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Bioaccumulation and risk assessment of per- and polyfluoroalkyl substances in wild freshwater fish from rivers in the Pearl River Delta region, South China

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ABSTRACT

Per- and polyfluoroalkyl substances (PFASs) are used in various industries, which results in their ubiquitous occurrence in the environment. This study determined the concentrations of eighteen PFASs in muscle and liver of nine wild freshwater fish species collected from rivers in the Pearl River Delta (PRD) region, South China, and assessed their bioaccumulation and potential health risks to local people. The results showed that eight and twelve PFASs were detected in the fish muscle and liver samples, respectively. Perfluorooctane sulfonate (PFOS) was found to be the predominant PFAS both in muscle and liver with its highest concentrations of 79 ng/g wet weight (ww) in muscle and 1500 ng/g ww in liver, followed by Perfluoroundecanoic acid (PFUnDA) and Perfluorotridecanoic acid (PFTrDA) with trace concentrations. The mean PFOS concentrations in fish muscle and liver tissues of the nine collected species ranged from 0.40 ng/g in mud carp to 25 ng/g in snakehead, and from 5.6 ng/g in mud carp to 1100 ng/g in snakehead, respectively. Significant positive correlations were found among PFASs both in water and fish, indicating a similar pollution source for these PFASs. In tilapia samples, PFOS concentrations showed an increasing trend with increasing length and weight, but no significant difference between genders. Bioaccumulation factors (log BAF) in fish for the PFASs were in the range from 2.1 to 5.0. The calculated hazard ratios (HR) of PFOS for all fishes were in the range of 0.05–2.8, with four out of nine species (tilapia, chub, leather catfish and snakehead) having their HR values more than 1.0. The results suggest that frequent consumption of these four fish species may pose health risks to local population.

1. Introduction

Per- and polyfluoroalkyl substances (PFASs), including perfluorinated carboxylates (PFCAs) and sulfonates (PFSAs), are a class of man-made organic chemicals widely used in industrial applications, such as carpet, metal plating, fire-fighting foams, semiconductor and food packaging, paper and other areas since the mid-20th century (Key et al., 1997; Giesy and Kannan, 2001; Moody and Field, 2000; Lewandowski et al., 2006). After almost a half century use, Giesy and Kannan (2001) first reported the occurrence of PFASs in wildlife, and this raised great concern over scientific community. Subsequently, high bioaccumulation was observed in biota for this group of chemicals; for example, a bioaccumulation factors (BAF) of 23,000 was found for perfluoro-*n*-tridecanoic acid (PFTrDA) in rainbow trout under laboratory exposure conditons (Martin et al., 2003). Meanwhile, adverse effects including hepatotoxicity, developmental toxicity, immunotoxicity and hormonal effects in animals have been proven because of exposure to PFASs (Lau et al., 2007; Peters and Gonzalez, 2011). As of their unique physicochemical properties and persistence, bioaccumulation (biomagnification) and toxic properties (PBT), ever since then, large amount of studies on PFASs especially PFOS and PFOA have been performed worldwide mainly on their occurrence and toxicity. Because of the properties of high solubility of PFASs, most of this group of chemicals would exist mainly in water phase, but some of PFASs could accumulate in fish. It proved ubiquity of this group of chemicals with ng/L levels in surface water (Hansen et al., 2002; Hong et al., 2013), ng/g levels in biota (Giesy and Kannan, 2001; Tao et al., 2006; Bloom et al., 2009), and ng/mL levels in human serum (Hansen et al., 2001). As a result, PFOS and its related chemicals were phased out in the

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United States in 2002, and listed to Annex B of Stockholm Convention which restricted its production and use worldwide UNEP (2009).

Previous studies have reported that air, drinking water, indoor dust and food are the primary pathways for human exposure to PFASs (Fromme et al., 2009;Vestergren and Cousins, 2009; Zhang et al., 2011; Knobeloch et al., 2012). Food consumption is believed to be the major pathway for human exposure to PFASs, contributing more than 60 percent of total lifetime exposure (Tittlemier et al., 2007). In particular, fish has been suggested as the most important source of PFASs exposed to humans through dietary route (Haug et al., 2010).

China is the largest fish production country, with the production volume of 47.5 million tons in 2008, and wild fish (14.8 million tons) accounted for 31.2 percent of the total production (Food and Agriculture Organization of the United Nations, 2010). As a highly urbanized region of the Pearl River Delta (PRD), it would consume more fish than other regions. Kannan et al. (1997) reported that in the PRD, consumption of contaminated fish is one of the major pathways for human exposure to organic pollutants. However, no large-scale study focusing on PFASs in wild freshwater fish samples has been performed in China until now. Moreover, there is scarce information on the risks of PFASs exposure via wild fish consumption in China, especially in the PRD region.

The objectives of this study were: (1) to investigate the contamination levels and profiles of eighteen PFASs (11 PFCAs, 5 PFSAs, 1 perfluoro-1-octansulfonamide (PFOSA) and 1*N*-ethylperfluoro-1-octanesulfonamido acetic acid (*N*-EtFOSAA)) in different fish species collected from rivers of the PRD region; (2) to evaluate the gender-, body weight- and length-related PFASs bioaccumulation in a model fish species (tilapia); and (3) to assess the potential risks of local people exposure to PFASs through fish consumption. The results from this study can help better understand the contamination of PFASs in the rivers of the PRD region and assist local governments to better manage the exposure risks.

2. Materials and methods

2.1. Chemical and reagents

Eighteen PFASs were examined in this investigation, with their full names, abbreviations and formula being given in Table S1. Purities of all the analytical standards were more than 95 percent. PFBA, PFPeA and PFH_XA were purchased from J&K Company (Guangzhou, China), Acros Organics (Geel, Belgium) and Tokyo Chemical Industries (Portland, OR, USA), respectively. PFOA and PFOS were obtained from Accustandard (New Haven, USA). PFHpA, PFNA, PFDA, PFD_oDA and PFTeDA were acquired from Alfa Aesar (Ward Hill, MA, USA), while PFUnDA, PFTrDA, PFBS and PFH_XS were obtained from Sigma-Aldrich (St. Louis, USA). PFHpS, PFDS, N-EtFOSAA and internal standards ($MPFH_XA$ ($^{13}C_2$-PFH_XA), MPFOA ($^{13}C_4$-PFOA), MPFNA ($^{13}C_5$-PFNA), MPFDA ($^{13}C_2$-PFDA), $MPFH_XS$ ($^{18}O_2$-PFH_XS), and MPFOS ($^{13}C_4$-PFOS)) were bought from Wellington laboratories (Guelph, ON, Canada). LC–MS grade ammonium acetate (> 99 percent) was purchased from CNW (Dusseldorf, Germany). Potassium hydroxide was obtained from Sigma-Aldrich (St. Louis, MO, USA). Ammonium hydroxide (10 percent) and acetic acid were bought from Fluka (Germany). HPLC grade methanol (MeOH) was purchased from Merck Corporation (Darmstadt, Germany). The cartridges used for purification were Oasis WAX cartridges (150 mg sorbent, 6 mL size) from Waters (Milford, MA, USA). Ultrapure water was supplied by a Milli-Q system from Millipore (Watford, UK). Individual stock solutions of the target analytes and internal standards were prepared in methanol and stored in polypropylene (PP) bottles at −18 °C.

2.2. Sample collection and sample pretreatment

The study area is shown in Fig. 1, which lists the location of sampling sites in the rivers of the PRD, South China. Fish samples were collected by electroshocking and netting from 11 monitoring sites in the year of 2011–2012. Surface water samples were also collected for two seasons at the same time. Three replicate water samples were collected from each site in each season using a clean stainless steel bucket or polypropylene containers and stored in polypropylene containers with narrow mouths and screw tops. Detailed information about the sampling sites and collected fish species are given in Table 1. The collected fish species in this study included tilapia (*Tilapia aurea*), crucian carp (*Carassius auratus*), common carp (*Cyprinus carpio*), leather catfish (*Clarias fuscus*), snakehead (*Ophicephalus argus*), grass carp (*Ctenopharynodon idellus*), chub (*Hypophthalmichthys molitrix*), mud carp (*Cirrhinus molitorella*), and bream (*Parabramis pekinensis*). All the collected fish samples were kept alive in cold water with oxygen supply and immediately transported to the laboratory after collection. Once arrived in the laboratory, those fish were anaesthetized and skins were removed, and the muscle samples were cut into small pieces. And only muscle and liver samples were used for this study. Each fish sample was individually wrapped in aluminum foil and then put in polyethylene bags. Then the muscle tissues were freeze-dried, ground to fine powder, wrapped in aluminum foil and stored at −18 °C until extraction. Liver samples were wrapped in aluminum foil directly and stored at −18 °C until extraction.

2.3. Sample extraction

The collected water samples were filtered using glass fiber filters (GFF, Whatman, O.D. 47 mm, 0.7 μm), stored in a cold room at 4 °C in darkness and extracted within five days. The water samples (500 mL each) were extracted by solid phase extraction (SPE) using Waters Oasis WAX Cartridges, which is adopted from a previous reported method (Taniyasu et al., 2005), with addition of the internal standards mixture (5 ng each) prior to extraction. Two different extraction methods (alkaline digestion and ion-pairing methods) were used for the extraction of muscle and liver samples in this study, respectively. For the muscle samples, a previous reported alkaline digestion method was used in the extraction (Taniyasu et al., 2005). In brief, 0.2 g of each dried muscle sample (approximately 1.0 g wet sample) was weighed into a 50 mL PP centrifuge tube, followed by addition of 5 ng of each internal standard. Then 10 mL of 10 mM KOH in methanol was added to the tube, which was shaken at 250 rpm for 16 h. After digestion and centrifugation, the supernatant was transferred to a 250 mL PP bottle and diluted to 200 mL with Milli-Q water, which was used for purification with an Oasis WAX cartridge. The cartridge was pre-conditioned with 4 mL 0.1 percent NH_4OH in MeOH, 4 mL MeOH and 4 mL Milli-Q water. After loading, the target compounds were eluted from the cartridge with 4 mL MeOH and 4 mL 0.1 percent NH_4OH in MeOH. Then the eluate was brought to dryness under a gentle stream of nitrogen, and then reconstituted in 500 μL methanol. The final extract was filtered through a 0.22 μm nylon filter into a 1 mL PP snap top vial with a polyethylene (PE) cap and stored in −18 °C until analysis.

For the liver samples, the ion-pairing liquid extraction method was applied in this study for PFASs as described elsewhere (Yeung et al., 2006). Briefly, 0.2 to 0.5 g of each wet liver sample was weighed into a 50 mL PP tube and homogenized by IKA T10 basic ULTRA-TUTTAX homogenizer (Germany) at 30,000 rpm with 2 mL Milli-Q water, 2 mL of 0.25 M sodium carbonate buffer and 1 mL of 0.5 M tetrabutylammonium hydrogen sulfate (TBAHS) solution. After completely homogenized, the PP tube was vigorously shaken for 5 min for extraction. After thorough mixing, 5 mL of methyl-*tert*-butyl ether (MTBE) was added into the tube, and the mixture was shaken again for 20 min. The organic and aqueous layers were separated by centrifugation at 3500*g* for 20 min, and an exact volume of 4 mL of MTBE was transferred into a 10 mL PP tube. Another 5 mL of MTBE was added into the remnant aqueous mixture again, followed by shaking and centrifuging with the above conditions, the supernatant was combined with the first one in the 10 mL PP tube. The MTBE extract was allowed to evaporate to dry under nitrogen and reconstituted in 500 μL of methanol. The final extract was filtered through a 0.22 μm nylon filter into a 1 mL PP snap top vial with a PE cap and stored in −18 °C until analysis.

2.4. Chemical analysis

High performance liquid chromatography–tandem mass spectrometry (LC–MS/MS) was used to determine the concentrations of the target PFASs in the extracts. The instrument used in the analysis was an Agilent 1200 HPLC system interfaced to an Agilent 6460 Triple Quadrupole mass spectrometer that was operated under electrospray negative ionization (ESI-) mode. A 5 μL aliquot of each sample extract was injected into the instrument. The target compounds were separated on a Betasil C18 column (2.1 mm i.d. × 50 mm length, 5 μm; Thermo Hypersil-Keystone, Bellefonte, PA, USA) with a pre-column (2.1 mm, 0.2 μm; Agilent Technologies). The mobile phase used consisted of 2 mM ammonium acetate aqueous solution (solvent A) and methanol (solvent B) at a flow rate of 250 μL/min. The gradient program of the mobile phase was given as follows: 10 percent B at 0 min, increasing linearly to 35 percent B at 0.1 min, 55 percent B at 7 min, and finally to 95 percent B at 17 min and kept for 1 min, then reversing to 10 percent B at 20 min. The capillary voltage was held at 3500 V. Dry and sheath gas flows were maintained at 6 and 12 L/min, respectively. Dry and sheath temperatures were kept at 325 and 350 °C, respectively. The mass spectrometer was operated under multiple reaction monitoring (MRM) mode. The MS/MS mass transition, fragmentor and collision energy of each compound are listed in Table S1.