Exposure of Perfluorinated Chemicals through Lactation: Levels of Matched Human Milk and Serum and a Temporal Trend, 1996–2004, in Sweden

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BACKGROUND: Only limited data exist on lactation as an exposure source of persistent perfluorinated chemicals (PFCs) for children.

OBJECTIVES: We studied occurrence and levels of PFCs in human milk in relation to maternal serum together with the temporal trend in milk levels between 1996 and 2004 in Sweden. Matched, individual human milk and serum samples from 12 primiparous women in Sweden were analyzed together with composite milk samples (25–90 women/year) from 1996 to 2004.

RESULTS: Eight PFCs were detected in the serum samples, and five of them were also above the detection limits in the milk samples. Perfluorooctanesulfonate (PFOS) and perfluorohexane-sulfonate (PFHxS) were detected in all milk samples at mean concentrations of 0.201 ng/mL and 0.085 ng/mL, respectively. Perfluorooctanesulfonamide (PFOSA), perfluorooctanoic acid (PFOA), and perfluorononanoic acid (PFNA) were detected less frequently.

DISCUSSION: The total PFC concentration in maternal serum was 32 ng/mL, and the corresponding milk concentration was 0.34 ng/mL. The PFOA milk level was on average 1% of the corresponding serum level. There was a strong association between increasing serum concentration and increasing milk concentration for PFOS ($r^2 = 0.7$) and PFHxS ($r^2 = 0.8$). PFOS and PFHxS levels in composite milk samples were relatively unchanged between 1996 and 2004, with a total variation of 20 and 32% coefficient of variation, respectively.

CONCLUSION: The calculated total amount of PFCs transferred by lactation to a breast-fed infant in this study was approximately 200 ng/day. Lactation is a considerable source of exposure for infants, and reference concentrations for hazard assessments are needed.

KEY WORDS: human exposure, LC-MS, PFC, PFOA, PFOS, Environ Health Perspect 115:226–230 (2007). doi:10.1289/ehp.9491 available via http://dx.doi.org/ [Online 28 November 2006]
women were collected each year between 1996 and 2004 and pooled into an annual composite sample. Donors originated from four regions in Sweden (Uppsala, Lund, Göteborg, Lycksele). All samples were from primiparous women and were collected in glass bottles during the third week after delivery and stored in plastic containers at −20°C. A summary of the sample information, including age of donors and number of pooled samples, is given in Table 1. The local ethics committee approved the design of this study, and informed consent was obtained from the study participants.

**Chemicals.** We purchased ammonium acetate (>99%, for high-performance liquid chromatography (HPLC)) from Fluka (Steinheim, Germany), formic acid (98–100%) from Scharlau (Barcelona, Spain), and methanol (HPLC) from Labscan (Dublin, Ireland). All water used was laboratory-methanol (HPLC) from Labscan (Dublin, Ireland) and distilled water. We used perfluorobutanesulfonate (PFBuS) were purchased from ABCR (Karlsruhe, Germany). 1H,1H,2H,2H-PFOS (THPFOS, purity unknown), and PFHxS (98%) were purchased from Interchim (Montluçon, France). 13C4-Labeled PFOA, 13C4-labeled PFOS, and 13C5-labeled PFNA were from Wellington Laboratories (Guelph, Ontario, Canada).

**Extraction.** The serum and milk samples were extracted using weak anion exchange, solid-phase extraction (Waters Oasis WAX, Milford, MA, USA) based on the method by Taniyasu and colleagues (Taniyasu et al. 2005). Internal standards (13C4-PFOA and 13C5-PFOS) and 2 mL formic acid/water (1:1) were added to 1 mL milk and 0.5 mL serum. The solution was sonicated for 15 min and centrifuged at 10,000 × g for 30 min. The supernatant was extracted and the perfluorinated compounds were eluted with 1 mL 2% ammonium hydroxide in methanol, after washing the column with 2 mL sodium acetate buffer solution, pH 4, and 2 mL 40% methanol in water. Sodium acetate buffer was not used for the serum samples. The final volume for the serum extracts was 500 μL. Milk extracts were further evaporated to 30 μL, and 20 μL 2-mM ammonium acetate in water was added. Finally, filtration through a Microsep YM-3 centrifugal filter (Millipore, Billerica, MA, USA) was conducted at 14,000 × g for 30 min. Performance standards, 13C4-PFNA and 7H-PFPHaP were added to both milk and serum extracts immediately before injection.

**Analysis.** We performed the analysis using an Agilent 1100 HPLC system coupled to an Agilent 1100 mass spectrometric detector (Agilent, Waldbronn, Germany) with an atmospheric electrospray interface operating in negative ion mode. Separation was performed on a Discovery HS C18 (50 mm length, 2.1 mm inner diameter, 3 μm particles, 120 Å pore size) column with a guard column of the same material (20 mm length, 2.1 mm inner diameter, 3 μm particles, 120 Å pore size) (Supelco, Bellefonte PA, USA). Both columns were kept at 40°C. An extra guard column (HyperCarb graphitic carbon, 4 mm length, 10 mm inner diameter, 5 μm particle size; Thermo Hypersil-Keystone, Bellefonte PA, USA) was inserted between the pump and injector to remove any fluorochromes originating from the HPLC system. Injection volume was 10 μL and the flow rate was set to 100 μL/min. The mobile phases consisted of 2 mM ammonium acetate in methanol and 2 mM ammonium acetate in water. HPLC gradient and MS settings have been described in detail elsewhere (Karmas et al. 2005).

**Quality assurance.** Quantification was performed using the internal standard method with standards dissolved in 35% methanol in water. We used 13C4-PFOS as internal standard for the sulfonates and PFOSA, and 13C4-PFOA for the carboxylates. We used 13C4-PFNA and 7H-PFPHaP to monitor the recovery of the internal standards. The recovery was on average 67%, within 50–130% for 78% of all milk samples and within 84–97% for all serum samples. An overview of the method performance is given in Table 2. Recoveries were evaluated by three or five replicate fortifications to a low-contaminated serum sample and a breast milk sample containing PFCs below the detection limit. Average recoveries were >50% for all compounds except for PFOSA, PFDA, PFUnDa, and PFDoDa (34–47%) and the coefficient of variation (CV) was 2–27% for multiple determinations. Containers used for storage of milk and serum samples were extracted with methanol and did not show traces of the target compounds. Procedural blank trace levels were detected for PFOA, PFOS, and PFNA (Table 3). In the case of blank levels, the mean blank signal plus 3 SDs of multiple blank injections were subtracted from the calculated concentrations in the samples. A blank corrected concentration was reported provided that the blank level was ≤50% of the uncorrected concentration. Detection limits for serum and breast milk were 0.1–1.1 ng/mL and 0.005–0.209 ng/mL, respectively. The selectivity of the mass
spectrumometry (MS) analysis was verified by triple quadrupole mass spectrometry (MS/MS) analysis. All breast milk samples (1 mL) were extracted in duplicates. The first sample extract was evaporated to 50 μL and injected on the LC-MS system (10 μL). The second extract was kept at 500 μL, of which 200 μL was injected on a column-switching LC system connected to a triple quadrupole MS system (Micromass QuattroII, Altrincham, UK). Further quality assurance was taken by successful participation in the first interlaboratory study on PFCs (Van Leeuwen et al. 2006).

Results

A summary of the results of 12 individual milked milk and serum samples is given in Table 3. Highest mean serum concentration was obtained for PFOS (20.7 ng/mL) followed by PFHxS (4.7 ng/mL), PFOA (3.8 ng/mL), PFNA (0.80 ng/mL), PFDA (0.53 ng/mL), PFUnDA (0.40 ng/mL), and PFOSA (0.24 ng/mL). PFDS was detected in only one serum sample (0.33 ng/mL). Of the eight PFCs found in the serum samples, five were detected in the matched milk samples at the current detection limits. PFOS and PFHxS were detected in all milk samples at mean concentrations of 0.201 ng/mL and 0.085 ng/mL, respectively. PFOSA was detected in eight milk samples with a mean concentration of 0.013 ng/mL, and PFNA was detected in two milk samples (0.020 and 0.014 ng/mL). Similar PFC occurrence and levels were found in the milk composite samples collected during the 8 years between 1996 and 2004 (Table 4).

Milk levels were lower compared with the matched serum levels on a volume basis (nanograms per milliliter). The mean ratio of milk to serum (M:S) concentration of PFOS available for excretion to milk is therefore about five times higher than the measured concentration in serum. The M:S ratio for PFOSA should therefore be close to that of PFOS if nearly all PFOSA were distributed to serum. The serum and milk pattern suggests that PFHxS is excreted to milk in a higher degree than PFOS and PFOSA. A preferential excretion of shorter, less hydrophobic PFCs is a possible explanation of the observed pattern, but could not be concluded in the present study because of the limited number of matched milk and serum samples.

The presented linear relationship between serum and milk levels suggests a partitioning process, which can be predicted from the PFC blood concentration on a volume basis. The steeper slope of PFHxS demonstrates the higher partition than PFOS to milk (Figure 1). The association between milk and serum concentrations could not be seen for PFOSA (r² < 0.1). The PFOSA ratio between milk and serum can be influenced by several parameters. First, it has been suggested that PFOSA can degrade to PFOA in biologic systems (Tomiy et al. 2003), which might affect the ratio between milk and serum. Second, PFOSA is partly lost during the separation of the red blood cells, which makes serum a poor matrix for determining PFOSA blood concentrations. Finally, relatively more milk and serum samples had levels of PFOSA close to the detection limit.

Discussion

The serum levels in the present study are similar to or lower than the levels found in a previous study on 17 Swedish human plasma samples collected in 1998–2000 from men and women (Kärkänen et al. 2004). The Swedish PFOS and PFOA blood levels are similar to levels in, for example, Canada, Australia, and some less-industrialized regions in Japan, but somewhat lower than reported blood levels in the United States (Harada et al. 2004; Karrman et al. 2006a; Kubwabo et al. 2004; Olsen et al. 2003).

Only one study originating from China has previously reported levels of several PFCs in human milk. The Swedish levels are comparable to human milk from China except for those for PFOSA, which was not included in the Chinese study (So et al. 2006). In addition to detected PFCs in the present study, PFHxS, PFDA, and PFUnDA were also found in human milk from China.

PFOSA was frequently detected in the milk samples, unlike PFNA, PFDA and PFUnDA, even though the latter were detected at higher concentrations in the serum samples. This is most likely caused by the fact that PFOSA concentrations in plasma have been found to be only about 20% of the whole blood concentration on a volume basis (Kärkänen et al. 2006b). The total blood concentration of PFOSA available for excretion to milk was obtained for PFOS (20.7 ng/mL) followed by PFHxS (4.7 ng/mL), PFOA (3.8 ng/mL), PFNA (0.80 ng/mL), PFDA (0.53 ng/mL), PFUnDA (0.40 ng/mL), and PFOSA (0.24 ng/mL). PFDS was detected in only one serum sample (0.33 ng/mL). Of the eight PFCs found in the serum samples, five were detected in the matched milk samples at the current detection limits. PFOS and PFHxS were detected in all milk samples at mean concentrations of 0.201 ng/mL and 0.085 ng/mL, respectively. PFOSA was detected in eight milk samples with a mean concentration of 0.013 ng/mL, and PFNA was detected in two milk samples (0.020 and 0.014 ng/mL). Similar PFC occurrence and levels were found in the milk composite samples collected during the 8 years between 1996 and 2004 (Table 4).

Milk levels were lower compared with the matched serum levels on a volume basis (nanograms per milliliter). The mean ratio between milk and serum (M:S) concentration of PFOS was 0.011 for PFOS, 0.021 for PFHxS, and 0.071 for PFOSA (Table 3). The M:S ratios for PFOA and PFNA are uncertain because only one and two milk samples, respectively, contained levels above the detection limit. Simple regression analysis and the Spearman’s correlation test of the matched serum and milk samples show a significant association (r² = 0.7–0.8, p < 0.05) between levels of PFOS and PFHxS in serum and milk (Figure 1). The percentage contribution of each of PFHxS, PFOA, PFOSA, and PFNA to the total concentration in serum and milk is given in Figure 2.

The limits of detection (LOD) in human milk were between 0.005 and 0.010 ng/mL, except for PFHxS and PFHpA, which were an order of magnitude higher (0.1 ng/mL) (Table 2). A relatively high blank level was obtained for PFOA (0.209 ng/mL). PFOA is reported in only one milk sample as a consequence of the high blank level and the quantification criteria.
For more fat-soluble, persistent organohalogen compounds, the levels in blood and milk are about the same when calculated on a fat basis and with a steady state assumption. On a volume basis, the ratio of lipophilic compounds in whole blood and milk is approximately 1:10, because of the higher lipid content in milk than in blood (Jensen and Slorach 1991). The lactational transfer of PFCs may be more similar to that of heavy metals. For example, the concentration of lead in milk has been found to be 5–10 times lower than that in blood (Jensen and Slorach 1991). Perfluorinated acids are generally believed to bind to serum albumin (Jones et al. 2003). It has been demonstrated that serum albumin in plasma has a large binding capacity for PFOA (6–9 binding sites per molecule and millimolar concentration in plasma) and the free fraction of PFOA in plasma was estimated to be <5% (Han et al. 2003). The reason for the relatively higher PFC concentration in human serum than in milk is unknown.

Excretion of PFCs into milk may be accomplished by two ways that have been identified as transport mechanisms for chemical contaminants: binding to milk protein (protein content ~ 1 g/100 mL milk) or to the surface of fat (fat content ~ 4 g/100 mL milk) (Jensen and Slorach 1991). The fat content in milk fluctuates but does not vary significantly during the course of lactation, unlike the total protein content, which was shown to decrease rapidly during the first month of lactation. The serum albumin content of milk was, however, stable during a 6.5-month period of lactation (Lönnerdal et al. 1976). Assessing the amount of PFCs transferred and adsorbed by an infant during the course of lactation involves several assumptions—for instance, the variation of PFC concentration in milk with time and the uptake efficiency of PFCs from milk by the infant. The total mean PFC concentration of all detected compounds in the present study was 32 ng/mL in serum and 0.34 ng/mL in milk. Hypothetically, a lactation of 600 mL/day and 100% uptake would produce an exposure burden for an infant (and maternal excretion) of 203 ng PFCs per day, corresponding to 34 μg PFCs after 6 months, given a constant PFC concentration in milk during 6 months. A risk assessment is unfeasible because of the lack of human hazard assessment of each of the detected PFCs and lack of reference intake levels or concentrations to compare with. However, So et al. (2006) used a reference dose (25 ng/kg/day) for PFOS estimated by the Environmental Working Group, based on the end point of increase in mammary fibroadenomas in a rat chronic toxicity study (Thayer and Houlihan 2002). Using the same assumptions (milk consumption 600 g/day, body weight 7 kg), two milk samples with the highest PFOS concentration in our study (0.465 and 0.337 ng/mL) exceed the reference dose and would therefore constitute a risk to the infant. However, there are several uncertainties that need clarification before any conclusions can be made.

This study contributes to PFC exposure risk assessments for infants, and the evaluation of lactation as an exposure pathway as well as a route for maternal excretion. Several studies indicate that females have lower blood concentrations of several PFCs than do males (Calafat et al. 2006a; Karrman et al. 2006a; Olsen et al. 2003). Elimination through lactation could be one explanation for this observation. However, a sex difference was observed also for 2- to 12-year-old children in the United States (Olsen et al. 2004).

PFOS and PFHxS were detected in composite milk samples collected each year between 1996 and 2003–2004 from four different regions in Sweden (Table 4). The variation of PFOS and PFHxS in the composite samples is remarkably small (a total variation of 20% and 32% CV, respectively), indicating that milk levels of PFOS and PFHxS have been constant in the last 8 years. Consequently, no clear temporal trend could be distinguished (Figure 3). However, the samples from 2001, 2003, and 2003–2004 were from regions different from the rest of the samples. Possible regional differences in human PFC levels in Sweden remain yet to be established. PFOS has been present in the Swedish environment at least since 1968, and the levels increased dramatically up to 1997 in guillemot eggs (Holmström et al. 2004). PFOS-related products were imported in Sweden until 2002 and will probably be used for a long period of time [KEMI (Swedish Chemicals Agency) 2004]. The global production of perfluorooctanesulfonamide fluoride started to decrease in 2001 after the phase-out decision by the major producer 3M (3M 2000). A possible effect of the action taken by governments and producers in terms of declining environmental and human concentrations needs to be monitored for several years to come because of the persistence of PFCs [PFOS half-life is approximately 5 years in humans (Olsen et al. 2005a)].
The relatively low levels of PFCs present in the human milk samples challenged the analysis. By reducing the volume of the milk sample extracts by a factor of 100, required detection limits were achieved. As a consequence, traces in the procedural blanks were seen for several of the compounds monitored (Table 2). A confident quantification of PFOA in the milk samples was hampered by a high procedural blank contamination. PFOA is usually the second highest PFC found in human milk, except in Korea where PFOA levels have been reported to exceed those of PFOS (Kannan et al. 2004). PFOA contributed up to 36% of the total PFC content in human milk from China (So et al. 2006). The selectivity of the single quadrupole MS method was successfully verified with triple quadrupole MS/MS analysis. Qualitative comparison indicated that MS/MS analysis demonstrated on average 50% higher concentrations compared with the single quadrupole MS analysis. However, different preconcentration methods were used for the different instruments, and the differences seen between the methods can be multifactorial.

Conclusions

The PFC level in human milk are about 1% of the corresponding level in serum. There is an indication that elimination of PFCs through lactation is compound-dependent and partitioning of PFCs into milk seems to relate to the concentration in maternal blood. A trend of PFC concentrations in milk between 1996 and 2004 could not be observed in the present study. Lactation is a considerable source of PFC exposure for infants. The present study indicates that approximately 200 ng PFCs per day may be transferred from a lactating mother to the infant. Reference concentrations as well as information on the infants’ uptake and excretion of PFCs during the lactation period are urgently needed for a full risk assessment. The ubiquitous presence and levels of PFOS in human milk justifies further monitoring of this class of contaminants in human milk worldwide.

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