RECENT 25 YEARS TRENDS OF PERFLUOROOCTANOATE AND PERFLUOROOCTANE SULFONATE LEVELS IN ARCHIVED JAPANESE SERUM SAMPLES DONATED BY THE KYOTO HUMAN SPECIMEN BANK

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Abstract
The PFOS and PFOA concentrations in historically recorded human serum samples in Kyoto were measured to study recent trends of them for 25years. The historical samples collected from 1983 to 1999 demonstrated that the PFOA concentrations in males and females from Kyoto have increased 4.4-fold and 4.3-fold at a rate of increase of 0.49 ng/ml/year and 0.42 ng/ml/year, respectively. In contract, serum concentrations of PFOS reached a plateau in the late 1980s. Those trends were clearly demonstrated by using of serum samples donated by the Kyoto Human Specimen Bank (KHSB).
Also, the widely collected 200 Japanese serum samples also donated by the KHSB were measured to investigate regional differences in both concentrations of PFOS and PFOA. The concentrations in serum [geometric mean (geometric standard deviation)](ng/ml) in 2003-2004 ranged from 7.6(1.6) in the town of Matsuoka in Fukui prefecture to 27.8(1.6) in Kyoto city, and ranged from 2.3(1.5) in Matsuoka to 14.5(1.3) in Osaka city for PFOS and PFOA, respectively

Introduction
Perfluorooctane sulfonate (PFOS) and perfluorooctanoate (PFOA) have recently received attention due to their widespread contamination in the environment, wildlife and humans. There have been several reports on PFOS and PFOA concentrations in human[1-3]. Recently, Olsen and associates reported that PFOS and other serum fluorochemical concentrations increased between 1974 and 1989, reaching a plateau level at 1989 in US[4].
However, we demonstrated that in Japan, serum levels of PFOS and PFOA increased by factors of 3 and 14, respectively, between 1977 and 2003[2].
In this study, we have expanded the sampling area to investigate past trends and geographical differences in the serum concentrations of PFOS and PFOA in Japanese. Archived historical serum samples donated by KHSB [5] were used.

Materials and Methods
Experimental design and study population: To evaluate the long‐term trend in PFOS and PFOA concentrations in serum, historical serum samples were obtained from KHSB which holds about 13000 samples collected over the last 20 years in Kyoto city[5]. Samples used were collected from those who lived in the Kinki district (including Kyoto city and neighboring cities) (Fig. 1). We chose five time points from 1983 to 1999 at 4-year intervals: 1983, 1987, 1991, 1995 and 1999. A total of 100 serum samples were randomly selected so as to obtain 20 persons per each time point with a sex ratio of 1:1 for people of ages between 20 and 60 years.

Figure 1. Serum Sampling sites in Japan.

Table1 Study area and study population

Sampling site		Year	Male				Female			
			N	Age[a]	(Range)		*N*	Age[a]	(Range)	
(Historical study)										
Kyoto		1983	10	37.3±9.0	(27-54)		10	36.8±10.2	(20-51)	
		1987	10	37.1±9.3	(24-55)		10	33.3±9.9	(20-53)	
		1991	10	40.5±11.7	(25-58)		10	34.8±13.6	(21-54)	
		1995	10	38.5±10.1	(22-54)		10	37.5±10.5	(22-54)	
		1999	10	40.6±11.8	(21-59)		10	38.2±12.7	(20-51)	
						ANOVA[b]				ANOVA[b]
(Geographical study)										
Tohoku	Akita city	2004	10	41.7±11.3	(26-58)	AB	10	37.9±11.8	(20-54)	A
	Sendai city	2003	10	41.4±12.0	(23-57)	AB	10	35.1±10.8	(21-51)	AB
Chubu	Takayama city	2003-2004	10	44.9±8.8	(33-56)	A	10	35.9±10.1	(22-54)	AB
	Matsuoka town	2004	10	38.9±9.8	(21-55)	AB	10	39.8±10.2	(26-56)	A
Kinki	Kyoto city	2003-2004	10	40.0±12.5	(20-55)	AB	10	36.2±11.9	(24-59)	AB
	Osaka city	2003	5	40.4±13.1	(22-58)	AB	8	28.0±8.4	(21-43)	B
	Nishinomiya city	2003-2004	12	33.9±12.9	(21-55)	B	15	38.7±8.1	(25-54)	A
Chugoku-Shikoku	Shimonoseki city	2004	10	34.4±10.2	(24-55)	B	10	39.0±10.7	(25-53)	A
	Kochi city	2004	10	39.7±10.7	(25-55)	AB	10	39.2±11.8	(23-58)	A
Okinawa	Naha city	2004	10	35.9±9.1	(27-52)	AB	10	33.9±8.9	(21-55)	AB
	Total		97	38.9±11.1			103	36.6±10.3		

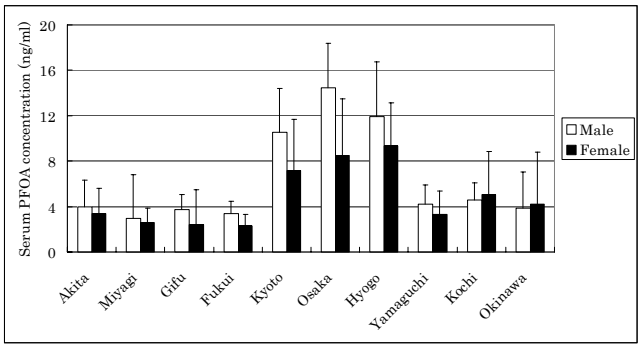
[a] Data are presented as mean ± standard deviation.
[b] Means with different letters (A and B) differed significantly ($p < 0.05$) but AB is located between A and B.

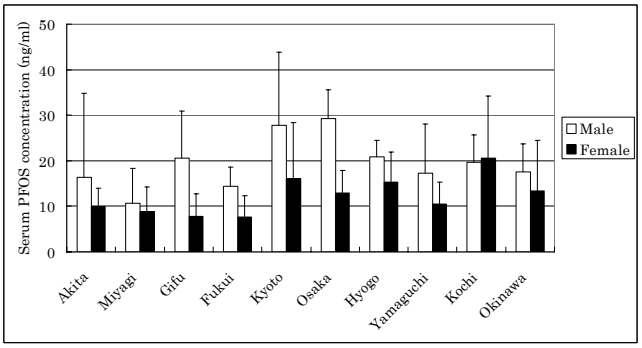
The age and sex of donors are summarized in Table 1.

Reagents: Heptadecafluorooctane sulfonic acid potassium salt (FW.538.22) and pentadecafluorooctanoic acid ammonium salt (FW.431.10) were purchased from Fluka (Milwaukee,WI) as an authentic standard for PFOS and PFOA, respectively. The internal standard ［1,2-$^{13}C_2$］perfluorooctanoate was donated by the Environmental Protection Agency of the USA (originally synthesized by Perkin Elmer, Boston, MA).

Determination of PFOS and PFOA in serum: The internal standard, ［1,2 - $^{13}C_2$］perfluorooctanoate was added to each serum sample prior to extraction. The serum samples were extracted using the method developed by Hansen et al.. Each extracted solution was analyzed by liquid chromatography-mass spectrometry (LC/MS) as previously reported. The limit of quantification (LOQ) and limit of detection (LOD) (ng/ml) were 0.1 and 0.03 for both analytes.

Statistics: All statistical analyses were carried out using SAS software (Version 8.2, SAS Institute, Inc.). $p<0.05$ was considered to be significant. Statistical analyses were conducted after logarithmic transformation of serum concentrations because the serum levels of PFOA and PFOS in these samples displayed skewed patterns.





Results and Discussion

PFOS and PFOA concentrations in 2003-2004: The range, median and geometric mean (GM) for PFOS and PFOA are presented in Table 2. All samples contained PFOS and PFOA at concentrations above the LOQ. There were significant geographical differences in PFOA and PFOS serum concentrations for both males and females (ANOVA: $p<0.001$) (Figure 2). Participants who lived in the Kinki district, i.e., Kyoto, Osaka and Hyogo prefectures, exhibited significantly higher serum PFOA levels in males (Tukey-Kramer's HSD test: $p<0.05$). Females in the Kinki district also had higher serum PFOA levels, followed by those from Chugoku-Shikoku and Okinawa (Tukey-Kramer's HSD test:

p<0.05).

Long-term serum concentrations of PFOS and PFOA: The concentrations of PFOS and PFOA in the serum collected in Kyoto between 1983 and 1999 are shown in Fig. 2. From 1983 to 1999, PFOA serum concentrations in Kyoto exhibited an annual increase of 0.49ng/ml for males and one of 0.42 ng/ml for females (Pearson's product moment correlation: r=0.672, p<0.001 for males and r=0.585, p<0.001 for females). In contrast, there was no obvious trend of increase in PFOS levels by linear regression (Pearson's product moment correlation: r=0.06, p=0.67 for males and r=0.212, p=0.13 for females).

Serum concentrations of PFOS and PFOA: The PFOS and PFOA serum concentrations in Japan are shown in Figure 2. Compared to previous reports on human serum concentrations in Japan, the rural areas Taiwa town and Yokote city, neighbor the cities Sendai and Akita, respectively, had lower GMs of serum PFOS concentrations.

To the best of our knowledge, the present study is one of the largest nationwide assessments of PFOA and PFOS levels in human serum in Japan yet conducted. In this study, we also confirmed that the serum levels of PFOA have steadily increased throughout urban areas in Japan, as well as in rural areas, as previously reported, albeit in somewhat restricted geographical areas[2]. This finding is in sharp contrast to a survey conducted in the USA[4]. Serum concentrations of PFOS, however, reached plateau levels in Japan in the late 1980s, which is consistent with the USA study[4]. The company 3M had once been one of the major manufacturers of PFOS and its derivatives in the world and continued constant production of PFOS between 1989 and 1999[4], a time period consistent with the stationary levels of serum PFOS in USA. On the other hand, the 3.5‐fold increase in fluoropolymer production in Japan was observed between 1983 and 1999, which requires PFOA is in accord with the trend of increasing serum concentration of PFOA.

Geographical differences in the serum levels of PFOA in 2003‐2004 were readily observed. At least two exposure routes play an important role in the exposure to PFOA in Japan; drinking water[6] and air‐horne dust[7] . It should be pointed out that exposure to PFOA via these routes are relatively higher in Kyoto area than in other areas[6,7]. Thus geographical differences may be attributable to differences in exposure intensities via these two routes. In addition, the relative population and proportion of industrial sectors were significant predictors serum concentrations of PFOA, a finding suggestive of a contribution of industrial sources of PFOA. In contrast, the proportion of industrial sectors was not a significant predictor for the serum concentrations of PFOS. Thus other factors should be taken into consideration. One report showed that a high fish consumption group had 2.6‐fold higher serum levels of PFOS to the reference population but to a lesser extent, i.e., 1.4‐fold for PFOA. A nationwide study to investigate exposure sources including air, food and water is needed to adequately account for the regional differences in exposure intensities.

We confirmed sex differences in the serum concentrations of PFOS and PFOA in 2003‐2004: the concentrations of PFOS and PFOA were higher in males than in females, as previously reported. It should also be noted that PFOS and PFOA serum concentrations were higher in males than in females by male‐female pair‐wise comparison even within the same geographical district, except in Naha city for PFOA. Furthermore, there was correlation between age and PFOS serum levels in serum in 2003‐2004.

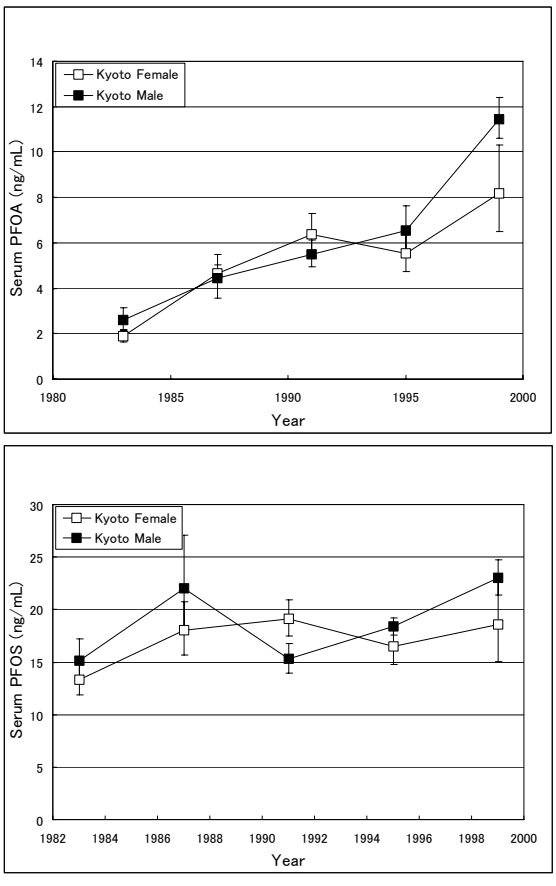


Figure 3. Time trends in PFOA and PFOS serum levels in Kyoto.

The present study has a major limitation. Historical samples in the Kyoto may not necessarily represent the entire Japanese population. Thus, the present study population for historical evaluation between 1983 and 1999 may not accurately represent the trend in serum concentrations in the general population in Japan. However, serum concentrations of PFOS and PFOA in 2003[2] were comparable to the serum levels in 1999, suggesting that the population selection bias may not be significant, if any.
The finding of increasing human serum concentrations of PFOA and higher levels of both PFOA and PFOA for Kinki residents raises an alarming signal. Further investigation will be required to specifically characterize the risk of exposure for residents in the Kinki district. It also needs to be determined whether or not an increasing trend of serum levels of PFOA is a global phenomenon.

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