MEASUREMENT OF PERFLUORINATED COMPOUNDS IN HUMAN MILK AND HOUSE DUST

Nakata A[1], Katsumata T[1], Iwasaki Y[1], Ito R[1] Saito K[1] Izumi S[2], Makino T[2], Kishi R[3], Nakazawa H[1]
[1] Department of Analytical Chemistry, Faculty of Pharmaceutical Sciences, Hoshi University, 2-4-41, Ebara Shinagawa-ku, Tokyo, 142-8501, Japan; [2] Department of Obstetrics and Gynecology, Tokai University School of Medicine, 143, Shimokasuya, Isehara-shi, 259-1193, Japan; [3] Department of Public Health, Hokkaido University Graduate School of Medicine, Department of Public Health, North 15 West 7 Kita-ku, Sapporo, 060-8501, Japan;

Abstract
The purpose of the present study is to evaluate the exposure sources of perfluorinated compounds (PFCs) for infants. Highly sensitive methods for measuring four kinds of PFCs (perfluorooctanesulfonate; PFOS, perfluorooctanoic acid; PFOA, perfluorononanoic acid; PFNA and perfluorohexanesulfonate; PFHxS) in house dust and human milk were developed using liquid chromatography tandem mass spectrometry (LC/MS/MS). The average recovery which was assessed at two different concentrations were more than 97.9% in house dust samples and 94.3 % in human milk samples, respectively. The detection limits of the methods for each PFCs were assessed as being 0.58 ng/g to 0.72 ng/g in house dust and 0.004 ng/mL to 0.1 ng/mL in human milk. To investigate the source of PFCs exposure for infants, house dust samples (n = 20) and breast milk samples (n = 51) were measured by the developed methods. PFOS and PFOA were detected in all dust samples (PFOS: 7.0 to 41 ng/g; PFOA: 18 to 89 ng/g). In human milk sample, PFOS was detected in all samples with the range from 0.008 to 0.401 ng/mL. Based on our experiments, house dust or human milk might be one of the human exposure sources of PFCs.

Introduction
Perfluoronated compounds (PFCs), such as perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA), which have their biological and chemical stability are widely used in the manufacture of plastic, electronics, textile, and construction material in the apparel, leather, and upholstery industries[1]. However, PFCs may be detrimental to rodent development possibly affecting thyroid hormone levels, so affect of PFCs on infant is concerned[2]. Moreover, environmental pollution and human exposure[3,4,5] of PFCs have been reported. According to these studies, PFCs level in human blood is significantly higher than that in environment. So, it is very important to elucidate human exposure of PFCs. House dust is a curious sample to give an assessment of PFCs exposure in infant who is exposed though maternal feeding as indoor environment. PFCs level of human milk is very low compared to that of human blood, but infant takes a lot of milk during lactational period, so it is required to estimate intake amount of PFCs. Although human exposure of PFCs is widely reported, very few information about exposure source of PFCs have indicated so far. In this study, we have developed an analytical method for the determination of PFCs in house dust and human milk by liquid chromatography tandem mass spectrometry (LC/MS/MS) to investigate exposure source of PFCs.

Materials and Methods
Chemicals: Perfluorohexane sulfonate (PFHxS; 98%) was purchased from Wellington Laboratories Inc., Japan. Perfluorooctane sulfonate (PFOS; 98%) were purchased from Wako Pure Chemicals Industries, Ltd., Japan. perfluorooctanoic acid (PFOA; >90%) and perfluorononanoic acid (PFNA; 95%) were purchased from Fuluka Chemie AG, Buchs, Switzerland. $^{13}C_4$-PFOS was purchased from Wellington Laboratories Inc., Japan. $^{13}C_2$-PFOA was purchased from PerkinElmer Inc., USA. Other chemicals were purchased from Wako Pure Chemicals Industries, Ltd., Japan Water was purified using a Milli-Q cartridge system (Millpore, Bedford, MA, USA).

Instrumentation and analytical conditions of LC/MS/MS:
LC/MS/MS analysis was performed using a Waters Quattro micro system. Separation was achieved on an Xbridge column (2.1 × 50 mm, 2.1 μm, Waters Inc., Japan). The column oven was maintained at 40°C. The separation was carried out using a mobile phase of 1.0 mM ammonium acetate in water/acetonitrile at a flow rate of 0.2 mL/min. The gradient profile of mobile phase was as follows: 0-10 min. using a linear increase from 10 to

70% acetonitrile solution, and holding at 70%. The conditions of MS/MS were as follows: the desolvation and source temperatures were set at 350 and 100°C, respectively; the capillary was held at a potential of 600 V relative to the counter electrode in the negative ion mode for all compounds. The cone gas and desolvation gas were 50 and 350 L/hr, respectively. The cone and collision voltage were 48 and 40 V for PFHxS, 60 and 65 V for PFOS, and 14 and 11 V for PFOA, 18 and 10 V for PFNA, respectively.

Sample preparation of human milk sample by solid phase extraction

Because of very low concentrations of PFCs in the sample matrix, the solid phase extraction (SPE) has been applied for the sample extraction and concentration. The average extraction recoveries for PFCs and relative standard deviation (R.S.D.) were over 94.3% and 10.3%, respectively. The milk samples were extracted using a solid-phase extraction cartridge (Waters Oasis WAX, Milford, MA, USA). Internal standards ($^{13}C_2$-PFOA and $^{13}C_4$-PFOS) and 15 mL of 0.1 mol/L formic acid were added to 5 mL of milk. The solution was sonicated for 15 min and centrifuged at 6000 rpm for 15 min. The supernatant was loaded onto the cartridge and rinsed by wash solvent. The PFCs were eluted from the SPE cartridge with 3 mL of 3% ammonium hydroxide in acetone. The extracts were concentrated by dry nitrogen stream, and finally, filtered through a nylon membrane filter. The final sample volume for the milk extracts was adjusted to 100 µL. This SPE method using the Oasis WAX® was successfully used for the pretreatment of PFCs in human milk samples.

Sample preparation of house dust sample by SFE

House dust sample were contained several compounds. It was necessary to clean up for the determination of PFCs. We tried to use Supercritical fluid extraction (SFE) method for PFCs extraction in house dust. First of all, the dust samples were collected from the dust bags of vacuum cleaners from residential homes in Japan, and were passed though two different size of sieves (mesh size: 1 mm and 75 µm). The relatively large solids and minute powders were removed. The selected dust samples having a particle size of 75 µm-1 mm were used for analysis. A 0.5 g of house dust was charged in cell for SFE and internal standard was added. Then, the samples were extracted for one hour by SFE at 0.5 mL/min with carbon dioxide as extraction solvent, and 4% v/v methanol were added as a modifier. The SFE extract was got through same procedure on human milk samples. The final volume for the dust extracts was adjusted to 2.5 mL.

Result and Discussion

Validation of the LC/MS/MS method:

To validate of the LC/MS/MS method, several experimental parameters, such as limit of detection (LOD) and limit of quantitation (LOQ), were examined. LOD was 0.08 to 0.15 ng/mL in house dust sample. LOQ was 0.004 to 0.1 ng/mL and 0.5 ng/mL in human milk and house dust, respectively (S/N = 10). The calibration curves were obtained by analyzing mixtures containing and the internal standard in the range from 0.2 to 20 ng/mL for PFCs, respectively. The average recoveries and relative standard deviation (R.S.D.) were over 94.3 and 10.3% for PFCs at the spiked. On the other hand, the average recoveries and relative standard deviation (R.S.D.) were over 93.4% and 8.6% for PFCs at the spiked. Our methods were enabled to prepare in the human milk and the house dust samples.

Determination of PFCs in human milk and house dust samples:

The proposed method was applied to the analysis of human milk samples from healthy mother volunteers. The representative chromatogram of the milk sample is shown in Fig. 1. PFCs were detected in all the samples, and PFOS from the milk samples ranged from 0.008 to 0.4 ng/mL, as shown in Table 1. The house dust samples were relatively high concentrations of PFCs were detected (Table 2).

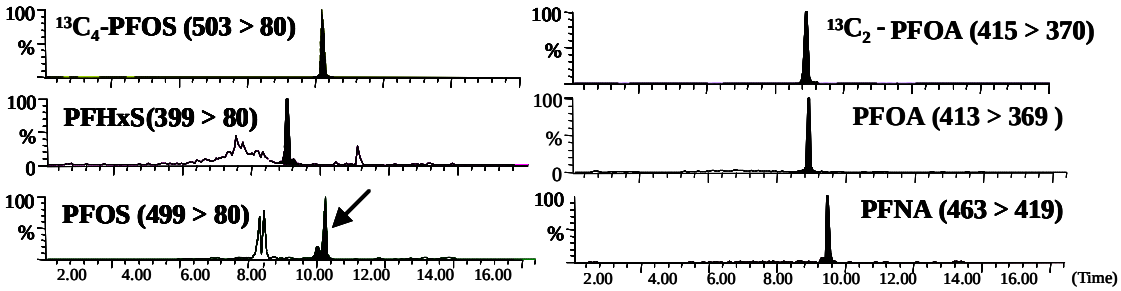

Fig. 1 Chromatogram of PFCs in the milk sample

Table 1 Concentration of PFCs in human milk samples

Compound	Detection rate (%)	Maximum (ng/mL)	Minimum (ng/mL)
PFHxS	64	0.025	N.D.
PFOS	100	0.401	0.008
PFOA	44	0.339	N.D.
PFNA	86	0.150	N.D.

Table 2 Concentration of PFCs in house dust samples

Compound	Detection rate (%)	Maximum (ng/g)	Minimum (ng/g)
PFHxS	40	5.5	N.D.
PFOS	100	41.0	7.0
PFOA	100	88.5	17.5
PFNA	100	69.0	5.5

Conclusion

The proposed LC/MS/MS method enabled the simultaneous determination of PFCs in human milk and house dust samples. In addition, interferences from sample matrice were removed by using the SPE for human milk or SFE for house dust, respectively. To investigate the source of PFCs exposure for infants, house dust samples (n = 20) and human milk samples (n = 51) were measured by developed methods. PFOS and PFOA were detected in all dust samples (PFOS: 7.0 to 41 ng/g; PFOA: 18 to 89 ng/g). Based on our results, house dust might be one of exposure sources of PFCs. Further studies are necessary to make sure the exposure source of PFCs for infant from indoor condition. In addition, PFOS was also detected in all samples in human milk (PFOS: 0.008 to 0.401 ng/g;). The infants will be exposed to PFCs from not only the umbilical cord blood but also human milk with maternal feeding.

Acknowledgments

We are grateful to participants who donated their human milk samples. This study was supported by grants from the Ministry of the Health, Labor and Welfare and Ministry of Educations, Culture, Sports, Science and Technology.

References

1. M. E. Austin, B. S. Kasturi, M. Barber, K. Kannan, P. MohanKumar, S. M. J. MohanKumar, *Environ. Health. Perspect.*, 2003;35*:*111:
2. J. R. Thiboeaux, R. G. Hanson, J. M. Rogers, B. E. Grey, B. D. Barbee, J. H. Richards, J. L. Butenhoff, L. A. Stevenson, C.Lau., *Toxiclol. Sci.*, 2003;74:369
3. K.Inoue, F.Okada, R.ITO, S.Kato, S.Sasaki, S.Nakajima, A.Uno, Y.Saijo, F.Sata, Y.Yoshimura, R.Kishi, H.Nakazawa : *Environ. Health Perspect.*, 2004;112:1204
4. A. Karrman, B. B. Van, U. Jarnberg, L. Hardell, G. Lindstrom, *Anal. Chem.*, 2005;77:864
5. M. K. SO, S. Taniyasu, N, Yamashita, J. P. Giesy, J. Zheng, Z. Fang, S. H. IM, P. K.S. Lam., *Environ. Health. Technol.*, 2005*;*38:4056