ACCUMULATION OF PERFLUORINATED COMPOUNDS IN AQUATIC ECOSYSTEM

Lee B[1], Eom I[1], Lee D[1], Kim P[1]*, Choi K[1], Cho H[2]

[1] Risk Assessment Division, National Institute of Environmental Research, Kyungseo-Dong, Seo-gu, Incheon 404-708, Republic of Korea; [2] Faculty of Marine Technology, College of Ocean Science & Technology, Chonnam National University, San 96-1, Dundeok-dong, Yeosu 550-749, Republic of Korea

Introduction

Perfluorinated Compounds (PFCs), artificially synthesized for the use of its unique properties, form bond at a molecular level (Giesy and Kannan, 2001). They are widely used as fluoropolymers, coating agents for food applications, cleaning agents for semiconductors, repellant agents, and coating additives, etc. With its surfactant–like property, they are also used as aqueous film forming foam for fire-fighting foams (Mawn et al., 2005; Begley et al., 2005). Since PFCs rarely decompose when entering into the environment due to its molecular stability, they have a capacity of long range transport and are therefore detected at polar regions or the oceans where pollutants do not exist (Giesy and Kannan, 2001; Yamashita et al., 2008).

The investigation of PFCs has been conducted at full scale mainly by developed countries starting from 2000s, as their bioaccumulation, hazards and risk started to be well known. In May 2005, 3 M, the largest manufacturer handling PFOS, declared to halt its manufacture of the substance, and major countries including Canada, the EU, USA and Japan even implemented regulations against PFOS (Yeung et al., 2009). At the 4th Conference of the Parties of the Stockholm Convention in May 2009, the parties agreed to include PFOSF, its salts and PFOSF in the list of POPs (UNEP, 2009). The release of PFCs into the environment occurs mainly around water systems (Hansen et al., 2002; Saito et al., 2003; Senthilkumar et al., 2007). In Korea, manufacture facilities of PFCs do not exist and therefore PFCs frequently used in daily lives and detected in rivers are judged to be released from waste water-disposal facilities.

In this study, we selected the rivers with high and low doses of PFCs and evaluated their residual and accumulative properties in organisms.

Materials and methods

Sampling

To investigate accumulative levels along the food chain, we selected Bockha stream of Namhan River where the level of PFCs is expected to be high, and Chungju-lake as a clean area. Sampling at each site was conducted twice in June and September 2010, and samples including phyto/zoo plankton, fish, water, and sediment were simultaneously collected at the same time. Phyto plankton were collected using phyto plankton net, and zoo plankton were vertically collected from bottom to surface layers using two types of NORPAC net with mesh size. *C.Auratus* and *S.scherzeri* were collected for fish: for *C.Auratus,* 22 and 34 individuals were collected in Chungju-lake and Bockha stream, respectively; for *S.scherzeri,* 33 and 28 individuals were collected in Chungju-lake and Bockha stream, respectively. After collection, the blood of fish was sampled from tail arterio or heart using syringe. The liver was autopsied after labeled livers from individuals were kept frozen at -20℃.

We used polypropylene (pp) bottles for sampling, not to use florinate polymer containers with indirect contamination potential and glass equipment with adsorption potential of PFCs when sampling water and sediment.

Pretreatment

River water samples (1000 mL) were collected without containing precipitate and suspension. The samples were added with 5 ng of $^{13}C_4$-PFOA and $^{13}C_4$-PFOS as internal standard for Solid Phase Extraction, and then loaded at 3 mL/min into a Solid Phase Cartridge type Waters Oasis® HLB 3 cc 60 mg (USA). Solid Phase Cartridge was used after activation with 6 mL of methanol and of Milli-Q water. After loading samples, Solid Phase Cartridge was cleaned up with 40% methanhol and completely dried. The target PFCs collected by dried

Cartridge were extracted using 6 mL of methanol (100%) and then blew down into 1 mL by nitrogen gas (99.999%) . The samples passed into autovial sample of a HPLC after filtering through a 0.2 μm Nylon filter.

For sediment, 5 g of dried samples in hood were applied with solid-liquid extraction method. The samples were put in 50 mL PP tube and then 5 ng of each two types of internal standards were added, finally extracting twice for 30 min using a sonicator, with 20 mL of methanol. Extracted solution was dried and processed as described for water samples.

The blood and liver samples from fish were applied with ion-pair extraction method. 1 mL of blood and 1 g liver samples in 15 mL pp tubes were homogenized by adding 2 mL of Milli-Q water and using a vortex. 1 ml of tetra-n-butylamonium hydrogensulfate solution 0.5 M (adjusted to pH 10), 2 ml of 0.25 M sodium carbonate buffer and 5 ml methyl-tert-butylether (MTBE) were then added to each tube. Next, the mixture was intensively shaken for 30 min. The organic and aqueous layers were separated by centrifuging at 3000 rpm in 20 min and an exact volume of upper layer of MTBE (4 ml) was transferred to a second tube. This extraction procedure was repeated twice and all MTBE layer was transferred to this second tube to attain a final extraction solvent for a biota sample. Extracted solution was blown down into 1 mL by nitrogen gas (99.999%). Finally, the samples passed into autovial sample of a HPLC after filtering through a 0.2 μm Nylon filter.

Analysis of PFCs

16 types of target PFCs include 11 types of perfluoro alkyl carboxylic acids (PFCAs) and 5 types of perfluoro alkyl sulfonates (PFASs). For quantitative analysis, internal standard method was used. The instrument used in this analysis includes Agilent 1100(USA) HPLC and HPLC-MS/MS interfaced with API 2000 tandem mass spectrometer (Applied Biosystems) triple-quadrupole mass spectrometer.

10 μL aliquot of the extracted sample was injected to a 2.1×12.5 mm (5 μm) guard column(Zorbax Eclopse XDB-C_8) connected serially to an 100×2.1mm(5μm) analytical column(Thermo Betasil® C_{18}). Temperature of analytical column was fixed at 35℃. Mobile phase used 2 mM ammonium acetate and Methanol as a gradient mode, and flow rate was 300 μL/min. To quantify the target PFCs in MS/MS, a multiple reaction monitoring mode was utilized.

For qualitative analysis, standard and retention time of samples were compared and MS/MS spectra mode was applied with Multiple reaction monitoring mode (MRM). MRM for 16 types of PFCs is as follows: PFBS (Perfluorinated butane sulfonate) 299>99, PFHxS (Perfluorinated hexane sulfonate) 399>99, PFHpS (Perfluorinated Heptane sulfonate) 449>99, PFOS (Perfluorinated octane sulfonate) 499>99, PFDS (Perfluorinated decane sulfonate) 599>99, $^{13}C_4$-PFOS 503>99, PFOSA (Perfluorinated octanesulfonyl amide) 498>78, PFHxA (Perfluorinated Hexanoic acid) 313>269, PFOA (Perfluorinated octanoic acid) 369>169, $^{13}C_4$-PFOA 372>172, PFNA(Perfluorinated nonanoic acid) 463>219, PFDA (Perfluorinated decanoic acid) 513>469, PFUnA (Perfluorinated undecanoic acid) 563>519, PFDoA (Perfluorinated dodecanoic acid) 613>569.

The calibration curve was drawn by 9 curve points of native external standard at 0.1, 0.2, 0.5, 1, 2, 5, 10, 20, 50, 100 ng/mL. The regression coefficient (r^2) of calibration curves for all target PFCs were higher than 0.99. Limit of detection (LOD) was 0.4~1.53 ng/g, and Limit of Quantification (LOQ) was 1.38~4.59 ng/g. For blood and liver, the recovery rates (%) of internal standards were in acceptable ranges of 75.0±5.1%, 73.6±5.8%, for $^{13}C_4$-PFOA and 73.9±6.4%, 80.4±4.9% for $^{13}C_4$-PFOS.

Results and discussion

PFCs in Media

The average concentrations of PFCs analyzed twice were as shown in Fig. 1. The PFCs frequently detected in the whole media included PFNA, PFDA, PFUnA, PFDoA, PFTrA, PFTeDA, and PFOS.

Of media including water, SS(suspended solid), sediment, and phyto/zoo plankton, SS was found to have the highest level of PFCs. The PFC levels in water and sediment were relatively lower than that of SS, indicating similar results as previous studies. In particular, carboxylate compounds with 8-12 carbon chains were mostly detected, and only PFOS among sulfonate compounds was detected higher compared with carboxylate compounds. In water, SS, sediment, phyto-, meso zoo plankton, carp liver, mandarin liver, carp blood, mandarin blood, the levels of PFOS were 0.0033, 54.15, 0.18, 2.07, 3.20, 15.41,19.38, 35.72, and 60.62 ppb. In analysis of concentrations in plankton, carboxylate compounds with large number of carbon chains (9-16) were mainly

detected in both phyto and zoo planktons. In particular, of PFCs in liver and blood of *C.Auratus* and *S.scherzeri*, PFOA and PFOS were detected high, especially higher in blood.

Based on the results, the level of PFOS increased in an order of water, plankton, *C.Auratus,* and *S.scherzeri.* For sulfonate compounds, only PFOS was detected in all media, and the levels of other compounds were below LOD. PFOS showed the most significant increase according to trophic level. For carboxylate compounds, the concentration was PFUnA>PFDoA=PFDA=PFTrA>PFTeA=PFNA.

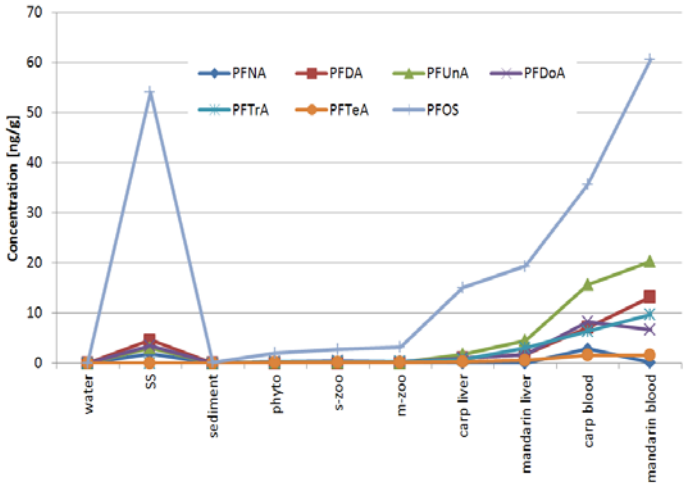

Fig. 1. PFCs in media

Bioconcentration factor by media

Table 1 shows bioconcentration factor (BCF) of PFCS by food chain. In general, PFOS and caroxylate compounds with more than 9 carbon chains such as PFNA show bioaccumulation. In this study, the results showed that bioconcentration by chemical was different with trophic level. In plankton, PFDoA with 12 carbon chains showed the highest bioconcentration, and in blood of *C.Auratus*, PFUnA with 11 carbon chains showed the highest bioconcentration, while in blood of *S.scherzeri,* PFDA with 10 carbon chains showed the highest bioconcentration. It is considered that these results have correlations with the presence of certain binding compounds in organisms, and further studies are required. In particular, as the lengthen of chains is longer, there is no direct correlation with bioconcentrations.

Table 1. Bioconcentration factor of PFCs by food chain

BCF	Phyto Plankton	s-zoo	m-zoo	carp liver	mandarin liver	carp blood	mandarin blood
PFNA	3600	2680	3550	170	620	35310	2380
PFDA	1220	1890	1890	39680	63560	271110	501990
PFUnA	2590	3310	1890	33870	90690	312310	406390
PFDoA	7150	8590	6360	20110	35290	164580	134753
PFTrA	4790	5860	6100	14280	61990	127740	194520
PFTeA	2470	3670	2170	5460	12200	33250	33030
PFOS	620	850	960	4580	5860	10810	18350

Conclusions

In zoo and phyto planktons, carboxylate compounds with 9-16 carbon chains were mainly detected, and among sulfonate compounds, PFOS was the only compound detected relatively high. For the liver and blood of

fish, the level of PFNA in blood of *C.Auratus* was 10 times higher in Bockha stream compared with Chung-ju lake. The level of PFDoA showed no significant difference among regions, whereas PFOS showed relatively higher level in Chung-ju lake. The level of PFOS increased in an order of water, plankton, *C.Auratus,* and *S.scherzeri*, and PFOS showed the most significant increase in trophic level. For carboxylate compounds, the concentration was PFUnA>PFDoA=PFDA=PFTrA>PFTeA=PFNA. In conclusion, the bioconcentration was different from PFCs, but the levels were 10^3 in plankton and 10^4 in liver and blood of fish.

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