PERFLUORINATED COMPOUNDS IN HUMAN MILK FROM SHANGHAI, CHINA

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Abstract

Two predominant perfluorinated compounds (PFCs), perfluorooctanesulfonate (PFOS) and perfluorooctanoate (PFOA), were detected in 20 human milk samples collected in 2007 from Shanghai, one of the largest cities in China. The concentration of PFOA ranged from 112pg/ml to 1061pg/ml with a geometric mean of 360pg/ml and a medium of 359pg/ml. The concentration of PFOS ranged from 19pg/ml to 1615pg/ml with a geometric mean of 92pg/ml and a medium of 90pg/ml. PFOA and PFOS were significantly correlated with each other in human milk from Shanghai. The dietary intake of PFOA and PFOS by breast-feeding infant from Shanghai was estimated. The geometric mean of estimated daily dietary intake of PFOA and PFOS were 34ng/kg of body weight and 9ng/kg of body weight respectively.

Introduction

In recent years, perfluorinated compounds (PFCs), referred to as "emerging Persistent Organic Pollutants" [1], posed increasing concern for their nature of resistance to degradation in the environment [2], global distribution [3, 4], environmental toxic effects and health risk to human [5, 6, 7]. An increasing number of studies have shown that human is widely exposed to PFCs, while perfluorooctanesulfonate (PFOS) and perfluorooctanoate (PFOA) are generally the most prevalent PFCs found in human [8]. The presence of PFCs in human milk samples was firstly reported by Kuklenyik et al. [9]. After that, PFCs were reported presenting in human milk samples from some studies [10, 11] which implied that the lactational transfer during breast-feeding might be an important pathway of the infant exposure to PFCs. In consideration of the potential risks posed to infants and children by exposure to PFCs in human milk, information on the levels of PFCs in human milk is needed.

Materials and Methods

Samples Collection: In 2007, 20 human milk samples were donated by mothers from various regions of Shanghai city, China. Locally resident time of donors was all more than 10 years. The average age of mothers at the time of sampling was 26 (ranging from 21 to 32) and babies' ages ≤ 8 weeks. Human milk was collected either using a breast pump or by hand expressing the milk into the pre-washed polypropylene container that was prepared for every mother. All samples were stored in the refrigerators at -20℃ until analysis.

Reagents and Chemicals: The standard solutions of PFOA and PFOS and the internal standard of $^{13}C_4$-PFOA and $^{13}C_4$-PFOS were all purchased from Wellington Laboratories. Methanol of high-performance liquid chromatography grade was purchased from J.T.Baker (Phillipsburg, USA). Milli-Q water was used throughout the study. Ammonium acetate and formic acid of HPLC grade were purchased from Dikma Pure (Richmond Hill, USA). Ammonium hydroxide (25%) of analytical grade was from Xin Guang (Beijing, China).

Extraction and Instrumental Analysis: The extractor lines were purged with water and methanol prior to each extraction run. Before spiking on 60 mg/3mL weak anion exchange cartridges (Waters Oasis WAX, Milford, MA, USA), internal standards ($^{13}C_4$-PFOA and $^{13}C_4$-PFOS, 10 μL of a 10 pg/μL solution in methanol) and 8 mL 2% formic acid in water were added to 2 mL human milk, followed by sonication and centrifugation. The supernatant was transferred to the cartridges preconditioned by passage of 2 mL methanol and 2 mL water. The cartridges were then washed with 2% formic acid in water and 2% formic acid solution/methanol (50:50). The target analytes were eluted by 2 mL 9% ammonium hydroxide in methanol which was evaporated to dryness, then methanol/water (50:50) was added to a final volume of 0.2 mL. The particles in the final solution were removed by filtration using nylon syringe filter.

Analytes were separated and quantified using UPLC-MS/MS. A 20 μL aliquot of the sample extract was injected with a full loop injection into a 2.1×50 mm BEH C18 column (1.7μm; Waters, USA). 2 mM ammonium acetate aqueous solution and methanol were used as mobile phases. The gradient was starting at 20% methanol and increasing linearly. At a flow rate of 0.4 mL/min, the gradient was increased to 90% methanol at 5 min, 100% methanol at 5.1 min, and was kept at that level until 6 min before reversion to original condition at 7 min, 2 min was needed for equilibration. The triple-quadrupole mass spectrometer was operated in the negative electrospray mode with multiple-reaction-monitoring (MRM). The capillary voltage was 0.95 kV. The temperatures of ion source and desolvation gas were 120 ℃ and 400 ℃, respectively. The cone gas flow was 50 L/h and desolvation gas flow was 800 L/h. The mass transitions were 413→369 for PFOA and 499→99 for PFOS. The limits of detection (LOD) of PFOA and PFOS were 14.2pg/ml and 1.5pg/ml respectively.

Results and Discussion

PFOA and PFOS were found above the LOD in all samples. The concentrations of PFOA and PFOS in samples were list in table 1. The concentration of PFOA ranged from 112pg/ml to 1061pg/ml with a geometric mean of 360pg/ml and a medium of 359pg/ml. The concentration of PFOS ranged from 19pg/ml to 1615pg/ml with a geometric mean of 92pg/ml and a medium of 90pg/ml. The sum of PFOA and PFOS in human milk ranged between 214 pg/ml and 2676pg/ml with a geometric mean of 475pg/ml and a medium of 467pg/ml. The exposure level of PFOA in samples was significantly higher than that of PFOS by almost four times ($P<0.05$), which was not observed in previous studies. However, in the sample HM18 which had the highest PFCs concentration, the concentration of PFOS was higher than that of PFOA. PFOA and PFOS were significantly correlated with each other in human milk samples from Shanghai($r=0.686$, $P<0.01$). The association between PFOA and PFOS in human milk suggests common sources or/and pathway for human exposures in Shanghai.

Concentrations of PFCs in human milk have been examined in a few of studies conducted in Sweden, China, Germany, USA and some Asian countries[10, 12, 13, 14, 15]. In Figure1, the exposure levels of PFOA and PFOS in human were compared between some recent studies. The PFOS level in human milk from Shanghai was about 50% lower than that from Japan and Sweden, while human milk PFOS from Shanghai was comparable with that from USA, Germany and Zhoushan which is a city near Shanghai. The extremely high PFOA level has been

observed in this study. The human milk PFOA from Shanghai was significantly higher than that from other studies, even higher than that from the North Rhine-Westphalian (NRW) Sauerland area in Germany[13], where highly PFOA contaminated industrial waste was mixed by a recycling company into a so called soil improver and disseminated by farmers on agricultural land.

No certain source for the high PFOA level in human milk from Shanghai was identified in our study. However, the high PFOA concentration and proportion in the water of Yangtze River collected in Shanghai[16] suggested possible association between surface water PFCs contamination and human exposure. The PFOA concentration (up to 260ng/ml) and proportion (87.8%) in surface water from Shanghai was comparable with that from PFCs case in NRW (Germany), where a high concentration of PFOA in water of the Ruhr River (446ng/ml) was detected after PFCs contamination in an upper tributary of Ruhr River, Mohne River that was impacted by the PFCs-containing soil improver[17]. In that case, a 4-8 folds increase in blood PFOA concentration was found in the residents near that river system[18].

Table 1 Concentrations of PFOA and PFOS in human milk samples (pg/mL) and daily EDIs of PFOA and PFOS by infants in Shanghai (ng/kg body weight)

	Concentration		EDI			Concentration		EDI	
	PFOA	PFOS	PFOA	PFOS		PFOA	PFOS	PFOA	PFOS
HM1	112	185	11	18	HM11	548	104	52	10
HM2	296	102	28	10	HM12	397	88	38	8
HM3	387	168	37	16	HM13	313	68	30	6
HM4	875	99	83	9	HM14	275	35	26	3
HM5	376	92	36	9	HM15	404	62	38	6
HM6	435	91	41	9	HM16	526	70	50	7
HM7	300	99	29	9	HM17	342	125	33	12
HM8	244	70	23	7	HM18	1061	1615	101	154
HM9	244	52	23	5	HM19	287	90	27	9
HM10	195	19	19	2	HM20	504	64	48	6

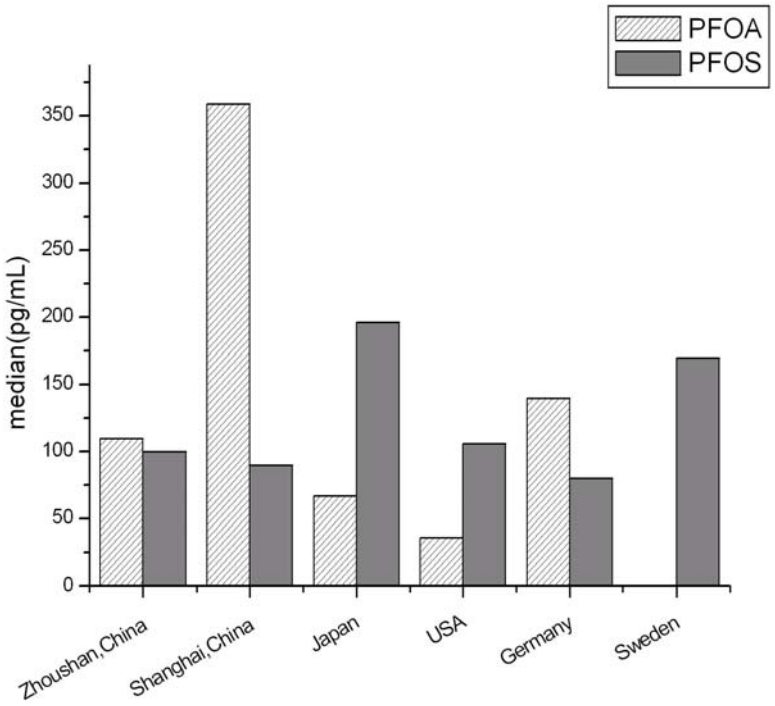

Figure1 Comparison of PFOA and PFOS in human milk from some studies

The daily estimated dietary intake (EDI) of PFOA and PFOS via human milk were calculated on the basis of levels of PFOA and PFOS in human milk samples, infant ingest data (742 mL/day) and body weight (7.8kg) data from the U.S.Environmental Protection Agency (23) assuming that human milk is the only food source for nursing infants (1-6 months old). The EDI of 20 infants from Shanghai was list in Table 1. The geometric mean of estimated daily dietary intake of PFOA and PFOS were 34 ng/kg of body weight and 9 ng/kg of body weight, respectively.

After the PFCs case in NRW, the provisional tolerable daily intake (TDI) values of 100ng/kg of body weight were derived for PFOA and PFOS from an assessment of their health effects performed in Germany by Federal Drinking Water Commission and the Federal Institute for Risk Assessment[19]. In 2008, European Food Safety Authority established the TDI value of 1500ng/kg of body weight and 150ng/kg of body weight for PFOA and PFOS respectively[20]. In our study, one infant's daily EDI of PFOS (154ng/kg) slightly exceeded both TDIs recommended by Germany and EU, while the daily EDI of PFOA (101ng/kg) for that infant slightly exceeded the TDI of PFOA recommended by Germany. Although the TDI refers to the lifetime tolerable daily intake and the period of breast feeding is relative short, the potential risk for infant derived from PFOA and PFOS exposure should be concerned in Shanghai due to infants' susceptibleness to chemical contaminants.

Acknowledgments

This research was funded by the National Nature Science of Foundation of China (20607021 and 20837003) and

National Support Program for Science and Technology (2007BAC27B02). We gratefully acknowledge the donors for collaborating with the study and voluntarily donating human milk samples.

References

1. Lohmann R., Breivik K., Dachs J. and Muir D. *Environmental Pollution* 2007; 150:150.

2. Villagrasa M., Lopez A. M. and Barcelo D. *Anal Bioanal Chem* 2006; 386:953.

3. Taniyasu S., Kannan K., So M. K., Gulkowska A., Sinclair E., Okazawa T. and Yamashita N. *J Chromatogr A* 2005; 1093:89.

4. Tao L., Kannan K., Kajiwara N., Costa M. M., Fillmann G., Takahashi S. and Tanabe S. *Environ Sci Technol* 2006; 40:7642.

5. Hekster F. M., Laane R. W. and de V. P. *Reviews of Environmental Contamination and Toxicology* 2003; 179:99.

6. Fei C., McLaughlin J. K., Tarone R. E. and Olsen J. *Environmental Health Perspectives* 2007; 115:1677.

7. Apelberg B. J., Witter F. R., Herbstman J. B., Calafat A. M., Halden R. U., Needham L. L. and Goldman L. R. *Environ Health Perspect* 2007; 115:1670.

8. Kannan K., Corsolini S., Falandysz J., Fillmann G., Kumar K. S., Loganathan B. G., Mohd M. A., Olivero J., Van W. N., Yang J. H. and Aldoust K. M. *Environ Sci Technol* 2004; 38:4489.

9. Kuklenyik Z., Reich A. J., Tully S. J. and Calafat M. A. *Environmental Science & Technology* 2004; 38:3698.

10. Karrman A., Ericson I., van Bavel B., Darnerud P. O., Aune M., Glynn A., Lignell S. and Lindstrom G. *Environmental Health Perspectives* 2007; 115:226.

11. Volkel W., Genzel Boroviczény O., Demmelmair H., Gebauer C., Koletzko B., Twardella D., Raab U. and Fromme H. *International Journal of Hygiene and Environmental Health* 2008; 211:440.

12. So M. K., Yamashita N., Taniyasu S., Jiang Q., Giesy J. P., Chen K. and Lam P. K. *Environmental Science & Technology* 2006; 40:2924.

13. Bernsmann T. and Furst P. *organohalogen compounds* 2008; 70:718.

14. Tao L., Kannan K., Wong C. M., Arcaro K. F. and Butenhoff J. L. *Environmental Science & Technology* 2008; 42:3096.

15. Tao L., Ma J., Kunisue T., Libelo E. L., Tanabe S. and Kannan K. *Environ Sci Technol* 2008; 42:8597.

16. So M. K., Miyake Y., Yeung W. Y., Ho Y. M., Taniyasu S., Rostkowski P., Yamashita N., Zhou B. S., Shi X. J., Wang J. X., Giesy J. P., Yu H. and Lam P. K. *Chemosphere* 2007; 68:2085.

17. Skutlarek D., Exner M. and Farber H. *Environ Sci Pollut Res Int* 2006; 13:299.

18. Holzer J., Midasch O., Rauchfuss K., Kraft M., Reupert R., Angerer J., Kleeschulte P., Marschall N. and Wilhelm M. *Environ Health Perspect* 2008; 116:651.

19. Roos P. H., Angerer J., Dieter H., Wilhelm M., Wolfle D. and Hengstler J. G. *Arch Toxicol* 2008; 82:57.

20. European Food Safety Authority. *The EFSA Journal* 2008; 653.