# DETERMINATION OF PERFLUORINATED COMPOUNDS IN HUMAN MILK

**T. Bernsmann and P. Fürst**

Chemical and Veterinary Control Laboratory, Joseph-König-Strasse 40, D - 48147 Münster, Germany

## Introduction

Perfluorinated compounds (PFC) are found in a wide range of products, because of their stability toward acids, bases and heat as well as for their inert and non adhering surface properties. They are used in many commercial products, such as paints, polishes, packaging, fire extinguishing foams and stain repellents[1]. Some PFC are persistent, bioaccumulative and toxic in animal studies. Perfluorinated sulfonates and carboxylates have been identified at low levels in the environment and in human serum, indicating their widespread presence[2].

Following the PFC case in the North Rhine-Westphalian (NRW) Sauerland area, where highly PFC contaminated industrial waste, which was mixed by a recycling company into a so called soil improver and disseminated by farmers on agricultural land, the Ministry of the Environment of NRW offered breast feeding women residing in this state an analysis of their milk for PFC. The Chemical and Veterinary Control Laboratory Münster (CVUA) was responsible for the analyses. The analytical methodology as well as the results of the first 203 individual samples analysed will be presented here.

## Materials and Methods

The method is adapted from Kärrman[3] and further developed.

*Chemicals and perfluorinated compounds*

- Native and $^{13}$C-labelled PFC standards were purchased from Wellington Laboratories: perfluoro-n-pentanoic acid (PFPA), perfluoro-n-hexanoic acid (PFHxA), perfluoro-n-heptanoic acid (PFHpA), perfluoro-n-octanoic acid (PFOA), perfluoro-n-nonanoic acid (PFNA), perfluoro-n-decanoic acid (PFDA), perfluoro-n-undecanoic acid (PFUnA), perfluoro-n-dodecanoic acid (PFDoA), perfluoro-1-butanesulfonate (PFBS), perfluoro-1-hexanesulfonate (PFHxS), perfluoro-1-heptanesulfonate (PFHpS), perfluoro-1-octanesulfonate (PFOS), perfluoro-1-decanesulfonate (PFDS)
- Solvents used were of quality grade “Nanograde” and purchased from Promochem, Germany
- Protease Typ XIV (Sigma L 1754-5G) and Lipase Typ VII (Sigma P 5147-1G) (50 mg/5 ml in 50 mmol ammonium acetate and adjusted to pH 7.5 with ammonia)

*Sample collection*

The mother’s milk was collected from the breast feeding woman herself into a clean 40 ml polypropylene tube (supplied by our institute) and dispatched to our laboratory. After arrival the samples were either analysed immediately or frozen at -18°C until analysis. Information about the number of breast fed children as well as the length of the respective breast feeding period was only partly available from the individual women.

*Extraction and analysis*

The frozen sample was allowed to thaw at room temperature. Three ml of the homogenised sample was transferred to a 15 ml polypropylene centrifuge tube, previously washed with methanol. An aliquot of 250 µl internal standard solution containing $^{13}$C-labelled PFHxA, PFOA, PFOS, PFDA und PFDoA (c = 0.002 µg/ml) was added. After thorough mixing, 1 ml of the lipase and 1 ml of the protease mixture were added and the pH adjusted to 7.5. This solution was incubated at 37°C over night to hydrolyse fat and protein bound PFC. The successful hydrolysis was indicated by the decrease of the pH to 6.9. After adding 2 ml methanol and adjustment of the pH to 6.5 the solution was sonicated for 15 minutes and centrifuged at 3500g for 10 minutes. The supernatant, which contains the PFC, was transferred onto a Strata X SW-solid phase extraction column (Phenomenex; 60 mg/3 ml) that was prewashed with 2 ml methanol and 2 ml water. After loading the column was washed with 2 ml water and 2 ml methanol and put under vacuum suction until dryness. The elution of the PFC was performed with 2 ml 2 %-$NH_3$ in Methanol. After adding 20 µl glycerol as a keeper the extract was evaporated in a gentle stream of nitrogen and finally reconstituted with 100 µl water/methanol (8/2). The extract was filtered through an 0.2 µm polypropylene filter into a polypropylene vial and then injected into an Agilent 1200 SL LC-System equipped with a ZORBAX SB C 18 column (Agilent, 100 x 2.1 mm, 1.8 µm). The column temperature was held at 50°C. A water/methanol mobile phase gradient with 2 mM ammonium acetate was delivered with a flow rate of 150 µl/min. The gradient started at 20 % methanol followed by a 15 min ramp to

95% methanol, a 5 min hold and then reverting to initial conditions. MS/MS-detection was performed with an Agilent 6410 triple quadrupole mass spectrometer equipped with an electrospray interface operating in negative ion mode with following settings: nebuliser gas temperature 300°C, nebuliser gas pressure 45 psi, drying gas flow 10 l/min and capillary voltage 3000 V. The multiple reaction monitoring (MRM) parameters are presented in Table 1.

Table 1: MRM parameters

| Compound | Precurser | Quantifier | Frag (V) | CE (V) | Qualifier | CE (V) |
|---|---|---|---|---|---|---|
| PFPA | 263 | 219 | 70 | 5 | | |
| PFBS | 299 | 99 | 145 | 50 | 80 | 50 |
| PFHxA | 313 | 269 | 70 | 4 | 119 | 16 |
| $^{13}C_2$-PFHxA | 315 | 270 | 60 | 4 | 119 | 17 |
| PFHpA | 363 | 319 | 77 | 4 | 169 | 15 |
| PFHxS | 399 | 99 | 160 | 50 | 80 | 50 |
| PFOA | 413 | 369 | 90 | 4 | 169 | 16 |
| $^{13}C_4$-PFOA | 417 | 372 | 95 | 5 | 172 | 17 |
| PFHpS | 449 | 99 | 170 | 45 | 80 | 80 |
| PFNA | 463 | 419 | 90 | 5 | 219 | 15 |
| PFOS | 499 | 99 | 190 | 50 | 80 | 50 |
| $^{13}C_4$-PFOS | 503 | 99 | 190 | 60 | 80 | 60 |
| PFDA | 513 | 469 | 105 | 6 | 219 | 15 |
| $^{13}C_2$-PFDA | 515 | 470 | 105 | 7 | 220 | 16 |
| PFUnA | 563 | 519 | 105 | 7 | 219 | 15 |
| PFDS | 599 | 99 | 190 | 95 | 80 | 105 |
| PFDoA | 613 | 569 | 100 | 8 | 169 | 18 |
| $^{13}C_2$-PFDoA | 615 | 570 | 100 | 8 | 169 | 19 |

Quantification:
Quantification was performed using the internal standard method. If no $^{13}$C-labelled PFC standard for an individual compound is available, the next higher $^{13}$C-labelled standard is used for quantification. The limit of detection (LOD) is defined as the signal to noise ratio of 3:1 of the qualifier ion and quantified against external calibration curves. The limit of quantification (LOQ) is defined as the concentration at which the RSD ≤ 20 %.

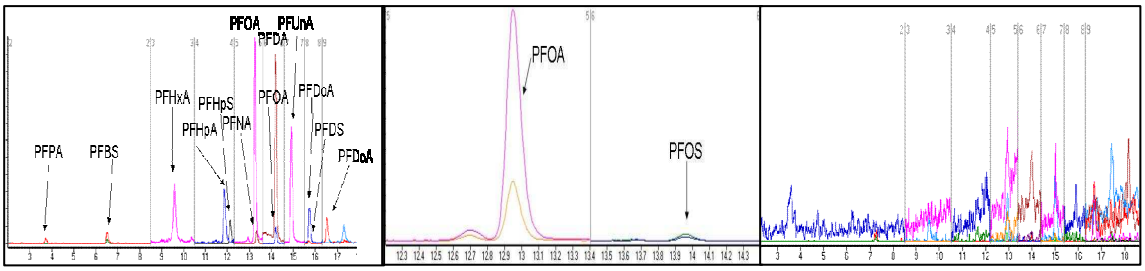
**Results and Discussion**

Table 2: Recoveries of spiking experiments. If recoveries in the table are below the LOQ, these were calculated only with the quantifier ion.

| Compound | n | Recovery | Recovery | Recovery | Recovery | Recovery | LOD | LOQ |
|---|---|---|---|---|---|---|---|---|
| | | 0.02 µg/l | 0.04 µg/l | 0.125µg/l | 0.5 µg/l | 1.25 µg/l | µg/l | µg/l |
| PFBS | 7 | | | 84.7 | 91.8 | 92.1 | 0.1 | 0.15 |
| PFPA | 7 | | | 60.3 | 65.7 | 80.1 | 0.1 | 0.20 |
| PFHxA | 7 | | | 85.4 | 87.0 | 93.0 | 0.05 | 0.10 |
| PFHpA | 7 | | | 51.5 | 43.2 | 88.1 | 0.05 | 0.10 |
| PFHxS | 7 | | | 97.5 | 92.3 | 103.6 | 0.05 | 0.10 |
| PFHpS | 7 | | | 90.5 | 87.2 | 93.0 | 0.05 | 0.10 |
| PFOA | 7 | 85.2 | 81.3 | 87.4 | 90.1 | 95.4 | 0.05 | 0.08 |
| PFNA | 7 | | | 78.5 | 83.7 | 98.5 | 0.05 | 0.10 |
| PFOS | 7 | 84.6 | 113.0 | 85.5 | 82.1 | 88.2 | 0.02 | 0.04 |
| PFDA | 7 | 67.9 | 105.6 | 85.4 | 88.4 | 94.9 | 0.04 | 0.08 |
| PFDS | 7 | | | 29.2 | 44.2 | 57.7 | 0.15 | 0.30 |
| PFUnA | 7 | | | 38.3 | 55.2 | 65.8 | 0.15 | 0.30 |
| PFDoA | 7 | | | 104.5 | 88.5 | 95.6 | 0.15 | 0.20 |

It is known that PFC in blood bind to serum albumin[4]. Therefore, a hydrolysis step using a lipase and a protease was implemented. The enzymes destroy the milk emulsion to release the PFC trapped in micelles and the chemically bound PFC. After the incubation the breaking of the emulsion was clearly visible. Another indication of the successful incubation was the change in the pH of the sample.

The method performance is demonstrated with a PFC free milk. The recoveries were calculated by standard addition of 5 concentration levels (0.02 µg/l to 1.25 µg/l) Results are presented in Table 2.




The first Figure shows a mother's milk spiked with PFC at 0.04 µg/l. Almost all PFC are already seen with the quantifier trace. Figure 2 shows a mother's milk with 0.14 µg/l PFOA and 0.04 µg/l PFOS (LOQ). The chromatogram of a PFC free milk is presented in Figure 3.

**Levels in human milk**

Data on the worldwide burden of breast milk with PFC are limited. Kuklenyik [5] published the first analyses of two different breast milk samples from the USA but they were below the LOD. Table 3 gives an overview on PFC levels in human milk samples that were analysed recently. The results of the analyses of Chinese[6], Swedish[7, 8], Japanese[9] and German[10] breast milk samples are in the same order of magnitude (Table 3). In contrast, the levels reported by Suchenwirth et al.[11] are considerably higher and differ significantly regarding the PFC profile. This unusual profile in combination with the high levels make the interpretation of that pilot study difficult.

Table 3: Median levels and ranges for PFC in human milk from different countries

| Authors | PFOA [µg/l] (range) | PFOS [µg/l] (range) | PFHxS [µg/l] (range) | Remarks |
|---|---|---|---|---|
| So et al[6] | 0.11 (0.05-0.21) | 0.10 (0.05-0.36) | 0.01 (0.004-0.10) | N=19; China 2004 |
| Kärrman et al.[8] | (< 0.209-0.492) | 0.17 (0.06-0.47) | 0.07 (0.03-0.17) | N=12; Sweden 2004 |
| Nakata et al.[9] | (LOD - 0.339) | (0.008 – 0.401) | (LOD – 0.025) | N=51, Japan; also PFNA was detected up to 0.15 µg/l |
| Völkel et al.[10] | (< 0.20-0.46) | 0.12 (0.03-0.31) | | N=57: Germany |
| Suchenwirth et al.[11] | 4.1-12.7 | | | N=12 pooled breast milk PFHxA: 7.5-12.5 µg/l |

In our investigation 203 human milk samples were analysed for PFC. In about 150 samples PFOA and PFOS were found at very low concentrations (Table 4). The results are comparable with the results of Kärrman et al.[7, 8], Nakata et al.[9], Völkel et al.[10] and So et al.[6]. In 2 samples low levels of PFHxS were found (0.18 µg/l and 0.16 µg/l; LOD 0.05 µg/l). All other PFC could not be detected. In most studies reported on humans with background exposure, the levels of PFOA and PFOS in blood plasma of women were in the range of 6-20 µg/l[3]. In our investigations of breast milk no levels for PFOS and PFOA above 1 µg/l were found. This confirms the low transfer of PFC into human milk as already shown by Kärrman et al.[7] who found a PFC ratio between human blood and human milk of around 100:1. Thus, in contrast to other persistent organic pollutants (POPs), human milk can not be considered as an appropriate bio indicator for PFC.

A first assessment of the health effects of PFOS and PFOA was performed in Germany by the Federal Drinking Water Commission and the Federal Institute for Risk Assessment[12]. They derived provisional tolerable daily intake (TDI) values of 0.1 µg/kg of body weight[12]. However, the TDI refers to the lifetime tolerable daily intake and therefore should not be applied to the relative short period of breast feeding.

Table 4: Statistical data of the 203 breast milk samples (*incl. half LOQ or half LOD, respectively)

| | PFOA (µg/l) | PFOA* LOQ: 0.08 µg/l; LOD: 0.05 µg/l (µg/l) | PFOS (µg/l) | PFOS* LOQ: 0.04 µg/l; LOD: 0.02 µg/l (µg/l) | ∑ PFOS PFOA (µg/l) | ∑ PFOS PFOA* (µg/l) |
|---|---|---|---|---|---|---|
| mean | 0.176 | 0.113 | 0.093 | 0.060 | 0.200 | 0.173 |
| median | 0.137 | 0.090 | 0.082 | 0.056 | 0.183 | 0.140 |
| maximum | 0.610 | 0.610 | 0.284 | 0.284 | 0.810 | 0.820 |
| minimum | 0.080 | 0.025 | 0.050 | 0.010 | 0.039 | 0.035 |
| $90^{th}$ percentile | 0.320 | 0.239 | 0.138 | 0.123 | 0.362 | 0.316 |
| N > LOQ | 113 | | 111 | | | |
| N > LOD | 162 | | 163 | | | |

Kraft et al.[13] performed a risk assessment for breast fed babies based on an average milk intake of 130 ml per kg body weight and an adsorption rate of 100 %. They derived a health based guidance value of 0.54 µg/l for the sum of PFOA and PFOS. This value was only slightly exceeded by three samples.

**References**


1 Kissa, E. Fluorinated Surfactants and Repellents, 2nd ed.; Marcel Dekker: New York, 2001
2 Calafa A.M. *Environ Sci Technol* 2006; 40: 2128-2134.
3 Kärrman A., van Bavel B., Järnberg U., Lindström G. *Organohalogen Compounds* 2004; 66: 4053-4057.
4 Jones, P.D., Newsted J.L. *Organohalogen compounds* 2003; 62: 311-314
5 Kuklenyik Z., Reich J.A., Tully J.S., Needham L.L., Calafat A.M. *Environ. Sci. Technol*. 2004 ; 38 : 3698-3704.
6 So MK., Yamashita N., Taniyasu S., Jiang Q., Giesy JP., Chen K. Lam PKS. *China Environ Sci Technol*. 2006; 40 (9): 2924 – 2929
7 Kärrman A., Ericson I., van Bavel B., Darnerud PO., Aune M., Glynn A., Lignell S., Lindström G. *Environ Health Perspect* 2007; 115: 226-230.
8 Kärrman A., Ericson I., van Bavel B., Lindström G. *Organohalogen Compounds* 2006; 68: 545-547
9 Nakata A., Katsumata T., Iwasaki Y., Ito R., Saito K., Izumi S., Makino T., Kishi R., Nakazawa H. *Organohalogen Compounds* 2007; 69: 2844-2846
10 Völkel W., Genzel-Boroviczény O., Demmelmair H., Gebauer C., Koletzko B., Verdugo-Raab U., Twardella D., Fromme H. *Int.J.Hyg.Environ.Health* (in press, *available online 17 September 2007*).
11 Suchenwirth RHR., Jürling H., Huppermann R., Bücking M. Perfluorierte Alkyl-Substanzen (PFAS) in der Muttermilch. Ergebnisse und vorläufige Bewertungen einer Pilotstudie des niedersächsischen Landesgesundheitsamtes. NGLA Umwelt und Gesundheit 2006; Report 3
12 Federal Drinking Water Commission Germany (2006): Opinion of 21.06.06, revised 13.07.06 www.umweltbundesamt.de/uba-info-presse/hintergrund/pft-im-trinkwasser.pdf (last call: 23.05.2008)
13 Kraft M., Bernsmann T., Hölzer J., Paul J., Krüger C., Quabeck G., Rauchfuss K., Wilhelm M. *Umweltmedizin in Forschung und Praxis* 2007; 12 (2): 79-86.