

Perfluorinated Compounds in Human Milk from Massachusetts, U.S.A.

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Perfluorinated compounds (PFCs), notably perfluorooctanesulfonate (PFOS) and perfluorooctanoic acid (PFOA), have been reported in human blood. Furthermore, the occurrence of PFCs in the blood of newborn babies, coupled with the need to study the potential association of PFC exposure with birth outcomes in neonates, suggests the need for determining the sources and magnitude of exposure in infants. In this study, nine PFCs were measured in 45 human breast milk samples collected in 2004 from Massachusetts, U.S.A. PFOS and PFOA were the predominant PFCs found at mean concentrations of 131 and 43.8 pg/mL, respectively. Comparison of the ratio of PFOS to PFOA in human milk with the ratios published for human serum from the U.S. female population suggested preferential partitioning of PFOA to milk. Concentrations of PFOA were significantly higher in the milk of mothers nursing for the first time ($n = 34$) than in the milk of mothers who have previously nursed ($n = 8$). Based on the estimated body weight and milk intake, the average and highest daily intakes of total PFCs by infants were 23.5 and 87.1 ng/kg bw, respectively. We found that the daily ingestion rates of PFOS and PFOA did not exceed the tolerable daily intake recommended by the U.K. Food Standards Agency. This is the first study to measure the occurrence of PFCs in human milk from the U.S.A.

Introduction

Perfluorinated compounds (PFCs), especially perfluorooctanesulfonate (PFOS) and perfluorooctanoic acid (PFOA), are widespread contaminants in human blood (1–5). Blood concentrations of PFOS and PFOA in the U.S. population are greater than those reported from several other countries (1, 2, 4, 6, 7). In the U.S.A., concentrations of PFOS and PFOA in the blood of 2–12-year-old children have been similar to the concentrations found in adults (8). The occurrence of PFCs in the blood of children makes it appropriate to investigate potential sources of exposure, such as lactational

transfer during breast-feeding. Maternal blood and human milk present potential sources of infant exposure to PFCs during gestation and lactation, respectively. Concentrations of PFOS in cord blood were significantly correlated with concentrations in maternal blood (9, 10). Similarly, transplacental transfer of PFOA was suggested as a pathway of PFOA exposure in neonates (10). PFOS and PFOA were also found in neonatal blood collected immediately after birth (11). Recent studies from China and Sweden have shown lactational transfer of PFCs (5, 12).

Exposure of PFOS and PFOA in pregnant laboratory rodents has been shown to cause developmental effects including reduced birth weight and reduced postnatal weight gain with associated delays in reaching developmental landmarks and reduced postnatal survival during early life stages (13–20). Cross-foster studies in laboratory rodents demonstrated that lactational exposure may contribute to reduced body weight gain in pups nursed by PFOS- or PFOA-exposed dams (19, 20). However, it should be noted that the developmental effects in rodents exposed to PFOS and PFOA occurred at concentrations 3 orders of magnitude higher than the concentrations measured in general populations.

Three recent studies have investigated the association of exposure levels of PFOS and PFOA with birth outcomes. Firstly, PFOS and PFOA concentrations in cord blood were negatively associated with small reductions in birth weight, ponderal index, and head circumference (21, 22). In a second study, maternal PFOA concentrations, but not PFOS concentrations, were negatively associated with birth weight in children from Denmark (23). The evidence of the association between exposure levels of PFOA and PFOS and changes in birth outcome remains uncertain when considering the small differences in the range of exposure and the modest magnitude of reported differences as well as potential metabolic and pharmacokinetic parameters that could effect both blood concentrations and birth outcomes (24). Lastly, a study involving workers of a perfluorooctanesulfonyl fluoride manufacturing facility did not find an association between potential maternal exposure to PFOA and PFOS and low birth weight of neonates, including 32 births from mothers in the highest exposure category with estimated geometric mean serum concentrations of 1300–1970 ng/mL (25).

Despite the reports of higher concentrations of PFCs in the U.S. general population than in other countries little is known regarding the exposure of infants to PFCs via breastfeeding in the U.S.A. The only study that determined PFCs in breast milk from the U.S.A. involved the analysis of two human milk samples, while that study was aimed at developing a method to determine PFCs in milk (26). No information is available on the daily intake of PFCs by infants through breastfeeding. In this study, 45 human milk samples collected from Massachusetts, U.S.A., were analyzed for nine PFCs including PFOS and PFOA. The influence of the age of mothers and infants on the concentrations of PFCs in the milk was investigated. The daily intake of PFCs through the ingestion of human milk was estimated to enable potential risk characterization in breast-fed infants.

Materials and Methods

Samples. Forty-five human milk samples were obtained between June and November 2004 from women residing in various towns in Massachusetts, U.S.A. Institutional Review Board approval was obtained for the sampling of human specimens for this study. Information including the mother's age, the baby's age at the sampling time, and the previous

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TABLE 1. Concentrations (pg/mL) of PFCs in Human Milk from Massachusetts, U.S.A. (*n* = 45)^a

	PFOS	PFOA	PFHxS	PFNA	PFHpA	PFDA	PFUnDA	PFDoDA	PFBS
mean	131	43.8	14.5	7.26					
median	106	36.1	12.1	6.97					
range	<32.0–617	<30.1–161	<12.0–63.8	<5.20–18.4	<10.0–23.4	<7.72–11.1	<4.99–8.84	<4.40–9.74	<10.0–19.8
no. of samples above LOQ	43	40	23	29	3	4	3	1	1

^a Mean and medians were not calculated for those compounds with more than 60% of the samples had values below LOQ.

nursing history was obtained from 42 participants. The average (range) ages of the mothers and babies at the time of sampling of milk were 33 (22–43) years and 6 (<1–17) months, respectively. Eight women had previously nursed one or more babies within the past 4 years, and 34 were nursing for the first time. Women were asked to pump all of the milk in one breast, and the milk (30–150 mL) was immediately transferred to a sterile glass bottle and transported to the laboratory. After dilution to 1:1 with phosphate-buffered saline, the milk was centrifuged at 100g for 15 min to remove cells (breast epithelial, immune system cells, and foam cells) in the milk. Analysis of selected milk samples (*n* = 4) before and after centrifugation yielded similar PFC concentrations, and therefore the effect of centrifugation on the concentrations of PFCs is negligible. All of the fat and two-thirds of the supernatant (skimmed milk) were then transferred to a clean amber-colored glass jar and stored at –20 °C until the analysis.

Sample Extraction. A solid-phase extraction method described by Kuklenyik et al. (26) was used, with some modifications, for the extraction of PFCs in milk. A total of 10 mL of human milk was mixed with 1 ng each of a mixture of internal standards (including ¹³C₄–PFOS, ¹³C₄–PFOA, ¹³C₂–PFNA, and ¹³C₂–PFDA) and was then sonicated with 14 mL of formic acid (90.2%) for 1 h in a 50-mL polypropylene (PP) tube. The milk samples were extracted using Oasis weak anion exchange cartridges (WAX; 6 cc, 150 mg; Waters, Milford, MA). The cartridges were preconditioned by passing 3 mL of 0.1% NH₄OH in methanol, followed by 6 mL of methanol and 6 mL of Milli-Q water, at a rate of 1 drop/s. The samples were then loaded onto the preconditioned cartridge at a rate of 1 drop/s. The cartridges were prevented from drying during the preconditioning and sample-loading steps. The cartridges were rinsed with 6 mL of Milli-Q water and 6 mL of 25 mM sodium acetate/acetic acid buffer (pH 4) immediately after passage of the sample and then cleaned by passing 6 mL of 40% methanol in Milli-Q water. The cartridges were dried under vacuum (–70 kPa) for 2 min to remove the residual water and were then eluted with 6 mL of 0.1% NH₄OH in methanol at a rate of 1 drop/s. The eluate was concentrated to 200 μL under a gentle stream of nitrogen and then transferred into a PP vial.

Instrumental Analysis. PFCs in human milk were identified and quantified with an Agilent 1100 series high-performance liquid chromatography coupled with an Applied Biosystems API 2000 electrospray triple-quadrupole mass spectrometer (ESI-MS/MS) (27). A total of 10 μL of the extract was injected onto a 100 × 2.1 mm Betasil C18 column (5 μm; Thermo Electron Corp., Bellefonte, PA) with a Javelin guard column (20 × 2.1 mm). The mobile phase was 2 mM ammonium acetate/methanol at a flow rate of 300 μL/min. The gradient started at 10% methanol, increased to 99% methanol at 10 min, and was held for 2 min before reverting back to 10% methanol. The API 2000 was operated in electrospray negative ionization mode, and the target compounds were determined by multiple reaction monitoring. Compound-specific MS/MS parameters and mass

transitions for each analyte are shown in the Supporting Information (Table S1).

Quality Assurance and Quality Control. Matrix-spike recoveries of individual PFCs through the analytical procedure were determined by the spiking of nine target compounds into bovine milk (4% fat content) as well as into human milk. PFCs were spiked at three levels of 40, 200, and 600 pg/mL into bovine milk (*n* = 5 for each spiking level) purchased from a local supermarket; they were spiked at the level of 200 pg/mL into human milk (*n* = 6). The samples were extracted and analyzed following the procedure described above. Recoveries of PFCs spiked into bovine milk ranged from 78 ± 12% (mean ± SD) to 99 ± 9% (Table S2 in the Supporting Information). Recoveries of PFCs spiked into human milk ranged from 80 ± 18% to 100 ± 8% for all PFCs, except for PFDoDA for which the recoveries were 62 ± 16%. Recoveries of the four ¹³C-labeled internal standards, which were spiked into all samples prior to extraction, ranged from 82 ± 11% to 99 ± 10% in bovine milk and from 74 ± 15% to 98 ± 26% in the human milk samples (Table S2 in the Supporting Information). Procedural blanks (*n* = 6) were prepared by substitution of 10 mL of Milli-Q water for the milk, followed by passage through the entire analytical procedure. The average contamination level in procedural blanks was 9.3, 9.0, and ≤2.3 pg/mL for PFOS, PFOA, and each of the other PFCs, respectively.

Quantification was performed using linear regressions (*r*² > 0.99 for all analytes) generated from an eight-point calibration standard prepared in methanol at concentrations ranging from 0.1 to 20 ng/mL. The matrix-related effect was insignificant for human milk samples, as we had confirmed from both human and bovine milk matrix-spike studies. Furthermore, the availability of labeled internal standards enabled an understanding of the matrix-related effects on a sample-by-sample basis. The limit of quantitation (LOQ) was determined as 3 times the highest concentration found in blanks. A value of a signal-to-noise ratio of <3 or a negative value after subtraction of the blank from each sample was treated as a nondetectable value. Reported concentrations in the human milk samples were minus the highest blank values and were not corrected for the recoveries of internal standards.

Statistical Analysis. Nondetectable concentrations were treated as zero, and concentrations detected, but which were below the LOQ, were assigned half the value of the LOQ for statistical analysis. Nonparametric statistical tests were applied to assess the statistical significance. The Mann-Whitney U test was applied to compare the PFC concentrations in the milk of mothers who were nursing for the first time with concentrations in the milk of mothers who had nursed previously (within 4 years). Spearman's correlation analysis was used to examine the relationship between the mother's age and PFC concentrations. Temporal changes of the PFC concentrations in milk were examined by simple regression.

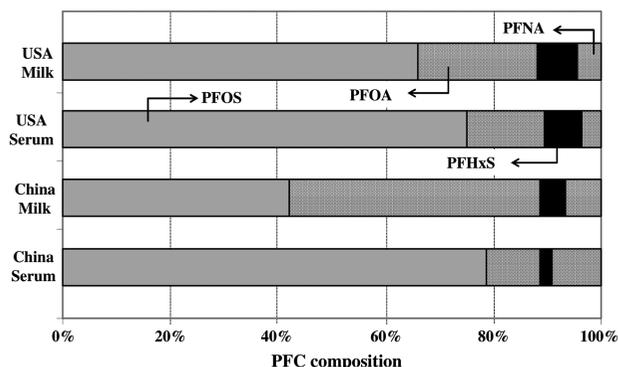


FIGURE 1. Composition (%) of PFOS, PFHxS, PFOA, and PFNA to the sum of total concentrations in milk and serum from female donors in the U.S.A. and Zhoushan, China. Data for milk and serum are not paired (4, 7, 12).

Results and Discussion

PFC Concentrations. PFOS was the predominant PFC found in the 45 human milk samples, followed by PFOA. PFOS and PFOA were detected in 100% and 98% of the human milk samples analyzed and were quantified in 96% and 89% of samples, at concentrations of 131 ± 103 pg/mL (mean \pm SD) and 43.8 ± 33.1 pg/mL, respectively (Table 1). PFHxS and PFNA were detected in 93% of the samples and were quantified in 51% and 64% of the samples, at mean concentrations of 14.5 ± 13.7 and 7.26 ± 4.70 pg/mL, respectively. PFHpA, PFDA, PFUnDA, and PFDoDA were found in only a few ($n \leq 4$) samples, at concentrations of <24 pg/mL.

In general, concentrations of PFOS and PFOA in human milk were 0.7% and 1.3%, respectively, of the concentrations reported for serum samples from the female U.S. blood donors from the National Health and Nutrition Examination Survey (NHANES) in 2003–2004 (7). Concentrations of PFOS in our milk samples were similar to concentrations reported in milk samples from China, Sweden, and Germany (12, 5, 28). Respective concentrations of PFOA and PFNA in human milk from the U.S.A. were 67% and 56%, respectively, lower than the concentrations reported for the Chinese milk samples. Long-chain PFCs such as PFDA and PFUnDA were detected in all of the milk samples from China but were rarely detected in our samples.

The human milk samples analyzed in our study have previously been tested for the presence of persistent organic pollutants such as DDT, polybrominated diphenyl ethers (PBDEs), and polycyclic musk fragrances (29, 30). Mean concentrations of Σ PFCs (sum of nine PFCs) were $1/8$ of the concentrations of PBDEs, $1/7$ of the concentrations of DDTs, and $1/40$ of the concentrations of polycyclic musks.

Composition of PFCs in Human Milk. The relative proportion of PFOS, PFHxS, PFOA, and PFNA concentrations in the milk samples is shown (Figure 1). PFOS and PFOA accounted for 67% and 22%, respectively, of the total PFC concentrations in the milk samples. In comparison with the profile of PFOS, PFHxS, PFOA, and PFNA in serum of the 2003–2004 U.S. female population (i.e., NHANES; 7) (Figure 1), the proportion of PFOA was enriched from 14% in serum to 22% in milk and the proportion of PFOS was attenuated from 75% in serum to 67% in milk. The proportion of PFHxS and PFNA did not change between milk and serum. However, a statistical comparison is not available because of the limited available information on serum samples. The enrichment of PFOA and attenuation of PFOS in human milk relative to that in serum has also been found in samples from China (Figure 1), although both milk and serum samples were from different individuals living in Zhoushan, China. A relatively higher proportion of PFOA in human milk than in serum

suggests a preferential partitioning of PFOA to milk. The differences in the partitioning of PFOS and PFOA between human milk and serum may be related to the concentrations of specific proteins to which these two PFCs bind and/or physicochemical properties such as water solubility. PFOA is reported to have a higher binding affinity for albumin at a 6:1 ratio (31), whereas PFOS binds to serum albumin at a 1:1 ratio (32).

PFC Concentrations and Nursing History. The PFC concentrations in the milk of mothers who were nursing for the first time ($n = 34$) were compared to concentrations in milk of those who had nursed previously ($n = 8$). We did not include an individual who had breastfed 10 years ago or two individuals with unknown information for this comparison. Concentrations of PFOA in the milk of mothers who had nursed previously were 49% lower than the concentrations in milk of those who were nursing for the first time, and this decrease was statistically significant ($p \leq 0.05$; Figure 2). Mean concentrations of PFOS, PFHxS, and PFNA in the milk of mothers who had nursed previously were 32%, 14%, and 30% lower than the concentrations in the milk of those who were nursing for the first time, although this difference was not statistically significant. In general, our finding suggests that PFOA and other PFCs are excreted in breast milk and that the concentrations of PFCs in milk decrease with a history of previous breastfeeding.

Temporal Changes in PFC Concentrations in Human Milk. In this study, the milk samples were collected from mothers who were at different time points of the lactation process. Based on the age of infants, obtained at the time of sampling (which was used as an indicator of the duration of nursing), milk samples of mothers who were nursing for the first time were selected for the evaluation of temporal changes in PFC concentrations during lactation. This comparison was restricted to those samples that were collected within the first 6 months of breastfeeding. Temporal changes in the concentrations of PFOS, PFOA, and Σ PFCs ($n = 25$) are shown (Figure 3). Concentrations of PFOS and Σ PFCs in milk increased during the first 6 months of breastfeeding, while the increase of PFOA concentrations in milk was not statistically significant. One sample collected at the 6 month of breastfeeding contained the highest concentration for PFOS and Σ PFCs. After removal of this outlier, the trend of the increase for Σ PFC concentrations remained marginally significant ($p = 0.07$). Our results suggest changes in the PFC concentrations in milk during the first 6 months of nursing.

A decline in the concentrations of dioxins, PCBs, and DDT in milk with the duration of breastfeeding has been reported (33, 34). Unlike lipophilic contaminants, PFCs bind to serum albumin (31). Changes in the composition of albumin/protein in breast milk can also contribute to changes in the PFC concentrations in milk. Albumin in human milk is provided by maternal circulation (35), and the albumin content of milk is thought to be stable during the period of lactation. Additionally, the increase of the PFC concentrations seen during the first 6 months of breastfeeding may be related to increased dietary exposures due to high energy demands and possibly changes in the consumption pattern of nursing mothers during breastfeeding. In one study, diet was shown to contribute to 61% of the total daily intake of PFCs (36). However, dietary habits of mothers were not obtained in this study. A limitation in our temporal comparison was that the data were obtained from samples collected from different mothers. Future studies of temporal changes in the PFC burden in breast milk collected from the same individuals over time during the lactation process will help in the identification of sources of PFC exposures in lactating mothers.

Correlation between the PFC Concentrations and the Mother's Age. Concentrations of PFOS, PFHxS, PFOA, and

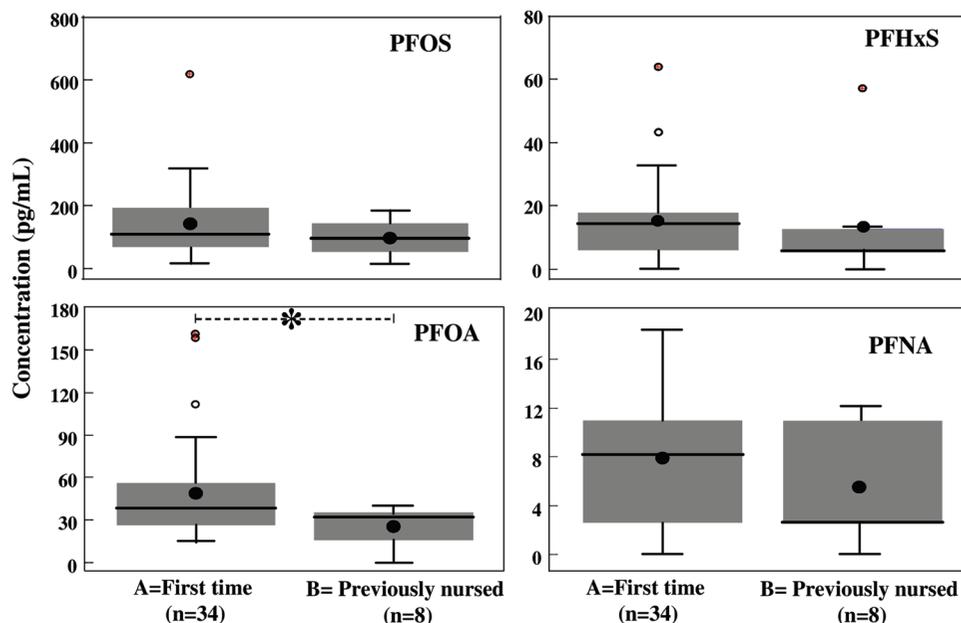


FIGURE 2. Box and Whisker plot of concentrations of PFOS, PFOA, PFHxS, and PFNA in the milk of mothers who were nursing for the first time (A) and those who had nursed previously (B). Black dots denote the mean, and longer black lines denote the median. The asterisk indicates a statistically significant ($p \leq 0.05$) difference between two groups of mothers.

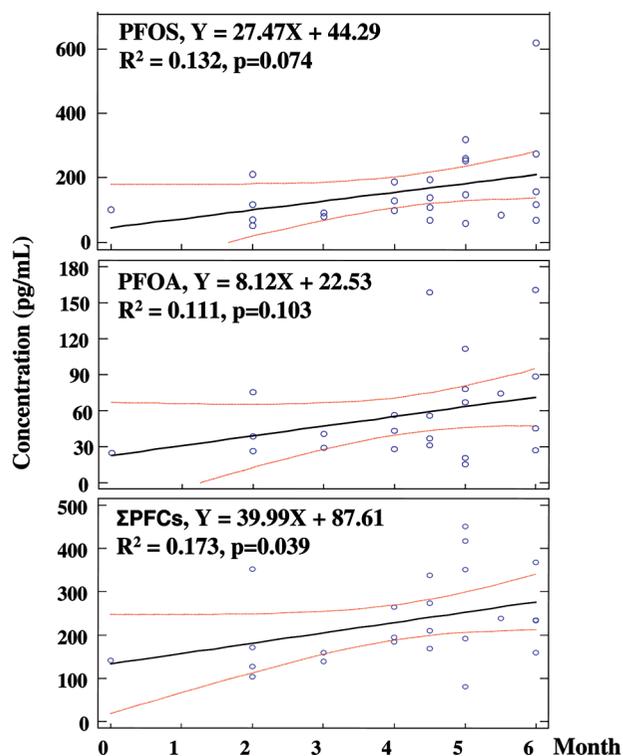


FIGURE 3. Temporal changes in the concentrations of PFOS, PFOA, and Σ PFCs in the first 6 months ($n = 25$) of breastfeeding from mothers who were nursing for the first time. Black solid lines are regression lines. Dashed lines are confidence intervals at the 95% level.

PFNA were significantly correlated with each other in human milk (Table S4 in the Supporting Information). These correlations are consistent with what was reported for serum samples from the U.S.A. (1, 7). The association among several PFCs in milk and in blood suggests exposures from common sources. A previous study found the lack of a relationship between PFHxS and PFOS in human milk from China (12); the discrepancy could suggest diverse PFC exposure patterns from one country to another. In our study, no correlation

was found between the concentrations of PFCs in human milk and the mother's age (Table S4 in the Supporting Information).

Daily Intake (DI) of PFCs through Breast Milk. DIs of PFOS, PFOA, PFHxS, and PFNA were calculated based on a generalized ingestion rate of milk by infants estimated at various ages and the measured PFC concentrations in milk. We also estimated the risk index (RI), based on the intake of PFCs by infants, by comparison with a recently reported tolerable DI (TDI). The DI was calculated as

$$DI_i \text{ (ng/kg of body weight/day)} = \frac{\text{milk consumption (mL/day)} \times \text{PFC concentration}_i \text{ (ng/mL)}}{\text{infant body weight (kg)}}$$

An infant's daily consumption of breast milk can vary depending on the child's age and its solid food intake. We calculated the DI by using an average daily milk consumption rate of 800 mL/day for an infant at 0–6 months old, 500 mL/day at 6–9 months, and 400 mL/day after 9 months (37). The body weight of infants was not recorded at the time of the sampling in our study; thus, the values were estimated using the U.S. infant growth chart, reported by the Centers for Disease Control and Prevention (38). RIs for PFOS and PFOA were calculated as $RI = DI_i/TDI_i$.

The TDIs for PFOS and PFOA were 300 and 3000 ng/kg of bw/day, respectively (35, 36). Those TDI values were recommendations of the U.K. Food Standards Agency Committee on Toxicology and were based on a thorough review of currently available toxicity studies (39, 40).

The respective average and the highest DIs of PFOS, PFOA, and Σ PFCs via breast milk were 14.7 and 65.4, 1.7 and 8.0, and 23.5 and 87.1 ng/kg of bw/day. The average intake of Σ PFCs in infants >6 months was $1/5$ of that in infants <6 months. In general, the increase in body weight and the decrease in milk consumption at ages after 6 months could explain the decrease in the calculated intake of PFCs after 6 months.

The calculated RI for PFOS was less than 0.22 for all of the infants, with an average value of 0.05. The calculated RI for PFOA was 40 times lower than the RI for PFOS. Earlier studies have reported RI values exceeding unity in 2 of 12 Swedish infants (5) and 1 of 19 Chinese infants (12) by using

a 10-fold lower reference dose (than that of the U.K. Food Standard Agency). The USEPA has not yet established the reference doses for PFOS or PFOA. The DI values of PFOS, PFOA, PFHxS, PFNA, and Σ PFCs and the RI values of PFOS and PFOA for infants at various ages are given in the Supporting Information (Table S3).

The RI based on the available TDI value should be interpreted with caution. Because of the lack of a consensus TDI value for PFCs, our assessment of risk from exposure through breastfeeding is not directly comparable to the reference values reported in previous toxicological studies. The extrapolation of laboratory animal data to derive TDI for infant risk assessment involves several uncertainties. Furthermore, the risks associated with PFC exposures via breastfeeding should be compared with the benefits of breastfeeding. It is generally known that breastfeeding is highly recommended for providing adequate nutrients, enhancing the immune system, and defending against infections in children.

Studies in rodents demonstrated that gestational exposure provides the strongest contribution to neonatal and developmental effects while lactational exposure provides supplementary contributions (19, 20). Developmental effects observed in the offspring of two strains of wild-type mice (13, 41) were not observed in offspring of Peroxisome Proliferator Activated Receptor Alpha (PPAR α) knockout mice when given PFOA through pregnancy (41). Because of quantitative and qualitative differences in the nuclear receptor PPAR α between humans and rodents, humans may be refractory to certain PPAR α -mediated effects as compared to rodents (42–44). This suggests that it would be important to develop a better understanding of the relevance to humans of the PPAR α -related developmental effects of PFCs.

In summary, this is the first study of the occurrence of PFCs in human milk from the U.S.A. PFC concentrations in the milk of American mothers from Massachusetts are $1/_{100}$ of those in U.S. female serum. Our results are comparable to the concentrations of PFCs reported previously in the milk of Chinese, Swedish, and German mothers. The relative proportions of PFOS and PFOA in milk were different from those found in blood, suggesting differences in partitioning of these two PFCs between milk and blood. PFC concentrations in human milk decreased with a history of previous nursing. DIs of PFOS and PFOA calculated for the milk samples did not exceed the recommended TDIs for risk estimation. In addition to breast milk, other potential exposure sources, such as indoor air, dust, infant formula, and solid food, should be investigated for a better understanding of exposure of PFCs in children.

Acknowledgments

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Supporting Information Available

Chemicals and standards and Tables S1–S3. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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