LEVELS OF PERFLUOROALKYLATED COMPOUNDS IN WHOLE BLOOD FROM SWEDEN

Anna Kärrman[1], Bert van Bavel[1], Ulf Järnberg[2], Lennart Hardell[1], Gunilla Lindström[1]

[1]Man-Technology-Environment Research Centre, Örebro University
[2]Institute of Applied Environmental Research, Stockholm University

Introduction

Historically the reports on perfluoroalkylated (PFA) compounds have been limited to mainly perfluorooctane sulfonate (PFOS) and perfluorooctanoate (PFOA) but recently a number of other, potentially bioaccumulating, perfluorinated acids were reported in wildlife and in human blood (1,2,3,4). PFOS is the PFA compound that has been reported most frequently and in the < 1-82 pg/µl range in human serum from the general population of several countries (5,6,7,8).

PFOS and its salts represents only a fraction of the total fluoroproduction but can occur as an impurity in other products and is suspected to be the stable degradation product from other perfluorinated compounds, for example derivatives of perfluorooctane sulfonamide (PFOSA) (9,10). Recent studies indicate that more volatile fluorotelomer alcohols (FTOHs) and sulfonamidoethanols can degrade biotic to different PFA compounds (11). FTOHs and sulfonamidoalcohols are used in both industrial and household applications and have been found in the trophospere (12). Since possible degradation pathways, distribution and exposure routes for this group of compounds are still under investigation; information of human exposure to a larger number of PFAs is valuable. In this study we analysed 66 whole blood samples from the Swedish general population with respect to 12 perfluorinated sulfonates and carboxylates (including perfluorooctane sulfonamide) with carbon chain length between 4 and 14.

Materials and methods

The whole blood material consists of sons of the age 19-46 (n=40) and mothers of the age 46-75 (n=26) drawn from the Swedish population registry (Stockholm, Sweden), and collected during 1997-2000. The blood samples were initially treated with Heparin and stored at -20°C. An aliquot of 0.75 ml whole blood, 7.5 µl internal standard (perfluoroheptanoic acid, PFHpA), 1.5 ml water and 1.5 ml

formic acid were mixed, sonicated and centrifugated before extracted on a solid phase extraction column (C18, 200 mg, 3 ml) which was prewashed with methanol and water. After washing the column with 40 v/v% methanol in water and vacuum suction until dryness, the PFAs were eluated with 0.5 ml methanol. After filtration the extracts were injected into an Agilent 1100 LC system with a Supelco Discovery HS C18 (50*2.1 mm, 3µm) column (Sigma-Aldrich) and a guard column of the same material. The instrument was equipped with a thermostated column department that was kept at 40°C. A water/methanol mobile phase gradient with 2 mM ammonium acetate (99%, pa for HPLC, Fluka) was delivered with a flow rate of 0.3 ml/min. The gradient started at 35% methanol followed by a 20 min ramp to 90% methanol, a 10 min hold followed by a 10 min washing sequence with 100% methanol, and then reverting to initial conditions allowing 7 min stabilisation time. Detection was performed with an Agilent 1100 MSD mass spectrometer equipped with an atmospheric electrospray interface operating at negative ion mode. Single ion monitoring was used measuring the [M-H] ion for sulfonic acids and [M-COOH] ion for carboxylic acids. Quantification was performed using relative response factors calculated from calibration curves of unextracted standards using PFHpA as internal standard.

Results

Of the 12 PFAs studied, PFOS, PFOA, PFOSA, perfluorohexane sulfonate (PFHxS) and perfluorononanoic acid (PFNA) were found in 92-100% of the samples and these results are presented in *Table 1*. Perfluorodecane acid (PFDA) and perfluoroundecane acid (PFUnDA) were found in about 65% of the samples at LOD(0.1)-0.7 pg/µl. Besides this, we were able to detect perfluorodecane sulfonate (PFDS), perfluorohexane acid (PFHxA), , perfluorododecane acid (PFDoDA and perfluorotetradecane acid (PFTDA) in 3-8% of the samples at LOD(0.3-0.5)-5 pg/µl. The only compound that we did not detect in this study was perfluorobutane sulfonate (PFBuS).

Materials and method procedure were controlled for possible contamination by extracting water with every batch. Recovery of the internal standard was evaluated by adding 7H-PFHpA to the extract prior to injection and resulted in 63-112% recovery. Recoveries of 12 PFAs were evaluated by standard addition to a sample at three different levels and were 64-112% except for perfluorobutane sulfonate (PFBuS), which was 27%. The limit of detection (LOD), defined as a signal to noise (S/N) ratio of three was 0.1-0.5 pg/µl except for PFBuS (2 pg/µl). The limit of quantification (LOQ), defined as the concentration at which repeated injections

resulted in RSD% ≤ 20% at a minimum S/N of 5 were 0.3-0.9 pg/µl except for PFBuS (3 pg/µl).

Table 1. Concentrations (pg/µl) of 5 PFA compounds in whole blood collected in Sweden, 1997-2000.

Whole blood	PFHxS	PFOA	PFOS	PFNA	PFOSA	ΣPFCA[1]	ΣPFSA[2]
All, n=66							
Range [3]	0.4-28.4 (66)	0.5-12.4 (66)	1.7-37.0 (66)	<0.1-1.9 (61)	0.4-22.9 (66)		
Arithmetic mean	2.3	2.7	18.2	0.4	4.1	3.0	24.6
Median	1.5	2.5	17.1	0.3	2.7		
Males, n=40							
Range	0.4-28.4	0.5-12.4	1.7-37.0	<0.1-1.9	0.8-22.9		
Arithmetic mean	3.0	2.9	18.4	0.4	4.6	3.3	26.0
Median	1.7	2.7	17.7	0.3	2.7		
Females, n=26							
Range	0.4-2.5	0.8-4.1	4.6-32.8	<0.1-1.0	0.4-9.5		
Arithmetic mean	1.3	2.3	17.8	0.4	3.3	2.6	22.3
Median	1.2	2.1	16.9	0.3	2.7		

[1] Sum of perfluorocarboxylic acids. [2] Sum of perfluorosulfonic acids incl. PFOSA. [3] Figures in parenthesis are the number of samples in where the compound was found.

Discussion

The results shows that the Swedish population is exposed to a large number of PFAs in conformity with the study performed on European parliament members (4). The concentrations of PFOS (18.2 pg/µl) and PFOA (2.9 pg/µl) in whole blood samples from Sweden are about a factor two lower than previously reported (34.9 and 4.6 pg/µl respectively) in serum samples of the USA population (13). Assuming that PFAs bind to plasma proteins (14,15) and that whole blood consist of about 50% plasma, the concentrations of PFOS and PFOA in the general population from Sweden and USA can be regarded as similar. The relationship between different compounds was studied in order to find eventual exposure patterns. The association between PFOS and PFOA was strongest (R^2 0,3-0,5), demonstrated in *Figure 1*.

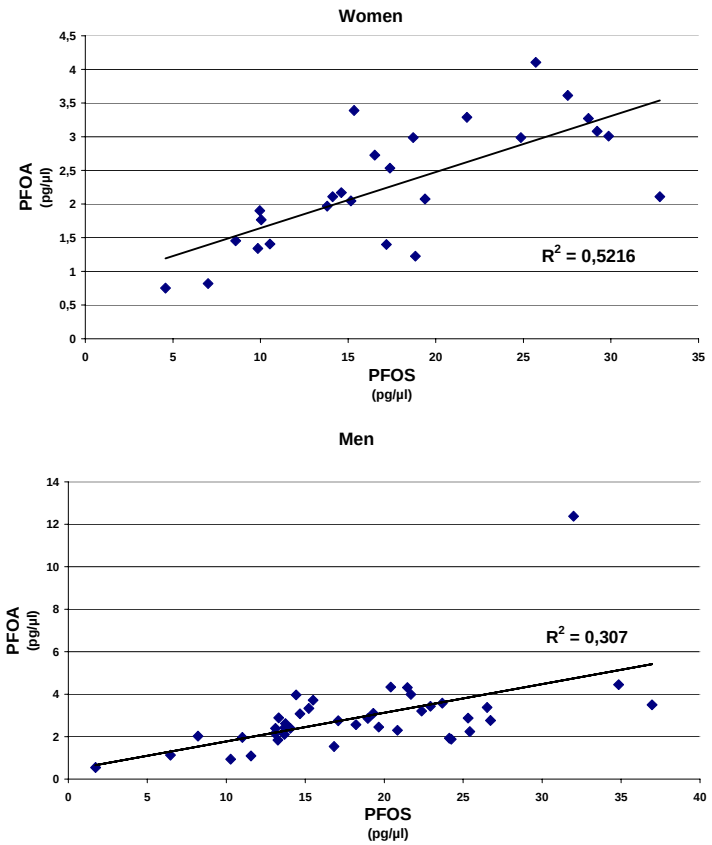

Figure 1. Relationship between PFOS and PFOA in whole blood samples from men and women from Sweden.

Since the cohort consists of young men and older women, conclusions about relationship between concentrations of PFAs and age or sex is somewhat difficult to draw but slightly higher concentrations were found in the young male group. No age dependent association could be seen. The mean and median were similar for PFOS, PFOA and PFNA indicating a distribution close to normal. One reason for not finding PFBuS can of course be the fact that the described method is not well suited for this compound, as indicated by low recovery and high LOD.

Acknowledgment
We would like to thank the Swedish EPA and Cancer- och Allergifonden (Stockholm, Sweden) for financial support.

References

1. Martin, J. W., Smithwick, M. M., Braune, B.M., Hoekstra, P.F., Muir, D.C, Mabury, S.A. (2003). Environmental Science and Technology ASAP 25/11.
2. Calafat, A.M., Kuklenyik, Z., Reich, J.A., Butenhoff, J.L., Needham, L.L. (2003). Organohalogen compounds 62: 319-322.
3. Moody, C. A., Martin, J. W., Kwan, W.C., Muir, D.C., Mabury, S.A. (2002). Environmental Science and Technology 36(4): 545-551.
4. WWF (2004). "Chemical check up-an analysis of chemicals in the blood of members of the European Parliament." WWFs European policy office.
5. Hansen, K.J., Clemen, L.A., Ellefson, M.E., Johnson, H.O. (2001). Environmental Science and Technology 35(4): 766-770.
6. Masunaga, S., K. Kannan, Doi, R., Nakanishi, J., Giesy, J.P. (2002). Organohalogen Compounds 59: 319-322.
7. 3M (2000). Draft initial assessment report Perfluorooctane sulfonic acid and its salts. EPA public docket AR226-0978.
8. Kannan, K., Kumar, K.S., Corsolini, S., Aldous, K.M. (2003). Organohalogen compounds 64: 29-32.
9. 3M (1999). Fluorochemical Use, Distribution and Release Overview**.** EPA docket AR226-0550
10. Tomy, G.T., Tittlemier, S.A., Palace, V.P., Budakowski, W.R., Braekevelt, E., Brinkworth, L., Friesen, K. (2003). Environmental Science and Technology ASAP Dec. 3.
11. Dinglasan, M.J.A., Ye, Y. Edwards, E.A., Mabury, S.A. (2004). Environmental Science and Technology ASAP April 16.
12. Stock, N.L., Lau, F. K., Ellis, D.A., Martin, J.W., Muir, D.C., Mabury, S.A. (2004). Environmental Science and Technology 38: 991-996.
13. Olsen, G.W., Church, T.R., Miller, J.P., Burris, J.M., Hansen, K.J., Lundberg, J.K., Armitage, J.B., Herron, R.M., Medhdizadehkashi, Z., Nobiletti, Z.B., O´Neill, E.M., Mandel, J.H., Zobel, L.R. (2003). Environmental Health Perspectives 111(16): 1892-1901.
14. Jones, P.D., Hu, W.,de Coen, W., Newsted, J.L., Giesy, J.P. (2003). Environmental Toxicology and Chemistry 22(11): 2639-2649.
15. Han, X., Snow, T.A., Kemper, R.A., Jepson, G.W. (2003). Chemical research in toxicology 16: 775-781.