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Concentrations of Perfluorinated Compounds in Tap Water and Human Serum from Osaka, Japan

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Abstract—Perfluorinated compounds (PFCs) are hydrophilic compounds found in tap water all over the world. The fact that tap water may be the main source of PFCs contamination in humans is of great concern. We analyzed 17 PFCs in the tap water of Osaka, Japan to estimate the human intake. In addition, we analyzed PFCs in the serum of 44 volunteers who lived or worked in Osaka, Japan, to investigate the contamination levels in the humans. Of PFCs detected in tap water, perfluorooctanoate (PFOA) concentration was presented at the highest concentration. PFOA, perfluorononanoate (PFNA), perfluoroundecanoate (PFUdA), perfluorodecanoate (PFDA), perfluorooctanesulfonate (PFOS), and perfluorohexanesulfonate (PFHxS) were detected in all human serum samples. We found that while short-chain PFCs were detected in tap water samples, these were not detected in any of the human serum samples tested. Conversely, while long-chain PFCs were not detected in tap water samples, these PFCs were detected in the human serum. Therefore, it appears that there are differences in the accumulation and metabolism, which are related to the carbon chain length.

Keywords: PFCs, tap water, human serum

INTRODUCTION

Perfluorinated compounds (PFCs) such as perfluorooctanesulfonate (PFOS) and perfluorooctanoate (PFOA) are very stable in the environment and thus accumulate in living organisms, including humans. Thus, there are increasing concerns relating to the possible effects that PFOS and PFOA may be exerting on the wider biosphere, and more specifically, on humans (Giesy and Kannan, 2001; Kannan *et al*., 2002, 2004). PFOS and PFOA have 8 carbon chains. Other PFCs exist that have either less or more than 8 carbon chains. These PFCs were detected in the aquatic environment and wildlife. Therefore, it is important to analyze and assess their potential to affect human health.

Due to relatively high water solubility, PFCs are difficult to remove by water treatment systems (Takagi *et al*., 2008; Mak *et al*., 2009). PFCs have been detected in tap water all over the world. Therefore, tap water may be the main source of human exposure to PFCs. In this study, we measured and characterized

the PFCs in tap water supplied in Osaka, Japan. In addition, we analyzed 17 PFCs in the human serum that was obtained from subjects who lived or worked in Osaka, Japan, to investigate the contamination levels in these participants.

MATERIALS AND METHODS

Reagents and chemicals

PFAC-MXB (native PFCs) and MPFAC-MXA (labelled PFCs) were purchased from Wellington Laboratories Inc. (Guelph, ON, Canada). $^{13}C_8$-PFOA was purchased in the syringe-form from Cambridge Isotope Laboratories, Inc. (Andover, MA, U.S.A.). Sep-pak plus HLB cartridge (Waters, Tokyo, Japan) were used in the solid phase extraction (SPE) of water samples.

Analytical method

Tap water samples were collected in Osaka, Japan, between September 2009 and December 2009, every 2 weeks. This tap water was treated by ozonation and activated carbon filtration. The volume of each water sample analyzed was 1 L. Residual chlorine was removed by sodium sulphite solution, and then the samples were stored at 4°C until analysis.

Human serum samples were collected from 23 men (age: 27–65 years) and 21 women (age: 26–60 years) volunteers who were living or working in Osaka, Japan, in November 2009. We collected blood from volunteers after obtaining their informed consent. Whole blood samples were left to stand for 3 h at room temperature then centrifuged at 3000 rpm for 10 min at 25°C. The serum fractions were transferred to vials and stored at –40°C prior to analysis. We obtained information from volunteers via questionnaires.

Analyte separation was performed using an Agilent 1100 series HPLC system. The extracts (5 μL) were injected into an Inertsil ODS-3 (150 × 2.1 mm i.d., 5 μm) column (GL Science; Tokyo, Japan) with a 10 mM ammonium acetate/acetonitrile mobile phase starting at 25% acetonitrile held for 2 min. At a flow rate of 200 μL/min, the gradient was increased to 95% acetonitrile at 22 min and held for 13 min. The column temperature was maintained at 40°C. For the quantitative determination, the HPLC system was interfaced to an AB Sciex API 3000 tandem mass spectrometer operated with the electrospray negative ionization mode.

RESULTS AND DISCUSSION

The concentrations of PFCs in tap water remained constant for 4 months (Table 1). The concentrations of perfluorodecanesulfonate (PFDS), perfluorobutanoate (PFBA), perfluoropentanoate (PFPeA), perfluorododecanoate (PFDoA), perfluorotridecanoate (PFTrDA), perfluorotetradecanoate (PFTeDA), perfluorohexadecanoate (PFHxDA), and perfluorooctadecanoate (PFOcDA) were below the method quantification limit (MQL) in all samples. Of perfluoroalkyl sulfonates (PFASs), PFOS was the highest in concentration, ranging from 0.51 to 1.0 ng/L. Perfluorobutanesulfonate (PFBS) and perfluorohexanesulfonate (PFHxS)

Table 1. Concentrations (ng/L) of PFCs in tap water samples from Osaka, Japan.

Month	*n*		PFBS	PFHxS	PFOS	PFDS	PFPeA	PFHxA	PFHpA	PFOA	PFNA	PFDA	PFUdA
Sep-09	10	Max	0.59	0.38	0.93	<0.13	0.56	3.2	3.2	13	3.5	0.58	0.33
		Mean	0.50	0.35	0.89	0.0	0.48	2.9	3.0	12	3.0	0.50	0.31
		Min	0.45	0.31	0.84	<0.13	0.41	2.6	2.7	11	2.6	0.45	0.29
Oct-09	18	Max	0.54	0.40	1.0	<0.13	0.57	3.2	3.2	14	3.6	0.64	0.40
		Mean	0.42	0.32	0.76	0.0	0.41	2.7	3.0	13	3.0	0.52	0.34
		Min	0.31	0.24	0.55	<0.13	0.26	2.4	2.7	12	2.6	0.39	0.29
Nov-09	17	Max	0.39	0.33	0.84	<0.13	0.57	2.8	3.0	15	3.1	0.56	0.32
		Mean	0.33	0.29	0.73	0.0	0.43	2.5	2.7	14	2.8	0.51	0.28
		Min	0.27	0.25	0.61	<0.13	0.31	1.6	2.1	12	2.3	0.43	0.24
Dec-09	19	Max	0.41	0.29	0.66	0.27	0.38	2.2	2.3	12	2.6	0.50	0.28
		Mean	0.37	0.25	0.62	0.03	0.34	1.9	2.1	11	2.3	0.44	0.25
		Min	0.35	0.21	0.51	<0.13	0.27	1.5	2.0	10	2.2	0.37	0.22

Table 2. PFCs intake from tap water.

	ng/day	ng/year
PFBS	0.8	287
PFHxS	0.6	214
PFOS	1.4	529
PFDS	0.0	0.0
PFHxA	4.8	1767
PFHpA	5.2	1909
PFOA	25.0	9128
PFNA	5.4	1985
PFDA	1.0	355
PFUdA	0.6	212
PFDoA	0.0	0.0
PFTrDA	0.0	0.0
PFTeDA	0.0	0.0
PFHxDA	0.0	0.0
PFOcDA	0.0	0.0

were detected at approximately the same level, with concentrations ranging from 0.31 to 0.59 ng/L and from 0.21 to 0.40 ng/L, respectively. PFOS concentration in tap water was lower than that previously reported (Takagi *et al.*, 2008). Therefore, production and consumption of PFOS-containing products may be decreased in Osaka.

Of perfluoroalkylcarboxylic acid (PFACs), PFOA was present in the highest concentration, ranging from 10 to 15 ng/L. The concentration ranges of perfluorohexanoate (PFHxA) perfluoroheptanoate (PFHpA) and perfluorononanoate (PFNA) were from 1.5 to 3.6 ng/L. The concentrations of perfluorodecanoate (PFDA) and perfluoroundecanoate (PFUdA) were low, ranging from 0.37 to 0.64 ng/L and from 0.22 to 0.40 ng/L, respectively. Compared to the findings in a previous report (Takagi *et al.*, 2008), the PFOA concentration in tap water has decreased in Osaka similar to that of PFOS.

The PFOS and PFOA provisional health advisory levels, as reported by the United States Environmental Protection Agency (U.S. EPA), are 200 and 400 ng/L, respectively (U.S. EPA, 2009). The concentrations of PFOS and PFOA in all water samples were lower than these values. The PFCs intake, assuming an intake of 2 L of tap water every day, is shown in Table 2. When the detected concentration was below the MQL, the concentration was assigned as a value of zero. The PFOA intake was the highest, at 25 ng/day from 2 L of tap water. The PFACs intake was 43 ng/day; 2.8 ng of PFASs (1.4 ng of PFOS) would be ingested every day from 2 L of tap water.

In the case of a 60 kg adult, the daily intake of PFOS was 0.000024 μg/kg body weight (bw)/day and that of PFOA was 0.00042 μg/kg bw/day. The tolerable daily intake (TDI) of PFOS and PFOA reported by the European Food Safety Authority (EFSA) are 0.15 and 1.5 μg/kg bw/day, respectively (EFSA, 2008). The projected intakes of PFOS and PFOA from tap water were 0.02 and

Table 3. Concentrations (ng/mL) of PFCs in human serum samples obtained from Osaka, Japan.

Sex	Age	*n*		PFHxS	PFOS	PFHpA	PFOA	PFNA	PFDA	PFUdA	PFDoA	PFTrDA
male	20–29	2	Max	1.8	14	<0.13	14	3.6	1.2	2.9	0.26	0.63
			Mean	1.7	13	0.0	10	3.6	1.1	2.5	0.13	0.45
			Min	1.6	12	<0.13	5.5	3.6	1.1	2.2	<0.15	0.27
	30–39	5	Max	1.5	12	<0.13	16	4.5	1.4	3.0	<0.15	0.34
			Mean	1.2	10	0.0	12	3.7	1.0	2.1	0.0	0.14
			Min	0.77	5.4	<0.13	3.0	1.5	0.44	1.1	<0.15	<0.05
	40–49	6	Max	2.7	21	<0.13	23	6.2	1.5	2.9	0.28	0.49
			Mean	1.9	15	0.0	13	4.0	1.0	2.0	0.050	0.25
			Min	1.1	9.6	<0.13	6.4	2.8	0.52	1.3	<0.15	<0.05
	50–59	9	Max	2.2	23	0.27	18	7.3	1.9	4.6	0.59	0.60
			Mean	1.5	13	0.030	12	4.3	1.2	2.5	0.12	0.33
			Min	0.81	7.6	<0.13	6.2	2.4	0.65	1.3	<0.15	<0.05
	60–	1	Max	1.7	23	0.28	15	5.3	2.0	4.6	0.49	0.84
			Mean	1.7	23	0.28	15	5.3	2.0	4.6	0.49	0.84
			Min	1.7	23	0.28	15	5.3	2.0	4.6	0.49	0.84
female	20–29	5	Max	0.91	9.1	0.39	8.6	3.8	1.8	3.9	0.41	0.78
			Mean	0.51	7.2	0.14	6.5	3.0	1.2	2.5	0.16	0.31
			Min	0.35	4.4	<0.13	4.5	2.2	0.89	1.5	<0.15	<0.05
	30–39	3	Max	1.2	9.4	<0.13	7.6	3.4	1.2	2.9	0.29	0.42
			Mean	0.86	8.5	0.0	6.2	3.4	1.1	2.4	0.10	0.35
			Min	0.53	7.4	<0.13	4.1	3.2	0.96	1.8	<0.15	0.30
	40–49	7	Max	1.7	24	0.49	17	6.2	2.3	5.2	0.59	0.74
			Mean	1.0	12	0.14	11	3.9	1.4	3.2	0.22	0.44
			Min	0.54	4.6	<0.13	6.5	2.2	0.65	1.3	<0.15	0.26
	50–59	6	Max	1.6	28	0.38	16	7.5	3.2	4.0	0.81	0.81
			Mean	1.1	15	0.11	13	4.6	1.6	3.0	0.32	0.54
			Min	0.77	7.3	<0.13	8.7	2.4	0.92	2.1	<0.15	0.31

0.03% of these values. Therefore, it seems that the concentrations of PFOS and PFOA detected in tap water in the current study pose no immediate risk to human health.

PFCs concentrations in human serum samples are shown in Table 3. The concentrations of PFOS were the highest, followed by PFHxS, of the PFASs. The concentration ranges of PFOS and PFHxS were from 4.4 to 28 and from 0.35 to 2.7 ng/mL, respectively. The concentrations of PFOS and PFHxS were detected in all human serum samples. PFBS and PFDS were below the MQL in all samples.

Of the PFACs, PFOA was present at the highest concentrations, ranging from 3.0 to 23 ng/mL. The concentration ranges of PFNA, PFDA, and PFUdA were from 1.5 to 7.5, from 0.44 to 3.2, and from 1.1 to 5.2 ng/mL, respectively. These PFACs were detected in all human serum samples. PFHpA, PFDoA, and PFTrDA were detected in some samples, and the detection ratios were 18, 39, and 80%, respectively. Other PFACs were not detected in any sample. The concentrations of PFHxS and PFOS were significantly lower in women than in

men (Mann-Whitney U-test, $p < 0.05$), the result that is in accordance with previous reports (Yeung *et al.*, 2006; Harada *et al.*, 2007). On the other hand, there were no significant differences between men and women with respect to the levels of PFOA, PFNA, PFDA, and PFUdA.

There were significant differences in the PFOA and PFHxS levels between women in their 20's and those in their 50's (Kruskal-Wallis test, $p < 0.05$). In women who were in their 50's, these PFCs were higher than in those in their 20's. However, in men, there were no differences in the concentrations of any PFCs across the generations.

In human serum samples, odd-numbered PFACs such as PFNA, PFUdA, and PFTrDA were higher than even-numbered PFACs such as PFDA and PFDoA. Ammonium perfluorononanoate is manufactured primarily in Japan. This product has corresponding odd-numbered PFACs (Prevedouros *et al.*, 2006). This may be the source of the odd-numbered PFACs in human serum in Osaka, Japan. Although long-chain PFCs were not detected in tap water samples, these PFCs had accumulated in the human serum. Therefore, it appears that there are differences in accumulation and metabolism between these PFCs according to carbon-chain length.

The geometric mean (GM) concentrations of PFOS and PFOA in human serum from Osaka city in 2003 were 29 and 15 ng/mL respectively in men (N = 5, age: 22–58 years) and 13 and 8.5 ng/mL in women (N = 8, age: 21–43 years) (Harada *et al.*, 2007). In our study, the GM concentrations of PFOS and PFOA in men living in the Osaka city (N = 15, age: 31–65 years) were 11 and 12 ng/mL, respectively. The GM concentrations of PFOS and PFOA in women (N = 5, age: 33–52 years) were 9.5 and 11 ng/mL, respectively. Compared with the findings in the report in 2004 (Harada *et al.*, 2007), the PFOS concentrations in men were reduced and the PFOS concentrations in women were at approximately the same level. On the other hand, the PFOA concentrations in both men and women was detected at approximately the same levels, as reported in 2004 (Harada *et al.*, 2007). The GM concentrations of PFOA in human serum from the Kinki region, including Osaka were 12 ng/mL in men and 8.5 ng/mL in women in 2004 (Harada *et al.*, 2007). The GM concentrations of PFOA from other regions were from 3.4 to 5.2 ng/mL in men and from 2.3 to 4.4 ng/mL in women (Harada *et al.*, 2007). The PFOA concentration in human serum from the Kinki region was significantly higher than that from other regions in 2004. Therefore, it is thought that, compared to the other regions in Japan, the PFOA levels in the human serum have remained at high concentrations in Osaka.

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