Fluorinated organic compounds (FOCs), such as perfluorooctane sulfonate (PFOS), perfluorooctanoate (PFOA), and perfluorooctanesulfonamide (PFOSA), are stable chemicals with a wide range of industrial and consumer applications (Renner 2001). Recent reports have indicated the presence of these environmental contaminants in wildlife and river water (Giesy and Kannan 2001; Kannan et al. 2001; Saito et al. 2003; Taniyasu et al. 2003). FOCs have been manufactured for > 50 years and are used as refrigerants, surfactants, and polymers and as components of paper coatings, fire retardants, adhesives, cosmetics, and insecticides (Key et al. 1997).

In the United States, PFOS is a stable FOC with many industrial applications. The amount of PFOS used in consumer application totals 5.6 million lb [U.S. Environmental Protection Agency (EPA) 2000]. These findings prompted the major manufacturer of PFOS in the United States, 3M Company, to halt production in the end of 2002 (Renner 2001). However, PFOS is still available on the Japanese market. A recent study of four FOCs in 65 human serum samples collected from several biologic supply companies in the United States detected PFOS (6.7–81.5 ng/mL), PFOA [limit of quantitation (LOQ), 35.2 ng/mL], and PFOSA (LOQ, 2.2 ng/mL) (Hansen et al. 2001). A study of human exposure to FOCs found that mean serum PFOS concentration was 17.7 ng/mL in 24 donors (Olson et al. 2003). In Japan, there is only one report of exposure in humans (Taniyasu et al. 2003), indicating that PFOS concentrations range from 2.4 to 14 ng/mL. Recently, a rapid and sensitive method for measuring 15 FOCs in human breast milk and serum has been developed (Calafat et al. 2003). The study of human exposure to FOCs requires information about the concentration of these toxicants in a non-occupationally exposed population. However, to our knowledge, there is no study of FOCs in human maternal and cord blood samples for fetal risk assessment. Human fetal and maternal exposure assessment studies are urgently needed because maternal and developmental toxicities of PFOS have been indicated (Lau et al. 2003; Thibodeaux et al. 2003).

The aim of the present study was to determine human cord and maternal serum concentrations of PFOS, PFOA, and PFOSA for fetal risk assessment. Although based on only a small sample set, our findings indicate the levels of FOCs to which Japanese women...
are exposed. We developed an easy, reliable, and high-throughput analytical method that uses liquid chromatography–electrospray mass spectrometry (LC-MS) coupled with online extraction to measure specific FOCs in human plasma and serum samples. This method enables the precise determination of standards in human blood samples and can be applied to the detection of PFOS, PFOA, and PFOSA in human blood samples for monitoring human exposure.

Materials and Methods

Clinical materials. We recruited subjects between February and July 2003 at Sapporo Toho Hospitals in Hokkaido, Japan. This study was conducted with all the subjects' written informed consent and was approved by the institutional ethical board for epidemiologic studies at the Hokkaido University Graduate School of Medicine. The data of physical and biologic examinations, laboratory tests, and questionnaires were recorded by the Department of Public Health, Hokkaido University Graduate School of Medicine. Blood was sampled from pregnant women (n = 15; Table 1) between gestation weeks 38 and 41 (mean ± SD, 39.7 ± 1.05). Cord blood samples were collected immediately after birth by using standard procedure, which included careful cleansing of the cord and strict puncture of the umbilical vein to avoid maternal contamination. Body mass index (BMI) was calculated from the information given in Table 1 (height, prepregnancy weight, and weight at delivery).

FOC analysis. PFOS [molecular weight (MW), 538.23; 98% purity], PFOA (MW, 414.07; > 90%), and PFOSA (MW, 199.14; 97%) were purchased from Wako Pure Chemical Inc. (Osaka, Japan), Fluka Chemie AG (Buchs, Switzerland), and ABCR GmbH & Co. (Im Schleiert, Germany). The internal standard (perfluorodecanoic acid) was purchased from Lancaster Company, Inc. (Morecambe, UK). Other reagents and solvents were of HPLC grade and were purchased from Wako Pure Chemical Inc. (Osaka, Japan). The distilled water purification system was Milli-Q gradient A 10 with an EDS polisher (Millipore, Bedford, MA, USA).

LC-MS with electrospray ionization was performed using an Agilent 1100 MSD-SL system (Agilent Technologies, Palo Alto, CA, USA). The working conditions were as follows: The drying nitrogen gas temperature was set at 350°C and was introduced into the capillary region at a flow rate of 12 L/min; the capillary was held at a potential of 3,500 V relative to the counter electrode in the negative-ion mode for all compounds. The fragmenter voltages were 220 V for PFOS, 130 V for PFOA, and 170 V for PFOSA during the chromatographic run. The direct injection volume was 30 µL. The column used was Inertsil C8-3 (2.1 × 100 mm, 5 µm; GL Sciences Inc., Tokyo, Japan) with a Mightysil RP-18 GP precolumn (2.0 × 5 mm, 5 µm; Kanto Chemical Inc., Osaka, Japan).

The column-switching LC-MS coupled with an on-line extraction system consisted of this LC-MS combined with an LC pump (Shimadzu LC-10 ADvp pump; Shimadzu, Kyoto, Japan) and Oasis HLB extraction column (20 × 2.1 mm, 25 µm; Waters Co., Milford, MA, USA). After a blood sample was injected by an autosampler, it was loaded onto the extraction column by flowing water/methanol (90/10, vol/vol) at a rate of 1.0 mL/min using a Shimadzu pump for 5 min. After on-line extraction for 5 min, the position of the switching valve was changed. This configuration connected the back-flashing extraction column to the analytical column and the MS detector in the flow path of the Agilent pump. After 20 min, the switching valve was returned to its original position. The run time for the assay of the sample mixture was 30 min. Gradient mobile phase of 1.0 mM ammonium acetate in water/acetonitrile (vol/vol) was used at a flow rate of 0.2 mL/min (5–15 min using a linear increase from 65 to 85% acetonitrile solution and holding at 85%).

In the quantitative procedure, standard solutions of PFOS, PFOA, and PFOSA were prepared in aqueous solution to cover the calibration range. Quantitative analysis was performed in the single ion monitoring mode to maximize sensitivity. PFOS, PFOA, and PFOSA concentrations in each sample were calculated relative to the internal standard added to the sample before direct analysis. Calibration curves of PFOS, PFOA, and PFOSA were performed daily for all samples with internal standard. We added 0.3 mL of sample to 0.3 mL of internal standard solution. The mixed sample was centrifuged at 3,000 × g for 10 min. The top clear layer was removed to the glass tube. This sample solution was filtered. This solution was analyzed by column-switching LC-MS. We analyzed quality-control materials (spiked samples in 25 ng/mL of PFOS) with each batch of samples on separated days. In the result, this material did not deviate from the 99% confidence interval (CI) (in this case, 99% CI, 24.17–25.53).

The method was developed previously. The compounds were separated by reverse-phase LC with a C3 column and detected by MS in the selected ion monitoring mode. When working in the selected ion monitoring mode, the m/z ions for PFOS, PFOA, and PFOSA were [M–K]– 499, [M–COOH]– 369, and [M–H]– 498. In addition, the m/z ion of the internal standard was designated as [M–COOH]– 469 in the negative ion mode.

The analysis of trace levels of PFOS, PFOA, and PFOSA in biologic samples is complicated by contamination, particularly by leaching from Teflon plastic. Thus, care must be taken to control contamination during experiments and, where possible, to eliminate the contamination. Investigations of PFOS, PFOA, and PFOSA contamination of the Milli-Q water system, the plastic tube, and the LC system produced negative results (below the limit of detection). We investigated whether the recoveries of PFOS, PFOA, and PFOSA (10 and 100 ng/mL) in the samples were 95%.

Table 1. Characteristics of mothers and infants (n = 15).

| Characteristics | Median (range) |
|----------------|----------------|
| Maternal age (years) | 28.4 (17–37) |
| Gestation (weeks) | 39.7 (28–41) |
| Maternal height (cm) | 157.2 (148–168) |
| Maternal prepregnancy weight (kg) | 50.3 (40–61) |
| Maternal weight at delivery (kg) | 60.4 (49.1–72) |

Infants

| Male (n [%]) | 8 (53.3) |
| Female (n [%]) | 7 (46.7) |
| Birth weight (g) | 3125.7 (2579–4162) |

Table 2. Concentrations (ng/mL) of FOC congeners (PFOS, PFOA, and PFOSA) in maternal and cord blood samples (n = 15).

| Sample no. | Maternal PFOS | Maternal PFOA | Maternal PFOSA | Fetal PFOS | Fetal PFOA | Fetal PFOSA |
|------------|---------------|---------------|---------------|------------|------------|------------|
| 1          | 10.4          | 0.7           | ND            | 3.9        | ND         | ND         |
| 2          | 17.6          | 2.3           | ND            | 5.3        | ND         | ND         |
| 3          | 9.5           | ND            | ND            | 3.9        | ND         | ND         |
| 4          | 7.9           | ND            | ND            | 2.4        | ND         | ND         |
| 5          | 12.8          | ND            | ND            | 4.7        | ND         | ND         |
| 6          | 5.4           | ND            | ND            | 1.6        | ND         | ND         |
| 7          | 7.9           | ND            | ND            | 2.3        | ND         | ND         |
| 8          | 9.5           | 0.5           | ND            | 3.0        | ND         | ND         |
| 9          | 7.9           | ND            | ND            | 2.5        | ND         | ND         |
| 10         | 5.8           | ND            | ND            | 2.1        | ND         | ND         |
| 11         | 9.9           | ND            | ND            | 2.8        | ND         | ND         |
| 12         | 8.1           | ND            | ND            | 2.5        | ND         | ND         |
| 13         | 4.9           | ND            | ND            | 1.7        | ND         | ND         |
| 14         | 8.3           | ND            | ND            | 2.8        | ND         | ND         |
| 15         | 6.9           | ND            | ND            | 1.6        | ND         | ND         |

ND, not detected; PFOS < 0.5 ng/mL; PFOA < 0.5 ng/mL; PFOSA < 1.0 ng/mL.
could be determined by this method. The average recoveries of PFOS, PFOA, and PFOSA ranged from 82.2 to 98.7%, with relative standard deviation < 5.2%. We used this method to assess FOC levels in human blood samples to obtain a reference range.

**Thyroid hormone estimation.** Thyroid-stimulating hormone (TSH) and free thyroxine (T₄) levels of newborns were measured in Sapporo City Institute of Public Health. Blood specimens on filter paper were collected from infants between 4 and 7 days of age. TSH and free T₄ were determined in single 0.3-cm disks punched from the same filter paper. Blood specimens from infants were collected by the Sapporo City Institute of Public Health.

Detection limits were for TSH, 0.5 µIU/mL, and for free T₄, 0.20 ng/dL, respectively. The concentrations of PFOS, PFOA, and PFOSA in the maternal and cord samples was 100% (30 of 30), 10% (3 of 30), and 0% (0 of 30), respectively. The percentage detection of PFOS, PFOA, and PFOSA in the maternal and cord samples was 100% (30 of 30), 10% (3 of 30), and 0% (0 of 30), respectively. The concentrations of PFOS in maternal serum samples ranged from 4.9 to 899 ng/mL with a range of 40–10,060 ng/mL (Olsen et al. 2003a), and 899 ng/mL with a range of 722–1,120 ng/mL (Olsen et al. 2003c). By contrast, PFOS concentrations in cord blood samples have not been reported so far. Therefore, we cannot compare our cord blood PFOS exposure levels with other data. However, a study of exposure to PFOS during pregnancy in the rat and mouse indicated that the amount of accumulated PFOS is proportional to the treatment dosage and the levels detected in fetal liver; in terms of concentration, the fetal liver level appears to contain approximately half as much PFOS as its maternal counterpart (Thibodeaux et al. 2003). Based on our study, the mean ratio of PFOS concentration in maternal blood to that in cord blood is 0.32 (range, 0.23–0.41). The studies of PFOS exposure during pregnancy in the rat and mouse (Lau et al. 2003; Thibodeaux et al. 2003) support our findings that PFOS accumulation can be measured in humans and that there is a high correlation between PFOS concentrations in maternal and cord blood.

On the other hand, PFOA was detected in a small number of maternal samples but not in cord samples (< 0.1 ng/mL). PFOA may pose a developmental risk to children at concentrations already found in the blood of women and children, according to a U.S. EPA preliminary risk assessment released in April 2003 (Renner 2003). Kannan et al. (2002a) measured FOCs, including PFOA, in bird livers collected from Japan and South Korea and found that the highest concentration of PFOA in the samples was 21 ng/g wet weight. PFOA has been found in environmental samples (Giesy and Kannan 2001; Kannan et al. 2002b). However, we do not know how people are exposed to PFOA according to a nonoccupational exposure assessment study. The occupational exposure levels of PFOA in humans are 1,780 ng/mL with a range of 40–10,060 ng/mL (Olsen et al. 2003a), and 899 ng/mL with a range of 722–1,120 ng/mL (Olsen et al. 2003c). By contrast, nonoccupational exposure to PFOA was found to be at trace levels (range of < 0.5–4.1 ng/mL, 15 of 21) in our other study (Okada et al. 2003). Therefore, we surmise that fetal exposure to PFOA is at trace levels. Recently, the dissociation constants for PFOA binding to human serum albumin (HSA) and the number of PFOA binding sites on HSA were determined (Han et al. 2003). At the same time, Han et al. (2003) predicted that PFOA bound to maternal blood protein may not be able to cross the placental barrier. The reasons for the trace levels of PFOA in fetal blood samples are nonoccupational exposure and the binding of PFOA to maternal blood protein.

![Figure 1](image1.png)

**Figure 1.** PFOS concentrations in maternal and cord blood samples ($r^2 = 0.876$, $y = 0.3332x - 0.0877$).

![Figure 2](image2.png)

**Figure 2.** Maternal age (A) and BMI (B) plotted against PFOS concentration in maternal blood samples ($n = 15$).

![Figure 3](image3.png)

**Figure 3.** Infants’ sex (A) and birth weight (B) plotted against PFOS concentration in cord blood samples ($n = 15$). Error bars indicate mean ± SD.

![Figure 4](image4.png)

**Figure 4.** Infants’ thyroid hormones levels [TSH and free T₄] plotted against PFOS concentration in cord blood samples ($n = 15$).
Like polychlorinated biphenyls, organochlorine pesticides, and polybrominated diphenyl ethers, PFOS may be able to cross the placental barrier to enter fetal circulation (Covaci et al. 2002; DeKoning and Karmaus 2000; Mazdai et al. 2003; Sala et al. 2001; Waliszewski et al. 2000). Our data suggest that the slope is approximately 0.33 (Figure 1), indicating that PFOS does not pass into the fetal circulation completely; that is, there does seem to be a barrier effect. In contrast to PFOS, however, PFOA and PFOSA cannot cross the placental barrier to enter fetal circulation. PFOS is known to exhibit developmental toxicity and postnatal effects, as has been demonstrated in experimental animal studies (Lau et al. 2003; Thibodeaux et al. 2003). In those studies, exposure to PFOS during pregnancy led to significant physiologic alterations in utero and via breast milk. A review. J Expo Anal Environ Epidemiol 10:285–293.

Giesy PJ, Kannan K. 2001. Global distribution of perfluorooctanoic acid in wildlife. Environ Sci Technol 35:1339–1342.

Hansen CJ, Clemen LA, Elford ME, Johnson HO. 2001. Compound-specific, quantitative characterization of organic fluorochromes in biological matrices. Environ Sci Technol 35:766–770.

Hu W, Jones PD, DeCraen W, King L, Fraker P, Newsted J, et al. 2003. Alterations in cell membrane properties caused by perfluorinated compounds. Comp Biochem Physiol C Toxicol Pharmacol 135:77–88.

Hu W, Jones PD, Ugham BN, Trosko JE, Lau C, Giesy JP. 2002. Inhibition of gap junctional intercellular communication by perfluorinated compounds in rat liver and dolphin kidney epithelial cell lines in vitro and Sprague–Dawley rats in vivo. Toxicol Sci 68:429–438.

Kannan K, Choi JW, Iseki N, Senthilkumar K, Kim DH, Giesy JP. 2002a. Concentrations of perfluorinated acids in livers of northern fulmars (Fulmarus glacialis) and rainbow trout (Oncorhyncus mykiss). Environ Sci Technol 36:2566–2571.

Key BD, Howell RD, Criddez CA. 1997. Fluorinated organics in the biosphere. Environ Sci Technol 31:2465–2454.

Lau C, Thibodeaux JR, Hanson RG, Rogers JM, Grey BE, Stanton ME, et al. 2003. Exposure to perfluorooctane sulfonate during pregnancy in rat and mouse. I. Maternal and perinatal evaluations. Toxicol Sci 34:202–209.

Luebker DJ, Hansen KJ, Bass NM, Butenholz JL, Seacet AM. 2002. Interactions of fluorochemicals with rat liver fatty acid-binding protein. Toxicology 176:175–185.

Mazdai A, Dodder NG, Abernathy MP, Hites RA, Bigby RM. 2003. Polybrominated diphenyl ethers in maternal and fetal blood samples. Environ Health Perspect 111:1249–1252.

Okada F, Ito R, Inoue K, Nakazawa N. 2003. Fluorinated organic compounds exposure in residents of Japan: development of liquid chromatography–mass spectrometry coupled with on-line extraction for determination of PFOS, PFOA and PFOSA in blood samples [Abstract]. In: Proceedings of the 6th annual meeting of Japan Society of Endocrine Disrupters Research, 2–3 December, 2003. Tsukuba, Ibaragi, Japan:Japan Society of Endocrine Disrupters Research. 66.

Olsen GW, Burris JM, Burlew MM, Mandel JH. 2003a. Epidemiologic assessment of worker serum perfluorooctanesulfonate (PFOS) and perfluorooctanoic acid (PFOA) concentrations and medical surveillance examinations. J Occup Environ Med 45:260–270.

Olsen GW, Hansen KJ, Stevenson LA, Burris JM, Mandel JH. 2003b. Human donor liver and serum concentrations of perfluorooctanesulfonate and other perfluorochemicals. Environ Sci Technol 37:888–891.

Olsen GW, Logan PW, Hansen KJ, Simpson CA, Burris JM, Burlew MM, et al. 2003c. An occupational exposure assessment of a perfluorooctanesulfonate fluoride production site: biomonitoring. AIHA J (Fairfax, VA) 64:651–658.

Renner R. 2001. Growing concern over perfluorinated chemicals. Environ Sci Technol 35:154A–160A.

Renner R. 2003. Concerns over common perfluorinated surfactant. Environ Sci Technol 37:201A–202A.

Salto N, Sasaki K, Nakatome K, Harada K, Yoshinaga T, Kuizumi A. 2003. Perfluorooctane sulfonate concentrations in surface water in Japan. Arch Environ Contam Toxicol 45:149–158.

Sala M, Ribas-Fito N, Cardo E, de Muga ME, Marco E, Mazon C, et al. 2001. Levels of hexachlorobenzene and other organochlorine compounds in cord blood: exposure across placenta. Chemosphere 43:895–901.

Seacet AM, Thomford PJ, Hansen KJ, Clemen LA, Eldridge SR, Elcombe CR, et al. 2003. Sub-chronic dietary toxicity of potassium perfluorooctanesulfonate in rats. Toxicology 183:117–121.

Seacet AM, Thomford PJ, Hansen KJ, Olsen GW, Case MT, Butenholz JL. 2002. Subchronic toxicity studies on perfluorooctanesulfonate potassium salt in cynomolgus monkeys. Toxicol Sci 68:249–264.

Taniyasu S, Kannan K, Horii Y, Hanani N, Yamashita N. 2003. A survey of perfluorooctane sulfonate and related perfluoro-organic compounds in water, fish, birds, and humans from Japan. Environ Sci Technol 37:2634–2639.

Thibodeaux JR, Hansen RG, Rogers JM, Grey BE, Barbee BD, Richards JH, et al. 2003. Exposure to perfluorooctane sulfonate during pregnancy in rat and mouse. I. Maternal and prenatal evaluations. Toxicol Sci 74:369–381.

U.S. EPA (U.S. Environmental Protection Agency). 2000. Perfluorocycloalkyl Sulfonates: Proposed Significant New Use Rule. Fed Reg 65(202):62319–62333.