ± 0.30
PEP	1.56 ± 0.09	1.69 ± 0.05	1.77 ± 0.09	1.87 ± 0.14	2.69 ± 0.27	4.06 ± 0.23
HA	1.53 ± 0.11	1.74 ± 0.05	1.69 ± 0.08	1.75 ± 0.13	2.45 ± 0.04	3.69 ± 0.37
TA	1.52 ± 0.09	1.75 ± 0.05	1.88 ± 0.04	2.18 ± 0.03	3.18 ± 0.08	5.08 ± 0.15
FA	1.52 ± 0.12	1.80 ± 0.11	1.94 ± 0.03	2.45 ± 0.25	3.62 ± 0.26	6.15 ± 0.34

3.2 Bioconcentration of PFASs in the larvae in the presence of DOM

The BCF_{ss} values were calculated based on the data obtained from the steady state condition. As shown in Table 1 and Table S2, the BCF_{ss} values of PFASs in the absence of DOM were in the order: PFDoA > PFUnA > PFDA > PFOS > PFNA > PFOA. This was in agreement with their perfluoroalkyl chain length except for PFOS. Both PFNA and PFOS had eight fluorinated carbons, but the BCF_{ss} value of PFOS was higher than PFNA. This is because sulfonate group is more hydrophobic than carboxylate group (Xiao et al., 2011). In addition, the BCF_{ss} values of PFASs in the absence of DOM were positively correlated with their calculated octanol-water partition coefficients ($\log K_{ow}$) ($P < 0.05$) (Fig. 2 and Table S3). This result agreed with our previous study on *Daphnia magna* (Dai et al., 2013). In addition, the BCF values obtained in this study (between 1.28 and 12.02 L·kg⁻¹) (Table 1 and Table S2) were much lower than previous studies about PFASs (between 4 and 18000 L·kg⁻¹) (Conder et al., 2008; Martin et al., 2003; Xia et al., 2013). This may be due to the lower trophic level organism and higher PFAS concentration. Many studies have shown that the BCF values of PFASs are connected with organism species, which differ in food sources, feeding types, uptake and excretion rates, and metabolism (Kannan et al., 2006; Naile et al., 2013a; Wang et al., 2011b). In this study, *Chironomus plumosus* larvae are benthic organism and belong to low trophic level creature. In addition, some investigators reported that PFAS bioconcentration had concentration dependency (Liu et al., 2011; Morikawa et al., 2006). For example, the body burden of PFASs in green mussels did not increase proportionally with the increase of PFAS concentration, while the BCF values decreased with increasing PFAS concentration (Liu et al., 2011). In the present study, the PFAS concentrations are higher than other studies (Liu et al., 2011; Xia et al., 2015a; Xia et al., 2013). Therefore, the obtained BCF values of PFASs were lower than other reports.

Fig. 2 Relationship between $\log K_{ow}$ and BCF_{ss} of PFASs in the absence of DOM (the data of $\log K_{ow1}$ were predicted by COSMOtherm model (Wang et al., 2011c); $\log K_{ow2}$ were calculated by SPARC model (Arp et al., 2006))

The effects of DOM on the PFAS accumulation in the larvae were dependent on DOM types and concentrations (Fig. 3 and Fig. S1). Upon bioconcentration equilibrium, the body burdens of PFASs with longer perfluoroalkyl chain, such as PFDoA, PFUnA, PFDA, and PFOS, decreased in the presence of PEP and HA, and the longer the perfluoroalkyl chain was, the more the body burden decreased at the same DOM concentration (Table S4). In addition, the decrease of PFAS body burden was larger in the presence of HA than PEP for PFDoA, PFUnA, PFDA and PFOS. For example, in the presence of $50 \text{ mg} \cdot \text{L}^{-1}$ HA and PEP, the body burden of PFDoA decreased by 51.1% and 38.0%, respectively. In the meantime, the body burdens of longer perfluoroalkyl chain PFASs decreased with the increase of PEP and HA concentrations. Correspondingly, the BCF_{ss} values of PFASs based on their nominal concentrations in water and their body burdens in the larvae also decreased with the increase of PEP and HA concentrations (Table S2). When the PEP and HA

concentrations increased from 0 to 50 mg·L⁻¹, the BCF_{ss} values of longer perfluoroalkyl chain PFASs decreased by 6.8%-38.0% and 12.6%-51.1%, respectively.

Fig. 3 Effects of DOM concentrations in the water system on the body burden of PFASs in *Chironomus plumosus* larvae after exposure for 10 days (steady state)

However, opposite to the effect of PEP and HA, FA and TA enhanced PFAS bioconcentration in the larvae. Upon bioconcentration equilibrium, the body burdens of longer perfluoroalkyl chain PFASs (PFDoA, PFUnA, PFDA, and PFOS) in the larvae increased with FA and TA concentrations. FA had a stronger effect on the PFAS bioconcentration than TA. For example, in the presence of 50 mg·L⁻¹ FA and TA, the body burden of PFDoA increased by 148.8% and 37.1%, respectively, compared to the control group without DOM. Similarly, the BCF_{ss} values of PFASs with longer perfluoroalkyl chain also increased with the concentrations of FA and TA (Table S2). When the FA and TA concentrations increased from 0 to 50 mg·L⁻¹, the BCF_{ss} values of longer perfluoroalkyl

chain PFASs increased by 7.5%-148.8% and 5.7%-37.1%, respectively.

Regarding to the PFASs with shorter perfluoroalkyl chain (PFOA and PFNA), upon bioconcentration equilibrium, the body burdens in the larvae did not change significantly with the variation of DOM concentration ($p > 0.05$). For example, when the PEP and HA concentrations rose from 0 to 50 mg·L⁻¹, the body burden of PFOA only decreased by 2.4% and 4.3%, respectively. In addition, regardless of DOM types and concentrations, the sequence of the PFAS body burden in the larvae was PFDoA > PFUnA > PFDA > PFOS > PFNA > PFOA, consistent with that in the control group. This indicates that the DOM addition did not change the bioconcentration sequence of individual PFAS.

3.3 Influencing mechanisms of different types of DOM on PFAS bioconcentration

3.3.1 Reduction effect of PEP and HA on PFAS bioconcentration

Many investigators considered that DOM-bound contaminants were lack of bioavailability, and the body burdens of chemicals in organisms were connected with their freely dissolved concentrations (Chen et al., 2010; Gouliarmou et al., 2012; Xia et al., 2015a). The relatively high partition coefficients (K_p) of PFASs between DOM (including humic substances and proteins) and water indicate a strong binding between PFASs and DOM (Xia et al., 2015a; Xia et al., 2013). Especially for PEP and HA, in this study, their relatively big aggregates with PFASs may be too large to be assimilated by the larvae. In addition, the binding between PFASs and DOM would make the freely dissolved concentration of PFASs decrease at the same time, and a higher DOM concentration would yield a lower freely dissolved PFAS concentration (Xia et al., 2013). Both big aggregations and reduced freely dissolved concentrations of PFASs would make k_u values decrease. Besides, the values of $BCF_{kinetic}$ based on k_u and k_e were almost the same as those of BCF_{ss} , which indicated that the body burdens of PFASs in the larvae were related not only to both k_u values but also to k_e values (Table 1). Although the k_e values decreased at the same time in the presence of PEP and HA, the k_u decreased more substantially than k_e (Table S5) and the reduction of k_u was

dominating. For example, compared to the control group without DOM, the k_u and k_e values of PFDoA decreased by 32.4% and 12.4% at 10 mg·L⁻¹ HA, respectively. Therefore, the body burdens of PFASs in the larvae decreased in the presence of PEP and HA, and there was a negative relationship between the body burdens of PFASs and the concentrations of these two types of DOM.

As for shorter perfluoroalkyl chain PFASs, such as PFOA and PFNA, the four types of DOM had no significant influence on their accumulation in the larvae. Some investigators indicated that the interaction between shorter perfluoroalkyl chain PFASs and proteins was weaker than the longer ones (Jones et al., 2003; Li et al., 2009), and the $\log K_p$ values of shorter perfluoroalkyl chain PFASs between DOM and water were lower than the longer ones (Xia et al., 2015a; Xia et al., 2013). Taking HA as an example, the K_p value of PFOA between HA and water was approximate one order of magnitude less than that of PFDoA (Xia et al., 2015a). Therefore, the change of the freely dissolved concentrations of shorter perfluoroalkyl chain PFASs might be less remarkable than the longer ones. Furthermore, the effect of DOM on the uptake and elimination rate constants of shorter perfluoroalkyl chain PFASs under the influence of DOM was comparable (Table 1 and Table S5). This resulted in the insignificant change of PFOA and PFNA bioconcentrations in the presence of DOM.

3.3.2 Enhancement effect of FA and TA on PFAS bioconcentration

Many researchers reported that an increased bioconcentration of organic compounds repeatedly occurred at low concentrations of DOM (Haitzer et al., 1998; Xia et al., 2015a; Xia et al., 2013). Our previous study showed that PFAS bioconcentration in *D. magna* increased at a low chicken egg albumin concentration level (1 mg·L⁻¹), but decreased at a high level (20 mg·L⁻¹) (Xia et al., 2015a). In this study, with the presence of FA and TA, the enhancement effect occurred not only at low DOM concentrations, but also at the high concentrations (Fig. 3 and Fig. S1), which was different from the previous studies. The mechanism about the enhancement of the organic pollutant bioconcentration caused by DOM remains unclear. As shown in Table 1 and Table S5, in

addition to k_u , the k_e values of PFASs also decreased in the presence of FA and TA, and k_e decreased more than k_u . For example, compared to the control group without DOM, the k_u values of PFDoA decreased by 7.2% at 10 mg·L⁻¹ FA while the k_e values decreased 27.0%. The reasons for the strong decrease effect of FA and TA on the elimination rate constants of PFASs from the larvae can be analyzed as follows. Smaller molecule weight DOM (below 3.5 kDa) can easily pass through cell membranes and be taken up by organisms (Timofeyev et al., 2007). In this study, the molecule weight of TA is 1701 and FA is several hundred to several thousands. Therefore, they could be taken up by the larvae in part at least. The absorbed DOM by the larvae might decrease k_e values by two ways. The one is that the absorbed DOM might bind with PFASs in the larvae, and the DOM-bound PFASs were too large to be eliminated from the larvae (Xia et al., 2015a), which caused the k_e values decrease. The other is that the absorbed DOM could inhibit the activity of P-glycoprotein, which has the function to increase the efflux of many xenobiotics from organisms (Rusiecka and Skladanowski, 2008; Timofeyev et al., 2007). For example, natural organic matters obviously decreased the elimination of rhodamine B from amphipod species, such as *Eulimnogammarus cyaneus* (Timofeyev et al., 2007). Some researchers reported that PFASs could be eliminated from HeLa cells by the P-glycoprotein (Rusiecka and Skladanowski, 2008). Therefore, the absorbed DOM could inhibit the elimination of PFAS from the larvae body. Both of the two ways resulted in the decrease of k_e values with the presence of FA and TA (Table 1), and the k_e values might decrease with the increase of FA and TA concentrations. Consequently, the body burdens of PFASs increased with the increase of FA and TA concentrations.

As discussed in the previous sections, the k_u values of PFASs in the larvae decreased in the presence of FA and TA; it means that the freely dissolved concentrations of PFASs decreased at the same time. However, the body burdens of some PFASs in the larvae increased. This finding suggests that the bioconcentration of PFASs may not simply depend on the freely dissolved concentrations of PFASs. It contradicts with our previous study that the equilibrium concentrations of PAHs in

fish positively correlated with their freely dissolved concentrations in the water (Xia et al., 2015c). We consider that two reasons may cause this result. Firstly, different from traditional HOCs such as PAHs, PFASs are a type of proteinophilic compounds (Borga et al., 2012; Jones et al., 2003; Vanden Heuvel et al., 1992). The bioconcentration equilibrium of PFASs may not be related to a partition between water and lipids in the larvae. The body burden of PFASs in aquatic organisms, such as green mussels, did not increase proportionally with the increase of PFAS concentration due to a nonlinear adsorption mechanism of PFASs in organisms (Liu et al., 2011). Secondly, the bond energy of C-F is so high that the PFASs are extremely difficult to be degraded in organisms (Conder et al., 2008). Therefore, the efflux of PFASs from the larvae may be the dominant way of their elimination. This route is related to multixenobiotic resistance system (MXR), which controls xenobiotics, such as PFASs and DOM, across the cell membrane (Rusiecka and Skladanowski, 2008; Timofeyev et al., 2007). Therefore, the inhibition effect of DOM on the PFAS elimination may enhance the PFAS bioconcentration in the larvae. However, more research should be conducted to study the mechanisms regarding the increase of PFAS bioconcentration in aquatic organisms under the influence of DOM with a small molecular weight.

4 Conclusions

The effects of four types of DOM on the bioconcentration of six PFASs in *Chironomus plumosus* larvae were studied in the present work. The DOM can form large DOM-PFAS aggregates and decrease the freely dissolved concentrations of PFASs, both of which made the uptake rate constants decrease. In the meantime, DOM could also decrease the elimination rates of PFASs from the larvae. The net effect of DOM on the PFAS bioconcentration in the larvae depended on the uptake and elimination rate constants. For the PEP and HA with relatively larger molecule weights, the decrease effect of uptake rate constants prevailed, leading to the reduction of PFAS bioconcentration with the increase of PEP and HA concentrations. For the FA and TA with relatively smaller molecule weights, the decrease effect of elimination rate constants dominated,

resulting in the enhancement of PFAS bioconcentration with the increase of FA and TA concentrations.

This study suggests that the molecule weights of DOM have important effects on the PFAS bioconcentration in aquatic organisms. Therefore, not only the concentration but also the molecule weight of DOM should be taken into consideration when studying PFAS bioconcentration in aquatic environment. In addition, more works should be carried out to investigate the uptake and elimination mechanisms of PFASs as well as their interaction with DOM in organisms.

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