Perfluorooctane sulfonate (PFOS) and other fluorochemicals in fish blood collected near the outfall of wastewater treatment plant (WWTP) in Beijing

Xuemei Li [a,d], Leo Wai Yin Yeung [b,c], Muqi Xu [a], Sachi Taniyasu [c], Paul K.S. Lam [b], Nobuyoshi Yamashita [c,**], Jiayin Dai [a,*]

[a] Key Laboratory of Animal Ecology and Conservation Biology, Institute of Zoology, Chinese Academy of Sciences, Datun Road, Chaoyang District, Beijing 100101, PR China
[b] Department of Biology and Chemistry, City University of Hong Kong, HK SAR, PR China
[c] National Institute of Advanced Industrial Science and Technology, 16-1 Onogawa, Tsukuba, Ibaraki 305-8569, Japan
[d] Graduate School of the Chinese Academy of Sciences, Beijing 100039, PR China

Distribution of PFCs reveals varied composition profiles in zooplankton and fish from a recipient water affected by WWTPs in Beijing.

ARTICLE INFO

ABSTRACT

Perfluorinated compounds (PFCs) were measured in zooplankton and five fish species collected from Gaobeidian Lake, which receives discharge from wastewater treatment plant (WWTP) in Beijing, China. The mean total PFCs in five fish were in the order: crucian carp > common carp > leather catfish > white semiknife carp > tilapia. Perfluorooctane sulfonate (PFOS) occurred at the greatest concentrations, with mean concentrations ranging from 5.74 to 64.2 ng/ml serum. Perfluorodecanoic acid (PFDA) was the second dominant PFC in fish samples except for common carp in which perfluorooctane sulfonamide (PFOSA) was dominant. A positive linear relationship ($r^2 = 0.85$, $p < 0.05$) was observed between ln PFOS concentrations (ln ng/ml) and trophic level (based on δ^{15}N) if tilapia was excluded. The risk assessment showed that PFOS might not pose an immediate risk to fish in Gaobeidian Lake.

© 2008 Elsevier Ltd. All rights reserved.

1. Introduction

PFCs are a group of emerging chemicals having abilities to repel both water and oil. The strong carbon–fluorine bond accounts for the strong thermal and chemical stability of these compounds. These characteristics make them ubiquitous in the environments such as lake water (Moody et al., 2002), seawater (Taniyasu et al., 2003), river water (So et al., 2007), sediment and sludge (Higgins et al., 2005), fish (Taniyasu et al., 2003; Yamashita et al., 2004), wildlife (Dai et al., 2006; Giesy and Kannan, 2001) and human (Kannan et al., 2004; Taniyasu et al., 2003; Yeung et al., 2006). To date, limited studies on PFCs were carried out in water (So et al., 2007), panda (Dai et al., 2006) and human (Yeung et al., 2006) in China. Results showed that the widespread occurrence of PFCs in China and perfluorooctane sulfonate (PFOS) was the major PFC in those samples.

Discharge of municipal wastewater treatment plants (WWTPs) is recognized as an important source of some organic contaminants including PFCs in aquatic environments (Loganathan et al., 2007; Sinclair and Kannan, 2006). Although some studies have reported the fate and biodegradation of PFCs in WWTPs (Higgins et al., 2005; Schultz et al., 2006; Wang et al., 2005), few studies have been conducted on the levels of PFCs in organisms in the recipient water. With the rapid development in China, more water from WWTPs will be recycled in the near future in Beijing, China. Therefore, studies on concentrations of PFOS and other PFCs in WWTP effluents are becoming more important. Gaobeidian Lake has a surface area of about 0.15 km^2 and its water mainly comes from Gaobeidian WWTP, which processes approximately 1 million tons of wastewater per day and discharges 30% of its effluent into Gaobeidian Lake. Water in Gaobeidian Lake is also used as a recycling coolant for the Beijing Guohua Thermal Power Plant. Consequently, the resultant water temperature in the lake is high (between 12 and 41 °C) corresponding to seasonal changes, and it is about 5–10 °C higher than the ambient water temperature. The water in Gaobeidian Lake first runs into Tonghui River and finally into the largest estuary, Bohai Bay in China (Fig. 1).

* Corresponding author. Tel.: +86 10 64807185; fax: +86 10 64807099.
** Corresponding author. Tel./fax: +81 29 8618335.
E-mail addresses: daijy@ioz.ac.cn (J. Dai), nob.yamashita@aist.go.jp (N. Yamashita).

The objective of this study is to determine the concentrations of PFCs in zooplankton and fish from Gaobeidian Lake. The risk assessment of PFCs for fish is also discussed in details.

2. Materials and methods

2.1. Sample collection

Zooplankton (dominating species were *Moina rectirostris*, *Moina micrura* and *Moina macrocopa*) was collected with a 30 μm net and was then concentrated by centrifugation. Five species of fishes including common carp (*Cyprinus carpio*), crucian carp (*Carassius auratus*), leather catfish (*Clarias lazera*), white semiknife carp (*Hemiculter leucisculus*), and tilapia (*Oreochromis niloticus*) were collected from Gaobeidian Lake during December 2005 to April 2007. The whole blood of common carp ($n = 6$), leather catfish ($n = 6$), tilapia ($n = 2$), white semiknife carp ($n = 2$) and the serum of crucian carp ($n = 13$) were sampled. One pooled sample of white semiknife carps was made of five individuals due to its small size. All samples were kept at −20 °C until analysis.

2.2. Reagents and chemicals

The potassium salt of PFOS, potassium salts of perfluorohexanesulfonate (PFHxS), perfluorooctane sulfonamide (PFOSA), perfluorononanoic acid (PFNA), perfluorooctanoic acid (PFOA), and perfluoroheptanoic acid (PFHpA) were purchased from Wellington Laboratories Inc. (ON, Canada). Unsaturated fluorotelomer carboxylate (8:2 FTUCA) and saturated fluorotelomer carboxylate (8:2 FTCA) were provided by Asahi Glass Co Ltd. (Tokyo, Japan). Perfluorohexanoic acid (PFHxA) was purchased from Wako Pure Chemical Industries Ltd (Osaka, Japan). Perfluorodecanoic acid (PFDA) and perfluoroundecanoic acid (PFUnDA) were purchased from Fluorochem Ltd (Derbyshire, UK). Perfluorobutanesulfonate (PFBS) was purchased from Chiron (Trondheim, Norway). Purities of all the analytical standards were ≥95%. Oasis® weak anion exchange (WAX; 6 cc, 150 mg, 30 μm) solid phase extraction (SPE) cartridges were purchased from Waters (Milford, MA). Milli-Q water was used throughout the whole experiment. Methanol (residual pesticide and PCB analytical grade), ammonium acetate (97%), ammonium solution (25%), acetic acid (99.9%), tetra-*n*-butyl ammonium hydrogen sulfate (TBA), methyl-*tert*-butyl ether (MTBE), sodium carbonate and sodium hydrogen carbonate (99%) were from Wako Pure Chemical Industries (Osaka, Japan).

2.3. PFC analysis

The samples were analyzed for 12 PFCs (including PFOS, PFHxS, PFBS, PFOSA, PFUnDA, PFDA, PFNA, PFOA, PFHpA, PFHxA, 8:2 FTCA and 8:2 FTUCA) using a high-performance liquid chromatography–tandem mass spectrometer (HPLC–MS/MS). After the samples were thawed at room temperature, extraction and analysis of PFCs were carried out with some modifications according to the protocol described elsewhere (Hansen et al., 2001; Taniyasu et al., 2005; Yeung et al., 2006). Extraction of PFCs from blood/serum/zooplankton samples was done using an ion pairing extraction method which was described elsewhere (Hansen et al., 2001) and the final ion pairing extract was then subjected for a further cleanup process using SPE-Oasis-WAX-method (Taniyasu et al., 2005). In brief, 0.5 ml/g whole blood/serum/zooplankton was mixed with 1 ml of 0.5 M TBA solution and 2 ml of 0.25 M sodium carbonate buffer (pH 10) and then added to a 15-ml PP tube for extraction. After

mixing, 5 ml of MTBE was added, and the mixture was shaken for 20 min at 250 rpm. The organic and the aqueous layers were separated by centrifugation at 3000 rpm for 15 min. MTBE (4 ml) was removed and transferred to a second new 15-ml PP tube. The extraction was repeated twice as described above, except that 5 ml of MTBE was removed each time, instead of 4 ml. All three extracts were combined in the second 15-ml PP tube. The final extract was concentrated under nitrogen gas after adding 1 ml MeOH. After that, 0.5 ml of the final solution was diluted into 100 ml Milli-Q water for SPE cleanup. All samples were then extracted using Oasis® WAX cartridge. The cartridge was first pre-conditioned by passing through the cartridge in the sequence of 4 ml 0.1% NH$_4$OH/MeOH, 4 ml MeOH and 4 ml water at a rate of 2 drops/s. Samples (100 ml) were passed through the pre-conditioned cartridges at a rate of 1–2 drop/s. The cartridges were prevented from drying at all times during sample loading. After loading all the samples, the cartridges were rinsed with 20 ml 0.01% NH$_4$OH/H$_2$O and then three times with 10 ml Milli-Q water to remove impurity. Then, they were washed by 4 ml 25 mM acetate buffer solution (pH 4). Residual water was completely removed from the cartridges by centrifugation at 3000 rpm for 2 min because water remaining in the cartridges would affect the concentration efficiency at a later process and, more importantly, affect the recovery of PFOSA (data not shown). The target analytes were eluted into two fractions. The first fraction (F1) and second fraction (F2) were eluted by 4 ml MeOH and 4 ml 0.1% NH$_4$OH/MeOH, respectively. F1 and F2 were concentrated to 0.5 ml, respectively, under a stream of high purity nitrogen.

2.4. Instrumental analysis

Analysis of the PFCs was performed using an HPLC–MS/MS. The separation of the analytes was performed by using an Agilent HP1100 liquid chromatograph (Agilent, Palo Alto, CA) that was interfaced with a Micromass Quattro Ultima Pt mass spectrometer (Waters Corp., Milford, MA) and operated in the electro-spray negative mode. A 10 μl extract was injected onto a guard column (XDB-C8, 2.1 mm i.d. × 12.5 mm, 5 μm; Agilent Technologies, Palo Alto, CA) connected sequentially to a Betasil C18 column (2.1 mm i.d. × 50 mm length; Thermo Hypersil-Keystone, Bellefonte, PA). The mobile phase is 2 mM ammonium acetate–MeOH as the mobile phase, starting at 10% MeOH. Detailed instrumental parameters were reported elsewhere (Taniyasu et al., 2005).

2.5. Quality control

Calibration curve was prepared with a series of concentrations of 0, 2, 10, 50, 200, 1000, 5000, and 20 000 pg/ml and the deviation of every point from the standard was less than 20%. Every sample, blank and recoveries were extracted in duplicates to make sure stable repeatability. Standard solutions (1000 pg/ml) were run every 10 sample injections during analysis to detect any deviation in the response of the system. Blanks and recoveries were checked following the same procedures as described above for every batch of extraction. The blanks were all below the limit of quantifications (LOQs) and the details on removing various interferences were described elsewhere (So et al., 2007; Yamashita et al., 2004). Matrix recoveries were also conducted. Recoveries of PFOS, PFHxS, PFBS, PFUnDA, PFDA, PFNA, PFOA, PFHpA, PFHxA, 8:2 FTCA, 8:2 FTUCA, and PFOSA were 104 ± 6, 107 ± 12, 104 + 9, 73 ± 13, 98 ± 7, 91 ± 8, 86 ± 8, 104 ± 14, 100 ± 11, 67 ± 8, 76 ± 7, and 28 ± 4%, respectively. The concentrations of PFCs in the real samples were not corrected by recoveries.

2.6. ^{15}N analysis

Zooplankton and muscle of fish were dried at 60 °C and ground to a fine powder with a mortar and pestle. The values of δ^{15}N were determined using DELTAplus XP. Stable nitrogen in the biota was expressed as δ^{15}N‰, where δ^{15}N‰ = $[(^{15}N_{sample}/^{14}N_{sample} - {}^{15}N_{standard}/^{14}N_{standard}) - 1] \times 1000$. Trophic levels of various fish species were assigned using the relationship described by Fisk et al. (2001): trophic level = $2 + (\delta^{15}N_{consumer} - \delta^{15}N_{plankton})$.

3. Results and discussions

3.1. Concentrations of PFCs in zooplankton and fish

The concentrations of PFCs in whole blood were converted to serum basis by multiplying the concentrations by a factor of 2 (Yeung et al., 2006). PFBS, PFHpA, PFHxA, 8:2 FTCA and 8:2 FTUCA were all below their LOQs (10 ng/ml for PFHpA, PFHxA and 8:2 FTCA, and 50 ng/ml for 8:2 FTUCA, respectively). Although the recoveries of PFOSA were low, it could be detected in the samples. The PFC concentrations in zooplankton and fish are summarized in Table 1 and the total PFCs were calculated based on the sum of seven PFCs in this study.

Zooplankton is part of the fish diet and is a key link in many food webs/chains. The mean PFOS and total PFCs were 4.18 and 5.44 ng/g
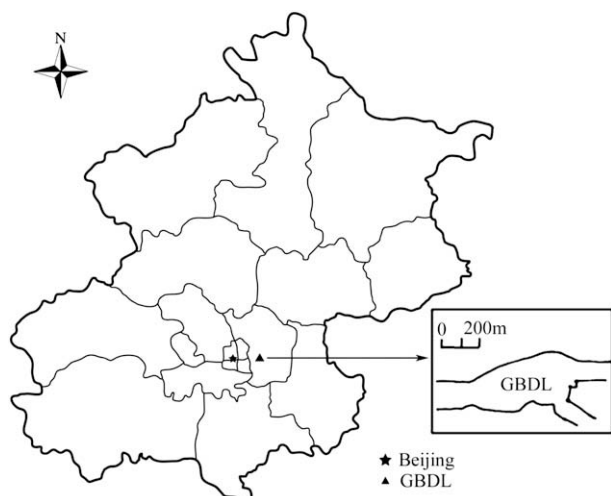

Fig. 1. Sampling location.

Table 1
PFC concentrations in zooplankton (ng/g wet weight) and fish (ng/ml serum) from Gaobeidian Lake

Compounds	Zooplankton[a] ($n = 1$)	Min–max (Mean)				
		White semiknife carp[a] ($n = 2$)	Tilapia ($n = 2$)	Leather catfish ($n = 6$)	Common carp ($n = 6$)	Crucian carp ($n = 13$)
PFOS	4.18	8.41–11.4 (9.88)	4.81–6.67 (5.74)	7.02–25.9 (12.9)	14.2–32.2 (32.2)	48.9–84.4 (64.2)
PFHxS	0.197	0.166–0.166 (0.166)	0.181–0.187 (0.184)	0.0150–0.156 (0.0620)	0.415–2.36 (1.17)	0.385–1.26 (0.793)
PFOSA	0.0470	1.03–5.79 (3.41)	0.105–2.83 (1.47)	1.27–5.07 (2.83)	2.68–16.4 (8.64)	0.342–2.01 (1.22)
PFUnDA	0.199	1.71–2.50 (2.10)	1.60–1.91 (1.75)	5.41–14.1 (7.89)	1.10–9.03 (3.87)	7.03–15.3 (11.0)
PFDA	0.615	5.21–10.3 (7.74)	2.95–4.27 (3.61)	5.10–15.5 (7.99)	1.40–11.7 (5.49)	9.72–25.4 (18.4)
PFNA	0.150	0.330–0.466 (0.398)	0.312–0.465 (0.398)	0.114–1.01 (0.385)	0.135–0.661 (0.369)	0.295–1.18 (0.612)
PFOA	0.0500	0.159–0.184 (0.172)	0.328–0.494 (0.411)	0.114–2.43 (0.814)	0.108–0.363 (0.201)	0.152–0.669 (0.344)
Total PFCs	5.44	17.0–30.7 (23.9)	10.3–16.8 (13.6)	20.7–64.2 (32.9)	23.6–100 (51.9)	76.4–120 (96.6)

[a] The samples from pooled samples.

wet weight, respectively (Table 1). The concentrations of PFCs in zooplankton were in the following rank order: PFOS > PFDA > PFUnDA > PFHxS > PFNA > PFOA > PFOSA (Fig. 2). The concentration of PFOS was higher than those (<LOQs to 0.2 ng/g wet weight; 1.8 ± 0.3 ng/g wet weight) in previous studies (Powley et al., 2008; Tomy et al., 2004). Meanwhile, the composition profiles of PFCs in this study were different from those of other studies (Powley et al., 2008; Tomy et al., 2004). Powley et al. (2008) reported that PFDA and PFDoDA were the predominant compounds, with a small contribution from PFOS in zooplankton in the Western Arctic. Besides, the concentration of PFOA was greater than that of PFOS in zooplanktons in Eastern Arctic (Tomy et al., 2004). These differences indicated that sources of contamination might be different in these environments.

The mean total PFCs concentrations in fish were in the following ranking order: crucian carp > common carp > leather catfish > white semiknife carp > tilapia (Table 1). Among these PFCs, PFOS was the predominant compound, which could be detected in all fish samples with the mean PFOS concentrations ranging from 5.74 ng/ml serum in tilapia to 64.2 ng/ml serum in crucian carp. PFOS can resist being degraded or metabolized due to its unique chemical and biological stability (Tomy et al., 2004). Other compounds such as N-ethyl perfluorooctanesulfonamidoacetate (PFOSAA), N-ethylperfluorooctanesulfonamide (N-EtFOSA), Perfluorooctanesulfinate (PFOSulfinate) and PFOSA might be transformed into PFOS (Loganathan et al., 2007). The discharges from municipal wastewater were believed to be the source of the PFOS in this study. The PFOS from fire-fighting foam was also discharged to the WWTP (Houde et al., 2006). All these factors might contribute to the high PFOS concentrations in the samples.

High PFOS concentrations were also reported in other studies (Kannan et al., 2002; Moody et al., 2002). The greatest reported

concentration of PFOS (72 900 ng/g wet weight) was measured in liver of common shiner (*Notropus cornutus*) from Etobicoke Creek (Moody et al., 2002), which was about 864 times greater than the greatest concentration (84.3 ng/ml) in crucian carp in this study. The high PFOS concentration measured by Moody et al. (2002) was a consequence of direct spill of PFOS-containing fire-fighting foams in the river and therefore it cannot be compared with the concentrations measured in this study. A substantially higher PFOS concentration (3250 ng/g wet weight) was also reported in the liver of the carnivorous and near-bottom feeder ornate jobfish (*Tropidinius amoenus*) from Okinawa, Japan (Taniyasu et al., 2003). In addition, other two relatively high PFOS concentrations were 923 and 296 ng/g wet weight in the muscle of fish from Belgian estuary and US Great Lakes, respectively (Kannan et al., 2001). In this study, the PFOS concentrations in fish blood were comparable with or higher than those from Japan (Taniyasu et al., 2003), Italy (Kannan et al., 2002) and USA (Keller et al., 2005), but were lower than those recorded in some fish such as blue gill (*Lepomis macrochirus*), conger eel (*Conger myriaster*), flatfish (*Pleuronectiformes pleuronectidae*), Japanese stingfish (*Sebastiscus marmoratus*), Largemouth bass (*Micropterus salmoides*) from Japan (Taniyasu et al., 2003). Generally, concentrations of PFOS in this study are comparable to those from other developed countries (such as USA and Japan).

Mean concentrations of PFHxS ranged from 0.0620 for leather catfish to 1.17 ng/ml serum for common carp in our study, which were comparable to the previous studies (Kallenborn et al., 2004; Kannan et al., 2002; Keller et al., 2005) but were lower than those in fish from Japan (Taniyasu et al., 2003).

PFDA was found to be the second dominant PFC in fish samples except for common carp (in which PFOSA was dominant). The mean concentrations of PFDA ranged from 3.61 in tilapia to 18.4 ng/ml serum in crucian carp. The ratios of PFDA/total PFCs ranged from 0.19 for crucian carp to 0.32 for white semiknife carp. A high PFDA concentration was also reported in another study, Moody et al. (2002) found that PFDA was predominant in liver of common shiner (32–390 ng/g wet weight) and contributed 23–46% to the total PFCs. Martin et al. (2003) also reported greater bioconcentration of longer chain PFCAs. PFDA is a representative of the perfluorinated carboxylic acids used as commercial wetting agents and flame retardants (Harris and Birnbaum, 1989), which might be to one of the contributing sources. Moreover, 1H,1H,2H,2H-perfluoro-1-dodecanol (10:2 FTOH) could be biodegraded to PFDA and PFUnDA, and thus 10:2 FTOH might be another source (Kannan et al., 2005; Loganathan et al., 2007).

Although PFOS and PFOA have received the most attention (Tomy et al., 2004), PFOA was not the major contaminant in this study. The greatest concentration of PFOA was 2.43 ng/ml serum in leather catfish. Concentrations of PFOA were approximately 20- to 200-fold lower than those of PFOS in the fish samples in our study. The relative contribution of PFOA was very low and the results in
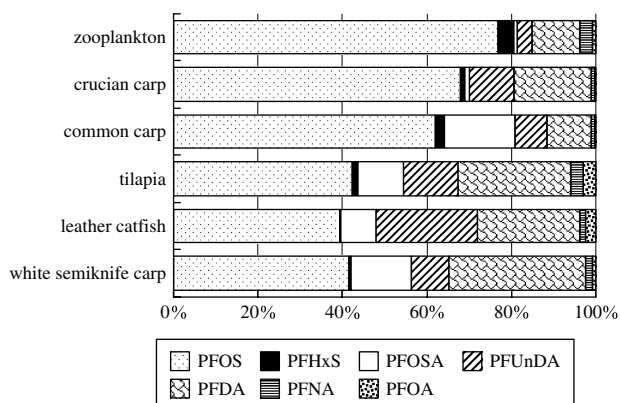

Fig. 2. PFC composition profiles in zooplankton and fish.

our study were similar to those of a previous study, in which PFOA generally contributed to a minor percentage to the total PFCs in several species of Arctic fish, birds and mammals (Keller et al., 2005). The concentrations of PFOA in this study were also comparable with some previous reports, for example, Arctic cod (*Gadus morhua*) from Sweden (<0.4 ng/g wet weight in liver) (Kallenborn et al., 2004). Martin et al. (2004a) reported that the PFOA concentrations were below 2 ng/g wet weight in tissues of some fish including liver of brook trout (*Salvelinus fontinalis*), liver of lake whitefish (*Coregonus clupeaformis*), whole body of lake trout, liver of pike (*Esox lucius*) from Canada (Martin et al., 2004a). In blood, the concentration of PFOA was also less than 2.5 ng/ml in bluefin tuna (*Thunnus thynnus*) from Mediterranean Sea, Italy (Kannan et al., 2002). Higher PFOA concentrations (6–91 ng/g wet weight) were found in liver of common shiner from Canada (Moody et al., 2002). In this study, the lower concentrations of PFOA in fish were different from those in human blood in which PFOA occurred at the second highest concentrations in several countries (Kannan et al., 2004) and the fourth highest concentration in China (Yeung et al., 2006). This difference might be explained by species-specific bioaccumulation, different sources of contamination, and/or different exposure pathways.

Compared to other fishes, PFOSA was high in common carp (8.64 ng/ml) in this study. It is known that PFOSA is used as the chemical building block in the industrial synthesis of numerous other PFOSA-type compounds (Tomy et al., 2004). The discharge of PFOSA together with the PFOSA-type compounds from the WWTP might enter into the aquatic environment and bioaccumulate in fish tissue. In addition, these PFOSA-type chemicals and precursors such as PFOSAA, *N*-EtFOSA, PFOSulfinate might be degraded into PFOSA that might be one of the reasons for the high PFOSA concentration in fish samples (Loganathan et al., 2007). It is worth noting that PFOSA is also thought to degrade to PFOS via the PFOSA intermediate (Tomy et al., 2004). The greatest PFOSA concentrations were found in liver of pike (*E. lucius*) (91 ± 37 ng/g wet weight) from Norway (Kallenborn et al., 2004) and whole slimy sculpin (*Cottus cognatus*) (150 ± 17 ng/g wet weight) from lake Ontario (Kallenborn et al., 2004; Martin et al., 2004b). Higher PFOSA percentage in common carp than in the other fish species in this study and this distinct presence of PFOSA in fish indicated limited biotransformation capacities for this compound in certain species of fish (Houde et al., 2006).

The greatest concentration of PFNA was 1.18 ng/ml serum in crucian carp. PFNA concentrations were equal to or greater than those of PFOA except for leather catfish (Table 1). The results were similar to a previous report, in which PFNA concentration was greater than PFOA concentrations in a variety of fish species from North America (Houde et al., 2006). PFNA also contributed more than PFOA in liver of brook trout and lake whitefish from Canada (Martin et al., 2004a), Arctic cod, perch (*Perca fluviatilis*) from Sweden, brown trout from Norway, eelpout (*Zoarces viviparous*), flounder (*Platichthys flesus*) from Denmark, pike from Finland (Kallenborn et al., 2004) and rainbow smelt (*Osmerus mordax*) from Canada (Martin et al., 2004b). Keller et al. (2005) suggested that PFNA was more bioaccumulative which might result in higher concentrations in biota than those of PFOA. This might account for the higher PFNA concentration as compared to that of PFOA in this study.

The greatest mean concentration of PFUnDA (11.0 ng/ml serum) was observed in crucian carp. PFUnDA concentrations ranged from 32 to 390 ng/g wet weight in liver of common shiner, which had the greatest concentrations of PFOS and PFDA (Moody et al., 2002). Atmospheric oxidation of 10:2 FTOH to PFUnDA might lead to the predominance of PFUnDA in biota. However, for biota living near sources such as wastewater effluents, this pattern could be obscured by direct sources of the PFCAs (Houde et al., 2006), which

could explain the dominance of PFUnDA in fish living in the recipient water affected by WWTPs in this study.

3.2. Correlations among PFCs

The correlations among PFCs in all samples were analyzed and summarized in Table 2. Significant correlations were observed among PFOS and other compounds (PFHxS, PFDA, PFNA, and PFUnDA). PFNA did not correlate well with PFOS as well as PFDA and PFUnDA in this study, which was similar to the previous study (Powley et al., 2008). No significant correlation between PFOS and PFOSA was found in this study. The positive correlations among PFOS and other compounds were also found in other studies (e.g. Powley et al., 2008; Yeung et al., 2006). Powley et al. (2008) showed that PFCAs (including PFUnDA, PFNA, PFDA, and PFDoA) were strongly correlated with PFOS in seal liver. Significant positive correlations were also found among PFCs (Table 2). The positive correlation suggested the possibility of a common pollution source for these compounds (So et al., 2007).

3.3. The relationship between PFCs and trophic levels

One-way analysis of variance (ANOVA) was used to examine the difference of PFCs in three fishes (leather catfish, common carp and crucian carp). Tilapia and white semiknife carp were not included because only two samples were measured for the two species. Statistical differences were found among theses fish species ($p < 0.05$). Some factors might affect the PFCs' bioaccumulation in fish, such as age, sex, trophic levels, feeding habit, habitat and so on. In this study, the relationship between sex/age and concentrations of PFOS could not be studied due to the limited sample size, and only trophic levels (based on δ^{15}N values) could be analyzed. No significant relationship ($p > 0.05$) could be observed between ln PFOS concentrations and trophic level when tilapia was considered. However, a positive linear relationship ($r^2 = 0.85$, $p < 0.05$) was observed between ln PFOS concentrations and trophic level when tilapia was excluded (Fig. 3). The results demonstrated that trophic levels could affect the bioaccumulation of PFOS in serum of fish. Tilapia showed a wide and consistent divergence from all other fish and the reasons might be: first, tilapia is an annual tropical fish and they were younger than the other fish species; second, only two tilapias samples were measured in this study; third, tilapia are benthic rather than pelagic fish, and another study has previously shown that the divergence between pelagic and benthic animals shows divergent biomagnifications patterns (Kidd et al., 2001). No significant trend was observed for PFHxS, PFOSA, PFUnDA, and PFOA ($p > 0.05$), which indicated that these PFCs will not be biomagnified in this study. Other studies also found that trophic levels could affect the concentrations of PFOS and there was a significant correlation between concentrations of PFOS and trophic levels

Table 2
Correlation analyses of PFCs in all samples from Gaobeidian Lake

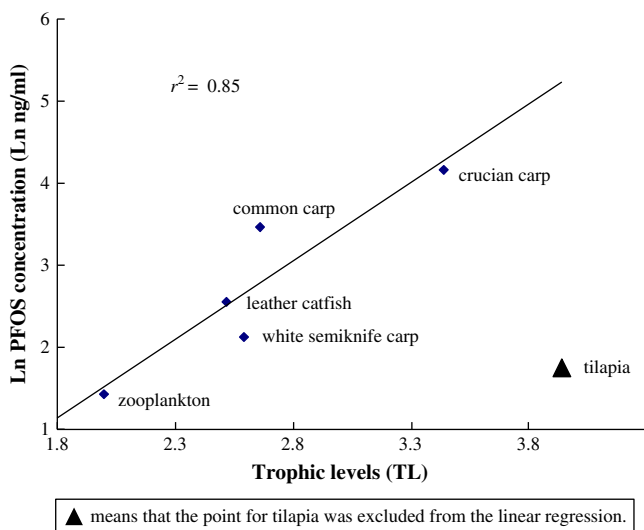| Compounds	r^2	p
PFOS–PFHxS	0.423	<0.01
PFOS–PFDA	0.607	<0.01
PFOS–PFNA	0.215	<0.05
PFOS–PFUnDA	0.484	<0.01
PFHxS–PFNA	0.154	<0.05
PFDA–PFNA	0.450	<0.01
PFDA–PFUnDA	0.815	<0.01
PFNA–PFOA	0.358	<0.01
PFNA–PFUnDA	0.387	<0.01
PFOA–PFUnDA	0.136	<0.05

Fig. 3. Relationships of trophic levels and PFOS concentrations.

(based on δ^{15}N) (Martin et al., 2004b; Tomy et al., 2004). Tomy et al. (2004) attempted to quantify the magnitude of biomagnifications or trophic transfer and found the significant relationship between PFOS concentration and trophic levels based on δ^{15}N. The trophic magnification factors (TMFs) and biomagnification factors (BMFs) based on PFOS concentrations in various tissues could be different. Different TMFs and BMFs among tissues could not be addressed in this study because the concentrations of PFCs in other tissues (such as liver, muscle and so on) were not measured. Moreover, there are no studies reporting the relationship between PFC concentrations in serum and trophic levels.

3.4. Risk assessment

PFOS might induce DNA breakage and/or affect DNA repair thereby disturbing the homeostasis of the "overall" DNA metabolism. PFOS also causes deleterious effects on membrane integrity, resulting in necrosis of liver cells (Hoff et al., 2003). In the latter study, Hoff et al. (2005) measured the serum alanine aminotransferase (ALT) activity and hepatic PFOS concentration in carp and eel collected from the field in Flanders (Belgium). Their results demonstrated that serum ALT activity had a significant positive correlation with hepatic PFOS concentration, which might suggest that PFOS induce hepatic damage in the species in the field.

The risk of PFOS to fish was evaluated according to the method described in a previous study (Yeung et al., 2006). The route of exposure could affect the "points of departure" for risk characterization and the exposure duration was longer than 1 year except for tilapia. Although there were no studies that reported the "points of departure" for serum of fish, some researches have reported that PFOS concentrations in blood were lower than those in liver and the two concentrations were highly correlated with each other (Dauwe et al., 2007; Kannan et al., 2001). The lowest observed effect concentration for serum ALT activity for a short term exposure experiment in carp was chosen as the conservation concentration (Hoff et al., 2003). The conservative concentration 561 (ng/g wet wt.)/ (ng/ml) was regarded as "point of departure" and used to assess the risk of PFOS to fish (Hoff et al., 2003). In this study, all the measured PFOS serum concentrations were lower than conservative concentration, which suggested that there would be no immediate risk to the fish to PFOS exposure. It should be noted that this was a preliminary risk assessment of PFOS on fish, a refined risk assessment should be undertaken with a larger sample size and more data points for the evaluation of risk. Besides, mixture effects of other PFCs were not in consideration. In future studies, it would be instructive to examine the combined/cumulative risks of all PFCs.

4. Conclusions

PFOS was the predominant contaminant among PFCs in this study. Different PFC composition profiles suggested species-specific bioaccumulation. Significant correlations between trophic levels and some PFCs were observed in this study (such as PFOS, PFDA and PFNA) when tilapia was excluded. Further work is necessary to determine the PFOS and related compounds in different tissues, such as muscle and liver, in order to assess the risk for human through the consumption of the contaminated fish. Time-related changes in concentrations of fluorinated compounds in species from Gaobeidian Lake also warrant in future research.

References

Dauwe, T., Van de Vijver, K., Coen, W.D., Eens, M., 2007. PFOS levels in the blood and liver of a small insectivorous songbird near a fluorochemical plant. Environmental International 33, 357–361.

Dai, J.Y., Li, M., Jin, Y.H., Saito, N., Xu, M.Q., Wei, F.W., 2006. Perfluorooctanesulfonate and perfluorooctanoate in red panda and giant panda from China. Environmental Science and Technology 40, 5647–5652.

Fisk, A.T., Hobson, K.A., Norstrom, R.J., 2001. Influence of chemical and biological factors on trophic transfer of persistent organic pollutants in the northwater polynya marine food web. Environmental Science and Technology 35, 732–738.

Giesy, J.P., Kannan, K., 2001. Global distribution of perfluorooctane sulfonate in wildlife. Environmental Science and Technology 35, 1339–1345.

Hansen, K.J., Clemen, L.A., Ellefson, M.E., Johnson, H.O., 2001. Compound-specific, quantitative characterization of organic fluorochemicals in biological matrices. Environmental Science and Technology 35, 766–770.

Harris, M.W., Birnbaum, L.S., 1989. Developmental toxicity of perfluorodecanoic acid in C57BL/6N mice. Fundamental and Applied Toxicology 12, 442–448.

Higgins, C.P., Field, J.A., Criddle, C.S., Luthy, R.G., 2005. Quantitative determination of perfluorochemicals in sediment and domestic sludge. Environmental Science and Technology 39, 3946–3956.

Hoff, P.T., Van Dongen, W., Esmans, E.L., Blust, R., De Coen, W.M., 2003. Evaluation of the toxicological effects of perfluorooctane sulfonic acids in the common carp (*Cyprinus carpio*). Aquatic Toxicology 62, 349–359.

Hoff, P.T., Campenhout, K.V., Van de Vijver, K., Covaci, A., Bervoets, L., Moens, L., Huyskens, G., Goemans, G., Belpaire, C., Blust, R., De Coen, W., 2005. Perfluorooctane sulfonic acid and organohalogen pollutants in liver of three freshwater fish species in Flanders (Belgium): relationships with biochemical and organismal effects. Environmental Pollution 137, 324–333.

Houde, M., Martin, J.W., Letcher, R., Solomon, K.R., Muir, D.C.G., 2006. Biological monitoring of polyfluoroalkyl substances: a review. Environmental Science and Technology 40, 3463–3473.

Kallenborn, R., Berger, U., Järnberg, U., 2004. Perfluorinated Alkylated Substances (PFAS) in the Nordic Environment. Nordic Council of Ministers, Copenhagen, Norway.

Kannan, K., Koistinen, J., Beckmen, K., Evans, T., Gorzelany, J.F., Hansen, K.J., Jones, P.D. , Helle, E., Nyman, M., Giesy, J.P., 2001. Accumulation of perfluorooctane sulfonate in marine mammals. Environmental Science and Technology 35, 1593–1598.

Kannan, K., Corsolini, S., Falandysz, J., Oehme, G., Focardi, S., Giesy, J.P., 2002. Perfluorooctanesulfonate and related fluorinated hydrocarbons in marine mammals, fishes, and birds from coasts of the Baltic and the Mediterranean Seas. Environmental Science and Technology 36, 3210–3216.

Kannan, K., Corsolini, S., Falandysz, J., Fillmann, G., Kumar, K.S., Loganathan, B.G., Mohd, M.A., Olivero, J., Wouwe, N.V., Yang, J.H., Aldous, K.M., 2004. Perfluorooctanesulfonate and related fluorochemicals in human blood from several countries. Environmental Science and Technology 38, 4489–4495.

Kannan, K., Yun, S.H., Evans, T.J., 2005. Chlorinated, brominated and perfluorinated contaminants in livers of polar bears from Alaska. Environmental Science and Technology 39, 9057–9063.

Keller, J.M., Kannan, K., Taniyasu, S., Yamashita, N., Day, R.D., Arendt, M.D., Segars, A. L., Kucklick, J.R., 2005. Perfluorinated compounds in the plasma of loggerhead and Kemp's ridley sea turtles from the southeastern coast of the United States. Environmental Science and Technology 39, 9101–9108.

Kidd, K.A., Bootsma, H.A., Hesslein, R.H., Muir, D.C.G., Hecky, R.E., 2001. Biomagnification of DDT through the benthic and pelagic food webs of Lake Malawi, East Africa: importance of trophic level and carbon source. Environmental Science and Technology 35, 14–20.

Loganathan, B.G., Sajwan, K.S., Sinclair, E., Kumar, K.S., Kannan, K., 2007. Perfluoroalkyl sulfonates and perfluorocarboxylates in two wastewater treatment facilities in Kentucky and Georgia. Water Research 41, 4611–4620.

Martin, J.W., Mabury, S.A., Solomon, K.R., Muir, D.C.G., 2003. Bioconcentration and tissue distribution of perfluorinated acid in rainbow trout (*Oncorhynchus mykiss*). Environmental Toxicology and Chemistry 22, 196–204.

Martin, J.W., Smithwick, M.M., Braune, B.M., Hoekstra, P.F., Muir, D.C.G., Mabury, S.A., 2004a. Identification of long-chain perfluorinated acids in biota from the Canadian Arctic. Environmental Science and Technology 38, 373–380.

Martin, J.W., Whittle, D.M., Muir, D.C.G., Mabury, S.A., 2004b. Perfluoroalkyl contaminants in a food web from Lake Ontario. Environmental Science and Technology 38, 5379–5385.

Moody, C.A., Martin, J.W., Kwan, W.C., Muir, D.C.G., Mabury, S.A., 2002. Monitoring perfluorinated surfactants in biota and surface water samples following an accidental release of fire-fighting foam into Etobicoke Creek. Environmental Science and Technology 36, 545–551.

Powley, C.R., George, S.W., Russell, M.H., Hoke, R.A., Buck, R.C., 2008. Polyfluorinated chemicals in a spatially and temporally integrated food web in the Western Arctic. Chemosphere 70, 664–672.

Schultz, M.M., Higgins, C.P., Huset, C.A., Luthy, R.G., Barofsky, D.F., Field, J.A., 2006. Fluorochemical mass flows in a municipal wastewater treatment facility. Environmental Science and Technology 40, 7350–7357.

So, M.K., Miyake, Y., Yeung, W.Y., Ho, Y.M., Taniyasu, S., Rostkowski, P., Yamashita, N., Zhou, B.S., Shi, X.J., Wang, J.X., Giesy, J.P., Yu, H., Lam, P.K.S., 2007. Perfluorinated compounds in the Pearl River and Yangtze River of China. Chemosphere 68, 2085–2095.

Sinclair, E., Kannan, K., 2006. Mass loading and fate of perfluoroalkyl surfactants in wastewater treatment plants. Environmental Science and Technology 40, 1408–1414.

Taniyasu, S., Kannan, K., Horii, Y., Hanari, N., Yamashita, N., 2003. A survey of perfluorooctane sulfonate and related perfluorinated organic compounds in water, fish, birds, and human from Japan. Environmental Science and Technology 37, 2634–2639.

Taniyasu, S., Kannan, K., So, M.K., Gulkowska, A., Sinclair, E., Okazawa, T., Yamashita, N., 2005. Analysis of fluorotelomer alcohols, fluorotelomer acids, and short- and long-chain perfluorinated acids in water and biota. Journal of Chromatography A 1093, 89–97.

Tomy, G.T., Budakowski, W., Halldorson, T., Helm, P.A., Stern, G.A., Friesen, K., Pepper, K., Tittlemier, S.A., Fisk, A.T., 2004. Fluorinated organic compounds in an Eastern Arctic marine food web. Environmental Science and Technology 38, 6475–6481.

Wang, N., Szostek, B., Buck, R.C., Folsom, P.W., Sulecki, L.M., Capka, V., Berti, W.R., Gannon, J.T., 2005. Fluorotelomer alcohol biodegradation – direct evidence that perfluorinated carbon chains breakdown. Environmental Science and Technology 39, 7516–7528.

Yamashita, N., Kannan, K., Taniyasu, S., Horii, Y., Okazawa, T., Petrick, G., Gamo, T., 2004. Analysis of perfluorinated acids at parts-per-quadrillion levels in seawater using liquid chromatography-tandem mass spectrometry. Environmental Science and Technology 38, 5522–5528.

Yeung, L.W.Y., So, M.K., Jiang, G.B., Taniyasu, S., Yamashita, N., Song, M.Y., Wu, Y.N., Li, J.G., Giesy, J.P., Guruge, K.S., Lam, P.K.S., 2006. Perfluorooctanesulfonate and related fluorochemicals in human blood samples from China. Environmental Science and Technology 40, 715–720.