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Levels of perfluoroalkyl substances (PFAS) in individual serum samples from first-time mothers in Uppsala 2012 and 2014

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Sammanfattning

Sedan 1996 har Livsmedelsverket regelbundet samlat in blodprover från förstföderskor i Uppsala för analys av persistenta halogenerade organiska miljöföroreningar (POP). Perfluorerade alkylsyror (PFAA) är en sådan substansgrupp. I vårt projekt undersöks hur exponering för PFAA från dricksvatten påverkar tidstrender av PFAA i blod hos exponerade kvinnor i Uppsalaområdet och om åtgärder för att rena dricksvattnet ger en önskad sänkning av blodhalterna av PFAA. Eftersom individuella prover analyseras kan tidstrender bland kvinnor som druckit förorenat vatten jämföras med trender bland kvinnor som inte druckit förorenat vatten. Individuella prover möjliggör också undersökning av vilka livsstilsfaktorer som påverkar PFAA-halten i kvinnornas blod. I följande rapport redovisas halterna PFAA i individuella blodserum insamlade 3 veckor efter förlossningen 2012 och 2014. I en kommande rapport redovisas resultaten från hela tidstrendsstudien 1996-2016. Sulfonsyrorna PFOS och PFHxS var de PFAA som förelåg i högst halter bland kvinnorna 2012 och 2014. Den relativt höga halten av PFHxS beror på att dricksvattnet i Uppsala varit förorenad med denna förening. Bland karboxylsyrorna minskade blodhalten med ökat antal kol i kolkedjan på PFAA-molekylen och PFOA förelåg i högst halter. Halterna av PFOS var positivt korrelerade med halter av så kallade långkedjiga karboxylsyror, sannolikt beroende på att fiskkonsumtion är en viktig gemensam källa för exponering. PFOS-halterna var inte korrelerade med PFHxS-halterna beroende på att dricksvatten har varit en signifikant källa för PFHxS men inte för PFOS.

Levels of perfluoroalkyl substances (PFASs) in individual serum samples from first-time mothers in Uppsala 2012 and 2014

Background

Manufacturing of perfluoroalkyl substances (PFASs) occurs globally and started over five decades ago. The major uses of PFASs include industrial processes (e.g. production of fluoropolymers), water and stain proofing agents, lubricants, paints and fire-fighting foams (Kissa 2001; Prevedouros et al. 2006). Perfluoroalkyl acids (PFAAs), with fully fluorinated carbon backbones, have been found globally in wildlife and in humans and are environmentally persistent (Giesy and Kannan 2001; Kissa 2001; Kannan et al. 2004; Houde et al. 2006).

For two of the most widely distributed PFASs, perfluorooctane sulfonic acid (PFOS) and perfluorooctanoic acid (PFOA), measures have been taken to decrease production and use. PFOS and related substances were phased out by their major manufacturer in 2002, and added to Annex B of the Stockholm Convention on Persistent Organic Pollutants in 2009. Several major manufacturers have also committed to reducing and eliminating the use of PFOA in products by 2015. These changes have resulted in a reduction of PFOS and PFOA levels in the blood of humans in most parts of the world. In Sweden, a study of PFAS temporal trends in pooled blood serum from first-time mothers in Uppsala County (POPUP study, 1996-2010), showed that levels of PFOS and PFOA had declined during the study period, with a faster decline of PFOS (8% per year) compared to PFOA (3% per year) (Glynn et al. 2012). However, not all PFASs are showing a decline. Temporal increases in levels of shorter-chained PFASs, including perfluorobutane sulfonic acid (PFBS) and perfluorohexane sulfonic acid (PFHxS), were observed (about 10% per year), as well as increases of longer-chained PFAS perfluorononanoic acids (PFNA), perfluorodecanoic acid (PFDA), perfluoroundecanoic acid (PFUnDA), perfluorododecanoic acid (PFDoDA) and perfluoorotridecanoic acid (PFTrDA) (around 4% per year) (Glynn et al. 2012; Gebbink et al. 2015). A follow-up study of PFAS temporal trends up to 2014 strongly indicates that the increasing trends of PFHxS and the long-chain PFAS in human serum are levelling off (Glynn et al. 2015), suggesting that exposure is not increasing anymore.

Despite showing indications of reduced exposure to certain PFASs, the aforementioned studies are limited by the use of pooled samples. Pooled samples do not provide information about the variation in exposure within a studied population, which is important for risk assessment purposes. Moreover, in the case of the POPUP study, some participants lived in areas with PFAS-contaminated drinking water, whereas others did not. Consequently it is likely that temporal trends of some PFASs differ depending on where the participants lived. Data from individual samples also makes it possible to identify dietary/life style factors that may explain some of the observed variation in exposure, thus giving information about possible sources of exposure.

The aim of the present study was to assess the variation in blood serum PFAS levels among the participants of the POPUP study recruited 1996-2014, to determine if there are differences in temporal trends of PFAS depending on area of living, and to examine the dietary/lifestyle factors that explains at least some of the variation in serum PFAS levels. In this first report, results from 2012 and 2014 are presented. In a second report in 2017 we will provide the full results of the findings from 1996-2016.

Material and methods

Recruitment and sampling

In the POPUP study (Persistent Organic Pollutants in Uppsala Primiparas), first-time mothers from the general population living in Uppsala County were recruited between 1996 and 2014 as described in Glynn et al. (2012). The participants donated a blood sample 3 weeks after delivery. Blood sampling was carried out using 9 ml Vacutainer® or Vacuette® serum tubes, and serum was stored at -20°C. The study was approved by the local ethics committee of Uppsala University, and the participating women gave informed consent prior to the inclusion in the study. In the present study we used individual samples collected 2012 and 2014 (Table 1).

Table 1. Personal characteristics of the participating mothers.

Variable		N	Mean	Median	Range
Age (yr)		60	29	30	20-38
Pre-pregnancy BMI (kg/m^2)		60	23.7	23.2	17.4-32.6
Weight gain during pregnancy (% of initial weight)		60	23.3	22.6	5.33-43.8
Weight reduction from delivery to sampling (%)[a]		60	8.71	8.63	2.15-15.4
Variable		**N**	**%**		
Education	**max 3-4 yr high school**	14	23		
	1-3 yr higher education	13	22		
	>3 yr higher education	33	55		
Smoking[b]	**non-smoker**	37	62		
	former smoker	19	32		
	smoker	4	7		

PFAS analyses

PFASs (Table 2) were analyzed as described in Gyllenhammar et al. (2015). In short, 0.5g of serum was spiked with internal standards and extracted with acetonitrile. The concentrated extract underwent dispersive clean-upon with graphitized carbon. Aqueous ammonium acetate and volumetric standards were added before instrumental analysis on an Acquity ultraperformance liquid chromatography system (UPLC) coupled to a Xevo TQ-S tandem mass spectrometer (MS/MS) (both from Waters Corp., Milford, MA, U.S.) operated in negative electrospray ionization, multiple reaction monitoring mode.

The instrumental method including optimized parameters is described in detail in Vestergren et al. (2012b). Quantification was performed by isotope dilution using a 5-point calibration curve (linear, 1/x weighting, excluding the origin) which was run before and after samples. For most targets, exactly matched isotopically labelled internal standards were available. For PFBS, PFTriDA, PFTeDA, and PFPeDA, a structurally similar internal standard was used (Table 2). For PFHxS and PFOS, branched and linear isomers were quantified separately. The Method quantification limits (MQLs) were 0.02 ng/g serum for PFHxA; 0.03 ng/g for PFHpA; 0.3 ng/g for PFOA; 0.01 ng/g for PFNA, PFDA, PFUnDA, and PFDoDA; 0.03 ng/g for PFTriDA, 0.1 ng/g for PFTeDA; 0.4 ng/g for PFPeDA, 0.15 ng/g for PFBS, 0.06 ng/g for PFHxS; and 0.1 ng/g for PFOS.

Table 2. PFAS included in the study.

Substance	No of carbons in fluorinated chain	Abbreviation	Internal Standards
Perfluoroalkyl sulfonic acids (PFSA)			
Perfluorobutane sulfonic acid	4	PFBS	$^{18}O_2$-PFHxS
Perfluorohexane sulfonic acid[a]	6	PFHxS	$^{18}O_2$-PFHxS
Perfluorooctane sulfonic acid[a]	8	PFOS	$^{13}C_4$-PFOS
Perfluoroalkyl carboxylic acids (PFCA)			
Perfluorohexanoic acid	5	PFHxA	$^{13}C_2$-PFHxA
Perfluoroheptanoic acid	6	PFHpA	$^{13}C_4$-PFHpA
Perfluorooctanoic acid	7	PFOA	$^{13}C_4$-PFOA
Perfluorononanoic acid	8	PFNA	$^{13}C_5$-PFNA
Perfluorodecanoic acid	9	PFDA	$^{13}C_2$-PFDA
Perfluoroundecanoic acid	10	PFUnDA	$^{13}C_2$-PFUnDA
Perfluorododecanoic acid	11	PFDoDA	$^{13}C_2$-PFDoDA
Perfluorotridecanoic acid	12	PFTriDA	$^{13}C_2$-PFDoDA
Perfluorotetradecanoic acid	13	PFTeDA	$^{13}C_2$-PFDoDA
Perfluoropentadecanoic acid	14	PFPeDA	$^{13}C_2$-PFDoDA

[a]Branced and linear isomers

A procedural blank extraction was performed with every batch of samples. MQLs were defined based on the quantified background contamination signals. In the absence of procedural blank contamination MDLs were defined as the lowest concentration in a serum sample giving a chromatographic signal with a signal-to-noise ratio of 3. Absolute recoveries of the internal standards (determined relative to $^{13}C_4$-PFOA) were on an average between 60% and 69%. Further method validation parameters are provided in Glynn et al. (2012).

Results and discussion

Concentrations were below the MQL in the majority of samples in the case of PFBS (0.15 ng/g serum), PFHxA (0.02), PFHpA (0.03), PFTeDA (0.1) and PFPeDA (0.4). Among the PFSAs, branched PFHxS were present at lowest concentrations and linear PFHxS and PFOS at the highest concentrations (Table 3). The median total PFOS concentration was 1.6-fold higher than the median total PFHxS concentration. In comparison, a nation-wide survey of PFAS concentrations in blood serum from adults in Sweden (2010-11) revealed median PFOS concentrations about 5-fold higher than median PFHxS concentrations (Bjermo et al. 2013). The relative differences in average PFOS and PFHxS concentrations between the two studies

are mainly due to PFHxS contamination of drinking water in Uppsala (Gyllenhammar et al. 2015). This contamination resulted in an increased temporal trend of PFHxS in blood serum between 1996 and 2014 among young women (Glynn et al. 2015). The concomitant temporal decrease in PFOS concentrations has consequently resulted in nearly equivalent average PFHxS and PFOS concentrations in Uppsala from 2012-2014.

Table 3. Concentrations of perfluoroalkyl sulfonic acids (PFSA) and perfluoroalkyl carboxylic acids (PFCA) (ng/g) in individual serum samples from nursing primiparous women in Uppsala, Sweden.

Substance	**N**	**N<MQL**	**Mean**	**Median**	**Range**
PFHxS (total)	60	0	4.2	3.5	0.53-13
(linear)	60	0	4.0	3.3	0.50-12
(branched)	60	7	0.22	0.16	<0.05-1.1
PFOS (total)	60	0	5.9	5.7	2.4-16
(linear)	60	0	4.2	4.0	1.8-14
(branched)	60	0	1.7	1.5	0.55-5.0
PFOA	60	0	1.8	1.7	0.71-3.8
PFNA	60	0	0.57	0.51	0.22-1.5
PFDA	60	7	0.22	0.19	<0.01-1.3
PFUnDA	60	4	0.26	0.24	<0.01-1.3
PFDoDA	60	24	0.032	0.024	<0.01-0.24
PFTriDA	60	8	0.036	0.029	<0.01-0.19

PFCAs were dominated by PFOA and there was a tendency of decreasing levels with increasing chain length of the homologues (Table 3). This is in agreement with the survey of PFAS levels among Swedish adults from 2010-11 (Bjermo et al. 2013).

Correlation analyses (Pearson) showed that total PFOS concentrations were significantly correlated with concentrations of long-chain PFCAs, with the exception of PFDoDA (Table 4). Almost 50% of the PFDoDA levels were below the MQL, which made the correlation results for this homologue uncertain. Fish consumption, as a main source of PFOS, PFNA, PFDA, PFUnDA and PFTriDA in Sweden, may contribute to the positive correlations among these homologues (Vestergren et al. 2012; Bjermo et al. 2013).

Table 4. Correlations between PFAA concentrations in individual serum samples from nursing primiparous women in Uppsala, Sweden. Statistically significant correlations ($p \leq 0.05$) in bold italics.

	Lin-PFHxS	Tot-PFHxS	Br-PFOS	Lin-PFOS	Tot-PFOS	PFOA	PFNA	PFDA	PFUnDA	PFDoDA	PFTriDA
Br-PFHxS	***0.84***	***0.86***	***0.37***	0.08	0.18	***0.27***	-0.089	-0.043	0.022	0.13	-0.017
Lin-PFHxS		***1.00***	***0.46***	0.19	***0.29***	0.22	-0.097	0.001	0.053	0.078	-0.002
Tot-PFHxS			***0.46***	0.19	***0.29***	0.23	-0.097	-0.002	0.051	0.082	-0.003
Br-PFOS				***0.71***	***0.85***	***0.44***	***0.34***	0.18	0.076	-0.066	0.051
Lin-PFOS					***0.97***	***0.40***	***0.70***	***0.66***	***0.60***	-0.082	***0.45***
Tot-PFOS						***0.44***	***0.63***	***0.55***	***0.47***	-0.083	***0.35***
PFOA							***0.47***	***0.33***	0.013	0.24	0.17
PFNA								***0.67***	***0.53***	-0.093	***0.34***
PFDA									0.67	-0.013	***0.37***
PFUnDA										-0.057	***0.52***
PFDoDA											***0.29***

The significant positive correlation between PFOS and PFOA concentrations is probably due to common sources of exposure other than fish (Vestergren et al. 2012; Glynn et al. 2013). Half-lives of PFOS and PFOA are long, estimated at 4-5 years (Olsen et al. 2007). Common historical sources of exposure, for example from consumer products in use prior to the phase-out of PFOS and PFOA, may contribute to the positive correlation.

PFHxS and PFOS concentrations were not correlated, except for a weak but significant positive correlation among branched isomers (Table 4). In Uppsala PFAS contamination of drinking water has resulted in clearly elevated concentrations of PFHxS, but not of PFOS, in serum of young women living in areas with contaminated water. This in spite of the fact that the drinking water has been contaminated with both PFHxS and PFOS at similar levels (about 2-fold higher PFHxS levels) (Gyllenhammar et al. 2015). The lack of correlation between PFHxS and PFOS concentrations strongly suggests that sources of PFOS exposure other than drinking water are important in Uppsala.

The significant positive correlation between the branched isomers of PFOS and PFHxS may be due to drinking water being a dominant common source of exposure to these isomers in Uppsala. The proportions of branched PFOS in the contaminated Uppsala drinking water was estimated to about 50% (Gyllenhammar et al. 2015), which is higher than the proportion in the technical mixture from the major PFOS manufacturer 3M (30%) (Benskin et al. 2010). The higher proportion of branched PFOS in drinking water in Uppsala may be due to a higher mobility of branched than of linear isomers in ground water from the point source of contamination.

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