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Odd-numbered perfluorocarboxylates predominate over perfluorooctanoic acid in serum samples from Japan, Korea and Vietnam

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ABSTRACT

Perfluorooctanoic acid (PFOA) has recently attracted attention as a potential health risk following environmental contamination. However, information detailing exposure to perfluorinated carboxylic acids (PFCAs) other than PFOA is limited. We measured the concentrations of PFCAs (from perfluorohexanoic acid to perfluorotetradecanoic acid) in serum samples obtained from patients in Japan (Sendai, Takayama, Kyoto and Osaka) between 2002 and 2009, Korea (Busan and Seoul) between 1994 and 2008 and Vietnam (Hanoi) in 2007/2008. Total PFCA levels (geometric mean) were increased from 8.9 ng mL^{-1} to 10.3 ng mL^{-1} in Japan; from 7.0 ng mL^{-1} to 9.2 ng mL^{-1} in Korea; and were estimated at 4.7 ng mL^{-1} in Vietnam. PFCAs of greater length than PFOA were significantly increased in Sendai, Takayama and Kyoto, Japan, and levels of long-chain PFCAs exceeded PFOA levels in serum. Among these PFCAs, perfluoroundecanoic acid (PFUnDA) was the predominant component (28.5%), followed by perfluorononanoic acid (PFNA 17.5%), perfluorodecanoic acid (PFDA 7.9%), perfluorotridecanoic acid (PFTrDA 6.1%) and perfluorododecanoic acid (PFDoDA 1.8%). Odd-numbered PFCAs (PFNA, PFUnDA and PFTrDA) were also observed in Korea and Vietnam and their presence increased significantly in Korea between 1994 and 2007/2008. The proportion of long-chain PFCAs in serum was relatively high compared to reports in Western countries. Further investigations into the sources and exposure routes are needed to predict the future trajectory of these serum PFCA levels.

1. Introduction

Perfluorinated compounds such as perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA) have recently attracted attention owing to widespread contamination of the environment, wildlife and humans by these chemicals (Houde et al., 2006). In 2002, after 50 years of production, 3M Company phased out their manufacture of PFOS (Renner, 2001). PFOA is considered to be a major component of perfluorocarboxylate (PFCA) emission. However, in Japan, PFCA emissions consisted of not only PFOA but also perfluorononanoic acid (PFNA) and perfluoroundecanoic acid (PFUnDA) (of which 25 and 7 metric tons, respectively, were emitted in 2000) (Prevedouros et al., 2006). These odd-numbered PFCAs (PFNA, PFUnDA and perfluorotridecanoic acid (PFTrDA)) were detected at higher concentrations in samples from local wildlife than similar even-numbered PFCAs (PFOA, perfluorodecanoic acid (PFDA) and perfluorododecanoic acid (PFDoDA), respectively) (Furdui et al., 2008). Although studies using human samples from Western countries showed that PFOA was the most prevalent (followed by PFNA, PFDA and PFUnDA) (Haug et al., 2009; Joensen et al., 2009; Kato et al., 2009), our previous study of Japanese women in the Miyagi prefecture showed that PFNA and PFUnDA (average: 2.8 and 5.4 ng mL^{-1}, respectively) were found at broadly similar serum concentrations to PFOA (average: 4.9 ng mL^{-1}) (Kärrman et al., 2009).

PFCAs with longer chains than PFOA have higher bio-concentration factors suggesting persistency in the environment (Martin et al., 2003). Temporal trends in serum levels after 2002 showed no apparent

Abbreviations: PFCAs, perfluorinated carboxylic acids; PFOS, perfluorooctane sulfonate; PFOA, perfluorooctanoic acid; PFHxA, perfluorohexanoic acid; PFHpA, perfluoroheptanoic acid; PFNA, perfluorononanoic acid; PFDA, perfluorodecanoic acid; PFUnDA, perfluoroundecanoic acid; PFDoDA, perfluorododecanoic acid; PFTrDA, perfluorotridecanoic acid; PFTeDA, perfluorotetradecanoic acid; IDLs, instrumental detection limits; MDLs, method detection limits; RSD, relative standard deviation; SD, standard deviation; GM, geometric mean; GSD, geometric standard deviation.

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decline of PFNA, PFDA or PFUnDA in Norway (Haug et al., 2009), although serum levels of PFOA and PFOS both decreased in the United States, Norway and Japan (Harada et al., 2010; Harada and Koizumi, 2009; Haug et al., 2009; Olsen et al., 2008). These findings suggest a possibility that the origin and source of exposure to long-chain PFCAs could differ from those of PFOA and PFOS.

In the present study, we investigated current serum concentrations of PFCAs in three Asian countries (Japan, Korea and Vietnam). We selected the cities of Busan and Seoul in Korea because they are comparably urban and industrialized to Osaka, Japan. To confirm the temporal trends in Japan and Korea, we used archived historical serum samples stored in the human specimen bank (Koizumi et al., 2005; Koizumi et al., 2009). Hanoi in Vietnam was selected to evaluate the development of PFCA contamination following recent industrialization.

2. Material and methods

2.1. Experimental design and study population

To evaluate geographical differences and temporal trends in Asian countries, we compared 521 samples collected from Japan (Sendai, Takayama, Kyoto and Osaka) between 2002 and 2009; Korea (Busan and Seoul) between 1994 and 2008; and in Hanoi, Vietnam between 2007 and 2008. Samples from Sendai and Takayama in 2008, Osaka, Busan, Seoul and Hanoi are identical to a previous analysis of PFOS and PFOA (Harada et al., 2010; Kärrman et al., 2009). A total of 521 serum samples with information on donor age, sex and residential history (>5 years in each area) were selected from the archived samples in Kyoto Human Specimen Bank (Koizumi et al., 2005; Koizumi et al., 2009) (Table 1). Serum was separated from cellular components and stored at −30 °C until analysis.

The study population in Osaka and Kyoto consisted of residents that had been intensely exposed to PFOA from a local industrial source (the fluoropolymer manufacturer, Daikin Company) (Harada et al., 2004, 2007, 2010; Kärrman et al., 2009; Niisoe et al., 2010). In contrast, there is no known potential industrial source of PFCAs that would affect sample populations in the other cities studied.

For historical comparisons, samples were selected so that age and gender were matched among time points, except for Busan in 2000 and Osaka (Table 1).

The research protocol for the present study was reviewed and approved by the Ethics Committee of the Kyoto University Graduate School of Medicine on 14 November 2003 (E25).

2.2. Reagents

Ammonium acetate (purity: >99% by HPLC) was purchased from Aldrich (Steinheim, Germany). Acetonitrile (LC–MS grade) and water (distilled LC–MS grade) were obtained from Kanto Chemicals (Tokyo, Japan). Acetic acid and benzyl bromide were purchased from Wako pure chemicals (Osaka, Japan). Mixture of native PFCAs, $^{13}C_4$-labeled PFOA and $^{13}C_5$-labeled PFNA were obtained from Wellington Laboratories (Guelph, Ontario, Canada).

2.3. Determination of PFCAs in serum

Perfluorohexanoic acid (PFHxA), perfluoroheptanoic acid (PFHpA), PFOA, PFNA, PFDA, PFUnDA, PFDoDA, PFTrDA and perfluorotetradecanoic acid (PFTeDA) were analyzed. Serum samples were subjected to a clean-up procedure using a dispersive carbon method described by Powley et al. (2005). Briefly, the serum samples (0.5 mL, except for Korean samples between 1994 and 2000, which were 0.25 mL) together with an internal standard ($^{13}C_4$-PFOA, 1 ng) were extracted with 5 mL of acetonitrile, followed by centrifugation at 1600×*g* for 15 min. The supernatants were transferred into new tubes with 25 mg of ENVI-Carb and 50 μL of acetic acid, and the solutions were mixed by vortexing for 30 s. After centrifugation at 1600×*g* for 15 min, the extracts were dried under a nitrogen stream. The residue was then re-dissolved in 100 μL of 100 mM benzyl bromide acetone containing the recovery performance standard $^{13}C_5$-PFNA (1 ng) for 1 h at 80 °C and transferred to an autosampler vial. Extracts were analyzed using gas chromatography–mass spectrometry (Agilent 6890GC/5973MSD, Agilent Technologies Japan, Ltd., Tokyo, Japan) in electron impact ionization mode using single ion monitoring. PFCA benzyl esters were separated on a DB-5MS column (30 m length, 0.25 mm i.d., 1 μm film thickness) with a helium carrier gas. Split-less injections (1 μL) were performed with the injector set at 220 °C, and the split was opened after 1.5 min. The initial oven temperature was 70 °C for 2 min, ramped at 20 °C min^{-1} to 100 °C, and then at 30 °C min^{-1} to 280 °C. Ion fragments ($[M]^+$) were monitored and used as quantification ions (Table 2).

Instrumental detection limits (IDL) were defined as the mass of analyte producing a peak with a signal-to-noise ratio of 3, and ranged from 1 pg (PFTeDA) to 0.25 pg (other PFCAs) (Table 2). Since blank samples (0.5 mL distilled water) contain no detectable concentrations, the method detection limit (MDL) value was considered to be equal to the IDL corresponding to 0.2 ng mL^{-1} for PFTeDA and 0.025 ng mL^{-1} for other PFCAs (Table 2).

Table 1
Study area and study population.

Sampling site	Population (×10^3)	Latitude and longitude	Year	n (%female)	Age[a]	(Range)
Japan						
Sendai	1031	38°17′04″ N 140°55′46″ E	2008	50 (100)	37.5 ± 9.44	(21–53)
	1023	–	2003	50 (100)	36.6 ± 10.1	(20–59)
Takayama	94 (65)[b]	36°08′13″ N 137°15′16″ E	2008	50 (100)	40.5 ± 4.78	(29–49)
	67	–	2003	50 (100)	39.9 ± 4.5	(31–45)
Kyoto	1466	35°01′18″ N 135°46′38″ E	2009	30 (50)	33.2 ± 14.7	(21–68)
	1469	–	2002	30 (50)	35.4 ± 11.3	(21–58)
Osaka	2652	34°45′31″ N 135°31′52″ E	2008	50 (100)	45.9 ± 8.92[A,*]	(30–63)
	2619	–	2004	10 (100)	60.9 ± 6.3[B]	(49–69)
Korea						
Busan	3711	35°14′39″ N 129°05′54″ E	2008	35 (100)	40.1 ± 6.44[A,*]	(18–49)
	3732	–	2000	30 (100)	35.4 ± 4.27[B]	(28–45)
	3961	–	1994	39 (100)	42.3 ± 4.65[A]	(34–52)
Seoul	10,421	37°27′52″ N 127°01′56″ E	2007	36 (100)	34.5 ± 8.24	(20–54)
	10,798	–	1994	24 (100)	38.0 ± 7.41	(24–51)
Vietnam						
Hanoi	6232	21°00′08″ N 105°49′50″ E	2007–2008	37 (100)	30.2 ± 5.76	(20–40)

* Means of age with different letters differed significantly ($p<0.05$ by Tukey's HSD test). For example, the letters A and B indicate that the corresponding values differ significantly at $p<0.05$.

[a] Data are presented as mean ± standard deviation.

[b] Takayama city merged with neighboring cities in 2005. Numbers in paretheses denote populations areas corresponding to those used in 2003.