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**Levels of poly- and perfluoroalkyl substances (PFAS) in
individual serum samples from first-time mothers in
Uppsala, Sweden: results from year 2020-2022, and
temporal trends for the time period 1996-2022**

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Levels of poly- and perfluoroalkyl substances (PFAS) in individual serum samples from first-time mothers in Uppsala, Sweden: results from year 2020-2022, and temporal trends for the time period 1996-2022

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<p>Rapporttitel Levels of poly- and perfluoroalkyl substances (PFAS) in individual serum samples from first-time mothers in Uppsala, Sweden: results from year 2020-2022, and temporal trends for the time period 1996-2022</p>	<p>Beställare Naturvårdsverket 106 48 Stockholm</p> <p>Finansiering Nationell hälsorelaterad miljöövervakning</p>
<p>Nyckelord för plats Uppsala, Sverige</p>	
<p>Nyckelord för ämne Perfluorerade alkylsyror, PFCA, PFSA, serum, kvinnor</p>	
<p>Tidpunkt för insamling av underlagsdata 1996-2022</p>	
<p>Sammanfattning</p> <p>Sedan 1996 har Livsmedelsverket regelbundet samlat in blodprover från förstfödorskor i Uppsala för analys av persistenta organiska miljöföroreningar (POP). Poly- och perfluorerade alkylsyror (PFAS) är en sådan substansgrupp. I följande rapport redovisas halter av PFAS i serum från förstfödorskor provtagna 2020-2022 samt tidstrender för perioden 1996-2022. PFOS förekommer i högst halt i serum följt av PFHxS och PFOA. Ca 60 % av förstfödorskorna, provtagna 2020-2022, hade serumhalter över den nivå som är önskvärd hos mammor för att skydda barnet mot hög exponering under foster- och amningsperioden. Resultaten visar att storskalig utfasning runt millenniumskiftet av PFOS och PFOA internationellt har resulterat i minskande exponering i befolkningen. Från 2000/2001 minskade halterna först årligen med 13 % för PFOS och 7 % för PFOA fram till år 2010, därefter har minskningen gått långsammare, i medeltal 5 % under perioden 2011-2022. På grund av dricksvattenföroreningar av PFAS i Uppsala har serumhalterna av PFHxS ökat i början av studien hos förstfödorskorna. Halterna låg som högst 2004-2011. Efter 2012, då åtgärder sattes in för att sänka halten PFAS i dricksvattnet har halterna i serum minskat med i medeltal 10 % per år. För PFNA, PFDA och PFUnDA sågs en ökande trend av serumhalter i början av studien fram till 2006-2008 men därefter sjunker halterna med i medeltal 2-4 % per år. Kvinnor med högst utbildning (mer än 3 år eftergymnasial utbildning) hade högre PFAS-halter än kvinnor med lägst utbildning (gymnasieskola). Störst skillnad observerades för PFHxS som till viss del sannolikt beror på att kvinnor med högst utbildning i högre grad bott inne i Uppsala stad och därigenom fått högre exponering via dricksvattnet, än kvinnor med lägst utbildning som bott längre från stadskärnan. Serumhalterna ökade med 1-2 % per år i ökande ålder för alla PFAS utom PFOA och PFDA, vilket antyder att äldre kvinnor hade något högre halter av dessa PFAS än yngre kvinnor. Det är viktigt att fortsätta följa trender av PFAS i POPUP för att se om exponeringen fortsätter att minska,</p>	

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INTRODUCTION

The Swedish Food Agency has conducted recurrent sampling of breastmilk and blood from primiparous women in Uppsala since 1996, in the so-called POPUP study (Persistent Organic Pollutants in Uppsala Primiparas). The Swedish Environmental Protection Agency has funded the study since the year 2000. The main aim of the study is to estimate the body burdens of persistent organic pollutants (POP) among pregnant and nursing women and to estimate temporal trends of the exposure of fetuses and infants. The following report presents results of analyses of per- and polyfluorinated substances (PFAS) in serum samples from the mothers.

PFAS have a wide range of applications and have been manufactured world-wide for many decades. Today thousands of PFAS are known to exist on the global market. Since the start of the 21st century, measures have been taken to reduce emissions of the most widely distributed PFAS, perfluorooctanesulfonate (PFOS) and perfluorooctanoate (PFOA). Within the EU, PFOS was regulated in 2008 and PFOS, PFOA and perfluorohexanesulfonate (PFHxS) (and their related compounds) were listed on the Stockholm Convention in 2009, 2019 and 2022, respectively. Humans are exposed to PFAS mainly via food and drinking water due to environmental contamination, but also via dust, air, and the use of products containing PFAS (EFSA 2020; Poothong et al. 2020). In Uppsala, drinking water has been contaminated with PFAS since at least 1996, due to the use of fire-fighting foams at a nearby military airport, resulting in elevated PFAS serum levels in mothers and children in the POPUP study (Gyllenhammar et al. 2015, Gyllenhammar et al. 2019). PFAS are also transferred through the placenta and into breastmilk, which represent important exposure routes for PFAS early in life (Gützkow et al. 2012, Gyllenhammar et al. 2018, Mondal et al. 2012).

Temporal trends of PFAS in first-time mothers have been published for earlier periods in pooled samples (Glynn et al. 2012; Gebbink et al. 2015; Gyllenhammar et al. 2017; Miaz et al. 2020) and individual samples (Glynn et al. 2017; Gyllenhammar et al. 2020) from the POPUP-study. The following report presents results of PFAS analyses in individual serum samples from first-time mothers sampled in 2020-2022 (according to agreement 215-21-003). The new data were used to establish updated temporal trends for the period 1996-2022.

MATERIALS AND METHODS

Recruitment and sampling

In the POPUP study first-time mothers from the general population living in Uppsala County were recruited between 1996 and 2022 as described in Glynn et al. (2012). Participants were randomly recruited among first-time mothers who were Swedish by birth and delivered at Uppsala University Hospital. In total, 90 women were recruited between 2020 and 2022 and the participation rate was 47%. The participants donated a blood sample 3 weeks after delivery. Blood sampling was carried out using 10 ml Vacutainer® or Vacuette® serum tubes, and serum was stored at the Swedish Food Agency at -20°C. Data on age, weight, length, lifestyle etc. of the mothers were obtained from questionnaires. The temporal trend study includes serum samples from in total 833 women sampled 1996-2022. 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.

PFAS analysis

PFAS (Table 1) 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-up with graphitized carbon. Aqueous ammonium acetate and recovery standards were added before instrumental analysis on an ultra-high pressure liquid chromatography system (UHPLC) coupled to a tandem mass spectrometer (MS/MS) operated in negative electrospray ionization, multiple reaction monitoring mode.

Quantification was performed by isotope dilution using an 8-point calibration curve (linear, 1/x weighting, excluding the origin) which was run before and after the 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. A procedural blank and control sample were included in each batch of samples. The samples were analyzed in different batches and the method quantification limits (LOQs) and detection limits (LODs) for the different analytical batches are provided in Table 1 and 2. Further method validation parameters are provided in Glynn et al. (2012).

Table 1. PFAS included in the study.

Compound	Abbreviation	IS	LOQ	LOD
Perfluorohexanoate	PFHxA	M4PFHpA	0.328	
Perfluoroheptanoate	PFHpA	M4PFHpA	0.328	
Perfluorooctanoate	PFOA	M4PFOA	0.328	0.241
Perfluorononanoate	PFNA	M5PFNA	0.328	0.113
Perfluorodecanoate	PFDA	M2PFDA	0.328	0.102
Perfluoroundecanoate	PFUnDA	M2PFUnDA	0.328	0.081
Perfluorododecanoate	PFDoDA	M2PFDoDA	0.328	
Perfluorotridecanoate	PFTriDA	M2PFDoDA	0.328	0.165
Perfluorotetradecanoate	PFTeDA	M2PFDoDA	0.328	
Perfluoropentadecanoate	PFPeDA	M2PFDoDA	0.328	
Perfluorobutanesulfonate	PFBS	18O2-PFHxS	0.290	
Perfluoropentanesulfonate	PFPeS	18O2-PFHxS	0.310	0.107
Perfluorohexanesulfonate lin.	lin-PFHxS	18O2-PFHxS	0.310	
Perfluorohexanesulfonate br.	br-PFHxS	18O2-PFHxS	0.310	0.105
Perfluoroheptanesulfonate	PFHpS	M4PFOS	0.313	0.101
Perfluorooctanesulfonate lin.	lin-PFOS	M4PFOS	0.313	
Perfluorooctanesulfonate br.	br-PFOS	M4PFOS	0.313	0.077
Perfluorononanesulfonate	PFNS	M4PFOS	0.313	
Perfluorodecanesulfonate	PFDS	M4PFOS	0.316	
Perfluorooctane sulfonamide	FOSA	M8FOSA	0.334	
Perfluorooctane sulfonamidoacetate	FOSAA	d3-MeFOSAA	4.092	
Ethyl perfluorooctane sulfonamidoacetate lin.	lin-EtFOSAA	d5-EtFOSAA	0.330	
Ethyl perfluorooctane sulfonamidoacetate br.	br-EtFOSAA	d5-EtFOSAA	0.330	
Methyl perfluorooctane sulfonamidoacetate lin.	lin-MeFOSAA	d3-MeFOSAA	0.335	
Methyl perfluorooctane sulfonamidoacetate br.	br-MeFOSAA	d3-MeFOSAA	0.335	
3:3 Fluorotelomer carboxylic acid	3:3 FTA (FPrPA)	M2PFHxA	13.935	
5:3 Fluorotelomer carboxylic acid	5:3 FTA (FPePA)	M4PFOA	0.328	
7:3 Fluorotelomer carboxylic acid	7:3 FTA (FHpPA)	M2PFDA	0.331	
4:2 Fluorotelomer sulfonate	4:2 FTS	M2 6:2 FTS	0.329	
6:2 Fluorotelomer sulfonate	6:2 FTS	M2 6:2 FTS	0.329	
8:2 Fluorotelomer sulfonate	8:2 FTS	M2 6:2 FTS	0.327	
Potassium 9-chlorohexadecafluoro-3-oxanonanoate-1-sulfonate	9Cl-PF3ONS	M2PFDA	0.328	
Potassium 11-chloroeicosafluoro-3-oxaundecane-1-sulfonate	11Cl-PF3OUdS	M2PFDA	0.328	
Dodecafluoro-3H-4,8,-dioxanonanoate	ADONA	M4PFOA	0.335	
6:2 Fluorotelomer phosphate diester	6:2 diPAP	M4 6:2/6:2 diPAP	0.338	
6:2/8:2 Fluorotelomer phosphate diester	6:2/8:2 diPAP	M4 8:2/8:2 diPAP	0.332	
8:2 Fluorotelomer phosphate diester	8:2 diPAP	M4 8:2/8:2 diPAP	0.330	

Table 2. Limits of quantification (LOQs) for the different analytical batches, analysed 2013-2022.

PFAS	Analytical batch									
	1	2	3	4	5	6	7	8	9	10 ^a
PFHpA	0.040	0.10	0.030	0.14	0.14	0.162	0.008	0.082	0.082	nd/0.328
PFOA	0.20	0.25	0.30	0.030	0.14	0.162	0.140	0.082	0.082	0.241/0.328
PFNA	0.050	0.10	0.010	0.030	0.040	0.162	0.008	0.082	0.082	0.113/0.328
PFDA	0.050	0.070	0.010	0.070	0.15	0.162	0.008	0.082	0.082	0.102/0.328
PFUnDA	0.050	0.050	0.010	0.020	0.060	0.036	0.008	0.082	0.082	0.081/0.328
PFDoDA	0.050	0.050	0.010	0.030	0.060	0.036	0.008	0.082	0.161	nd/0.328
PFTriDA	0.050	0.050	0.030	0.020	0.040	0.036	0.008	0.082	0.082	0.165/0.328
PFBS	0.010	0.010	0.15	0.090	0.10	0.186	0.007	0.279	0.072	nd/0.290
PFPeS	-	-	-	-	-	-	0.008	-	0.078	0.107/0.310
lin-PFHxS	0.010	0.10	0.060	0.020	0.040	0.036	0.008	0.227	0.078	nd/0.310
br-PFHxS	0.010	0.010	0.050	0.020	0.040	0.036	0.008	0.078	0.078	0.105/0.310
PFHpS	-	-	-	-	-	-	0.008	-	0.078	0.101/0.313
lin-PFOS	0.010	0.50	0.10	0.030	0.040	0.034	0.008	0.078	0.078	nd/0.313
br-PFOS	0.010	0.20	0.020	0.030	0.040	0.15	0.008	0.078	0.078	0.077/0.313

^aLOD/LOQ

- = not analysed

nd = not determined

Statistical analyses

Statistical analyses were performed using the software package STATA version 17.0. When serum concentrations were below LOD, levels were set to $\text{LOD}/\sqrt{2}$. Temporal trends were investigated for the study period 1996-2022. Linear regression models with sampling year modeled with restricted cubic splines (henceforth called cubic spline models) were used to analyse associations between log-transformed serum concentrations and sampling year. The temporal trends in Figure 1 were based on cubic spline models that were adjusted for the covariates maternal age, pre-pregnancy body mass index (BMI), weight gain during pregnancy (%), weight loss from delivery to time of sampling (%), education and smoking. The number of knots for the cubic spline bases were determined by fitting models with 3 to 7 knots and the model that provided the lowest Akaike information criterion (AIC) was preferred. The temporal trends expressed as mean percentage change per year in Table 5 were assessed using linear regression models with sampling year modeled with linear splines (henceforth called linear spline models) and adjustment for the same covariates and with specified knots. The specified knots were placed at the year after the year with the highest geometric mean levels compared to 1996 (when two years had the same level the last year of the two were chosen). For PFOA, PFOS and the sum of PFOA, PFNA, PFHxS and PFOS (PFAS4), also another specified knot was added at year 2011, as a plateau was observed for PFOA and PFAS4 around that year and

a change in decreasing rate for PFOS. For PFHxS, the time period 2012-2022 was also evaluated as PFAS drinking water contamination was discovered in Uppsala in July 2012 and after that measures were taken to mitigate the levels. The associations between PFAS levels and the covariates age, BMI, weight gain during pregnancy, weight loss after delivery, education level and maternal smoking were also assessed in the cubic spline models. As a consequence of the logarithmic transformation, the associations between sampling year and serum concentrations, and between covariates and concentrations, were presented as percent change of concentrations per year, and percent change per unit of the covariate, and not as change in absolute levels.

Table 3. Population characteristics of mothers sampled 2020-2022 (n=90).

Variable	Mean ±SD	Median	(Min-Max)
Age (year)	31.9 ± 3.4	31.6	(25.5-42.7)
Pre-pregnancy BMI (kg/m ²) ^{ab}	24.0 ± 4.3	23.3	(18.5-47.3)
Weight gain during pregnancy (%) ^b	21.1 ± 8.3	21.0	(0-50.8)
Weight reduction from delivery to sampling (%) ^b	8.4 ± 2.9	8.6	(2.1-14.7)
Variable	n	%	
Education	Max 3-4 years of high school	7	8%
	1-3 years of higher education	33	37%
	>3 years of higher education	50	56%
Smoking ^c	Non-smoker	77	86%
	Former smoker	11	12%
	Smoker	2	2%

^aBody mass index

^bn=88

^cWomen who stopped before pregnancy are considered to be former smoker. Women who smoked during pregnancy are defined as smoker even if they stopped during the first or second month of pregnancy.

RESULTS AND DISCUSSION

Characteristics of the first-time mothers sampled 2020-2022 are shown in Table 3. Among perfluoroalkyl carboxylic acids (PFCAs) the mean level in serum sampled 2020-2022 was highest for PFOA (0.98 ng/g serum) and declined in the order PFOA>PFNA>PFDA~PFUnDA (Table 4). For PFTriDA most women had serum levels below LOD (86 out of 90). All women had serum levels above LOD for the sulfonic acid lin-PFHxS, and except of one participants for lin-PFOS, and br-PFOS with median levels ranging from 1.2 to 2.8 ng/g (Table 4). For PFPeS and PFHpS, which were analysed in serum from the POPUP-mothers since 2017 only, results appeared consistent with the previous report, with most women having serum levels below LOD (Table 4). In all mothers, the levels of PFHxA, PFDoDA, PFTeDA, PFPeDA, PFBS, PFNS, PFDS, FOSA, FOSAA, lin-Et-FOSAA, br-Et-FOSAA, lin-MeFOSAA, br-MeFOSAA, FPrPA, FPePA, FHpPA, 4:2 FTS, 6:2 FTS, 8:2 FTS, 9Cl-PF3ONS, 11Cl-PF3OUdS, ADONA, 6:2 diPAP, 8:2 diPAP and 6:2/8:2 diPAP were below LOQ.

In September 2020, the European Food Safety Authority (EFSA) published a scientific opinion on health risks of PFAS in Food (EFSA 2020). In the risk assessment EFSA established a new tolerable weekly intake (TWI) of 4.4 ng/kg body weight/week for the sum of the four PFAAs; PFOA, PFNA, PFHxS, and PFOS. A serum level of 6.9 ng/mL was estimated to be the maternal body burden attained after a maternal life-time intake (35 yrs) at TWI before pregnancy. This serum level was considered safe and would not cause levels in the child that would be of health concern after pregnancy and 1 year of breastfeeding. Compared to the safe maternal level established by EFSA, a mean of 8.7 ng/mL and median of 7.6 ng/mL for PFAS4 was observed in the present study (Table 4). In total, 61% of the women had serum levels above the safe level during the period 2020-2022. A comparison to the EFSA safe serum limit for PFAS4 (EFSA TWI), for the whole period 1996-2022 are shown in Figure 1. The drinking water in Uppsala has in some areas of the city been contaminated with PFHxS, and to a lesser degree with PFOS, PFOA and PFBS (Gyllenhammar et al. 2015). The contamination of PFAS was discovered in 2012, however as the PFAS4 are known to have long half-lives (EFSA 2020), levels in Uppsala women may still be affected by the earlier contamination. Therefore, the results are probably not comparable to mothers in Sweden with normal/lower background exposure.

Table 4. Concentrations of PFAS (ng/g) in individual serum samples from nursing primiparous women in Uppsala County 2020-2022 (n=90).

PFAS	n<LOQ	n<LOD	Mean ^a	±SD	Median	Range
PFOA	5	2	0.98	0.66	0.86	<0.24-5.21
PFNA	22	9	0.53	0.31	0.49	<0.11-1.55
PFDA	69	28	0.22	0.16	0.21	<0.10-1.08
PFUnDA	73	36	0.20	0.18	0.17	<0.08-0.99
PFTriDA	90	86				<0.17-0.25
PFPeS	88	77				<0.11-0.43
lin-PFHxS	0		2.15	1.77	1.62	0.39-9.49
br-PFHxS	86	71	0.11	0.09	<0.11	<0.11-0.54
tot PFHxS			2.26	1.82	1.71	0.47-9.78
PFHpS	86	61				<0.10-0.50
lin-PFOS	1	1	3.53	3.08	2.77	<0.11-25.46
br-PFOS	2	1	1.36	0.92	1.19	<0.09-7.87
tot PFOS			4.88	3.95	4.04	0.21-33.34
PFAS4 ^b			8.65	4.64	7.59	2.49-35.86

^aWhen calculating means data below LOD was replaced with LOD/√2.

^bSum of the four PFAS: PFOA, PFNA, tot PFHxS, and tot PFOA

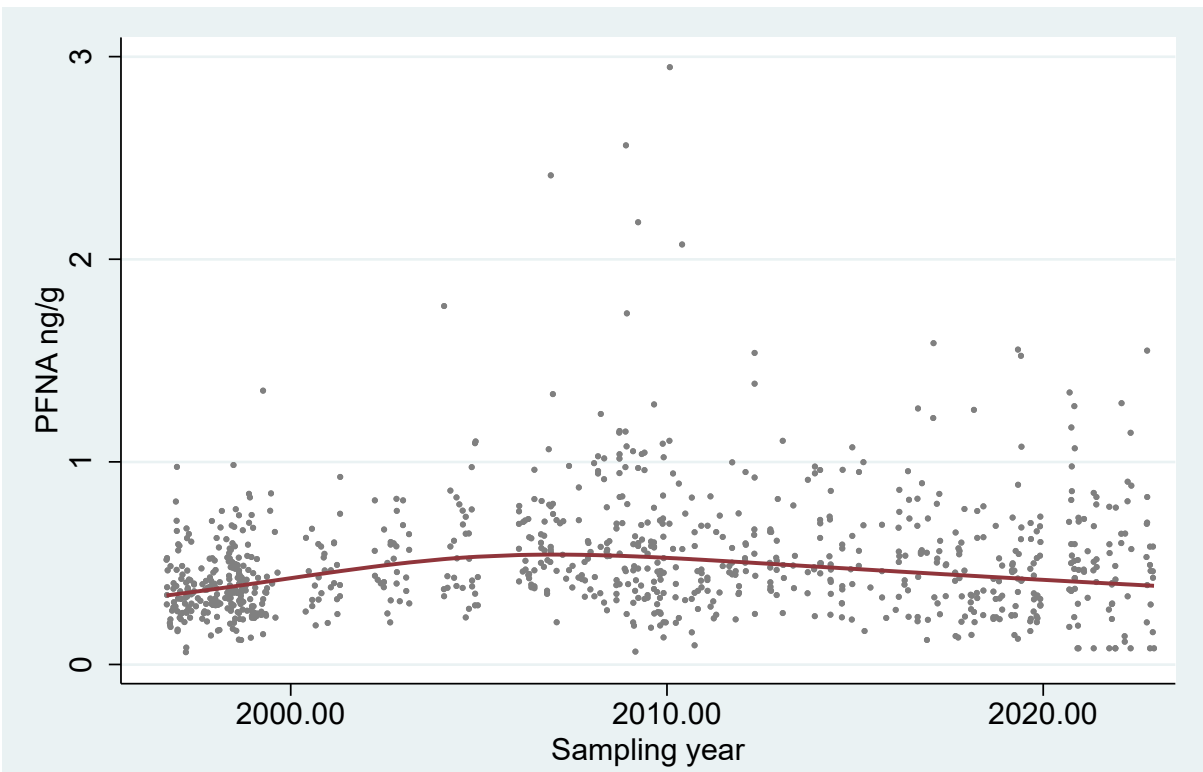
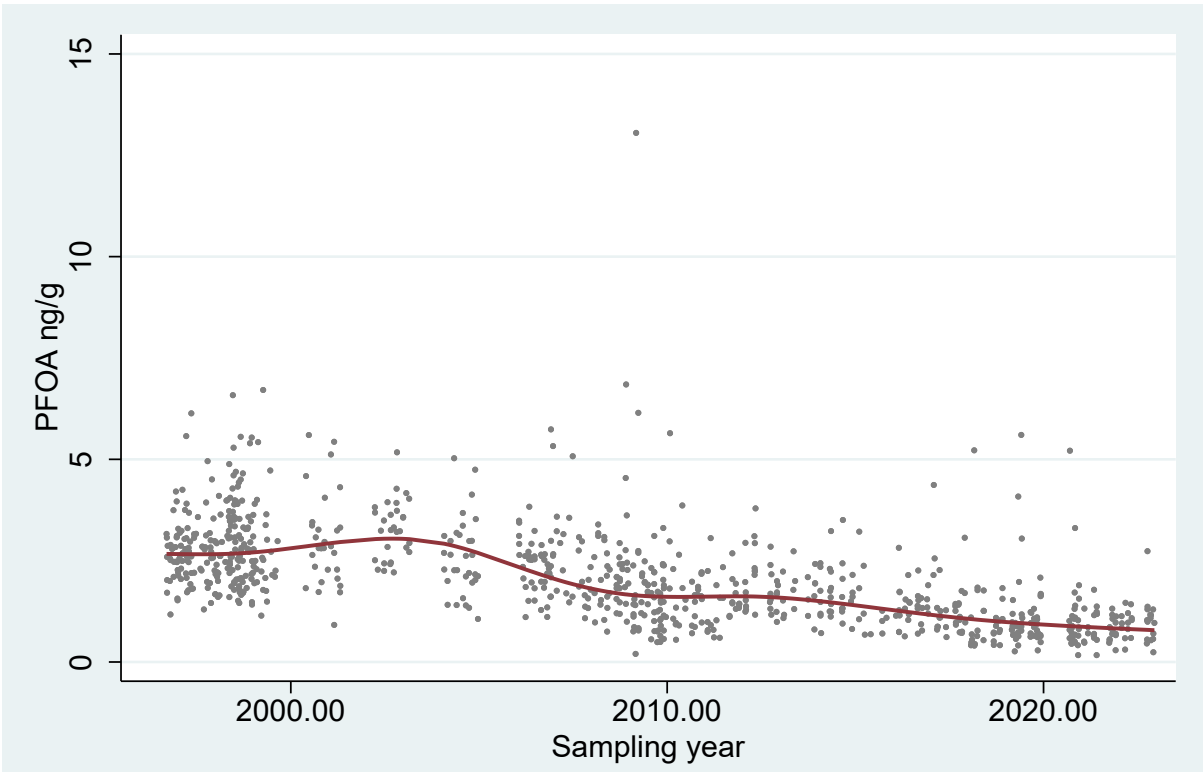
Linear (lin), branched (br), sum of lin and br (tot).

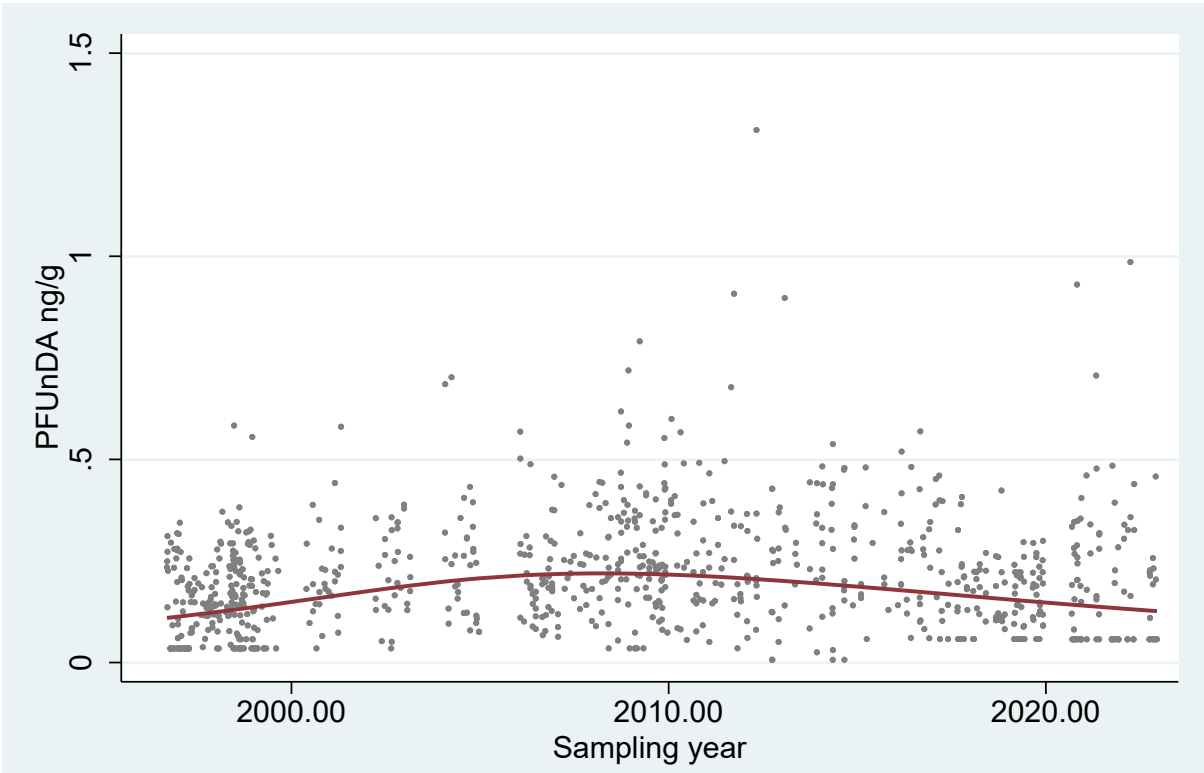
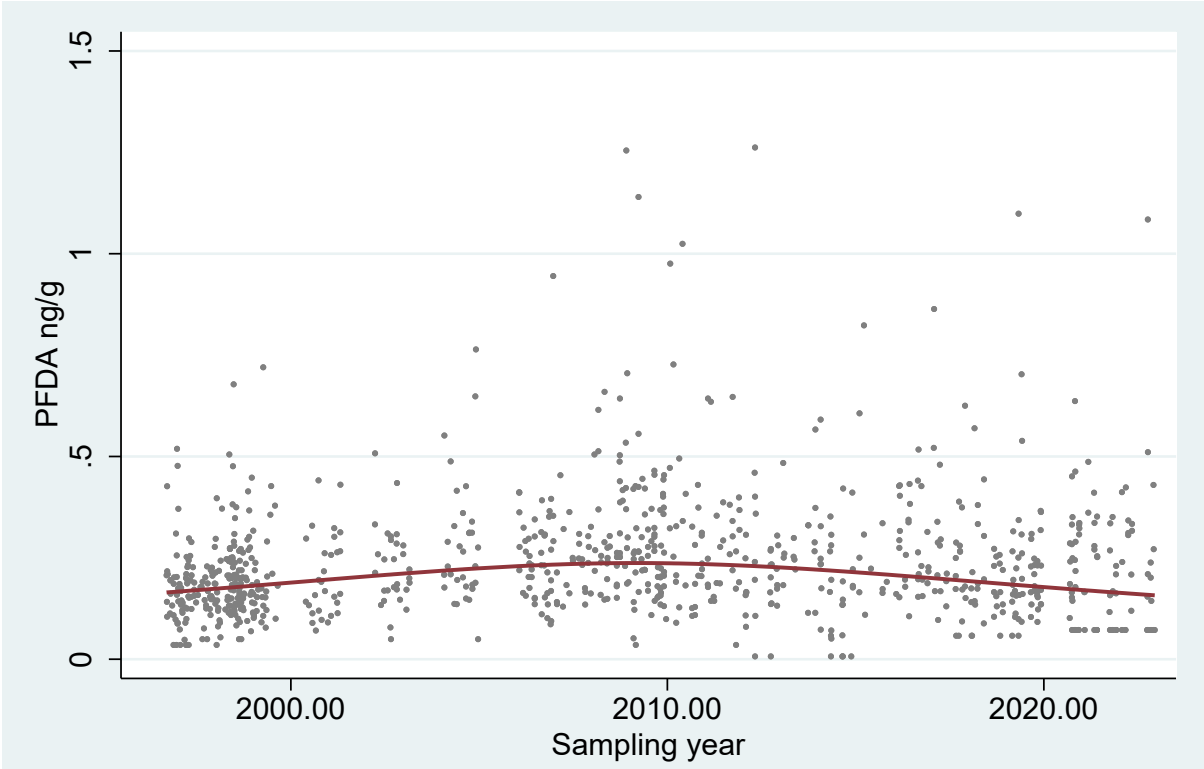
Temporal trends

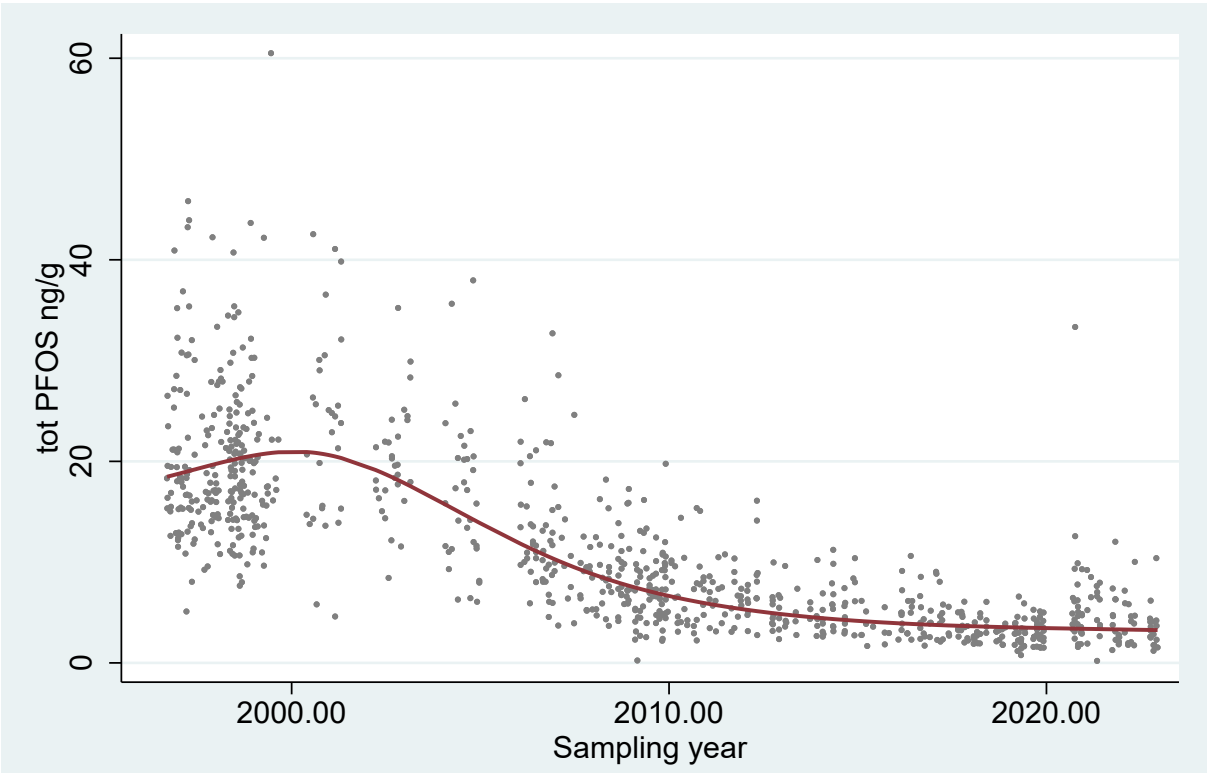
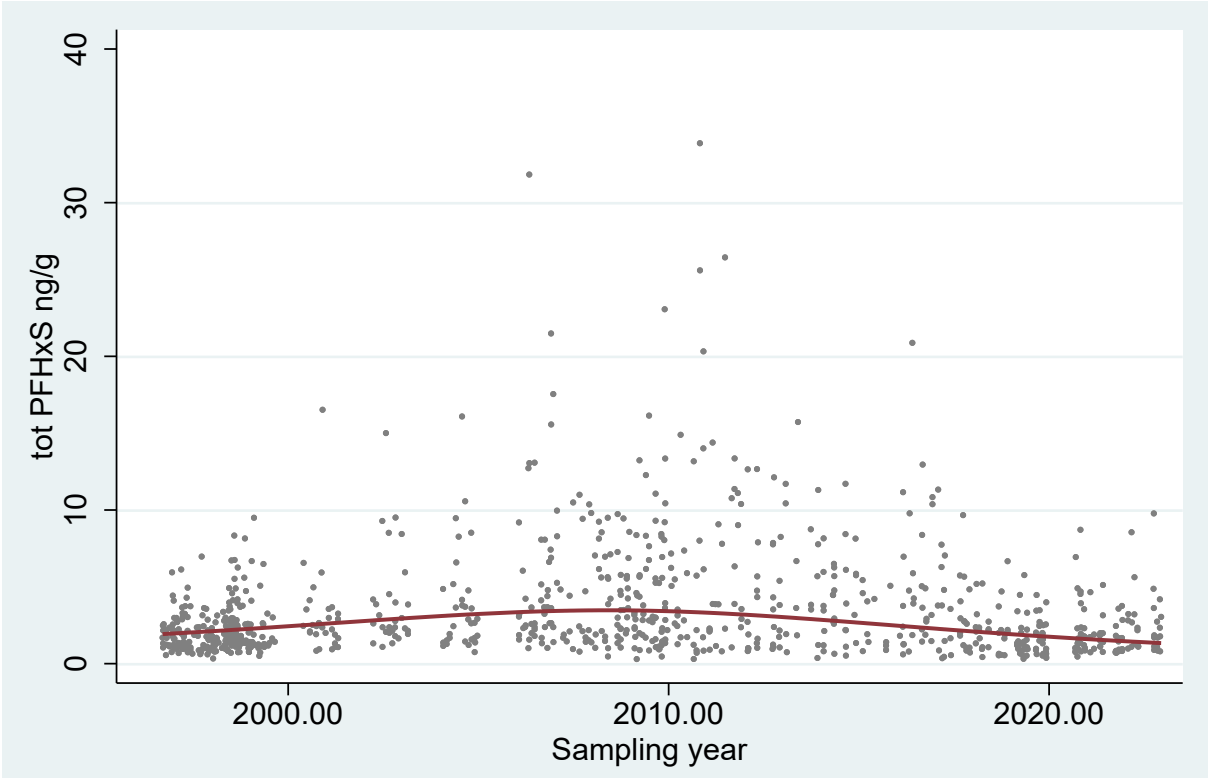
The temporal trend analysis utilized PFAS serum levels from mothers sampled 1996-2022. In total, analyses have been performed on 10 different batches during an 11 year period and the LOD/LOQs varied between analytical runs (Table 3). The results of the spline analyses show temporal trends in PFAS levels, adjusted for possible temporal changes in personal characteristics associated with serum PFAS concentrations (Fig 1, Table 5). All PFAS, except for PFAS4, showed an increasing temporal trend (average 2-5% per year) during the first years of the study (Table 5). PFOA and PFOS showed increasing trends during 1996-2000/2001 and after that the levels started to decrease. PFOS levels decreased much faster during 2000/2001-2010 than the levels of PFOA (13% per year vs 7% per year, respectively) which is consistent with the almost complete phase-out of PFOS and related compounds. However, after 2010 the declining trends slowed down, dropping at a rate of 5% per year thereafter for both substances. PFNA, PFDA and PFUnDA showed similar trends, with mean concentrations increase around 3-5% per year until 2006/2008 and thereafter declining around 2-4% per year. For PFHxS, the highest serum levels were observed between 2004 and 2011 with a 70-80% increase of the geometric mean concentrations compared to 1996 (data not

shown). In 2008, the highest concentration was observed (83% higher geometric mean compared to 1996) with an increasing trends before and a decreasing trend after that year. When evaluating the period after the drinking water contamination was mitigated in 2012, serum levels have decreased around 10% per year for PFHxS (Table 4). The temporal trend for PFAS4 was similar to PFOS and PFOA, which are the main contributors to the sum of the four PFAS (in mean 74%).

PFAS temporal trends have previously been studied in pooled samples from the POPUP cohort 1997-2017 (Miaz et al. 2020). The results in the present study are in concordance with Miaz et al. (2020) which reported change point years for PFOA and PFOS occurring in 2001 and 2002, respectively, and around 2004-2008 for the long-chained PFCAs (PFNA, PFDA and PFUnDA). For PFHxS, the change point year was around two years later (2010-2011) in Miaz et al. (2020) than in the present report. The differences might be explained by the use of pools in the previous study and individual samples in the present report and the difference in statistical methods. In the Danish population, the median concentrations of PFOS and PFOA increased from 1988 until the late 1990s and thereafter decreased until 2021 (Hull et al. 2023). In females from the U.S. within the National Health and Nutrition Examination Survey (NHANES), geometric mean serum levels of PFAS were in the same range as in the present study and PFOS, PFOA and PFHxS have declined continuously during 1999-2018 (Sonnenberg et al. 2023). PFNA and PFDA levels reported in NHANES also increased up until 2009-2010 and 2005-2006, respectively, and thereafter declined.







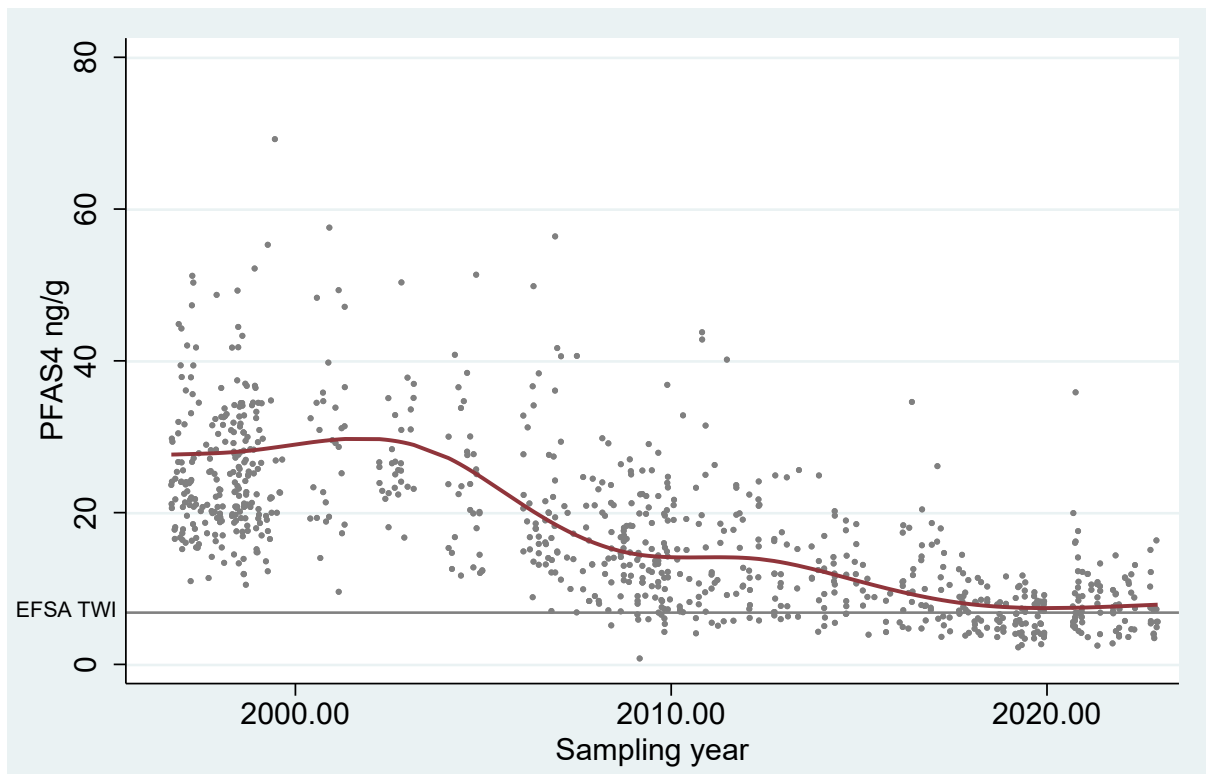


Figure 1. Temporal trends of PFAS in serum from first-time mothers in Uppsala, sampled 1996-2022. Red line = Adjusted cubic spline trend, adjusted for maternal age, BMI, weight increase, weight decrease, education level and smoking status. Grey line = safe serum level for mothers from the risk assessment by EFSA.

Table 5. Mean change in PFAS concentrations in serum during different periods between 1996 and 2022, using linear spline models adjusted for age, BMI, weight gain during pregnancy, weight change between delivery and sampling, educational level and smoking.

PFAS	n	% <LOD ^a	Years	Mean change per year % (SE)	p	Years	Mean change per year % (SE)	p	Years	Mean change per year % (SE)	p	R ²
PFOA	833	0.2	1996-2001	2.3 (0.96)	0.017	2002-2010	-7.2 (0.62)	<0.001	2011-2022	-5.4 (0.64)	<0.001	0.47
PFNA	833	1	1996-2006	4.2 (0.59)	<0.001	2007-2022	-2.4 (0.49)	<0.001				0.11
PFDA	833	7	1996-2008	3.1 (0.65)	<0.001	2009-2022	-3.1 (0.57)	<0.001				0.096
PFUnDA	833	13	1996-2008	5.2 (0.76)	<0.001	2009-2022	-4.2 (0.64)	<0.001				0.15
tot PFHxS	831	0	1996-2008	4.8 (0.72)	<0.001	2009-2022	-6.9 (0.70)	<0.001				0.21
						2012-2022 ^b	-9.9 (0.98)	<0.001				
tot PFOS	832	0.1	1996-2000	3.6 (1.6)	0.019	2001-2010	-13 (0.58)	<0.001	2011-2022	-4.9 (0.68)	<0.001	0.69
PFAS4 ^c	831		1996-2001	0.7 (1.1)	0.48	2002-2010	-8.9 (0.65)	<0.001	2011-2022	-5.4 (0.60)	<0.001	0.57

^aData below LOD was replaced with LOD/ $\sqrt{2}$.

^bPercent change in concentrations of tot PFHxS during 2012-2022 after mitigation of levels in drinking water.

^cSum of the four PFAS: PFOA, PFNA, tot PFHxS, and tot PFOA

Determinants of PFAS levels

In the adjusted cubic spline models it was possible to determine the associations between personal characteristics, included as independent variables in the analysis, and PFAS concentrations. For each determinant, the association with PFAS is adjusted for possible influence of the other covariates on the association. All results are shown in Table 6.

Age was positively associated with serum levels of PFNA, PFUnDA, PFHxS, PFOS and PFAS4 with an increase of 1-2% of PFAS level per year of increased age. Tot PFHxS and PFAS4 were inversely related to BMI and the serum concentration decreased on average with 1-2% per unit increase in BMI, suggesting that overweight women had slightly lower serum concentrations than women with low BMI. PFAS levels were not associated with an increase in weight during pregnancy or weight loss after delivery. Education level was positively associated with serum PFAS concentrations, except for PFOA, with on average 11-54% higher concentrations among women with the highest education level. PFHxS showed the largest difference between women with “only high school education” and women with “more than 3 years of higher education”, in mean 54% higher levels. In addition the mean levels for women with “1-3 years of higher education” were 18% higher compared to “only high school education”. Since PFHxS is associated with drinking water contamination it is speculated that some of the association with education may be related to place of living in Uppsala City. Higher education level is also associated with higher income that was shown to be one of the most important determinant together with nationality, parity and breast feeding history in a recent review of determinants of legacy PFAS concentrations in pregnant mothers (McAdam and Bell 2023). In addition, higher education level is associated with higher fish consumption, which is in turn associated with increased PFAS levels (Berger et al. 2009, Christensen et al. 2017, Papadopoulou et al. 2019). No significant associations were observed between PFAS levels and women that reported that they are former smokers. For PFDA and PFOS, women that smoked during pregnancy had significantly lower serum concentrations than non-smoking women. There may be other personal characteristics among smokers that cause decreased PFAS concentrations in serum.

The variation of the independent variables in the regression model explained 10-70% of the variation in PFAS concentrations (Table 6), showing that there are important determinants of serum concentrations not studied by us. The highest R^2 was observed for PFOA, PFOS and PFAS4, mainly due to the large between-sampling year variation in concentrations.

Table 6. Percent change (mean (standard error) in PFAS serum concentration per unit change in covariates included in the cubic spline model and coefficient of determination (R^2) of the whole model also including the covariate “sampling year”.

PFAS	n	Age	BMI (kg/m ²)	Weight gain	Weight loss	Education ^a		Smoking ^b		R ²
						2	3	2	3	
PFOA	833	ns	ns	ns	ns	ns	ns	ns	ns	0.48
PFNA	833	1.3 (0.48)	ns	ns	ns	ns	11 (5.8)	ns	ns	0.11
PFDA	833	ns	ns	ns	ns	ns	17 (7.8)	ns	-18 (8.0)	0.095
PFUnDA	833	2.0 (0.62)	ns	ns	ns	ns	23 (9.0)	ns	ns	0.16
tot PFHxS	831	1.9 (0.75)	-1.9 (0.76)	ns	ns	18 (8.8)	54 (11)	ns	ns	0.21
tot PFOS	832	1.3 (0.46)	ns	ns	ns	ns	14 (5.2)	ns	-12 (5.1)	0.69
PFAS4 ^c	831	1.2 (0.43)	-1.2 (0.43)	ns	ns	ns	19 (4.9)	ns	ns	0.58

^aThe variable “Education” included women with high school education (1, reference group), women with 1-3 years of higher education (2) and women with more than 3 years of higher education (3).

^bWomen that had never smoked was reference group for the variable “smoking”, group (2) women who had stopped smoking before pregnancy and (3) women who smoked during pregnancy or stopped smoking during the 1st trimester of pregnancy.

^cSum of the four PFAS: PFOA, PFNA, tot PFHxS, and tot PFOA

ns=not significant $p>0.05$.

CONCLUSION

Temporal trends for PFOS and PFOA are declining as a result of international regulation and phase-out initiatives. Due to drinking water contamination, serum concentrations of PFHxS have been increasing in the mothers from Uppsala. The decreasing serum levels of PFHxS after 2012 indicates that the measures to reduce the levels of PFAS in Uppsala's drinking water have been effective at reducing exposure. Concentrations of PFNA, PFDA and PFUnDA have been increased about 3-5% per year during the first part of the study 1996-2006/2008, but after a peak in mean levels, the trend was decreasing around 2-4% per year 2006/2008-2022. Although PFAS levels are decreasing, still 61% of the Uppsala mothers sampled 2020-2022 had PFAS4 levels above the safe serum level established by EFSA. It is important to follow-up the trends of PFAS in the future in the POPUP mothers to confirm if the exposure of the population continues to decrease.

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