TROPHIC MAGNIFICATION AND ISOMER FRACTIONATION OF PERFLUOROALKYL SUBSTANCES IN THE FOOD WEB OF TAIHU LAKE, CHINA

Lingyan Zhu, Chengdong Zhuang, Shuhong Fang, Xinwei Chen

College of Environmental Science and Engineering, Tianjin Key Laboratory of Environmental Remediation and Pollution Control, Key Laboratory of Pollution Processes and Environmental Criteria, Ministry of Education, Nankai University, Tianjin, P.R. China 300071

Introduction

Perfluoroalkyl substances (PFASs), including perfluorosulfonates (PFSAs) and perfluorocarboxylates (PFCAs), are widely used in various consumer products.[1, 2] They are identified in wildlife all around the world, even in remote Arctic wildlife. Two primary processes, electrochemical fluorination (ECF) and telomerization, have been used to synthesize PFASs. The ECF products are comprised of complex isomeric mixtures with rather consistent compositions: 70-80% linear and 20-30% branched isomers.[3,4] Telomerization method, which was developed by Dupont in the 1970s and produces strictly linear geometry, becomes the predominant method to manufacture PFOA.[5]

Many laboratory studies and field investigations demonstrated that PFASs, especially for PFOS and long-chain PFCAs, could be bioaccumulated in various organisms. Among the studies regarding to PFAS biomagnification,[6-8] most of them were about marine food webs and high trophic level organisms (TL >5), such as seabirds and marine mammals, were included, and inconsistent biomagnification results were observed. A few of them were about PFAS biomagnification in fresh water food webs and all mostly in Lake Ontario. Moreover, most of the studies took PFASs as single compounds without further identification of their isomers. Sparse information is available for the fractionation of the isomers along the food web.

The current study aimed to: (1) investigate the accumulation levels of PFASs and homologue profiles in the various aquatic organisms; (2) to study the isomeric profiles of PFOA, PFOS and perfluorooctane sulfonamide (PFOSA) in the water and biota samples in Taihu Lake; (3) to evaluate bioaccumulation and trophic magnification of PFASs and isomeric discrimination in aquatic organisms.

Materials and methods

The sampling took place in May, 2012 at Meiliang area of Taihu Lake. Surface water (n=5), phytoplankton [pooled samples (p)=3], zooplankton (p=3), invertebrates [including Taihu Lake shrimp (n=30), white shrimp (n=6), freshwater mussel (n=3), pearl mussel (n=3)], fishes [including minnow (n=10), silver carp (n=3), whitebait (n=60), crucian (n=6), lake saury (n=10), carp (n=3), mongolian culter (n=20), mud fish (n=10), Chinese bitterling (n=60), gobies (n=30)] were collected. The samples were brought to the laboratory immediately for further pretreatment (stored at -20 °C). Homogenized plankton, muscle tissue of fish and shrimp, and soft parts of the bivalves were freeze dried at -60 °C for 48 h and stored at -20 °C for further extraction.

Filtered water was concentrated using Cleanert PEP cartridges (made of polydivinylbenzene on which the surface is functionalized with vinyl prolidone, 500 mg/6 mL, Bonna-Agela Technologies, China). Zooplankton, invertebrates and fishes were extracted with 10 mM NaOH in methanol, followed by purification with Pesti-Carb (made of graphitized carbon by a distinct surface modification process, 500 mg/6 mL, Bonna-Agela Technologies, China) and PWAX (a weak anion exchange SPE cartridge, 500 mg/6 mL, Bonna-Agela Technologies, China) method. Phytoplankton samples were extracted by DCM and methanol and followed by purification with Pesti-Carb and PWAX cartridges. PFASs and the isomers were separated and quantified by UPLC-MS/MS (electrospray ionization in negative mode) with a FluoroSep-RP Octyl column.

Results and discussion

Concentrations and profiles of PFASs in the water and biota samples

The concentration of total PFASs (∑PFASs) in the water phase of Meiliang area of Taihu Lake was 70.8 ± 5.7 ng/L, with the mean concentration of ∑PFOA (30.5 ± 3.0 ng/L) as the highest, followed by perfluorohexanoate (PFHxA) (16.6 ± 0.81 ng/L), ∑PFOS (13.7 ± 1.9 ng/L), perfluorobutanesulfonate (PFBS) (3.76 ± 0.32 ng/L) and PFHpA (2.94 ± 0.31 ng/L). The concentrations of long chain PFCAs, such as PFDA, PFUnA and PFDoA in water were very low, with the concentrations less than 1 ng/L. The relatively high concentrations of these short-chained PFSAs and PFCAs might be due to their lower K_{oc} as compared to longer homologues (C>7). In addition, it suggests that the production volume of short-chained PFCAs and PFSAs, which are being used as alternatives of longer PFASs due to their lower toxicity, is increasing since the voluntarily phase out of PFOS and related products by 3M.

The concentration of total PFASs (∑PFASs) ranged from 5.19 to 165 ng/g ww (wet weight) in the biota samples collected from Taihu Lake. As compared to water samples, the profiles of PFASs in the organisms are quite different, as shown in Table 1. ∑PFOS and PFUnA were predominant in the biota samples with concentrations in the range of 2.04-94.9 and 0.602-59.9 ng/g ww. For most of the organisms, the concentration of PFOS was much higher than PFUnA except planktons and pearl mussel. They were followed by some PFCAs with long carbon chain (C>8), such as PFDA (0.222-14.7 ng/g ww), PFNA (0.0858-11.0 ng/g ww), ∑PFOA (<MDL-8.65 ng/g ww) and PFDoA (0.0553-3.31 ng/g ww). PFOS, PFNA, PFDA, PFUnA and PFDoA were detected in all the biota samples, while the short-chained PFASs were less frequently detected. The detection frequency of perfluoroheptanoate (PFHpA) was 75%, perfluorohexanesulfonate (PFHxS) was 44%, PFBS was 38%, and PFHxA was only 19% despite its rather high concentration in water. This suggests that they have higher bioaccumulation potential than the short ones.

PFOA isomer profiles

In water samples, *n*-PFOA was the predominant isomer, with a proportion of 83.3%, followed by *iso*-PFOA (7.93%), 5*m*-PFOA (4.75%), 4*m*-PFOA (4.13%). Despite that the proportion of *n*-PFOA was slightly higher than in historical 3M ECF PFOA (ca. 78.0%), the isomeric profile still agreed with the signature of the 3M ECF product (78.0% *n*-PFOA, 10.1% *iso*-PFOA, 3.90% 4*m*-PFOA, 3.12% 5*m*-PFOA) and TPFOA provided by Wellington Laboratories (79.0% *n*-PFOA, 9.00% *iso*-PFOA, 4.00% 4*m*-PFOA, 4.50% 5*m*-PFOA). The presence of branched PFOA isomers (including *iso*-, 4*m*- and 5*m*-PFOA) in water phase implies that the ECF PFOA product makes significant contribution to the PFOA in Taihu Lake. Considering that branched isomers are more soluble than *n*-PFOA, the proportion of *n*-PFOA in water would be lower than in ECF product. However, the result was opposite. This suggests that there is an input of *n*-PFOA from an additional source: telomerized PFOA, which contains strictly linear PFOA isomer.

In the biota samples, *n*-PFOA was still predominant, and the proportion of *n*-PFOA was within a range of 91.9-100%, even higher than in water. The most frequently detected branched isomer was 4*m*-PFOA with a detection frequency of 12.5%, while *iso*-PFOA and 5*m*-PFOA were only found in whitebait, in which their contributions (0.45%, 0.25%) were significantly lower than in the ECF products (10.1%, 3.12% respectively). The much higher proportion of *n*-PFOA in biota than in water suggests preferential enrichment of *n*-PFOA relative to the branched isomers or preferential elimination of branched ones in biota.

PFOS and PFOSA isomer profiles

Unlike PFOA, which likely has both telomerization and ECF inputs, PFOS is primarily produced by ECF method. Thus, comparing the distribution profile of PFOS isomers in the environment samples with that in commercial products could give some information with respect to isomer discrimination processes. In water samples, *n*-PFOS was the predominant isomer with a proportion of 41.6%, followed by 3+5*m*-PFOS (23.9%), m_2-PFOS (12.5%) and 4*m*-PFOS (10.3%) (Figure 1). The total branched PFOS isomers contributed 58.4%, which is much higher than the branched content in 3M ECF standard (27.2%) and Jinfu K-PFOS (30.8%, a commercial product manufactured in China). This suggests that *n*-PFOS is depleted while branched isomers are enriched in water. This could be explained by preferential sorption of *n*-PFOS to sediment.[9] In addition, *n*-PFOS could also be adsorbed by suspended particulate matter due to its high organic matter content.

Similar to PFOA, *n*-PFOS was also predominant in biota samples, and displayed much higher proportion than in water: 78.6% (in minnow) to 95.5% (in white shrimp). It was followed by 3+5*m*-PFOS (mean percentage of 4.14%), 4*m*-PFOS (2.92%), *iso*-PFOS (2.82%), m_2-PFOS (0.900%), 1*m*-PFOS (0.534%). These results suggest that *n*-PFOS is preferentially assimilated or bioconcentrated in biota. The *n*-PFOS proportion in benthic biota, including invertebrates (89.8-95.5%) and benthic fishes, such as crucian (93.8%), mud fish (92.3%) and Chinese bitterling (93.4%), was higher than in the pelagic fishes (such as minnow (78.6%), sliver carp (87.7%),

lake trout (86.6%)). This could be explained by the higher proportion of *n*-PFOS in sediment than in water and ingestion of sediment is an important source of PFASs for the benthic biota.

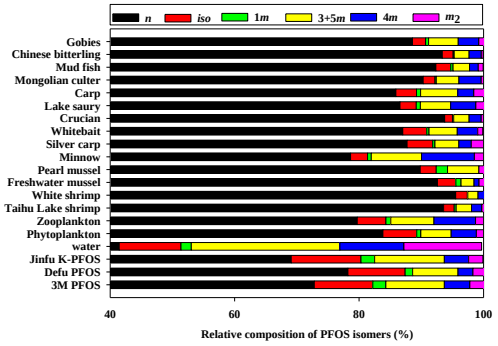

Figure 1

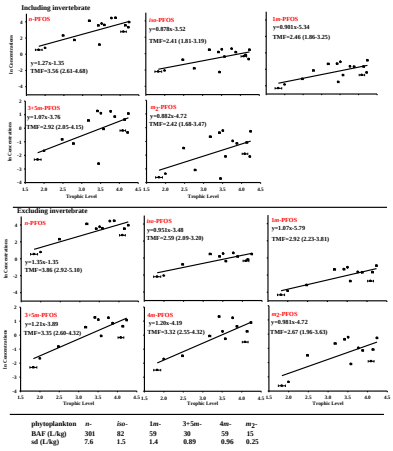

Figure 2

Bioaccumulation and biomagnification of PFASs

The low detection frequency of PFHxA in biota samples indicates that it has very low potential for bioaccumulation, which is in line with the previous reports. For PFCAs with longer perfluorinated carbon chain length (C8-C12), they were found in all the organisms.

$\delta^{15}N$ and $\delta^{13}C$ have been used to determine trophic relationships in many studies. $\delta^{15}N$ is often used to determine trophic level position due to its stable enrichment in food web. Overall, the species displayed an overlap in $\delta^{13}C$ throughout the food web, suggesting that the some species shared similar carbon sources. The results indicate that predator-prey relationship exist among the studied food web.

Linear regression was used to evaluate the association between PFAS concentrations and TLs among the seven most frequently detected PFASs, including ∑PFOA, PFNA, PFDA, PFUnA, PFDoA, ∑PFOS and PFOSA. Only PFDA, PFDoA and ∑PFOS displayed positively linear relationship with TLs (p<0.05) if the invertebrates were all included. Thus, the TMFs were calculated for these three compounds, and they were all greater than 1 (TMF = 2.43 for PFDA, 2.68 for PFDoA and 3.46 for ∑PFOS), indicating that PFCAs with long carbon chain length and PFOS are biomagnified in the food chain. If the invertebrates were not included, significant linear relationship with TLs (p<0.05) was observed for all of them except PFOSA (see Figure S6, Table S4). The TMFs were calculated to be 2.13, 2.19, 2.53, 2.25, 3.19 and 3.74 for ∑PFOA, PFNA, PFDA, PFUnA, PFDoA and ∑PFOS. For PFCAs, the TMF increased with the perfluoroalkyl chain length except for PFUnA, suggesting that TMF increases with their log K_{ow}. It was reported that the log K_{ow}s of PFASs increase with their carbon chain length and the estimated log K_{ow} values of PFOS and PFCAs with 7-11 perfluorinated carbon chains are in the range of 3.6-7.1. Among all the PFASs, PFOS displayed the highest TMF value, which is in agreement with some previous studies. The relatively higher TMF of PFOS may be attributable to metabolism of its precursors,[10] such as *N*-Methyl perfluorooctane sulfonamide (MeFOSA), *N*-Ethyl perfluorooctane sulfonamide (EtFOSA), *N*-Methyl perfluorooctane sulfonamidoethanol (MeFOSE) and *N*-Ethyl perfluorooctane sulfonamidoethanol (EtFOSE). All of these compounds are the precursors of a wide variety of commercial products and are produced in large quantities.

Bioaccumulation and Biomagnification of PFOS Isomers

Figure 2 lists the BAFs of individual PFOS isomers in phytoplankton. The BAF was 301 ± 7.6, 82 ± 1.5, 59 ± 1.4, 30 ± 0.89, 59 ± 0.96 and 15 ± 0.25 for *n*-PFOS, *iso*-, 1*m*-, 3+5*m*-, 4*m*- and m_2-PFOS, respectively. Linear PFOS displayed much higher bioaccumulation ability than the branched isomers. Among all the isomers, m_2-PFOS displayed the lowest BAF value, suggesting it is very difficult to be bioaccumulated in biota.

Statistically significant correlation was observed between the ln concentrations of all the isomers and the TLs of biota (p<0.05) except 4*m*-PFOS if all the organisms were included. Thus TMFs of all PFOS isomers were calculated and they were greater than unit Figure 2) with the highest TMF observed for *n*-PFOS (3.56), followed

by 3+5*m*-PFOS (2.92), 1*m*-PFOS (2.46), m_2-PFOS (2.42) and *iso*-PFOS (2.41). If invertebrates were excluded, the TMFs could be calculated for all the isomers, and they were 3.86 for *n*-PFOS, 3.35, 3.32, 2.92, 2.67 and 2.59 for 3+5*m*-, 4*m*-, 1*m*-, m_2- and *iso*-PFOS branched isomers.

Due to lack of knowledge on physicochemical properties of PFOS isomers, we could only speculate the hydrophobicity of isomers by their elution behaviors on the FluoroSep-RP Octyl column, which was used for the separation in the present study. The PFOS isomers were eluted in the order of: m_2-, 1*m*-, 3*m*-, 4*m*-, 5*m*-, *iso*- and *n*-PFOS in present study and previous study. In general, more hydrophobic isomers would be eluted later. Thus, the order of hydrophobicity of the isomers is in line with the TMFs found in present studies (except *iso*-PFOS).
The results in present study indicates that linear isomers of PFOA and PFOS are preferentially accumulated in the organisms. In addition, linear PFOS was more trophic magnified than branched isomers, suggesting that the composition of PFOS isomers would change at different trophic levels. It has been reported that the toxicity of PFASs can also be isomer-specific. Experimental research on rats and mice found that the body weight was more affected by pure *n*-PFOA than 80% linear/20% branched PFOA mixture. In addition, O'Brien et al. also reported that technical PFOS (comprised of 65% linear and 35% branched isomers) induced greater transcriptionally response than pure *n*-PFOS in the cultured chicken embryonic hepatocyte. These suggest that the PFASs may display different toxic effects to the organisms at different trophic levels. However, studies are sparse on the toxicities of PFASs isomers due to the lack of individual isomer standards, especially on the aquatic organisms. Further research on PFAS isomers is warranted to properly understand the their ecological risk.

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