Sveriges lantbruksuniversitet
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SWEDISH ENVIRONMENTAL PROTECTION AGENCY

NATIONAL ENVIRONMENTAL MONITORING COMMISSIONED BY THE SWEDISH EPA

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

Contract no. 215-19-004

Intraindividuell trend av PFAS bland kvinnor och barn

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2021-04-29

NATIONELL MILJÖÖVERVAKNING PÅ UPPDRAG AV NATURVÅRDSVERKET

ÄRENDENNUMMER NV-01623-19
AVTALSNUMMER 215-19-004
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
Sverige

Nyckelord för ämne
PFAS, blodserum, dricksvatten, exponering, kvinnor, barn, upprepade mätningar

Tidpunkt för insamling av underlagsdata
1996-2019

Sammanfattning
I den här studien undersöktes hur serumhalterna av PFOA, PFNA, PFDA, PFUnDA, PFHxS (linjär och grenade) och PFOS (linjär och grenade) förändrats hos unga kvinnor och deras förstfödda barn (POPUP-kohorten) under en 12-årsperiod av upprepade provtagningar (2-4 gånger). Två grupper av mammor provtogs för första gången tre veckor efter förlossningen. De som provtogs första gången 1996-99 följdes upp en gång efter 12 år (N=57) och de som lämnade första provet 2004-2011 följdes upp 2-3 gånger 4-12 år senare (N=13-44). Under åren 1996-99 provtogs spädbarn vid 3 månaders ålder och följdes upp 12 år senare (N=31). Under åren 2008-2015 togs första provet på barnen när de fyllt 4 år eller 8 år. 4-åringarna följdes upp vid 8 år (N=34) eller vid 8 och 12 års ålder (N=11) och 8-åringarna följdes upp vid 12 års ålder (N=34). De intra-individuella PFAS-trenderna, bland både mammor och deras barn, verkar generellt ha påverkats av de åtgärder som vidtagits för att begränsa tillverkning/användning av ämnena under de senaste årtiondena. Utfasning/förbud av PFOA och PFOS, med start runt 2000, bidrog till i medeltal sjunkande intra-individuella serumhalter med ökande ålder både hos mammor och barn. Åtgärder för att begränsa produktion/användning av PFNA, PFDA och PFUnDA kom senare än för PFOS och PFOA. Det resulterade i ökade intra-individuella serumhalter mellan första provet (1996-199) och provet 12 år senare bland både mammor och barn. För deltagare med första provtagning senare under studieperioden, 2004-2011 för mammor och 2008-2015 för barn, observerades en tendens av i medeltal sjunkande halter med ökande ålder. Undantag var PFNA and PFUnDA hos mammorna, för vilka något ökande eller stabila halter observerades. Bland mammorna provtagna första gången 1996-1999, sågs i medeltal en ökning av intra-

individuella PFHxS-halter under 12-årsperioden, troligtvis på grund av de dricksvattenföroreningar av PFAS som förekommit i Uppsala. Bland barnen observerades dock ingen skillnad mellan första provtagningen vid 3 månaders ålder och 12 år senare. Bland mammorna som provtogs för första gången 2004-2011, tycks ökningen ha följts av en minskning efter att rening av PFAS från Uppsalas dricksvatten infördes 2012. En minskning av intra-individuella PFHxS-halter observerades också hos barnen mot slutet av studieperioden. Bland mammorna kan skillnader i intra-individuella PFAS-trender mellan deltagare sannolikt till viss del förklaras av hur många barn kvinnorna fött under uppföljningsperioden, eftersom elimineringen av PFAS ur kroppen ökar under graviditet, förlossning och amning.

Intra-individual temporal trends of perfluoroalkyl substances among mothers and their first-born children repeatedly sampled during 12 years.

Summary

This report describes how the serum concentrations of highly fluorinated substances (PFASs) have changed among young women and their children (POPUP cohort) who have been sampled repeatedly over a 12-year period. In a cohort of first-time mothers recruited in 1996-1999 and 2004-2011 and their first-born children from Uppsala (POPUP), PFOA, PFNA, PFDA, PFUnDA, PFHxS (linear and branched) and PFOS (linear and branched) were studied. The mothers, who were first sampled 3 weeks after delivery of their first child in 1996-99, were only followed up once after 12 years. This was also true for their 3 month old first-born babies. Starting in 2004, the mothers were followed up 4, 8 and 12 years after the first sampling 3 weeks after delivery, and their children were sampled at 4, 8 and 12 years of age (no 3-month-sampling) starting in 2008.

Among the mothers, serum concentrations of individual PFASs were in most cases significantly correlated ($p \leq 0.05$) between sampling occasions, with Spearman's correlation coefficients (r) of 0.14-0.91 (N = 14-71). The strongest correlations were observed 4-12 years after delivery. Among the children, who were first sampled 1996-1999 at 3 months of age and then followed up with a sampling at 12 years of age (N = 31), no significant correlations were observed (r = -0.14-0.35, $p > 0.05$). The correlations between PFASs in samples 4, 8 and 12 years after birth (N = 14-36), on the other hand, were significant in many cases (r = 0.28-0.81).

Differences in intra-individual trends were observed between the participating mothers and may depend, among other things, on how many children the mothers gave birth to during the follow-up. PFAS concentrations decreased by an average of 30-50% per child born during

follow-up (not for PFUnDA). This is probably due to an increased elimination of PFASs linked to pregnancy, childbirth and breastfeeding.

Despite these individual differences in intra-individual trends of specific PFASs, some general conclusions could be made about intra-individual trend patterns for different PFASs. Among mothers who were sampled for the first time in 1996-1999, and then followed up with a sampling 12 years later, the PFOA and branched/linear PFOS concentrations on average decreased with increasing age, while concentrations of PFNA, PFDA, PFUnDA and branched/linear PFHxS increased. Among mothers with initial sampling starting in 2004, PFOA and PFOS concentrations on average still decreased with increasing age 4-12 years after the initial sampling, while PFDA initially decreased and thereafter stabilized at lower levels. This suggests that intra-individual cumulative PFOA and PFOS exposure was lower than cumulative elimination from the body throughout most of the follow-up period (decreasing serum levels), whereas the differences between exposure and elimination of PFDA decreased towards an equilibrium at the end of follow-up. When initial sampling started in 2004, PFNA levels were on average unchanged with increasing age, while PFUnDA levels increased towards a plateau at the end of the 12 year follow-up. For PFHxS, the concentrations seemed to decrease with increasing age towards the end of the follow-up. The homologue-specific differences in intra-individual trends may be due to differences in timing of international measures to limit production/use of the studied PFASs. Actions against PFNA, PFDA and PFUnDA came later (end of the 2000s-beginning of 2010s) than for PFOS and PFOA (beginning-middle of the 2000s). The special case of drinking water PFHxS contamination in Uppsala, with remediation in 2012, most probably also contributed to the observed PFHxS intra-individual trends.

Among the first-born children, decreasing PFOA and PFOS concentrations were in general observed throughout the follow-up from 3 months to 12 years of age. The PFHxS concentrations on average did not differ between the samples taken at 3 months of age (start 1996-1999) and 12 years later. Among children with first sampling at 4 years of age, with first sampling starting 2008-2015, slightly declining concentrations were observed with increasing age.

PFNA, PFDA and PFUnDA concentrations increased with age among children sampled at 3 months of age 1996-99 and 12 years later. However, among children sampled for the first time at 4 years of age starting in 2008-2015, intra-individual concentrations of PFNA and PFDA decreased slightly up to 12 years of age. PFUnDA also seemed to decrease slightly on average after an initial period of increasing/stable levels.

Taken together, the results strongly suggests that the intra-individual trends of the PFASs, studied in both mothers and their children, were influenced by the measures taken to limit PFAS production and use in recent decades. Phasing out of PFOA and PFOS during the 2000s has generally contributed to declining serum levels with increasing age in both mothers and children. Measures to limit PFNA, PFDA and PFUnDA production/use came later than for PFOS and PFOA, which resulted in a combination of increasing serum concentrations with increasing age at the beginning of the follow-up, followed by a flattening/decrease during the latter part. The tendency of an age-dependent increase in PFHxS concentrations among mothers, and to some extent also in their children, were followed by a reduction of concentrations, on average, after the pollution of Uppsala's drinking water were remediated in 2012.

Introduction

Per- och polyfluoroalkyl substances (PFASs) are surface active man-made chemicals with global use. Long-range transport of PFASs occurs in the atmosphere and in oceans, but also due to world-wide trade of products containing PFASs [1, 2]. Certain PFASs (precursors) are degraded to perfluoroalkyl acids (PFAAs), that are very persistent in the environment and are detected in wild animals and humans all over the world [2, 3]. Food is an important PFAA exposure source for humans, but drinking water (DW) also contribute to exposure, especially in areas with PFAS contamination of raw water from point-sources [4-10].

In Uppsala, DW contamination of PFAA was discovered in 2012, and thereafter the DW producer initiated measures to mitigate DW contamination [4, 11]. A cross-sectional study of temporal trends of serum concentrations of PFAAs among first-time mothers 1996-2019 showed that concentrations of the major PFAA-contaminant in the DW, PFHxS, increased up to the end of the 2000s to the beginning of the 2010s and decreased thereafter [12, 13]. Some other PFAAs showed diverging trends unrelated to DW contamination. PFOA and PFOS decreased more or less during the whole study period, whereas PFNA, PFDA and PFUnDA initially increased but began to decrease during the middle/end of the 2000s [12, 13].

Cross-sectional temporal trends, designed as in the case of POPUP with sampling of new study participants of the same sex and age each year, mainly show changes in general exposure during the study period. It is likely that elimination, the other major factor determining PFAA serum concentrations, is more or less constant over the years. The cross-sectional approach does however not give information about temporal trends in individuals (intra-individual trend), which may both be affected by changes in exposure and elimination. Knowledge about intra-individual trends is important for a better understanding of how different efforts to reduce emissions of PFASs into the environment affects human body burdens of PFAAs.

We report intra-individual temporal trends of PFAAs in blood serum from POPUP mothers sampled at delivery of their first child and 4, 8 and 12 years later. Determinants of changes in intra-individual trends, such as number of children born (parity), breast-feeding,

BMI, and fish consumption were investigated. Finally, intra-individual trends of serum concentrations were investigated in the first-born POPUP children sampled at 3 months, 4, 8 and 12 years of age.

Table 1. Characteristics of mothers that had their first child 3 weeks before the first sampling (Start) and were followed up after 4, 8 and 12 years. Years in parenthesis represent time period for each sampling.

Start (1996-2011)	N	Median (range)
Maternal age start (yrs)	127	29 (21-38)
Pre-pregnancy BMI (kg/m^2)	127	22.7 (18.2-39.4)
Fish consumption (times/month)	127	6 (0-34)
4 years (2008-2015)		
BMI (kg/m^2)	45	22.9 (18.2-37.0)
No. of children from 1st sampling	45	1 (0-2)
Total nursing from 1st sampling (months)	43	15 (3-37)
Fish consumption (times/month)	45	8 (0-19)
8 years (2008-2019)		
BMI (kg/m^2)	43	23.2 (18.4-45.6)
No. of children from 1st sampling	43	1 (1-2)
Total nursing from 1st sampling (months)	38	19 (4-61)
Fish consumption (times/month)	45	8 (0-17)
12 years (2008-2019)		
BMI (kg/m^2)	69	23.6 (20.1-38.1)
No. of children from 1st sampling	38	1 (1-3)
Total nursing from 1st sampling (months)	68	19 (3-82)
Fish consumption (times/month)	71	5 (0-33)

Materials and methods

Recruitment and sampling

First-time mothers were randomly recruited during pregnancy (1996-99) or shortly after pregnancy (2000-2015). In a subgroup of the children, serum samples were collected at 3 months of age 1996-1999. In 2008 a follow-up study on the mothers and their first-born children were initiated, with the aim to follow intra-individual temporal PFAS trends in mother and children 4, 8 and 12 years after the first sampling close to delivery. The mothers answered a self-administered questionnaire about dietary habits and other life-style factors (Table 1). A midwife/nurse took blood samples from both the mother and first-born child at home using 9 ml Vacutainer® or Vacuette® 6 serum tubes and serum was stored at -20°C.

The study was approved by the local ethics committee in Uppsala, Sweden, and the participating women gave informed consent prior to the inclusion of the children in the study.

PFAS analyses

PFASs (Table 2) were analyzed as described in Gyllenhammar et al. [4]. In short, 0.5 g of serum was spiked with internal standards and extracted twice with acetonitrile. The concentrated extract underwent dispersive clean-upon with graphitized carbon. Aqueous ammonium acetate and volumetric standards were added before instrumental analysis on an Acquity ultraperformance liquid chromatography system (UPLC) coupled to a Xevo TQ-S tandem mass spectrometer (MS/MS) (both from Waters Corp., Milford, MA, U.S.) operated in negative electrospray ionization, multiple reaction monitoring mode. Quantification was performed by isotope dilution using a 5-point calibration curve (linear, 1/x weighting, excluding the origin) which was run before and after samples. For most targets, exactly matched isotopically labelled internal standards were available (Table 2). For PFHxS and PFOS, branched and linear isomers were quantified separately. A procedural blank and control sample were included in each batch of samples.

Table 2. PFASs included in the study.

Substance	No of carbons in fluorinated chain	Acronym[1]	Internal Standards
Perfluoroalkyl sulfonic acids (PFSA)			
Perfluorohexane sulfonic acid[a]	6	PFHxS	$^{18}O_2$-PFHxS
Perfluorooctane sulfonic acid[a]	8	PFOS	$^{13}C_4$-PFOS
Perfluoroalkyl carboxylic acids (PFCA)			
Perfluorooctanoic acid	7	PFOA	$^{13}C_4$-PFOA
Perfluorononanoic acid	8	PFNA	$^{13}C_5$-PFNA
Perfluorodecanoic acid	9	PFDA	$^{13}C_2$-PFDA
Perfluoroundecanoic acid	10	PFUnDA	$^{13}C_2$-PFUnDA

[1]Buck et al. [14].

Calculations and statistical analyses

The significance level in all analyses was set to $p \leq 0.05$. Concentrations below limit of quantification (LOQ) were set to LOQ/√2. Cross-sectional temporal PFAS trends were analyzed using the sampling occasion with the highest number of participants, i.e. the first sample from the mothers 1996-2011 (N=128), and the samples from the follow-up of the children at 12 years of age 2008-2019 (N=88). Log-linear regression analyses of associations between ln-transformed serum PFAS concentrations and sampling year were performed.

Intra-individual correlations of PFAS concentrations were investigated by use of Spearman´s rank-order correlation analysis. Paired T-test was used to analyze differences between paired samples.

GLM Repeated Measure analysis was used to investigate the log-linear intra-individual temporal trends of serum PFAS concentrations among the mothers. Covariates included in the regression models were sampling year, number of children born during the follow up, body mass index (BMI), total number of months of nursing, and fish consumption.

Results and Discussion

PFAS concentrations

At the first sampling, maternal linPFOS showed the highest median concentrations followed by brPFOS, PFOA and linPFHxS (Table 3). At the 12-year sampling linPFOS still showed the highest median concentrations, but was now followed by linPFHxS and brPFOS. This shows that the PFAS concentration pattern changed, most likely due to diverging temporal trends of serum concentrations of different PFASs during the study period. Among the children, PFOA and linPFOS showed the highest median concentration at 3 months of age followed by brPFOS and linPFHxS (Table 3). At the 12-year sampling the highest median concentration was observed for linPFOS, followed by PFOA, brPFOS and linPFHxS.

Table 3. PFAS concentrations in serum at the different sampling periods.

PFAS	N	Median (range)	N	Median (range)
Mothers start 1996-2011			Children start 1996-1999 (3 months old)	
PFOA	122	2.28 (0.561-5.64)	31	7.38 (1.34-15.5)
PFNA	122	0.410 (0.0843-2.95)	31	0.316 (0.104-1.06)
PFDA	122	0.202 (0.0250-0.976)	31	0.107 (0.0350-0.263)
PFUnDA	122	0.175 (0.0250-0.791)	31	0.0859 (0.0250-0.181)
brPFHxS	122	0.119 (0.0050-2.17)	31	0.118 (0.0189-0.556)
linPFHxS	122	2.11 (0.363-25.1)	31	1.79 (0.414-5.69)
brPFOS	122	4.71 (1.05-18.8))	31	4.52 (0.870-11.8)
linPFOS	122	9.54 (1.97-28.0)	31	7.34 (1.36-14.6)
4 years 2008-2015			4 years 2008-2015	
PFOA	45	0.811 (0.305-5.66)	41	2.52 (0.861-8.34)
PFNA	45	0.474 (0.248-2.87)	41	0.697 (0.323-2.93)
PFDA	45	0.288 (0.103-1.73)	41	0.260 (0.0500-0.540)
PFUnDA	45	0.303 (0.0410-0.852)	41	0.175 (0.0750-0.770)
brPFHxS	45	0.0943 (0.0390-1.34)	41	0.147 (0.0071-1.65)
linPFHxS	45	1.99 (0.714-1.34)	41	4.25 (0.550-34.9)
brPFOS	45	1.22 (0.595-2.90)	41	1.44 (0.512-4.24)
linPFOS	45	2.85 (1.45-6.66)	41	2.25 (0.871-7.12)
8 years 2008-2019			8 years 2008-2019	
PFOA	45	0.910 (0.332-2.15)	67	1.76 (0.400-4.00)
PFNA	45	0.610 (0.234-1.37)	67	0.678 (0.287-2.13)
PFDA	45	0.338 (0.122-0.792)	67	0.253 (0.0500-0.626)
PFUnDA	44	0.344 (0.0931-0.834)	67	0.191 (0.0400-0.524)
brPFHxS	45	0.109 (0.0390-1.10)	67	0.0700 (0.0150-1.29)
linPFHxS	45	1.86 (0.537-11.4)	67	1.95 (0.639-17.8)
brPFOS	45	1.09 (0.278-2.35)	67	1.40 (0.543-4.66)
linPFOS	45	3.13 (0.863-8.49)	67	2.92 (1.12-18.8)
12 years 2008-2019			12 years 2008-2019	
PFOA	71	1.33 (0.405-6.53)	89	1.85 (0.406-3.71)
PFNA	71	0.550 (0.168-2.46)	89	0.520 (0.0400-3.92)
PFDA	69	0.256 (0.114-0.891)	88	0.221 (0.0400-0.507)
PFUnDA	71	0.251 (0.0750)-0.821)	89	0.110 (0.0400-0.490)
brPFHxS	71	0.179 (0.0110-2.68)	89	0.0535 (0.00500-1.01)
linPFHxS	71	2.78 (0.252-29.2)	89	1.17 (0.298-12.4)
brPFOS	71	1.71 (0.399-4.85)	89	1.62 (0.431-4.42)
linPFOS	71	3.81 (0.907-13.7)	89	3.15 (0.833-9.72)

Significant positive correlations were observed between maternal PFAS concentrations at the different sampling occasions (Table 4). Comparisons of correlations between the different sampling occasions were complicated by the fact that different sampling occasions did not include the same participants, and also not the same number of participants. Nevertheless, when looking at the correlations for the first sampling it seemed to be weakest when correlated with the 12-year follow-up, with a few exceptions (Table 4). Correlations between the initial sampling and the 4-year follow-up were in many cases weaker than correlations between the 4-, 8- and 12-year samplings. This could at least partially be due to

the tendencies of larger intra-individual changes in concentrations during the first 4 years of follow-up in comparison to the later follow-up periods (see below). The long half-lives of the studied PFASs in serum, being on average more than a year [15], contributed to the relatively strong correlations during the 4 to 12 years of follow-up.

*Table 4. Mothers. Spearman´s correlation coefficients between intra-individual concentrations of PFASs at different sampling occasions after repeated sampling of participants for 12 years. Number of participants within parenthesis in the PFOA columns. Coefficients in **bold** r>0.7.*

	4 years	8 years	12 years
PFOA start	0.376* (45)	0.504* (45)	0.283* (71)
4 years		0.619* (45)	0.363 (14)
8 years			**0.705*** (14)
PFNA start	0.429*	0.515*	0.278*
4 years		**0.785***	**0.833***
8 years			**0.899***
PFDA start	0.674*	**0.725***	0.289*
4 years		**0.708***	0.681*
8 years			**0.797***
PFUnDA start	0.715*	0.618*	0.268*
4 years		0.666*	**0.881***
PFUnDA 8 years			0.549
brPFHxS start	0.593*	0.358*	0.265*
4 years		**0.825***	**0.824***
8 years			**0.866***
linPFHxS start	0.739*	0.546*	0.351*
4 years		**0.788***	**0.846***
8 years			**0.866***
brPFOS start	0.628*	0.364*	0.137
4 years		0.660*	0.182
8 years			0.335
linPFOS start	0.601*	0.447*	0.385*
4 years		**0.716***	0.675*
8 years			**0.912***

*p≤0.05

Comparisons between studies of repeated PFAS measurement in blood are complicated by differences in study design and in study populations. Nevertheless, in a study of plasma PFAS concentrations among 100 women from Norway [16], with a mean time between two pregnancies (and sampling) of 18 months (range: 3-53 months), the Pearson´s correlation coefficient for natural log concentrations of PFOA was 0.50, for PFNA 0.39, PFDA 0.60,

PFUnDA 0.71, PFHxS 0.74, and PFOS 0.80 [16]. In our study the corresponding Pearson´s correlation coefficients for natural log concentrations at the first sampling 3 weeks after delivery of the first child and 4 year sampling were similar, except for PFNA (stronger) and linPFOS (weaker); 0.44 (PFOA), 0.66 (PFNA), 0.75 (PFDA), 0.70 (PFUnDA), 0.72 (linPFHxS), and 0.53 (linPFOS). These correlations were, as also in the case of the Spearman correlation analyses (Table 4), all statistically significant ($p \leq 0.05$).

*Table 5. Children. Spearman´s correlation coefficients between intra-individual concentrations of PFASs at different sampling occasions after repeated sampling of participants for 12 years. Number of participants within parenthesis in the PFOA columns. Coefficients in **bold** r>0.7.*

	8 years	12 years
PFOA start (3 months old)		-0.137 (31)
4 years	**0.717*** (34)	0.587* (14)
8 years		**0.725*** (36)
PFNA start		0.134
4 years	0.491*	0.393
8 years		0.525*
PFDA start		0.142
4 years	0.398*	0.276
8 years		0.604*
PFUnDA start		0.168
4 years	0.349*	0.502
PFUnDA 8 years		0.474*
brPFHxS start		0.346
4 years	0.539*	0.517
8 years		0.442*
linPFHxS start		0.345
4 years	**0.822***	0.644*
8 years		**0.883***
brPFOS start		0.092
4 years	0.477*	**0.802***
8 years		0.616*
linPFOS start		0.044
4 years	0.567*	0.560
8 years		**0.805***

*$p \leq 0.05$

Among the children, correlations between concentrations measured during infancy (age: 3 months) and 12 years later were not statistically significant, whereas correlations between the 4-, 8- and 12-year samplings in most cases were statistically significant (Table

5). The non-significant correlations between concentrations at the 3-month and 12-year samplings may be due to changes in sources of cumulative exposure from *in utero* transfer and breast-feeding during infancy to food and drinking water after the nursing period. The stronger correlations during the childhood period from 4 to 12 years of age suggests that the influence of *in utero* and breast milk exposure had weakened in relation to other exposure sources during childhood, and that the individual exposure levels were relatively stable during this childhood period.

Table 6. Cross-sectional temporal trends of PFASs in maternal (1996-2011, N=128) serum, sampled 3 weeks after delivery, and serum of their first-born 12-year-old child (2008-2019, N=88).

PFAS	% change (mean (SE))	
	Mothers (1996-2011)	Children (2008-2019)
PFOA	-4.0 (0.64)*	-7.9 (0.92)
PFNA	3.5 (0.76)	-3.8 (1.7)
PFDA	4.5 (0.94)	-4.4 (1.3)
PFUnDA	11 (1.3)	ns
br-PFHxS	9.2 (1.6)	ns
lin-PFHxS	7.5 (1.1)	ns
br-PFOS	-9.0 (0.71)	-8.6 (1.3)
lin-PFOS	-7.8 (0.67)	-5.8 (1.5)

ns=no statistically significant trend (p>0.05)

Cross-sectional temporal trends

In cross-sectional studies, temporal trends of serum PFAS concentrations are generally a net result of cumulative PFAS exposure and cumulative elimination before the cross-sectional sampling. Since new participants are sampled at each sampling time-point, the observed temporal trend is influenced by a birth cohort effect on cumulative exposure. As an example, POPUP mothers (average age ≈30 years) sampled year 1996, 3 weeks after delivery of their first child, were born around 1966, whereas 30-year-old mothers sampled in 2011 were born 15 years later around 1981. It is not likely that the elimination rates (half-lives) of the studied PFASs changed significantly in the sampled age group of primiparous women during study period. Consequently, differences in PFAS concentrations among mothers from the two sampling occasions was mainly due differences in cumulative exposure. As an example, a lower average PFAS concentration among mothers sampled 2011 than among mothers

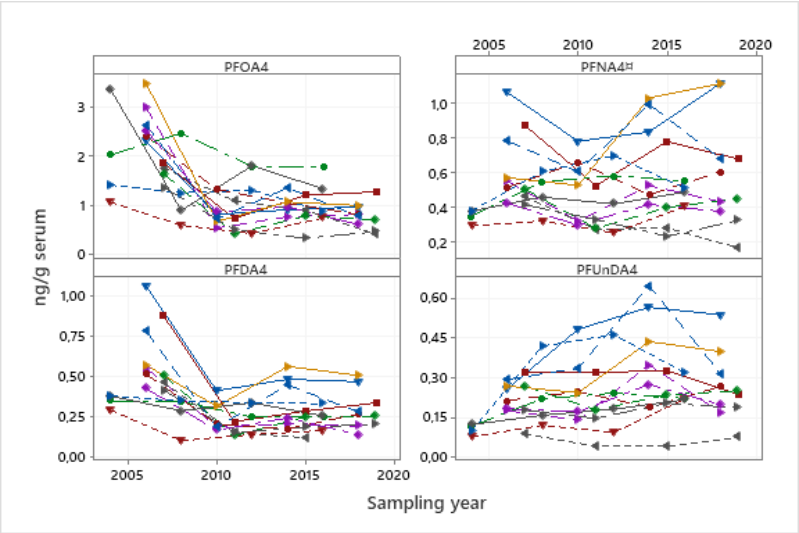
sampled year 1996 would mainly be a result of a decreased cumulative exposure of first-time mothers during the period 1981-2011 than during 1966-1996. In line with the results of the previous cross sectional study on POPUP mothers 1996-2010 [17], PFOA concentrations decreased on average about 4% per year in the present study, and lin- and brPFOS about 8-9% per year (Table 6). Increasing temporal trends (3.5%-11%) were observed for PFNA, PFDA, PFUnDA and lin- and br-PFHxS, as also observed in the previous cross-sectional study [17].

The observed decreasing trends of PFOS and PFOA were mainly due to phase-out of production and use of these PFASs and related compounds by the main manufacturers in North America and Europe the 2000s [18]. This has led to decreased exposure from food [19], and most likely also from some other possible exposure sources [18]. The slower decrease for PFOA was due to a slower phase-out than of PFOS. For the PFCAs with 8 or more carbons in the fluorinated carbon chain, the increasing trends most likely illustrates a much later phase-out of production of these and related PFASs than of PFOS and PFOA [18]. The increase in PFHxS isomer concentrations, observed both in the present study and in Glynn et al. [17], was mainly due to exposure from PFHxS-contaminated drinking water in Uppsala [4], which was mitigated in 2012, a year later than the final sampling in the cross-sectional trend (Table 6).

Among the 12-year-old children cross-sectional sampled 2008-2019 (Table 6), similar decreasing cross-sectional trends of PFOS and PFOA were observed as among mothers (1996-2011) and in an extended cross-sectional study on POPUP children 2008-2019 [13, 20]. This suggests that the decrease in human exposure to these PFASs continued after 2011 in Uppsala, as also have been shown in extended temporal trend studies of POPUP mothers 1996-2019 [12, 13]. In the children, PFNA and PFDA showed declining trends 2008-2019, which is in line with the results of the extended trend study on mothers, where declines were suggested from around 2005, and in children [13, 20]. The non-significant trend of PFUnDA and PFHxS among the 12-year-old children were most likely due to a combination of a relatively late change from increasing to decreasing cumulative exposure during the study

period (around 2010) in comparison to PFNA and PFDA and a low statistical power to detect temporal trends (N=88).

A)



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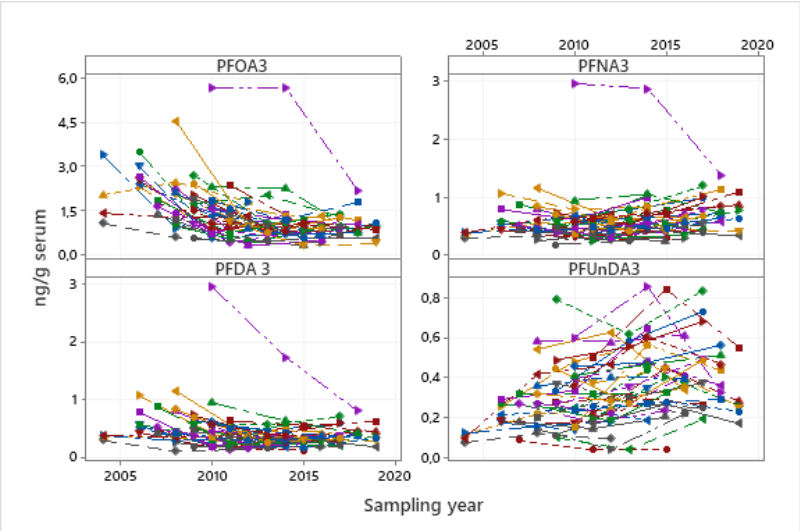


*Figure 1. Serum concentrations of PFCAs in mothers from the POPUP cohort with **A)** 4 measurements starting 3 weeks after delivery of their 1st child and ending 12 years later (N=13), and **B)** mothers with 3 repeated samplings during the first 8 years after delivery (N=44).*

Intra-individual trends

As mentioned above, in the cross-sectional temporal trend POPUP study, where new primiparous mothers are recruited at each sampling and sampling always takes place 3 weeks after delivery, temporal changes in serum PFAS concentrations were mainly due to birth cohort-dependent changes in cumulative exposure before sampling. In intra-individual temporal trend studies, however, individual changes in serum PFAS concentrations can both be due to changes in cumulative exposure and cumulative elimination during the study period. In this case, cumulative elimination can for instance be influenced age-dependent physiological changes in elimination.

Mothers - PFCAs

Plots of repeated measurement data for PFCAs, including the 13 mothers with data from all 4 sampling occasions (12 years) and the 44 mothers with 3 repeated samplings (8 years), show that intra-individual trends clearly differed between participants (Fig. 1). However, there was a tendency of an overall decline of PFOA and PFDA during the 8-12 years of sampling. Less consistent changes were suggested for PFNA and PFUnDA (Fig. 1).

Paired t-test analyses showed, on average, decreased individual PFOA concentrations for mothers with only one measurement 12 years after the first sampling 1996-1999, whereas PFNA, PFDA and PFUnDA concentrations increased (Fig. 2). The continuously decreased overall human exposure to PFOA in Sweden during the last 2 decades, as shown in the cross-sectional POPUP temporal trends [12, 13], most probably contributed to the average age-dependent intra-individual decline in PFOA concentrations among POPUP mothers. The concurrently increasing PFNA, PFDA and PFUnDA exposure in Sweden up to the middle/end of the 2000s [18] may in part explain the increased intra-individual concentrations during the 12-year follow-up with the first samples taken 1996-1999.

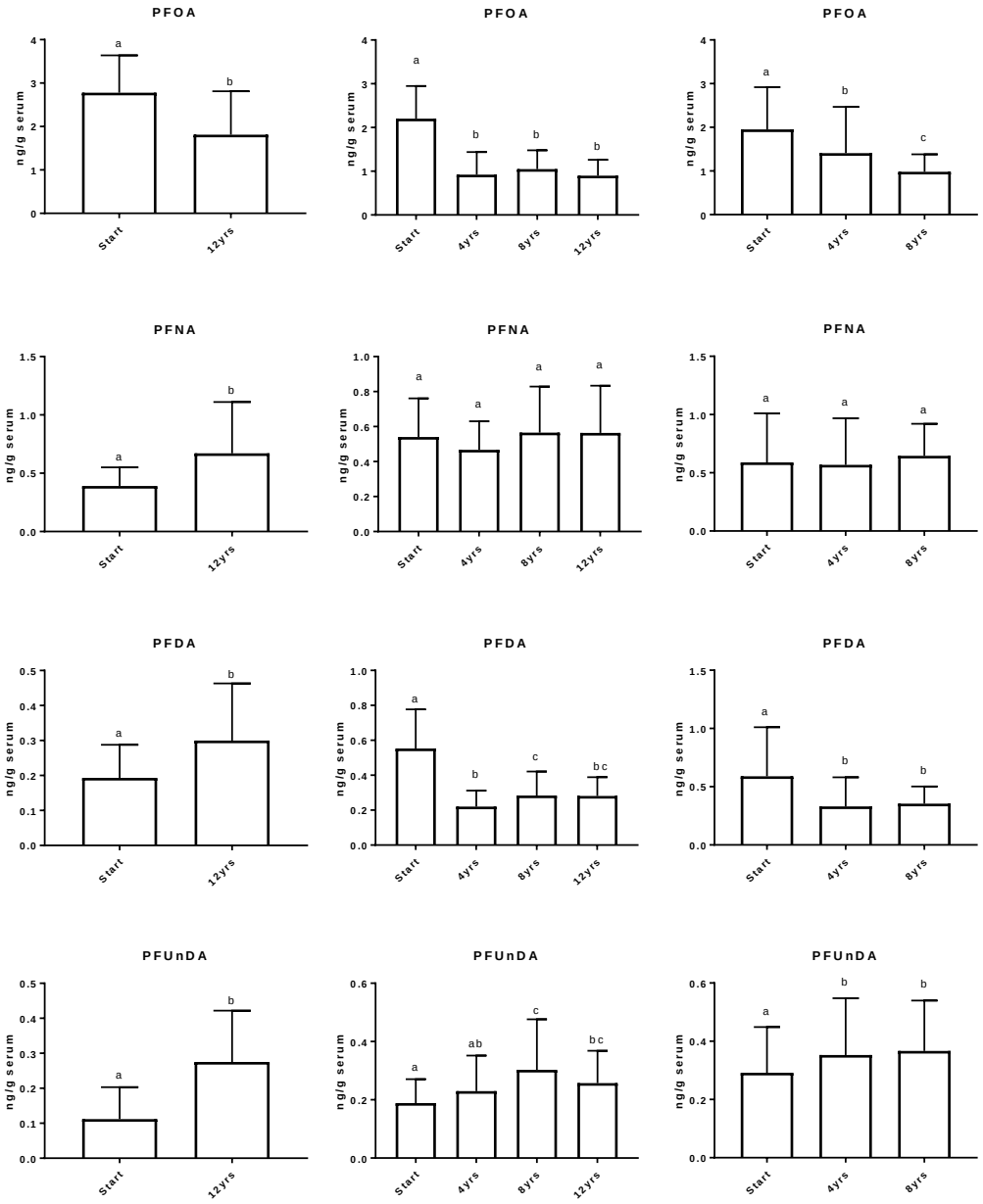


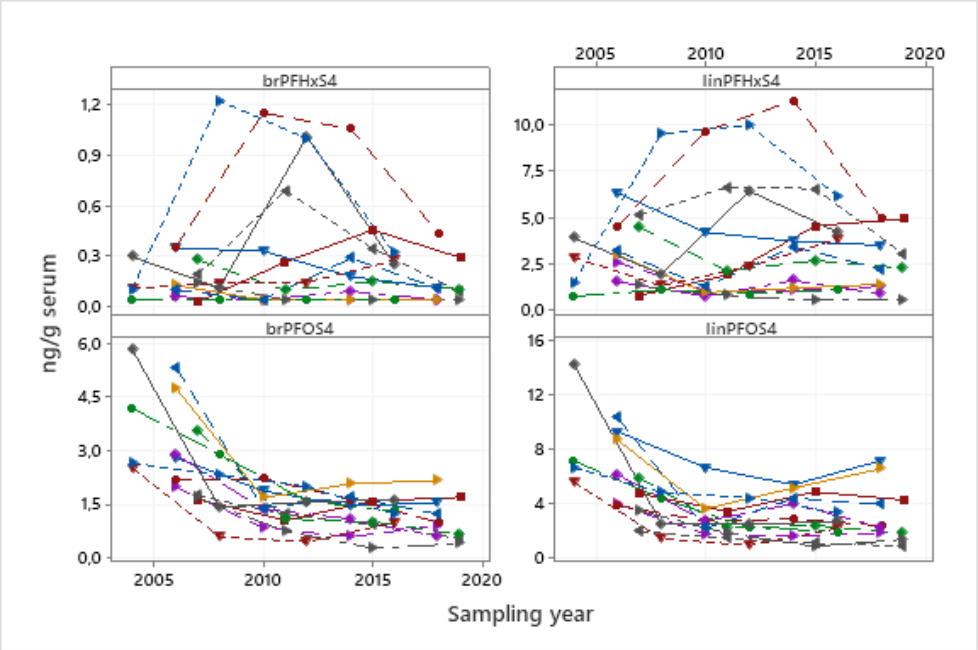
Figure 2. Paired t-test of intra-individual differences in maternal serum PFCA concentrations during 12 years of repeated measurements. Some mothers were only followed up 12 years after the first sampling 3 weeks after delivery of their first child (1996-1999) (N=57). Results are also shown for mothers with 3 (N=44) and 4 (N=13) repeated measurements, starting 2004-2011 and 2004-2007, respectively. Different letters denotes statistically significant differences (p≤0.05).

Among mothers with 3-4 repeated measurements, the decreasing cumulative exposure to PFOA during the last decades most probably also contributed to the initial decline of PFOA during the first 4 years of follow-up (Fig. 2). Mothers with 4 repeated samples seemed to have reached steady-state after the first 4 years, i.e cumulative exposure=cumulative elimination, but among mothers with 3 measurements the decline continued after the first follow-up sampling (Fig. 2). This difference in intra-individual trend pattern of PFOA may be due to a considerably lower number of mothers with 4 repeated samples (N=13), i.e. low statistical power to detect changes in concentrations and results being more sensitive to chance findings. Nevertheless, collectively these results suggests that overall elimination was higher than exposure during the 12 years of follow-up, thus causing (on average) decreasing intra-individual trends.

For PFNA, PFDA and PFUnDA average intra-individual trends differed (Fig. 2). Similarly to what was observed for PFOA, PFDA, concentrations declined during the first 4 years of follow-up. In this case, however, concentrations seemed to stabilize after first 4 years of follow-up, suggesting that serum concentrations had reached an overall steady-state. For PFNA, intra-individual concentrations did not change significantly during the 12 years of follow-up, whereas PFUnDA concentrations seemed to increase during the first 4 years and thereafter reached a plateau (Fig. 2). Homologue-dependent differences in temporal changes of cumulative exposure could contribute to the observed differences, as illustrated in the cross-sectional POPUP study 1996-2019 [12, 13]. In this case the increase in temporal trend of serum PFDA ended around 2004, followed by PFNA (2007) and PFUnDA (2008).

The overall decreasing intra-individual trend of PFOA among POPUP mothers (Fig. 2) is in line with an annual 6% decrease in intra-individual PFOA concentrations among 75 women from the USA, 45-56 years of age at base-line, repeatedly sampled four times 1999-2011 [21]. Similarly to the POPUP women first sampled 1996-1999 and followed-up 12 years later, an increasing intra-individual PFNA trend (16% per year) was observed among the US women [21]. In this study increasing concentrations of PFDA and PFUnDA were also suggested, although many samples had concentrations below the limits of detection [21].

A)



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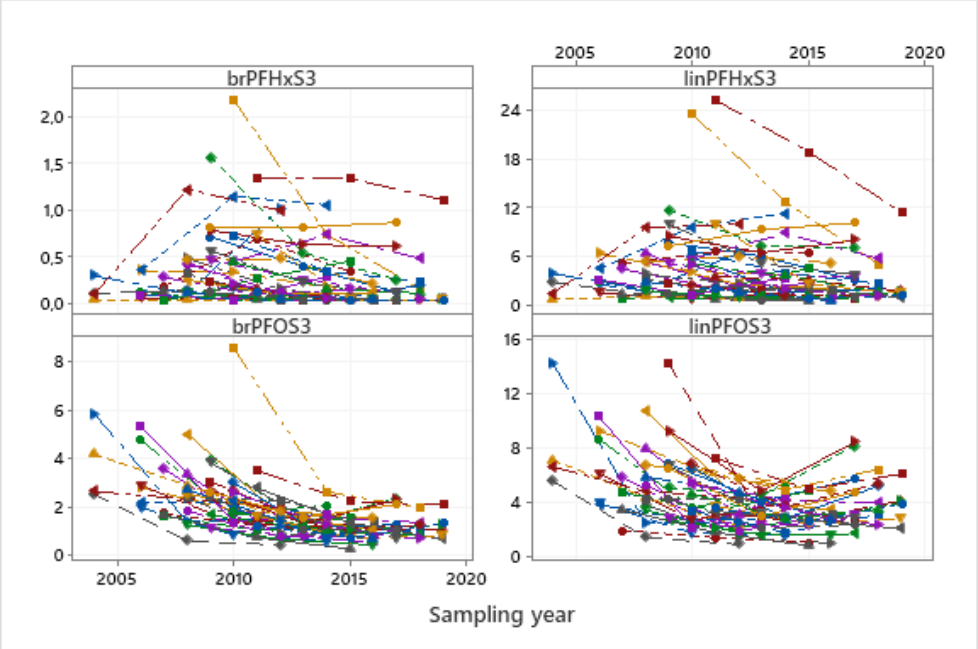

*Figure 3. Serum concentrations of PFSAs in mothers from the POPUP cohort with **A)** 4 measurements starting 3 weeks after delivery of their 1st child and ending 12 years later (N=14), and **B)** mothers with 3 repeated samplings during the first 8 years after delivery (N=44).*

Mothers - PFSAs

Plots of repeated measurement data for PFSAs, among the 14 mothers with 4 sampling occasions (12 years) and the 44 mothers with 3 repeated samplings (8 years) show that, similarly to PFCAs, intra-individual trends differed markedly between participants (Fig. 3). However, there was a tendency towards an initial decline of br- and linPFOS, with the decline levelling off towards the end of the follow-up periods, suggesting serum concentrations reached steady-state. For some participants the concentration seemed to increase during the last 4 years of follow-up (Fig. 3). Less consistent changes were observed for br- and linPFHxS, with some mothers showing large variations with a peak in the middle of the study period around 2010 and then a decline. This peak coincided with the time period when PFHxS-contamination of Uppsala drinking water was remediated [12].

Paired t-test analyses showed, on average, increased individual br- and linPFHxS concentrations for mothers with only one measurement 12 years after the first sampling 1996-1999, whereas for br- and linPFOS the concentrations decreased (Fig. 4). The PFHxS-contamination of Uppsala drinking water was most likely the reason for the increased intra-individual PFHxS concentrations between 1996-1999 (first sampling) and 2008-2010 (12-year follow-up [12]. The effect of mitigation of the drinking water contamination in 2012 is illustrated among the mothers with 4 repeated sample, by the tendency of an increase in average intra-individual PFHxS concentrations during the first 8 years and thereafter a decrease (Fig. 4). However, there were large variations in intra-individual trends, as illustrated in Fig. 3, and large standard errors of the PFHxS concentrations at the different sampling occasions (Fig. 4). Thi is likely due to the significant differences in PFHxS-contamination of drinking water in different drinking water districts in Uppsala, with mothers working in one district and living in another, and/or moving from one district to another during the follow-up [4]. Among mothers with 3 repeated samples, the intra-individual PFHxS concentrations on average declined with increasing age with an indicated slower decline after the first 4 years of follow-up (Fig. 4). Differences in time period for initial sampling, in relation to the mitigation of PFHxS-contamination of the drinking water, may at least partly explain the differences in intra-individual trends between mothers with 4 repeated

samples (starting 2004-2007) and those with 3 (2004-2011). The large difference in number of mothers sampled may also have contributed (4 repeated samples N=14, 3 repeated samples N=44).

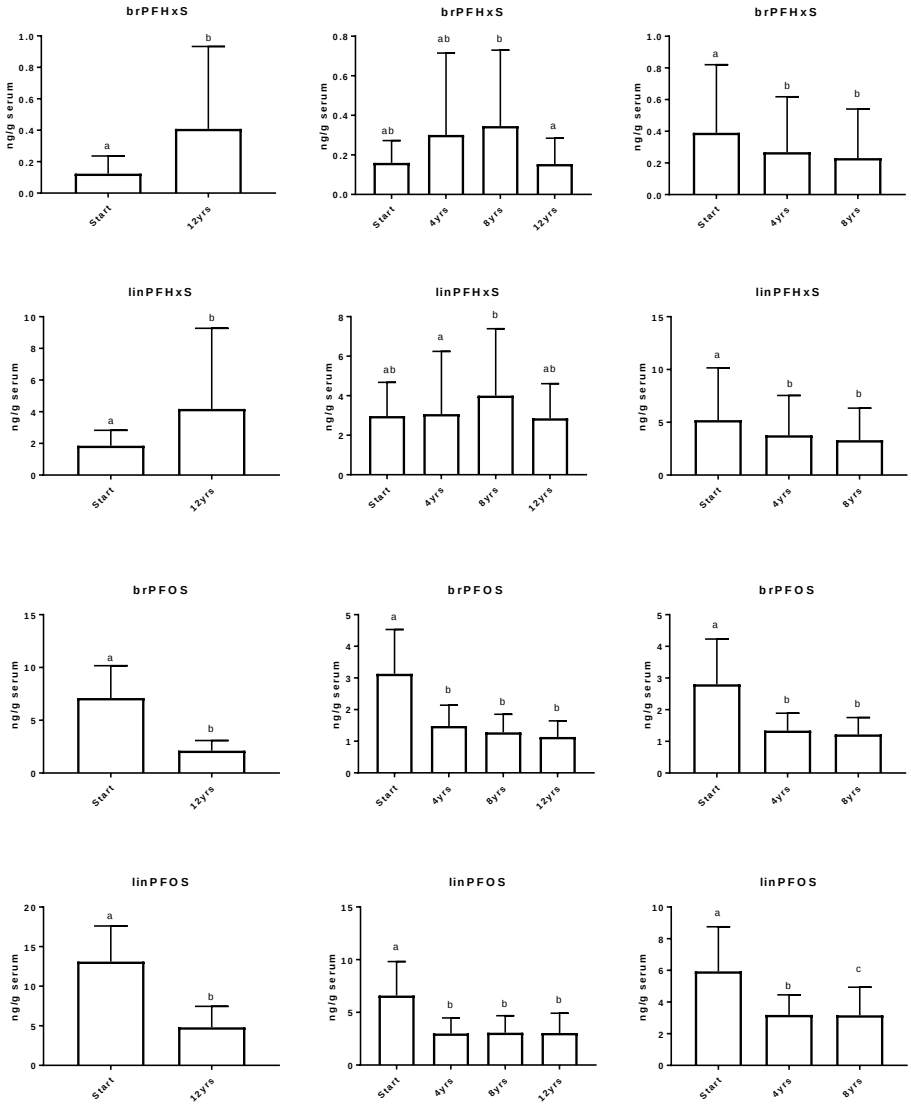

Figure 4. Paired t-test of intra-individual differences in maternal serum PFSA concentrations during 12 years of repeated measurements. Some mothers were only followed up 12 years after the first sampling 3 weeks after delivery of their first child (1996-1999) (N=57). Results are also shown for mothers with 3 (N=44) and 4 (N=13) repeated measurements, starting 2004-2011 and 2004-2007, respectively. Different letters denotes statistically significant differences (p≤0.05).

Intra-individual concentrations of branched and linear PFOS isomers decreased after the initial sampling of the mothers after delivery of their first child (Fig. 4). This is in line with the decrease in general PFOS exposure observed in the cross-sectional trends among the POPUP mothers 1996-2019 [12, 13]. However, after the first 4 years of decline, the intra-individual concentrations became more stable, on average reaching steady-state. For linPFOS, however, a slight increase in average concentrations was observed after 8 years of follow-up among mothers with 3 repeated samples. Steady state concentrations, or even a slight increase of linPFOS concentrations, at the end of the follow-up, does not fit with the observed decreasing cumulative exposure in the cross-sectional trend studies [12, 13]. Inclusion of more participants in the study are needed to enable firm conclusions about if this increase is due to chance or not.

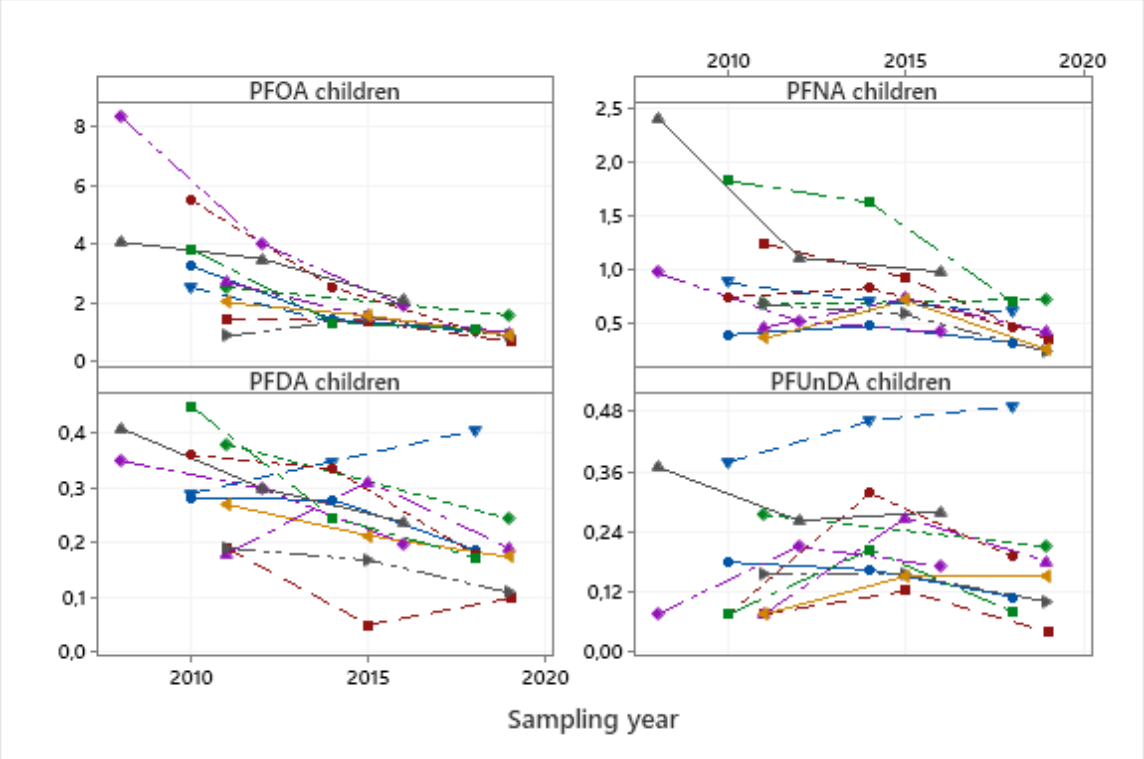

Figure 5. Serum concentrations of PFCAs in children from the POPUP cohort with 3 measurements starting at 4 years of age and ending at 12 years of age (N=11).

Children - PFCAs

Plots of repeated measurement data for PFCAs among the 11 children with data from 3 sampling occasions, starting at the age of 4 years and ending at 12 years of age, show a tendency of an overall decline of PFOA, PFNA and PFDA with age during the 8 years of sampling (Fig. 5). Less consistent changes were suggested for PFUnDA.

Paired t-test analyses showed, on average, age-dependent decreases in individual PFOA concentrations in the children during the 12 years of follow-up (Fig. 6). Similar to observations among the mothers, the generally decreasing PFOA exposure in Sweden over recent decades most likely contributed to lower serum PFOA concentrations, as shown in the cross-sectional trend analyses (Table 6). Although age-dependent changes in elimination of PFASs cannot be excluded during infancy/childhood, a much higher cumulative PFOA exposure early in life, due to trans-placental transfer and breastfeeding, than during the rest of the childhood most likely also contributed to the decline [22]. Consequently, cumulative elimination became higher than cumulative exposure after the end of breastmilk exposure period resulting in decreased serum concentrations. Growth dilution during childhood, due to increased body distribution volumes of PFAS, may also have contributed. However, an earlier cross-sectional study on POPUP children showed non-significant or very weak associations between serum PFAS concentrations and body weight during infancy and childhood [23]. A continuous decrease in intra-individual PFOA concentrations after the end of the nursing period is supported by pharmacokinetic (PK) modelling of prenatal and postnatal PFOA exposure [22].

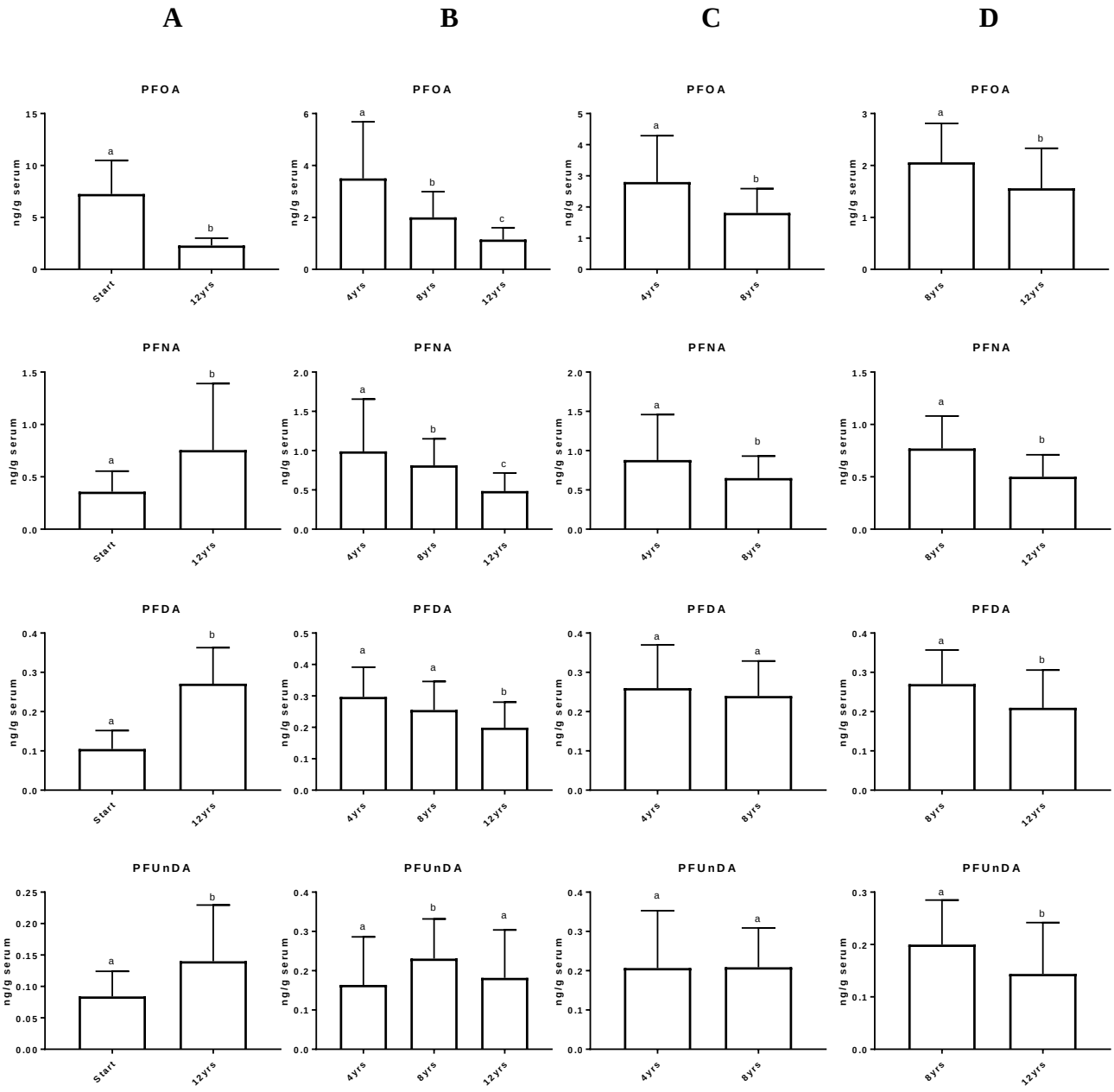

Figure 6. Paired t-test of intra-individual differences in serum PFCA concentrations in children during 12 years of repeated measurements. Some children were only followed up ***A)*** *12 years after the first sampling at 3 months of age 1996-1999 (N=31). Results are also shown for children with* ***B)*** *3 repeated measurements (N=11) starting at 4 years of age (2008-2011), and 2 repeated measurements between* ***C)*** *4-8 (N=34, 2008-2015) and* ***D)*** *8-12 years of age (N=34, 2008-2015). Different letters denotes statistically significant differences (p≤0.05).*

In contrast to PFOA, PFNA, PFDA and PFUnDA showed increased concentrations among children sampled 12 years after the first measurement at 3 months of age (1996-1999, Fig. 6). In this case, early life cumulative exposure through placental transfer and breastfeeding was apparently lower than subsequent cumulative exposure during childhood. In the cross-sectional study of POPUP children [23], maternal serum PFOA concentrations at delivery were positively associated with serum concentration in their children at 4-12 years of age, but not for PFNA and PFDA (PFUnDA not studied). This supports the hypothesis that early life exposure has a much larger impact on child serum PFOA concentrations during childhood than in the case of PFNA, PFDA and PFUnDA. The general increase in human exposure to these PFASs in Sweden for almost a decade after the first sampling of the children (1996-1999) most probably contributed to the observed increase in concentrations from infancy to 12 years of age [18].

In contrast, intra-individual PFNA, PFDA and PFUnDA concentrations among children sampled from 4 years of age, starting in 2008, did not increase up to 12 years of age (Fig. 6). Instead, PFNA and PFDA concentrations decreased with age, also to some extent for PFUnDA. These differences in intra-individual trends between children first sampled during infancy and those first sampled after infancy is most likely due to differences in sampling periods. The infant group sampling started in 1996-1999 had a follow-up period with many years of general increases in PFNA, PFDA and PFUnDA exposures (Table 6) [18]. The 4-year sampling started 2008, with years of generally decreasing exposures to PFNA and PFDA during the follow-up period, also to some extent for PFUnDA (Table 6) [18].

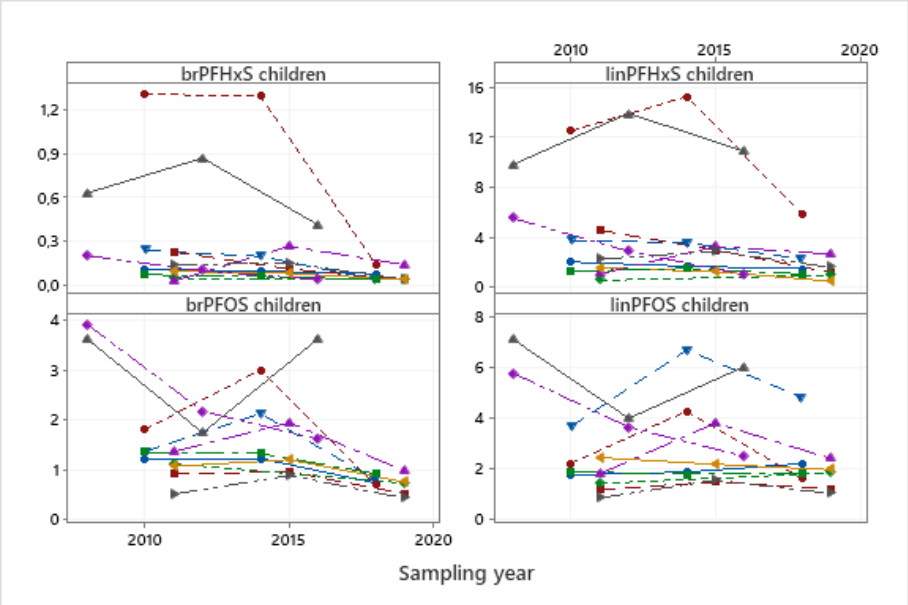

Figure 7. Serum concentrations of PFSAs in children from the POPUP cohort with 3 measurements starting at 4 years of age and ending at 12 years of age (N=11).

Children – PFSAs

Among children with 3 repeated samples between 4 and 12 years of age, diverging intra-individual trends of PFHxS and PFOS were found, with some children having high concentrations in comparison to the rest of the participants, especially of PFHxS (Fig. 7). The individuals with high PFHxS concentrations most likely were exposed to contaminated drinking water up to 2012 [23].

Paired t-tests showed do significant differences in serum PFHxS concentrations between samples taken at 3 months and 12 months of age (Fig. 8). Similarly to PFOA, PFOS concentrations decreased during the same period. This difference could at least partly be due to the larger contribution of drinking water exposure for PFHxS than for PFOS during childhood after the breastfeeding period [23]. For PFOS, with less influence of drinking water exposure, the decrease in serum concentrations was probably due to a combination of considerably lowered cumulative exposure after the breast-feeding period ended, and a general temporal decrease in general exposure, as shown in the cross-sectional trends (Table 6). PK modelling of prenatal and postnatal PFOS exposure predicted high cumulative PFOS

exposure early in life, resulting in decreased exposure and subsequent also decreased serum concentrations after cessation of breastfeeding.[22]

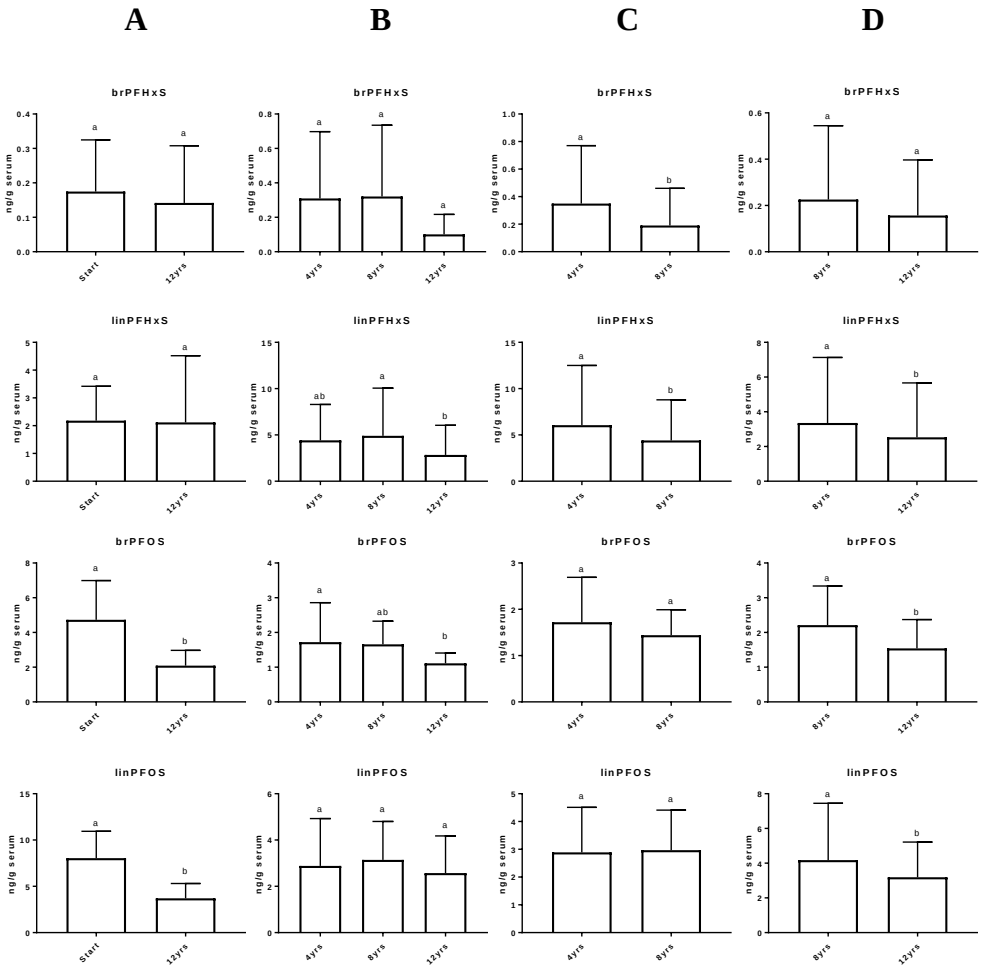

Figure 8. Paired t-test of intra-individual differences in serum PFSA concentrations in children during 12 years of repeated measurements. Some children were only followed up ***A)*** *12 years after the first sampling at 3 months of age 1996-1999 (N=31). Results are also shown for children with* ***B)*** *3 repeated measurements (N=11) starting 2008-2011 at 4 years of age, and 2 repeated measurements* ***C)*** *4-8 years starting 2008-2015 (N=34) and* ***D)*** *8-12 years of age (2008-2015, N=34). Different letters denotes statistically significant differences (p≤0.05).*

Among 4-12 years old children, intra-individual PFHxS and PFOS concentrations appeared to decrease towards the end of the follow up period. The remediation of the drinking water contamination 2012, and the general decrease in exposure from other sources (Table 6) most certainly contributed to this observation [12].

Table 7. Intra-individual temporal trends of PFCAs and PFSAs among mothers with 3 repeated serum samples during 8 years of follow-up (N=44) after the initial sample 3 weeks after delivery of the first child (2004-2011).

PFAS	% change per unit change in covariate					
	Unadjusted year	Adjusted[a] year	Additional children	BMI (kg/m^2)	Nursing Months	Fish consumption g/d
3 measurements 8 years (N=44)						
PFOA	**-8.2 (0.99); <0.001**	-2.1 (2.3); 0.378	**-38 (14); 0.009**	-5.3 (2.9); 0.076	0.13 (0.69); 0.854	
PFNA	**1.6 (0.72); 0.026**	**4.6 (1.7); 0.008**	**-28 (11); 0.010**	-1.4 (2.2); 0.520	0.67 (0.51); 0.199	0.49 (0.51);0.523
PFDA	**-5.8 (0.96); <0.001**	0.95 (2.1): 0.661	**-42 (13); 0.002**	0.40 (2.8); 0.886	0.30 (0.65); 0.643	1.7 (0.98); 0.088
PFUnDA	**2.9 (0.92); 0.002**	3.0 (2.2); 0.190	-3.1 (14); 0.822	3.5 (2.9); 0.236	0.21 (0.68); 0.758	1.0 (1.0); 0.319
br-PFHxS	**-9.6 (2.0); <0.001**	-2.0 (4.8); 0.675	-44 (30); 0.141	0.41 (6.2); 0.948	-0.23 (1.4); 0.871	
lin-PFHxS	**-6.3 (1.3); <0.001**	0.07 (4.7); 0.712	--33 (19); 0.081	-2.6 (3.9); 0.505	-0.31 (0.88); 0.731	
br-PFOS	**-10 (0.94); <0.001**	-2.9 (2.1); 0.162	**-37 (13); 0.004**	-5.1 (2.7); 0.059	-0.25 (0.62); 0.693	0.55 (0.93); 0.559
lin-PFOS	**-6.6 (0.99); <0.001**	2.2 (2.1); 0.286	**-51 (13); <0.001**	**-5.8 (2.7); 0.038**	-0.041 (0.63); 0.948	-0.34 (0.95); 0.725

[a]Adjusted means (standard error); p-value **(bold)**, determined from GLM Repeated Measures analyses including year of sampling, number of additional children, BMI, months of nursing, and fish consumption (not PFOA and PFHxS) in the model.

Determinants of intra-individual trends

Mothers

As was the case in paired t-test analyses of the mothers with 3 repeated samplings during 8 years starting 2004-2011, the GLM Repeated Measures analyses showed a general declining un-adjusted intra-individual trend of PFOA, PFDA, brPFHxS, linPFHxS, brPFOS and linPFOS concentrations, on average 6-10% per year of age (Table 7). In contrast, for PFNA and PFUnDA the intra-individual concentrations increased on average with 2-3% per year of age. After adjustment for number of additional children during follow-up, BMI, months of nursing, and fish consumption (not PFOA and PFHxS), only PFNA showed a significant intra-individual temporal trend. Number of additional children born during the follow-up period was inversely related to maternal serum concentration during follow-up. The average

serum concentrations decreased 30-50% per additional child, except for PFUnDA, although some of the relations were not statistically significant. Consequently, in addition to an average decline in exposure, observed declining un-adjusted intra-individual trends of some of the PFCAs and PFSAs were explained by increased elimination in connection to additional pregnancies during follow-up.

Several other studies have reported inverse relations between serum/plasma concentrations of long-chain PFASs in women and parity, most likely due to loss of PFASs by placental transfer during the pregnancy, loss of blood during delivery, and transfer of PFASs to breast-milk during nursing [21, 24, 25]. In the present study, months of nursing was not associated with PFAS concentrations (Table 7), although nursing has been shown to be a significant route of elimination of long-chain PFASs in larger studies [26-28]. The small size of our study and the inclusion of parity and nursing in the same model may be reasons behind the non-significant nursing associations.

For adults, fish consumption is a significant source of exposure to PFNA, PFDA, PFUnDA and PFOS in Sweden [19, 26]. In the analyses of repeated sampling no significant associations with fish consumption were observed, most probably due to small changes in rate of fish consumption during the 8 year follow-up (Table 7). It could be hypothesized that an increase in BMI would result in an increased distribution volume of the PFASs, thus causing “dilution” of serum concentrations. However, the changes in BMI were generally small and the study most probably was too small to detect minor changes in serum concentrations due to BMI changes. The only exception was linPFOS, with significantly decreasing concentrations with increased BMI during the follow-up, in line with the “dilution” hypothesis. A similar trend was observed for PFOA and brPFOS although not statistically significant. These findings may however be due to chance due to the small size of the study (Table 7).

Conclusions

Differences in intra-individual trends of certain PFASs were observed between the participating mothers and may depend, among other things, on an increased elimination of PFASs linked to pregnancy, childbirth and breastfeeding during the follow up period. Despite these individual differences, more general intra-individual trend patterns were also observed, strongly suggesting that the intra-individual trends of the PFASs were influenced by the measures taken to limit PFAS production and use in recent decades. Phasing out of PFOA and PFOS during the 2000s has generally contributed to declining serum levels with increasing age in both mothers and children. Measures to limit PFNA, PFDA and PFUnDA production/use came later than for PFOS and PFOA, which instead resulted in a combination of increasing serum concentrations with increasing age at the beginning of the follow-up, starting 1996-1999, followed by stable concentrations or even decreases among participants with the follow-up initiated 2004-2015. The tendency of an age-dependent increase in PFHxS concentrations among mothers, and to some extent also in their children, were followed by reductions of concentrations, on average, after the pollution of Uppsala's drinking water was remediated in 2012.

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