THE RANGES OF POLYFLUORINATED COMPOUNDS IN SERUM FROM A GROUP OF HIGH CONSUMERS OF FISH FROM A CONTAMINATED LAKE IN NORWAY

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Introduction

Polyfluorinated compounds (PFCs) have been used the last 50 years in many commercial applications including surfactants, lubricants, paints, polishes, food packaging and fire-retarding foams. Concerns about the persistence and bioaccumulative properties of PFCs were raised when the widely used surfactant perfluorooctylsulfonate (PFOS) was found to be ubiquitous in wildlife and human populations worldwide[1-5]. Some of the PFCs have been suggested to induce developmental, reproductive and other toxic effects in animal studies[6-8].

During the last five years, extensive research has focused on PFOS and perfluorooctanoate (PFOA). However, very little is known about environmental levels and fate of other PFCs, and human exposure pathways of PFCs are at present still unclear. The PFCs are generally not soluble in fat, and other dietary sources are expected for PFCs than for persistent organic pollutants (POPs) such as dioxins, PCBs and brominated flame retardants (BFRs). However, a recent study by Falandysz[9] showed that consumption of seafood highly influenced the human body burden of several PFCs. The aim of this study was to investigate the blood levels of PFCs in a group of high consumers of inland fish from a contaminated lake in Norway.

Materials and Methods

Chemicals

Perfluorooctane sulphonamide (PFOSA), *N*-ethyl-perfluorooctane sulfonamide (Et-PFOSA), *N*-methyl-perfluorooctane sulfonamide (Me-PFOSA), 2-(*N*-ethyl-perfluorooctane sulfonamido) ethanol (Et-PFOSA-EtOH), 2-(*N*-methyl-perfluorooctane sulfonamido) ethanol (Me-PFOSA-EtOH), 2-(*N*-ethyl-perfluorooctane sulfonamido) acetic acid (Et-PFOSA-AcOH), 2-(*N*-methyl-perfluorooctane sulfonamido) acetic acid (Me-PFOSA-AcOH), potassium perfluorobutane sulfonate (PFBuS), potassium perfluorohexane sulfonate (PFHxS), potassium perfluorooctane sulfonate (PFOS) and ammonium perfluorooctanoate (PFOA) were provided by 3M Co. (Saint Paul, MN, USA). Perfluoropentanoic acid (PFPeA), perfluorohexanoic acid (PFHxA), perfluoroheptanoic acid (PFHpA), perfluorononanoic acid (PFNA), perfluorodecanoic acid (PFDeA), perfluoroundecanoic acid (PFUA), and perfluorododecanoic acid (PFDoA) were purchased from Oakwood Products (West Columbia. SC, USA). 1,2-$^{13}C_2$-Perfluorooctanoic acid ($^{13}C_2$-PFOA), was provided by Dupont Co. (Wilmington, DE, USA). $^{18}O_2$-Perfluorooctanesulfonate ($^{18}O_2$-PFOS) and $^{18}O_2$-perfluorooctanesulfonamide ($^{18}O_2$-PFOSA) were purchased from Research Triangle Institute (Research Triangle Park, NC, USA). D_3-*N*-methyl-perfluorooctane sulfonamide (d_3-Me-PFOSA), D_5-*N*-ethyl-perfluorooctane sulfonamide (d_5-Et-PFOSA) and N-1,2-^{13}C-perfluorodecanoic acid (^{13}C-PFDeA) were from Wellington Laboratories (Guelph, Ontario, Canada). HPLC grade methanol, acetonitrile, and water were from Caledon Laboratories (Georgetown, Ontario, Canada); glacial acetic acid was from Sigma-Aldrich (St. Lois, MO, USA); formic acid was from EM Science (Gibbstown, NJ, USA); and ammonium hydroxide was from J.T. Baker (Phillipsburg, NJ, USA).

Serum samples

This study was conducted on a sub group of 35 serum samples from a study, organised by the Norwegian Institute of Public Health, to investigate the body burdens of POPs in high consumers of in land fish caught in a contaminated lake in Norway. The participants were recruited among local hobby fishermen and women. Participants provided serum and urine samples, and filled in detailed questionnaires regarding personal background data and dietary habits, both concerning their regular diet and intake of fish from the lake. The project was approved by the Regional Committees for Medical Research Ethics.

Sample preparation and quantitative determination
The serum samples were extracted and analyzed according to the method described by Kuklenyik et al. [10] comprising an on-line solid phase extraction (SPE) coupled to HPLC-MS/MS. In brief, 200 µl of serum were diluted with 0.1 M formic acid (500 µl) containing internal standard solution in methanol (50 µl). Of this mixture 400 µl were injected and the analytes concentrated on a C18 SPE column (HySphere HD C18, 7 µm, 10 mm x 1 mm; Spark Holland, Plainsboro, NJ, USA), reconditioned and reused 10-12 times. The chromatographic separation was performed on a Betasil C8 column (5 µm, 3 x 50 mm, ThermoHypersil, Keystone, Bellefonte, PA, USA) and the analytes detected using negative-ion TurboIonSpray ionization MS/MS. The method has been described in detail elsewhere[10]. In addition to the 35 serum samples, quality control (QC) samples and procedural blanks were analysed, and found within pre-characterized limits.

Results and Discussion
The participants in this study consisted of a total of 18 men and 17 women, their mean age being 59.4 and 51.5 years, respectively (median age 59.5 and 57 years). The PFCs serum concentrations are presented in Table 1. As can be seen, PFOS, PFHxS, PFOA, PFNA, PFDeA and PFUA were found in all of the samples, PFOSA in most of the samples, and PFDoA and Me-PFOSA-AcOH in about half of the samples. The other nine PFCs were not observed above the detection limits. There were no significant differences between the serum concentrations observed in men compared to women for any of the PFCs, except for PFOS, which was significantly higher in men compared to women. This is in accordance with the results presented in a recent study by Harada et al.[4], where higher concentrations were observed in men.

PFOS was the PFC found at highest concentration in all the samples, contributing to about 75% of the total concentration of the PFCs measured in this study. The next most abundant PFC observed was PFOA. The levels of PFOS and PFOA in all the individual samples are shown in Figure 1. The present mean PFOS level in these Norwegian samples is in close agreement with levels that have been found in human samples in Sweden[11], however almost ten times lower concentrations were found in a previous screening study on a limited number of samples from Norway[12]. A relatively large variation has been observed in PFCs levels in human populations worldwide[3].

Concentrations of PFOS correlated with the concentrations of the other detected PFCs except Me-PFOSA-AcOH. As an example, the correlations between PFOS and PFOA/PFDeA are shown in Figure 2. Significant correlations were also found between PFNA, PFDeA, PFUA, PFDoA and PFOSA, however PFHxS and PFOA were only significantly correlated to each other in addition to PFOS.

Table 1. Concentrations of the PFCs in ng/ml serum in the 35 samples.

	Men (n=18)					Women (n=17)					
	mean	**median**	**min**	**max**	**No. det.**	**mean**	**median**	**min**	**max**	**No. det.**	**LOD**
PFOS	34	31	17	62	18	25	27	11	45	16	0.2
PFHxS	1.2	0.98	0.53	2.0	18	1.1	0.96	0.32	2.2	17	0.1
PFOA	3.5	3.2	1.3	7.4	18	3.3	3.3	1.8	5.4	17	0.2
PFNA	1.6	1.2	0.79	4.6	18	1.3	1.4	0.56	2.3	17	0.2
PFDeA	0.81	0.65	0.26	3.1	18	0.53	0.43	0.23	1.1	17	0.2
PFUA	1.8	1.3	0.20	7.7	18	1.2	0.89	0.21	2.5	17	0.2
PFDoA	0.31	0.14	0.14	1.6	8	0.19	0.14	0.14	0.40	7	0.2
PFOSA	0.12	0.09	0.04	0.60	14	0.09	0.07	0.04	0.18	14	0.05
Me-PFOSA-AcOH	0.22	0.14	0.14	0.53	8	0.42	0.27	0.14	2.0	13	0.2

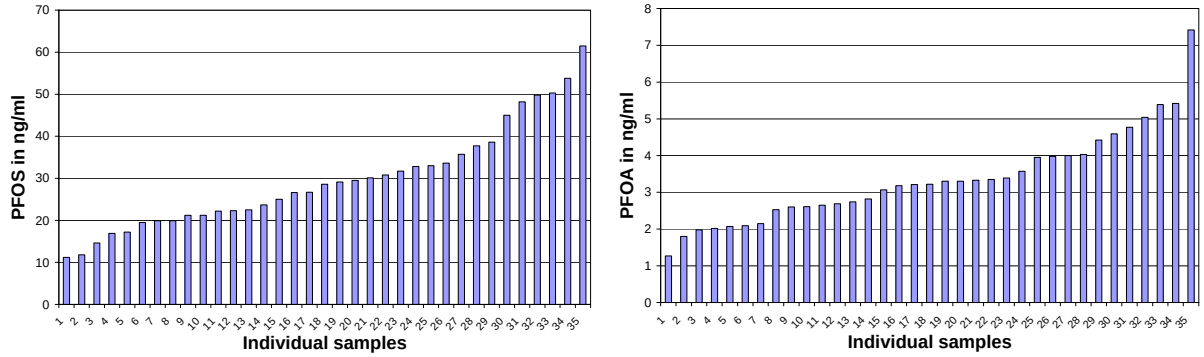

Figure 1. The concentration of PFOS and PFOA in ng/ml serum in the 35 individual samples.

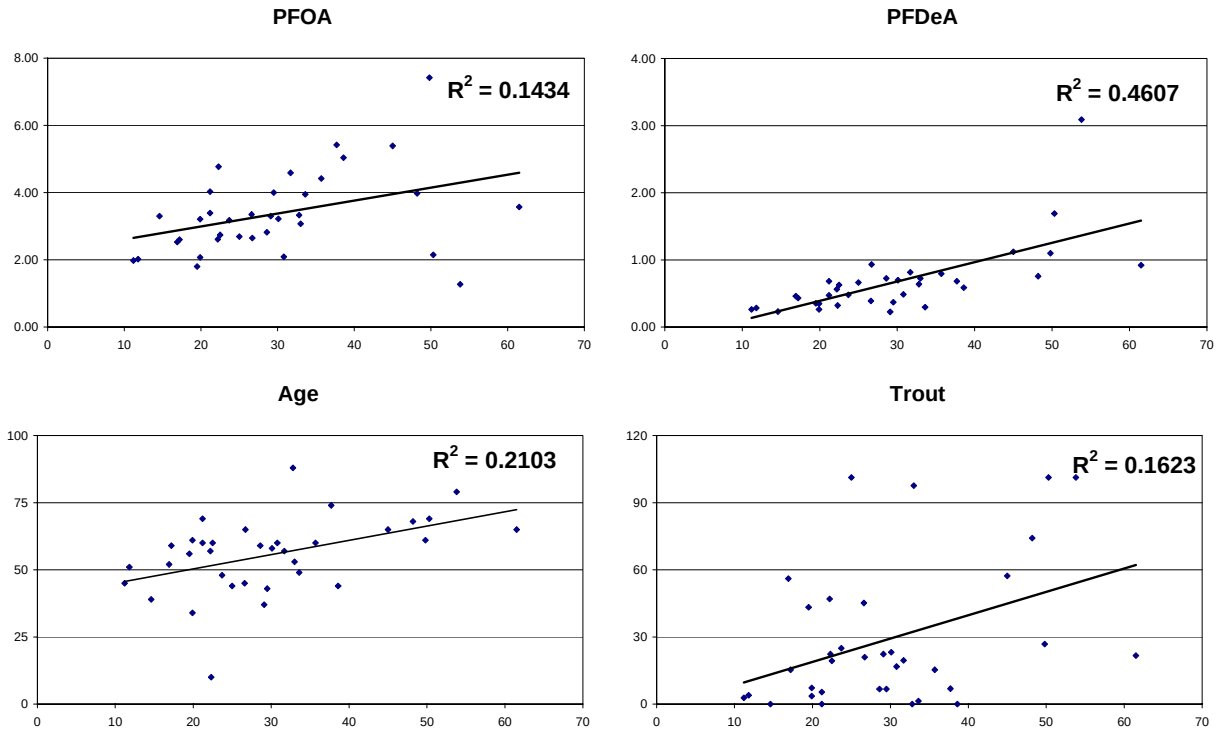

Figure 2. The relationship of the serum concentration of PFOS in ng/mL (X-axis) in the 35 samples and the concentration of PFOA, PFDeA, age of the subjects and their intake of trout in g/day (Y-axis).

Fish consumption

Trout was by far the fish the participants had consumed most frequently, followed by perch and pike. From the questionnaire consumption data, the estimated mean intake of trout was 32.8 g/day (median 21.7g/day), with a quite wide range (1.4 -101g/day). For comparison, consumption of freshwater fish in Norway is, in general, low and the median intake of those who eat such fish is 4.4 g/day. The trout in this lake has previously been shown to be relatively highly contaminated with PCBs and BFRs[13]; PFCs have also been reported, although at background levels[14]. The blood levels of PCBs and BFRs of the participants in this study have been shown to be significantly and strongly correlated to the intake of trout[15], also while taking the age and gender into consideration. As can been seen from Figure 2, the serum PFOS concentrations are also correlated to the participants age and intake of trout from the lake, indicating that fish consumption might be an exposure source for PFCs. Adjusted statistical evaluations of the serum levels of the individual PFCs and corresponding data from the questionnaires will be performed to identify factors that influence the observed variation in concentrations.

To summarise, this study on 35 hobby fishermen and women, shows a clear association between the concentrations of PFOS in serum and the subjects' age and intake of freshwater fish. PFOS was the PFC found at highest concentration in all samples, and concentrations of most of the PFCs were strongly correlated.

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