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PFAS in first-time mothers from Sweden: temporal trends and the impact from fish/seafood consumption and drinking water exposure

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ABSTRACT

Per- and polyfluoroalkyl substance (PFAS)-contaminated drinking water has been a significant source of human exposure to PFAS in Uppsala, Sweden. Herein, we investigated temporal trends of PFAS in serum samples collected three weeks after delivery from first-time mothers in Uppsala (1996–2022; n = 869), to determine whether efforts to remediate drinking water contamination have reduced maternal PFAS exposure. In addition, the impact of fish/seafood consumption as an exposure source was evaluated. PFAS were analysed by ultra-high performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS) and temporal trends were evaluated using adjusted cubic spline models.

Linear (lin) and branched (br) perfluorooctane sulfonate (PFOS) and perfluoroctanoate (PFOA) showed declining temporal trends, likely due to international regulation and phase-out initiatives. Later initiatives to restrict use and emissions of perfluorononanoate (PFNA), perfluorodecanoate (PFDA) and perfluoroundecanoate (PFUnDA) likely explained the initial increased concentrations by 3–7% per year, up to 2007 or 2010, followed by decreasing trends, on average 2–3% per year. Drinking water contamination was likely responsible for the increase in serum br and lin perfluorohexanesulfonate (PFHxS) concentrations early in the study period, followed by a decline over the last decade associated with remediation of the drinking water contamination around 2012. However, even after remediation, drinking water appeared to contribute to perfluoropentanesulfonate (PFPeS) and PFHxS. Fish/seafood consumption was significantly associated with serum levels of lin PFOS, PFNA, PFDA, and PFUnDA.

Overall, PFAS exposure among first-time mothers in Uppsala has declined, resulting in a marked reduction in the proportion of mothers exceeding the serum reference value established by the European Food Safety Authority. Nevertheless, 54% of the mothers sampled from 2018 to 2022 still exceeded this level, showing that efforts to reduce PFAS exposure must continue for many years to come.

1. Introduction

Per- and polyfluoroalkyl substances (PFAS) have a wide range of applications and have been manufactured globally since the 1950 s. Today, thousands of PFAS are known to exist on the global market (OECD, 2018). Since the discovery of PFAS in the environment early in the 21st century (Giesy and Kannan, 2001), measures have been taken to reduce emissions of the most widely distributed PFAS, perfluorooctanesulfonate (PFOS) and perfluorooctanoate (PFOA) (US EPA, 2000, US EPA, 2006). Within the EU, PFOS was regulated in 2008 under REACH, followed by inclusion of PFOS, PFOA and perfluorohexanesulfonate (PFHxS) (and related compounds) in the Stockholm Convention in 2009, 2019 and 2022, respectively (Stockholm Convention, 2009, Stockholm Convention, 2019, Stockholm Convention, 2022). PFAS with shorter fluorinated carbon chains than the long-chain PFAS mentioned above have been widely used as replacements due to regulatory measures and voluntary industry actions (Brendel et al., 2018).

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In background-exposed populations, humans are mainly exposed to PFAS via food and drinking water arising from environmental contamination, but also via dust, air, and the use of products containing PFAS (EFSA, 2020, Poothong et al., 2020). PFAS are also transferred through the placenta and into breastmilk, which represent important exposure routes for PFAS early in life (Gutzkow et al., 2012, Gyllenhammar et al., 2018, Mondal et al., 2014). Fish consumption is a significant source of dietary exposure to long-chain PFAS with carbon chain lengths > 7, such as PFOS and perfluoro-nonanoate (PFNA), −decanoate (PFDA), and –undecanoate (PFUnDA) (Bjermo et al., 2013, Christensen et al., 2017, Papadopoulou et al., 2019, Nystrom et al., 2022, Wang et al., 2024). Drinking water can be a major source of human exposure to both short- (carbon chain length ≤ 7) and long-chain PFAS, and contamination has led to elevated blood levels in human populations in numerous places (Shin et al., 2011, Ingelido et al., 2018, Johanson et al., 2023, Xu et al., 2021, Kotlarz et al., 2020). Even low concentrations of PFAS in drinking water (<10 ng/L of individual PFAS) can lead to significant accumulation in the human body over time (Nystrom-Kandola et al., 2023). In the City of Uppsala, Sweden, drinking water has been contaminated with PFAS since at least 1996, mainly due to the use of aqueous film forming foams (AFFF) at fire-fighting training areas, resulting in elevated PFAS serum levels in mothers and children (Gyllenhammar et al., 2015, Gyllenhammar et al., 2019).

The Swedish Food Agency has conducted recurrent cross-sectional sampling of breastmilk and blood from primiparous women in Uppsala since 1996, in the so-called POPUP study (Persistent Organic Pollutants in Uppsala Primiparas). An investigation between 1996 and 2011 showed that women in districts of Uppsala supplied with PFAS-contaminated drinking water during the study period had higher levels of PFHxS and perfluorobutane sulfonate (PFBS) in serum than women living in other districts which were not expected to be contaminated (Gyllenhammar et al., 2015). Following discovery of the drinking water contamination in 2012, it was immediately mitigated by the drinking water producer (Gyllenhammar et al., 2015). A study of PFAS temporal trends in pooled serum samples (1996–2017) showed that PFBS and PFHxS levels started to decline soon after the remediation was introduced (Miaz et al., 2020). However, follow-up investigations of PFAS trends in individuals from the POPUP cohort have not been performed. The major advantage of assessing individuals is that both their location- and duration of residence in Uppsala can be taken into account, thereby offering a more accurate assessment of the impact of drinking water remediation on PFAS body burdens.

The main objective of the present work was to estimate PFAS body burden temporal trends among individual POPUP mothers sampled three weeks after delivery, thus estimating exposure of fetuses and infants in the different districts of Uppsala before and after remediation of the drinking water contamination. In addition, the impact of PFAS exposure from fish/seafood consumption on maternal PFAS body burdens was evaluated. Serum levels were also compared to the serum PFAS level representing the Tolerable Weekly Intake (TWI) established by the European Food Safety Authority (EFSA), to evaluate potential health risks (EFSA, 2020).

2. Material and methods

2.1. 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) and Gyllenhammar et al. (2015). Participants were randomly recruited among first-time mothers who were born in Sweden and who delivered at Uppsala University Hospital. In total, 1109 women were recruited between 1996 and 2022 and the participation rate was on average 50 %. PFAS levels have been analysed in serum samples from 869 of the mothers and the number of samples from each year is described in the Supplemental Information (Table S1). The blood samples were taken at home by a midwife three weeks after delivery. Blood sampling was carried out using 10 ml Vacutainer® or Vacuette® serum tubes. Serum was stored at the Swedish Food Agency at −20 °C, which has been shown to keep PFAS stable during storage (Kato et al., 2013). The study was approved by the local ethics committee of Uppsala University, and the participating women gave informed consent prior to their inclusion in the study.

2.2. Questionnaire data

All women completed a questionnaire during the home visit from the midwife, providing information about their age, weight, height, education, smoking habits, and residence history. After the visit, a food frequency questionnaire was sent out to the mothers, asking about fish and shellfish consumption during the year before their pregnancy. The questions were divided into different fish categories of seafood normally consumed in Sweden, such as lean marine fish (cod species, flatfish), salmon, canned tuna, herring, freshwater fish (pike, perch, zander), fish roe, crab, and shellfish. The answers were used to calculate the total daily consumption of fish/seafood in g/day using the same portion sizes as in Glynn et al. (2011). The mothers were also divided into three groups consuming fish/seafood < 1 time/week, 1–2 times/week or > 2 times/week calculated using a portion size of 125 g.

2.3. Drinking water district

Hydraulic models of the water distribution pipeline network have been used to simulate spreading of PFAS in drinking water in Uppsala city and have been published previously (Gyllenhammar et al., 2015). In the present study, all mothers were assigned to different drinking water districts in Uppsala based on their home address at the time of sampling (see Supplemental Information Table S2). The drinking water districts have changed over the years due to modifications in the water production and distribution system. For each participating mother, the water distribution model covering the sampling date was used.

Mothers living in districts receiving up to 10 % and districts receiving 10–90 % of drinking water from the water works with elevated PFAS levels (Gyllenhammar et al. 2015) were categorised separately. If they were sampled before July 2012, these districts were defined as"Medium PFAS" (up to 10 %) and "High PFAS" (10–90 %). After July 2012 (following remediation), these districts were defined as"Medium PFAS after remediation" and"High PFAS after remediation". In total 28 women were, prior to 2007, living in the district that received the highest proportion of PFAS-contaminated drinking water (90–100 %) (Gyllenhammar et al. 2015). These women were combined with the "High PFAS" group in order to increase sample size.

In temporal trend analyses, women were also divided into the groups "no PFAS", which included women living outside Uppsala or in the"Low PFAS" district that had not received PFAS-contaminated drinking water; and "PFAS", which included women in the "High PFAS"- and "High PFAS after remediation" groups, (Supplemental Information Table S2).

2.4. PFAS analysis

PFAS were analyzed in 297 of the women sampled 1996–1999 and 2008–2011, as described in Gyllenhammar et al. (2015). In the present study, PFAS were additionally analyzed in 572 first-time mothers, sampled between 1996 and 2022 using the same method. In short, 0.5 g of serum was spiked with isotopically labelled internal standards and extracted with acetonitrile. The concentrated extract underwent dispersive clean-up with graphitized carbon. Aqueous ammonium acetate and recovery standards were added prior to instrumental analysis on a Waters ultra-high performance liquid chromatograph (UPLC) coupled to a Waters Xevo TQ-S tandem mass spectrometer (MS/MS) operated in negative electrospray ionization, multiple reaction monitoring mode. Quantification was performed by isotope dilution using an

Table 1
Population characteristics of first-time mothers (n = 869) sampled 1996–2022, in Uppsala County.

Variable		Mean	±SD	Median	Min-Max	Missing
Age (year)		29.8	4.0	29.7	19.3–45.3	0
Pre-pregnancy BMI[a] (kg/m^2)		23.4	3.6	22.7	16.2–47.3	7
Weight gain during pregnancy (%)		23.6	8.5	23.1	−7.9–61.7	6
Weight reduction from delivery to sampling (%)		9.2	3.2	9.0	−1.0–22.0	41
Fish/seafood consumption (g/day)		33	24	29	0–220	46
Variable		**n**	**%**			
Education	Max 3–4 years of high school	214	25			
	1–3 years of higher education	194	22			
	>3 years of higher education	458	53			
	Missing	3				
Smoking[b]	Non-smoker	670	77			
	Former smoker	122	14			
	Smoker	74	9			
	Missing	3				
Fish/seafood consumption	<1 time/week	218	27			
	1–2 times/week	305	37			
	>2 times/week	300	37			
	Missing	46				
Drinking water district[c]	Outside of Uppsala	214	25			
	Low PFAS	366	42			
	Medium PFAS	40	5			
	High PFAS[d]	128	15			
	Medium PFAS after remediation	29	3			
	High PFAS after remediation	91	10			
	Missing	1				
Years in Uppsala[e]	0	107	13			
	1–3	106	13			
	4–6	164	20			
	7–9	216	26			
	≥ 10	242	29			
	Missing	34				

[a] Body mass index.
[b] Women 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.
[c] Drinking water has been contaminated with PFAS mainly in the southern parts of Uppsala City. All mothers have been categorized into the districts presented in Gyllenhammar et al. 2015 according to their home address at sampling.
[d] In total 28 mothers that lived in the PFAS area before 2007, receiving drinking water with > 90 % of PFAS-contaminated water, were grouped together with the High PFAS area receiving 10–90 % of PFAS-contaminated drinking water.
[e] The number of years that the mothers had lived in Uppsala before sampling.

8-point calibration curve (linear, 1/x weighting, excluding the origin). A full list of target analytes and their matching internal standards is provided in table S3 of the Supplemental Information. A total of 20 PFAS were analysed in all batches; in addition, three batches were analyzed using an extended target list of 32 PFAS (n = 219; Supplemental Information Table S4). Methods limits of quantification (LOQs) were based on the lowest point on the calibration curve showing a signal to noise ratio of > 3. In total, analyses were performed on 10 different batches during an 11-year period and the LOQs varied between analytical runs (Supplemental Information Table S4). Each batch of samples was extracted with procedural blanks and quality control samples (spiked human samples) and some also included a sample of NIST standard reference material (SRM) 1957. The average recoveries of these quality control samples ranged between 74 – 125 % for the 20 PFAS analysed in all batches (Supplemental Information Table S5).

2.5. Statistical analyses

Statistical analyses were performed using R version 4.5.0 (), with the addon packages Epi (Carstensen et al., 2024), stdReg (Sjolander and Dahlqwist, 2021), and emmeans (LENTH, 2025). When serum PFAS concentrations were below LOQ, levels were set to LOQ divided by $\sqrt{2}$ (HORNUNG and AND REED, 1990). Statistical analyses were performed for individual PFAS as well as for the sum of PFOA, PFNA, PFHxS, and PFOS (PFAS4) according to the TWI established by EFSA (EFSA, 2020). Temporal trends were investigated for the entire study period (i.e. 1996–2022) for PFOA, PFNA, PFDA, PFUnDA, branched (br) PFHxS, linear (lin) PFHxS, br PFOS, lin PFOS and PFAS4. Univariable analysis of the temporal trends was performed using linear regression models, with the log-transformed yearly geometric mean value of the PFAS as the outcome and sampling year modeled with restricted cubic splines (henceforth referred to as cubic spline models). Knots for the cubic splines were selected according to HARREL, (2001) recommended percentiles and the number of knots for the different PFAS were determined by fitting models with 3 to 7 knots and the model that provided the lowest Akaike information criterion (AIC) was prioritized (Supplemental Information Table S6). Multivariable analysis of the temporal trends was based on multivariable 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, fish/seafood consumption, and smoking. The temporal trends expressed as mean percentage change per year were assessed for the same covariates at the year with the highest geometric mean levels compared to the first year 1996, and after that year and the last year 2022. For PFOA, PFOS and PFAS4, trends were evaluated until 2012 from the year with the highest geometric mean and 2012–2022, as a plateau was observed for PFOA and PFAS4 and a change in decreasing rate for PFOS, around 2012. 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 remedial measures were introduced. In a sensitivity analysis, temporal trends were investigated for the first-time mothers living in the districts with the highest PFAS contamination

Table 2
Concentrations of PFAS (ng/g serum) in individual serum samples from first-time mothers sampled three weeks after delivery, in Uppsala County 1996–2022.[a]

PFAS	n	<LOQ	Mean[b]	±SD[b]	Median	Min-Max
PFHpA	869	662 (76 %)	0.022	0.045	<LOQ	<LOQ-0.40
PFOA	869	2 (0.2 %)	2.05	1.18	1.88	<LOQ-13.05
PFNA	869	9 (1 %)	0.52	0.30	0.46	<LOQ-2.95
PFDA	869	60 (7 %)	0.24	0.15	0.21	<LOQ-1.26
PFUnDA	869	117 (13 %)	0.21	0.14	0.19	<LOQ-1.31
PFBS	869	623 (72 %)	0.018	0.049	<LOQ	<LOQ-0.80
PFPeS[c]	219	160 (73 %)	0.054	0.11	<LOQ	<LOQ-0.79
br PFHxS	867[d]	173 (20 %)	0.23	0.33	0.11	<LOQ-5.32
lin PFHxS	867[d]	0	3.33	3.42	2.18	0.27–32.72
tot PFHxS[b]	867		3.56	3.68	2.32	0.32–33.88
PFHpS[c]	219	124 (57 %)	0.072	0.092	<LOQ	<LOQ-0.50
br PFOS	868[e]	2 (0.2 %)	3.65	3.02	2.66	<LOQ-19.64
lin PFOS	868[e]	1 (0.1 %)	7.63	3.02	5.78	<LOQ-40.86
tot PFOS[b]	868		11.27	8.84	8.48	0.21–60.50
PFAS4[b, f]	867		17.41	10.48	15.57	0.79–69.27

Linear (lin), branched (br).
[a] PFAS included in the statistical analysis. Other PFAS in Supplemental Information Table S4.
[b] Data below LOQ was replaced with the lowest LOQ for each compound/$\sqrt{2}$.
[c] Analysed during the years 2017–2022.
[d] PFHxS not analysed in 2 samples.
[e] PFOS not analysed in 1 sample.
[f] Sum of the four PFAS: PFOA, PFNA, br and lin PFHxS, br and lin PFOS.

in drinking water ("PFAS", n = 219), and for the participants living in the "no PFAS" district outside or in the north of Uppsala "Low PFAS" (n = 580) in order to compare the temporal trends in the different districts (Supplemental Information Table S2). The associations between PFAS levels and the covariates age, BMI, weight gain during pregnancy, weight loss after delivery, education level, fish/seafood consumption, and maternal smoking were also assessed in the cubic spline models. In a sensitivity analysis, drinking water district was also adjusted for and the results are presented in the Supplemental Information. As a consequence of the logarithmic transformation, the associations between sampling year and serum PFAS concentrations, and between covariates and concentrations, were presented as percent change of PFAS concentrations per year, and percent change per unit of the covariates, and not as change in absolute levels.

Differences in serum levels of PFOA, PFNA, PFDA, PFUnDA, br and lin PFHxS, br and lin PFOS, and PFAS4 between fish/seafood consumption categories, drinking water districts and number of years living in Uppsala during the last 10 years before delivery, were calculated and compared using multiple linear regression, adjusting for the relevant covariates age, pre-pregnancy BMI, weight gain during pregnancy, weight loss after delivery, education, smoking and sampling year, to control for potential confounding. All confidence intervals and p-values were adjusted for the number of comparisons using Tukey's honestly significant difference (HSD) test (TUKEY, 1949). In the analyses of serum PFAS levels among mothers from different drinking water districts, number of years in Uppsala was included as a covariate. In addition, in analyses of both drinking water district and PFAS levels in relation to number of years in Uppsala also fish/seafood consumption were included as a covariate in the regression models. For PFAS detected in the contaminated drinking water but with low detection rates in serum (i.e. PFHpA, PFBS, PFPeS, and PFHpS), results for differences between drinking water districts are presented in the Supplemental Information.

3. Results and discussion

3.1. Serum PFAS concentrations

Characteristics of the first-time mothers sampled 1996–2022 are shown in Table 1. Median PFAS concentrations in serum over the entire study period were highest for lin PFOS (5.8 ng/g serum) followed by br PFOS > lin PFHxS > PFOA > PFNA > PFDA > PFUnDA > br PFHxS (Table 2). Serum levels of PFBS have been reported previously from mothers in POPUP (Gyllenhammar et al 2015), but more recently, all measurements have been below LOQ, likely due to a higher LOQ during recent years (Supplemental Information Tables S3 and S4). Out of all 869 samples (1996–2022), 28 % were above the LOQ for PFBS (Table 2). For PFPeS and PFHpS, which have only been analyzed in serum from the POPUP mothers since 2017, 27 % and 43 % of analysed samples, respectively, had levels above LOQ (Table 2). Among the remaining PFAS, the most frequently detected were PFTriDA (32 %, n of analyzed samples = 869), followed by PFHpA (24 %, n = 869), PFDoDA (21 %, n = 869), PFDS (15 %, n = 869), FOSA (8 % n = 869), PFTeDA (3 %, n = 869), 8:2 FTS (3 %, n = 219), 9Cl-PF3ONS (1 %, n = 219), and PFHxA (0.5 %, n = 869). In all mothers, the serum levels of PFPeDA (n = 716), PFNS, 11Cl-PF3OUdS, ADONA, 3:3 FTA, 5:3 FTA, 7:3 FTA, 4:2 FTS, 6:2 FTS, 6:2 diPAP, 8:2 diPAP, and 6:2/8:2 diPAP (n = 219) were below LOQ (Supplemental Information Table S7).

3.2. Temporal trends

The results of the spline analyses show temporal trends in PFAS levels between 1996 and 2022, adjusted for possible temporal changes in covariates associated with serum PFAS concentrations (Figs. 1 and 2, Table 3). PFOS and PFOA showed no apparent trends during the first part of the study (1996–2000 for PFOS and 1996–2003 for PFOA) before starting to decline. PFOS decreased slightly faster than PFOA during the early 2000 s (mean 6 % per year and 5 % per year, respectively), and showed a steeper overall trend (Fig. 1). This pattern is consistent with the almost complete phase-out of PFOS and related compounds starting in the year 2000 in the USA, and the slower stepwise phase-out of PFOA production (US EPA, 2000, US EPA, 2006). After 2012 the declining trends slowed down for PFOS, and the mean rates were 4 % and 3 % per year for br PFOS and lin PFOS, respectively (Table 3), suggesting that the initial effect of the phase-out (i.e. reduction in primary emission sources) on temporal trends was diminished and that secondary sources of environmental contamination may have become more important for the temporal trends in human exposure. PFNA, PFDA, and PFUnDA showed similar trends, but with a slightly later peak than for PFOS and PFOA; mean concentrations increased around 3–7 % per year until 2007 for PFNA, and 2010 for PFDA and PFUnDA and thereafter declined around 2–3 % per year. This change coincided with the agreement between the US FDA and industry to phase out production and use of the long-chained PFAS by 2011 (FDA, 2017), which may also have influenced exposure levels in other countries.

For PFHxS, the highest geometric mean concentration was observed in 2009 (both br and lin PFHxS), with increasing trends before and decreasing trends after that year (Fig. 1, Table 3). This peak in PFHxS concentrations coincided with a change in the water distribution system in Uppsala in 2007, after which the drinking water in the district with the highest PFAS pollution was diluted with drinking water with lower PFAS contamination (Gyllenhammar et al. 2015). When evaluating the period after the drinking water contamination was discovered and mitigated in 2012, serum levels decreased around 5 % per year for PFHxS (Table 3). The temporal trends for PFAS4 were similar to PFOS and PFOA, which is expected given that these two compounds are the main contributors to the sum of the four PFAS (mean contribution 74 %

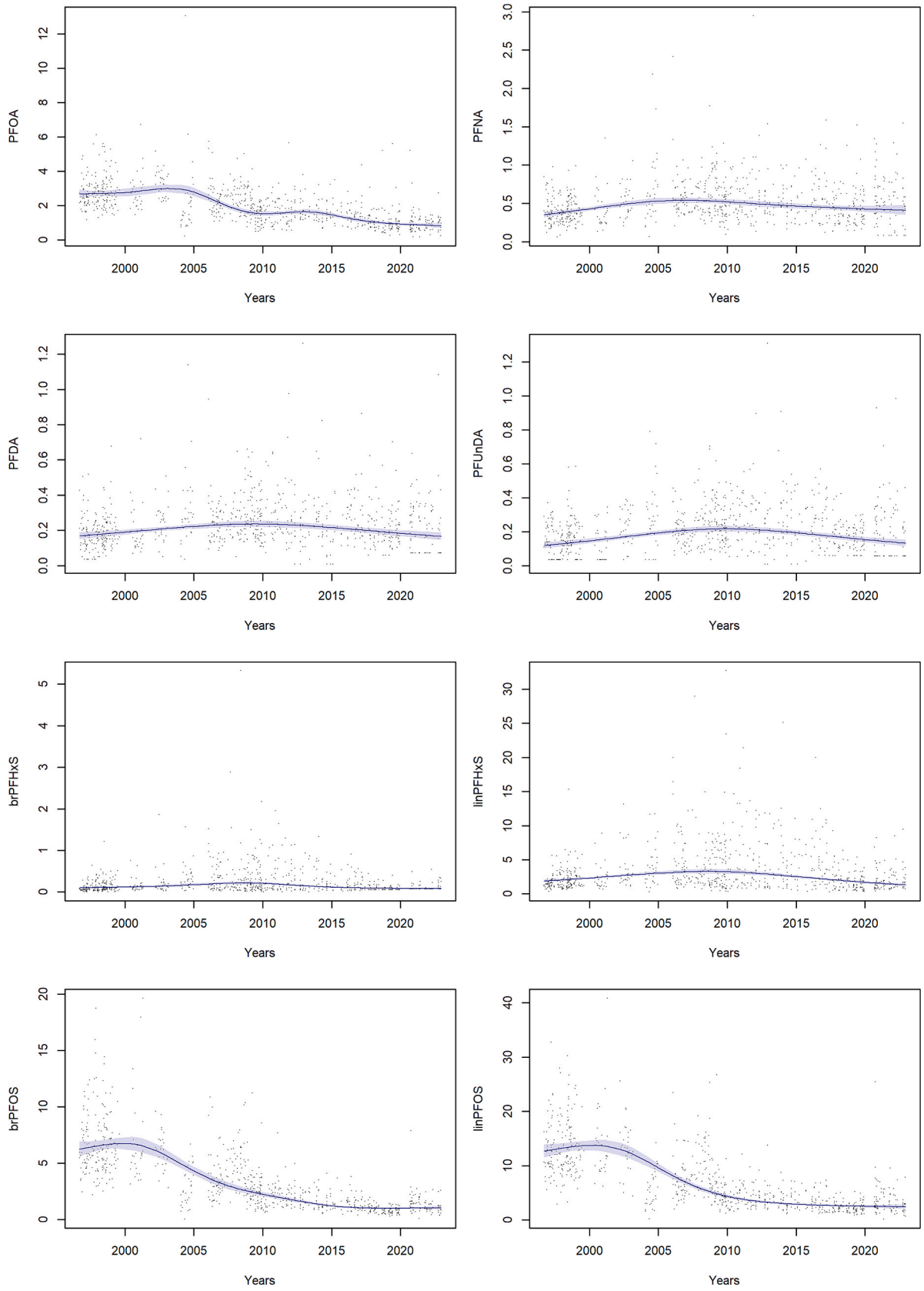

Fig. 1. Temporal trends of PFAS in serum from first-time mothers in Uppsala, sampled 1996–2022. Blue line = cubic spline trend with 95 % CI, adjusted for maternal age, pre-pregnancy BMI, weight increase during pregnancy, weight decrease between delivery and sampling, fish/seafood consumption, education level, and smoking status. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

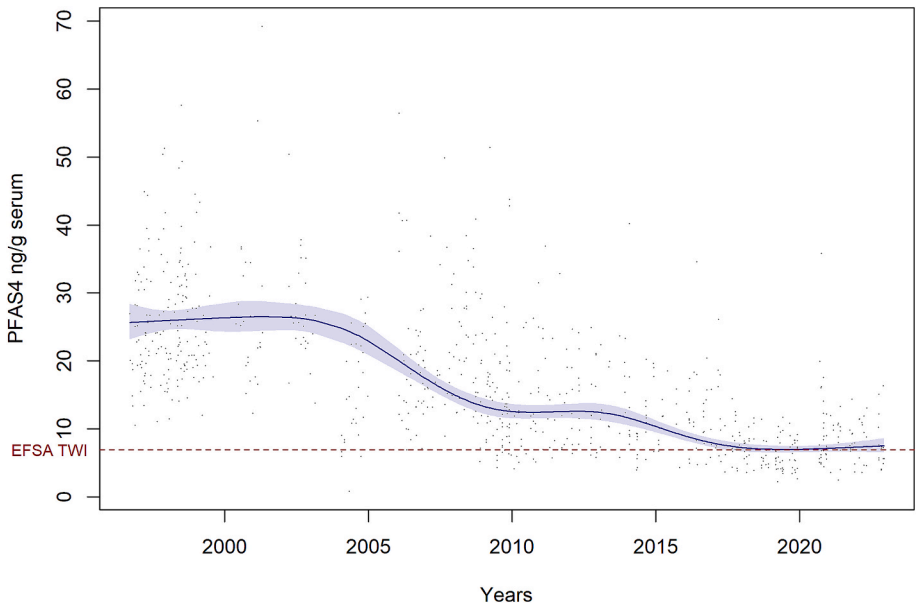

Fig. 2. Temporal trends of PFAS4 (sum of PFOA, PFNA, PFHxS and PFOS) in serum from first-time mothers in Uppsala, sampled 1996–2022. Blue line = cubic spline trend with 95 % confidence interval, adjusted for maternal age, pre-pregnancy BMI, weight increase during pregnancy, weight decrease between delivery and sampling, fish/seafood consumption, education level, and smoking status. Red line = estimated safe serum level for mothers in the calculation of the tolerable weekly intake (TWI) from the risk assessment by EFSA (2020). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 3
Adjusted mean change in PFAS concentrations in serum during different periods between 1996 and 2022[a], from cubic spline models adjusted for age, BMI, weight gain during pregnancy, weight change between delivery and sampling, fish/seafood consumption, educational level, and smoking.

PFAS	n	Years[b]	Mean change per year % (95 % CI)	Years[b]	Mean change per year % (95 % CI)	Years[b]	Mean change per year % (95 % CI)
PFOA	792	1996–2003	1.7 (−0.63/4.4)	2003–2012	−5.2 (−5.7/−4.5)	2012–2022	−4.7 (−5.3/−3.9)
PFNA	792	1996–2007	5.6 (3.6/7.8)	2007–2022	−1.5 (−2.2/−0.8)		
PFDA	792	1996–2010	3.1 (1.5/5.0)	2010–2022	−2.3 (−3.0/−1.4)		
PFUnDA	792	1996–2010	6.6 (4.1/9.6)	2010–2022	−3.0 (−3.8/−2.1)		
br PFHxS	790	1996–2009	9.1 (4.7/15)	2009–2022	−4.7 (−5.3/−4.1)		
				2012-2022[c]	−5.2 (−6.1/−4.0)		
lin PFHxS	790	1996–2009	6.5 (4.2/9.3)	2009–2022	−4.4 (−4.9/−3.7)		
				2012-2022[c]	−5.3 (−6.0/−4.5)		
br PFOS	791	1996–2000	2.7 (−1.6/7.8)	2000–2012	−6.1 (−6.4/−5.9)	2012–2022	−4.3 (−5.1/−3.2)
lin PFOS	791	1996–2000	2.7 (−0.6/6.6)	2000–2012	−6.2 (−6.4/−6.0)	2012–2022	−3.0 (−4.1/−1.8)
PFAS4[d]	790	1996–2001	0.8 (−3.0/5.4)	2001–2012	−4.8 (−5.2/−4.2)	2012–2022	−4.2 (−4.9/−3.4)

Linear (lin), branched (br).
[a] Data below LOQ was replaced with LOQ/$\sqrt{2}$.
[b] Specified years for the mean change per year.
[c] Percent change in concentrations of tot PFHxS during 2012–2022 after mitigation of PFAS levels in drinking water.
[d] Sum of the four PFAS: PFOA, PFNA, br and lin PFHxS, br and lin PFOS.

during the whole study period). The contribution from the different PFAS to the total sum has changed from 1996 to 2022 (Fig. 3). In 2000, the sum of br and lin PFOS accounted for 78 % of the sum of the 8 most detected PFAS (PFOA, PFNA, PFDA, PFUnDA, PFHxS (br and lin), and PFOS (br and lin)), however in 2020 the contribution had decreased to 51 %. At the same time, contributions from long-chained carboxylic acids (PFCAs, i.e. PFNA, PFDA, and PFUnDA) had increased from 3 % in the year 2000 to 11 % in 2020 and PFHxS (br and lin) from 9 % (2000) to 26 % (2020).

An investigation into temporal trends in the"PFAS" and "no PFAS" water districts of Uppsala, revealed that serum br and lin PFHxS levels increased between 1996 and 2008–2009 more rapidly in the former compared to the latter district (Supplemental Information Table S8). In non-contaminated areas, the adjusted mean increase was 6.0 % for br PFHxS and 4.2 % for lin PFHxS, compared to 8.5 % and 6.3 %, respectively, in the PFAS district (Supplemental Information Table S8). Following the remediation of PFAS in the drinking water in 2012, mothers from the PFAS district experienced a more rapid decrease in br and lin PFHxS (−7.8 % and −5.9 %, respectively) compared to mothers in the no PFAS districts (−3.2 % and −4.7 %, respectively). Furthermore, PFOA, br and lin PFOS, and PFAS4 displayed a somewhat faster decline in the PFAS district (−4 to −5%), compared to the no PFAS district (−3 to −4%) (Supplemental Information Table S8), suggesting that remediation of PFAS in the drinking water may have affected the trends of PFAS other than PFHxS. Studies of outgoing drinking water from the waterworks receiving the PFAS-contaminated raw water have shown that conventional treatment steps of raw water in the waterworks before 2012, such as aeration, softening, sand filtration, chlorine disinfection, and granular active carbon (GAC) filters for pesticide removal, did not remove detected PFAS (including PFHxS, PFOS, and

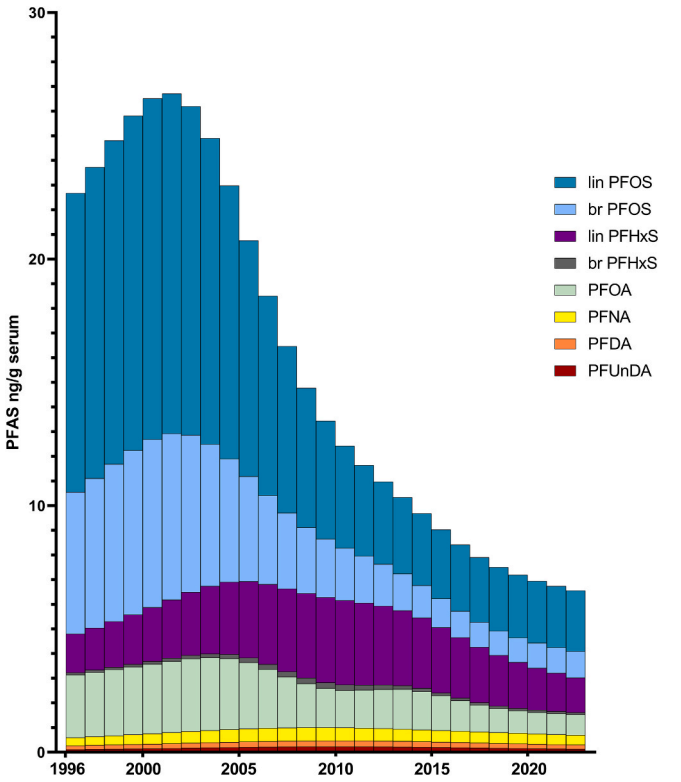

Fig. 3. Geometric mean levels of PFAS, generated in the cubic spline analysis of PFAS-concentrations in serum from first-time mother sampled 1996–2022.

PFOA) from the drinking water (Belkouteb et al., 2020). The pesticide GAC filters were not regenerated often enough to provide effective removal of PFAS via sorption. However, after installation of GAC filters for PFAS remediation in the plant after 2012, the concentrations of these PFAS decreased considerably in the drinking water (Belkouteb et al., 2020).

PFAS temporal trends have previously been studied in pooled samples from the POPUP cohort between 1997 and 2017 (Miaz et al., 2020). The general 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 one-two years later (2010–2011) in Miaz et al. (2020) compared to the present study.

In serum of adolescents from southern Sweden similar cross-sectional temporal trends of PFOS, PFOA and other long-chained PFCAs were observed from 2000 to 2017 (Norén et al., 2021). In contrast to the POPUP cohort, PFHxS displayed declining trends for the entire study period in southern Sweden (Norén et al., 2021), further illustrating the influence of drinking water contamination on the temporal trends in Uppsala. Noren et al. (2021) attributed the decline in PFHxS body burdens of adolescents to phase-out of production of PFHxS and related substances by the main manufacturer at the same time as PFOS (FDA, 2015; FDA, 2017; Stockholm Convention, 2018). In a Danish population, including both children and adults, cross-sectional median concentrations of PFOS and PFOA increased from 1988 until the late 1990 s and thereafter decreased until 2021 (Hull et al., 2023). In females from the U.S. within the National Health and Nutrition Examination Survey (NHANES), PFAS geometric mean serum levels were in the same range as in the present study, and PFOS, PFOA, and PFHxS declined continuously from 1999 to 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. Declining trends of PFAS4 plasma levels have also been reported in German adults, up to 2019 (Gockener et al., 2020). This shows that the elimination of legacy PFAS production by the main U.S. manufacturers and the following international efforts to further eliminate environmental and human exposure have had a pronounced positive impact on human background exposure to the legacy PFAS. However, as shown in the present study, and many others, environmental pollution around PFAS hotspots will continue to influence on human exposure to PFAS independently of the legislative efforts to reduce PFAS production/use (Cousins et al., 2016).

3.3. Determinants of PFAS levels

In the adjusted cubic spline models it was possible to determine associations between personal characteristics (included as independent variables in the analysis) and PFAS concentrations. For each determinant, the association with PFAS was adjusted for possible influences by the other covariates on the associations, including sampling year. All results are shown in Table 4. The variation of the independent variables in the regression model explained 10–70 % of the variation in PFAS concentrations (Table 4), showing that there are important determinants of serum concentrations not captured in the present study. The highest R^2 was observed for PFOA, PFOS and PFAS4, mainly due to the large between-sampling year variation in concentrations.

Age was positively associated with serum levels of PFNA, PFUnDA, lin PFHxS, lin PFOS and PFAS4, with an increase of 1–2 % in PFAS concentrations per year of increased age (Table 4). This may be due to the long biological half-lives of long-chain PFAS, combined with a birth cohort effect, where individuals born in earlier years may have accumulated higher PFAS levels over time due to historically greater environmental exposure. POPUP only included primiparous women which excluded the possible influence of maternal characteristics such as parity and breastfeeding that may decrease the PFAS levels among women (McAdam and Bell, 2023). PFOA, lin PFHxS, and PFAS4 were inversely related to pre-pregnancy BMI and the serum concentration decreased on average with 1–2 % per unit increase in BMI, suggesting that women that were overweight before pregnancy had slightly lower serum concentrations 3 weeks after delivery than women with low pre-pregnancy BMI (Table 4). In a review of previously published studies, McAdam and Bell (2023), concluded that none of the reviewed studies showed associations between maternal PFOA levels and pre-pregnancy BMI, and for PFOS and PFHxS mixed results were reported. Negative associations between maternal PFAS levels and pre-pregnancy BMI due to dilution effects related to increased BMI were by McAdam and Bell (2023) proposed to be less likely due to the distribution pattern of PFAS in the body, i.e. blood, liver and kidneys. Unmeasured or inadequately measured factors (i.e., residual confounding) may partly explain the associations observed in the POPUP cohort. PFNA was the only PFAS significantly associated with weight changes during and after pregnancy, with levels inversely related to weight gain during pregnancy and positively related to weight loss after delivery, supporting that the BMI relations with PFOA and PFHxS was not due to changes in body composition during pregnancy and the early nursing period. Sensitivity analyses showed that the weight change associations did change after adjustment of the results for drinking water district and also PFOA and PFAS4 were inversely associated with pregnancy weight gain (Supplemental Information Table S9).

Education level was positively associated with serum PFAS concentrations, except for PFOA and PFNA, with on average 11–73 % higher concentrations among women with the highest education level compared to the lowest (Table 4). Br and lin PFHxS levels were also significantly different between women with “only high school education” and those with “1-3 years of higher education,” with average levels being 31 % and 18 % higher compared to “Max 3–4 years of high school”, respectively. Since PFHxS is associated with drinking water contamination it is speculated that some of the association with education may be related to home address. In a sensitivity analysis when

Table 4
Adjusted percent change (mean (95% confidence interval) 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	Pre-pregnancy BMI	PregnancyWeight gain	Weight loss after delivery	Education[a]		Smoking[b]		Fish/seafood consumption	R^2
		years	kg/m^2	%	%	2	3	2	3	g/day	
PFOA	792	*0.51 (−0.34/1.4) p = 0.24*	**−1.1 (−2.0/ −0.14) p = 0.025**	*−0.43 (−0.88/0.012) p = 0.057*	*0.41 (−0.69/ 1.5) p = 0.47*	*−4.3 (−5.2/ 15) p = 0.38*	*8.7 (−0.39/ 19) p = 0.061*	*1.6 (−7.6/ 12) p = 0.75*	*5.0 (−6.9/ 18) p = 0.43*	*0.005 (−0.12/ 0.13p = 0.95*	0.48
PFNA	792	**1.5 (0.51/ 2.5) p = 0.003**	*−0.99 (−2.1/ 0.079) p = 0.069*	**−0.60 (−1.1/ −0.10) p = 0.019**	**1.3 (0.020/ 2.6) p = 0.046**	*5.4 (−5.5/ 17) p = 0.34*	*9.5 (−0.80/ 21) p = 0.071*	*−0.16 (−10/11) p = 0.98*	*−4.3 (−17/ 9.8) p = 0.53*	**0.33 (0.19/ 0.48) p < 0.001**	0.14
PFDA	792	*1.1 (−0.036/ 2.3) p = 0.057*	*−1.2 (−2.5/ 0.11) p = 0.072*	*−0.40 (−1.0/0.21) p = 0.20*	*0.54 (−0.98/ 2.1) p = 0.49*	*7.3 (−6.0/ 22) p = 0.30*	**16 (3.2/ 31) p = 0.014**	*−5.2 (−17/ 8.1) p = 0.43*	*−7.5 (−22/9.3) p = 0.36*	**0.40 (0.22/ 0.58) p < 0.001**	0.12
PFUnDA	792	**2.0 (0.62/ 3.3) p = 0.003**	*−1.3 (−2.7/ 0.17) p = 0.084*	*0.079 (−0.61/0.77) p = 0.82*	*0.45 (−1.2/ 2.2) p = 0.60*	*12 (−3.6/ 30) p = 0.14*	**17 (1.9/ 34) p = 0.025**	*−6.1 (−19/ 8.9) p = 0.41*	*−8.3 (−24/11) p = 0.37*	**0.80 (0.60/1.0) p < 0.001**	0.22
br PFHxS	790	*−0.57 (−2.4/1.3) p = 0.55*	*−0.87 (−2.9/ 1.2) p = 0.41*	*−0.027 (−1.0/0.95) p = 0.96*	*0.27 (−2.1/ 2.7) p = 0.83*	**31 (6.7/ 62) p = 0.010**	**73 (43/ 109) p < 0.001**	*5.4 (−14/ 30) p = 0.62*	*−16 (−35/ 9.5) p = 0.20*	*0.11 (−0.17/ 0.39) p = 0.46*	0.18
lin PFHxS	790	**2.1 (0.57/ 3.6) p = 0.006**	**−2.2 (−3.8/ −0.59) p = 0.008**	*−0.44 (−1.2/0.33) p = 0.26*	*−0.59 (−2.5/1.3) p = 0.54*	**18 (0.33/ 39) p = 0.046**	**57 (35/ 82) p < 0.001**	*0.025 (−15/18) P = 0.99*	*−9.1 (−26/12) p = 0.37*	*−0.045 (−0.27/ 0.18) p = 0.69*	0.21
br PFOS	791	*0.81 (−0.19/1.8) p = 0.11*	*−0.61 (−1.7/ 0.49) p = 0.27*	*−0.23 (−0.75/0.29) p = 0.38*	*1.1 (−0.20/ 2.4) p = 0.098*	*5.0 (−6.0/ 17) p = 0.39*	**12 (−1.7/ 24) p = 0.022**	*−8.7 (−18/ 1.9) p = 0.11*	*−12 (−24/ 0.73) p = 0.063*	*0.029 (−0.12/ 0.18) p = 0.70*	0.68
lin PFOS	791	**1.5 (0.51/ 2.4) p = 0.002**	*−1.0 (−2.0/ 0.019) p = 0.054*	*−0.44 (−0.93/0.048) p = 0.077*	*1.0 (−0.18/ 2.3) p = 0.094*	*7.7 (−3.1/ 20) p = 0.17*	**11 (1.4/ 23) p = 0.025**	*−7.1 (−16/ 3.2) p = 0.17*	*−12 (−23/ 0.81) p = 0.066*	**0.34 (0.20/ 0.48) p < 0.001**	0.66
PFAS4[c]	790	**1.3 (0.45/ 2.1) p = 0.003**	**−1.3 (−2.2/ −0.37) p = 0.006**	*−0.41 (−0.85/0.024) p = 0.064*	*0.37 (−0.71/ 1.5) p = 0.51*	*8.1 (−1.5/ 19) P = 0.10*	**19 (9.1/ 29) p < 0.001**	*−5.3 (−14/ 4.0) p = 0.25*	*−8.1 (−18/ 3.4) p = 0.16*	**0.13 (0.005/ 0.26) p = 0.041**	0.57

Linear (lin), branched (br).

[a] The 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).

[b] 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.

[c] Sum of the four PFAS: PFOA, PFNA, br and lin PFHxS, br and lin PFOS.

drinking water district was added to the model, the associations were almost the same but lin PFHxS levels were not significantly different between "only high school education" and those with "1-3 years of higher education" (p = 0.081) (Supplemental Information Table S9). In a recent review of determinants of legacy PFAS concentrations in pregnant mothers, higher income was shown to be one of the most important determinant together with nationality, parity and breast feeding history (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). In POPUP, the education association was adjusted for fish consumption and only first-time mothers were included. Maternal PFAS exposure was not associated with smoking, which have also been concluded in the review by McAdam and Bell (2023).

3.3.1. Fish/seafood consumption

The adjusted cubic spline models showed that levels of PFNA, PFDA, PFUnDA, lin PFOS, and PFAS4 increased with increased fish/seafood consumption (Table 4). Further, when dividing fish/seafood consumption into categories, PFNA, PFDA, PFUnDA, and lin PFOS levels were higher among participants eating more fish/seafood (Fig. 4). The adjusted means of serum levels for PFNA and lin PFOS were higher in mothers eating fish more than once a week compared to those eating less (Table 3). Significantly higher levels of PFDA and PFUnDA were observed across all three categories of fish/seafood consumption (<1 time/week, 1–2 times/week, and > 2 times per week), indicating that even small differences in fish consumption can affect serum levels (Fig. 4). PFOA, br PFHxS, lin PFHxS, and br PFOS were not associated with fish/seafood (Table 4, Supplemental Information Fig. S1). The isomeric difference of PFOS associations with fish/seafood consumption has also been observed among adolescents from Sweden (Nystrom et al., 2022). It was hypothesized that this difference may be due to higher bioaccumulation of lin PFOS than of br PFOS in fish, resulting in lin PFOS enrichment in fish tissues (Sharpe et al., 2010, Houde et al., 2008, Fang et al., 2016). Among the adolescents, and similar to POPUP, lin PFOS, PFNA, PFDA and PFUnDA concentrations in serum increased with increasing long-term seafood consumption (Nystrom et al., 2022).

In a study of mothers from six European birth cohorts, a maternal intake of fish four or more times per week was associated with 42 % higher PFUnDA blood levels compared to mothers who ate fish less than twice per week (Papadopoulou et al., 2019). In a pregnancy cohort study from the U.S. PFOS, PFNA, and PFDA were positively associated with fish/seafood consumption whereas no associations were found for

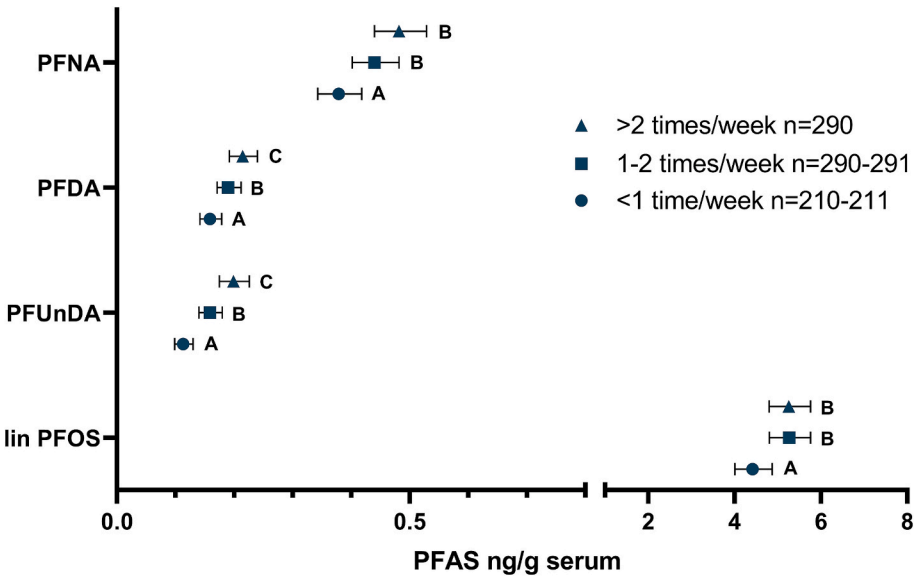

Fig. 4. Adjusted means (95 % confidence interval) of PFAS concentrations in first-time mothers sampled 1996–2022 according to fish/seafood consumption. The means were calculated and compared using multiple linear regression followed by Tukey's honestly significant difference test, and were adjusted for age, pre-pregnancy BMI, weight gain during pregnancy, weight loss after delivery, education, smoking, and sampling year. Bars not sharing the same letter were significantly different ($p < 0.05$).

PFHxS and PFOA (Wang et al., 2024). As fish are an important part of a healthy diet, continued monitoring and research are essential to better understand PFAS exposure through fish consumption and to develop strategies for reducing possible negative impacts on public health.

3.3.2. *Drinking water district*

Adjusted mean serum levels of PFAS for the different drinking water districts are presented in Fig. 5 and Supplemental Information Fig. S2. The PFAS levels in the drinking water from the waterworks using contaminated raw water have decreased after the start of remediation in 2012. First, the affected water sources were shut down until 2014. With granular activated carbon treatment in place from 2014 to 2023 the sum of 11 PFAS averaged 22 ng/L in the drinking water from the water works receiving contaminated water, and the sum of PFAS4 13 ng/L. The predominant PFASs were PFBA, PFPeA, PFHxA, PFHpA, PFOA, PFBS, PFPeS, PFHxS, and PFOS (Sorengard et al., 2022, Uppsala Vatten, 2024). This could be compared to the other waterworks in Uppsala with PFAS4 levels ranging from 1.8 to 2.7 ng/L (Uppsala Vatten, 2024).

When examining the entire period (1996–2022), lin and br PFHxS stood out with the highest adjusted mean concentrations in serum of mothers living in the High PFAS district (Fig. 5a). This shows that PFHxS exposure from drinking water in this district was high enough to be the major contributor to the total PFHxS exposure from all possible sources among the women. Adjusted mean concentrations of PFAS4 in serum did not markedly differ between water districts during the whole study period but was higher in High PFAS district compared Outside of Uppsala and Low PFAS districts (Fig. 5a). This was mainly due to PFAS4 being dominated by PFOA and lin PFOS, which did not differ between districts (Supplemental Information Fig. S2a). During the period after remediation from August 2012, the adjusted mean PFHxS levels in serum among mothers in the Medium PFAS and High PFAS districts decreased (Fig. 5a). Consequently, PFAS remediation in the affected water works starting 2012 had clear positive effects on PFHxS exposure.

Prior to PFAS remediation (i.e. 1996-July 2012), the adjusted mean lin and br PFHxS levels in serum were 2- and 3-fold greater, respectively, in the High PFAS district than in the other districts (Fig. 5b). As in the case of PHFxS levels during the whole study period (1996–2022), mothers from the Medium PFAS district did not show markedly higher br and lin PFHxS concentrations, illustrating that the exposure to ≤ 10 % of the PFAS-polluted drinking water at home was not high enough to markedly affecting PFHxS body burdens. In this case, PFAS4 levels were higher in High PFAS district compared to Outside of Uppsala and Low PFAS districts (Fig. 5b), and similar to the findings for the whole study period, PFOS and PFOA levels did not differ between districts (Supplemental Information, Fig. S2b). During this period, High PFAS district mothers also had markedly higher adjusted mean concentrations of PFBS than mothers from the other districts, confirming the observations in Gyllenhammar et al. (2015) that the highly PFAS-contaminated drinking water was a significant source of PFBS exposure (Supplemental Information Fig. S2b. Levels of PFHpA were also highest among women from the High PFAS district but only significantly different to the Medium PFAS district (Supplemental Information Fig. S2b).

Among mothers sampled 2017–2022, starting 5 years after remediation, the difference in adjusted mean lin PFHxS levels between the High PFAS district mothers and Low PFAS district mothers had decreased to 1.6-fold (Fig. 5c) and the difference for br PFHxS had decreased to 1.7-fold, further supporting a clear positive effect of PFAS remediation of contaminated drinking water. A faster decline of serum levels of br PFHxS than of lin PFHxS was also reflected in the more rapid cross-sectional temporal decline of br PFHxS levels than of lin PFHxS levels in the PFAS districts than in the no PFAS districts, after remediation (Supplemental Information Table S8). To the best of our knowledge, no study has published human serum half-lives of br PFHxS, but hypothetically our results suggest a shorter half-life of br PFHxS than of lin PFHxS. Despite remediation of the drinking water at least 5 years before the study period 2017–2022, br and lin PFHxS still showed the highest adjusted mean serum levels in mothers from the High PFAS drinking water area (Fig. 5c). The High PFAS district also had the highest adjusted mean concentration of PFPeS, a PFAS only analysed in serum from mothers sampled in recent years (Supplemental Information Fig. 2c). PFPeS and PFHxS have estimated serum half-lives around 0.6–2.5 and 2.9–4.5 years, respectively (Xu et al., 2020, Li et al., 2022). The relatively long half-lives of these PFASs may be a reason for the higher levels in the High PFAS district many years after remediation. However, even after the PFAS remediation the PFHxS and PFPeS concentrations in drinking water in the High PFAS district were higher than in the Low PFAS district, thus drinking water may still have been a source of exposure in Medium and High PFAS districts thus affecting serum PFHxS

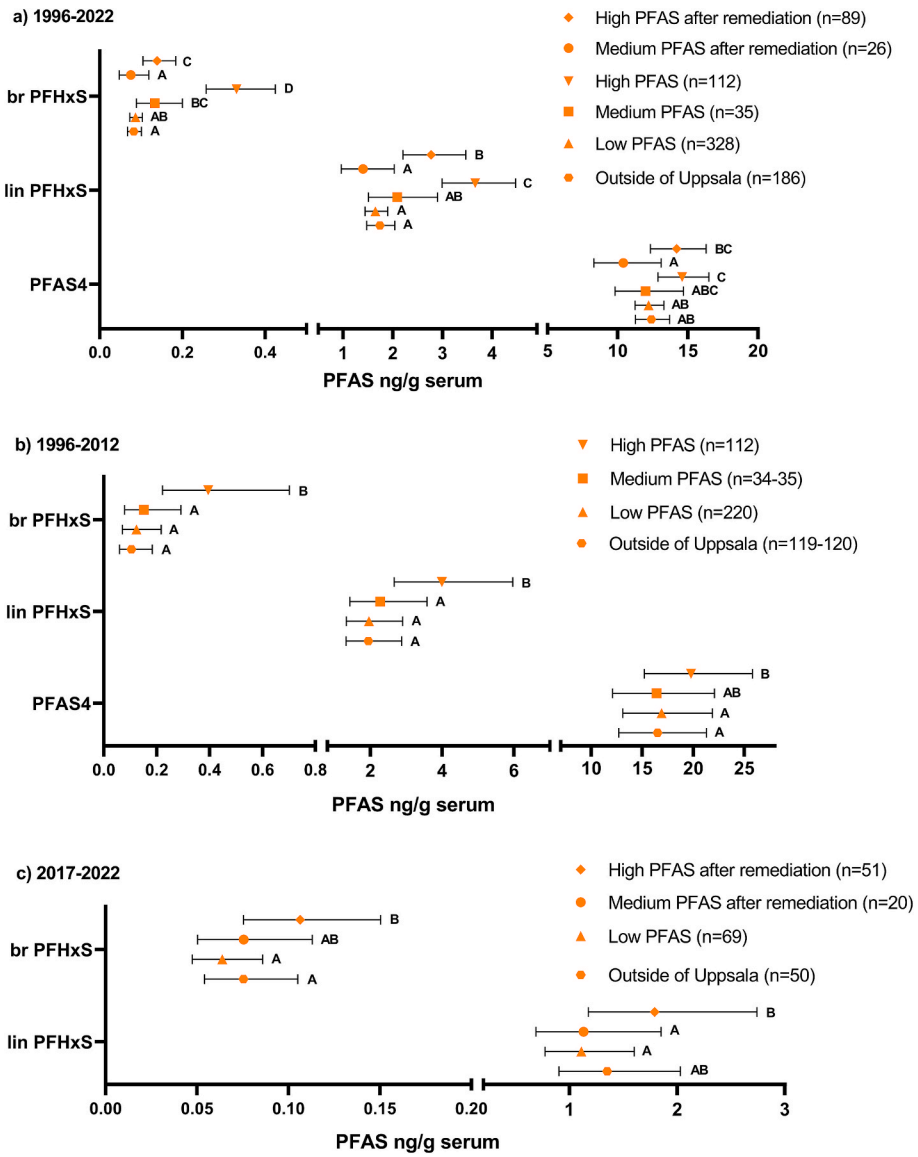

Fig. 5. Adjusted means (95 % confidence interval) of PFAS concentrations in first-time mothers according to drinking water district of living in the Uppsala City sampled a) 1996–2022, b) 1996-July 2012, and c) 2017–2022. The means were calculated and compared using multiple linear regression followed by Tukey's honestly significant difference test, and were adjusted for age, pre-pregnancy BMI, weight gain during pregnancy, weight loss after delivery, education, smoking, fish/seafood consumption, sampling year and number of years in Uppsala during the 10 years before delivery. Bars not sharing the same letter were significantly different ($p < 0.05$).

and PFPeS levels in residents of these areas. PFOA, PFHpS, PFOS, and PFAS4 levels did not differ between the districts (Supplemental Information, Fig. S2c). In 2017–2022 PFHpA and PFBS were not sufficiently detected to make comparisons between districts possible.

3.3.3. *Years living in Uppsala*

Adjusted mean serum PFAS levels for the number of years the mothers lived in Uppsala over the last 10 years before delivery are presented in Fig. 6 and Supplemental Information Fig. S3. The results for the entire study period show that mothers living 7–10 or more years in Uppsala had significantly higher adjusted mean levels of br PFHxS, lin PFHxS and PFAS4 compared to 0–3 years or 0–6 years (Fig. 6). Further, br PFOS was higher in women living 7–10 or more years in Uppsala compared to women that had lived less than a year in Uppsala, while lin PFOS was higher in women that had lived 7–9 years in Uppsala compared to women that have lived 0–3 years (Fig. 6). We speculate that the high proportion of br PFOS in relation to lin PFOS in the PFAS-contaminated Uppsala drinking water may have contributed to this observation (Gyllenhammar et al., 2015). Two studies of pooled serum samples from POPUP mothers showed that the proportion of br PFOS increased, similar to br PFHxS, between 1996 and 2010 (Glynn et al., 2012, Liu et al., 2015). Among mothers sampled 2017–2022, the highest adjusted mean levels of br PFHxS, lin PFHxS and PFAS4 were observed in the group of women that have lived 10 or more years in Uppsala (Fig. 6).

3.4. *Serum levels in comparison to the risk assessment by EFSA*

In 2020, 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 PFAS4. The TWI was based on lowered titres of diphtheria antibodies in 1 year old toddlers after in utero and breast-feeding exposure to PFAS4 (EFSA, 2020). 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 the TWI before pregnancy. This serum level is considered safe and

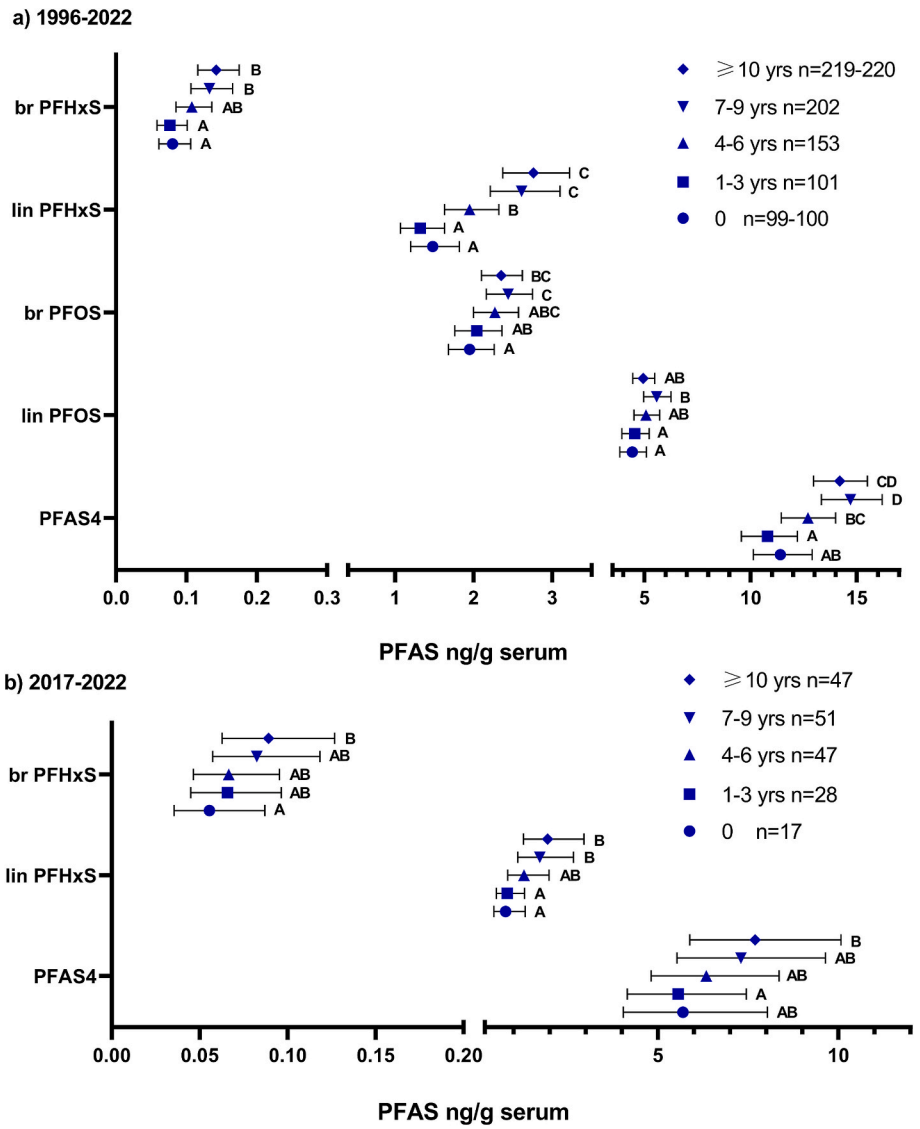

Fig. 6. Adjusted means (95 % confidence interval) of PFAS concentrations in first-time mothers according to number of years of living in the Uppsala during the last 10 years before delivery, sampled a) 1996–2022 and b) 2017–2022. The means were calculated and compared using multiple linear regression followed by Tukey's honestly significant difference test, and were adjusted for age, pre-pregnancy BMI, weight gain during pregnancy, weight loss after delivery, education, smoking, fish/seafood consumption, and sampling year. Bars not sharing the same letter were significantly different ($p < 0.05$).

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 17 ng/mL and median of 16 ng/mL for PFAS4 was observed in the present study, assuming a relative density of 1 for serum (Table 2). A comparison with the EFSA safe serum limit for PFAS4 (EFSA TWI) over the entire period from 1996 to 2022 is shown in Fig. 2. The figure indicates that while all women had levels above the safe serum limit before 2007, from 2017 to 2022, 44 % of the mothers were below 6.9 ng PFAS4/mL, showing a positive trend. As PFAS4 concentrations in maternal serum increased with the number of years living in Uppsala (Fig. 6), long-term drinking water PFAS exposure most probably contributed to the proportion of mothers above the serum PFAS4 TWI level (35 % of the women who had lived 0–3 years in Uppsala compared to 65 % of the women that had lived in the city 10 or more years). Consequently, the results are probably not representative for mothers in the general population in Sweden with background exposure from drinking water. The considerable decline in PFHxS exposure after remediation of the drinking water in 2012 contributed significantly to the declining proportion of mothers exceeding 6.9 ng PFAS4/mL serum during the last decade in Uppsala.

4. Conclusion

PFOS and PFOA levels in serum from first-time mothers living in Uppsala have declined between 1996 and 2022 as a result of international regulation and industrial/governmental phase-out initiatives. Due to drinking water contamination, serum PFHxS concentrations increased in the mothers between 1996 and 2009. However, our results show that remediation of contaminated drinking water in one of the Uppsala waterworks starting 2012, has been reducing maternal PFHxS exposure. Nevertheless, the long half-life of PFHxS and residual contamination of the remediated drinking water most likely contributed to slightly higher average PFHxS concentrations among women in certain Uppsala areas during the later part of the study period. Concentrations of PFNA, PFDA, and PFUnDA increased about 3–7 % per year during the first part of the study (1996 up to 2007 or 2010), but after that the levels decreased around 2–3 % per year, showing that international efforts to reduce production and use of these PFAS during the most recent decade have reduced human exposure in Sweden. For these long-chain PFAS, as well as lin PFOS (but not br PFOS), fish/seafood consumption was a significant contributor to exposure. This suggests

that PFOS isomers may have somewhat different sources of exposure. The international efforts to reduce exposure to PFAS4 and the remediation of PFAS contaminated drinking water in Uppsala have resulted in a marked decrease in the proportion of first-time mothers exceeding health-based safe serum PFAS4 levels. However, 54 % of the Uppsala mothers sampled 2018–2022 had PFAS4 concentrations above the safe serum level established by EFSA, showing that efforts to diminish PFAS exposure needs to continue for many years to come.

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CRediT authorship contribution statement

Irina Gyllenhammar: Writing – original draft, Visualization, Validation, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Conceptualization. **Jonathan P. Benskin:** Writing – review & editing, Validation, Resources, Methodology, Investigation. **Merle Plassmann:** Writing – review & editing, Validation, Methodology, Investigation. **Martin Kruså:** Validation, Methodology, Investigation. **Philip McCleaf:** Writing – review & editing, Methodology. **Pernilla Hedvall Kallerman:** Writing – review & editing, Project administration. **Erik Lampa:** Writing – review & editing, Methodology. **Anders Glynn:** Writing – review & editing, Methodology, Investigation, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envint.2025.109671.

Data availability

The authors do not have permission to share data.

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