# QUANTITATIVE ANALYSIS OF SERUM AND BREAST MILK FOR PERFLUOROCHEMICAL SURFACTANTS

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***Introduction***

In 1999, the US Environmental Protection Agency (EPA) began investigating perfluorooctane sulfonate (PFOS), a widely used perfluorochemical, after scientific data provided by 3M indicated that PFOS was persistent and bioaccumulative and had been detected in the blood of the nonoccupationally exposed general population and in wildlife.[1-4] Furthermore, data from toxicological studies indicated that PFOS was capable of producing systemic toxicity, including neonatal toxicity, which was related to body burden.[5-7] PFOS has been used as a surfactant in numerous applications and could have been generated by the environmental or metabolic degradation of perfluorooctanesulfonyl fluoride or substituted perfluorooctanesulfonamide chemistries used as surfactants, insecticides, and soil, stain, grease, or water protection for paper, carpet, upholstery, and textile. In May 2000, 3M, the sole manufacturer of PFOS in the United States and the principal manufacturer worldwide, announced it was discontinuing its perfluorooctanyl chemistries, including the production of PFOS. In June 2000, EPA identified possible health concerns related to perfluorooctanoic acid (PFOA) and fluorinated telomers. PFOA is used primarily to produce its salts, which are used in the production of fluoropolymers and fluoroelastomers. The major fluoropolymers manufactured using PFOA salts are polytetrafluoroethylene (PTFE) and polyvinylidine fluoride (PVDF). PTFE is used in soil, stain, grease, and water resistant coatings on textiles and carpet; in nonstick coatings on cookware; in personal care products; and in several industries (e.g., automotive, mechanical, aerospace, chemical, electrical, medical, and building and construction). PVDF is used primarily in electrical/electronics, building and construction, and chemical processing industries.

In April 2003, EPA released a preliminary risk assessment on PFOA, which indicated potential human exposure to low levels of PFOA in the United States.[8] Based on animal data,[9-10] risk of developmental and other adverse effects associated with human exposures to perflurorinated chemicals could exist, but more data regarding human exposure to these chemicals and adverse health effects are needed.

Understanding human exposure to perfluorochemicals requires information about the concentration of these toxicants in the nonoccupationally exposed population and on the pharmacokinetic data of these compounds. To help obtain the needed information, we developed a rapid and sensitive method for measuring 15 perfluorinated chemicals, including PFOS and PFOA, in breast milk and serum using high-performance liquid chromatography-tandem mass

spectrometry (HPLC-MS/MS). We used this method to measure the levels of perfluorochemicals in PFOS-dosed rats and in people nonoccupationally exposed to these compounds.

***Methods and Materials***

The analytical method used for the analysis of breast milk and serum samples is an adaptation of a published method.[11] The perfluorochemicals were extracted from the biological matrix (i.e., serum or breast milk) using an automated solid phase extraction (SPE) procedure, separated from other extracted components in the SPE eluate by reverse phase HPLC, and detected by negative ion TurboIonSpray® ionization-tandem mass spectrometry (HPLC-MS/MS) using a multiple reaction monitoring experiment. The TurboIonSpray® ionization source is a variant of the electrospray source and converts liquid-phase ions into gas-phase ions. Perfluorooctane sulfonamide (PFOSA), N-methyl-perfluorooctane sulfonamide (Me-PFOSA), N-ethyl-perfluorooctane sulfonamide (Et-PFOSA), 2-(N-ethyl-perfluorooctane sulfonamido) ethyl alchohol (Et-PFOSA-EtOH), 2-(N-ethyl-perfluorooctane sulfonamido) acetic acid (Et-PFOSA-AcOH), perfluorohexane sulfonic acid (PFHxS), perfluorooctane sulfonic acid (PFOS), and tetrahydro-perfluorooctane sulfonic acid (THPFOS) were kindly provided by 3M Company (Saint Paul, MN). Perfluoropentanoic acid (PFPeA), perfluorohexanoic acid (PFHxA), perfluoroheptanoic acid (PFHpA), perfluorooctanoic acid (PFOA), perfluorononanoic acid (PFNA), perfluorodecanoic acid (PFDeA), perfluoroundecanoic acid (PFUA), and perfluorododecanoic acid (PFDoA) were purchased from Oakwood Products (West Columbia, SC). THPFOS was used as an internal standard. The breast milk or serum samples (1 mL) were spiked with internal standard (10 ng/mL), diluted with 0.1M formic acid (3 mL), and automatically extracted on a Zymark RapidTrace Station (Zymark Corporation, Hopkinton, MA) using 60 mg/3 mL Oasis-HLB columns (Waters Corporation, Milford, MA). After washing the columns with formic acid, 50:50 MeOH:formic acid, and 1% aqueous ammonium hydroxide (only the serum samples) to eliminate interfering matrix components, the perfluorochemicals were eluted from the column with 1 mL of 1% ammonium hydroxide in acetonitrile. The SPE eluate was evaporated to dryness using a Turbovap (Zymark Corporation, Hopkinton, MA), and the residue was resuspended in a methanol/water solution containing acetic acid (200 µL) and transferred to a polypropylene autosampler vial for HPLC-MS/MS analysis. Forty microliters of the reconstituted SPE extract was injected into an Agilent 1100 high performance liquid chromatograph (Agilent Technologies, Wilmington, DE) coupled to an 4000 triple quadrupole mass spectrometer (Applied Biosystems, Foster City, CA). The analytes were separated from the other SPE extract components on a Betasil phenyl column (5 µm, 50 mm × 2 mm, Keystone, Bellefonte, PA) using a water/methanol solvent gradient. The HPLC analysis run was 10 minutes. During the first 3 minutes following the injection, a switching valve in the 1100 HPLC system directed the postcolumn flow to waste; then the automatic switching valve directed the postcolumn flow to the mass spectrometer where the analytes were detected and quantified using negative ion TurboIonSpray-MS/MS. During the analysis, specific precursor and product ion combinations for each eluting analyte were monitored.

All of the samples, blanks, standards, and quality control materials were processed identically. We used the peak area ratio of each analyte to THPFOS (i.e., response factor, [RF]) for quantification. Eight standard concentrations (0.1, 0.5, 1.0, 2.5, 5.0, 10.0, 25.0, and 50.0 ng/mL), which encompassed the entire linear range of the method, were used to construct calibration curves of RF versus standard amount for each analyte. The calibration curve weighted by the reciprocal of the standard amount was used for quantification. The limits of detection (LODs) were in the low nanogram per milliliter range (~ 0.5 ng/mL) in 1 mL of serum or breast milk.

***Results and Discussion***

Perfluorochemicals, including PFOS and PFOA, distribute primarily in the liver and, to a lesser extent, in the blood. Studies on laboratory rats indicate that PFOS tends to bind to proteins and does not bioconcentrate in the lipid fraction of the tissues.[6] Protein-bound chemicals are less likely than lipid-soluble chemicals to passively diffuse into breast milk.[12] There are no reports of PFOS in breast milk; therefore, the degree of partitioning of PFOS between blood and breast milk is not known. However, indirect evidence from animal cross-fostering studies suggests that lactational exposure to PFOS may occur.[6] Because of the potential health impact of perfluorochemicals to nursing mothers and their children, it is important to determine whether PFOS and other perfluorinated chemicals partition into breast milk and if so, to monitor breast milk for these contaminants.

Several years ago, 3M scientists conducted a cross-fostering study using Sprague-Dawley rats to determine whether the adverse effects observed in pups resulted from in utero or lactational exposure to PFOS.[6] Some of the rats were administered PFOS by gavage, others (used as controls) were not. Samples of blood, milk, and liver from selected rats and pups were collected and analyzed for PFOS, but only the measurements of PFOS in serum were performed. Samples from other tissues were stored.[6] Using our method, we analyzed some of the stored breast milk and serum samples from the 3M cross-fostering study.[6] We found that the PFOS serum concentrations in the two treated animals (Table 1) were significantly higher than in the eight control rats (80 ng/mL ± 106 ng/mL). Similarly, we measured significant amounts of PFOS (ppm levels) in the breast milk samples from two rats, the PFOS-dosed dams (Table 1); no PFOS was detected in the breast milk samples of the control rats. The concentrations of PFOS in the breast milk of the two treated dams were lower than the corresponding serum concentrations. These data suggest that PFOS can transfer to breast milk and therefore confirm that lactational exposure to PFOS may occur.

Table 1. Levels of PFOS paired serum-breast milk samples from dosed rats

| | PFOS in serum (µg/mL)[a] | PFOS in serum (µg/mL) | PFOS in breast milk (µg/mL) |
|---|---|---|---|
| Rat 1 | 218 | 196 | 100 |
| Rat 2 | 97.5 | 116 | 13.7 |

[a] Measurements performed by 3M

We also applied our SPE-HPLC-MS/MS method to measure the levels of perfluorochemicals in two pooled serum samples from non-occupationally exposed people. We found several perfluorochemicals at concentrations above the LOD. PFOS was found at the highest levels (25.3 ng/mL ± 0.1 ng/mL), followed by PFOA (7.9 ng/mL ± 6.7 ng/mL), Et-PFOSA-AcOH (4.0 ng/mL ± 0.2 ng/mL), PFOSA (2.2 ng/mL ± 0.4 ng/mL), PFHxS (1.9 ng/mL ± 0.5 ng/mL), PFNA (1.3 ng/mL ± 0.1 ng/mL), and PFUA (1.6 ng/mL ± 0.3 ng/mL).

In summary, we have developed a fast and sensitive method to measure 15 perfluorochemicals in serum and breast milk. We found PFOS in the breast milk of rats dosed with PFOS. These data indicate that, even though PFOS is tightly bound to proteins in the plasma, PFOS can be incorporated into the breast milk and therefore can be transferred to the nursing child. We also detected several perfluorochemicals in pooled human serum samples, thus confirming human exposure to these compounds. We plan to use this method for measuring perfluorochemical levels in serum of residents of the United States. These population data on exposure to

perfluorochemicals will serve an important role in public health by helping to set research priorities and by establishing a nationally representative baseline of exposure to which population levels can be compared.

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