Health Risks in Infants Associated with Exposure to Perfluorinated Compounds in Human Breast Milk from Zhoushan, China

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Recent studies have reported the ubiquitous distribution of perfluorinated compounds (PFCs), especially perfluorooctanesulfonate (PFOS) and perfluorooctanoic acid (PFOA), in wildlife and human whole blood or serum. In 2003 a solid phase extraction method was developed, which allowed the measurement of PFCs in human breast milk. In the present study, PFCs in samples of human breast milk from 19 individuals from Zhoushan, China, were analyzed by modifying a previously established method, based on weak-anion exchange extraction. PFOS and PFOA were the two dominant chemicals detected in all the milk samples. Concentrations of PFOS and PFOA ranged from 45 to 360 ng/L and 47 to 210 ng/L, respectively. The maximum concentrations of other PFCs were 100 ng/L for perfluorohexanesulfonate (PFHxS), 62 ng/L for perfluorononanoate (PFNA), 15 ng/L for perfluorodecanoate (PFDA) and 56 ng/L for perfluoroundecanoate (PFUnDA). Statistically significant correlations between various PFCs suggested a common exposure source to humans. No statistically significant correlation was found between concentrations of either PFOS or PFOA and maternal age, weight, or infant weight. Rate of consumption of fish was found to be positively correlated with PFNA, PFDA, and PFUnDA concentrations. Daily intake of PFOS for the child via breast milk with greater PFOS concentrations exceeded the predicted conservative reference dose in 1 of 19 samples, indicating that there may be a small potential risk of PFOS for the infants in Zhoushan via the consumption of breast milk.

Introduction

Perfluorinated compounds (PFCs), possessing properties of repelling both water and oil, have been widely used in a range of industrial and consumer products including fire-fighting foams, floor polishes, shampoos, as well as surface protectors in carpets, leather, paper, and food containers (*1*). Even though PFCs were found in human tissues over 30 years ago (*2*), it was only recently that more specific and sensitive methods have allowed for the identification and quantification of PFCs in tissues of wildlife and humans (*3*). Perfluorooctanesulfonate (PFOS) and perfluorooctanoate (PFOA) are found globally in tissues of wildlife such as fish, shrimp, crab, harbor porpoise, bird, and polar bear (*4*−*7*). This ubiquitous distribution, together with the potential toxicities of some PFCs, have led to various government agencies to begin assessing the potential environmental risks of these compounds (*8*). The potential for redistribution and limited degradation of these chemicals throughout the environment heighten the necessity of continual monitoring of PFCs in the environment, not only in wildlife species, but also the human population (*5*).

Compound-specific analysis of PFCs has allowed the detection of these chemicals in human blood or serum. PFOS concentrations of up to 12 800 ng/mL were detected in serum samples of donors working in a fluorochemical manufacturing plant (*9*). Lesser concentrations of PFCs have been found in sera of the general population, including children. A large-scale study has been undertaken to measure PFOS, perfluorohexanesulfonate (PFHxS), PFOA, and perfluorooctanesulfonamide (PFOSA) in 473 human blood, serum, or plasma samples from several countries. The greatest PFOS and PFOA concentrations were 164 ng/mL from Kentucky, U.S. and 256 ng/mL from Korea, respectively. Concentrations of PFHxS and PFOSA were comparatively less than that of PFOS (*10*). A striking finding was the detection of PFCs in serum samples of children. The maximum PFOS concentration was 515 ng/mL (*11*). This concentration was comparable to levels found in workers in 3M's Sumitomo plant in Japan (*12*).

While some PFCs, such as PFOS, have been demonstrated to alter cellular homeostasis and to cause toxicity (*5, 13*−*16*), and many studies have demonstrated toxicities of PFCs in animals (*17*−*19*), the relationship between PFC exposure and its corresponding adverse health effects in humans has still not been clearly established. A retrospective cohort mortality study has reported an increased number of deaths from bladder cancer for employees working in a perfluorooctanesulfonyl fluoride based fluorochemical production facility (*20*). However, there are uncertainties in drawing a definite conclusion from this study. Studies demonstrating toxicities of PFCs in animals include a sub-chronic dietary exposure study which showed liver hypertrophy, decreased serum cholesterol and glucose, increase in hepatic palmitoyl Co A oxidase, and alanine aminotransferase in rats of the high dose exposure group (*19*). Maternal toxicity, including reduced body weight gain and feed consumption, was found in dosed pregnant rats and rabbits (*17*). The developmental toxicity of perfluoroalkyl acids and their derivatives has also been described (*21*). Neuroendocrine effects, such as increased serum corticosterone level and norepinephrine concentrations in the paraventricular nucleus of the hypo-

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thalamus, were also observed in rats associated with PFOS exposure (*22*). The occurrence of PFCs in human tissues, together with their toxicities reported in animal studies, indicates at least a potential for toxic effects but the exact concentration necessary to elicit adverse effects remains unknown.

Previous studies have focused on determining concentrations of PFCs in whole blood or blood serum in humans (*10*, *23*). It was not until 2003 that a solid phase extraction (SPE) method was developed, which allowed measurement of PFCs in human breast milk (*24*). This method has not only provided important additional data for human exposure assessment, but also improved our understanding of the partitioning of PFCs into breast milk and the potential exposure of breast-fed infants. The statistically significant correlation between PFOS concentrations in maternal and cord blood in human suggests that PFOS may be able to pass through the placental barrier and enter the fetal circulation (*25*). Although it is too early to draw a definitive conclusion, signs of toxicity, such as delays in eye opening and increase in liver weight, were observed in rat and mouse pups exposed prenatally to PFOS (*26*). Therefore, an understanding the maternal transfer of PFCs to prenatal child is becoming increasingly important.

In the present study, PFCs were identified and quantified in human breast milk samples collected from 19 primiparas mothers in Zhoushan, China. Health risks of infants via the consumption of mothers' breast milk were assessed by estimating the health indexes based on the USEPA guidelines (*27*). The relationship between the PFC exposure and diet habits was also investigated.

Materials and Methods

Sample Collection. Human breast milk samples were collected during 2004 from 19 volunteer donors from a local hospital in Zhoushan, China. Questionnaires were used to collect information such as age, body weight, occupation, dietary habit of the donors and body weight of infants at the time of sample collection. Approximately 20–30 mL milk samples were collected from each donor and were stored in 50 mL polypropylene (PP) tubes. Disposable PP laboratory ware, instead of glassware, was used to prevent potential loss of analytes to the glass surfaces. All the samples were kept at −20 °C before extraction.

Chemicals and Standards. Potassium salts of PFOS, PFHxS, and perfluorobutanesulfonate (PFBS) were provided by Dr. Kannan of the State University of New York. Perfluorohexanoic acid (PFHxA), PFOA, and perfluorononanoic acid (PFNA) were purchased from Wako Pure Chemical Industries Ltd. Perfluoroheptanoic acid (PFHpA), perfluorodecanoic acid (PFDA), and perfluoroundecanoic acid (PFUnDA) were purchased from Fluorochem Limited. We purchased 8:2 fluorotelomer acid (8:2 FTCA) and 8:2 fluorotelomer unsaturated acid (8:2 FTUCA) from Asahi Glass Co. Ltd. (Tokyo, Japan). We obtained $1,2^{13}$C-labeled PFOA from Perkin-Elmer (Boston, MA), and it was used as an internal standard (lot no. 3507-195; CUS1073000MG). Purities of all the analytical standards were ≥95%. Milli-Q water was used throughout the whole experiment. Methanol (HPLC grade), ammonium acetate, ammonium solution, and formic acid (25%, HPLC grade) were purchased from Wako Pure Chemical Industries, Ltd (Osaka, Japan). Nylon mesh filters (0.2 μm, 25 mm i.d.) were purchased from Iwaki (Tokyo, Japan). Formic acid was pre-filtered through Oasis weak-anion exchange (WAX) cartridge (0.2 g, 6 cm³) (Waters Corp., Milford, MA) prior to use.

Sample Preparation. The analytical method was modified from that of Kuklenyik et al. (*24*), based on weak-anion exchange SPE extraction. Frozen samples were thawed and allowed to return to room temperature before extraction.

Human breast milk samples were extracted by placing 2.0 mL of milk in a 15 mL PP tube containing 3.0 mL of 0.1 M formic acid. 10 μL of 100 ng/L internal standard solution (^{13}C−PFOA) was spiked into each sample. The mixture was vortexed, sonicated for 15 min, and then subjected to solid-phase extraction.

Sample Extraction. PFCs were concentrated from human breast milk by use of the Oasis WAX cartridges (0.2 g, 6 cm³). Prior to loading the sample onto the WAX cartridge, the cartridge was preconditioned by passing the following sequence of solutions through the cartridge: 4.0 mL of 0.1% NH_4OH in methanol, 4.0 mL methanol, followed by 4 mL Milli-Q water at a rate of 2 drops/sec. The sample mixture was then loaded onto the cartridge and eluted at a rate of 1 drop/sec. The cartridge was prevented from drying at all times during the loading of sample mixture. The cartridge was then washed with 4.0 mL 25 mM of acetate buffer, followed by 15 mL of 60% methanol/water and 1 mL methanol. Finally, the cartridge was eluted with 4.0 mL 0.1% NH_4OH (target fraction) at a rate of 1 drop/sec to elute target compounds. The volume of the target fraction was reduced to 1.0 mL under a gentle stream of high-purity nitrogen gas. The eluate was then passed through a 0.2 μm 25 mm nylon mesh filter and then transferred into a PP vial for chemical analysis.

Instrumental Analysis. PFCs in human breast milk were identified and quantified by use of high-performance liquid chromatography with high-resolution, electrospray tandem mass spectrometry (HPLC−MS/MS). Separation of analytes was performed by an Agilent HP1100 liquid chromatograph (Agilent, Palo Alto, CA) interfaced with a Micromass Quattro II mass spectrometer (Waters Corp., Milford, MA) operated in electrospray negative mode. A 10 μL aliquot of extract was injected onto a Keystone Betasil C_{18} column (2.1 mm i.d. × 50 mm length, 5 μm) with 2 mM ammonium acetate and methanol as the mobile phases starting at 10% methanol at a flow rate of 300 μL/min. The gradient was increased to 100% methanol at 10 min before reverting to the original conditions at 13 min. The desolvation gas flow and temperature were kept at 610 L/h and 450 °C, respectively.

Data Analysis. Acquisition analysis for all of the samples, blanks, and QC materials were performed using MassLynx software of the Micromass Quattro II system. Each ion of interest in the chromatogram was automatically selected and integrated. Concentrations of target analytes were quantified by using calibration curves, constructed using external standards of six different concentrations (2, 10, 50, 200, 1000, 10 000 ng/L). The standard calibration curves showed strong linearity (correlation coefficients >0.99).

Daily Intake Estimation and Risk Assessment. Health risks of infants through the consumption of mothers' breast milk were assessed by estimating the hazard indices (HI) based on the USEPA guidelines (*27*). The HI is estimated by comparing daily exposure concentration to infants with the reference dose (RfD). RfD values for PFCs have not yet been established by any government agencies. However, potential RfDs for PFOS and PFOA have been estimated based on rat chronic carcinogenicity studies and rat multigenerational studies, respectively, whereas RfDs for the other PFCs are not available at this time. On this basis, the RfD derived for PFOS was 0.025 μg/kg/d and that for PFOA was 0.333 μg/kg/d (*28*). The exposure level (Average Daily Intake; ADI) was calculated (eq 1). The average milk consumption rate

$$\text{ADI } (\mu g/\text{kg body weight/d}) = \text{consumption}$$
$$(\text{g/kg body weight/d}) \times \text{dietary concentration } (\mu g/g) \tag{1}$$

and body weight for one-year old infants were assumed to be 600 g/d and 7.3 kg, respectively (*29*). It was further assumed

TABLE 1. Recoveries (%), Blank (ng/L) and LOQs (ng/L) for Individual PFCs

			recovery (%)	
analyte	LOQ (ng/L)	blank (ng/L) ($n = 7$)	spike ($n = 7$) (Mean)	matrix-spike ($n = 6$) (Mean)
PFBS	1.0−5.0	<1.0−1.1	79−92 (86)	79−93 (88)
PFHxS	1.0−10	<1.0	86−97 (92)	86−106 (96)
PFOS	1.0−3.6	<1.0−3.6	88−102 (93)	82−99 (89)
PFHxA	1.0−10	<1.0−1.0	80−97 (88)	81−123 (105)
PFHpA	5.0−10	<5.0	85−102 (92)	81−97 (87)
PFOA	21−27	21−27	87−102 (92)	83−101 (88)
PFNA	1.0−10	<1.0−2.3	83−102 (92)	72−94 (81)
PFDA	1.1−2.5	1.1−2.5	83−99 (92)	73−93 (85)
PFUnDA	2.2−5.0	<1.0−2.8	83−106 (94)	56−98 (78)
8:2 FTCA	5.0−50	<5.0−8.7	57−88 (70)	50−96 (68)
8:2 FTUCA	1.0−2.0	<1.0	56−87 (69)	61−105 (82)

that the daily breast milk consumption remains relatively constant over an infant's life after the first month. The HI was calculated as follows (eq 2):

$$\text{hazard index} = \text{average daily exposure}/\text{RfD} \qquad (2)$$

A HI value less than unity indicates that the chemical involved is less likely to pose a significant health risk to the consumers (infants). However, HI greater than unity would indicate that exposure concentration exceeds RfD, and thus a potential risk may exist.

Statistical Analysis. All the food consumption data were log-transformed to achieve normality. A Pearson correlation analysis was used to examine the correlations between PFHxS, PFOS, PFOA, PFNA, PFDA, and PFUnDA concentrations and various parameters including maternal age, weight, infant weight, and food consumption data. Statistical significance was accepted at $P < 0.05$.

Results and Discussion

Quality Assurance and Control. Quantification of various PFCs is limited by detection limits that are determined by contamination of the sample from various laboratory appliances, including septa of autosampler vials as well as the internal parts of HPLC instrument. Thus, to achieve better sensitivity, all equipment items were checked for possible sources of contamination prior to sample analysis. Telfon-containing materials were completely avoided during the analysis. The analytical instrument was modified by replacing parts that may constitute a source of contamination. The details of the blank checks and the subsequent optimization have been described elsewhere (30).

To ensure the accuracy of the extraction and analytical procedures, procedural blanks, procedural recovery, and matrix-spiked recovery tests were conducted with each set of samples analyzed. Procedural blanks were carried out by performing the whole extraction procedure using the same amount of water, instead of milk samples. Concentrations of PFHxS, PFHpA, and 8:2 FTUCA were less than the limits of quantification (LOQs), which were defined as the smallest mass of standard injected, which resulted in a reproducible measurement of peak areas consistent with the calibration curve, with a signal-to-noise ratio equals to or greater than 3. Only trace concentrations were detected for all the analytes except PFOA for which 21−27 ng/L was measured in the procedural blanks (Table 1). Overall, the present method was able to achieve low LOQ values and blank levels for PFCs including, to a lesser extent, PFOA. The method is, therefore, suitable for the analysis of milk samples with comparatively low PFC concentrations.

Recovery tests were carried out by spiking external standard (100 ng/mL; 10 μL) into water (procedural recovery) and milk samples (matrix-spike recovery), and then extracted following the same procedures for real samples. The results of the procedural recovery and matrix-spike recovery tests are listed (Table 1). In general, most of the compounds, except for two precursor compounds (8:2 FTCA and 8:2 FTUCA), had mean recoveries greater than 86% and 78% in procedural recovery and matrix-spike recovery tests, respectively (Table 1).

PFC Concentrations in Human Breast Milk. The modified WAX cartridge solid phase extraction was used for the analysis, and the concentrations of all PFCs are summarized (Table 2). Concentrations of the two precursor compounds (8:2 FTCA and 8:2 FTUCA), PFBS and PFHxA in most of the samples were less than LOQs while PFHxS, PFOS, PFOA, PFNA, PFDA, and PFUnDA were detected in all samples. Concentrations and frequency of occurrence of PFHpA was comparatively lower than the other compounds. Among all, PFOS and PFOA were the two dominant chemicals found in the milk samples. Concentrations of PFOS ranged from 45 to 360 ng/L and those of PFOA ranged from 47 to 210 ng/L. The maximum concentrations of other PFCs were 100 ng/L for PFHxS, 62 ng/L for PFNA, 15 ng/L for PFDA, and 56 ng/L for PFUnDA (Table 2).

To our knowledge, the present study is the first report on a range of PFC concentrations in human breast milk in China. Many studies to date have focused on monitoring PFCs in whole blood, blood serum, or plasma in humans in an attempt to predict the extent of human exposure to PFCs. There is a general lack of information on the distribution of PFCs in other tissue compartments in humans. The detection of PFOS, PFHxS, and PFOA in seminal plasma samples from donors in Sri Lanka indicated that PFCs could be distributed to body fluids other than blood (31). Detectable concentrations of PFPeA (1.6 μg/L) and PFHxA (0.82 μg/L) were found in two human breast milk samples (24). However, insufficient information regarding these two milk samples and the small sample size make interpretation difficult. The detection of PFCs in Chinese milk samples demonstrated that various PFCs were able to accumulate, to some extent, in breast milk. It is noted, however, that the maximum concentrations of PFOS (360 ng/L) and PFOA (210 ng/L) detected in human breast milk samples were far less than those reported in human blood or serum samples. Average concentrations of PFOS and PFOA in human serum samples from 1998 to 2000 were 17 000−53 000 ng/L and 3000−17 000 ng/L, respectively (18). More specifically, our recent study detected 5400−26 000 ng/L for PFOS and 570−3300 ng/L for PFOA in whole blood of volunteer donors from Zhoushan in China (32). Similarly, concentrations of other long-chained perfluorocarboxylates in breast milk samples were lower than those found in blood samples of different individuals from Zhoushan. The measured mean concentrations in blood samples from Zhoushan population were 370, 1300, 490, and 1500 ng/L for PFHxS, PFNA, PFDA, and PFUnDA, respectively (32). The concentrations of various PFCs in breast milk samples were comparable to those found in seminal plasma in Sri Lanka population (31). The presence of PFCs in breast milk would mean that there may be a potential risk for infants via milk consumption. Monitoring of PFCs in human milk is thus important for exposure and risk assessments.

Composition Profile of Various PFCs. The profile of relative concentrations of PFHxS, PFOS, PFOA, PFNA, PFDA, and PFUnDA is shown in Figure 1a. PFOS and PFOA contributed up to 42 and 36%, respectively, of the total composition, whereas the rest of the compounds comprised less than 10%. Since the present study is the first survey of PFCs in human breast milk, data for global scale comparison are not available. The composition profile of PFHxS, PFOS,

TABLE 2. Concentrations of Individual PFC in Human Breast Milk Samples

sample no.	PFBS	PFHxS	PFOS	PFHxA	PFHpA	PFOA	PFNA	PFDA	PFUnDA	8:2 FTCA	8:2 FTUCA
						concentration (ng/L)					
1	1.4 ± 2.0	14 ± 0.80	89 ± 9.1	<1.0	<5.0	90 ± 9.7	15 ± 2.4	5.9 ± 0.10	17 ± 2.7	<5.0	<1.0
2	<5.0	100 ± 1.9	220 ± 15	<5.0	3.0 ± 4.1	120 ± 12	28 ± 2.9	8.0 ± 0.40	22 ± 1.0	<5.0	<1.0
3	<5.0	11 ± 0.50	120 ± 3.6	<5.0	2.6 ± 3.6	100 ± 3.0	20 ± 0.30	8.7 ± 0.80	22 ± 0.90	<5.0	<1.0
4	<5.0	32 ± 2.3	100 ± 6.2	<5.0	5.6 ± 0.10	110 ± 2.4	16 ± 1.1	6.6 ± 0.0	12 ± 0.90	<5.0	<1.0
5	2.5 ± 0.50	9.3 ± 0.40	45 ± 3.5	<1.0	<5.0	110 ± 3.4	13 ± 1.2	5.3 ± 0.0	7.6 ± 0.70	<5.0	<1.0
6	<5.0	33 ± 2.6	140 ± 7.4	<5.0	5.3 ± 0.50	110 ± 6.8	16 ± 0.50	6.3 ± 1.0	17 ± 0.10	<5.0	<1.0
7	<5.0	9.6 ± 1.7	160 ± 9.9	<5.0	<5.0	120 ± 2.6	21 ± 1.0	8.1 ± 0.30	21 ± 0.50	<5.0	<1.0
8	<1.1	31 ± 0.50	45 ± 0.30	<1.0	<5.0	52 ± 2.9	7.3 ± 0.50	4.8 ± 0.10	11 ± 0.60	<5.0	<1.0
9	<1.0	8.7 ± 1.3	140 ± 4.0	<1.0	<5.0	69 ± 3.9	16 ± 0.20	6.2 ± 0.20	14 ± 1.5	<5.0	<1.0
10	<2.0	19 ± 1.3	360 ± 50	<10	<10	190 ± 12	62 ± 1.8	15 ± 1.6	56 ± 8.4	<50	<2.0
11	<1.0	32 ± 1.8	180 ± 10	<1.0	<5.0	91 ± 6.0	18 ± 1.3	6.1 ± 1.2	23 ± 0.60	<5.0	<1.0
12	<5.0	11 ± 1.0	66 ± 2.7	<5.0	<5.0	47 ± 1.4	6.3 ± 0.20	4.8 ± 0.40	9.1 ± 0.30	<5.0	<1.0
13	<1.1	17 ± 1.2	78 ± 2.2	<1.0	<5.0	77 ± 4.3	15 ± 0.40	7.3 ± 0.10	22 ± 0.0	<5.0	<1.0
14	<5.0	4.0 ± 5.6	130 ± 2.7	<5.0	5.6 ± 1.0	140 ± 8.2	24 ± 0.60	11 ± 0.70	32 ± 0.90	<5.0	<1.0
15	<5.0	8.2 ± 0.70	57 ± 4.9	<5.0	5.8 ± 0.30	110 ± 5.1	9.3 ± 1.3	3.8 ± 0.20	8.8 ± 0.40	<5.0	<1.0
16	<5.0	28 ± 0.0	78 ± 9.0	<5.0	<5.0	75 ± 3.9	11 ± 0.60	7.3 ± 1.0	17 ± 1.0	<8.7	<1.0
17	<5.0	6.1 ± 1.5	87 ± 1.9	7.6 ± 0.40	<5.0	89 ± 2.9	14 ± 0.70	7.4 ± 0.40	16 ± 0.20	<8.7	<1.0
18	<5.0	4.1 ± 5.7	80 ± 6.4	<5.0	<5.0	110 ± 0.60	10 ± 0.10	6.1 ± 0.60	17 ± 2.7	<5.0	<1.0
19	<5.0	11 ± 0.0	120 ± 7.8	<5.0	6.7 ± 0.0	210 ± 4.8	22 ± 1.4	8.8 ± 0.40	18 ± 1.6	<5.0	<1.0
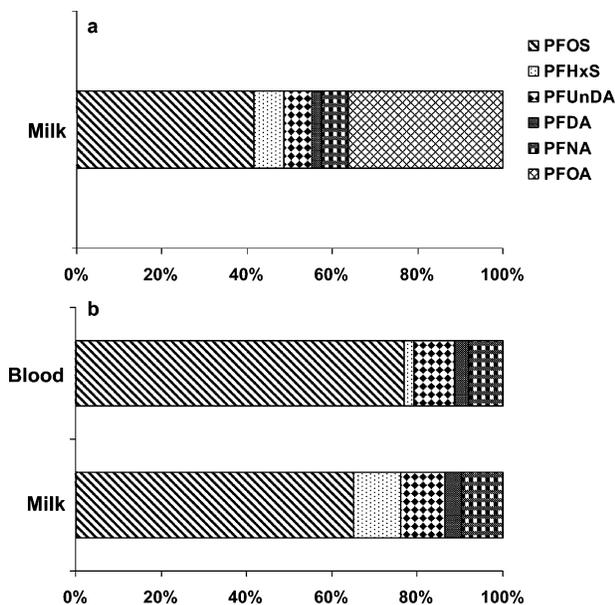
FIGURE 1. Composition profile of various PFCs in human breast milk (data from the present study) and blood samples (data from ref 32) from Zhoushan population. (a) Composition profile of PFOS, PFHxS, PFOA, PFNA, PFDA, and PFUnDA; and (b) Composition profile of PFOS, PFHxS, PFNA, PFDA, and PFUnDA.

TABLE 3. Correlation Coefficient of Pearson Correlation Analysis of Perfluorosulfonates and Perfluorocarboxylates

PFCs	r	P value
PFOS:PFHxS	0.31	0.20
PFOS:PFBS	−0.33	0.17
PFOS:PFUnDA	0.77	0.00[b]
PFOS:PFDA	0.64	0.0030[b]
PFOS:PFNA	0.87	0.00[b]
PFOS:PFOA	0.51	0.024[a]
PFUnDA:PFDA	0.75	0.00[b]
PFUnDA:PFNA	0.78	0.00[b]
PFUnDA:PFOA	0.47	0.045[a]
PFDA:PFNA	0.82	0.00[b]
PFDA:PFOA	0.59	0.0080[b]
PFNA:PFOA	0.72	0.00[b]

[a] Correlation is significant at the 0.05 level. [b] Correlation is significant at the 0.01 level.

PFNA, PFDA, and PFUnDA observed in the human breast milk in this study was similar to that of the blood samples from Zhoushan population (Figure 1b) (32). Although the blood and milk samples were not collected from the same individuals, the above finding suggests that there might be a common exposure source of PFCs to Zhoushan population.

Correlations Among PFCs. Statistically significant correlations were found among perfluorocarboxylates of different chain length (Table 3). The strongest correlation was found between PFNA and PFDA. These findings support the hypothesis that telomer alcohols could degrade to yield various perfluorinated acids including PFHxA, PFHpA, PFOA, PFNA, and other short-chained carboxylic acids (33). Similarly, PFOS was found to be positively correlated with various perfluorocarboxylates (Table 3). These correlations suggest that there might be a common exposure pathway of perfluorocarboxylates and perfluorosulfonates to humans. Our previous study showed a significant positive correlation between PFOS and PFHxS concentrations in human blood (32), such a relationship, however, was not found in the milk samples.

Correlations Between PFCs and Characteristics of Donors. Correlations between various PFCs (PFOS, PFOA, PFNA, PFDA, and PFUnDA) and maternal age, maternal and infant weight, and diet consumption pattern were investigated. The average maternal age was 25.8. The average maternal weight and infant weight were 60.4 and 3.4 kg, respectively. Neither PFOS nor PFOA concentrations were significantly correlated with any of these parameters. These results were similar to previous studies in which no age-related differences in PFOS and PFOA concentrations were found in human serum or blood samples (10, 25). This observation was different from that of other traditional persistent organic pollutants. For example, a positive correlation has been reported between concentrations of organochlorine pesticides in human milk and the maternal age (34). Such a difference could probably be due to the affinity of PFCs to lipoproteins rather than neutral lipids (35).

Correlations Between PFCs and Diet Consumption Pattern. The detection of PFCs in both occupationally and nonoccupationally exposed humans raises concern over the potential seriousness of the problem. Concentrations of PFOS and PFOA in blood sera have been reported to exceed the estimated reference concentrations in more than 50 and 5% of 599 children, respectively (11). Even now, it is still unclear

as to how children or the general population are exposed to PFCs. Understanding the potential exposure pathways is critically essential and important in conducting a comprehensive human health risk assessment. Various potential pathways leading to human exposure to PFCs have been proposed. One possible pathway is through exposure to indoor dust. An average of 200 ng/g, dw of PFOS and 380 ng/g, dw of PFOA were found in vacuum cleaner dust from Japanese homes (*36*). Furthermore, humans can be exposed to PFCs via respiration, food, and water consumption. It has been estimated that $10-100$ pg PFOS/d would be inhaled by an average person in Japan, and this may lead to an increase in plasma PFOS concentrations (*37*). Detection of PFOS in surface water in Ara ($13-39$ ng/L), Tama ($0.70-160$ ng/L) and Yodo ($0.90-27$ ng/L) rivers, serving as the sources of drinking water for more than eight million people in Japan, indicates that people can be exposed to PFCs through drinking contaminated water (*38*). Market basket surveys also detected the presence of PFCs in several food items including milk, ground beef, green beans, apples, and bread (*39*). A Canadian diet study detected PFCs in fast food composites in which the greatest concentration of N-Et PFOSA (24 000 pg/g, ww) was found in a pizza composite (*40*). To explore whether food consumption could be one of the possible exposure pathways for PFCs in humans in China, diet consumption data were collected from all donors in the present study. Dietary data indicated that the major food items included fish, shrimp and crab, clam, poultry, pork and beef, vegetables, eggs, and dairy products. In general, there is no correlation between concentrations of PFOS, PFOA, PFNA, PFDA, PFUnDA, and diet consumption data, except for some long-chained perfluorocarboxylates (PFNA, PFDA, and PFUnDA) which were found to have a significant positive correlation with fish consumption data ($r > 0.5$, $P < 0.05$). However, it is not possible to draw any definitive conclusion due to the relatively small sample size in the present study. Zhoushan is one of the coastal cities in China with little industry. It is likely that people in this area may be exposed to PFCs through the use of PFC-related consumer products and/or via consumption of various food products contaminated with PFCs. To have a more comprehensive exposure study, measurement of concentration of PFCs in various environmental compartments, including food, water, and air, may possibly identify important exposure pathways to human receptors.

Hazard Assessment of PFOS and PFOA Exposure to Infants. Health risks of infants through the consumption of mothers' breast milk were assessed. The daily intake of PFOS to the child from the mother with the greatest exposure would be 0.030 μg/kg/day, and that for PFOA would be 0.017 μg/kg/day. HIs for PFOS were calculated to be greater than unity in one out of 19 milk samples, whereas those for PFOA were all approximately 2 orders of magnitude less than unity. These results suggest that there may be a potential risk of PFOS for some infants in Zhoushan via the consumption of mothers' breast milk. However, it must be noted that uncertainty factors (UF$_A$: animal to human; UF$_H$: average human to sensitive human; UF$_L$: LOAEL to NOAEL; UF$_S$: sub-chronic to chronic; UF$_D$: database insufficiency; MF: modifying factor) have been applied in deriving the RfDs, and thus the actual risks may have been overestimated. When assessing the relative risks of trace concentrations of residues in human breast milk, the potential risks of neonatal exposure to these chemicals through lactation needs to be considered relative to the known benefits of breast feeding. Human breast milk is a good source of nutrition and it can provide essential nutrients including fats, carbohydrates, and proteins to infants and is important in developing immune competence (*41*). Nonetheless, it is prudent to undertake further investigations to clarify this issue.

Supporting Information Available

Three tables summarizing (1) characteristics of 19 volunteer donors and their corresponding infants ($n = 19$); (2) multiple-reaction-monitoring analysis of PFCs indicating precursor and product negative ion masses and MSMS conditions; (3) correlation between PFOS, PFOA, PFNA, PFDA, and PFUnDA and individual food items; and (4) daily exposure of infant to PFOS and PFOA through consumption of mother's breast milk and the corresponding hazard indices. This material is available free of charge via the Internet at http://pubs.acs.org.

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Health risks in infants associated with exposure to perfluorinated compounds in human breast milk from Zhoushan, China

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(5 Pages, 4 tables)

Table 1: Characteristics of 19 volunteer donors and their corresponding infants (n=19).

Characteristics	Range (Mean)	
	Mothers	**Infants**
Age	23-38 (25.8)	-
Height (cm)	155-166 (160.0)	48-51 (49.9)
Weight (kg)	46-78 (60.4)	2.8-3.8 (3.4)

Table 2: Multiple-Reaction-Monitoring Analysis of PFCs indicating Precursor and Product Negative Ion Masses and MSMS Conditions

Analyte	Abbreviation	Precursor ion	Product ion	Collision energies (eV)	Cone Voltages (V)
perfluorobutanesulfonate	PFBS	298.7	79.7	25	35
perfluorohexanesulfonate	PFHxS	398.7	79.7	30	70
perfluorooctanesulfonate	PFOS	498.6	79.7	35	90
perfluorohexanoate	PFHxA	312.8	268.8	7	35
perfluoroheptanoate	PFHpA	362.8	318.8	8	35
perfluorooctanoate	PFOA	413	368.7	10	35
perfluorononanoate	PFNA	462.7	418.8	10	35
perfluorodecanoate	PFDA	512.8	468.8	10	35
perfluoroundecanoate	PFUnDA	563	519	10	35
8:2 fluorotelomer acid	8:2 FTCA	477	393	20	35
8:2 fluorotelomer unsaturated acid	8:2 FTUCA	457	393	10	35

Table 3: Correlation between PFOS, PFOA, PFNA, PFDA and PFUnDA, and individual food items.

| Food items | Correlation | | | | | | | | | |
| | PFOS | | PFOA | | PFNA | | PFDA | | PFUnDA | |
	r	P value	r	P value	r	P value	r	P value	r	P value
Fish	0.43	0.067	0.35	0.14	0.51	0.026*	0.67	0.0020**	0.65	0.0030**
Shrimp and crab	0.18	0.46	0.43	0.064	0.14	0.57	0.098	0.69	-0.068	0.78
Clam	0.13	0.60	0.45	0.055	0.13	0.59	0.084	0.73	0.049	0.84
Poultry	-0.089	0.73	0.034	0.89	-0.12	0.63	0.023	0.93	0.019	0.94
Pork, beef	0.037	0.88	0.14	0.56	0.16	0.51	-0.011	0.96	-0.077	0.75
Vegetables	-0.53	0.023	-0.30	0.22	-0.51	0.032	-0.40	0.10	-0.44	0.068
Eggs	0.14	0.56	-0.098	0.69	0.15	0.53	0.16	0.50	-0.040	0.87
Dairy products	0.098	0.69	0.23	0.34	0.16	0.51	0.037	0.88	-0.23	0.34

* Correlation is significant at the 0.05 level.

** Correlation is significant at the 0.01 level.

Table 4: Daily exposure of infant to PFOS and PFOA through consumption of mother's breast milk and the corresponding hazard indices.

Sample no.	Daily consumption (µg/kg/day)		Hazard Index (HI)	
	PFOS	PFOA	PFOS	PFOA
1	0.007	0.007	0.29	0.02
2	0.018	0.009	0.73	0.03
3	0.010	0.008	0.39	0.02
4	0.009	0.009	0.34	0.03
5	0.004	0.009	0.15	0.03
6	0.012	0.009	0.46	0.03
7	0.013	0.010	0.52	0.03
8	0.004	0.004	0.15	0.01
9	0.011	0.006	0.44	0.02
10	0.030	0.016	1.19*	0.05
11	0.015	0.007	0.59	0.02
12	0.005	0.004	0.22	0.01
13	0.006	0.006	0.26	0.02
14	0.011	0.011	0.43	0.03
15	0.005	0.009	0.19	0.03
16	0.006	0.006	0.26	0.02
17	0.007	0.007	0.29	0.02
18	0.007	0.009	0.26	0.03
19	0.010	0.017	0.38	0.05

* Daily intake of PFOS exceeded the corresponding RfD (0.025 µg/kg/day).