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Perfluoroalkyl acids in serum from nursing women living in an area in Sweden with drinking water contamination

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Sammanfattning

Under perioden 1996 till 2011 har Livsmedelsverket samlat in blodserum från förstföderskor i Uppsala län. Ett av syftena med studierna är att undersöka hur halterna av vissa persistenta organiska miljögifter (POP) förändras med tiden. I denna rapport utvärderas blodserumnivåer för 14 perfluoroalkylsyror; (PFAA) varav tio perfluoroalkylkarboxylsyror (PFCA), fyra perfluoroalkansulfonsyror (PFSA) och perfluorooktansulfonamiden FOSA , från prover tagna 1996-1999 och 2008-2011 (n=297). Syftet med studien var att utvärdera om det finns signifikanta skillnader i blodnivåer av PFAA mellan kvinnor som bor inom olika områden i Uppsala stad, för att därigenom försöka bedöma om dricksvattenexponering påverkar nivåerna av PFAA i blod. Undersökningar av PFAA i grundvattenbrunnar och dricksvatten i Uppsala har tidigare visat att kontaminerat vatten främst distribuerats till de södra delarna av Uppsala. Kontaminerade brunnar har nu tagits ur produktion. Högre blodnivåer av perflurohexansulfonsyra (PFHxS) och perfluorbutansulfonsyra (PFBS) hittades i främst det södra området av Uppsala, både 1996-1999 (ej PFBS) och 2008-2011, vilket tyder på att konsumtion av dricksvatten är en viktig källa för exponering. Halterna av PFHxS var i allmänhet högre i Uppsala stad 2008-2011 än 1996-1999, men ej bland deltagare boende utanför Uppsala. Liknande resultat sågs för PFBS, vilket antyder att kontaminerat dricksvatten ligger bakom de ökande blodhalter av substanserna som tidigare observerats i Uppsala. Vi såg också samband mellan fiskkonsumtion och ökade nivåer av PFOS från kvinnor 2008-2011, vilket indikerar att fiskkonsumtion som exponeringskälla har ökat i betydelse sen 1990-talet.

Perfluoroalkyl acids in serum from nursing women living in an area in Sweden with drinking water contamination

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ABSTRACT
Perfluoroalkyl acids (PFAAs) are a group of persistent organic substances that are surface active and water and fat/oil repellent. Due to the wide use and persistence of PFAAs they are present everywhere in the environment, and humans are exposed via food, drinking water and the use of products containing PFAAs and related compounds. We investigated blood serum levels of ten perfluoroalkyl carboxylic acids (PFCAs), four perfluoroalkane sulfonic acids (PFSAs), and perfluoroctane sulfonamide (FOSA) in 297 women from Uppsala County, Sweden, sampled three weeks after delivery during 1996-1999 and 2008-2011. The aim of the study was to determine if there were significant differences in PFAA serum levels between the women living in different districts in the City of Uppsala, in attempt to ascertain the impact of drinking water exposure on blood levels of PFAA. Earlier results on drinking water analyses suggested that the drinking water distributed mainly to the Southern district of Uppsala was contaminated with high levels of perfluorohexane sulfonic acid (PFHxS) and perfluorooctane sulfonic acid (PFOS), and lower levels of perfluorobutane sulfonic acid (PFBS). The contaminated water wells have now been taken out of production. Another aim was to evaluate associations between blood PFOS levels and fish consumption, suggested to be a significant source of PFOS exposure. When comparing serum levels of PFAA in women from different city districts, significantly higher concentrations PFBS and PFHxS were found in the Southern and/or the Western districts both in 1996-1999 and 2008-2011. Higher levels of PFHxS were observed in 2008-2011 than in 1996-1999 in all Uppsala districts, but not in women from areas outside of the City of Uppsala. These results suggest that drinking water exposure to PFHxS and PFBS gave a major contribution to the total exposure to the compounds mainly in the Southern district but also to some extent in other districts of Uppsala. Levels of PFOS in serum did not differ between districts, and the levels were lower in 2008-2011 than in 1996-1999 suggesting that the drinking water contamination of PFOS has not yet resulted in increased serum levels in women consuming the contaminated water. Total fish consumption and lean fish and shellfish consumption were positively associated with serum concentrations of lin-PFOS ($p<0.05$) in the group of women from 2008-2011. Our results strongly suggest that drinking water exposure to perfluorohexane sulfonic acid (PFHxS) and perfluorobutane sulfonic acid (PFBS) is an important factor behind the increasing temporal trends of the levels of these compounds in blood serum from young women in Uppsala. Our results further suggested that the relative importance of fish consumption as human exposure pathway for PFOS has increased since the 1990s.

INTRODUCTION

Perfluoroalkyl acids (PFAAs) are a group of persistent organic substances that are surface active and water and fat/oil repellent. The PFAA molecule has a carbon chain, on which all hydrogen atoms have been substituted with fluor atoms. In the end of the carbon chain a functional group is positioned, and if this group is a carboxylic acid then the PFAAs are called perfluoroalkyl carboxylic acids (PFCAs) and if the functional group is a sulfonic acid the PFAAs are called perfluoroalkane sulfonic acids (PFSAs). PFAAs and related compounds are used as surfactants in industrial processes, in treatment of textiles and paper, in products such as lubricants, paint and firefighting foams. Due to the wide use and persistence of PFAAs they are present everywhere in the environment, and humans are exposed via food, drinking water and the use of products containing PFAAs and related compounds (Vestergren, 2011).

In a study of temporal trends of PFAAs in blood from young women living in Uppsala, Sweden between 1996 and 2010, it was observed that the levels of the PFSAs perfluorobutane sulfonic acid (PFBS) and perfluorohexane sulfonic acid (PFHxS) increased during the study period (Glynn et al., 2012). Concomitantly, the levels of other PFSAs, such as perfluorooctane sulfonic acid (PFOS) and perfluorodecane sulfonic acid (PFDS) decreased. Moreover, a decreasing temporal trend of the PFCA perfluorooctanoic acid (PFOA) was observed. The variation in temporal trends of different PFAAs may at least partially be due to phase-out of production and use of certain PFAAs and related compounds, and a concomitant phase-in of production and use of new (shorter chain) replacement PFAAs. However, the diverging temporal trends could also be due to variation in drinking-water exposure to the compounds.

In 2012 Livsmedelsverket and ITM analyzed PFAAs in a limited number of drinking water samples from different areas of Uppsala and from regions outside Uppsala (Glynn, 2012), see Fig. 1. Elevated levels of PFHxS and PFOS, and to some extent also of PFBS, PFHxA and PFOA were detected in a few samples from the southern and central part of the City of Uppsala. Background levels were detected in other samples from the central, western and northern part of the city, and in areas outside Uppsala. A risk assessment of PFAA exposure from the water, based on the limited data on PFAA levels in water, suggested that the PFHxS-contamination could cause a substantial PFHxS exposure of the consumers in comparison with the average intake from food (Glynn, 2012). In further investigations of the ground water wells in Uppsala the drinking water producer and supplier in Uppsala, Uppsala Vatten, found PFAA contamination restricted to a few wells that mainly supplied water to a drinking water plant in southern Uppsala (Ahlgren, 2013). Taken together the results suggest that there could be differences in the PFAA exposure of consumers from different districts of Uppsala. The source of the contamination is likely fire extinguishing foam that has been used at fire drills at Ärna airport, placed nearby Uppsala (Glynn, 2012, Ahlgren, 2013).

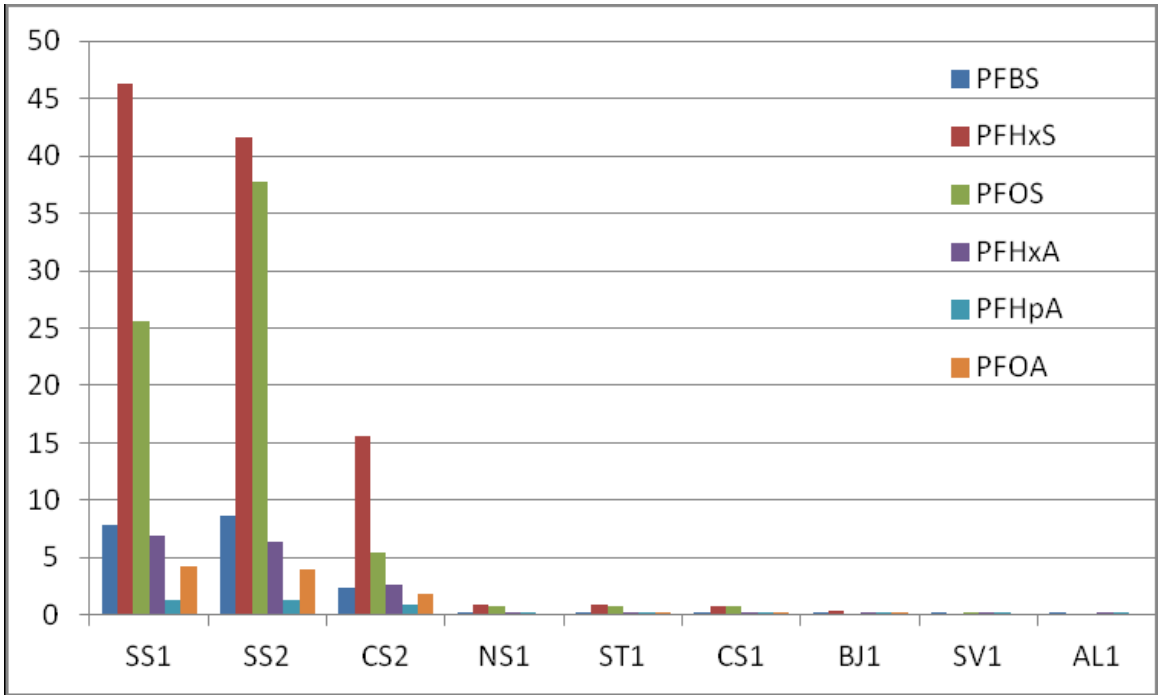


Figure 1. Levels of PFAAs (ng/L) in drinking water samples from Glynn, 2012. SS=Sunnersta, The Southern district, CS=The central Uppsala, NS=Gränby, The Northern district, ST=Stabby, the Western district, and BJ, SV, and AL= Outside of Uppsala.

The aim of our study was to determine if there were significant differences in PFAA serum levels between young women living in different districts in the City of Uppsala 1996-1999 and 2008-2011, in an attempt to ascertain the impact of drinking water exposure on blood levels of PFAA. In the temporal trend study (Glynn et al., 2012) increasing blood serum levels of PFHxS and PFBS were detected from 2004 and onwards among the Uppsala women. If the drinking water PFAA contamination played a role in the increasing temporal trends of PFHxS and PFBS, then the temporal trend study consequently would suggest that exposure through contaminated drinking water became the driving force for increased serum levels around and after year 2004. Based on this it may be hypothesized that between-district variation of PFAA exposure, and thus also of blood PFAA levels, should be more pronounced among Uppsala women sampled 2008-2011 than among women sampled 1996-1999.

Our study also aimed at looking at the possibility of between-district differences in temporal trends of certain PFAA. For instance, if drinking water PFAA is an important determinant for the increased temporal trend of PFHxS levels in blood among young women in the Uppsala region then it may be possible that districts affected by the drinking water contamination show increasing temporal trends whereas districts not affected by the contamination do not.

Furthermore, fish consumption is an important source of human exposure to PFOS in Sweden (Vestergren et al., 2012a). The decreasing temporal trend of PFOS blood levels among the

Uppsala women from 1996 and onwards strongly suggests that a significant source of PFOS exposure, other than fish consumption, has been eliminated before or around the turn of the century. In our study we therefore hypothesize that the association between fish consumption and blood PFOS levels will be stronger in the later part of the study period than in the earlier part.

MATERIALS AND METHODS

Study participants and study design

Biobanked blood serum samples from women in the POPUP cohort (Persistent Organic Pollutants in Uppsala Primiparas) were used. For details about recruitment, collection of data regarding personal characteristics see Glynn et al. (2007) and Lignell et al. (2009). The study was focused on samples from the beginning of the study period 1996-1999 and the end 2008-2011 and approximately 150 samples from each period were analyzed for PFAAs (Table 1). Blood was sampled from the women 3 weeks after delivery of their first child.

Table 1. Characteristics of the mothers participating in the study (n = 297).

Characteristics	Sampling years	n	Mean (range)	%
Age[a] (years)	1996-1999	147	28.6 (21-41)	
	2008-2011	150	30.2 (21-40)	
Pre-pregnancy BMI[a]	1996-1999	146	23.8 (18-36)	
(body mass index) (kg m^{-2})	2008-2011	150	23.1 (18-40)	
Weight gain during pregnancy[a] (%)	1996-1999	147	22.0 (5-42)	
	2008-2011	150	23.7 (-6-49)	
Weight loss from delivery to sampling[ab] (%)	1996-1999	130	10.2 (-1.0-21)	
	2008-2011	150	9.3 (-0.2-5)	
Primiparous mothers	1996-1999	147		100
	2008-2011	141		94
Smoking habits[a]				
Non-smoker	1996-1999	122		83
	2008-2011	140		95
Smoking during pregnancy	1996-1999	25		17
	2008-2011	7		5

[a]Variables included in the linear regression and general linear model analyses. [b]Weight of the mother just before the delivery (W_d), weight of the mother at the time of blood sampling (W_s), and the birth weight of the child(W_b) were used to calculated this weight loss: $[(W_d-W_s-W_b)/W_d]x100$.

Table 1 continued. Characteristics of the mothers participating in the study (n = 297).

Characteristics	Sampling years	n	Mean (range)	%
Food consumption (g day^{-1})				
Fish, total	1996-1999	145	23 (0-107)	
	2008-2011	150	38 (0-131)	
Fatty fish, total	1996-1999	145	6 (0-29)	
	2008-2011	150	14 (0-83)	
Fatty Baltic Sea fish	1996-1999	145	1 (0-18)	
	2008-2011	150	1 (0-17)	
Fatty fish, non Baltic Sea	1996-1999	145	5 (0-29)	
	2008-2011	150	13 (0-83)	
Fresh water fish	1996-1999	144	0.5 (0-10)	
	2008-2011	150	0.8 (0-18)	
Lean fish, shellfish	1996-1999	145	17 (0-78)	
	2008-2011	150	24 (0-77)	
City districts[a]				
Outside of Uppsala city	1996-1999	51		35
	2008-2011	28		19
Central Uppsala	1996-1999	28		19
	2008-2011	56		37
Eastern Uppsala	1996-1999	25		17
	2008-2011	20		13
Northern Uppsala	1996-1999	6		4
	2008-2011	6		4
Western Uppsala	1996-1999	17		12
	2008-2011	25		17
Southern Uppsala	1996-1999	20		14
	2008-2011	15		10

[a] Variables included in the linear regression and general linear model analyses.

PFAA analyses

Standards and reagents

Abbreviations of perfluoroalkyl substances used in this study are according to Buck et al. 2011. The target analytes were PFHxA, PFHpA, PFOA, PFNA, PFDA, PFUnDA, PFDoDA, PFTrDA, PFTeDA, PFPeDA, PFBS, PFHxS, PFOS, PFDS and FOSA (Table 2). For simplicity, FOSA is also included in the term PFAAs in this report, and grouped together with the PFSAs. All employed analytical standard compounds including mass-labeled standards were purchased from Wellington Labs. All solvents and reagents were of highest commercial purity and employed as received.

Table 2. Perfluoroalkyl acids (PFAAs)

Substance	**Formel**	**Acronym**
Perfluorohexanoic acid	$C_5F_{11}COOH$	PFHxA
Perfluoroheptanoic acid	$C_6F_{13}COOH$	PFHpA
Perfluorooctanoic acid	$C_7F_{15}COOH$	PFOA
Perfluorononanoic acid	$C_8F_{17}COOH$	PFNA
Perfluorodecanoic acid	$C_9F_{19}COOH$	PFDA
Perfluoroundecanoic acid	$C_{10}F_{21}COOH$	PFUnDA
Perfluorododecanoic acid	$C_{11}F_{23}COOH$	PFDoDA
Perfluorotridecanoic acid	$C_{12}F_{25}COOH$	PFTrDA
Perfluorotetradecanoic acid	$C_{13}F_{27}COOH$	PFTeDA
Perfluorobutane sulfonic acid	$C_4F_9SO_3H$	PFBS
Perfluorohexane sulfonic acid	$C_6F_{13}SO_3H$	PFHxS
Perfluorooctane sulfonic acid	$C_8F_{17}SO_3H$	PFOS
Perfluorodecanoic sulfonic acid	$C_{10}F_{21}SO_3H$	PFDS
Perfluoroctane sulfonamide	$C_8H_2F_{17}NO_2S$	FOSA

Extraction and clean-up

Sample extraction and clean-up followed the procedure described in detail in Glynn et al. (2012). In short, an aliquot of 0.5 g of human serum was spiked with eight mass-labeled internal standards. The target analytes were extracted (and proteins precipitated) with acetonitrile in an ultrasonic bath. Following centrifugation the supernatant extract was removed and concentrated under nitrogen. The concentrated extract underwent dispersive clean-up on graphitized carbon and glacial acetic acid. The sample was centrifuged and a volume of 0.5 mL of the clear supernatant extract was added to 0.5 mL of aqueous ammonium acetate. Before instrumental analysis the volumetric standards $^{13}C_8$-PFOA and $^{13}C_8$-PFOS were added.

Instrumental analysis and quantification

Aliquots of the final extracts were injected on an Acquity ultra performance liquid chromatography system (UPLC) coupled to a Xevo TQ-S tandem mass spectrometer (MS/MS) (both from Waters Corp., Milford, MA, USA) operated in electrospray ionization in the negative ion mode. The instrumental method including optimized parameters are described in detail in Vestergren et al. (2012b). Quantification was performed in selected reaction monitoring chromatograms using the internal standard method. An external solvent-based linear calibration curve was applied for calculation of relative response factors. Authentic mass-labeled internal standards were not available for all analytes. Therefore, $^{13}C_4$-PFOA was used as internal standard for PFHpA, $^{13}C_2$-PFDoDA for PFTrDA, PFTeDA and PFPeDA, $^{18}O_2$-PFHxS for PFBS and $^{13}C_4$-PFOS was used as internal standard for PFDS and FOSA. For PFHxS, PFOS, PFDS and FOSA, which showed quantifiable signals of branched isomers, the sum of branched isomers and the linear isomer were quantified separately.

Analytical quality control

To monitor the background contamination from the complete method, a procedural blank extraction was performed with every batch of samples. Method detection limits (MDLs) were defined based on the quantified background contamination signals. In the absence of procedural blank contamination MDLs were defined as the lowest concentration in a serum sample giving a chromatographic signal with a signal-to-noise ratio of 3. Compound specific MDLs are given in Table 2 and Table 3. Absolute recoveries of the stable isotope mass-labeled internal standards were on an average between 60 and 69%. All absolute recoveries were calculated using a solvent based calibration standard. The consistency of recoveries between different PFAA homologues showed that there was no significant matrix effect associated with the analytical method.

Data handling and statistical analyses

The participants were assigned to different districts of Uppsala depending on their home address (Table 1). The City of Uppsala was divided into a central, northern, eastern, western and southern district (See map, Fig. 2). Moreover, participants living outside the City of Uppsala were assigned to a separate group. General linear model (GLM) analysis was used in order to investigate possible differences in levels of PFAAs between 1996-1999 and 2008-2011 using the whole data set, and also looking at potential differences in within-district PFAA levels between 1996-1999 and 2008-2011. Covariates in the GLM analyses of trends using the whole dataset were age of the women, pre-pregnancy BMI, weight change during pregnancy, weight change between delivery and blood sampling, and home district. In the analyses of within-district trends, analyses were done for each district separately, using the data from both study periods. In this case covariates were age of the women, pre-pregnancy BMI, weight change during pregnancy, and weight change between delivery and blood sampling.

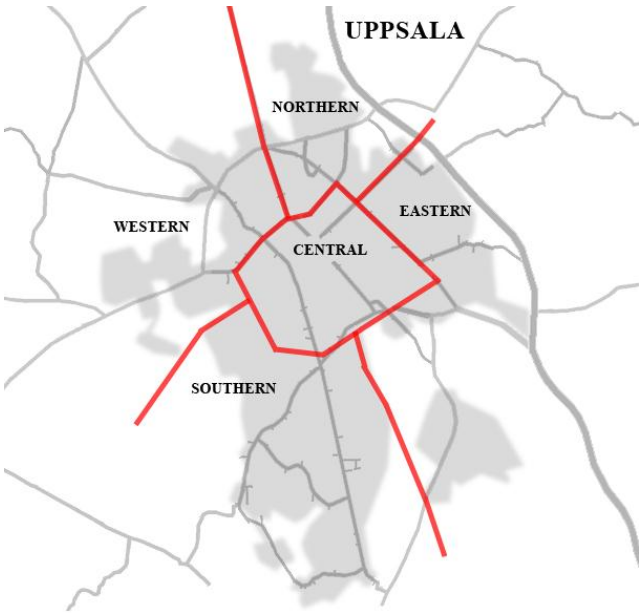


Figure 2. Map over Uppsala city divided into a central, northern, eastern, western and southern district.

In the analyses of between-district differences in PFAA levels for 1996-1999 and 2008-2011 separately, covariates included in the GLM analyses were age of the women, pre-pregnancy BMI, weight change during pregnancy, and weight change between delivery and blood sampling. Finally, the associations between fish consumption (g/day during the year before pregnancy) and PFOS levels were analyzed by multiple linear regression with the covariates age of the women, pre-pregnancy BMI, weight change during pregnancy, weight change between delivery and blood sampling, and district of living. In this case the analyses of data from 1996-1999 and 2008-2011 were done separately. The level of significance was set to $p \leq 0.05$.

RESULTS AND DISCUSSION

Concentrations of PFAAs in serum

The characteristics of the study population differed somewhat between the two study periods. A few multiparous women participated in 2008-2011 and smoking during pregnancy seemed to be more common in 1996-1999 (Table 1). Fish consumption appeared to be higher in 2008-2011, due to higher consumption of both lean and fatty fish. More women from areas outside the City of Uppsala participated in 1996-1999, mostly due to a selective recruitment of women living in areas along the Baltic Sea coast during this period.

Concentrations of PFAAs in serum samples are presented in Table 3 (PFCAs) and in Table 4 (PFSAs). PFOA showed the highest median concentration among the PFCAs from both sampling periods, with median levels above 1 ng/g serum. Among the PFSAs, PFHxS and PFOS showed the highest median concentrations. For PFHxS and PFOS, levels of linear isomers were higher than those of branched isomers, both 1996-1999 and 2008-2011. Most PFAAs did not reach levels above 10 ng/g serum, but for PFOA (2008-2011, n=1), totPFHxS (2008-2011, n=20), and totPFOS (1996-1999, n=139, and 2008-2011, n=22) certain women had levels >10 ng/g.

Table 3. Levels of PFCAs (ng/g) in serum from the participating mothers.

Substance	MDL[a]	Sampling years	n	Median (range)	<MDL (%)
PFHxA	0.3	1996-1999	147		100
		2008-2011	134		100
PFHpA	0.04	1996-1999	147	0.060 (<MDL-0.400)	28
		2008-2011	134	0.045 (<MDL-0.296)	43
PFOA	0.2	1996-1999	147	2.6 (1.2-6.7)	0
		2008-2011	149	1.5 (0.2-13)	0
PFNA	0.05	1996-1999	147	0.37 (0.062-1.4)	0
		2008-2011	149	0.46 (0.064-2.2)	0
PFDA	0.05	1996-1999	147	0.19 (<MDL-0.72)	5
		2008-2011	149	0.26 (<MDL-1.1)	2
PFUnDA	0.05	1996-1999	147	0.14 (<MDL-0.58)	34
		2008-2011	149	0.23 (<MDL-0.91)	5
PFDoDA	0.05	1996-1999	147	<MDL (<MDL-0.22)	82
		2008-2011	149	<MDL (<MDL-0.25)	67
PFTrDA	0.05	1996-1999	147	<MDL (<MDL-0.19)	98
		2008-2011	149	<MDL (<MDL-0.20)	68
PFTeDA	0.05	1996-1999	147	<MDL (<MDL-0.38)	91
		2008-2011	149	<MDL (<MDL-0.58)	96
PFPeDA	0.05	1996-1999	147		100
		2008-2011	149		100

[a]Method detection limit.

Table 4. Levels of PFSAs (ng/g) in serum from the participating mothers.

Substance	MDL[a]	Sampling years	n	Median (range)	<MDL (%)
PFBS	0.01	1996-1999	132	0.019 (<MDL-0.21)	31
		2008-2011	134	0.027 (<MDL-0.80)	19
br-PFHxS	0.01	1996-1999	146	0.089 (<MDL-0.76)	3
		2008-2011	148	0.25 (<MDL-2.2)	1
lin-PFHxS	0.01	1996-1999	146	1.7 (0.36-8.9)	0
		2008-2011	148	3.5 (0.29-33)	0
Tot PFHxS	0.01	1996-1999	146	1.8 (0.37-9.5)	0
		2008-2011	148	3.7 (0.32-34)	0
br-PFOS	0.01	1996-1999	146	6.2 (2.2-19)	0
		2008-2011	146	2.3 (0.70-8.6)	0
lin-PFOS	0.01	1996-1999	146	12 (2.9-28)	0
		2008-2011	147	4.4 (0.21-12)	0
Tot PFOS	0.01	1996-1999	146	18 (5.1-46)	0
		2008-2011	147	6.6 (0.21-20)	0
br-PFDS	0.005	1996-1999	146	0.014 (<MDL-2.2)	40
		2008-2011	148	<MDL (<MDL-0.13)	56
lin-PFDS	0.005	1996-1999	146	<MDL (<MDL-0.82)	55
		2008-2011	148	<MDL (<MDL-0.045)	77
lin-FOSA	0.01	1996-1999	147	<MDL (<MDL-0.095)	76
		2008-2011	149	<MDL (<MDL-0.012)	99

[a]Method detection limit.

Determinants of PFAA levels in serum

Evaluation of the results of the linear regression analyses showed that levels of PFAAs from the two sampling periods in some cases were associated with age of the mother, pre-pregnancy BMI, weight gain during pregnancy, and weight loss after delivery, Table 5. The multiple linear regression model explained 9-45% of the variation of PFAA levels, showing that some of the lifestyle/medical factors were major determinants of serum PFAA levels. Only PFHxS and PFOS levels were positively associated with age of the women. This suggests that these compounds are accumulating with age, which could be due to the long half-lives of PFOS and PFHxS in blood of humans (5-7 years) (Olsen et al., 2007).

Table 5. Adjusted geometrical percent changes (SE) in serum levels[a] of PFAAs per unit change of the independent variables in the multiple linear regression model including the variables "age", "pre-pregnancy BMI", "weight gain during pregnancy" and "weight loss after delivery".[bc]

Substance	Sampling years	Age years	BMI[d] kg m^{-2}	Weight gain[e] %	Weight loss[f] %	R^{2g} %
PFHpA	1996-1999					
	2008-2011		-4 (1.4)			20
PFOA	1996-1999					
	2008-2011		-5 (1.3)		4 (1.6)	20
PFNA	1996-1999				3 (1.2)	14
	2008-2011		-5 (1.3)		4 (1.6)	21
PFDA	1996-1999				3 (1.5)	20
	2008-2011		-5 (1.3)			21
PFUnDA	1996-1999			3 (1.2)		15
	2008-2011		-7 (1.5)			23
PFBS	1996-1999		6 (2.7)			20
	2008-2011					
Br-PFHxS	1996-1999					
	2008-2011					
Lin-PFHxS	1996-1999	2 (1.2)				45
	2008-2011	5 (2.1)	-4 (2.2)			25
Tot PFHxS	1996-1999					
	2008-2011	5 (2.1)	-4 (2.2)			26
br-PFOS	1996-1999					
	2008-2011	2 (1.1)	-4 (1.2)		4 (1.5)	15
lin-PFOS	1996-1999	2 (1.0)				9
	2008-2011	2 (1.2)	-4 (1.3)		4 (1.5)	18
Tot PFOS	1996-1999					
	2008-2011		-4 (1.3)		4 (1.6)	19
br-PFDS	1996-1999					
	2008-2011			-2 (0.9)		14
lin-PFDS	1996-1999				6 (2.9)	7
	2008-2011					

[a]Logarithmically transformed concentration data were used. [b]City district and smoking habits were also included in the multiple linear regression model. [c]All reported changes are statistically significant ($p<0.05$). [d]Pre-pregnancy BMI (body mass index). [e]Weight gain during pregnancy. [f]Weight loss from delivery to blood sampling. [g]Coefficient of determination for the whole multiple linear regression model.

More consistent inverse associations with pre-pregnancy BMI were observed for both PFCAs and PFSAs, but this association was evident only among women sampled 2008-2011, Table 5. The number of study participants was the same for the two study periods so this did not affect the power of the statistical analyses. However, the ranges of serum PFAA levels were in most cases wider in 2008-2011 than in 1996-1999, which may have increased the power to detect significant associations. The inverse association with pre-pregnancy BMI is possibly due to a dilution effect where PFAAs are distributed into a larger body. Positive associations were observed between levels of PFOA, PFNA, PFDA, PFOS and PFDS and weight loss during the three weeks between delivery and sampling, mostly among women sampled 2008-2011. This may in opposite be due to a concentration of the PFAAs in the body when losing weight.

Between-district differences in PFAA levels

When comparing serum levels of PFAA (least square means and SE from the GLM analysis) in women from different city districts, significantly higher concentrations were found in the Southern and/or the Western districts for some substances, both in 1996-1999 and 2008-2011 (Fig. 3 and Fig. 4). In women sampled in 1996-1999, PFHxS showed a significantly higher adjusted mean concentration in the Southern district compared to all other districts (Fig. 3). The Western district was also higher compared to the Northern district and compared to women living outside of Uppsala. In women sampled in 2008-2011, similar results were found for PFHxS in the Southern district, but not for the Western district, (Fig. 4). The pattern was similar for both branched and linear PFHxS. The results support the hypothesis that women living in the Southern district would have higher PFHxS levels due to the drinking water problem in this district. Interestingly, the women in the Southern district had higher PFHxS levels already in 1996-1999 suggesting that the contamination problem was evident already during the 1990s.

For PFDA and PFBS in 1996-1999, women in the Southern district had significantly higher concentrations than women in the Western and Eastern districts (Fig. 3). In samples taken during 2008-2011, PFBS levels in the Southern district were higher than in all other studied districts of Uppsala. PFDA levels, as well as PFNA levels, were higher in the western district compared to women living outside of Uppsala in 2008-2011 (Fig. 4). The results for PFBS are similar to those of PFHxS, suggesting that the drinking water exposure also has been a significant source of PFBS exposure for women living in the Southern district. A similar pattern is seen also for PFHpA in 2008-2011 (Fig. 4).

Elevated levels of PFOS were also detected in the PFAA contaminated Uppsala drinking water, although at lower levels than PFHxS (Glynn, 2012, Ahlgren, 2013). Contrary to the case of PFHxS, we did not find any significant differences in blood PFOS levels between Uppsala districts. However, there is a large difference in the contribution of drinking water to total PFHxS and PFOS exposure in Sweden. For PFHxS drinking water makes a much larger contribution to the total intake than for PFOS (Vestergren et al., 2012a). Our results suggest

that the contamination of the Uppsala drinking water with PFOS did not lead to significantly higher serum levels in exposed individuals.

In 1996-1999, none of the study participants had PFHxS levels above 10 ng/g, whereas in 2008-2011 20 women had levels ranging between 10 and 34 ng/g. Of these women n=6 lived in the Southern Uppsala district, n=6 lived in the Central district, n=3 lived in the Western respectively in the Eastern district, n=2 lived outside of Uppsala and none lived in the Northern district. In this study only data about home districts at the time of sampling was available and evaluated. It is possible that certain women in the study have been working or spending a considerable amount of time in another district, or have moved shortly before the study, and consequently have consumed drinking water from more than one district.

A risk assessment of PFAAs in the Uppsala drinking water has been made (Glynn, 2012) concluding that the currently measured levels in the drinking water probably not would be a significant health risk for the water consumers. This was based on the risk assessment of PFOS by the European Food Safety Authority 2008 (EFSA, 2008). The risk assessment is however uncertain since newer toxicological and epidemiological data suggests that some PFAA, among those PFOS, are more toxic than previously thought. Moreover, if the levels of PFHxS in the ground water are increasing in the long-term, which has been indicated in the temporal trend study, it might reach levels in the future that are of concern for the water consumers. The distribution of the contaminated water has now been stopped, but the PFAA contamination of the ground water could be a future problem for the drinking water supply in Uppsala if the contamination spreads further in the ground water aquifer. Further studies of the temporal trends and spatial distribution of the PFAA contamination of the ground water in Uppsala are needed in order to draw firm conclusions about future health risks associated with the PFAAs.

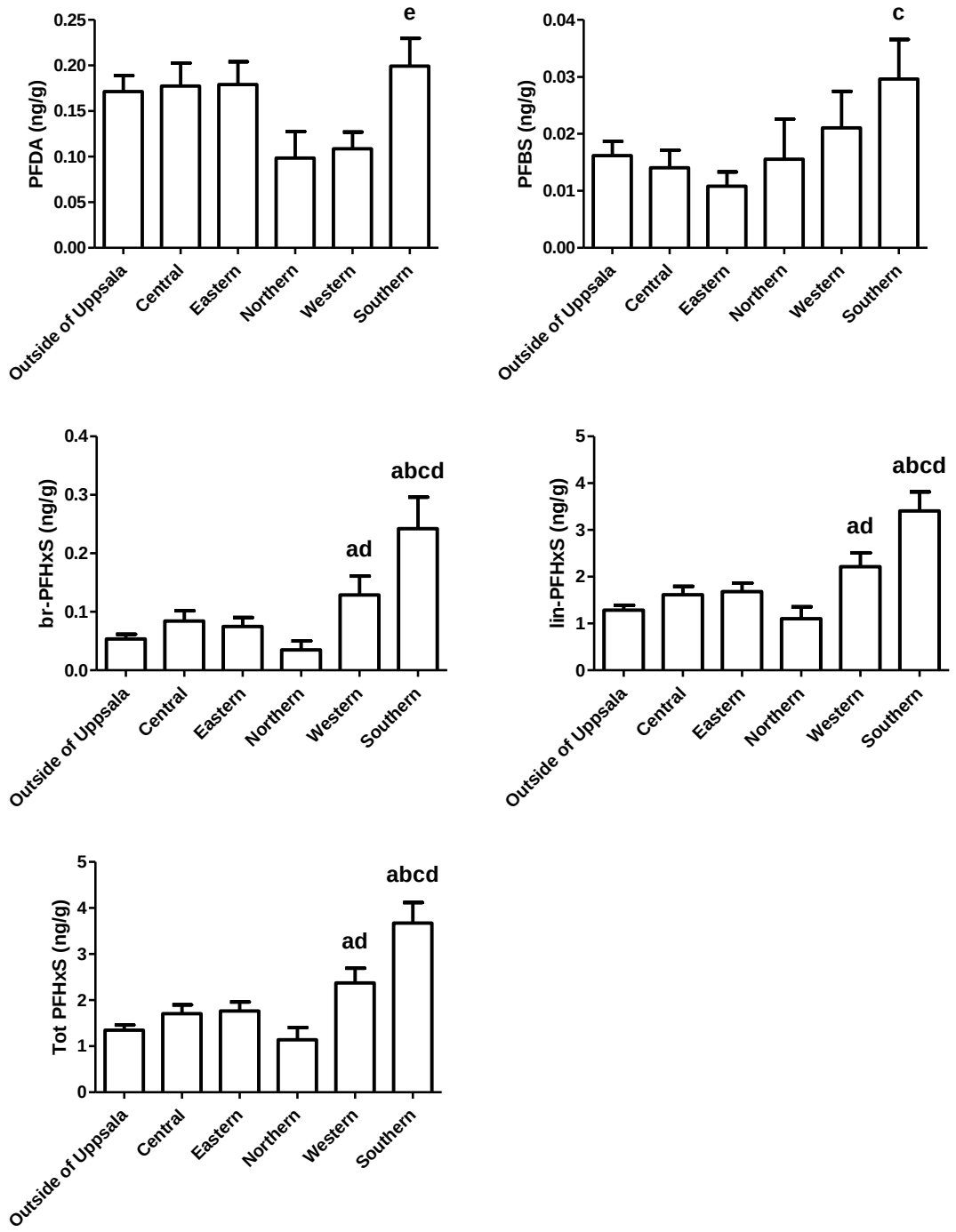
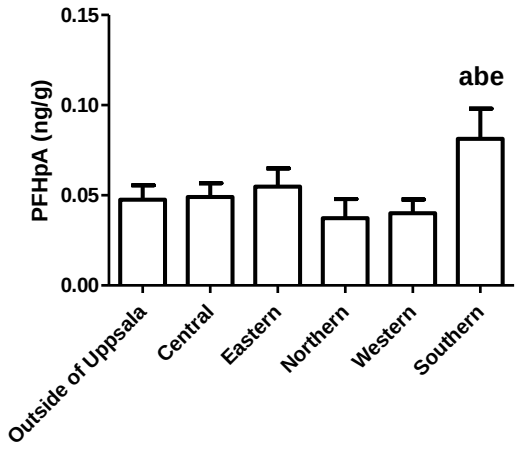
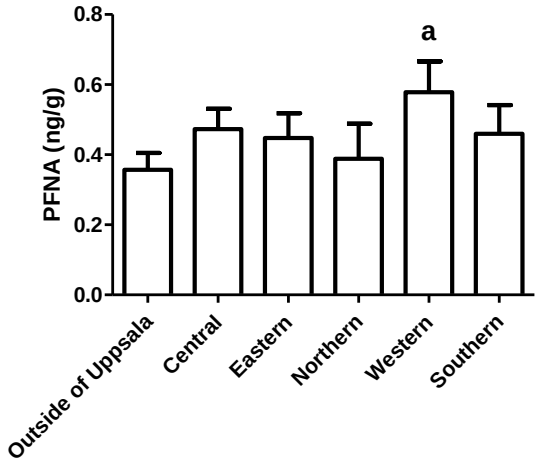
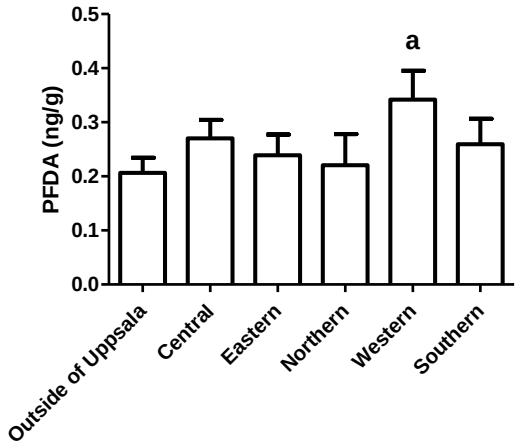
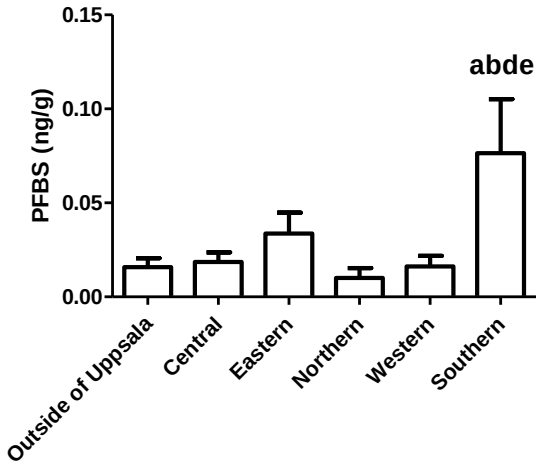


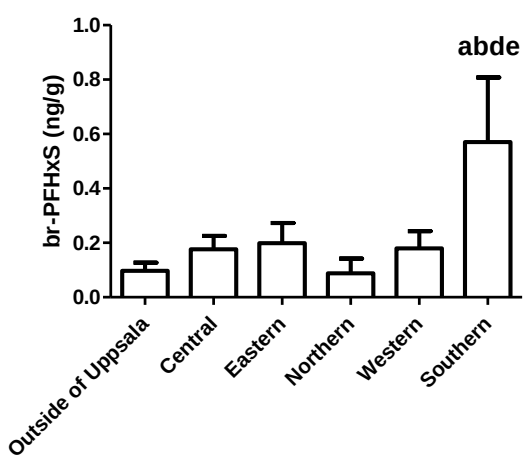
Figure 3. PFAA concentrations in serum (least square means and SE from the General linear model (GLM)) in mothers from different city districts in Uppsala 1996-1999. In the GLM analyses the covariates "age", pre-pregnancy BMI", "weight gain during pregnancy", "weight loss after delivery", and "smoking habits" were included in the GLM model. a = significantly different ($p<0.05$, Tukey Simultaneous Tests) from Outside of Uppsala, b = significantly different from Central Uppsala, c = significantly different from East of Uppsala, d = significantly different from Northern Uppsala, e = significantly different from Western Uppsala.

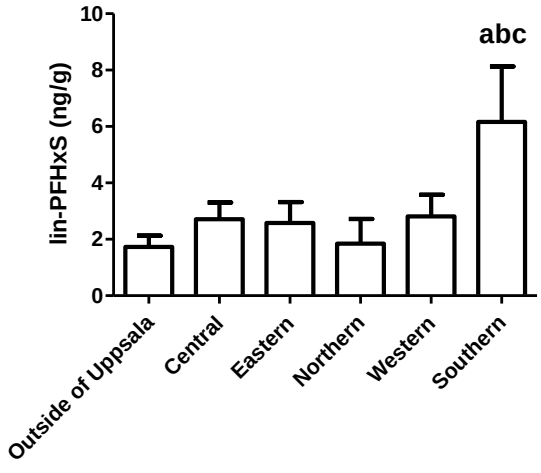

PFHpA (ng/g)
0.15
0.10
0.05
0.00
abe
Outside of Uppsala
Central
Eastern
Northern
Western
Southern


PFNA (ng/g)
0.8
0.6
0.4
0.2
0.0
a
Outside of Uppsala
Central
Eastern
Northern
Western
Southern


PFDA (ng/g)
0.5
0.4
0.3
0.2
0.1
0.0
a
Outside of Uppsala
Central
Eastern
Northern
Western
Southern


PFBS (ng/g)
0.15
0.10
0.05
0.00
abde
Outside of Uppsala
Central
Eastern
Northern
Western
Southern


br-PFHxS (ng/g)
1.0
0.8
0.6
0.4
0.2
0.0
abde
Outside of Uppsala
Central
Eastern
Northern
Western
Southern


lin-PFHxS (ng/g)
10
8
6
4
2
0
abc
Outside of Uppsala
Central
Eastern
Northern
Western
Southern

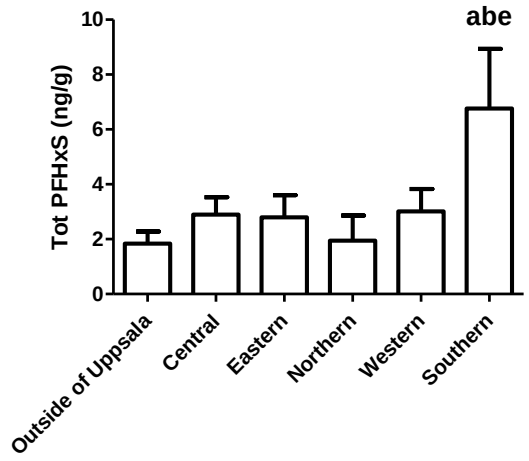


Figure 4. PFAA concentrations in serum (least square means and SE from the General linear model) in mothers from different city districts in Uppsala 2008-2011. In the GLM analyses the covariates "age", pre-pregnancy BMI", "weight gain during pregnancy", "weight loss after delivery", and "smoking habits" were included in the GLM model. a = significantly different ($p<0.05$, Tukey Simultaneous Tests) from Outside of Uppsala, b = significantly different from Central Uppsala, c = significantly different from East of Uppsala, d = significantly different from Northern Uppsala, e = significantly different from Western Uppsala.

Overall changes in blood levels of PFAAs

Concentrations of PFHpA and PFOA were significantly lower during the period 2008-2011 compared to 1996-1999 (Fig. 5), strongly suggesting that exposure to these PFCAs has decreased among the young women in the Uppsala area. The decline in PFOA levels corroborates with observations from other European countries and North America (Spliethoff et al., 2008, Haug et al., 2009, Kato et al., 2011, Glynn et al., 2012). In our study, with higher statistical power than in the earlier temporal trend study (Glynn et al., 2012), PFHpA levels were shown to be lower in 2008-2011 compared to 1996-1999, which were not seen in the previous study (Glynn et al., 2012). PFHpA and PFOA were shown to be elevated in the drinking water (Fig. 1) and is likely a source of exposure, thus the overall decline is governed by a stronger decline of other exposure pathways than the increase due to the water contamination. For PFNA, PFDA, and PFUnDA the concentrations have in opposite been increasing (Fig. 5), which is in line with some studies from Europe and North America, but not with other studies from the same areas (Spliethoff et al., 2008, Haug et al., 2009, Kato et al., 2011, Olsen et al., 2011). Our results further strengthen the observation that the exposure of young women from Uppsala to long-chain PFCAs have increased since the middle of the 1990s (Glynn et al., 2012).

Similarly as in the temporal trend study of the Uppsala women (Glynn et al., 2012), increasing levels of PFBS and PFHxS between the two sampling periods and decreasing levels for PFOS and PFDS were observed (Fig. 6). In the case of PFHxS levels of both branched and linear isomers increased during the study period. For PFOS and PFDS both branched and linear isomers showed decreasing levels (Fig. 6). The overall decline in PFOS levels is supported by the observations of declining PFOS levels in human blood from several areas of the world since the turn of the century (Spliethoff et al., 2008, Haug et al., 2009, Kato et al., 2011, Glynn et al., 2012). Declining levels of PFDS are in agreement with the previous study (Glynn et al., 2012) and to our knowledge no other study has reported declining temporal trends of PFDS. In earlier studies evaluating temporal trends of PFHxS, both decreasing and increasing levels have been reported (Spliethoff et al., 2008, Haug et al., 2009, Kato et al., 2011).

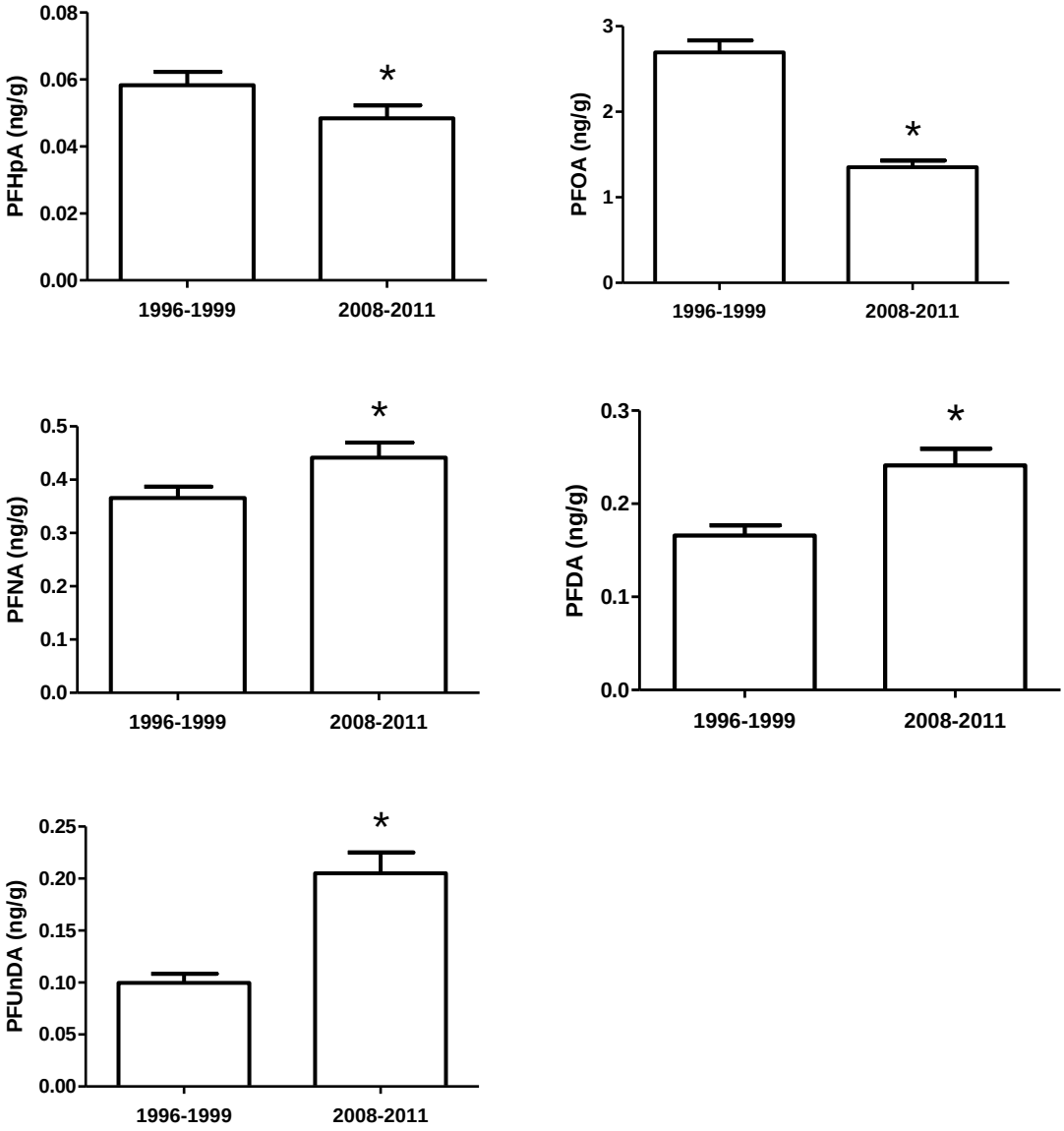


Figure 5. PFCA concentrations (ng/g) in serum (least square means and SE from the general linear model (GLM) analysis) in mothers in Uppsala County. In the GLM analyses the covariates "age", "pre-pregnancy BMI", "weight gain during pregnancy", "weight loss after delivery", "smoking habits", and "city district" were included in the GLM model.
*Significantly different from 1996-1999 $p<0.05$, Tukey Simultaneous Tests.

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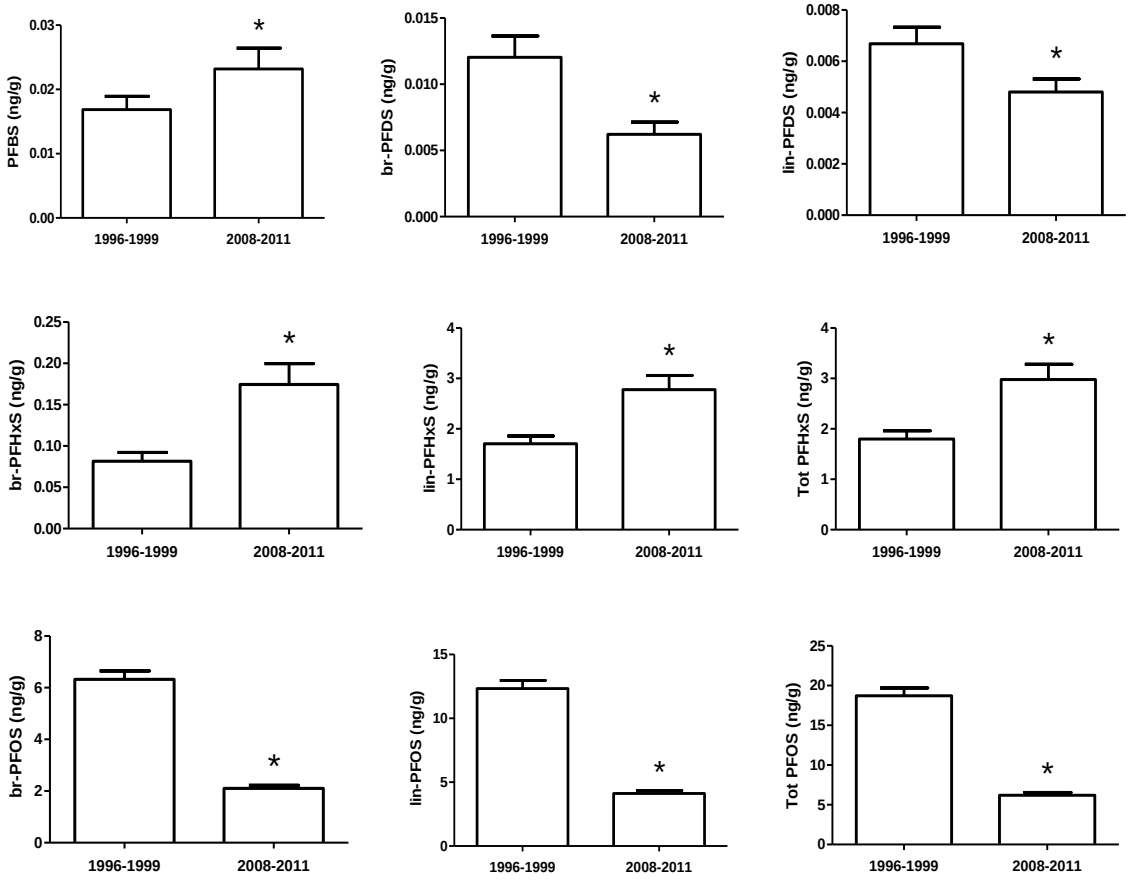

Figure 6. PFSA concentrations (ng/g) in serum (least square means and SE from the general linear model (GLM) analysis) in mothers in Uppsala County. In the GLM analyses the covariates "age", "pre-pregnancy BMI", "weight gain during pregnancy", "weight loss after delivery", "smoking habits", and "city district" were included in the GLM model.
*Significantly different from 1996-1999 $p<0.05$, Tukey Simultaneous Tests.

District-specific changes in PFAA levels

In Table 6 the least square means of PFBS, PFHxS, and PFOS for 1996-1999 and 2008-2011 are presented for the six different districts separately. PFOS is decreasing in all districts suggesting that the drinking water exposure to PFOS has not markedly affected the decreasing trend in exposure (Glynn et al., 2012). For PFHxS the women living in all City of Uppsala districts had significantly higher levels in 2008-2011 compared to 1996-1999. Women living outside of Uppsala did not show this difference in levels. For PFBS the pattern is similar as for PFHxS, with a tendency of increasing levels between 1996-1999 and 2008-2011, but only statistically significant for the Eastern and Southern districts (Table 6). This suggests that the drinking water exposure is an important factor to consider in the overall increasing temporal trend of PFHxS and PFBS observed among Uppsala women (Glynn et al., 2012). The similar temporal trends in all City of Uppsala districts could be at least partially due to women working in other areas than they are living, as well as some women having moved between districts after the drinking water contamination started. Moreover, the water distribution system in Uppsala is highly interconnected making it possible that contaminated drinking water was periodically not only distributed to the southern part of the city but also to the other areas.

Table 6. PFSA concentrations (ng/g) in serum (least square means and SE from the general linear model (GLM) analysis) in mothers in Uppsala County divided into their home district. In the GLM analyses the covariates "age", "pre-pregnancy BMI", "weight gain during pregnancy", "weight loss after delivery", and "smoking habits" were included.

Substance	Sampling years	Outside of Uppsala	Central	Eastern	Northern	Western	Southern
PFBS	1996-1999	0.016 (0.003)	0.023 (0.009)	0.011 (0.003)	0.011 (0.007)	0.027 (0.012)	0.021 (0.007)
	2008-2011	0.014 (0.004)	0.030 (0.011)	0.036 (0.012)*	0.011 (0.007)	0.028 (0.012)	0.047 (0.023)*
Br-PFHxS	1996-1999	0.048 (0.010)	0.106 (0.038)	0.069 (0.021)	0.027 (0.020)	0.0110 (0.060)	0.277 (0.069)
	2008-2011	0.076 (0.022)	0.212 (0.066)*	0.182 (0.067)*	0.188 (0.130)	0.231 (0.141)	0.629 (0.216)*
Lin-PFHxS	1996-1999	1.2 (0.14)	1.8 (0.53)	1.6 (0.34)	0.70 (0.36)	2.2 (0.68)	3.6 (0.93)
	2008-2011	1.5 (0.25)	3.2 (0.80)*	2.6 (0.66)	3.1 (1.5)*	3.8 (1.3)*	6.7 (2.4)*
Tot PFHxS	1996-1999	1.2 (0.15)	1.9 (0.57)	1.7 (0.36)	0.73 (0.38)	2.3 (0.74)	3.9 (0.98)
	2008-2011	1.6 (0.27)	3.4 (0.86)*	2.8 (0.72)*	3.3 (1.66)*	4.0 (1.41)*	7.4 (2.6)*
br-PFOS	1996-1999	5.5 (0.44)	6.4 (0.97)	7.3 (0.92)	7.5 (2.5)	7.6 (1.6)	6.8 (1.1)
	2008-2011	1.7 (0.98)*	2.3 (0.30)*	2.1 (0.32)*	2.6 (0.82)*	3.2 (0.76)*	2.9 (0.64)*
lin-PFOS	1996-1999	11 (0.98)	13 (2.2)	14 (1.7)	16 (3.4)	14 (2.6)	13 (2.0)
	2008-2011	3.0 (0.36)*	4.7 (0.67)*	4.6 (0.70)*	4.6 (0.93)*	6.3 (1.3)*	5.3 (1.1)*
Tot PFOS	1996-1999	17 (1.6)	20 (3.0)	21 (2.6)	23 (5.7)	22(4.0)	20 (3.0)
	2008-2011	4.4 (0.60)*	7.0 (0.93)*	6.7 (0.99)*	7.3 (1.7)*	9.7 (2.0)*	8.3 (1.7)*

*Significantly different from 1996-1999 $p<0.05$, Tukey Simultaneous Tests.

Consumption of fish and serum levels of PFOS

Evaluation of the results of the multiple linear regression analyses showed that total fish consumption and lean fish and shellfish consumption were positively associated with serum concentrations of lin-PFOS ($p<0.05$) in the group of women from 2008-2011. br-PFOS and tot-PFOS were not significantly associated ($p=0.15$-0.63). Dietary intake has been estimated to be an important pathway of human exposure to PFOS and fish products are nowadays the main contributor (about 80%) (Trudel et al., 2008, Vestergren et al., 2012a). The finding of a positive association in the later part of the study period, but not in the earlier part, corroborates our hypothesis that fish consumption would be more strongly associated with PFOS levels in the later part due to the phase-out of production for PFOS after 2000. It is likely that it was a stronger contribution of product-related exposure to PFOS during 1996-1999.

CONCLUSIONS

Our results strongly suggest that drinking water exposure to PFHxS and PFBS is an important factor behind the increasing temporal trends of the levels of these compounds in blood serum from young women in Uppsala. For PFOS the drinking water contamination has so far not been the driving factor for the serum time trend. Our results further strengthen the observation that the exposure of young women from Uppsala to long-chain PFCAs has increased since the middle of the 1990s and that the relative importance of fish consumption as human exposure pathway for PFOS has also increased since the 1990s.

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