

CIRCULATING LEVELS OF PERFLUORINATED ALKYL SUBSTANCES (PFAS) IN PLASMA FROM ELDERLY MEN AND WOMEN FROM SWEDEN AND THE INFLUENCE OF GENDER

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Introduction

Perfluoroalkyl substances (PFASs) are used in a wide variety of industrial and commercial applications[1]. PFASs are widely distributed and can be found in all environmental compartments worldwide. In humans perfluoroalkyl sulfonates (PFASs) such as perfluorooctane sulfonic acid (PFOS) and perfluorohexane sulfonic acid (PFHxS) are typically found in the highest concentrations[2]. While among the perfluoroalkyl carboxylates (PFCAs) perfluorooctanoic acid (PFOA) and perfluorononanoic acid (PFNA) are commonly detected in the highest concentrations [2]. Some PFASs and PFCAs, such as PFOS and PFOA, are persistent and remain in humans for years. Previous animal studies have pointed towards a gender specific difference in the ability to eliminate PFOA[3]and it was shown that the half-life of PFOA was higher among the male rats resulting in higher PFOA concentrations in male rats. In humans reports on gender differences in PFASs levels has been conflicting and both significant gender differences in PFASs concentrations have been observed[4, 5], however, there are also studies in which gender differences were absent [6]. Further studies are required to understand possible gender differences in exposure to PFAS among men and women from the general population and subsequent toxicological effects.

The aim of this study was to determine a broad range of PFCAs and PFASs including structural PFOS isomers in 1 016 (50% women) participants from the Prospective Investigation of the Vasculature in Uppsala Seniors (PIVUS) cohort and assess the concentrations of selected fluorinated compounds from a gender perspective.

Materials and methods

Participants

The participants in the PIVUS study were randomly selected from the general population in the community of Uppsala, Sweden. Invitation letters were sent between April 2001 and June 2004 and within two months of each of the participants 70th birthday. The target sample was 2, 025 participants out of which 1, 016 participated and overall participation rate was estimated at 50%.

Sample collection

Blood serum and plasma were collected in the morning after an overnight fast over-night fast. After the blood plasma samples were collected (1-2 mL vials), the vials were placed in freezers (-20 °C) until used for chemical analysis. The study was approved by the Ethics Committee of the University of Uppsala and the participants gave written informed consent.

Chemicals

Ammonium acetate (>99%, p.a. for HPLC), methanol (HPLC grade) and water (HPLC grade) were purchased from Fluka (Steinheim, Germany). Acetonitrile (HPLC) was purchased from Labscan (Dublin, Ireland). Formic acid (98-100%) was purchased from Scharlau (Barcelona, Spain). Ammonium hydroxide (25% in water). ¹³C-labeled internal standard mix (PFCAs and PFASs in methanol) and ¹³C-labeled recovery standards (PFCAs and PFASs in methanol) were acquired from Wellington Laboratories (Guelph, Ontario, Canada).

Sample preparation

Briefly, frozen serum was allowed to thaw at room temperature and then vortex mixed for 10 s. Internal standards and 150 µL serum were loaded onto the Ostro Sample Preparation 96-well plate 25mg (Waters) followed by 450 µL acetonitrile with 1% formic acid. The sample was mixed thoroughly by aspirating three

times with a pipette. Samples were filtered using a 15□ vacuum manifold for 5-10 minutes. The extract was transferred to vials containing recovery standard and evaporated down to 250 μL using nitrogen. Finally, 750 μL 0.1 M formic acid in water was used to dilute the sample prior to the instrumental analysis.

Instrumental analysis

Analyses were performed on a UPLC-MS/MS system (Waters Premier, Waters Manchester) by injecting a 250 μL aliquot of the sample onto a C18 (2.1×20mm, 2.5 μm) trap column connected to a C18 (2.1×100 mm, 1.7 μm) analytical column by a 6-port column switch valve. Analytes were analyzed on a MS/MS system run in electrospray ionization mode (ESI). Quantitative analysis of the PFASs was performed using the internal standard method and a matrix matched calibration using bovine serum.

Quality assurance and quality control

The method precision and accuracy were validated using standard reference material (SRM) 1957 serum samples (n=54) obtained from the National Institute for Standards and Technology (NIST) and a well characterized in-house quality control (QC) reference plasma sample (n=103).

Statistical analyses

To evaluate possible gender differences in POPs concentrations the final dataset was analyzed using Wilcoxon's rank-sum (Mann-Whitney) test. Differences for which $p < 0.05$ and < 0.01 were considered statistically significant. Multiplicity was adjusted using Holm's method (Holm, 1979).

Results and discussion

The method showed good linearity ($R^2 < 0.995$) at concentrations ranging from 0.01 to 60 ngmL⁻¹ and low method detection limits (MDLs) ranging between 0.01-0.17 ngmL⁻¹ depending on the analyte. The precision of the developed method was good, with within-run (n=7) and between-run (n=103) coefficients of variation between 2% and 20% for most compounds including PFOS (2%, 8%) and its structural isomers (2-6% and 4-8%). The method showed good conformity with NIST SRM 1957. The analytical method employed to all plasma samples was successfully validated in terms of recovery, precision, and reproducibility.

In total, 1016 participants, 509 women and 507 men were successfully analysed for PFASs and the results are presented in Table 1. A wide range of the studied PFASs with varying concentrations were observed in the PIVUS cohort. The detection rates for all compounds were relatively high depending on the analytes and ranged from 19-100% of the samples (see Table 1).

From Table 1 can be seen that all analytes were detected in more than 50% of the samples, except for perfluorotridecanoic acid (PFTTrDA), perfluorobutane sulfonic acid (PFBS), and perfluorodecane sulfonic acid (PFDS) that were detected in 34%, 16%, and 24% of the samples, respectively. A wide range of concentrations were detected in the cohort. Overall, the concentrations detected are comparable to the concentrations found in other studies from Sweden [7] and Norway [6].

Isomer specific analysis of structural PFOS isomers was performed to explore the possibilities of identifying branched PFOS isomers and possible sources of exposure. Isomer specific quantification is reported here for 25 samples. Branched PFOS isomers were observed in all samples quantified and the isomeric pattern is illustrated in Figure 1.

Table 1. Concentrations of selected PFAS including structural PFOS isomers in plasma samples from men and women from the PIVUS cohort

Analyte	PIVUS n=1 016			
	Concentration (ngmL ⁻¹)			Detection rate (%)
	25th	Median	75th	
PFHpA	0.047	0.067	0.106	75
PFOA	2.55	3.33	4.39	99
PFNA	0.530	0.713	0.974	99
PFDA	0.261	0.325	0.411	90
PFUnDA	0.257	0.286	0.400	82
PFDoDA	0.036	0.039	0.056	36
PFTTrDA	0.035	0.048	0.066	43
PFBuS	0.045	0.085	1.00	19
PFHxS	1.61	2.08	3.45	99
L-PFOS	10.1	13.4	17.8	99
PFOSA	0.078	0.115	0.172	84
PFOS isomers	PIVUS sub-sample n=25			
	Concentration (ngmL ⁻¹)			Detection rate (%)
	25th	Median	75th	
L-PFOS	7.90	11.8	15.4	100
1-PFOS	0.377	0.402	0.631	100
6/2-PFOS	1.42	1.85	2.28	100
3/4/5-PFOS	2.34	2.75	3.50	100
4.4/4.5/5.5-PFOS	0.077	0.095	0.113	100
∑PFOS	12.7	17.3	21.6	100

The isomer pattern of PFOS showed similarities to patterns previously observed in Sweden and Australia in which the branched isomers constituted to about 30-42% of the total PFOS [8]. But as shown in Figure 1, there were large interindividual variations in the isomer pattern which opens the way to discriminate the various sources of exposure. The inter-individual variations might both be due to the differences in exposure or biological factors and need to be further elucidated.

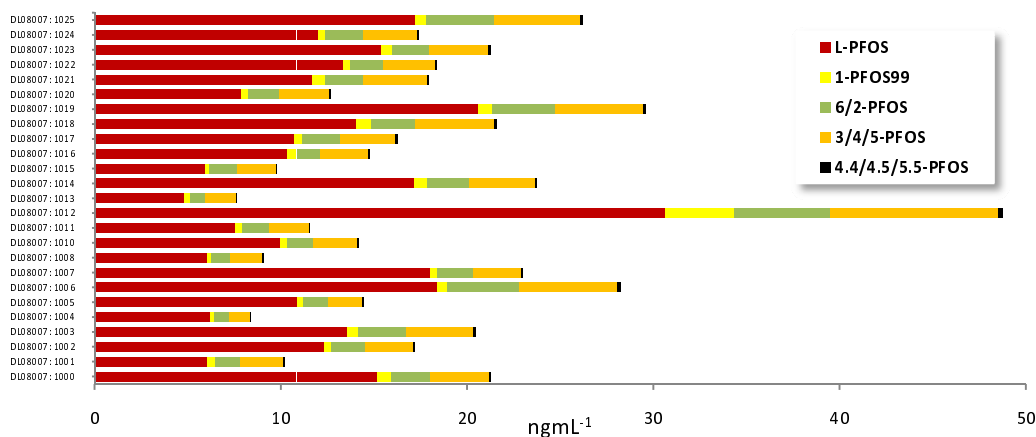
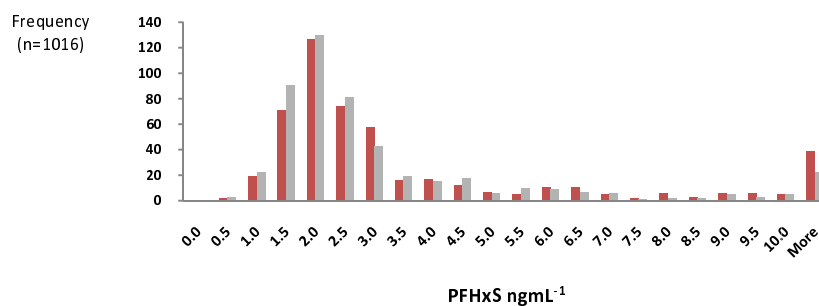
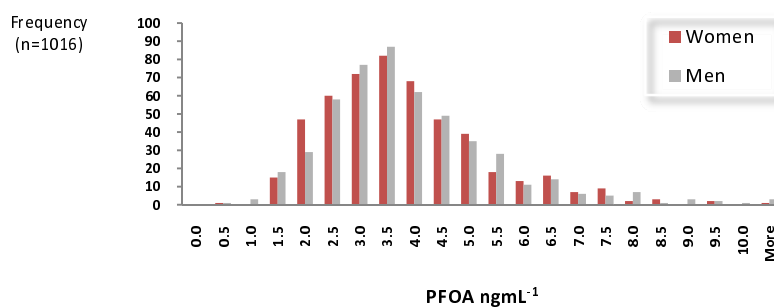


Figure 1. The pattern of structural PFOS isomers in 25 men and women from Sweden.

Analyses of gender differences were performed for each of the PFCAs and PFASs included in the study. The results from the analyses show significant gender differences for only three out of the fourteen studied PFAS. More specifically, the PIVUS men were found to have significantly ($p < 0.01$) higher plasma concentrations of perfluoroheptanoic acid (PFHpA) and L-PFOS when compared to the PIVUS women. On the other hand, the PIVUS women were found to have significantly ($p < 0.05$) higher levels of PFHxS in concordance with a previous study of residents from Catalonia [4]. However, in contrast to the study of Catalonian residents which observed higher concentrations of PFOA in women [4], there were no significant gender differences in the concentrations of PFOA among the men and women in the PIVUS cohort. No significant differences were observed for all the other PFAS compounds.



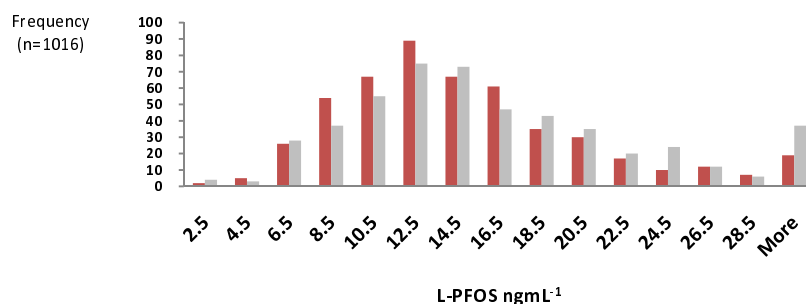


Figure 2. Histogram showing the distribution of PFOA, PFHxS, and L-PFOS in relation to gender.

References

- [1] A.B. Lindstrom, M.J. Strynar, E.L. Libelo, *Environmental Science and Technology*, 45 (2011) 7954-7961.
- [2] K. Kato, L.-Y. Wong, L.T. Jia, Z. Kuklennyik, A.M. Calafat, *Environ Sci Technol*, 45 (2011) 8037-8045.
- [3] N. Kudo, M. Katakura, Y. Sato, Y. Kawashima, *Chem-Biol Interact*, 139 (2002) 301-316.
- [4] I. Ericson, M. Gómez, M. Nadal, B. van Bavel, G. Lindström, J.L. Domingo, *Environ Int*, 33 (2007) 616-623.
- [5] A. Karrman, J.F. Mueller, B. Van Bavel, F. Harden, L.M.L. Toms, G. Lindstrom, *Environ Sci Technol*, 40 (2006) 3742-3748.
- [6] L.S. Haug, C. Thomsen, G. Bechert, *Environ Sci Technol*, 43 (2009) 2131-2136.
- [7] A. Glynn, U. Berger, A. Bignert, S. Ullah, M. Aune, S. Lignell, P.O. Darnerud, *Environ Sci Technol*, 46 (2012) 9071-9079.
- [8] A. Kärman, I. Langlois, B. van Bavel, G. Lindstrom, M. Oehme, *Environ Int*, 33 (2007) 782-788.