Perfluoroalkyl Acids in Marine Organisms from Lake Shihwa, Korea

Hoon Yoo · Nobuyoshi Yamashita · Sachi Taniyasu · Kyu Tae Lee ·
Paul D. Jones · John L. Newsted · Jong Seong Khim · John P. Giesy

Abstract To our knowledge, this is the first report of concentrations of perfluorooctanesulfonate (PFOS) and other perfluoroalkyl acids (PFAs) in marine organisms from the industrialized region of Korea. Concentrations of eight PFAs were determined in three species of fish (mullet, shad, and rockfish) and three species of marine invertebrates (blue crab, oyster, and mussel) from Lake Shihwa, Korea. This is an area in which relatively great concentrations of PFAs in water and in adjacent industrial effluents have been reported. PFOS was the dominant PFA in marine organisms and most PFOS concentrations were greater than the sum of all other PFAs. The mean concentrations of PFOS were 8.1×10 and 3.6×10 ng/g, wet weight in liver and blood of fish, respectively. Perfluorocarboxylic acids (PFCAs) were also found in fish, but their concentrations were 10-fold less than those for PFOS. Of the PFCAs measured in fish, concentrations of the longer-chain perfluoroundecanoic acid (PFUnA) were the greatest. Concentrations of PFOS in soft tissues of blue crabs decreased as a function of distance from the shore where inputs from the industrialized areas are discharged into Lake Shihwa. PFOS was the only PFA detectable in mussels and oysters with a mean of 0.5 ± 0.2 and 1.1 ± 0.3 ng/g, wet weight, respectively. Concentrations of PFUnA were positively correlated with perfluorodecanoic acid (PFDA) in both the liver and blood of fish, which suggests a common source of these two PFCAs in this area. Hazard quotients developed for fish species were all less than 1.0 for fish collected in Lake Shihwa.

Perfluorinated alkyl acids (PFAs) have been commonly used in industrial applications since the mid-1940 s, including as surface protection for carpets and leather, as surface-active components in fire-fighting foams, and as processing aids in the production of fluorinated polymers (Giesy and Kannan 2002; Kissa 2001). Global monitoring

H. Yoo · J. L. Newsted · J. P. Giesy
Zoology Department, National Food Safety and Toxicology Center, and Center for Integrative Toxicology, Michigan State University, East Lansing, MI 48824, USA

H. Yoo · N. Yamashita · S. Taniyasu
Potential Pollutants Group, National Institute of Advanced Industrial Science and Technology, Onogawa 16-1, Tsukuba, Ibaraki 305-8569, Japan

H. Yoo (✉)
National Exposure Research Laboratory, USEPA,
960 College Station Road, Athens, GA 30605, USA
e-mail: Yoo.Hoon@epamail.epa.gov

K. T. Lee
Institute of Environmental Protection and Safety,
NeoEnbiz Co., Rm 1306, Bucheon DaewooTechnopark Bldg. A,
Dodang dong 187-7, Bucheon, Gyeonggi, Korea

P. D. Jones · J. S. Khim · J. P. Giesy
Department of Biomedical and Veterinary Biosciences
and Toxicology Centre, University of Saskatchewan, Saskatoon,
SK, Canada

J. L. Newsted
ENTRIX, Inc., 4295, Okemos Road, Suite 101, Okemos,
MI 48864, USA

J. S. Khim
Division of Environmental Science and Ecological Engineering,
Korea University, Seoul 136-713, South Korea

J. P. Giesy
Biology and Chemistry Department, City University
of Hong Kong, Kowloon, Hong Kong, SAR, China

of PFA contamination has found that perfluorooctanesulfonate (PFOS) and other related PFAs are ubiquitously distributed in various tissues of animals, including invertebrates, fish, birds, and mammals irrespective of location, and some of them are able to biomagnify to upper-trophic-level organisms (Giesy and Kannan 2001; Kannan et al. 2005; Sinclair et al. 2006; Van de Vijver et al. 2003). As of the date of this investigation, PFOS had been found to be the predominant compound among PFAs analyzed with then-current analytical techniques, whereas another toxicological component of interest, perfluorooctanoate (PFOA) was persistent in human serum but not in tissues of wildlife (Houde et al. 2006; Sinclair et al. 2006). Although there are concerns about the ecological risks of this emerging persistent organic contaminant, few exposure data are available for the assessment of risks associated with PFAs in wildlife from Korea (Kannan et al. 2002; Yoo et al. 2008).

Lake Shihwa is an artificial saltwater lake, located on the west coast of Korea, that has been receiving industrial wastewater discharges from the Shihwa and Banweol Industrial Complexes (SBICs; approximate total industrial area $= 31$ km^2) since its construction in 1994 (Fig. 1). Although the lake was originally planned to supply freshwater to SBICs and nearby agricultural areas, the severe deterioration of lake-water quality in the mid-1990s (Jung et al. 1997) prompted evaluations of environment impact. Those research efforts indicated moderate to relatively great concentrations of trace metals and persistent organic pollutants such as PCBs, PAHs, and alkylphenols in waters and sediments from this region (Khim et al. 1999; Li et al. 2004). Recently reported concentrations of PFOS in waters of the lake (mean $= 1.3 \times 10$ ng/L) and tributary streams (mean $= 8.9 \times 10$ ng/L) have suggested that local discharges are important sources of PFAs, or their precursors, to the lake (Rostkowski et al. 2006).

Lake Shihwa (surface area $= 56.5$ km^2, drainage basin $= 476.5$ km^2) provides habitats for aquatic animals and birds, particularly migratory species. Because significant quantities of PFAs were observed in Lake Shihwa, it was deemed prudent to determine concentrations of PFAs in aquatic animals of Lake Shihwa and assess the potential risks that these compounds might pose to those organisms and organisms that might eat them. In the present study, concentrations of PFOS and other PFAs were measured in fish, crab, mussel, and oyster from Lake Shihwa. Because PFAs preferentially accumulate in the blood and liver, fish blood and samples of liver and/or hepato-pancreas were collected at four locations in Lake Shihwa. For marine invertebrates, soft tissues were retrieved and analyzed for quantification. Additionally, the current status of PFOS contamination in this region was evaluated and compared to concentrations of PFOS that have been reported to occur in fish from other parts of the world.

Materials and Methods

Sample Collection

Fish and blue crabs were collected in May 2006 at two locations near the SBICs and at two locations near a water-exchange gate in Lake Shihwa (Fig. 1). Stationary fish nets were set up at sampling locations for 3 days. Immediately after net fishing, all biota samples were stored in iceboxes and transported to the laboratory, where samples of blood

Fig. 1 Map showing sampling locations in Lake Shihwa study. Fish and blue crabs were collected at four locations (Stations 1–4) and mussels and oysters were collected at one location (Station 5). Currently, Lake Shihwa water is exchanged with seawaters of Gyeonggi Bay via a water-exchange gate during a flood tide
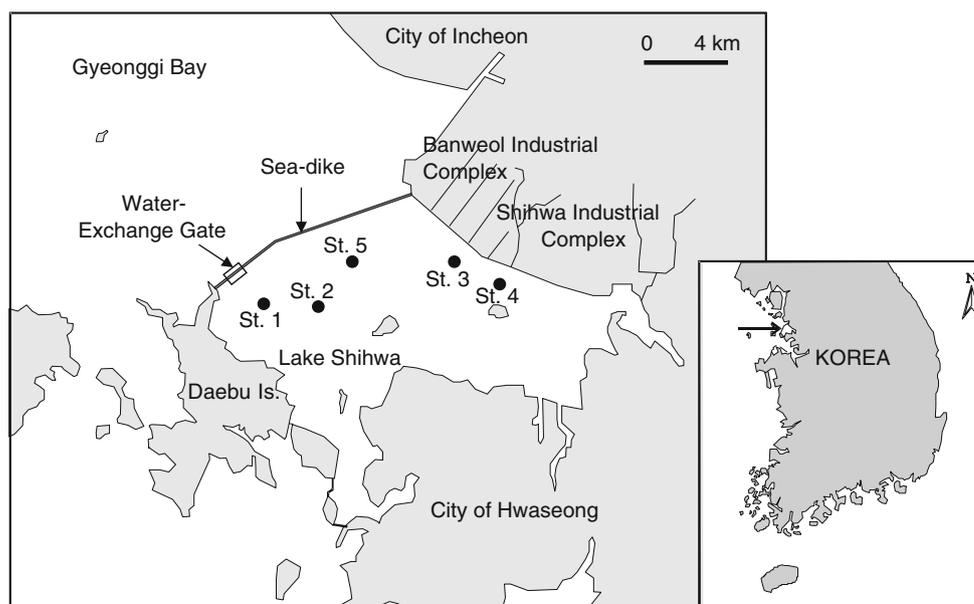
and liver of fish and samples of soft tissues of invertebrates were retrieved. Unfortunately, cotrapped jelly fish caused the mortality of several fish, resulting in only one or two fish species being available at each location. All masses and concentrations in this report are on a wet weight (ww) basis. Three composite samples (two fish per composite) for liver and blood of mullet (mean fish weight = 35.4 ± 4.5 g), rockfish (mean fish weight = 85.2 ± 10.9 g), and shad (mean fish weight = 38.2 ± 6.0 g) were prepared. Blood was collected using a heparinized syringe and transferred to a 15-mL polypropylene tube.

Mussels and oysters were collected from a barge ship located in the middle of the lake (Fig. 1). Soft tissues of blue crab (three individuals at each of four locations) and marine invertebrates (six mussels and four oysters) were pooled for PFAs analysis. Tissues were then mechanically homogenized with a vortex mixer and kept at –20°C until extraction. To avoid cross-contamination, the homogenizer probe and parts were thoroughly rinsed with Milli-Q water and methanol (MeOH) between sample homogenizations.

Chemicals and Standards

The standard mixture of PFA used in this study contained two perfluoroalkyl sulfonates, including PFOS and perfluorohexanesulfonate (PFHS), as well as five PFCAs, including perfluorododecanoic acid (PFDoA), perfluoroundecanoic acid (PFUnA), perfluorodecanoic acid (PFDA), perfluorononanoic acid (PFNA), PFOA, and 2-(N-ethylperfluorooctane sulfonamido) acetic acid (N-EtFOSAA) at 10 ng/mL for each standard. The standard mixtures were supplied by the National Institute of Advanced Industrial Science and Technology (AIST; Tsukuba, Japan). [$^{13}C_4$]-PFOS and [$^{13}C_2$]-PFOA obtained from Perkin Elmer (Boston, MA) were used as recovery internal standards. Purities of all analytical standards were ≥95%. Pesticide-grade MeOH, ammonium acetate, and ammonia solution (25%) were purchased from Wako Pure Chemicals (Osaka, Japan). Milli-Q® (Bedford, MA) water was used throughout the analyses.

Sample Preparation

Samples of biota were extracted with a modified ion-pairing extraction method (Giesy and Kannan 2001). An aliquant of blood (0.5 mL) or tissue homogenate (0.5 g, ww) was diluted fivefold with Milli-Q water. One milliliter of diluted blood was then transferred into a 15-mL polypropylene tube for liquid–liquid extraction. Extraction solutions and solvent were pipetted into the sample in the following sequence: 1 mL of 0.5 M ion-pairing agent (tetrabutyl ammonium hydrogensulfate), 2 mL of 0.25 M extraction buffer (sodium carbonate + sodium bicarbonate), and 5 mL of methyl tert-butyl ether (MTBE). Samples were

vigorously shaken for 20 min, followed by centrifugation for 15 min at 2000 rpm. The separated organic phase was transferred to a second 15-mL polypropylene tube. This MTBE extraction step was repeated twice and all combined MTBE supernatants were evaporated to near-dryness under a gentle stream of nitrogen. The sample was then redissolved in 1 mL of MeOH and filtered with a 0.45-μm nylon filter for instrumental analysis. For liver and soft tissue samples, a modified solid-phase extraction (SPE) was used as an additional cleanup step (Taniyasu et al. 2005). Following the ion-pairing extraction described earlier, a 0.5-mL aliquot of unfiltered extracts was diluted with 100 mL Milli-Q water. The resulting water-extract mixture was passed through an Oasis WAX® cartridge (0.2 g, 6 cm^3) at an elution rate of 1 drop/s. After sample loading was completed, the cartridge was first washed with 4 mL of pH 4 buffer (170 parts 25 mM of acetic acid to 30 parts of 25 mM ammonium acetate) and then with 4 mL of MeOH. The fraction containing PFAs of interest was obtained by eluting with 4 mL of MeOH containing 0.1% NH$_4$OH.

Instrumental Analysis and QA/QC

Separation and quantification of PFAs in samples of biota were performed using an Agilent HP 1100 liquid chromatograph (HPLC) (Agilent, Palo Alto, CA) interfaced with a Micromass Quattro II mass spectrometer (MS/MS) (Waters Corp., Milford, MA) operated in negative electrospray ionization mode. Ten-microliter aliquots of extract were injected onto a Keystone Betasil C$_{18}$ column (2.1 mm inner diameter × 50 mm length, 5 μm particle size), with 2 mM ammonium acetate and MeOH as the mobile phase at a flow rate of 0.3 mL/min and a gradient starting at 10% MeOH ramping to 100% MeOH in 10 min. Detailed instrumental parameters can be found in the work of Taniyasu et al. (2005). External calibration standards were prepared in MeOH at concentrations of 0.01–10 ng/mL and were used to quantitate PFAs in the extracts. Acquired data were deemed to be acceptable if the quality control (QC) standards measured with every 10 injections were within 30% of theoretical value; otherwise samples were run again with a newly constructed calibration curve. The limit of quantification (LOQ) was determined using the lowest acceptable standard within ± 20% of the theoretical value, signal–noise ratio, and dilution factor. LOQs were 0.3 ng/g for all analytes for all sample types, except PFOA (LOQ = 0.5 ng/g).

To ensure the quality of data, procedural blanks and procedural recovery standards were prepared with each extraction batch and matrix-spiked recoveries were tested for each type of biota. All target PFAs in procedural blanks were less than the LOQ. Mean recoveries from each homogenate (0.5 g or 0.5 mL) spiked with 10 ng PFAs were 90% for PFOS, 88% for PFHS, 81% for PFDoA, 97% for PFUnA,

106% for PFDA, 101% for PFNA, 80% for PFOA, and 78% for N-EtFOSAA. Mean matrix-spike recoveries for two internal standards, $[^{13}C_4]$-PFOS and $[^{13}C_2]$-PFOA, were 91% and 78%, respectively. Concentrations of PFAs were not adjusted for matrix recoveries. For calculation of mean concentrations, values determined to be less than the LOQ were assigned a value of half the LOQ.

Data and Statistical Analysis

A regional comparison of PFOS contamination in fish between Korea and Japan was tested using a nonparametric Mann-Whitney U-test without data transformation. Differences in PFOS concentrations of blue crab among sampling locations were investigated by one-way analysis of variance (ANOVA). Possible associations between PFA concentrations in fish were investigated using a Pearson correlation analysis. All statistical analyses were performed with the SYSTAT® 11 Package (SYSTAT Software Inc., Richmond, CA).

Results and Discussion

PFA Concentrations in Fish and Marine Invertebrates

Concentrations of two sulfonated PFAs, five PFCAs, and N-EtFOSAA were detected in the blood and liver of three fish (rockfish, shad, and mullet) (Table 1). PFOS was the predominant PFA in fish sampled from Lake Shihwa. Concentrations of PFOS in all individual fish were greater than the sum of all other seven PFAs monitored. In 12 out of 18 individual fish, the liver contained greater concentrations of PFOS than did blood. The liver sample of a mullet captured at Station 4 had 2.5×10^2 ng PFOS/g, which was the greatest concentration of PFAs determined in this study; meanwhile, blood samples from mullet at Station 3 contained a concentration as great as 9.3×10 ng PFOS/mL. Analyzed by species, mullet ($1.9 \times 10^2 \pm 3.2 \times 10$ ng/g) accumulated 6.8–8.2-fold greater concentrations of PFOS in their livers than did rockfish or shad (Fig. 2). In contrast, the concentration of PFOS in the blood of mullets compared to other species was 1.9-fold greater than that of rockfish and 3.0-fold greater than that of shad. PFHS was detectable in about half of the samples analyzed, and concentrations in the liver (< LOQ–2.0 ng/g ww) and blood (< LOQ–1.6 ng/mL) were similar. N-EtFOSAA was found at approximately the same concentration as the PFCAs in all samples of fish (Table 1). N-EtFOSAA, which degrades to more persistent PFOS, occurs often in the effluents from wastewater treatment and major rivers (Boulanger et al. 2004; Plumlee et al. 2008). Therefore, the presence of N-EtFOSAA in fish species indicates inputs of this precursor via wastewaters.

Table 1 Mean PFAs concentrations [ng/g (ww)] in the tissues of fish collected from Lake Shihwa, Korea

Location	Species	PFOS	PFHS	PFDoA	PFUnA	PFDA	PFNA	PFOA	N-EtFOSAA
Fish liver									
Station 1	Rockfish	2.2×10	1.1	1.1	1.4	0.7	0.7	1.1	1.7
	Shad	2.8×10	0.1	0.6	1.8	1.3	0.5	0.3	0.5
Station 2	Rockfish	2.5×10	1.5	0.8	1.2	0.6	0.6	1.7	1.4
Station 3	Shad	2.9×10	0.1	0.5	1.1	1.3	0.6	0.6	0.4
	Mullet	1.8×10^2	1.0	1.4×10	2.4×10	1.0×10	2.9	1.7	1.3×10
Station 4	Mullet	2.1×10^2	1.3	1.6×10	2.6×10	1.3×10	3.8	1.7	1.5×10
Min		1.8×10	<0.3	0.3	0.9	0.5	<0.3	<0.3	0.3
Max	All (n = 18)	2.6×10^2	1.6	1.9×10	3.1×10	1.5×10	4.2	2.2	2.0×10
Mean		8.1×10	0.9	5.4	9.2	4.6	1.5	1.2	5.4
Fish blood									
Station 1	Rockfish	2.8×10	1.0	2.2	3.6	1.9	1.4	1.6	2.6
	Shad	2.2×10	0.4	1.3	1.9	1.4	0.8	0.8	0.8
Station 2	Rockfish	3.1×10	1.4	2.8	3.6	1.9	1.9	3.1	3.3
Station 3	Shad	1.7×10	0.2	0.2	0.8	1.0	0.4	0.5	0.2
	Mullet	7.5×10	0.5	5.6	1.1×10	6.8	1.4	0.9	3.3
Station 4	Mullet	4.1×10	0.4	5.7	1.1×10	6.3	1.5	0.6	3.0
Min		1.5×10	<0.3	<0.3	0.7	0.8	0.3	0.3	<0.3
Max	All (n = 18)	9.3×10	2.0	8.7	1.5×10	8.9	2.7	5.2	5.3
Mean		3.6×10	0.6	3.0	5.4	3.2	1.2	1.3	2.2

Note: Values are given in the mean of three individuals and values below the LOQ were assigned as a half of LOQ in calculation of the means
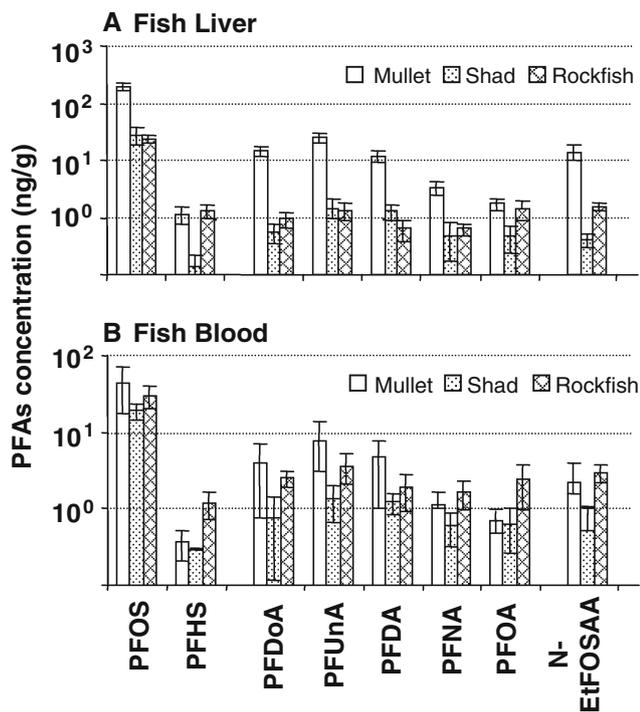
Fig. 2 Comparison of PFA concentrations in mullet, shad, and rockfish collected from Lake Shihwa. Mean concentrations were location-averaged ($n = 6$ fish). Error bars represent one standard deviation

In fish, the longer-chain PFCAs, such as PFDoA and PFUnA, occurred at greater concentrations than did PFNA and PFOA (Table 1). Concentrations of PFUnA in the livers of mullets were relatively great [$(2.5 \times 10) \pm 4.5$ ng/g]. This was the greatest concentration of the PFCAs, followed by PFDoA > PFDA > PFNA > PFOA. Concentrations of PFCAs in rockfish and shad were only detectable in the liver (<1.5 ng/g). The mean concentration of PFOA in livers averaged across all fishes was 1.2 ± 0.7 ng/g. In contrast to

the pattern observed for PFOS, greater concentrations of PFCAs were measured in the blood than liver (12 out of 18 individuals). PFUnA had the greatest concentration ($1.1 \times 10 \pm 2.7$ ng/mL) of PFCAs measured in the blood of mullets followed by PFDA > PFDoA > PFNA > PFOA. In contrast, concentrations of PFCAs in blood of other fish species were less than those in the mullet, with concentrations ranging from 1.6 to 3.6 ng/mL in rockfish and from 0.6 to 1.4 ng/mL in shad.

Mean PFA concentrations in marine invertebrates are summarized in Table 2. Whereas blue crabs were available at four locations (Stations 1–4), mussels and oysters were obtainable at one site in the middle of the lake (Station 5). Similar to PFA accumulation patterns in fish, PFOS was the predominant PFA analyzed in these marine organisms. PFOS concentrations in the soft tissue of crabs from Station 3 [9.6 ± 1.0 ng/g (ww)] and Station 4 [8.3 ± 0.1 ng/g (ww)] contained significantly greater PFOS concentrations than those from Stations 1 and 2 ($p < 0.05$). PFOS concentrations in mussel and oyster collected from Station 5 were 0.6 ± 0.2 ng/g (ww) and 1.1 ± 0.3 ng/g (ww), respectively. In blue crab samples, concentrations of PFDoA ranged from 1.4 to 6.2 ng/g (ww) and PFUnA concentrations ranged from 1.4 to 1.9 ng/g (ww). PFOA was detected in all crab tissues, but at quantifiable concentrations that ranged from 0.3 to 0.8 ng/g (ww). With the exception of PFOS and PFOA in oysters, all concentrations of the targeted PFCAs, PFHS, and N-EtFOSAA in mussel and oyster were less than their respective LOQ values.

Profiles of Relative Concentration of PFA in Marine Organisms

Profiles of relative concentrations of PFA in the three species of fish included in this study were similar to those reported in other studies (Hart et al. 2008; Houde et al.

Table 2 PFAs concentrations (ng/g wet wt) in the soft tissues of marine invertebrates collected from Lake Shihwa, Korea

Location	Species	PFOS	PFHS	PFDoA	PFUnA	PFDA	PFNA	PFOA	N-xEtFOSAA
Station 1	Blue crab	5.3	–[a]	1.5	1.9	0.8	0.4	0.6	–
Station 2	Blue crab	4.5	–	1.4	1.6	0.6	0.4	0.5	–
Station 3	Blue crab	9.6	–	1.5	1.6	0.8	0.3	0.3	–
Station 4	Blue crab	8.3	–	1.6	1.4	0.7	0.5	0.7	–
Min	All ($n = 12$)	4.6		1.3	1.4	0.6	<LOQ	0.3	
Max		1.1×10		2.0	2.6	0.9	0.6	0.8	
Mean		7.4	–	1.6	1.8	0.8	0.4	0.6	–
Station 5	Mussel	0.6	–	–	–	–	–	–	–
Station 5	Oyster	1.1	–	–	–	–	–	0.3	–

Note: Values are given in the mean of three individuals and values below the LOQ were assigned as a half of LOQ in calculation of the means

[a] Because all individual values were below the LOQ, the mean values were not calculated

2006), with PFOS accounting for the largest fraction of the total mass of PFAs (Fig. 3). The proportions of PFAs contributed by PFOS ranged from 72% to 85% in the liver and from 64% to 78% in blood. The next most prevalent PFA was the longer-chain PFUnA, which accounted for 5–13% and 4–9% of the total PFA concentrations in blood and the liver, respectively. The greater contribution of PFUnA in fish was also reported in the livers of tuna from East Asia at times equivalent to PFOS concentration (Hart et al. 2008). The dominance of PFOS in tissue samples was also observed in water samples taken from Lake Shihwa (Fig. 3).

Although PFOA is often a major PFA in water samples and is also one of the persistent PFAs found in human blood (Kannan et al. 2004; Yamashita et al. 2004), its contribution to the total concentration of PFAs in blood of fish was less than 2.5% (Fig. 3). The ratios of PFOS to total PFCAs (ΣPFCA) concentrations in liver ranged from 3.2 in mullet to 6.5 in shad, whereas for blood, the ratios ranged from 2.1 (mullet) to 4.1 (shad). In blue crabs, the ratio of PFOS to total PFCA ranged from 1.0 to 2.2.

Comparison of PFOS Concentrations to Other Studies

Beginning in 2001, concentrations of PFAs in wildlife have been surveyed, especially in marine mammals in North America and northern Europe (Giesy and Kannan 2001; Houde et al. 2006)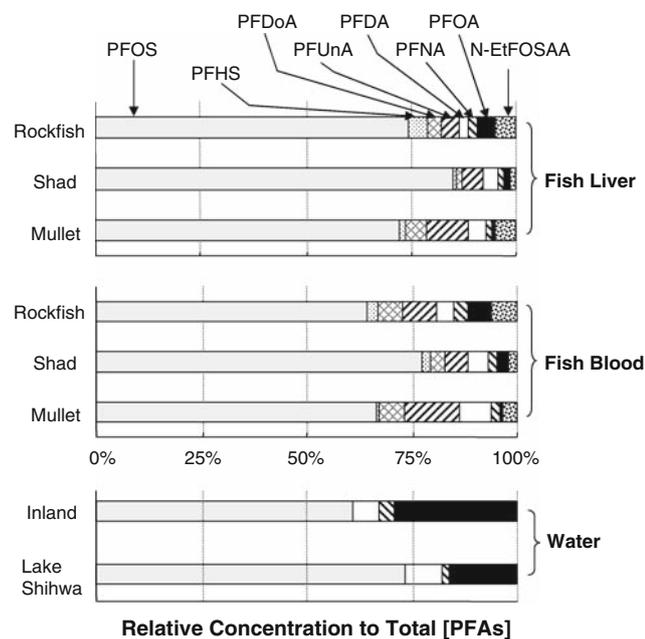. To our knowledge, this is the first report of contamination by PFOS and other perfluorinated alkyl compounds of marine organisms from industrialized areas of Korea.

The PFOS concentrations in fish species surveyed in this study are similar to those reported in fish from Japan (Fig. 4). In fish species collected around Japan, the median hepatic PFOS concentration was 1.4×10 ng/g (ww) (Nakata et al. 2006; Taniyasu et al. 2003). Fish from Lake Shihwa had a median concentration of 3.1×10 ng PFOS/g (ww) in the liver and this value was not statistically different from that of Japanese fish (Mann-Whitney U-test, $p < 0.05$). A recent survey reported contamination by PFOS and other PFAs in various fish species from major rivers in east central United States (Ye et al. 2008). On a whole-fish homogenates basis, the median PFOS concentration in fish was about 3.7×10 ng/g (ww), which is approximated to 1.8×10^2 ng/g (ww) in the liver using a steady-state concentration ratio of carcass to liver in rainbow trout (Martin et al. 2003). This value is about fivefold greater than PFOS levels in Lake Shihwa fish. For fish blood, there was also no statistical difference between median PFOS concentrations for Lake Shihwa
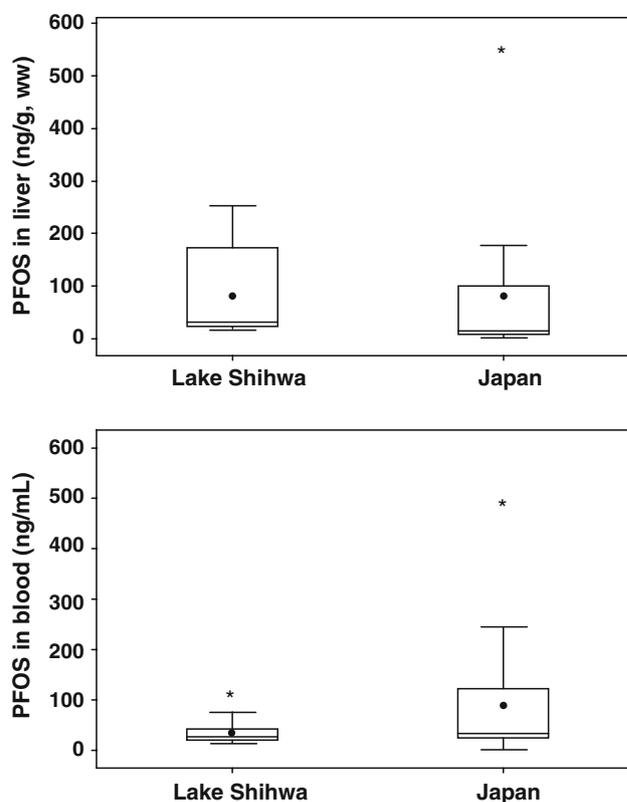
Fig. 3 Relative composition of PFAs analyzed in the liver and blood of mullet, shad, and rockfish collected from Lake Shihwa. Analysis of PFAs composition in Lake Shihwa waters was included for a reference using previous data (Rostkowski et al. 2006)

Fig. 4 Regional comparison of PFOS concentrations in tissues of fish from Japan and Lake Shihwa. The straight line represents the median and the filled circle represents the mean. The 25th and 75th percentiles define the box (asterisks are outliers). No statistical significance was found (Mann-Witney U-test: $p < 0.05$)

$(2.7 \times 10$ ng/mL) and Japan $(3.4 \times 10$ ng/mL). The mean blood-borne PFOS concentrations in five fish species collected near the outfall of a wastewater treatment plant in Beijing, China ranged from 5.7×10 to 6.4×10 ng/mL, demonstrating an increasing PFOS concentration with trophic level (Li et al. 2008). Overall, the current degree of PFOS contamination in fish from Lake Shihwa is comparable to those from neighboring countries, but it is lower than in fish from US waters (Giesy and Kannan 2001).

Source Estimation of PFAs in Lake Shihwa

The spatial distribution of PFOS concentrations in marine organisms demonstrates that biota samples collected from sites close to the outlets of inland creeks (Stations 3 and 4) were more contaminated than those sampled at sites away from the release sources (Stations 1 and 2) (Tables 1 and 2). The greatest mean concentration of PFOS in the livers of fish from Lake Shihwa was measured in mullet sampled at Station 4 $[2.1 \times 10^2$ ng/g (ww) liver]), whereas concentrations were $\sim$ 10-fold less in the livers of fish collected at Stations 1 and 2. Because PFAs generally occur in the water phase rather than in association with particulates and sediments, concentrations of PFOS in fish and blue crab were tentatively attributed to exposure to water-borne PFOS. Concentrations of PFOS in the waters at Stations 3 and 4 were reported to be 1.3×10 and 1.8×10 ng/L, respectively (Rostkowski et al. 2006). Although concentrations of PFOS in water near Stations 1 and 2 are not available, previously reported concentrations of PFOS at locations further from the known effluents would indicate that they should be lower at these two stations. In addition, Stations 3 and 4 are close to wastewater releases from inland channels containing greater concentrations of PFOS $(2.0 \times 10$ to 6.5×10^2 ng/L in streams receiving effluents from SBICs) (Rostkowski et al. 2006). A comparison of blue crab data collected at all four locations also showed that PFOS concentrations in soft tissues decreased as a function of distance from the shore and the inputs from the urbanized and industrialized areas where wastewaters are discharged into Lake Shihwa (Table 2).

Although PFOA was the second most abundant PFA in seawater samples $[1.7–1.1 \times 10$ ng/L] of the Lake Shihwa (Rostkowski et al. 2006), PFOA concentrations in fish were among the lowest of the targeted eight PFAs measured in this study. This observation was similar to that reported for other monitoring studies (Sinclair et al. 2006; Taniyasu et al. 2003). In general, PFOA has a lesser bioaccumulation potential than longer-chain PFCAs and PFOS (Martin et al. 2003). The occurrence of perfluorooctanesulfoamide (PFOSA) in fish was reported elsewhere (Houde et al. 2006; Sinclair et al. 2006), but

this analyte was not quantified in our samples. However, detection of N-EtFOSAA in marine biota suggests that precursor compounds for PFOS exist in this region and therefore, to a lesser degree, contribute to the overall accumulation of PFOS in fish.
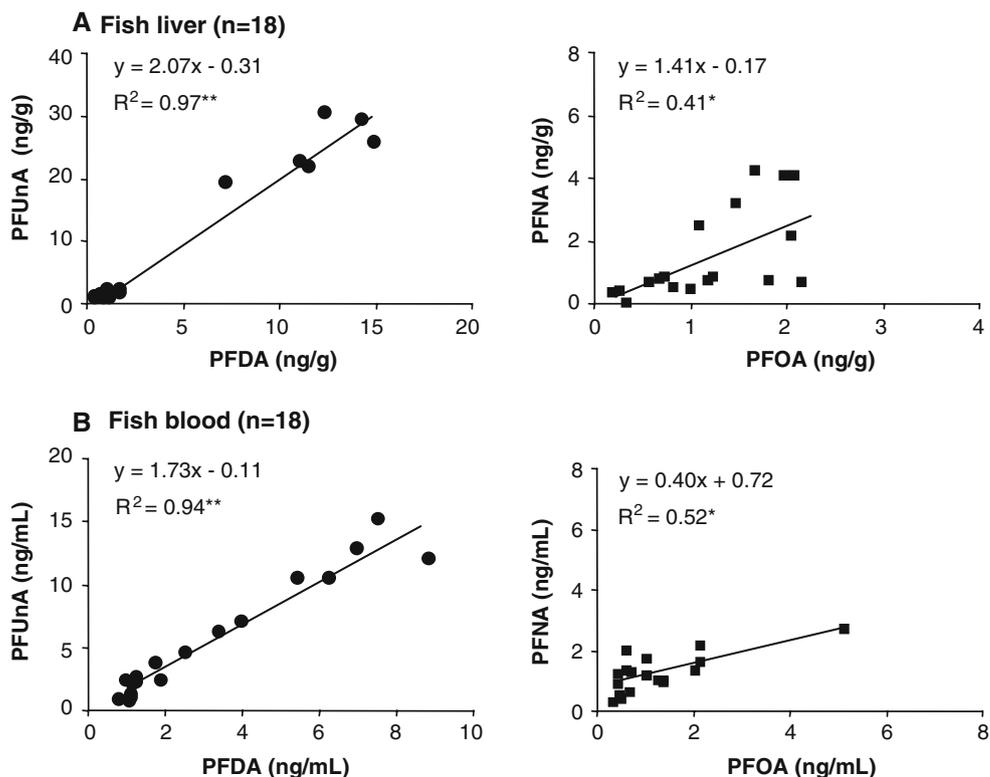
A significant positive correlation was observed between concentrations of PFUnA and PFDA in both the liver and blood of fish (Fig. 5). This result is indicative of the existence of a common source for PFCAs exposure in the Lake Shihwa area. A degradation study showed that the ubiquitous fluorotelomer alcohols (FTOHs) in the atmosphere could be a source for PFCAs (Ellis et al. 2004; Hart et al. 2008); for example, oxidation of $CF_3(CF_2)_7$ $CH2CH2OH$ into PFNA and PFOA was reported in smog-chamber experiments (Ellis et al. 2004). The observation of greater concentration of PFUnA than PFDA in Fig. 5 is consistent with a bioconcentration study result that PFUnA was more bioaccumulative than PFDA (Martin et al. 2003).

BCFs for PFAs in Fish

Bioconcentration factors (BCFs) were estimated for fish by dividing a PFA concentration in fish by that in seawater of Lake Shihwa reported previously (Rostkowski et al. 2006). Although the bioaccumulation factor (BAF) is a more accurate estimate for describing bioavailability from a field exposure, in this report we use BCF terminology for convenience. BCF values were calculated for five PFAs (PFOS, PFHS, PFDA, PFNA, and PFOA) for mullet collected at Stations 3 and 4, due to a lack of data on PFA concentrations in seawater at the outer sampling sites. The mean values of the BCF for PFOS were estimated to be about 3700 and 12,400 in mullet blood and liver, respectively. These estimates are comparable to mean BCF values of fish collected in Tokyo Bay, Japan (9200 for blood; 7700 for liver) (Taniyasu et al. 2003) but less than BCF values reported for fish liver tissue from a spill area in Etobicoke Creek in Toronto (6300–125,000) (Moody et al. 2002). BCF values for the six-carbon PFHS were less than those for PFOS (mean: 700 for blood and 850 for liver). Laboratory exposure experiments showed that uptake rate of PFOS was about 40 times greater than PFHS, whereas depuration rates were comparable to each other.

The site-specific BCF values of estimated PFCAs were greater than those determined under laboratory conditions; for example, the BCF value for PFOA, which is ubiquitous in water, was in the range 100–230 in mullet samples, whereas laboratory-derived BCFs were only 8–27 in rainbow trout (Martin et al. 2003). Similarly, the field-determined BCF for PFDA (9300 in blood and 17,000 in liver) in this study was 10-fold larger than that estimated for rainbow trout. Each fish species can have different

Fig. 5 Plot of an odd-chain PFCA and an even-chain PFCA in the liver (**a**) and blood (**b**) of fish collected from Lake Shihwa. A circle stands for the relationship between PFUnA and PFDA and a square represents for PFNA and PFOA. (* $p < 0.05$, ** $p < 0.01$)

A Fish liver (n=18)

$y = 2.07x - 0.31$
$R^2 = 0.97**$

$y = 1.41x - 0.17$
$R^2 = 0.41*$

B Fish blood (n=18)

$y = 1.73x - 0.11$
$R^2 = 0.94**$

$y = 0.40x + 0.72$
$R^2 = 0.52*$

toxicokinetics for PFCA accumulation, resulting in different BCF values. Alternatively, this observation might suggest the existence of unidentified precursors of PFCAs in Lake Shihwa area, which make significant contributions to the overall bioaccumulation of persistent PFCAs in marine organisms.

Screening-Level Hazard Assessment of PFOS Exposure to Fish

Because PFOS accounted for the majority of the PFAs measured, the screening-level hazard assessment of PFOS exposure to fish was evaluated. An earlier hazard assessment based on water-borne PFOS concentrations revealed that neither waters of inland streams nor Lake Shihwa waters exceed water-quality guideline values that are protective of aquatic species (Rostkowski et al. 2006). As another line of evidence, hazard quotients (HQs) from present tissue PFOS residues were used to evaluate the ecological risk of PFOS exposure to fish in Lake Shihwa. The HQ was calculated as a ratio of exposure concentration (PFOS tissue concentration) to effect concentration (critical PFOS body residue) (Giesy et al. 1999). A conservative estimate of a lethal dose (LD_{50}) on whole-body concentration was determined to be 163 mg PFOS/kg in fish from a 28-day PFOS-induced mortality study in bluegill (Beach et al. 2006). Prior to calculating the HQ, the concentrations of PFOS in liver were converted to whole-carcass PFOS

concentrations. The conversion value for PFOS (concentration ratio of hepatic to whole body = 4.9) was obtained from a water-only exposure study with rainbow trout, assuming a steady-state condition of a partitioning (Martin et al. 2003). Using this approach, the whole-body concentrations of PFOS in fish were approximated to be in the range of 3.9×10 to 5.1×10 ng/g. The calculated HQs were considerably less than 1.0 for all fish tested ($n = 18$); for example, the HQ calculated from the largest PFOS in fish was estimated to be at most 0.0003. This simple hazard-assessment result using body residue is consistent with that predicted previously from PFOS water data in this area (Rostkowski et al. 2006; So et al. 2004). These results suggest that current concentrations of PFOS in fish living in Lake Shihwa do not exceed concentrations that would cause chronic adverse effects. However, the HQ obtained for PFOS here should be interpreted with caution, because toxicological benchmark values used in this study were developed from a single-exposure study with different species and, more importantly, there is little information on the chronic effects of PFOS on fish populations other than mortalities.

References

Beach SA, Newsted JL, Coady K, Giesy JP (2006) Ecotoxicological evaluation of perfluorooctanesulfonate (PFOS). Rev Environ Contam Toxicol 186:133–174. doi:10.1007/0-387-32883-1_5

Boulanger B, Vargo J, Schnoor JL, Hornbuckle KC (2004) Detection of perfluorooctane surfactants in great lakes water. Environ Sci Technol 38:4064–4070. doi:10.1021/es0496975

Ellis DA, Martin JW, De Silva AO et al (2004) Degradation of fluorotelomer alcohols: a likely atmospheric source of perfluorinated carboxylic acids. Environ Sci Technol 38:3316–3321. doi:10.1021/es049860w

Giesy JP, Kannan K (2001) Global distribution of perfluorooctane sulfonate in wildlife. Environ Sci Technol 35:1339–1342. doi:10.1021/es001834k

Giesy JP, Kannan K (2002) Perfluorochemical surfactants in the environment. Environ Sci Technol 36:147A–152A. doi:10.1021/es022253t

Giesy JP, Solomon KR, Coats JR, Dixon KR, Giddings JM, Kenaga EE (1999) Chlorpyrifos: ecological risk assessment in North American aquatic environments. Rev Environ Contam Toxicol 160:1–29

Hart K, Kannana K, Tao L, Takahashi S, Tanabe S (2008) Skipjack tuna as a bioindicator of contamination by perfluorinated compounds in the oceans. Sci Total Environ 403:215–221. doi:10.1016/j.scitotenv.2008.05.035

Houde M, Martin JW, Letcher RJ, Solomon KR, Muir DCG (2006) Biological monitoring of polyfluoroalkyl substances: a review. Environ Sci Technol 40:3463–3473. doi:10.1021/es052580b

Jung HS, Choi KW, Kim DS, Kim CS (1997) Status of environmental conditions of Shihwa Lake in the west coast of Korea and strategies for its remediation. Ocean Policy Res 12:123–163 (in Korean)

Kannan K, Choi JW, Iseki N et al (2002) Concentrations of perfluorinated acids in livers of birds from Japan and Korea. Chemosphere 49:225–231. doi:10.1016/S0045-6535(02)00304-1

Kannan K, Corsolini S, Falandysz J et al (2004) Perfluorooctanesulfonate and related fluorochemicals in human blood from several countries. Environ Sci Technol 38:4489–4495. doi:10.1021/es0493446

Kannan K, Tao L, Sinclair E, Pastva SD, Jude DJ, Giesy JP (2005) Perfluorinated compounds in aquatic organisms at various trophic levels in a Great Lakes food chain. Arch Environ Contam Toxicol 48:559–566. doi:10.1007/s00244-004-0133-x

Khim JS, Villeneuve DL, Kannan K et al (1999) Alkyphenols, polycyclic aromatic hydrocarbons, and organochlorines in sediment from Lake Shihwa, Korea: instrumental and bioanalytical characterization. Environ Toxicol Chem 18:2424–2432. doi:10.1897/1551-5028(1999)018<2424:APAHAO>2.3.CO;2

Kissa E (2001) Fluorinated surfactants and repellents, 2nd edn. Marcel Dekker, New York

Li D, Kim M, Oh JR, Park J (2004) Distribution characteristics of nonylphenols in the artificial Lake Shihwa, and surrounding creeks in Korea. Chemosphere 56:783–790. doi:10.1016/j.chemosphere.2004.01.008

Li X, Yeung LWY, Xu M, et al. (2008) Perfluorooctane sulfonate (PFOS) and other fluorochemicals in fish blood collected near the outfall of wastewater treatment plant (WWTP) in Beijing. Environ Pollut. doi:10.1016/j.envpol.2008.03.008

Martin JW, Mabury SA, Solomon KR, Muir DCG (2003) Bioconcentration and tissue distribution of perfluorinated acids in rainbow trout (oncorhynchus mykiss). Environ Toxicol Chem 22:196–204. doi:10.1897/1551-5028(2003)022<0196:BATDOP>2.0.CO;2

Moody CA, Martin JW, Kwan WC, Muir DCG, Mabury SA (2002) Monitoring perfluorinated surfactants in biota and surface water samples following an accidental release of fire-fighting foam into Etobicoke Creek. Environ Sci Technol 36:545–551. doi:10.1021/es011001+

Nakata H, Kannan K, Nasu T, Cho HS, Sinclair E, Takemura A (2006) Perfluorinated contaminants in sediments and aquatic organisms collected from shallow water and tidal flat areas of the Ariake Sea, Japan: environmental fate of perfluorooctane sulfonate in aquatic ecosystems. Environ Sci Technol 40:4916–4921. doi:10.1021/es0603195

Plumlee MH, Larabee J, Reinhard M (2008) Perfluorochemicals in water reuse. Chemosphere 72:541–1547. doi:10.1016/j.chemosphere.2008.04.057

Rostkowski P, Yamashita N, So MK et al (2006) Perfluorinated compounds in streams of the Shihwa Industrial zone and Lake Shihwa, South Korea. Environ Toxicol Chem 25:2374–2380. doi:10.1897/05-627R.1

Sinclair E, Mayack DT, Roblee K, Yamashita N, Kannan K (2006) Occurrence of perfluoroalkyl surfactants in water, fish, and birds from New York State. Arch Environ Contam Toxicol 50:398–410. doi:10.1007/s00244-005-1188-z

So MK, Taniyasu S, Yamashita N et al (2004) Perfluorinated compounds in coastal waters of Hong Kong, South China, and Korea. Environ Sci Technol 38:4056–4063. doi:10.1021/es049441z

Taniyasu S, Kannan K, Hori Y, Hanari N, Yamashita N (2003) A survey of perfluorooctane sulfonate and related perfluorinated organic compounds in water, fish, birds, and humans from Japan. Environ Sci Technol 37:2634–2639. doi:10.1021/es0303440

Taniyasu S, Kannan K, So MK et al (2005) Analysis of fluorotelomer alcohols, fluorotelomer acids, and short- and long-chain perfluorinated acids in water and biota. J Chromatogr A 1093:89–97. doi:10.1016/j.chroma.2005.07.053

Van de Vijver KI, Hoff PT, Das K et al (2003) Perfluorinated chemicals infiltrate ocean waters: link between exposure levels and stable isotope ratios in marine mammals. Environ Sci Technol 37:5545–5550. doi:10.1021/es0345975

Yamashita N, Kannan K, Taniyasu S et al (2004) Analysis of perfluorinated acids at parts-per-quadrillion levels in seawater using liquid chromatography–tandem mass spectrometry. Environ Sci Technol 38:5522–5528. doi:10.1021/es0492541

Ye X, Strynar MJ, Nakayama SF et al (2008) Perfluorinated compounds in whole fish homogenates from the Ohio, Missouri, and Upper Mississippi Rivers, USA. Environ Pollut. doi:10.1016/j.envpol.2008.03.014

Yoo H, Kannan K, Kim SK, Lee KT, Newsted JL, Giesy JP (2008) Perfluoroalkyl acids in the egg yolk of birds from Lake Shihwa, Korea. Environ Sci Technol 42:5821–5827. doi:10.1021/es800447d