PERFLUORINATED ORGANIC COMPOUNDS IN SEDIMENT, AQUATIC ANIMALS INCLUDING SHARK FROM GEORGIA COAST, USA

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Abstract

Sediment and aquatic animals were collected from pond, lake, river and estuarine ecosystems in Savannah, Georgia Shelf (south Atlantic Bight), and the LCP Superfund Site in Brunswick, GA (USA). Samples were analyzed for 19 perfluorinated compounds (PFCs) such as perfluorinated sulfonates (PFSs), perfluorinated carboxylic acids (PFCAs), perfluorinated sulfonamidoacetic acid (N-MeFOSAA and N-EtFOSAA) and fluoroteromer unsaturated calboxylic acids (FTUCA). PFOS were the predominant contaminant with a concentration range of ND-320 followed by its isomer ND-75, PFHxA (ND-40) and PFNA (ND-23) on ng/g wet wt. Liver and muscle of Catfish collected from the Ogeechee River and Lake Mayer had greater concentrations. Sharks contained lower PFCs than other aquatic wildlife.

Introduction

Detection of perfluorinated compounds (PFCs), from biotic and a-biotic samples have been increasing since the 1970's when these compounds came in to use for various industrial and domestic applications[1-2]. Kannan's studies[2-5] discovering wide spread occurrence of these chemicals in the early 2000's triggered several researchers to focus on the distribution of these chemicals in the environment. Since then there have been several hundreds of quality studies carried out all over the world. There are >30 different PFCs studied to date. Among them perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA) were widely detected in biological and environmental samples. The major concern about these emerging chemicals are that they are: 1) persistent, 2) ubiquitous in nature and found even in Arctic and Antarctic, 3) several PFC compounds detected in humans, 4) several PFCs detected in wildlife and 5) laboratory toxicological studies showing that these chemicals can produce developmental toxicity to human and wildlife. Toxicological studies of mammals with PFOS and PFOA have suggested that hepatotoxicity, carcinogenicity, immunotoxicity, and developmental toxicity may be associated with chemical exposure. PFCs also have been shown to impact survival at birth, postnatal growth and induced hormone/enzyme functions.

One of our recent studies[6] reported elevated concentrations of some PFCs in wastewater treatment plant samples and their consistent discharge into the Savannah River Estuary. Therefore, we assume considerable contamination of PFC aquatic wildlife including species constituting seafood for the local community. Consequently, we analyzed PFCs in sediment and fish collected from various aquatic ecosystems of the Georgia coast. Particularly, we analyzed PFOS, isomer-PFOS, PFBS, PFHxS, PFDS, PFHxA, PFHpA, PFOA, PFNA, PFUnA, PFDoA, N-EtFOSAA, N-MeFOSAA, 6:2 FTUCA, 8:2 FTUCA, 10:2 FTUCA, PFHpS, PFPeS and PFNS for the first time.

Materials and Methods

Surface sediment samples were collected from the 10 USEPA[7] sampling stations at LCP Superfund Site during October 2006. Surface and core sediments (5 stations) were collected from Savannah River Estuary on 22nd August 2007. Various aquatic organisms including file shell, white shrimp, fishes and shark were collected in the Ogeechee River estuary in Richmond Hill, Skidaway Island, Lake Mayer, pond by Savannah Mall, St. Simons Island, Wassaw Sound, the Savannah River mouth and the Georgia Shelf in June 2006 to July 2007.

Immediately after collection, species and age were determined in the field, samples were wrapped in aluminum foil, packed in zip-lock bags and transported to the lab in a cooler and stored at -20^{o}C until dissection. For each species, 1-9 individuals were selected and then dissected. The liver and muscle tissue were separated from fish species, while soft tissue was separated from file shell and shrimp. For each species liver and muscle tissue were mixed and homogenized in a stainless steel homogenizer and stored in a freezer until chemical analysis. Only individual liver and muscle tissues of two shark species were analyzed.

Sediment samples were dried over-night under clean conditions. Approximately 10-g dry sediment was weighed in a clean 50 mL PP tube, mixed with 20 mL MeOH and spiked with ^{13}C-PFOA and ^{13}C-PFOS as an internal standard[8]. The sample was shaken in an orbital shaker for 10-min at 300 rpm, then ultra sonificated for 30 min. The resultant slurry is centrifuged for 15-min at 2500 rpm. MeOH layer was collected in a clean flask and 20-mL of MeOH was added to sediment and the extraction repeated again. Combined extracts were then vapo-trap extracted 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 leur lock nylon filter and analyzed by LC-MS/MS.

About 1-g of muscle and liver were homogenized (in clean PP tubes) with 5-mL of ultra pure water[8]. 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 ^{13}C-PFOS 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 3000 rpm. The MTBE layer was transferred into a clean PP tube and extraction was continued with addition of 5-mL of MTBE into tissue extract. The combined MTBE layer was purged under a gentle stream of nitrogen to dryness and reconstituted with 1-mL of MeOH which was filtered through a leur lock nylon filter and analyzed by LC-MS/MS. The lipid rich liver samples were reconstitute with 4-mL hexane, loaded in to a 5-g silicagel column, dried for 20 min. using a vacuum pump, eluted with 95% acetonitrile in pure water which was then evaporated and reconstituted with 1-mL of MeOH for LC-MS/MS analysis.

Instrumental analysis was performed using a high-performance liquid chromatograph (Agilent 1100LC, Agilent Technologies Palo Alto, CA) interfaced with a tandem mass spectrometer (Micromass Quattro, Waters, Milford, MA) operated in the electrospray negative-ion mode (HPLC-MS/MS). A 10-µL aliquot of extract was injected into a Zorbax Eclipse XDB C18 column (2.1 mm i.d. × 150 mm length, particle size = 5 µm) with 10 mM ammonium acetate/acetonitrile as the mobile phase starting at 50% acetonitrile at a flow rate of 200 µL/min. The gradient was increased to 100% acetonitrile at 5 min before reverting to 25% at 15 min. The desolvation gas flow rate and temperature were maintained at 500 L/h and 350˚C, respectively. The recoveries of spiked internal standards were in the range of (^{13}C-PFOA; 68-86% [sediment] and 73-94% [biota]) and (^{13}C-PFOS; 78-96% [sediment] and 74-110% [biota]). Obtained data were not corrected for the recovery. For sediment and fish liver, 1 and 5 procedural blanks were analyzed but only PFOA and PFNA were detected in some blanks at ND-0.1 and ND-0.2 ng/g, respectively (Table 1).

Results and Discussion

Detection of PFOS, Isomer PFOS, PFBS, PFHxS, PFDS, PFHxA, PFHpA, PFOA, PFNA, PFUnA and PFDoA in sediment was 100, 60, 60, 40, 20, 40, 60, 60, 20, 40 and 20%, respectively. Concentrations of PFCs in sediments were very low with ND to 1.8 ng/g ww (Table 1). Sediment core (12-15 cm depth) collected from Savannah River Estuary contained 1.8 ng/g PFHpA. PFOS were detected in all samples however, only at very low levels (0.1 to 0.8 ng/g dw). This study clearly shows that sediment collected from Savannah River and the LCP Superfund Site has less contamination profiles of PFCs. Although we report higher amounts of sewage discharge of some PFCs from President Street WWTP[6], contamination of sediment was significantly low (Savannah River Estuary Station 4 in Table 1). PFCs seem to be retained in the water column rather than in sediments[8-9]. WWTP sludge samples retain considerable levels of PFCs[6]. Physico-chemical behavior of

individuals PFC may vary and thus we suggest further study with large number of sediment samples from different localities.

Table 1. Concentrations of PFCs in sediment (ng/g dry weight [dw]) and fish (ng/g wet weight [ww]).

Sample Name	Sample	PFOS	Is-PFOS*	PFBS	PFHxS	PFDS	PFHxA	PFHpA	PFOA	PFNA	PFUnA	PFDoA
Blank (n=2)	*Blank*	*ND*	*ND*	*ND*	*ND*	*ND*	*ND*	*0.1*	*0.1*	*ND*	*ND*	*ND*
Sediment SR (n=5)		0.3-0.8	0.1-0.2	0.1-0.1	ND-0.3	ND	ND-0.6	ND-1.8	ND-0.2	ND-0.1	ND-0.2	ND-0.1
Sediment LCP (n=10)		0.1-0.4	ND	ND	ND-0.3	ND-0.2	ND-0.2	0.1-0.4	ND-0.2	ND	ND	ND
Blank (n=5)	*Blank*	*ND-0.1*	*ND*	*ND*	*ND*	*ND*	*ND*	*ND-0.1*	*ND-0.1*	*ND-0.2*	*ND*	*ND*
sawtooth pen clam (n=2)	T	33	8.6	ND	1.7	0.70	2.8	0.90	11	16	2.0	0.40
white shrimp (n=3)	T	32	8.6	0.30	0.40	1.5	1.5	0.10	2.5	4.2	5.1	0.80
white shrimp (n=4)	T	23	5.7	0.30	0.20	0.60	0.10	0.10	0.90	3.0	3.4	0.60
unidentified eel (n=1)	M	6.3	0.8	ND	ND	0.30	ND	ND	ND	ND	0.2	0.20
unidentified eel (n=1)	L	16	3.4	ND	0.20	2.9	ND	0.10	0.10	0.10	1.3	0.80
oyster toadfish (n=3)	M	2.9	0.70	ND	ND	0	0.10	0.10	0.30	0.70	0.30	0.10
oyster toadfish (n=3)	L	7.5	1.9	ND	0.20	0.40	11	ND	1.7	0.40	0.40	0.10
oyster toadfish (n=1)	M	8.6	2.3	ND	ND	0.10	0.10	0.20	0.70	2.0	2.4	0.40
oyster toadfish (n=1)	L	11	2.3	ND	ND	0.20	3.2	0.10	ND	0.40	1.0	0.30
unidentified snapper (n=3)	M	13	3.2	ND	ND	5.3	0.50	0.20	0.30	0.40	1.0	0.70
unidentified snapper (n=4)	M	12	2.9	ND	0.10	7.1	0.20	0.10	0.20	0.50	0.60	0.30
unidentified catfish (n=3)	M	33	7.60	ND	ND	1.4	1.6	0.10	0.40	1.7	1.5	0.30
unidentified catfish (n=3)	L	261	66	0.10	0.20	14	11	0.20	0.80	4.7	8.2	1.5
unidentified catfish (n=8)	M	41	8.0	0.80	2.00	2.1	0.50	0.30	0.30	0.80	0.70	0.40
unidentified catfish (n=8)	L	318	75	2.1	3.4	0.30	40	0.20	2.6	3.9	1.1	0.50
Atlantic croaker (n=4)	M	0.40	0.10	ND	0.10	ND	0.40	0.10	0.20	0.40	ND	ND
southern kingfish (n=5)	M	2.1	0.50	ND	ND	0.30	6.1	0.10	0.50	0.50	0.40	0.10
southern kingfish (n=5)	L	5.1	1.2	ND	ND	0.40	1.0	0.10	0.30	0.30	0.60	0.20
southern stingray (n=2)	M	3.0	0.80	ND	ND	0.10	0.10	0.20	1.0	0.80	0.70	0.30
southern stingray (n=2)	L	22	4.9	ND	ND	0.10	0.90	0.10	1.2	2.7	1.0	0.20
Atlantic croacker (n=5)	M	4.8	1.2	ND	0.10	0.10	0.10	0.10	0.30	0.90	0.80	0.20
silver perch (n=9)	M	0.40	0.10	ND	ND	0.20	0.20	0.10	0.30	0.30	0.20	0.10
southern kingfish (n=3)	M	4.7	1.2	ND	0.10	0.20	0.30	0.20	1.0	1.2	0.80	0.20
spot (n=3)	M	4.3	1.0	0.10	0.10	0.90	0.50	0.20	0.70	19	0.40	ND
inshore lizardfish (n=1)	M	0.40	0.10	ND	ND	0.10	0.10	1.1	0.70	23	ND	ND
inshore lizardfish (n=1)	L	1.2	0.30	0.40	ND	0.10	4.6	0.40	ND	0.20	0.20	0.10
tomtate (n=4)	M	0.10	ND	ND	ND	ND	0.10	0.10	0.50	0.20	ND	ND
tomtate (n=4)	L	0.20	ND	0.10	0.20	ND	20	0.10	1.1	0.20	0.10	0.10
sea robin (n=4)	M	4.5	1.0	ND	0.70	0.40	2.7	0.10	1.2	9.4	1.8	0.20
black sea bass (n=3)	M	3.3	1.0	ND	0.10	0.20	0.30	0.10	0.70	1.2	0.90	0.30
large mouth bass (n=1)	M	1.9	0.50	0.30	ND	0.10	0.10	ND	0.40	0.20	0.10	0.20
ASNS (n=5)	M	ND-1.1	ND-0.3	ND	ND	ND-0.5	ND-0.1	ND-0.2	ND-1.0	ND-0.5	ND-0.2	ND
ASNS (n=5)	L	1.1-2.8	0.3-0.6	ND-0.1	ND-0.1	0.1-0.3	0.8-3.4	0.2-0.3	0.1-1.5	0.3-1.5	0.1-1.5	ND-0.2
bonnethead shark (n=3)	M	ND-0.4	ND-0.1	ND	ND	ND-0.2	ND-0.1	ND-0.1	ND-0.4	ND-0.2	ND-0.1	ND-0.1
bonnethead shark (n=3)	L	0.2-6.3	0.1-1.4	ND-0.6	ND-0.1	ND-0.1	0.3-5.8	ND-0.6	0.4-0.7	1.0-1.4	0.1-2.6	0.1-0.6

ND=not detected; T, M, L, denote tissue, muscle, liver, respectively; ASNS= Atlantic sharpnose shark.

SR and LCP = Savannah River and Linden Chemicals and Plastic Superfund Site.

Common names of fishes can be obtained from: Nelson JS, Crossman EJ, Espinosa-Pérez H, Findley LT, Gilbert CR, Lea RN, Williams JD, 2004. Common and Scientific Names of Fishes from the United States Canada and Mexico 6th Edition, American Fisheries Society, 386 p.

Detection of PFOS, isomer-PFOS, PFBS, PFHxS, PFDS, PFHxA, PFHpA, PFOA, PFNA, PFUnA and PFDoA in fish tissue were 95, 86, 27, 48, 80, 55, 82, 86, 95, 84 and 70%, respectively. PFHpS, PFPeS and PFNS were not detected in any of aquatic wildlife analyzed in this study (Table 1). While, N-MeFOSAA, N-EtFOSAA and fluoroteromer unsaturated calboxylic acids (FTUCA) cannot be reported due to the low recovery rates (<50%) due to interference of matrix effect in the sample extract and low reliability of the data. Liver of catfish collected in Ogeechee River and Lake Mayer contained higher PFOS concentrations of 318 and 261 ng/g ww, respectively (Table 1). The second major PFC compound was PFOS-isomer which accumulated in the range of ND-75 ng/g ww. Among PFCAs, perfluorinated hexanoic acid (PFHxA) was the predominant contaminant (ND-40 ng/g) followed by perfluorinated nonanoic acid (PFNA; ND-23 ng/g) then PFOA (ND-11 ng/g), the latter one is usually greater among PFCAs in other studies[3-5]. Inshore lizard fish and sawtooth pen clam contained maximum PFNA and PFOA, respectively. This is the first study reporting contamination of isomer-PFOS, PFHxA and PFNA in fish tissue collected from the Georgia coast. Contamination of other PFC compounds in the fish tissue was found to be negligible. Muscle tissues contained lower PFCs than livers in all analyzed aquatic species. White shrimp and sawtooth pen clam soft tissue contained greater PFCs than shark liver. These results show that sharks can metabolize PFCs greater than lower trophic animals such as bivalves (sawtooth pen clam) and crustaceans (white shrimp). Hammerhead shark liver from Japan also showed lower levels of PFCs than other fish and marine mammals[9].

In this study isomer PFOS is second major contaminant followed by its precursor. PFOS contains several isomers, which are detectable in biota[10-11]. These isomers are usually not completely separated and reported as additional signal peaks[11]. Based on current high precision analytical methods, it is possible to separate and quantify different PFCs such as perfluorinated sulfonates from perfluorinated carboxylates and different carbon chain lengths. Particularly, hundreds of structural PFOS isomers ($C_8F_{17}SO_3^-$) are theoretically obtained. In this study we detected PFOS isomer with m/z (498.6 > 79.6) which is the second most predominant PFC contaminant in the wildlife samples. It should be worth indicating that resolution (Rs) of isomer PFOS and parent PFOS peaks is calculated to be 0.76. It is likely that this isomer is an impurity of perfluorinated sulfonates and therefore further study is warranted.

The results also indicate that due to a high dispersion ratio, PFCs were minimal in wildlife collected from estuaries and Atlantic Ocean compared to those from the Ogeechee River and Lake Mayer. Catfish were found to accumulate larger amounts of PFCs than the other aquatic predator species such as sea bass, Atlantic croaker, Atlantic spot, unidentified snapper, oyster toadfish, two shark species etc., Catfish commonly consumed by locals. Considering greater PFCs contamination, intake of this fish from the Ogeechee River and Lake Mayer leads to excess intake of PFCs. Contamination of the Ogeechee River is probably due to discharge of treated sewage water from the Georgetown area. Similarly contamination of catfish collected from Lake Mayer is due to sewage water mixing from neighborhood channels and streams to this lake. Furthermore atmospheric deposition and lake contamination by recreating people cannot be ignored. PFOS concentrations with the fish collected from this study were compared to other studies reported so far. Fish liver collected from Japan showed greatest concentrations followed by fish bile in Colombia, Belgium, Finland, Lake Michigan in USA[12]. Liver of catfish analyzed in this study showed comparable concentration with those of liver from the Great Lakes, however, they were several times higher than liver of fish from Mississippi, Florida, South Carolina and New York[12].

From the toxicological point of view, in fish, serum alanine aminotransferase (ALT), but not aspartate aminotransferase (AST) was shown to correlate positively with PFOS liver concentration, indicating that PFOS might contribute to the induction of hepatic damage[13]. Analysis of total carbohydrate, lipid and protein content of bib (*Trisopterus luscus*) liver tissue revealed a positive correlation between the protein content and the PFOS liver concentration. Analysis of the relation between the PFOS liver concentrations and biological samples such as fork length and serum activity of ALT and AST allowed a preliminary effect evaluation of PFOS on fish in

an estuarine marine environment. Based on this information and our results, further study is under way in our laboratory.

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