LIVSMEDELS VERKET

NATUR VÅRDS VERKET
SWEDISH ENVIRONMENTAL PROTECTION AGENCY

NATIONAL ENVIRONMENTAL MONITORING COMMISSIONED BY THE SWEDISH EPA

Report to the Swedish EPA (the Health-Related Environmental Monitoring Program)

Contract no. 215-18-001

Levels of perfluoroalkyl substances (PFAS) in individual serum samples from first-time mothers in Uppsala, Sweden: results from year 2017-2019, and temporal trends for the time period 1996-2019

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2020-11-30

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Rapporttitel
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Beställare
Naturvårdsverket
106 48 Stockholm

Finansiering
Nationell hälsorelaterad miljöövervakning

Nyckelord för plats
Uppsala

Nyckelord för ämne
Perfluorerade alkylsyror, PFCA, PFSA, serum, kvinnor

Tidpunkt för insamling av underlagsdata
1996-2019

Sammanfattning
Sedan 1996 har Livsmedelsverket regelbundet samlat in blodprover från förstföderskor i Uppsala för analys av persistenta halogenerade organiska miljöföroreningar (POP). Poly- och perfluorerade alkylsyror (PFAS) är en sådan substansgrupp. I vårt projekt undersöks bland annat hur exponering för PFAS har förändrats sedan stora ändringar i produktion av kemikalierna skett runt millennieskiftet. I följande rapport redovisas halter av PFAS i serum från förstföderskor provtagna 2017-2019 samt tidstrender för perioden 1996-2019. PFOS förekommer i högst halt i serum följt av PFHxS och PFOA. Hälften av förstföderskorna, provtagna 2017-2019, hade serumhalter över den nivå hos mammor som är önskvärd för att skydda barnet mot hög exponering under foster- och amningsperioden, fastställd av EFSA 2020. Serumhalter av PFUnDA har ökat cirka 2 % per år under hela studieperioden, medan det för PFNA observerades en ökning av halter fram till omkring år 2008 och därefter sjunker halterna. På grund av dricksvattenföroreningar av PFAS har serumhalterna av PFBS och PFHxS ökat hos förstföderskorna från Uppsala. Efter år 2010-2011 har halterna i serum börjat minska (change-point year), vilket överensstämmer med att åtgärder för att sänka halten PFAS i dricksvattnet har satts in sedan föroreningen upptäcktes i juli 2012. Resultaten visar att storskalig utfasning av PFOS och PFOA internationellt har resulterat i minskande exponering i befolkningen och serumhalterna minskade årligen med 5 % för PFOA och 9 % för PFOS under studieperioden i Uppsala. Kvinnor med högst utbildning (mer än 3 år eftergymnasial utbildning) hade 10-70 % högre PFAA-halter än kvinnor med lägst utbildning (gymnasieskola). Störst skillnad observerades för PFBS och 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 av flera av de långkedjiga karboxylsyrorna minskade med 1-3 % per enhetsökning av BMI, vilket antyder att överviktiga kvinnor hade något lägre halter av dessa PFAA än kvinnor med lågt BMI. Det är viktigt att fortsätta följa trender av PFAS i POPUP för att se om exponeringen fortsätter att minska, och för de ämnen som inte visar någon nedåtgående trend om nivåerna kommer att plana ut för att sedan minska.

Levels of perfluoroalkyl substances (PFAS) in individual serum samples from first-time mothers in Uppsala, Sweden: results from year 2017-2019, and temporal trends for the time period 1996-2019

Background

With funding from the Swedish Environmental Protection Agency (EPA), the Swedish National Food Agency (NFA) has recruited first-time mothers in Uppsala since 1996 with the aim to estimate the body burdens of POPs among pregnant and nursing women and to estimate temporal trends of the exposure of fetuses and infants. The study, known as POPUP (Persistent Organic Pollutants in Uppsala Primiparas), has analysed poly- and perfluoroalkyl substances (PFAS) in serum samples from the mothers from 1996 onwards.

PFAS have been manufactured world-wide for many decades, for uses in industrial processes (e.g. production of fluoropolymers), as water and stain proofing agents, and in lubricants, paints and fire-fighting foams (Kissa 2001; Prevedouros et al. 2006). Today over 4000 PFAS are known to exist on the global market and some of them, such as perfluoroalkyl acids (PFAAs), are very persistent in the environment and are detected worldwide in humans and wildlife (Giesy and Kannan 2001; Kissa 2001; Kannan et al. 2004; Houde et al. 2006). Since the start of the 21st century measures have been taken to decrease and stop production and use of the most widely distributed PFAS, perfluorooctane sulfonic acid (PFOS) and perfluorooctanoic acid (PFOA). 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 PFAAs and related compounds (Vestergren et al. 2012a; Poothong et al. 2020). In Uppsala, drinking water has been contaminated with PFAAs since at least 1996, most probably due to contamination from point sources (fire-fighting training areas) resulting in elevated PFAAs serum levels in mothers and children in the POPUP study (Gyllenhammar et al. 2015, Gyllenhammar et al. 2019). PFAS transfer over the placenta and into breastmilk are important exposure routes for PFAS early in life, and the relative importance of these pathways seems to depend on carbon chain length and functional groups of PFAS homologues (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) from the POPUP-study. The following report presents results of PFAS analyses in individual serum samples from first-time mothers sampled in 2017-2019 (according to agreement 215-18-001). The new data were used to establish updated temporal trends and possible change points (CP) for the period 1996-2019.

Material 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 2019 as described in Glynn et al. (2012). 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 -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. Personal characteristics of the participating mothers during 2017-2019 are shown in Table 1.

Table 1. Personal characteristics of the participating mothers during 2017-2019.

Variable		**n**	**Mean**	**Median**	**Range**
Age (yr)		110	30.4	30.2	21.9-45.3
Pre-pregnancy BMI (kg/m^2)		110	23.6	22.6	17.9-44.1
Weight gain during pregnancy (% of initial weight)		110	25.4	25.3	-1-57.1
Weight reduction from delivery to sampling (%)[a]		110	9.0	8.9	0.16-14.8
Variable		**n**	**%**		
Education	max 3-4 yr high school	15	13.6		
	1-3 yr higher education	27	24.5		
	>3 yr higher education	68	61.8		
Smoking	never	92	83.6		
	former smoker	16	14.5		
	smoker*	2	1.8		

*Those that stopped smoking during the first trimester were included in the smoker group.

PFAS analyses

PFAS (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-up 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 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.

Table 2. PFAS included in the study.

Compound	Abbreviation	IS	LOQ
Perfluorobutanoate	PFBA	M4PFBA	0.037
Perfluoropentanoate	PFPeA	M2PFDoDA	0.043
Perfluorohexanoate	PFHxA	M4PFHpA	0.082
Perfluoroheptanoate	PFHpA	M4PFHpA	0.082
Perfluorooctanoate linear isomer	lin-PFOA	M4PFOA	0.082
Perlfuorooctanoate branched isomers	br-PFOA	M4PFOA	0.082
Perfluorononanoate	PFNA	M5PFNA	0.082
Perfluorodecanoate	PFDA	M2PFDA	0.082
Perfluoroundecanoate	PFUnDA	M2PFUnDA	0.082
Perfluorododecanoate	PFDoDA	M2PFDoDA	0.161
Perfluorotridecanoate	PFTriDA	M2PFDoDA	0.082
Perfluorotetradecanoate	PFTeDA	M2PFDoDA	0.082
Perfluoropentadecanoate	PFPeDA	M2PFDoDA	0.082
Perfluorohexadecanoate	PFHxDA	M2PFDoDA	1.152
Perfluorooctadecanoate	PFOcDA	M2PFDoDA	3.878
Perfluorobutanesulfonate	PFBS	18O2-PFHxS	0.072
Perfluoropentanesulfonate	PFPeS	18O2-PFHxS	0.078
Perfluorohexanesulfonate lin.	lin-PFHxS	18O2-PFHxS	0.078
Perfluorohexanesulfonate br.	br-PFHxS	18O2-PFHxS	0.078
Perfluoroheptanesulfonate	PFHpS	M4PFOS	0.078
Perfluorooctanesulfonate lin.	lin-PFOS	M4PFOS	0.078
Perfluorooctanesulfonate br.	br-PFOS	M4PFOS	0.078
Perfluorononanesulfonate	PFNS	M4PFOS	0.078
Perfluorodecanesulfonate lin.	lin-PFDS	M4PFOS	0.080
Perfluorodecanesulfonate br.	br-PFDS	M4PFOS	0.080
Perfluoroundecanesulfonate	PFUnDS	M4PFOS	0.080
Sodium Dodecafluoro-3H-4,8,-dioxanonanoate	NaDONA	M4PFOA	0.084
Potassium 9-chlorohexadecafluoro-3-oxanonanoe-1-sulfonate	9Cl-PF3ONS	M2PFDA	0.082
Potassium 11-chloroeicosafluoro-3-oxaundecane-1-sulfonate	11Cl-PF3OUdS	M2PFDA	0.145
3:3 Fluorotelomer carboxylic acid	3:3 FTA (FPrPA)	M2PFHxA	0.286
5:3 Fluorotelomer carboxylic acid	5:3 FTA (FPePA)	M4PFOA	0.098
7:3 Fluorotelomer carboxylic acid	7:3 FTA (FHpPA)	M2PFDA	0.330
4:2 Fluorotelomer sulfonate	4:2 FTS	M2 6:2 FTS	0.082
6:2 Fluorotelomer sulfonate	6:2 FTS	M2 6:2 FTS	0.374
8:2 Fluorotelomer sulfonate	8:2 FTS	M2 6:2 FTS	0.084
Perfluorooctane sulfonamide lin,	lin-FOSA	M8FOSA	0.087
Perfluorooctane sulfonamide br.	br-FOSA	M8FOSA	0.084
Perfluorooctane sulfonamidoacetate lin.	lin-FOSAA	d3-MeFOSAA	1.183
Perfluorooctane sulfonamidoacetate br.	br-FOSAA	d3-MeFOSAA	0.292
Methyl perfluorooctane sulfonamidoacetate lin.	lin-MeFOSAA	d3-MeFOSAA	0.294
Methyl perfluorooctane sulfonamidoacetate br.	br-MeFOSAA	d3-MeFOSAA	0.294
Ethyl perfluorooctane sulfonamidoacetate lin.	lin-EtFOSAA	d5-EtFOSAA	0.272
Ethyl perfluorooctane sulfonamidoacetate br.	br-EtFOSAA	d5-EtFOSAA	0.082
6:2 Fluorotelomer phosphate diester	6:2 diPAP	M4 6:2/6:2 diPAP	0.296
6:2/8:2 Fluorotelomer phosphate diester	6:2/8:2 diPAP	M4 8:2/8:2 diPAP	0.082
8:2 Fluorotelomer phosphate diester	8:2 diPAP	M4 8:2/8:2 diPAP	0.082

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 (limit of quantification, LOQs) for the different analytical batches are provided in Table 3. Absolute recoveries of the internal standards (determined relative to 13C8-PFOA) were on an average between 60% and 69%. Further method validation parameters are provided in Glynn et al. (2012).

Table 3. Limits of quantification (LOQs) for the different analytical batches, analysed 2013-2020.

PFAS	**Analytical batch**								
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
PFHpA	0.040	0.10	0.030	0.14	0.14	0.162	0.008	0.082	0.082
PFOA	0.20	0.25	0.30	0.030	0.14	0.162	0.140	0.082	0.082
PFNA	0.050	0.10	0.010	0.030	0.040	0.162	0.008	0.082	0.082
PFDA	0.050	0.070	0.010	0.070	0.15	0.162	0.008	0.082	0.082
PFUnDA	0.050	0.050	0.010	0.020	0.060	0.036	0.008	0.082	0.082
PFDoDA	0.050	0.050	0.010	0.030	0.060	0.036	0.008	0.082	0.161
PFTrDA	0.050	0.050	0.030	0.020	0.040	0.036	0.008	0.082	0.082
PFBS	0.010	0.010	0.15	0.090	0.10	0.186	0.007	0.279	0.072
PFPeS	-	-	-	-	-	-	0.008	-	0.078
lin-PFHxS	0.010	0.10	0.060	0.020	0.040	0.036	0.008	0.227	0.078
br-PFHxS	0.010	0.010	0.050	0.020	0.040	0.036	0.008	0.078	0.078
PFHpS	-	-	-	-	-	-	0.008	-	0.078
lin-PFOS	0.010	0.50	0.10	0.030	0.040	0.034	0.008	0.078	0.078
br-PFOS	0.010	0.20	0.020	0.030	0.040	0.15	0.008	0.078	0.078

- = not analysed

Statistical analyses

For statistical analyses, concentrations below LOQ were replaced with LOQ/√2. PFAA temporal trends were investigated by log-linear regression analysis of yearly geometric mean values for the whole period 1996-2019. As a consequence of the logarithmic transformation, the associations between sampling year and PFAS concentrations are presented as percent change of concentrations per year, and not as change in absolute levels. To estimate a possible change-point (CP) in the temporal trends we used the technique described in Sturludottir et al. (2015). Briefly, the whole time-series was repeatedly divided into two parts with at least four years in each part. A series of generalized least squares (GLS) regression models, allowing for different time trends on each side of the possible CP, were fitted and the log-likelihood values were compared to a null model that did not allow a CP. If a model with a CP showed a sufficiently better fit to the data than the null model, the CP model was accepted. The

associations between PFAS levels and the covariates age, BMI, weight gain during pregnancy, weight loss after delivery, education level, and maternal smoking were assessed using linear mixed models with random intercepts for sampling year to account for the hierarchical nature of the data.

Results and discussion

Among perfluoroalkyl carboxylic acids (PFCAs) the mean level in serum sampled 2017-2019 was highest for lin-PFOA (1.2 ng/g serum) and declined in the order lin-PFOA>PFNA>PFDA~PFUnDA (Table 4). For PFHpA and PFTrDA most women had serum levels below LOQ. All women had serum levels above LOQ for the sulfonic acids, lin-PFHxS, lin-PFOS, and br-PFOS with median levels ranging from 1.2 to 3.7 ng/g (Table 4). For the first time PFPeS and PFHpS was analysed in POPUP mothers and the results showed that most women had serum levels below LOQ (Table 4). For PFBS and 9Cl-PF3ONS only one serum sample had levels over LOQ. In all mothers, the levels of PFBA, PFPeA, PFHxA, br-PFOA, PFDoDA, PFTeDA, PFPeDA, PFHxDA, PFOcDA, PFNS, lin-PFDS, br-PFDS, PFUnDS, lin-FOSA, br-FOSA, lin-FOSAA, br-FOSAA, lin-MeFOSAA, br-MeFOSAA, lin-Et-FOSAA, br-Et-FOSAA, 11Cl-PF3OUdS, NaDONA, HFPO-DA, FPrPA, FPePA, FHpPA, 4:2 FTS, 6:2 FTS, 8:2 FTS, 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). EFSA has 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. When comparing the safe maternal level established by EFSA with the results in the present study the sum of the four PFAA ($PFAA_4$) was in mean 7.4 and median 7.0 (Table 4). In total, 51% of the women had serum levels above the safe levels during the period 2017-2019. The drinking water in Uppsala have 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 PFAAs was discovered in 2012, however as the $PFAA_4$ 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 2017-2019.

PFAS	n	n<LOQ	Mean[a]	Median	Range
PFHpA	110	106			<0.082-0.20
lin-PFOA	110	0	1.2	1.0	0.26-5.60
PFNA	110	0	0.5	0.4	0.13-1.59
PFDA	110	7	0.2	0.2	<0.082-1.10
PFUnDA	110	16	0.2	0.2	<0.082-0.46
PFTrDA	110	101			<0.082-0.14
PFBS	110	109			<0.072-0.08
PFPeS	110	81			<0.078-0.43
lin-PFHxS	110	0	2.3	1.8	0.27-10.87
br-PFHxS	110	69			<0.078-0.47
tot PFHxS	110	69			0.32-11.34
PFHpS	110	63			<0.078-0.29
lin-PFOS	110	0	2.5	2.2	0.43-7.32
br-PFOS	110	0	0.9	0.9	0.29-2.49
tot PFOS	110	0	3.4	3.2	0.77-9.04
9Cl-PF3ONS	110	109			<0.082-0.20
sum $PFAA_4$[b]	110	NA	7.4	7.0	2.26-26.14

[a]When calculating means data below LOQ was replaced with LOQ/√2.
[b]Sum of the four PFAA; lin-PFOA, PFNA, tot PFHxS, and tot PFOA
Linear (lin), branched (br), sum of lin and br (tot). NA = not applicable

Temporal trends

The temporal trend analysis utilized PFAA serum levels from mothers sampled 1996-2019. In total, analyses have been performed on 9 different batches during an 8 year period and the LOQs varied between analytical runs (Table 3). The results of the multivariate analyses show temporal trends in PFAA levels, adjusted for possible temporal changes in personal characteristics associated with serum PFAA concentrations (Table 5, Fig. 1-2). PFUnDA, PFTrDA, and PFBS showed increasing temporal trends during the study period 1996-2019, but for PFBS a CP was observed around year 2011. The trend before 2011 was increasing but not significant. After the CP the PFBS levels have decreased with 17% per year (mean) (Table5). For PFHxS a CP was observed 2009-2011 with significant increasing trends before and decreasing trend after the CP. The results of PFBS and PFHxS shows that serum levels have decreased after the drinking water contamination was mitigated in 2012. The results showed an increasing trend for PFNA, on average 5% per year, before the CP in 2008 (Table

5, Fig. 1). After the CP, 2008-2019, a significant downward trend was observed for PFNA of 7% per year (mean). PFOA and PFOS showed decreasing temporal trends over the entire study period with, respectively, 5 and 9% annual mean decrease per year (Table 5, Fig. 1). PFOS levels have decreased much faster than the levels of PFOA which is consistent with the almost complete phase-out of PFOS and related compounds. No temporal trend was observed for PFHpA and PFDA. Figure 1 shows that the results for PFBS, PFHpA and PFTrDA are more uncertain than the results for the other PFAAs, due to generally very low concentrations and omission of data from analytical batches with high LOQs. Results of PFPeS and PFHpS, (decreasing trend for PFHpS) are also very uncertain due to that and because of low n values (Table 5, Fig. 5).

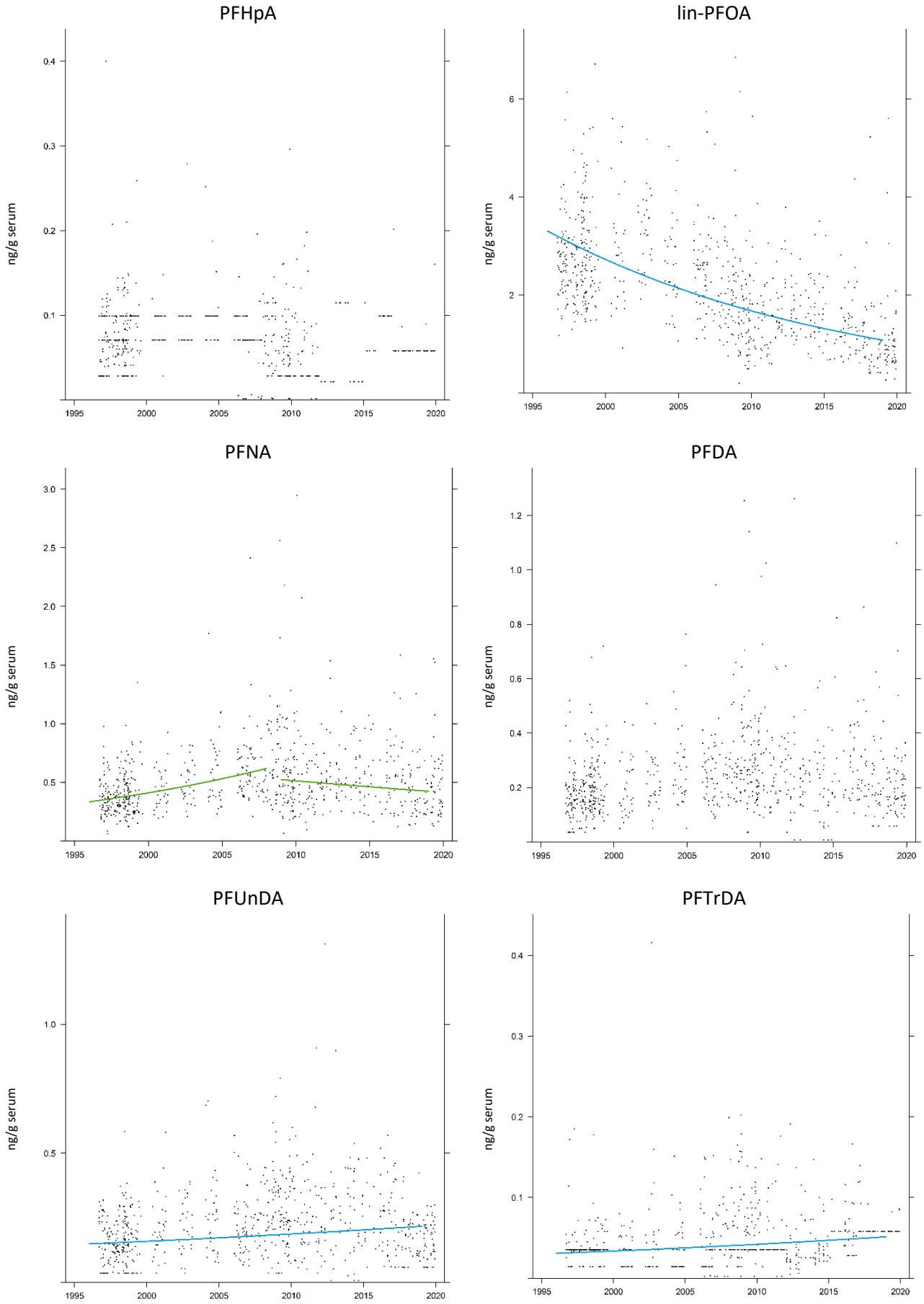
PFAS temporal trends have previously been studied in pooled samples from the POPUP cohort 1997-2017 (Miaz et al. 2020). The temporal trends for the whole period in the present study are in concordance with Miaz et al. (2020) for PFOA (-5% per year), PFUnDA (2% per year), and PFOS (-9% per year) (mean values). In the present study we observed an annual increase in concentration of PFTrDA (2%) and PFBS (7%), whereas no temporal trend was observed in the pooled trend analysis (Miaz et al. 2020). In the pooled temporal trend analysis significant temporal trends were found for PFHpA (-5%), PFNA (2%), PFDA (3%), and lin-PFHxS (4%) (mean values) (Miaz et al. 2020) which were not observed in the present study (Table 5). In Miaz et al. (2020) significant CPs were observed for all PFAAs evaluated in the present study and for PFNA and PFHxS the results were similar. For PFOS, a later CP year was observed around 2003-2004 compared to Miaz et al. (2020) reporting a CP around year 2001.

In the present study individual samples were evaluated and temporal trends were adjusted for potential confounders (Table 6). The adjustment could be one reason for the differences between the present study and the previous temporal trend study of pooled samples from the POPUP-mothers (Miaz et al. 2020) and that samples from year 2018 and 2019 were included. Another difference is that analyses have occurred in total 9 batches with different LOQs in the present study, which might also affect the statistical analysis, as can be seen in figure 1 for PFHpA, PFTrDA, PFBS, and possibly also for br-PFHxS.

Table 5. Annual change in PFAS concentrations in serum 1996–2019 and significant change points (CP) in the temporal trends. Adjusted for age, BMI, weight gain during pregnancy, weight change between delivery and sampling, educational level and smoking.

PFAS	n	Mean change per year (%)	95% CI	p	R^2	Change point year (CP)	Mean change before CP (%)	p	Mean change after CP (%)	p
PFHpA	742	-1.6	-4.2/1.1	0.23	0.023	ns				
PFOA	742	**-4.7**	**-5.7/-3.8**	**<0.001**	**0.81**	ns				
PFNA	742	0.87	-0.16/1.9	0.095	0.085	**2008**	**5.3**	**<0.001**	**-7.0**	**<0.001**
PFDA	742	1.0	-0.38/2.5	0.15	0.055	ns				
PFUnDA	742	**1.7**	**0.16/3.2**	**0.032**	**0.16**	ns				
PFTrDA	742	**2.2**	**1.0/3.5**	**<0.001**	**0.38**	ns				
PFBS	742	**7.7**	**4.0/12**	**<0.001**	**0.45**	**2011**	2.5	0.19	**-17**	**0.003**
PFPeS[a]	129	2.6	-7.6/14	0.60	0	ns				
lin-PFHxS	740	0.90	-1.1/2.9	0.36	0	**2011**	**5.8**	**<0.001**	**-16**	**<0.001**
br-PFHxS	740	0.31	-2.1/2.8	0.79	0	**2009**	**8.5**	**<0.001**	**-18**	**<0.001**
tot PFHxS	740	0.86	-1.1/2.9	0.39	0	**2010**	**6.0**	**<0.001**	**-15**	**<0.001**
PFHpS[a]	129	**-7.6**	**-11/-3.6**	**0.0053**	**0.71**	ns				
lin-PFOS	741	**-9.0**	**-10/-7.8**	**<0.001**	**0.91**	**2004**	1.3	0.22	**-9.7**	**<0.001**
br-PFOS	741	**-9.8**	**-11/8.6**	**<0.001**	**0.93**	**2003**	1.7	0.18	-13	**<0.001**
tot PFOS	741	**-9.2**	**-10/-8.1**	**<0.001**	**0.92**	**2004**	0.82	0.38	**-9.8**	**<0.001**

[a]2006-2019


PFHpA
lin-PFOA
PFNA
PFDA
PFUnDA
PFTrDA
ng/g serum
1995
2000
2005
2010
2015
2020

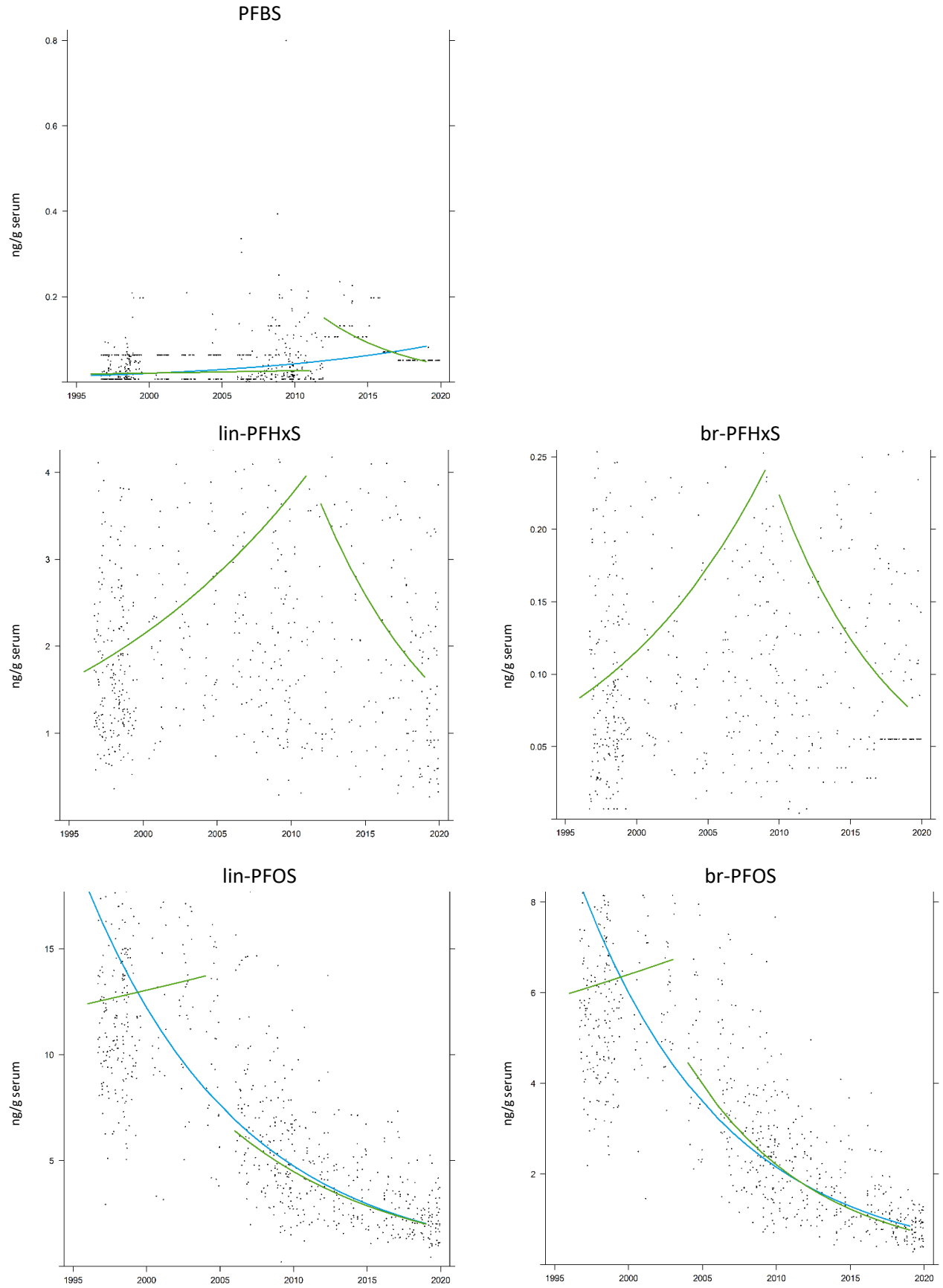


Figure 1. Temporal trends of PFAA in serum from first-time mothers in Uppsala 1996-2019. Blue line = temporal trend, green line = temporal trend before or after change point year.

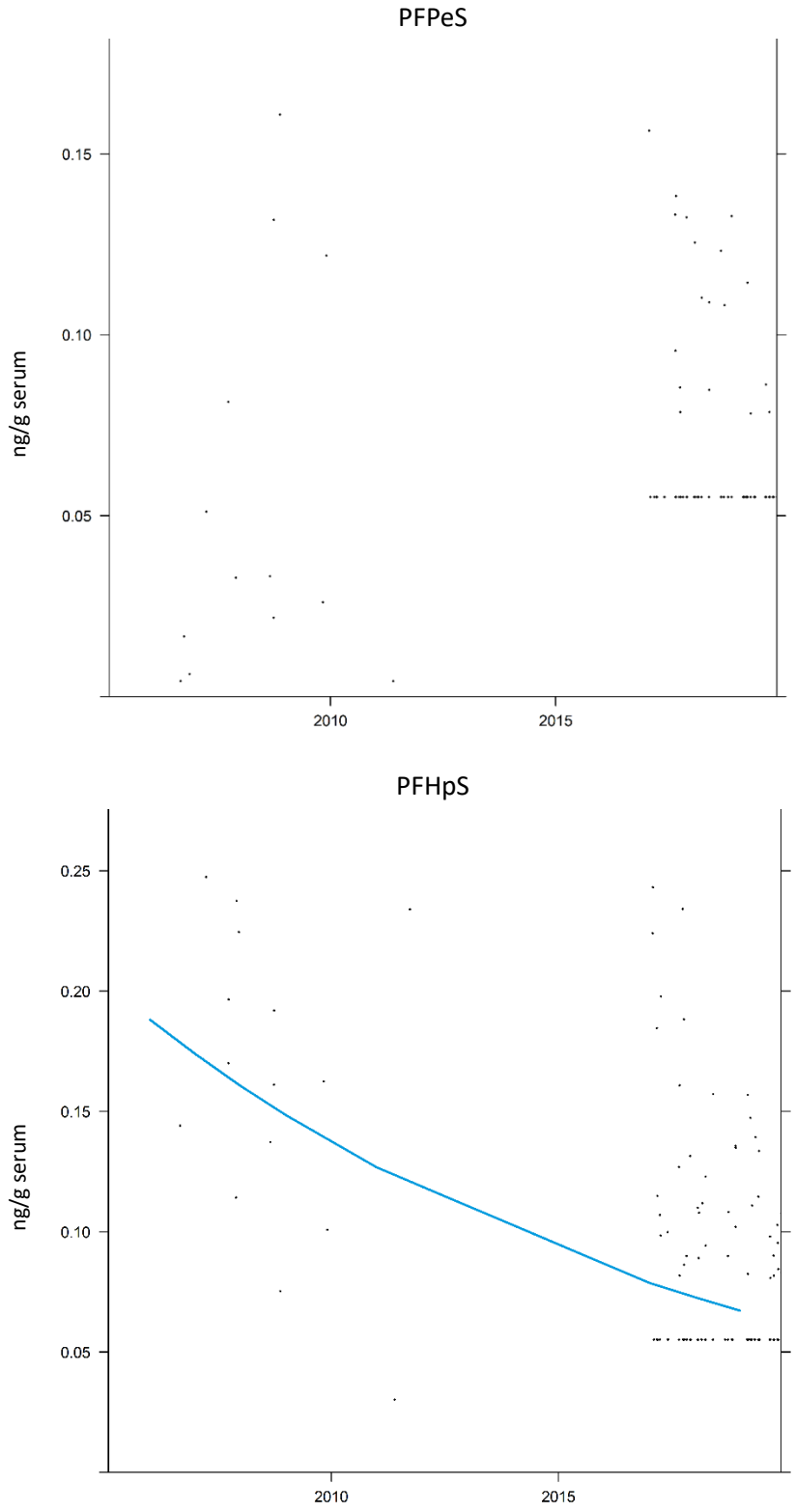


Figure 2. Levels of PFPeS and PFHpS in serum from first-time mothers in Uppsala 1996-2019. Blue line = temporal trend.

Determinants of PFAA levels

In the multivariate regression analysis it is possible to determine the associations between personal characteristics, included as independent variables in the regression models, and PFAA concentrations. For each determinant the association with PFAA is adjusted for possible influence of the other covariates on the association.

Age was positively associated with serum levels of PFNA, PFDA, PFUnDA, PFTrDa, and PFOS with an increase of 1-2% of PFAA level per year of increased age. The lin-PFHxS, tot PFHxS, and the PFCAs were inversely related to BMI, except in the case of PFHpA and PFNA (Table 6). The serum concentration decreased on average with 1-3% per unit increase in BMI, suggesting that overweight women had slightly lower serum concentrations than women with low BMI. PFAA levels were not associated with an increase in weight during pregnancy or weight loss after delivery, except for a significant association between weight loss and PFPeS and PFHpS. Education level was positively associated with serum PFAA concentrations, except for PFHpA, PFOA, and PFPeS, with on average 13-60% higher concentrations among women with the highest education level (Table 6). PFBS and PFHxS showed the largest difference between women with only high school education and women with more than 3 years of higher education, 40% and 60% increase, respectively. Since these two PFAAs are associated with drinking water contamination it may be 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 fish consumption that is known to be associated with increased PFAA levels (Berger et al. 2009, Christensen et al. 2017, Papdopoulou et al. 2019). No significant associations were observed between PFAA levels and women that reported stopping smoking before pregnancy (Table 6). For PFOS and PFDA, 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 PFAA concentrations in serum.

The variation of the independent variables in the regression model explained 2-90% of the variation in PFAA concentrations (Table 6), showing that there are important determinants of serum concentrations not studied by us. The highest R^2 was observed for PFOS and PFOA, mainly due to the large between-sampling year variation in concentrations.

Table 6. Percent change in PFAA serum concentration per unit change in covariates included in the multiple regression analyses (mean (standard error)), and coefficient of determination (R^2, %) of the whole regression model also including the covariate "sampling year".

PFAS	**n**	**Age**	**BMI (kg/m^2)**	**Weight gain**	**Weight loss**	**Education**		**Smoking**		**R^2**
						2	**3**	**2**	**3**	Including sampling year
PFHpA	742	ns	ns	ns	ns	ns	ns	ns	ns	0.023
PFOA	742	ns	-1.4 (0.005)	ns	ns	ns	ns	ns	ns	0.81
PFNA	742	1.0 (0.005)	ns	ns	ns	ns	17 (0.048)	ns	ns	0.085
PFDA	742	1.3 (0.006)	-1.4 (0.007)	ns	ns	ns	23 (0.060)	ns	-18 (0.082)	0.055
PFUnDA	742	2.2 (0.007)	-1.7 (0.008)	ns	ns	ns	27 (0.069)	ns	ns	0.16
PFTrDA	742	2.3 (0.006)	-2.6 (0.007)	ns	ns	ns	16 (0.062)	ns	ns	0.38
PFBS	742	ns	ns	ns	ns	ns	40 (0.091)	ns	ns	0.45
PFPeS	129	ns	ns	ns	6.7 (0.029)	ns	ns	ns	ns	0
lin-PFHxS	740	ns	-2.2 (0.009)	ns	ns	ns	60 (0.076)	ns	ns	0
br-PFHxS	740	ns	ns	ns	ns	ns	72 (0.099)	ns	ns	0
tot PFHxS	129	ns	-2.0 (0.009)	ns	ns	ns	60 (0.076)	ns	ns	0
PFHpS	741	ns	ns	ns	4.8 (0.017)	34 (0.13)	37 (0.12)	ns	ns	0.71
lin-PFOS	741	1.5 (0.005)	ns	ns	ns	ns	18 (0.045)	ns	-15 (0.061)	0.91
br-PFOS	741	0.96 (0.004)	ns	ns	ns	ns	13 (0.044)	ns	-12 (0.059)	0.93
tot PFOS	742	1.3 (0.004)	ns	ns	ns	ns	16 (0.044)	ns	-14 (0.059)	0.92

The variable "Education" included women with high school education (1, reference group), women with 1-3 years of higher eductation (2) and women with more than 3 years of higher education (3). Women 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. 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 PFBS and PFHxS have been increasing in the mothers from Uppsala. At around year 2010-2011 levels had started to decrease (CP) which is consistent with the initiation of efforts to mitigate the contamination in July 2012. Concentrations of PFUnDA have been increased about 2% per year during the entire study period, 1996-2019, but for PFNA a cessation of the increase was observed around 2008 and thereafter the levels are decreasing. 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 at the same rate and for those substances that do not show a downward trend if the levels will level out and then decrease.

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