

OTHER HALOGENATED POPs OF CONCERN

LEVELS OF PERFLUOROOCTANE SULFONATE (PFOS) AND OTHER
RELATED COMPOUNDS IN THE BLOOD OF JAPANESE PEOPLEShigeki Masunaga¹, Kurunthachalam Kannan², Rikuo Doi³, Junko Nakanishi^{1,4} and John P. Giesy²¹Graduate School of Environment and Information Sciences, Yokohama National University, 79-7, Tokiwadai, Hodogaya, Yokohama 240-8501, Japan²National Food Safety and Toxicology Center, Michigan State University, East Lansing, MI 48824, U.S.A.³Department of Hygiene, School of Medicine, Yokohama City University, 3-9, Fuku-ura, Kanazawa, Yokohama 236-0004, Japan.⁴Research Center for Chemical Risk Management, National Institute of Advanced Industrial Science and Technology, 16-1 Onogawa, Tsukuba, Ibaraki 305-8569, Japan

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Introduction

Despite of the situation that fluorinated organic compounds has been widely used for various purposes, contamination by these compounds in environmental or biological media has not been well documented compared with chlorinated or brominated compounds. Recently, however, with the development of analytical technique for these compounds, biological surveys became possible.

Olsen *et al.*¹⁾ found perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA) in the blood sera of employees in the fluorochemical manufacturing industry. Hansen *et al.*²⁾ reported the levels of PFOS, PFOA, perfluorooctanesulfonamide (PFOSA) and perfluorohexane sulfonate (PFHS) in human sera. The structures of these compounds are shown in Fig. 1. Studies of these compounds in wildlife from various places in the world have been performed^{3,4,5)}. They revealed that PFOS was the most prevalent in wildlife tissues among the fluorinated organics studied (PFOS, PFOSA, PFHS, and PFOA). PFOS was detectable in most samples (liver and plasma) including those from remote marine regions at concentrations greater than 1 ng/g, suggesting widespread distribution of these compounds on a global scale.

Perfluorooctane sulfonate (PFOS)	$\text{CF}_3\text{-CF}_2\text{-CF}_2\text{-CF}_2\text{-CF}_2\text{-CF}_2\text{-CF}_2\text{-CF}_2\text{-SO}_3^-$
Perfluorohexane sulfonate (PFHS)	$\text{CF}_3\text{-CF}_2\text{-CF}_2\text{-CF}_2\text{-CF}_2\text{-CF}_2\text{-SO}_3^-$
Perfluorooctanesulfonylamide (PFOSA)	$\text{CF}_3\text{-CF}_2\text{-CF}_2\text{-CF}_2\text{-CF}_2\text{-CF}_2\text{-CF}_2\text{-SO}_2\text{-NH}_2$
Perfluorooctanoic acid (PFOA)	$\text{CF}_3\text{-CF}_2\text{-CF}_2\text{-CF}_2\text{-CF}_2\text{-CF}_2\text{-CF}_2\text{-COOH}$

Figure 1. Structures of PFOS and related compounds

In this study, we measured the PFOS, PFOSA and PFOA in Japanese blood samples. In Japan, major fluorochemical manufacturers produce derivatives of PFOA, not PFOS derivatives. This is different from that in the US, where PFOS derivatives are primarily used. Thus, the fluorochemical exposures in Japanese population may be different from that in the US.

Materials and methods

Ethical approval of the research plan

As this study involves collection of human blood samples, study plan was submitted to the ethics

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committee belonging to the School of Medicine, Yokohama City University. The plan was approved by the committee on Feb. 19, 2001.

Blood samples

Volunteers of blood donor were from the Yokohama National University and Yokohama City University. Thus, the most of the donors are students or workers in these two universities. About 10 ml of blood was drawn into a Vacutainer (Becton Dickinson, Rutherford, NJ, USA) from each volunteer's arm by a professional nurse at Kanagawa Prefectural Institute of Preventive Medicine on March 6, 2002. The samples were refrigerated without any preservatives and analyzed for fluorochemicals.

Analysis

Extraction and analytical procedure were basically the same as those of Hansen *et al.*²⁾. One ml of whole blood, 5 ml of internal standard, 1 ml of 0.5 M tetrabutylammonium solution (adjusted to pH 10), and 2 ml of 0.25 M sodium carbonate buffer were added to 15-ml polypropylene tube for extraction. After through mixing, 5 ml of methyl *tert*-butyl ether (MTBE) were added to the solution and mixed for 20 min. The organic and aqueous layers were separated by centrifugation and 4.0 ml of MTBE was removed from the solution. The aqueous mixture was rinsed with MTBE and separated two more times. Then all the rinses were combined in a second polypropylene tube. The solvent was allowed to evaporate under nitrogen gas before being reconstituted in 0.5 ml of methanol. The sample was vortex mixed for 30 seconds and passed through a 0.2-mm nylon mesh filter into a vial. Ten ml of sample was, then, injected into high performance liquid chromatograph (Hewlett-Packard HP1100) equipped with Keystone Betasil C₁₈ column (50 x 2 mm, particle size 5 mm). The mobile phase was 2 mM ammonium acetate/methanol (300 ml/min) with gradient from 45% methanol to 90% in 9 min. A tandem mass spectrometer (Micromass Quattro II) was coupled with the liquid chromatograph and operated in the atmospheric pressure electrospray negative ion mode.

Results and discussion

Blood samples from 26 people, 24 Japanese and two foreigners from Asian countries, were analyzed. Two foreigners had lived in Japan more than a few years. The analytical results are shown in Table 1. Levels of PFOS and PFOSA were in the range of 2.0 - 20.2 and <1.3 - 4.8 ng/ml whole blood, respectively. PFOA concentrations were less than limit of quantitation (LOQ) of 3.35 ng/ml in whole blood. The distribution of PFOS concentration for all samples is shown in Figure 1. The arithmetic and geometric means of PFOS concentrations were 8.1 and 6.9 ng/ml, respectively. Average concentrations in female samples were slightly higher than that in male samples. The difference, however, was not significant. Figure 2 shows the age dependence of PFOS and PFOSA concentrations. PFOS concentration increased as the blood donor became older, especially for females. Its correlation, however, was weak. Age dependent accumulation was more uncertain for PFOSA because more than half of the samples did not contain quantifiable concentrations. The relationship between PFOS and PFOSA was not significant.

Hansen *et al.*²⁾ measured PFOS, PFOA, PFHS and PFOSA levels of 65 human serum samples. Their results showed that range of PFOS concentrations was 6.7 - 81.5 (average: 28.4) ng/ml serum. For comparison, our data were converted into serum basis. Human blood consists of about half volume of fluid (serum) and half volume of haemocytes. Thus, we can multiply whole blood based data by a factor of two, if the target compound is assumed not present in the haemocyte fraction of blood. As it is probable that haemocyte fraction also contains PFOS, multiplication by a factor of two may overestimate the serum based data. So, the arithmetic mean of 8.1 ng/ml on a whole blood basis for PFOS can be approximately 16.2 mg/ml on a serum basis. This value is about half of that reported for

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Table 1. PFOS, PFOSA and PFOA concentrations in whole blood from Japan*

Sample No.	Male/ Female	PFOS (ng/ml)	PFOSA*** (ng/ml)	Sample No.	Male/ Female	PFOS (ng/ml)	PFOSA*** (ng/ml)
1	Male	10.2	4.3	14	Female	3.3	< LOQ
2	Female	20.2	< LOQ	15	Male	7.2	< LOQ
3	Female	12.8	3.0	16	Male	8.6	< LOQ
4	Female	12.8	2.4	17	Male**	2.0	< LOQ
5	Female	3.1	< LOQ	18	Male	6.2	1.5
6	Male	8.1	3.2	19	Male	5.2	< LOQ
7	Male	15.2	2.7	20	Male	2.4	< LOQ
8	Male	6.8	2.3	21	Male	5.9	< LOQ
9	Female	6.1	1.4	22	Female	9.1	3.6
10	Female	8.9	2.9	23	Female	11.7	NA
11	Male**	6.3	< LOQ	24	Female	7.7	NA
12	Male	2.3	< LOQ	25	Female	15.0	< LOQ
13	Male	9.0	4.8	26	Male	4.1	< LOQ

	PFOS (ng/ml)	PFOSA (ng/ml)
Range (All samples)	2.0 - 20.2	<1.3 - 4.8
Arithmetic Mean (All samples)	8.1 (n=26)	1.7 (n=24)****
Geometric Mean (All samples)	6.9 (n=26)	1.2 (n=24)****
Arithmetic Mean (Japanese only)	8.4 (n=24)	1.8 (n=22)****
Geometric Mean (Japanese only)	7.3 (n=24)	1.3 (n=22)****
Arithmetic Mean (Japanese males)	7.0 (n=13)	1.8 (n=13)****
Arithmetic Mean (Japanese females)	10.1 (n=11)	1.8 (n=9)****

* PFOA was less than the LOQ (limit of quantitation) of 3.35 ng/ml for all samples.

** Foreigner from Asian country who had lived in Japan for a few years.

*** NA = not analyzed. LOQ (limit of quantitation) = 1.28 ng/ml.

**** Mean was calculated assuming that <LOQ samples had half of LOQ concentration (0.64 ng/ml).

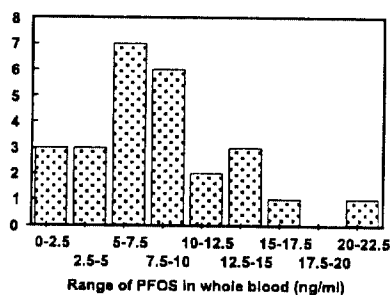


Figure 2. Distribution of PFOS concentration

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the US sera ²⁾. This shows, on the one hand, that the Japanese are less exposed to PFOS compared with Americans, probably because major Japanese producers of perfluorinated surfactants do not use PFOS derivative as intermediates. On the other hand, it shows that Japanese are exposed to PFOS probably through imported products and/or global scale environmental transport. Further study of exposure route is necessary to understand the causes.

The samples measured in this study are quite limited in number, both area-wise and age-wise. Thus, the present results may not necessarily represent general Japanese population. However, this is the first study of PFOS level in Japanese people and provides a rough estimate of present status.

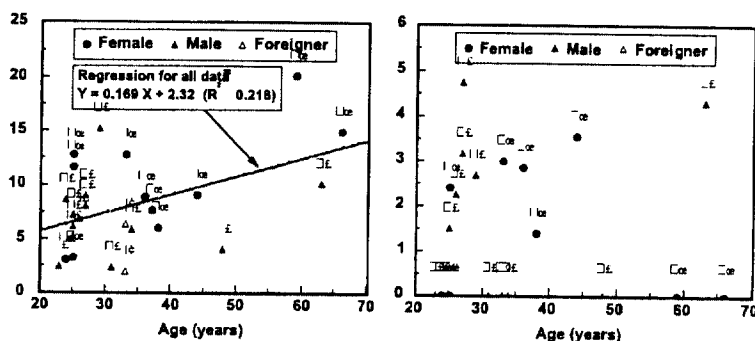


Figure 3. Age dependence and PFOS (left) and PFOSA (right) concentration in blood

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References

1. Olsen G.W., Burris, J.M., Burlew, M.M., Mandel, J.H. (2000) Drug Chem. Toxicol. 23, 603-620.
2. Hansen, K.J. Clemen, L.A., Ellefson, M.E., Johnson, H.O. (2001) Environ. Sci. Technol. 35, 766-770.
3. Giesy, J.P., Kannan, K. (2001) Environ. Sci. Technol. 35, 1339-1342.
4. Kannan, K., Koistinen, J., Beckmen, K., Evans, T., Gorzelany, J.F., Hansen, K.J., Jones, P.D., Helle, E., Nyman, M., Giesy, J.P. (2001) Environ. Sci. Technol. 35, 1593-1598.
5. Kannan, K., Hansen, S.P., Franson, C.J., Bowerman, W.W., Hansen, K.J., Jones, P.D., Giesy, J.P. (2001) Environ. Sci. Technol. 35, 3065-3070.