Bull Environ Contam Toxicol (2007) 79:427–431
DOI 10.1007/s00128-007-9243-2

Perfluorinated Compounds in River Water, River Sediment, Market Fish, and Wildlife Samples from Japan

Kurunthachalam Senthilkumar · Etsumasa Ohi · Kenneth Sajwan · Takumi Takasuga · Kurunthachalam Kannan

Received: 6 March 2007 / Accepted: 28 June 2007 / Published online: 20 July 2007

Abstract Perfluorinated organic compounds (PFCs) such as PFOS, PFOA, PFBS, PFH×S, PFOSA and PFDoA were determined in river water, river sediment, liver of market fish and liver of wildlife samples from Japan. Concentrations of PFOA and PFOS in water samples were 7.9–110 and <5.2–10 ng/L. Only PFOA were detected in sediment from Kyoto river at 1.3–3.9 ng/g dry wt. Among fish, only jack mackerel showed PFOA and PFOS at 10 and 1.6 ng/g wet wt. Wildlife liver contained PFOSA, PFOS, PFDoA, PFOA and PFH×S in the range of 0.31–362, 0.15–238, <0.03–28, >0.07–7.3 and <0.03–1.5, respectively, on ng/g wet wt. Cormorants showed maximum accumulation followed by eagle, raccoon dog and large-billed crow.

Keywords PFCs · River water · River sediment · Market fish · Cormorant · Racoon dog · Eagle · Large-billed crow

K. Senthilkumar · K. Sajwan
Department of Natural Sciences and Mathematics, Savannah State University, 3219 College Street, Savannah, GA 31404, USA

K. Senthilkumar · E. Ohi · T. Takasuga
Shimadzu Techno-Research, #1, Nishinokyo-Shimoaicho, Nakagyo-ku, Kyoto 604-8436, Japan

K. Kannan
Wadsworth Center, New York State Department of Health and Department of Environmental Health Sciences, School of Public Health, State University of New York at Albany, Albany, New York, USA

K. Senthilkumar (✉)
Department of Natural Sciences and Mathematics, Savannah State University, PO Box 20600, Savannah, GA 31404, USA
e-mail: kskumar@savstate.edu

Several studies have reported the occurrence of emerging perfluorinated compounds (PFCs) such as perfluorooctane sulfonate (PFOS; $C_8F_{17}SO_3^-$) and perfluoroctanoate (PFOA; $C_7F_{15}CO_2^-$) in human blood from the general population and in the tissues of wildlife from aquatic/terrestrial environments (Giesy and Kannan 2001; Kannan et al. 2004; Senthilkumar et al. 2005; Houde et al. 2006). Among several PFCs, PFOS is the predominant compound found in biota (Senthilkumar 2005; Houde et al. 2006), while PFOA was predominant in environmental matrices (Senthilkumar 2005). PFOS and PFOA have been shown to elicit toxic effects in exposed laboratory animals (Austin et al. 2003; Kennedy et al. 2004). An understanding of the sources and pathways of human and environmental exposures is needed to control and manage the release of these emerging toxic contaminants.

Several studies from Japan have shown elevated concentrations of PFOS and PFOA in water collected from various rivers, lakes and ponds (Taniyasu et al. 2003; Saito et al. 2004). The fish from Lake Biwa showed elevated concentrations of PFCs (Taniyasu et al. 2003). These studies also suggested the need for further studies on PFCs in Kanto regions (Kyoto, Osaka, Nara and Kobe) in Japan because several of these rivers ultimately deposit into Lake Biwa. Consequently, in this study we measured selected PFC compounds such as perfluorobutane sulfonate (PFBS), perfluorohexane sulfonate (PFH×S), PFOA, PFOS, perfluorooctane sulfonylamide (PFOSA), and perfluorododecanoic acid (PFDoA) in river water and river sediment from Kyoto and Osaka. Livers of market fish and wildlife samples were collected from different areas in Japan.

Materials and Methods

River water and river sediment were collected from rivers (Kamogawa, two stations in Ujigawa [Station 1: Rokujizoue Hashi and Station 2: Yamashina Hashi] in February 2005, Tenjingawa and Katsuragawa in March 2005) in Kyoto city, Japan (gawa denotes river in Japanese" and therefore Kamogawa indicates Kamo River; hashi denote bridge). River sediment was also collected from an undisclosed river [four stations] from Osaka in December 2003. Water and sediment samples were collected in clean polypropylene (PP) bottles and clean large mouth amber bottles, respectively, which were wrapped in aluminium foil. Various fish species were purchased in retail fish markets scad (n = 3) and rainbow trout (n = 3) originating from fish farms in the Kanto region, also sandfish (n = 7) originating from the Ishikawa area of the Japanese Sea, jack mackerel (n = 3) originating from Kyushu Prefecture and sardine (n = 3) originating from the Seto Inland Sea) all in fresh condition, were dissected, and individual fish livers were combined based on their species and homogenated for analysis were used for this preliminary analysis. Livers homogenates of cormorants (n = 5), raccoon dog (n = 2), large-bill crow (n = 2), and eagle (n = 2) that accidentally died, were also were used for this preliminary analysis. Livers of individual raccoon dogs, and birds were analyzed separately. Native standards used in this study were a gift from Professor K. Kannan, State University of New York, Empire State Plaza, PO Box 509, Albany, NY 12201-0509, USA. ^{13}C-PFOA was a gift from Professor A. Koizumi, Department of Health and Environmental Science, Kyoto University Graduate School of Medicine, Kyoto, Japan.

Sample cleanup and chemical analysis of river water, river sediment, and livers of market fish and wildlife samples have been illustrated in the following sections;

River water: After water samples were equilibrated at room temperature, 500-mL of river water was taken in a clean flask, residual chlorine was reduced by adding 200 μL of 250 mg/mL sodium thiosulfate and a known amount of ^{13}C-PFOA (internal standard). This sample was shaken well and passed through a conditioned Oasis HLB cartridge (Waters, Milford, MA, USA]. Cartridges were eluted with 10-mL methanol (MeOH) which was collected in a clean poly propylene (PP) tube. The extract was concentrated under a gentle stream of nitrogen to 1-mL, which was filtered through a syringe filter (mesh size of 0.2 μm) and analyzed by LC-MS/MS.

River sediment: Sediment samples were dried overnight under clean conditions. Approximately 15 g dry sediment was weighed in a clean 50-mL PP tube, mixed with 20 mL MeOH and spiked with ^{13}C-PFOA as an internal standard. The sample was shaken in a orbital shaker for 10 min at 300 rpm, then ultra sonificated for 30 min. The resultant slurry was centrifuged for 15 min at 2,500 rpm. The MeOH layer was collected in a clean flask and 20 mL of MeOH was added to sediment. The extraction was repeated again. Combined extracts were then rotary evaporated and transferred to a clean 10-mL PP tube and purged under a gentle stream of nitrogen to 1 mL, which was filtered through a syringe filter and analyzed by LC-MS/MS.

Livers of wildlife samples: About 1 g of liver was homogenized (in clean PP tubes) with 5 mL of ultra pure water. From this homogenate, 1 mL was taken for analysis. The homogenate was mixed with 1 mL of 0.5 M tetrabutylammonium hydrogen sulfate (pH was adjusted to 10), 2 mL of 0.25 M sodium carbonate/sodium bicarbonate buffer (Na_2CO_3/$NaHCO_3$) a known amount of ^{13}C-PFOA, and 5-mL methyl-tert-butyl ether (MTBE). The sample was shaken in an orbital shaker for 20 min at 300 rpm and then centrifuged for 20 min at 3,000 rpm. The MTBE layer was transferred into a clean PP tube and extraction was continued with the addition of 5-mL of MTBE into the liver extract. The combined MTBE layer was purged under a gentle stream of nitrogen until dried, and was re-constituted with 1 mL of MeOH, which was filtered through a syringe filter and analyzed by LC-MS/MS. For sediment and fish liver samples, three procedural blanks were analyzed; for water samples, five blanks were analyzed.

Instrumental analysis was performed using a high-performance liquid chromatograph interfaced with a tandem mass spectrometer (HPLC-MS/MS). Ammonium acetate of 10 mM (10-mM AA) and 100% acetonitrile were delivered at 0.2-μL/min with the Waters 626LC system pump and Waters 717 Plus Auto sampler. All teflon tubing was replaced with poly ethyl ether ketone (PEEK) tubing. Aliquot (20 μL) of sample was injected onto Inertsil ODS-3 (5 μm; 2.1 × 150 mm) column (GL Sciences Inc., Japan). The column temperature was set at 40°C. The gradient method was adapted for 10-mM AA and 100% acetonitrile mobile phase. The detector was an Applied Biosystems API-4000 tandem mass spectrometer operated in an electrospray interface in the negative ionization mode. The electron multiplier was set at 1.5 kV while the nebulizer gas was nitrogen. In order to determine the LC-MS/MS method detection limits, we analyzed 1-ng/mL standard mixture ten times. The quantification limits (QL) for PFOS, PFOSA, PFHxS, PFOA, PFDoA and PFBS were 3.0, 2.0, 3.0, 0.9, 3.0, and 2.0 ng/mL, respectively. QL were further divided by the used sample weight. The recoveries of ^{13}C-PFOA spiked into water sediment and wildlife liver samples ranged from 91% to 114%, 81% to 109% and 80% to 108%, respectively. Minimum six calibration points (0.1, 1, 5, 10, 50 and 100 ng/mL) of all PFCs were freshly prepared for each batch and used to calculate the sample concentrations which gives the $r^2 = 0.999$.

Results and Discussion

Concentrations of PFOA and PFOS were detected at greater levels in all river water samples (Table 1). PFBS, PFH×S, PFOSA and PFDoA were less than quantification limit (QL). The samples were collected during one time point and therefore further studies should be conducted by looking at monthly or seasonal variations in concentrations. Saito et al. (2004) analyzed PFOA and PFOS from several rivers in the Hokkaido, Kanto, Chubu, Kinki, Chugoku and Kyushu regions. PFOA and PFOS concentrations in their study were 10 to 100-fold lower (0.97–21 ng/L PFOA and 0.89–5.73 ng/L PFOS) than those observed in the present study. However, Saito et al. (2004) reported elevated PFOA and PFOS (4.5–67,000 ng/L PFOA and 1.5–526 ng/L PFOS) for river water from the Osaka region. Kyoto is one of the biggest cities in Japan, with several industries are located at main river basins such as Ujigawa and later part of the Katsuragawa. Most of Kyoto's rivers ultimately deposit in Lake Biwa (the largest lake in Japan) or to the Osaka Bay. Lake Biwa fish contained more elevated PFCs than fish from other regions (Taniyasu et al. 2003). Therefore, the Osaka and Kyoto regions in Japan are considered one of the major PFCs sources for Lake Biwa.

Concentrations of PFCs in river sediment from Kyoto and Osaka were very low (Table 2). Only the Tenjin and Katsura rivers showed detection limits of PFOS and PFOA. PFBS, PFH×S, PFOSA and PFDoA that were less than quantification limit. Most of the river sediments were comprised mainly of sand and silt, and thus sediment with organic matter should be collected during sampling. Furthermore, Katsura river water was collected immediately after rainfall, and therefore observed concentrations may not indicate the original concentrations in river. In addition the differences in the flow of river water may wash freshly deposited PFCs from the organic debris; therefore, the distribution of the surfactant nature of PFCs in sediment needed further investigation. Nakata et al. (2006) reported 0.09–0.14 (PFOS), 0.84–1.1 (PFOA), 0.33–0.55 (PFH×S) and <1.5 (PFOSA) ng/g wet weight in sediments from the Ariake Sea in Japan. Higgins and Luthy (2006) reported sorption of PFCs on sediments. Based on their conclusion, sedimentation does not appear to be a major loss mechanism for PFCs; however, this does not necessarily mean that sediments are unimportant in determining the ultimate fate and distribution of PFCs in the environment.

Studies also reported that for the global mass balance of PFOA, sedimentation and burial were cited as one of only two major removal mechanisms (Prevedouros et al. 2006). Investigations have noted either increased concentrations of PFCs in benthic organisms (Nakata et al. 2006). Further investigations into the bioavailability of sediment-bound anionic PFCs to benthic organisms should help evaluate where sediments will serve as long-term stationary sources of PFCs to the biosphere.

Concentrations of PFCs in fish liver were lower than the limit of quantitation, except in jack mackerel from Kyushu Prefecture in Japan (Table 3). Only PFOA and PFOS were detected at considerable limits. Scad and rainbow trout were collected from fish farms in which later we discovered that the water sources to these farms come from the mountains. Therefore concentrations in both these species were negligible. The detection limit for the livers of sandfish and sardine is not known. Taniyasu et al. (2003) reported low levels of PFCs in fish from other parts of Japan when compared to Lake Biwa, Tokyo and Osaka Bay. Further studies are being performed with large numbers of fish species collected from various bodies of water in the Kyoto region. On the other hand, considerable levels of PFCs were noticed in fish collected from Osaka Bay, Lake Biwa (Taniyasu et al. 2003). Continued monitoring of PFCs is needed in and around the Osaka regions due to high levels in present water, fish, air and humans.

Concentrations of PFCs in livers of cormorants, raccoon dogs, eagles and large-bill crows were shown in Table 4. Except PFBS all other PFCs were quantified in the order of great cormorant < eagle < raccoon dog < large-bill crow. PFOSA was a major accumulant in cormorants and one eagle followed by PFOS, PFDoA, PFOA and PFH×S. This is the first report demonstrating greater concentrations of PFOSA in birds and raccoon dog. Greater PFOSA concentrations in mussel and oyster have been reported by So et al. (2005). Similarly PFOSA was greater in pilot whale at concentrations similar to or higher than those of PFOS

Table 1 Concentrations of PFCs in water (ng/L) from Kyoto area river

Name of River	PFBS	PFHxS	PFOA	PFOS	PFOSA	PFDoA
Kamo River	NA	<6.6	36	4.1	<3.7	<6.8
Uji River Station-1	NA	<6.6	100	8.7	<3.7	<6.8
Uji River Station-2	NA	<6.6	110	10	<3.7	<6.8
Tenjin River	<1.7	<6.6	39	4.7	<3.7	<6.8
Katsura River	<1.7	<6.6	7.9	<5.2	<3.7	<6.8

NA denote not analyzed

Table 2 Concentrations of PFCs in sediment (ng/g dry wt.)

Name of River	PFBS	PFHxS	PFOA	PFOS	PFOSA	PFDoA
Detection limit	0.7	1.0	0.26	0.8	0.6	1.0
Quantification limit	2.2	3.3	0.87	2.6	1.8	3.4
Kamo River	<0.7	<1.0	1.6	<1.9	2.2	0.94
Uji River St-1	<0.4	<0.5	1.3	<0.33	<0.1	0.66
Uji River St-2	<0.2	<0.3	3.9	<1.4	<1.5	0.85
Tenjin River	<1.0	<1.4	2.1	11	6.5	2.4
Katsura River	<1.1	<1.6	2.3	<2.2	<0.85	1.7
Osaka River Station-1	<0.1	<0.2	<0.1	<1.0	<1.0	<0.2
Osaka River Station-2	<0.2	<0.2	<0.1	3.8	2.4	<0.2
Osaka River Station-3	<0.1	<0.1	<0.1	6.4	4.1	<0.2
Osaka River Station-4	<0.1	<0.2	<0.1	<1.7	<0.71	<0.2

Table 3 Concentrations of PFCs in fish liver (ng/g wet wt.)

Name of fish (Japanese)	PFBS	PFHxS	PFOA	PFOS	PFOSA	PFDoA
Detection limit	0.7	1.0	0.26	0.8	0.6	1.0
Quantification limit	2.2	3.3	0.87	2.6	1.8	3.4
Scad	<7.5	<11	0.39	<8.7	<6.2	<11
Sand fish	<6.4	<9.4	<2.5	<7.4	<5.2	<9.7
Jack Mackerel	<5.8	<8.5	10	1.6	<4.7	1.3
Rainbow trout	<6.4	<9.4	<2.5	<7.4	<5.3	<9.7
Sardine	<6.3	<9.3	<2.5	<7.3	<5.2	<9.6

Table 4 Concentrations of PFCs in wildlife (ng/g wet wt.)

	Gender	PFBS	PFH×S	PFOA	PFOS	PFOSA	PFDoA
Cormorants	M	<0.09	1.1	1.2	154	310	8.4
	F	<0.09	1.5	7.3	238	362	21
	M	<0.09	0.27	1.3	95	150	26
	M	<0.09	<0.06	0.64	35	87	11
	F	<0.09	1.2	3.2	113	262	28
Racoon dog	F	<0.03	0.16	3.5	33	3.7	6.5
Racoon dog	M	<0.03	0.14	6.1	19	14	9.6
Eagle	F	<0.03	0.01	1.1	61	30	6.1
Eagle	M	<0.03	0.40	3.0	25	59	3.7
Large-bill crow	F	<0.03	0.10	0.60	13	1.7	0.10
Large-bill crow	M	<0.03	<0.03	>0.07	0.15	0.31	<0.03

(Bossi et al. 2005). All these reports are in accordance with Kannan et al. (2002), who found concentrations of PFOSA 1–5 fold greater than those of PFOS in liver of mink, otter and marine mammals. In general, PFOSA was distributed sporadically in certain species and locations. Kannan et al. (2002) also pointed out that PFOSA was an intermediate in the production of several perfluorinated compounds and also a metabolic product in mammals of *n*-ethyl perfluorooctanesulfonamide (*N*-EtFOSA), an insecticide used for the control of cockroaches, termites and ants. The presence of PFOSA at high concentrations in wildlife samples in this study may indicate different sources of exposure of PFOS and PFOSA. PFOSA was found at higher levels in effluent than in influent in waste water plants from Georgia and Kentucky in USA (Loganathan et al. 2007).

PFDoA was a greater accumulant than PFOA this unique feature shows that PFDoA is not metabolized yet in the liver of selected wildlife species. However, with this limited number of samples we cannot come to any final conclusion. Average PFCs in two female cormorants were

greater than in three males. Taniyasu et al. (2003) reported PFCs in livers of wildlife from Japan. PFH×S, PFBS were not detected in most of samples while PFOS in carrion crow (68–1200), mallard (493), pintail duck (239–497), seagull (230), black-eared kite (180) and cormorant (170–650) were present on ng/g wet weight basis. It is apparent that higher tropic animals accumulated larger amounts of PFCs when compare to fish. Bioaccumulation of PFCs in humans is also possible and therefore human samples such as blood or fat must be included in future studies.

Acknowledgments We thank Prof. April Gentry Department of English and Ms Compton S. Sania Department of Natural Science, Savannah State University for critical reading of the manuscript.

References

Austin ME, Kasturi BS, Barber M, Kannan K, Mohankumar PS, Mohankumar SMJ (2003) Neuroendocrine effects of perfluorooctane sulfonate in rats. Environ Health Perspect 111:1485–1489

Bossi R, Riget FF, Dietz R, Sonne C, Fauser P, Dam M, Vorkamp K (2005) Preliminary screening of perfluorooctane sulfonate (PFOS) and other fluorochemicals in fish, birds and marine mammals from Greenland and the Faroe Islands. Environ Pollut 136:323–329

Giesy JP, Kannan K (2001) Perfluorochemical surfactants in the environment. Environ Sci Technol 35:1339–1345

Higgins C, Luthy RG (2006) Sorption of perfluorinated surfactants on sediments. Environ Sci Technol 40:7251–7256

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

Kannan K, Corsolini S, Falandysz J, Oehme G, Focardi S, Giesy JP (2002) Perfluorooctanesulfonate and related fluorinated hydrocarbons in marine mammals, fishes, and birds from coast of the Baltic Sea and the Mediterranean Sea. Environ Sci Technol 36:3210–3216

Kannan K, Corsolini S, Falandysz J, Fillmann G, Senthil Kumar K, Loganathan BG, Ali Mohd M, Olivero J, Van Wouwe N, Yang JH, Aldous KM (2004) Perfluorooctanesulfonate and related fluorochemicals in human blood from several countries. Environ Sci Technol 38:4489–4495

Kennedy GL, Butenhoff JL, Olsen GW, O'Connor JC, Seacat AM, Perkins RG, Biegel LB, Murphy SR, Farrar DG (2004) The toxicology of perfluorooctanoate. Crit Rev Toxicol 34:351–384

Loganathan BG, Sajwan KS, Sinclair E, Senthil Kumar K, Kannan K (2007) Perfluoroalkyl sulfonates and perfluorocarboxylates in two wastewater treatment facilities in Kentucky and Georgia. Water Res (in press)

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

Prededouros K, Cousins IT, Buck RC, Korzeniowski SH (2006) Sources, fate and transport of perfluorocarboxylates. Environ Sci Technol 40:32–44

Saito N, Harada K, Inoue K, Sasaki K, Yoshinaga T, Koizumi A (2004) Perfluorooctanoate and perfluorooctane sulfonate concentrations in surface water in Japan. J Occu Health 46:49–59

Senthilkumar K (2005) Fluorinated organic chemicals: a review. Res J Chem Environ 9:50–79

Senthilkumar K, Kannan K, Ohi E, Koizumi A, Takasuga T (2005) Occurrence of perfluorinated contaminants in water, sediment and fish from Kyoto Area, Japan. Organohalogen Compd 67:229–231

So MK, Taniyasu S, Lam PKS, Zheng GJ, Giesy JP, Yamashita N (2005) Alkaline digestion and solid phase extraction method for perfluorinated compounds in mussels and oysters from South China and Japan. Arch Environ Contam Toxicol 50:240–248

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 humans from Japan. Environ Sci Technol 37:2634–2639