LIVSMEDELS VERKET

NATUR VÅRDS VERKET
SWEDISH ENVIRONMENTAL PROTECTION AGENCY

NATIONAL ENVIRONMENTAL MONITORING COMMISSIONED BY THE SWEDISH EPA

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

Temporal trends of poly- and perfluoroalkyl substances (PFASs) in pooled serum samples from first-time mothers in Uppsala 1997-2016

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2017-11-01

NATIONELL
MILJÖÖVERVAKNING
PÅ UPPDRAG AV
NATURVÅRDSVERKET

ÄRENDENNUMMER NV-00110-15
AVTALSNUMMER 2215-15-001
PROGRAMOMRÅDE Hälsorelaterad MÖ
DELPROGRAM Biologiska mätdata – organiska ämnen

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Rapporttitel
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Beställare
Naturvårdsverket
106 48 Stockholm

Finansiering
Nationell hälsorelaterad miljöövervakning

Nyckelord för plats
Uppsala

Nyckelord för ämne
PFAS, poly- och perfluorerade alkylsubstanser, PFAA, prekursorer, blodserum, tidstrend, kvinnor

Tidpunkt för insamling av underlagsdata
1997-2016

Sammanfattning
Sedan 1996 har Livsmedelsverket regelbundet samlat in prover från förstföderskor i Uppsala (POPUP-studien) för analys av persistenta halogenerade organiska miljöföroreningar (POPar). I följande rapport redovisas halterna av poly- och perfluorerade ämnen (PFAS) i blodserum insamlade 3 veckor efter förlossningen 1997- 2016. Prover från ungefär 30 kvinnor per år delades upp i 3 samlingsprover per provtagningsår (9-10 prover per samlingsprov). Nya resultat från 2015 och 2016 är hopslagna med tidigare publicerade data från POPUP där samma analysmetod och laboratorium har använts. Halten av långkedjiga karboxylsyror (8-12 kol) ökade med runt 3 % per år. För PFNA, PFDA och PFUnDA sågs ett trendbrott runt 2004 och därefter ses ingen trend. För PFDoDA sågs ett liknande trendbrott runt 2011. PFTrDA hade inget trendbrott utan halterna har ökat hela tiden under studieperioden. Det är viktigt att följa upp resultaten för de långkedjiga karboxylsyrorna för att se att halterna planar ut och börjar minska. Tillverkningen av sulfonsyran PFOS och liknande substanser i världen upphörde i stort sett runt 2002. Detta har resulterat i sjunkande halter av substansen (ca 8 % per år). Även prekursorer till PFOS visar en nedåtgående trend som också är snabbare än för PFOS, 17-26 % per år. Halterna av andra prekursorer låg i allmänhet under kvantifieringsgränserna, även om de i vassa fall var mätbara. Tillverkningen av en karboxylsyra kallad PFOA har minskat, men inte ännu fasats ut helt, och minskningen av denna substans i kvinnornas blod går därför långsammare (ca 3 % per år). Befolkningen i Uppsala utsattes fram till 2012 för förhöjda halter av den mycket bioackumulerbara sulfonsyran PFHxS i dricksvattnet. Detta har resulterat i ökade blodhalter hos förstföderskor under studieperioden (ca 5 % per år). Ett trendrott sågs också 2011och därefter ses ingen trend. Detta antyder att de åtgärder som sattes in för att rena vattnet i Uppsala 2012 satte stopp för den ökade trenden av PFHxS. Även här krävs en dock en uppföljning framöver för att säkerställa att den minskade exponeringen från dricksvatten efter 2012 verkligen har resulterat i sänkta blodhalter av PFHxS och hur snabbt det går.

INTRODUCTION

Poly- and perfluoroalkyl substances (PFASs) are highly fluorinated organic compounds that have been manufactured for more than 50 years. PFASs are used in industrial processes and in products such as water and stain proofing agents, lubricants, paints and fire-fighting foams. Over 3000 PFASs are known to exist on the global market. Some PFAS, such as perfluoroalkyl carboxylic acids (PFCAs) and perfluoroalkyl sulfonic acids (PFSAs) are non-biodegradable in the environment and are detected worldwide in humans and wildlife.

In the present report we updated PFAS data from Uppsala first-time mothers for the period 1997-2016, expanding on the previous temporal trend study by Gebbink et al. (2015) and Glynn et al (2015) covering the period 1997-2014. In total 44 different PFASs were analysed in samples from 2015 and 2016, including 11 PFCAs, 4 PFSAs, 6 perfluoroalkyl sulfonamides (FASAs), 3 fluorotelomer sulfonates (FTSs), 3 fluorotelomer acids (FTAs), 15 polyfluoroalkylphosphate esters (PAPs), and 1 alternative (F-53B). In the present study statistical analyses of temporal trends and possible change points (CPs) on the temporal trends have been performed.

MATERIALS AND METHODS

Recruitment and sampling

In the POPUP study (Persistent Organic Pollutants in Uppsala Primiparas), first-time mothers from the general population living in Uppsala County were recruited between 1996 and 2016 as described in Glynn et al. (2012). The participants donated a blood sample 3 weeks after delivery. Blood sampling was carried out using 9 ml Vacutainer® or Vacuette® serum tubes, and serum was stored at -20°C. The study was approved by the local ethics committee of Uppsala University, and the participating women gave informed consent prior to the inclusion in the study.

In the present study we used banked pooled serum samples from 2015 and 2016 for analysis of PFAS. For each year of recruitment, 3 pooled serum samples were prepared, with serum from 10 individual mothers in each pool (Table 1).

Table 1. Composition of the pooled serum samples used for analyses of PFAS. Samples analyzed in the present study in bold.

Sampling year	No of pools	N in each pool	Age range (yrs)
1997	3	10	21-33
1998	3	10	22-34
2000	3	10	21-37
2002	3	10	24-37
2004	3	10	21-34
2006	3	10	19-40
2007	3	9-10	21-39
2008	3	10	20-35
2009	3	10	22-39
2010	3	10	20-41
2011	3	9-10	21-37
2012	3	10	20-38
2013	3	10	22-39
2014	3	10	20-38
2015	**3**	**10**	**21-37**
2016	**3**	**10**	**24-36**

PFAS analyses

PFASs analyzed in the present study are provided in Table 2, and included both PFAAs and PFAA precursors. The methods have been described previously in Gebbink et al. (2015). Briefly, serum samples (1 g) were spiked with 50 µL of a solution containing isotopically-labeled internal standards. Following addition of 3 mL of acetonitrile, samples were vortex-mixed, sonicated for 15 min, and then centrifuged for 10 min at 3000 rpm. The organic phase was transferred to a separate tube and the extraction procedure was repeated twice. The combined sample extracts were reduced to ~1ml under a stream of nitrogen, fortified with 10 mL of water, and then loaded onto weak anion exchange (WAX) cartridges (Waters, 150 mg, 6 mL) which had been pre-conditioned with 6 mL each of 2% NH_4OH in methanol, methanol, and water. The cartridges were rinsed with 1 mL of 1% formic acid in water and 2 mL of water, and then dried under vacuum. Neutral PFASs were subsequently eluted with 1 mL methanol (fraction 1). Cartridges were rinsed with an additional 2 mL of MeOH which was discarded. Ionic PFAS were subsequently eluted with 4 mL of 2% ammonium hydroxide in methanol (fraction 2). Fraction 1 was filtered and then transferred to a microvial containing 50 µl recovery standards ($^{13}C_8$-PFOS and $^{13}C_8$-PFOA). Fraction 2 was evaporated to dryness under nitrogen, re-dissolved in methanol, and then filtered, prior to transferring to a microvial containing 50 µl recovery standards ($^{13}C_8$-PFOS and $^{13}C_8$-PFOA).

Instrumental analysis was carried out by ultra performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS) using a BEH C18 (50×2.1 mm, 1.7 μm particle size, Waters) analytical column operated under gradient elution conditions. Mobile phases consisted of 95% water and 5% methanol (solvent A) and 75% methanol, 20% acetonitrile, and 5% water (solvent B). Both mobile phases contained 2 mM ammonium acetate and 5 mM 1-methyl piperidine. The column temperature was maintained at 40°C, and the injection volume was 5 μl. The mass spectrometer was operated in negative ion electrospray ionization (ESI−) mode.

Quantification was performed using an internal standard approach. For each compound a nine point calibration curve was prepared, which was linear over the entire concentration range (r^2 values were typically greater than 0.9). The linear isomer and the sum of branched isomers of PFHxS, PFOS and PFOA were chromatographically separated and quantified individually. Branched isomers were quantified using the linear isomer calibration curve. For quantification of the sum of branched PFOS isomers an average of the response obtained with the product ions m/z 80 and 99 was used.

Each batch of samples included a method blank, QC sample, and sample of NIST SRM1957. When blank contamination was observed the method quantification limits (MQLs) was determined as the mean plus three times the standard deviation of the quantified procedural blank signals. For other compounds the MQL was determined as the concentration in serum sample giving a peak with a signal-to-noise ratio of 10.

Table 2. PFASs included in the study.

Substance	No of carbons containing a F atom	Acronym[a]
Perfluoroalkyl sulfonic acids (PFSAs)		
Perfluorobutane sulfonic acid	4	PFBS
Perfluorohexane sulfonic acid[b]	6	PFHxS
Perfluorooctane sulfonic acid[b]	8	PFOS
Perfluorodecane sulfonic acid	10	PFDS
Perfluoroalkyl carboxylic acids (PFCAs)		
Perfluorobutanoic acid	3	PFBA
Perfluoropentanoic acid	4	PFPeA
Perfluorohexanoic acid	5	PFHxA
Perfluoroheptanoic acid	6	PFHpA
Perfluorooctanoic acid[b]	7	PFOA
Perfluorononanoic acid	8	PFNA
Perfluorodecanoic acid	9	PFDA
Perfluoroundecanoic acid	10	PFUnDA
Perfluorododecanoic acid	11	PFDoDA
Perfluorotridecanoic acid	12	PFTrDA
Perfluorotetradecanoic acid	13	PFTeDA
Perfluoroalkane sulfonamides (FASAs)		
Perfluorooctane sulfonamide[b]	8	FOSA
N-Methyl perfluorooctane sulfonamide[b]	8	MeFOSA
N-Ethyl perfluorooctane sulfonamide[b]	8	EtFOSA
Perfluorooctane sulfonamidoacetic acid[b]	8	FOSAA
N-Methyl Perfluorooctane sulfonamidoacetic acid[b]	8	N-MeFOSAA
N-Ethyl Perfluorooctane sulfonamidoacetic acid[b]	8	N-EtFOSAA
Fluorotelomer sulfonates (FTSs)		
4:2 Fluorotelomer sulfonate	4	4:2 FTS
6:2 Fluorotelomer sulfonate	6	6:2 FTS
8:2 Fluorotelomer sulfonate	8	8:2 FTS
Fluorotelomer acids (FTAs)		
3-Perfluoropropyl propanoic acid (3:3)	3	FPrPA
3-Perfluoropropyl propanoic acid (5:3)	5	FPePA
3-Perfluoropropyl propanoic acid (7:3)	7	FHpPA
Polyfluoroalkylphosphates (PAPs)		
4:2 Fluorotelomer phosphate monoester	4	4:2 monoPAP
6:2 Fluorotelomer phosphate monoester	6	6:2 monoPAP
8:2 Fluorotelomer phosphate monoester	8	8:2 monoPAP
10:2 Fluorotelomer phosphate monoester	10	10:2 monoPAP
4:2 Fluorotelomer phosphate diester	4, 4	4:2/4:2 diPAP
4:2/6:2 Fluorotelomer phosphate diester	4, 6	4:2/6:2 diPAP
6:2 Fluorotelomer phosphate diester	6, 6	6:2/6:2 diPAP
6:2/8:2 Fluorotelomer phosphate diester	6, 8	6:2/8:2 diPAP
8:2 Fluorotelomer phosphate diester	8, 8	8:2/8:2 diPAP
6:2/10:2 Fluorotelomer phosphate diester	6, 10	6:2/10:2 diPAP
8:2/10:2 Fluorotelomer phosphate diester	8, 10	8:2/10:2 diPAP
6:2/12:2 Fluorotelomer phosphate diester	6, 12	6:2/12:2 diPAP
10:2 Fluorotelomer phosphate diester	10, 10	10:2/10:2 diPAP
8:2/12:2 Fluorotelomer phosphate diester	8, 12	8:2/12:2 diPAP
6:2/14:2 Fluorotelomer phosphate diester	6, 14	6:2/14:2 diPAP
Alternatives		
2-(6-chloro-dodecafluorohexyloxy)-tetrafluoroethane sulfonate	8	F-53B

[a]Acronyms are according to (Buck et al., 2011). [b]Branched and linear isomers

Calculations and statistics

PFAS levels below MQL were recalculated to MQL/√2 or reported values over the detection limit (MDL) were used. To test for significant changes in PFAS concentrations over the whole period, 1997-2016, log-linear regression analyses were carried out.

To estimate a possible change-point (CP) in the temporal trends we used a technique similar to that suggested by Sturludottir et al. (2015). Prior to the change-point analysis, the data was screened for outliers. Observations with a residual from a regression line covering the whole period was excluded if the residual exceeded 3 times the interquartile range (IQR) of all the residuals ('the outer fence'). This is a conservative approach and only a few observations were excluded from the CP test. To detect the CP, the whole time-series was repeatedly divided into two parts with at least three years in each part and log-linear regression lines were fitted to each part and the residual variance was recorded for each combination. The combination of regression lines that gained the smallest variance was compared with a log-linear regression line for the whole study period and the mean for the whole time period with F-tests. The degrees of freedom were down-adjusted to compensate for the less restrained situation with two regression lines compared to a single regression line. Only one change-point was searched for because the time-series were generally too short for several change-points. The median concentration for the tested change-point year was included in both parts of the time series. This is a conservative approach which reduces the influence of abrupt changes from one year to the next but may also reduce the chance to detect significant trends on either side of the change-point. The two parts may not necessarily point in different directions (increasing- decreasing) and may not show significant slopes separately (only significant regressions lines were plotted) but they still show a significant decrease in residual variance, i.e. they explain significantly more of the variation in contaminant concentration than the mean or a regression line for the whole period. For time-series without a significant CP, log-linear regression was still carried out. A three-year unweighted moving average smoother was also applied for comparison with the change-point analysis.

RESULTS AND DISCUSSION

PFAS homologues occurring at the highest levels in serum samples from first-time mothers from Uppsala 2015 and 2016 was lin-PFHxS, with mean concentrations of 4.5 ng/g, followed by lin-PFOS (3.2 ng/g), br-PFOS (1.6 ng/g), and lin-PFOA (1.5 ng/g) (Table 3 and 4). Levels were below MQL or MDL for all samples in the case of PFBA, PFPeA, PFHxA, PFHpA, br-PFOA, PFTeDA, PFDS, FOSAA, br-MeFOSAA, br-EtFOSAA, 4:2 FTS, and 6:2 FTS and almost all samples for 8:2 FTS and F-53B (Table 5). Due to large variations in MQL between batches for br-PFOA the results are not presented in the report. FOSA, MeFOSA, EtFOSA, and all 15 PAPs were below MDL in all samples 2015-2016 (MDL 0.02-1.8 ng/g). FPrPA, FPePA, and FHpPA were analyzed for the first time in samples 2015-2016 and all were below MDL, 0.2-0.8 ng/g serum.

Table 3. Concentrations of perfluoroalkyl carboxylic acids (PFCAs) (ng/g) in pooled blood serum samples from primiparous women in Sweden. Results in bold generated in the present study, other data from Gebbink et al. (2015) and Glynn et al (2015).). Reported levels <MQL but above MDL in italics.

Year	PFBA	PFPeA	PFHxA	PFHpA	br-PFOA	lin-PFOA	PFNA	PFDA	PFUnDA	PFDoDA	PFTrDA	PFTeDA
1997	<0.3	<0.1	<0.05	0.031	0.050	2.25	0.29	0.14	0.14	0.026	0.038	<0.002
1997	<0.3	<0.1	<0.05	0.038	0.070	2.53	0.34	0.18	0.14	0.025	0.022	<0.002
1997	<0.3	<0.1	<0.05	0.023	0.040	2.19	0.26	0.14	0.11	0.017	0.016	<0.002
1998	<0.3	<0.1	<0.05	0.034	0.060	2.47	0.37	0.18	0.17	0.018	0.021	<0.002
1998	<0.3	<0.1	<0.05	0.022	0.040	2.58	0.39	0.23	0.21	0.031	0.028	<0.002
1998	<0.3	<0.1	<0.05	0.040	0.050	2.33	0.36	0.19	0.14	0.016	0.019	<0.002
2000	<0.3	<0.1	<0.05	0.019	0.040	2.59	0.39	0.19	0.17	0.023	0.026	<0.002
2000	<0.3	<0.1	<0.05	0.028	0.050	2.64	0.39	0.20	0.23	0.030	0.037	<0.002
2000	<0.3	<0.1	<0.05	0.043	0.050	2.54	0.31	0.17	0.16	0.020	0.027	<0.002
2002	<0.3	<0.1	<0.05	0.11	0.050	2.85	0.45	0.24	0.22	0.031	0.037	<0.002
2002	<0.3	<0.1	<0.05	0.045	0.030	2.64	0.43	0.24	0.23	0.027	0.021	<0.002
2002	<0.3	<0.1	<0.05	0.050	0.040	2.84	0.41	0.23	0.18	0.024	0.022	<0.002
2004	<0.3	<0.1	<0.05	0.044	0.040	2.31	0.38	0.20	0.18	0.026	0.030	<0.002
2004	<0.3	<0.1	<0.05	0.039	0.030	2.47	0.66	0.38	0.30	0.040	0.046	<0.002
2004	<0.3	<0.1	<0.05	0.029	0.020	2.67	0.56	0.37	0.29	0.038	0.044	<0.002
2006	<0.3	<0.1	<0.05	0.058	0.040	2.08	0.54	0.25	0.21	0.026	0.022	<0.002
2006	<0.3	<0.1	<0.05	0.032	0.030	2.18	0.52	0.29	0.25	0.034	0.045	<0.002
2006	<0.3	<0.1	<0.05	0.038	0.020	1.99	0.46	0.24	0.24	0.029	0.032	<0.002
2007	18	<1.44	3.5	0.24	0.063	2.72	0.57	0.24	0.18	0.032	0.020	<0.18
2007	17	<1.44	0.84	0.14	0.081	2.67	0.75	0.35	0.26	0.047	0.027	<0.18
2007	10	<1.44	0.33	0.093	<0.045	1.90	0.55	0.31	0.25	0.043	0.025	<0.18
2008	<0.3	<0.1	<0.05	0.038	0.030	1.65	0.56	0.26	0.25	0.036	0.049	<0.002
2008	<0.3	<0.1	<0.05	0.014	0.020	1.82	0.51	0.28	0.24	0.031	0.039	<0.002
2008	<0.3	<0.1	<0.05	0.014	0.020	2.19	0.72	0.39	0.26	0.035	0.039	<0.002
2009	19	<1.44	1.1	0.16	<0.045	1.82	0.59	0.26	0.25	0.036	0.028	<0.18
2009	7.9	<1.44	0.33	0.073	<0.045	2.27	0.63	0.27	0.26	0.040	0.032	<0.18
2009	7.9	<1.44	0.22	0.072	<0.045	2.30	0.58	0.27	0.28	0.045	0.034	<0.18
2010	<0.3	<0.1	<0.05	0.014	0.010	1.61	0.63	0.31	0.28	0.032	0.038	<0.002
2010	<0.3	<0.1	<0.05	0.030	0.020	1.93	0.75	0.38	0.31	0.040	0.047	<0.002
2010	<0.3	<0.1	<0.05	0.021	0.020	1.79	0.60	0.38	0.31	0.042	0.042	<0.002
2011	<2.9	<1.44	0.047	0.080	0.041	2.39	0.65	0.32	0.31	0.051	0.042	<0.18
2011	<2.9	<1.44	0.057	0.046	<0.045	1.61	0.53	0.26	0.30	0.038	0.038	<0.18
2011	8.7	<1.44	0.37	0.057	<0.045	1.76	0.48	0.28	0.33	0.044	0.049	<0.18
2012	<0.3	<0.1	<0.05	0.026	0.010	1.28	0.48	0.27	0.23	0.027	0.030	<0.002
2012	<0.3	<0.1	<0.05	0.030	0.020	1.71	0.56	0.27	0.25	0.031	0.030	<0.002
2012	<0.3	<0.1	<0.05	0.022	0.010	1.40	0.54	0.29	0.27	0.033	0.038	<0.002
2013	6.8	<1.44	0.15	0.046	<0.045	1.66	0.54	0.32	0.32	0.050	0.035	<0.18
2013	4.9	<1.44	0.19	0.056	<0.045	1.87	0.50	0.24	0.20	0.025	<0.023[2]	<0.18
2013	5.3	<1.44	0.29	0.059	<0.045	1.50	0.49	0.27	0.29	0.041	0.031	<0.18
2014	18	<1.44	0.82	0.078	<0.045	1.43	0.62	0.35	0.29	0.042	0.022	<0.18
2014	13	<1.44	0.47	0.093	<0.045	1.57	0.46	0.25	0.18	0.036	<0.023	<0.18
2014	12	<1.44	0.49	0.076	<0.045	1.33	0.54	0.31	0.28	0.037	0.025	<0.18
2015	**<0.2**	**<0.09**	**<0.06**	**<0.02**	**<0.08**	**1.73**	**0.64**	**0.33**	**0.36**	***0.06***	***0.07***	**<0.02**
2015	**<0.2**	**<0.09**	**<0.06**	**<0.02**	**<0.08**	**1.43**	**0.48**	**0.28**	**0.33**	***0.04***	***0.05***	**<0.02**
2015	**<0.2**	**<0.09**	**<0.06**	**<0.02**	**<0.08**	**1.52**	**0.59**	**0.35**	**0.32**	***0.04***	***0.05***	**<0.02**
2016	**<0.2**	**<0.09**	**<0.06**	**<0.02**	**<0.08**	**1.32**	**0.52**	**0.23**	**0.24**	**<0.03**	***0.04***	**<0.02**
2016	**<0.2**	**<0.09**	**<0.06**	**<0.02**	**<0.08**	**1.51**	**0.55**	**0.33**	**0.31**	***0.04***	***0.06***	**<0.02**
2016	**<0.2**	**<0.09**	**<0.06**	**<0.02**	**<0.08**	**1.35**	**0.45**	**0.25**	**0.24**	**<0.03**	***0.04***	**<0.02**

Table 4. Concentrations of perfluoroalkyl sulfonic acids (PFSAs) (ng/g) in pooled blood serum samples from primiparous women in Sweden. Results in bold generated in the present study, other data from Gebbink et al. (2015) and Glynn et al (2015).). Reported levels <MQL but above MDL in italics.

Year	PFBS	br-PFHxS	lin-PFHxS	tot-PFHxS	br-PFOS	lin-PFOS	tot-PFOS	PFDS
1997	0.025	0.17	1.97	2.14	5.37	9.86	15.2	0.047
1997	0.017	0.13	1.56	1.69	6.47	12.2	18.7	0.047
1997	0.017	0.12	1.53	1.64	6.52	12.0	18.5	0.081
1998	0.024	0.16	2.15	2.31	6.94	12.4	19.3	0.020
1998	0.020	0.14	1.85	1.99	6.78	13.5	20.3	0.091
1998	<0.009[2]	0.09	1.31	1.40	5.92	11.3	17.2	0.008
2000	0.014	0.11	1.85	1.96	5.57	11.2	16.8	<0.005
2000	0.029	0.21	2.30	2.51	6.63	12.4	19.0	0.006
2000	0.025	0.22	2.94	3.16	7.27	12.3	19.6	0.011
2002	<0.009	0.25	2.76	3.01	5.78	10.6	16.4	0.013
2002	<0.009	0.12	2.32	2.44	6.63	11.6	18.2	0.013
2002	<0.009	0.16	2.62	2.78	5.80	10.0	15.8	0.013
2004	0.019	0.21	2.32	2.53	4.10	7.36	11.5	0.022
2004	0.011	0.25	3.39	3.64	4.81	9.99	14.8	0.017
2004	<0.009	0.22	2.88	3.10	5.34	10.3	15.6	0.012
2006	0.058	0.47	4.15	4.62	5.35	9.62	15.0	0.006
2006	0.048	0.52	5.99	6.51	4.07	7.26	11.3	0.005
2006	0.022	0.17	2.06	2.23	3.71	6.32	10.0	<0.005
2007	0.058	0.52	5.23	5.75	4.04	7.06	11.1	<0.019
2007	0.046	0.38	4.79	5.16	5.50	9.71	15.2	<0.019
2007	0.041	0.34	4.05	4.38	2.89	8.84	8.73	<0.019
2008	0.056	0.43	3.90	4.33	3.21	5.30	8.51	0.007
2008	0.023	0.33	3.51	3.85	3.49	5.30	8.80	0.007
2008	0.019	0.34	4.01	4.35	3.11	5.33	8.44	0.012
2009	0.029	0.36	3.77	4.13	2.34	5.05	7.39	<0.019
2009	0.029	0.54	7.40	7.93	3.15	5.72	8.87	<0.019
2009	0.041	0.38	4.36	4.73	3.02	5.30	8.31	<0.019
2010	<0.009	0.21	2.36	2.57	2.21	3.75	5.96	0.009
2010	0.038	0.58	5.94	6.52	2.69	4.73	7.41	0.005
2010	0.019	0.45	5.19	5.65	2.35	4.13	6.48	0.007
2011	0.023	0.39	5.61	6.01	2.81	5.12	7.93	<0.019
2011	0.058	0.49	6.30	6.79	1.99	4.16	6.15	<0.019
2011	0.024	0.38	5.38	5.75	1.88	4.29	6.16	<0.019
2012	<0.009	0.13	1.87	2.00	1.90	3.71	5.61	<0.005
2012	0.020	0.39	4.60	5.00	2.51	3.94	6.45	<0.005
2012	<0.009	0.32	4.60	4.91	2.44	4.29	6.73	<0.005
2013	<0.016	0.30	5.01	5.30	1.55	3.55	5.10	<0.019
2013	0.030	0.34	5.00	5.34	1.66	3.10	4.75	<0.019
2013	0.029	0.28	4.50	4.79	1.50	3.68	5.18	<0.019
2014	0.024	0.21	3.41	3.62	1.31	2.99	4.30	<0.019
2014	0.033	0.35	4.76	5.11	2.06	3.55	5.61	<0.019
2014	<0.016	0.18	3.17	3.35	1.54	3.17	4.71	<0.019
2015	***0.04***	**0.22**	**4.10**	**4.32**	**1.53**	**3.22**	**4.75**	**<0.02**
2015	**<0.01**	**0.21**	**4.63**	**4.85**	**1.53**	**3.46**	**4.99**	**<0.02**
2015	***0.02***	***0.17***	**3.52**	**3.69**	**1.44**	**3.10**	**4.54**	**<0.02**
2016	***0.03***	**0.31**	**6.01**	**6.32**	**1.36**	**2.58**	**3.94**	**<0.02**
2016	***0.03***	**0.18**	**3.57**	**3.75**	**1.62**	**3.79**	**5.41**	**<0.02**
2016	***0.03***	**0.21**	**5.33**	**5.54**	**1.80**	**3.29**	**5.09**	**<0.02**

Table 5. Concentrations of perfluoroalkane sulfonamides (FASAs) (ng/g) in pooled blood serum samples from primiparous women in Sweden. Results in bold generated in the present study, other data from Gebbink et al. (2015) and Glynn et al (2015).). Reported levels <MQL but above MDL in italics.

Year	FOSAA	br-MeFOSAA	lin-MeFOSAA	br-EtFOSAA	lin-EtFOSAA
1997	0.39	0.026	0.36	0.003	0.76
1997	0.59	0.008	0.25	0.010	0.94
1997	0.50	0.018	0.30	0.028	1.28
1998	0.54	0.020	0.26	0.021	1.52
1998	0.42	0.035	0.34	0.014	0.73
1998	0.52	0.015	0.23	0.003	0.87
2000	0.33	0.020	0.34	<0.002	0.37
2000	0.55	0.031	0.34	0.007	0.36
2000	0.40	0.033	0.34	0.009	0.54
2002	0.32	0.019	0.25	<0.002	0.24
2002	0.46	0.026	0.46	0.002	0.17
2002	0.46	0.021	0.46	<0.002	0.20
2004	0.12	0.004	0.11	<0.002	0.051
2004	0.12	0.004	0.12	<0.002	0.060
2004	0.21	0.012	0.15	<0.002	0.13
2006	0.087	0.002	0.064	<0.002	0.033
2006	0.062	0.001	0.063	<0.002	0.020
2006	0.11	0.010	0.091	<0.002	0.023
2007	0.055	0.007	0.062	<0.02	0.024
2007	0.049	<0.004	0.051	<0.02	0.023
2007	0.089	0.013	0.155	<0.02	0.029
2008	0.072	<0.001	0.072	<0.002	<0.002
2008	0.090	0.002	0.058	<0.002	<0.002
2008	0.075	0.002	0.039	<0.002	0.010
2009	0.037	0.006	0.054	<0.02	0.023
2009	0.020	<0.004	0.035	<0.02	0.018
2009	0.034	0.006	0.038	<0.02	0.037
2010	0.031	<0.001	0.015	<0.002	0.003
2010	0.038	<0.001	0.022	<0.002	0.010
2010	0.041	0.001	0.035	<0.002	0.004
2011	0.024	<0.004	0.030	<0.02	0.015
2011	0.010	<0.004	0.021	<0.02	0.013
2011	0.014	<0.004	0.035	<0.02	<0.015
2012	0.041	0.002	0.028	<0.002	<0.002
2012	0.043	0.003	0.029	<0.002	0.014
2012	0.036	0.001	0.021	<0.002	0.009
2013	0.031	0.008	0.045	<0.02	<0.015
2013	0.012	0.006	0.017	<0.02	0.020
2013	0.009	<0.004	0.015	<0.02	0.010
2014	<0.016	<0.004	0.014	<0.02	<0.015
2014	0.014	<0.004	0.057	<0.02	0.010
2014	<0.016	<0.004	0.015	<0.02	0.0088
2015	**<0.01**	**<0.004**	***0.015***	**<0.004**	***0.004***
2015	**<0.01**	**<0.004**	**0.022**	**<0.004**	**<0.004**
2015	**<0.01**	**<0.004**	***0.014***	**<0.004**	***0.007***
2016	**<0.01**	**<0.004**	***0.014***	**<0.004**	***0.006***
2016	**<0.01**	**<0.004**	***0.013***	**<0.004**	***0.004***
2016	**<0.01**	**<0.004**	***0.011***	**<0.004**	**<0.004**

Table 6. Concentrations of fluorotelomer sulfonates (FTSs) and the alternative F-53B (ng/g) in pooled blood serum samples from primiparous women in Sweden. Results in bold generated in the present study, other data from Gebbink et al. (2015) and Glynn et al (2015).). Reported levels <MQL but above MDL in italics.

Year	4:2 FTS	6:2 FTS	8:2 FTS	F-53B
2007	<0.008	<0.50	0.028	0.007
2007	<0.008	<0.50	0.054	0.010
2007	<0.008	0.45	0.053	0.014
2009	<0.008	<0.50	0.018	0.022
2009	<0.008	<0.50	0.028	0.020
2009	<0.008	<0.50	0.025	0.021
2011	<0.008	<0.50	0.016	0.018
2011	<0.008	<0.50	0.019	0.016
2011	<0.008	0.62	0.024	0.018
2013	<0.008	<0.50	0.021	0.019
2013	<0.008	<0.50	0.012	0.006
2013	<0.008	<0.50	0.018	0.013
2014	<0.008	<0.50	0.021	0.010
2014	<0.008	<0.50	0.011	0.015
2014	<0.008	<0.50	0.005	0.012
2015	**<0.04**	**<4**	**<0.012**	***0.013***
2015	**<0.04**	**<4**	**<0.012**	**<0.012**
2015	**<0.04**	**<4**	**<0.012**	**<0.012**
2016	**<0.04**	**<4**	***0.015***	***0.028***
2016	**<0.04**	**<4**	**<0.012**	**<0.012**
2016	**<0.04**	**<4**	**<0.012**	**<0.012**

Temporal trends

Temporal trends were evaluated for the PFASs with sufficient data, both for the whole sampling period 1997-2016, in table 7, and for possible change points (CP) presented in the figures 1-3.

Table 7. Annual change (standard error) in concentrations of PFAS in serum 1997–2016.

Compound	n	Change (%) Mean (SE)	R^2 (%)	p
PFHpA	48			ns
lin-PFOA	48	-3.3 (0.34)	65	<0.001
PFNA	48	2.7 (0.47)	42	<0.001
PFDA	48	2.8 (0.49)	41	<0.001
PFUnDA	48	3.4 (0.47)	53	<0.001
PFDoDA	48	2.8 (0.64)	29	<0.001
PFTrDA	48	2.5 (0.82)	15	0.004
PFBS	48			ns
br-PFHxS	48	3.6 (1.1)	18	0.002
lin-PFHxS	48	5.4 (0.81)	50	<0.001
Tot PFHxS	48	5.3 (0.82)	48	<0.001
br-PFOS	48	-8.5 (0.43)	86	<0.001
lin-PFOS	48	-7.9 (0.35)	91	<0.001
Tot PFOS	48	-8.1 (0.36)	91	<0.001
FOSAA	48	-22 (0.80)	92	<0.001
lin-MeFOSAA	48	-17 (0.88)	87	<0.001
lin-EtFOSAA	48	-26 (1.7)	79	<0.001
8:2 FTS	21	-15 (2.1)	69	<0.001

Significant declining temporal trends were observed for all PFCAs above MQL, 1997-2016, except for lin-PFOA which decreased (Table 7). The results are similar to previously reported temporal trends from 1996-2010 (Glynn et al. 2012) and from 1997-2014 (Glynn et al 2015). Serum concentrations of lin-PFOA declined by 3.3% per year, and PFNA, PFDA, PFUnDA, PFDoDA, and PFTrDA increased by around 3% per year (Table 7). No trend was observed for PFHpA (Table 7, Figure 1). For lin-PFOA a significant CP was observed around year 2002 with an increasing trend before that year and a decreasing trend after (Figure 1). PFNA, PFDA, and PFUnDA levels increased during the first years of the study but all three PFCAs had a CP around year 2004 and after that no significant trend could be observed

(Figure 1). Similar pattern was observed for PFDoDA but the CP was later, around year 2011. For PFTrDA no CP was observed (Figure 1).

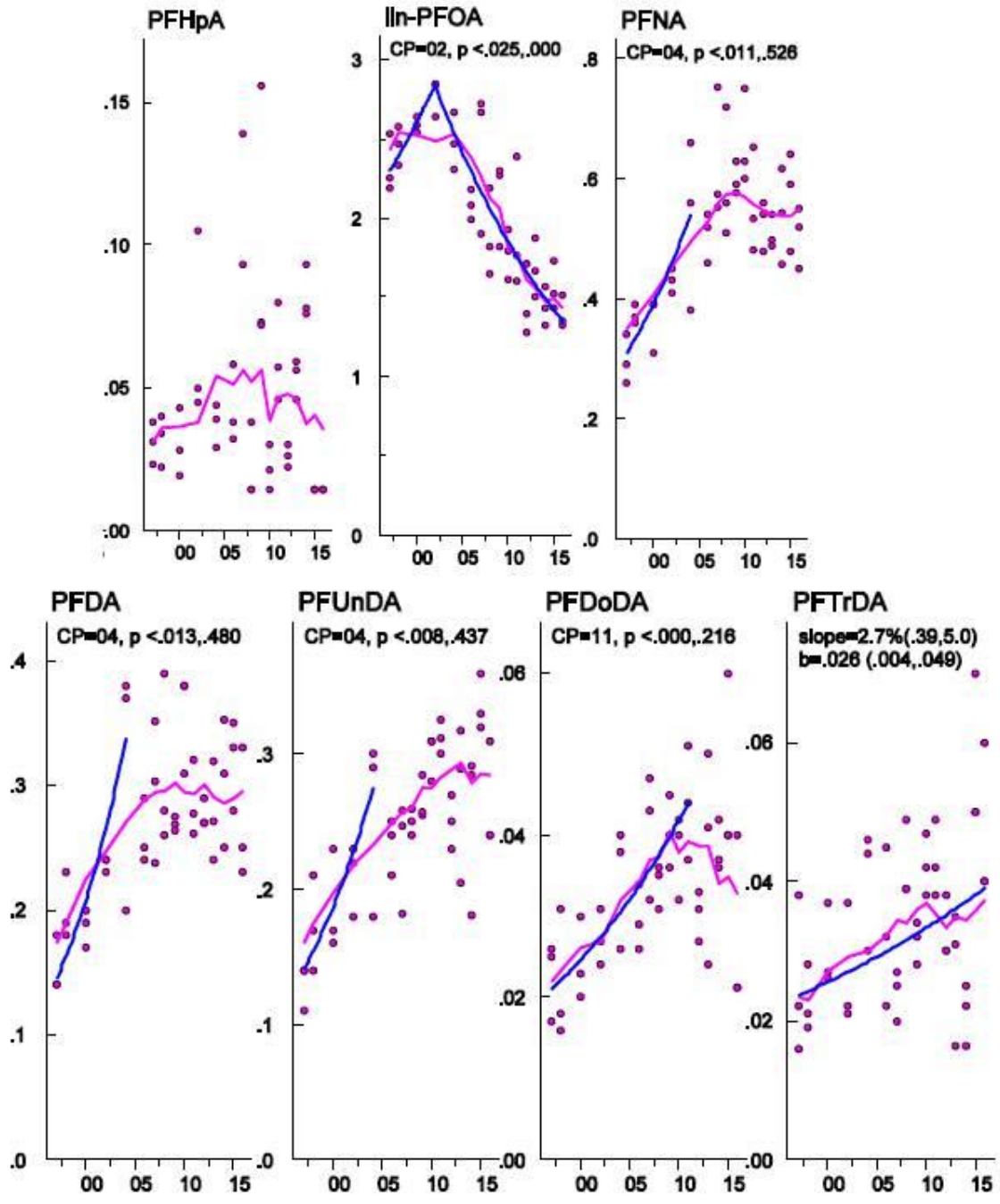

Figure 1. Levels of PFCAs (n=48), in pooled serum samples from first-time mothers in Uppsala, Sweden. The blue lines represent regression lines obtained from the CP-analyses or in cases where the CP analysis is not significant a regression line for the whole period. Purple lines display a three-year unweighted moving average smoother.

No statistically significant temporal trend for PFBS could be detected in the present study in accordance with previous studies (Gebbink et al 2015, Glynn et al. 2015). In Glynn et al. (2012), an increase of PFBS by 11% ($p<0.001$) per year was reported between 1996 and 2010. As pointed out by Gebbink et al. (2015), the differences in observed trends could be due to differences in composition of the pooled samples, since each pool from the early study period was composed of more than 10 individual samples in Glynn et al. (2012) and since the present study is lacking pools from 1996 (too few individual samples). Moreover, the analytical methods differed between studies. A study of differences in PFAS levels in individual serum from the Uppsala mothers between 1996-1999 and 2008-2011, with high statistical power, has shown that the population in Uppsala has experienced increased PFBS exposure since 1996 (Gyllenhammar et al. 2015). The increase in PFBS and PFHxS levels during the study period is due to exposure of the study participants to these PFAAs from contaminated drinking water in Uppsala (Gyllenhammar et al. 2015). In 2012 the polluted drinking water production wells were taken out of production in Uppsala. Interestingly, a CP is observed for both br-PFHxS and lin-PFHxS around year 2011 after which no increase is observed (Figure 2).

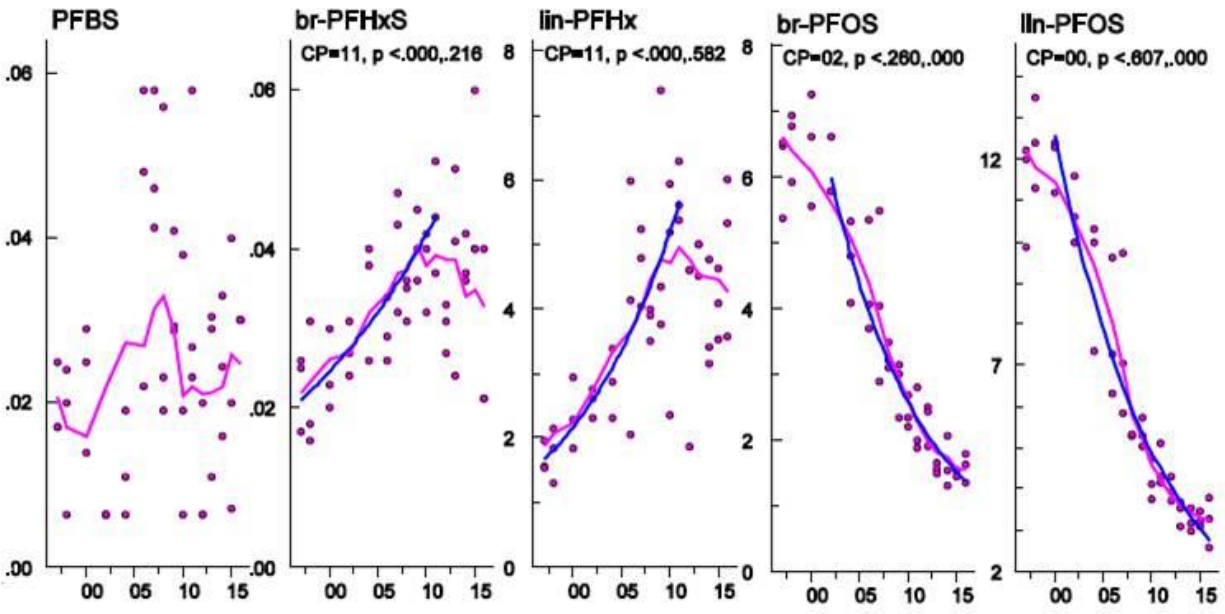

Figure 2. Levels of PFSAs (n=48), in pooled serum samples from first-time mothers in Uppsala, Sweden. The blue lines represent regression lines obtained from the CP-analyses or in cases where the CP analysis is not significant a regression line for the whole period. Purple lines display a three-year unweighted moving average smoother.

Serum levels of PFOS decline by around 8% per year over the entire sampling period. Non-significant CPs were observed early in the time series, for example the year 2000 for lin-PFOS and 2002 for br-PFOS (Figure 2). The decrease in PFOS is a reflection of the phase-out of PFOS-related production. The slower decline in PFOA levels may be due to a slower world-wide phase-out of production of PFOA and PFOA-related compounds.

The PFOS precursors FOSAA, lin-MeFOSAA, and lin-EtFOSAA, all declined at a higher rate (17-26%), compared to PFOS (Table 7). This was also observed in earlier studies (Gebbink et al. 2015). lin-EtFOSAA had a CP around the year 2007 and after that no significant trend was observed (Figure 3). The PFOA precursor 8:2 FTS was only analyzed in 21 samples 2007-2016 but showed a decreasing trend around 15% per year (Table 7), in accordance with the on-going phase-out of PFOA and related compounds.

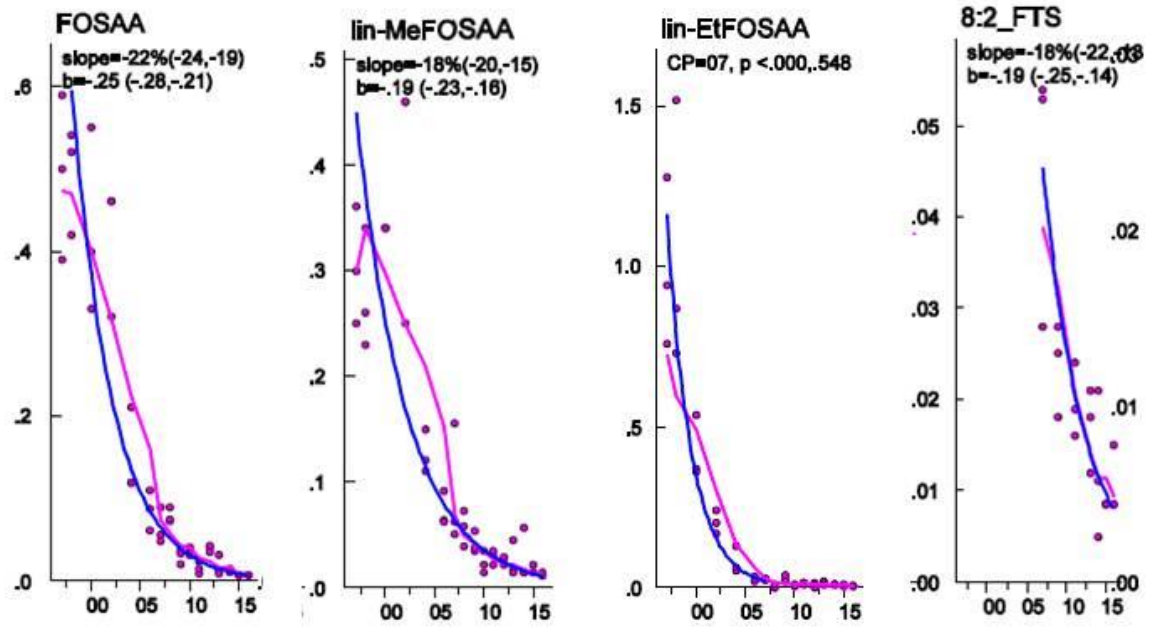

Figure 3. Levels of FOSAA, lin-MeFOSAA, and lin-EtFOSAA (n=48), and 8:2 FTS (n=21), in pooled serum samples from first-time mothers in Uppsala, Sweden. The blue lines represent regression lines obtained from the CP-analyses or in cases where the CP analysis is not significant a regression line for the whole period. Purple lines display a three-year unweighted moving average smoother.

F53-B is an alternative to PFOS manufactured exclusively in China. In the present study with only 21 samples, no temporal trend was seen for F53-B (data not shown). Levels of the precursors and new/alternative PFASs were low or below MDL in the present study, however only a few PFASs were analyzed and it is possible that there are other precursors that are more relevant. As there are over 3000 known PFASs on the global market it is probable that the total PFAS exposure reported here is underestimated. Future studies should expand the number of PFASs included in chemical analysis and also compare the sum concentration of fluorine from individual PFASs determined by LC-MS/MS to that obtained from combustion ion chromatography, a technique which measures the total concentration of fluorine from all organic substances containing a fluorine atom present in a sample. Without such measurements, the extent to which human exposure to PFASs is being underestimated remains unknown.

CONCLUSION

Temporal trends for PFOS, PFOA, and PFOS-precursors are declining as a result of international regulation and phase-out initiatives. PFOS-precursors are declining at a faster rate compared to PFOS. Due to drinking water contamination, serum concentrations of PFHxS have been increasing in the mothers from Uppsala. At around year 2011 levels had stopped increasing (CP) which is consistent with the initiation of efforts to mitigate the contamination in July 2012. Concentrations of long-chained PFCAs have been increased about 3% per year during the entire study period. For PFNA, PFDA, and PFUnDA a cessation of the increase was observed around 2004 and thereafter no trend was seen. A similar pattern was observed for PFDoDA with a CP around year 2011. Concentrations of PFTrDA have been increasing throughout the study period and no CP was observed. It is important to follow-up the trends of PFHxS and the long-chained PFCAs in the future to confirm that the exposure of the population is leveling off and decreasing.

Concentrations of precursors, such as FOSAAs, FTSs, FTAs and the PFAS alternative F53-B were generally below MQL. Increasing the number of PFASs included in the chemical analysis and also analyses total organic fluorine are necessary in order to estimate the amount of unknown PFAS for a more complete picture of human exposure to PFASs.

ACKNOWLEDGEMENT

The Swedish EPA (Environmental Protection Agency) is acknowledged for financial support. Appreciation is expressed to the participating women and to Marie Walterzon, Monica Rudin, Marianne Leimar, and Johanna Elwinger, the midwives who assisted in recruitment, interviewing, and sample collection in 2015 and 2016. Ellen Edgren and Jane Karlsdotter are appreciated for technical assistance.

REFERENCES

Buck RC,Franklin J, Berger U, Conder JM, Cousins IT, de Voogt P, Astrup Jensen AA, Kannan K, Mabury SA, van Leeuwen SPJ. 2011. Perfluoroalkyl and Polyfluoroalkyl Substances in the Environment: Terminology, Classification, and Origins. Integr Environ Assess Manag 7, 513–541.

Gebbink WA,Glynn A, Berger U. 2015. Temporal changes (1997-2012) of perfluoralkyl acids and selected precursors (including isomers) in Swedish human serum. Environ Pollut 199, 166-173.

Glynn A, Berger U, Bingert A, Ullah S, Aune M, Lignell S, Darnerud PO. 2012. Perfluorinated alkyl acids in blood serum from primiparous women in Sweden: Serial sampling during pregnancy and nursing, and temporal trends 1996-2010. Environ Sci Technol 46, 9071-9079.

Glynn A, Benskin J, Lignell S, Gyllenhammar I, Aune M, Cantillana T, Darnerud PO, Sandblom O. 2015. Temporal trends of perfluoroalkyl substances in pooled serum samples from first-time mothers in Uppsala 1997-2014. Report to the Swedish EPA (the Health-Related Environmental Monitoring Program)

Gyllenhammar I, Berger U, Sundström M, McCleaf P, Eurén K, Eriksson S, Ahlgren S, Lignell S, Aune M, Kotova N, Glynn A. 2015. Influence of contaminated drinking water on perfluoralkyl acid levels in human serum – A case study from Uppsala, Sweden. Environ Res 140, 673-683.