LEVELS OF PERFLUORINATED CHEMICALS IN MATCHED SAMPLES OF HUMAN BREAST MILK AND SERUM

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Introduction

An increasing number of studies show that humans are exposed to a large number of perfluorinated chemicals (PFCs) [1-3]. Human exposure pathways are currently being investigated. Studies from USA and Australia indicate that younger people have equal or higher PFC serum levels than adults and elderly people [4,5]. The extent of perinatal exposure to PFCs in the general population is not clear today. A cross-fostering study on Sprague-Dawley rats indicated that perfluorooctane sulfonate (PFOS) can be transferred to pups through lactation [6]. A study on two breast milk samples from USA showed the presence of PFCs in human milk [7]. PFOS was not detected (detection limit 0.3 ng/mL) but one sample contained 1.6 ng/mL perfluoroheptanoic acid (PFHpA) and the other sample 0.8 ng/mL perfluorohexanoic acid (PFHxA). Recently it was reported that low levels (10-592.6 pg/mL) of PFOS, perfluorooctanoic acid (PFOA), perfluorohexane sulfonate (PFHxS) and perfluorononanoic acid (PFNA) were found in breast milk from women living in China [8].

PFOS and PFOA are suggested to bind to serum albumin and not to lipids [9,10]. The lactational transfer mechanism and the PFC quantity of body burden or blood levels transferred to breast milk are not clear. This study reports occurrence and levels of PFCs in breast milk from Swedish primipara women together with their current blood serum levels at the time of donation.

Materials and Methods

Individual breast milk and serum samples from 12 women were collected in Uppsala, Sweden in the year 2004. All samples were from primipara women and were collected during the third week after delivery and stored at –20°C in glass bottles.

The serum and milk samples were extracted using weak anion exchange, solid-phase extraction (Waters Oasis® WAX) and analyzed by liquid chromatography (LC) coupled to a single quadrupole mass spectrometer (MS). Internal standards ($^{13}C_4$-PFOA and $^{13}C_4$-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 x g for 30 minutes. The supernatant was extracted and the perfluorinated compounds were eluted with 1 mL 2% ammonium hydroxide in methanol. 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 Microcon YM-3 centrifugal filter (Millipore, Billerica, MA, USA) was conducted at 14000 x g for 30 min. Performance standards, $^{13}C_5$-PFNA and *7H*-PFHpA, were added to both milk and serum extracts immediately before injection.

Analysis was perfomed using an Agilent 1100 HPLC system (Agilent, Waldbronn, Germany) coupled to an HP 1100 mass spectrometric detector (MSD, Waldbronn, Germany) with an atmospheric electrospray interface operating in negative ion mode. Separation was performed on a Discovery HS C18 (50 x 2.1 mm, 3 µm) column with a guard column of the same material (Supelco, Bellefonte PA, USA). Both columns were kept at 40 ºC. An extra guard column (HyperCarb 4 x 10 mm, 5µm) was inserted between the pump and injector to remove any fluorochemicals originating from the HPLC system. Injection volume was 10 µl and the flow rate was set to 300 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 [11].

Quantification was performed using the internal standard method with non-extracted standards dissolved in 35% methanol in water. $^{13}C_4$-PFOS was used as internal standard for the sulfonates and PFOSA. $^{13}C_4$-PFOA was used for the carboxylates. Recoveries were evaluated by three or five replicate fortifications to a low-contaminated serum sample and a non-contaminated breast milk sample. Average recoveries were >50% for all compounds except for PFOSA, PFDA, PFUnDA and PFDoDA (34-47%) and the coefficent of variation was 2-27% for multiple determinations. Procedural blank trace levels were detected for PFOA, PFOS and PFNA. In the case of blank levels, the mean blank signal plus three standard deviations 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 equal or less than 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. All breast milk samples were extracted in duplicates and the second extract volume was kept at 500 µl and 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 1st interlaboratory study on PFCs [12].

Results and discussion

A summary of the results of 12 individual milk and serum samples is given in Table 1.

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 only detected in one sample (0.33 ng/mL). The serum levels are similar to the levels found in previous studies on Swedish human plasma [13].

Table 1. PFC breast milk and serum levels (ng/mL) of primipara Swedish women, 2004.

Serum	**PFHxS**	**PFOS**	**PFOSA**	**PFOA**	**PFNA**	**PFDA**	**PFUnDA**
N > LOD	12	12	9	12	12	12	12
Range	1.8-11.8	8.2-48.0	<0.10-0.49	2.4-5.3	0.43-2.5	0.27-1.8	0.20-1.5
Mean	4.7	20.7	0.24	3.8	0.80	0.53	0.40
Std dev	2.9	10.5	0.16	1.0	0.55	0.41	0.35
Median	4.0	18.7	0.19	3.8	0.63	0.43	0.28
Milk							
N > LOD	12	12	8	1[a]	2	0	0
Range	0.031-0.172	0.060-0.470	<0.007-0.030	<0.209[b]-0.492	<0.005-0.020	<0.008	<0.005
Mean	0.085	0.201	0.013	-	0.017	-	-
Std dev	0.047	0.117	0.009	-	-	-	-
Median	0.070	0.166	0.010	-	-	-	-
Ratio S/M	**57**	**113**	**22**	**8**	**92**	**-**	**-**
RSD%	30	30	93	-	52	-	-

[a] Eleven additional samples were above blank level but was not reported due to blank levels > 50% of the concentrations detected. [b] Blank level

PFOS and PFHxS were detected in all milk samples at the mean concentrations 0.201 ng/mL and 0.085 ng/mL, respectively. PFOSA was detected in eight samples with the mean concentration 0.013 ng/mL and PFNA was detected in two samples (0.020 and 0.014 ng/mL). The limits of detection (LOD) were between 0.005 ng/mL and 0.010 ng/mL, except for PFHxA and PFHpA, which were an order of magnitude higher (0.1 ng/mL). A relatively high blank level was obtained for PFOA (0.209 ng/mL) with the consequence that the blank level was higher than 50% of the detected concentration in all samples except one.

The mean ratio between serum and breast milk (s/m) concentration was 113:1 for PFOS, 57:1 for PFHxS and 23:1 for PFOSA (Table 1). The s/m ratios for PFOA and PFNA are uncertain since only one, respectively two breast milk samples contained levels above the detection limit. Regression analysis of the matched serum and milk samples show a clear positive association (regression coefficient (r^2) 0.7-0.8) between levels of PFOS and PFHxS in serum and milk (Figure 1). This relationship could not be seen for PFOSA (r^2 <0.1).

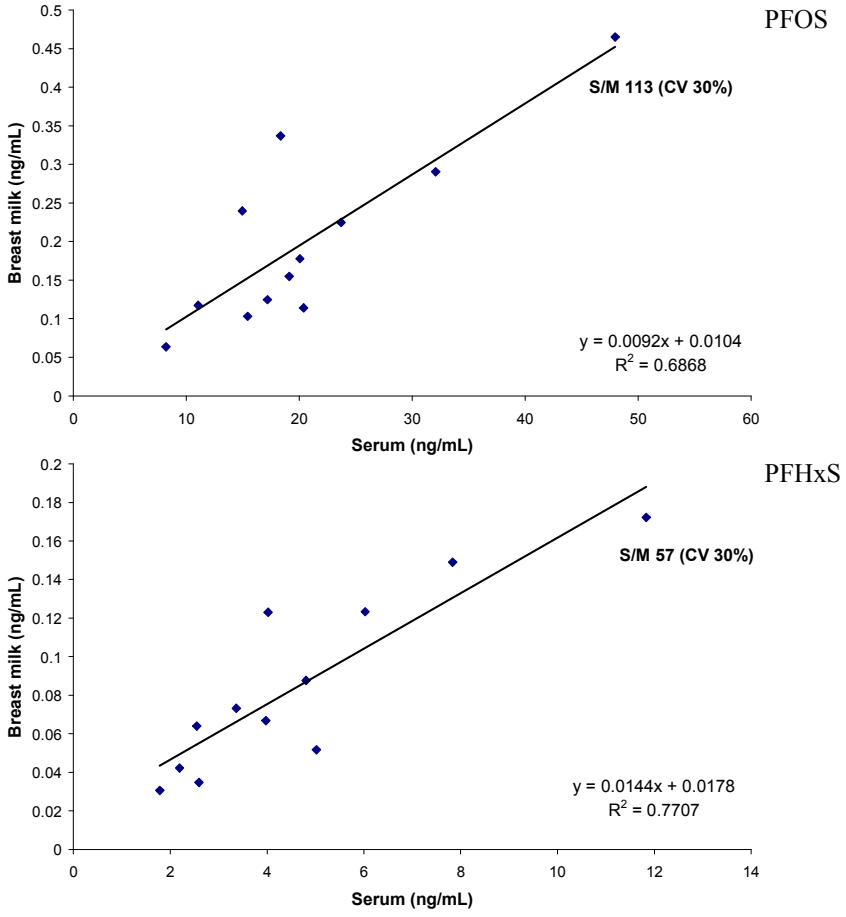

Figure 1. Regression analysis of PFOS and PFHxS levels in serum and breast milk from primipara women from Sweden, 2004

The selectivity of the MS method was successfully verified with MS/MS analysis. Qualitative comparison indicated that MS/MS analysis resulted in on average 50% higher concentrations compared to the single quadrupole MS analysis. It should be noted that different pre-concentration methods were used as well and the differences seen between the methods can be multi-factorial.

Presented results suggest that the concentration of PFOS and PFHxS in breast milk can be predicted from the serum concentrations. The ratio between serum and milk was different for different compounds. No conclusion can be made regarding PFOA since the relatively high procedural blank level hampered quantification. PFOSA levels in milk did not correlate to serum levels like for PFOS and PFHxS. Compared to PFOS and PFHxS, relatively more samples had levels of PFOSA close to the detection limit. It has been suggested that PFOSA can degrade to PFOS in biological systems[14], which might effect the level ratio between milk and serum. Moreover, it should be considered that PFOSA concentrations, in contrast to PFOS and PFHxS, are lower in serum as compared to that in whole blood[3].
Suggested here is that an equilibrium takes place, resulting in a higher serum to milk ratio with higher body burden, i.e. a greater relative transfer to milk at high exposure. For more fat-soluble, persistent organohalogens 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 whole blood and milk is approximately 1:10, due to the higher lipid content in milk compared to blood[15]. The lactational transfer of PFCs may be more similar to that for heavy metals. For example, concentration of lead in milk has been found to be 5-10 times lower than that in blood[15].

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