ELEVATED LEVELS OF PERFLUOROCARBOXYLATES IN BLOOD FROM TECHNICIANS USING FLUORINATED SKI WAX

Nilsson, Helena[1], Kärrman, Anna[1], Westberg, Håkan[1,2], van Bavel, Bert[1], Lindström, Gunilla[1].

[1] MTM Research Centre, Department of Natural Sciences, Örebro University, SE-701 82 Örebro, Sweden
[2] Department of Occupational and Environmental Medicine, Örebro University Hospital, SE-701 85 Örebro, Sweden

Introduction

Human exposure to perfluorinated chemicals (PFCs) is of great concern not the least since recent epidemiological studies point at adverse developmental effects at the background exposure levels in general populations [1, 2]. The levels and distribution of PFCs in blood from 66 Swedish citizens collected 1997-2000 were found to be in the same range as for other known general populations [3]. But, with the exception of a few subjects, including a person who reported frequent use of fluorinated ski wax. This called for further exposure assessment related to ski wax.

PFCs are used in a variety of applications due to unique surfactant properties that create oil- and water resistance to surfaces. Ski wax is applied to preventing adhesion of snow, ice, and moisture that slow down the ski's movement. Many different additives are used to enhance the water repellence of a ski-wax formulation. Among the most popular are fluorinated chemical components, formulas that have resulted in numerous US patents (www.patentstorm.us). Fluorinated ski wax is often a blend between a wax matrix, for example paraffin, and carbon fluoride. The exact contents of the fluorinated additives are usually not disclosed by producers.

The working environment for professional service technicians in cross-country skiing was studied in the season 2007/2008. This report presents the first part of the project, analysis of PFC blood levels of eight technicians during the course of FIS World Cup competitions.

Materials and Methods

Blood sampling. Individual blood samples from eight men working as service technicians for the Swedish Ski team and the US Ski Team in cross-country skiing were collected during the winter and spring of 2007/2008. The service technicians are Swedish citizens except for one who is Estonian. Median age is 36 years (range 27-51 years). The first sampling occasion was in September 2007 (pre-season). Sampling at FIS World Cup competitions were conducted in Kuusamo, Finland (December 2007) followed by Val di Fiemme, Italy (January 2008), Otepää, Estonia (February 2008), and finally in Holmenkollen, Norway (March 2008). All samples were stored in blood containers at -20 °C prior to analysis.

Analytical procedure. PFCs including sulfonates C4, C6, C8 (PFOS), 6:2 tetrahydro PFOS and carboxylates C6, C7, C8 (PFOA), C9, C10, C11, C12, C14 were analysed using weak anion exchange, solid phase extraction (Waters Oasis® WAX) and ultra-performance chromatography coupled to a tandem mass spectrometer (Acquity UPLC-Quattro Premier XE MS/MS). The sample extraction has been described elsewhere [4]. Briefly, internal standards ($^{13}C_4$-PFOA and $^{13}C_4$-PFOS, Wellington Laboratories) and 2 mL formic acid/water (1:1v/v) were added to 0.5 mL whole blood. After sonication and centrifugation, the supernatant was extracted using Oasis WAX and the perfluorinated compounds were eluted with 1 mL 2% ammonium hydroxide in methanol. The volume of the blood extract was adjusted to 200 µl using N_2. Performance standard ($^{13}C_5$-PFNA, Wellington Laboratories) and 300 µl 2 mM ammonium acetate in water were added prior to analysis. Analysis was performed using an Acquity UPLC coupled to an Quattro Premier XE (Waters Corporation, Midford) with an atmospheric electrospray interface operating in negative ion mode (ES-MS/MS). Separation was performed on an Acquity BEH C18 2.1 x 50mm, 1.7 µm kept at 50°C. An extra guard column (Waters prototype) 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 400 µl/min. A gradient program delivering mobile phases

consisted of 2 mM ammonium acetate in methanol and 2 mM ammonium acetate in water was employed. Multiple reaction monitoring was employed using molecular ion $[M]^-$ for sulfonates and $[M\text{-}COOH]^-$ for carboxylates and measuring two product ions for each compound.

Quantification was performed using the internal standard method with non-extracted standards dissolved in 30 % methanol in water. $^{13}C_4$-PFOS was used as internal standard for the sulfonates and $^{13}C_4$-PFOA was used for the carboxylates. The mean recoveries for internal standards $^{13}C_4$-PFOS and $^{13}C_4$-PFOA in all samples were 71% and 74%, respectively. 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 (S/N 3) for 12 PFCs in whole blood were 0.005-0.33 ng/mL.

Results and Discussion

Results for PFOS and the carboxylates PFOA, PFNA, PFDA, PFUnDA, PFDoDA, and PFTDA are presented in Table 1. In addition, PFHxS (0.05-2.02 ng/mL), PFHxA (0.1-6.86 ng/mL), and PFHpA (0.06-11.5 ng/mL) were detected in all samples. Levels of PFOS showed no time trend and were for the eight individuals (median of the five samples) between 0.59 and 28.9 ng/mL. A large variation was seen in PFOA levels, with the lowest whole blood level at 3.56 ng/mL (individual 1) and the highest at 466 ng/mL (individual 8). A time trend with increasing levels during the course of competition season was seen for individuals with initial low levels of PFOA (<10 ng/mL) but not for the others with initial PFOA levels between 93 and 444 ng/mL (Figure 1). Generally, blood levels of carboxylates decreases with increasing chain length, C8>C9>C10>C11>C12>C14, and there is a significant correlation between PFOA concentration and those of all other carboxylates ($p \leq 0.0001$). The strongest correlation was with PFNA and PFUnDA (R^2 0.9399 and 0.6378). Levels of PFNA were found to be second highest and between 10-27% of corresponding PFOA levels except for individual 2 (13-69%). No clear age trend in the levels was observed. However an association between number of years working as professional service technician and PFOA levels could be seen. Technicians with five years or less in the profession had pre-season PFOA levels <7 ng/mL, with one exception. However, this association might be biased since these individuals most likely have been active in cross-country skiing before being professional service technicians.

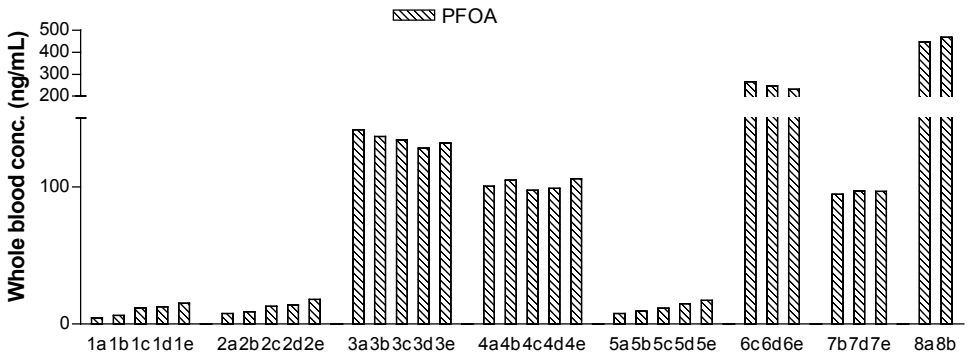

Figure 1. PFOA whole blood concentrations (ng/mL) for service technicians (1-8) at different sampling occasions, a) Sep07, b) Dec07, c) Jan08, d) Feb08, e) Mar08

Median levels in whole blood samples from a general Swedish population, n=66 collected 1997-2000, were 17 ng/mL for PFOS, 2.5 ng/mL for PFOA, and 0.3 ng/mL for PFNA [3]. PFDA and PFUnDA were detected in only few samples at levels close to the detection limit. PFTDA was not detected in any sample. The highest PFOA level in present study (465 ng/mL) is 186 times higher than median and 37 times higher than maximum

concentration found in the 1997-2000 study. Ammonium perfluorooctanoate (APFO) exposed workers in a 3M plant was reported to have serum PFOA levels ranging between 100-81300 ng/mL (1997, n=74)[5]. Figure 2 shows levels of selected PFCs in a Swedish general population, the service technicians (present study) and 3M workers.

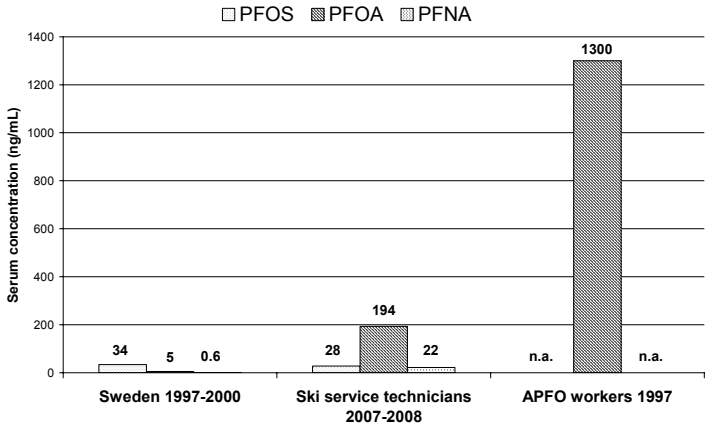

Figure 2. Comparison of median serum* concentrations of PFOS, PFOA and PFNA in human blood from a general Swedish population (n=67)[3], present study, 3M workers in APFO plant [5].
* Whole blood concentrations from present study and the Swedish study was multiplied with a factor of 2.
n.a. not analysed

Fluorinated ski-wax products emit perfluorinated carboxylates. Five of the eight technicians had blood PFOA levels in the lower range of occupational exposed workers in a 3M plant. Remaining three technicians showed an increase in PFOA levels with proceeding ski season. Their PFOS levels were all within the range for a general Swedish population. Although multiple sources and routes of exposure are possible, emission/breakdown of PFCs during ski-wax application and subsequent air exposure for the technicians is a likely explanation for the elevated carboxylate levels.

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Table 1. PFCs in whole blood (ng/mL) from eight service technicians in cross country skiing, 2007/2008.

Individual 1	**September 07**	**December 08**	**January 08**	**February 08**	**March 08**
PFOS	0.57	0.65	0.85	0.56	0.47
PFOA	3.56	6.03	10.8	11.6	14.1
PFNA	0.95	1.27	1.73	2.02	2.22
PFDA	1.25	2.08	3.64	4.71	5.40
PFUnDA	0.21	0.37	0.40	0.61	0.63
PFDoDA	0.21	1.19	1.00	1.60	1.72
Individual 2	**September 07**	**December 08**	**January 08**	**February 08**	**March 08**
PFOS	25.5	26.3	24.3	23.2	15.8
PFOA	6.80	8.11	12.0	13.2	18.0
PFNA	4.72	5.23	5.47	5.81	17.2
PFDA	3.55	4.15	5.24	6.66	26.6
PFUnDA	0.41	0.54	0.53	0.65	2.58
PFDoDA	0.16	0.57	0.82	1.19	3.96
Individual 3	**September 07**	**December 08**	**January 08**	**February 08**	**March 08**
PFOS	17.9	16.9	16.2	14.4	15.9
PFOA	142	137	134	128	132
PFNA	16.5	16.5	15.3	14.9	17.3
PFDA	20.6	23.0	21.2	20.3	26.7
PFUnDA	1.44	1.96	1.70	1.56	2.58
PFDoDA	1.06	2.57	2.31	2.18	1.29
Individual 4	**September 07**	**December 08**	**January 08**	**February 08**	**March 08**
PFOS	12.6	13.4	13.4	12.3	13.4
PFOA	99.1	103	96.2	97.7	105
PFNA	16.0	17.0	16.2	17.0	19.6
PFDA	8.05	7.83	8.00	8.23	10.8
PFUnDA	1.44	1.06	1.09	1.20	1.79
PFDoDA	1.23	1.00	1.34	1.37	2.03
Individual 5	**September 07**	**December 08**	**January 08**	**February 08**	**March 08**
PFOS	15.0	15.2	13.7	15.0	14.1
PFOA	6.42	8.20	10.3	13.5	15.9
PFNA	1.89	2.19	2.22	3.07	3.45
PFDA	1.39	2.11	2.45	4.38	4.60
PFUnDA	0.55	0.64	0.52	0.91	0.93
PFDoDA	0.28	0.67	0.91	1.70	2.07
Individual 6	**September 07**	**December 08**	**January 08**	**February 08**	**March 08**
PFOS	-	30.2	-	28.8	27.5
PFOA	-	265	-	245	230
PFNA	-	58.5	-	53.5	50.6
PFDA	-	28.0	-	24.4	24.7
PFUnDA	-	4.26	-	3.20	3.82
PFDoDA	-	4.06	-	3.24	4.20
Individual 7	**September 07**	**December 08**	**January 08**	**February 08**	**March 08**
PFOS	-	11.9	-	11.6	11.4
PFOA	-	93.0	-	95.4	95.1
PFNA	-	10.7	-	11.0	11.8
PFDA	-	8.51	-	8.82	10.8
PFUnDA	-	0.89	-	1.21	1.19
PFDoDA	-	0.57	-	0.96	1.12
Individual 8	**September 07**	**December 08**	**January 08**	**February 08**	**March 08**
PFOS	7.81	8.28	-	-	-
PFOA	444	465	-	-	-
PFNA	117	118	-	-	-
PFDA	12.4	13.5	-	-	-
PFUnDA	2.55	2.88	-	-	-
PFDoDA	2.33	2.34	-	-	-