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Levels of poly- and perfluoroalkyl substances (PFAS) in serum from children at 4, 8 and 12 years of age, in Uppsala, Sweden: results from year 2020-2021 and temporal trends for the time period 2008-2022

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Sammanfattning
Sedan 1996 har Livsmedelsverket årligen samlat in modersmjölk och blod från förstföderskor i Uppsala för analys av persistenta organiska miljöföroreningar (POP). Sedan 2008 har mor-barn-paren tillfrågats att vara med i en uppföljande studie när barnet uppnått 4, 8 och 12 års ålder. I följande rapport redovisas halterna av 32 poly- och perfluorerade alkylsubstanser (PFAS) i serum från 4-, 8- och 12-åriga barn provtagna under perioden 2020-2022. Resultaten visar att de flesta analyserade PFAS, 22 av 32 stycken, inte hade mätbara halter i serum. Bland de detekterbara PFAS hade PFOS, PFHxS och PFOA de högsta halterna följt av de långkedjiga karboxylsyrorna PFNA, PFDA och PFUnDA. För att studera tidstrender av PFAS slogs resultaten ihop med redan insamlad data från barn i samma studie från 2008-2019. Resultaten visade att medelhalterna har minskat med 6 % per år för PFOA, 4 % för PFNA, 4 % för PFDA och 3 % för PFOS under tidsperioden 2008-2022. För PFHxS och PFUnDA fanns ingen signifikant trend under samma period. Eftersom det är känt att Uppsalas dricksvatten varit kontaminerat av PFAS, studerades också tidstrenden för PFHxS efter att åtgärder för att minska halterna sattes in från 2012. Resultaten visade att barnens halter av PFHxS har sjunkit i medeltal 6 % per år, 2013-2022, vilket indikerar att åtgärderna har haft effekt och exponeringen har minskat. Skillnader i serumhalter mellan kön och ålder utvärderades också. De yngre barnen (4- och 8-åringar) visade inte på några skillnader i halter mellan pojkar och flickor. I 12-åringar hade däremot pojkarna högre serumhalter av PFOA, PFNA, PFDA, PFOS och PFAS4 jämfört med flickorna. I jämförelsen mellan åldersgrupper var PFOA-halterna högre i 4-åringarna jämfört med 8-åringarna och för PFDA och PFUnDA hade 8-åringarna högre halter jämfört med 12-åringarna.

Introduction

With funding from the Swedish Environmental Protection Agency, the Swedish Food Agency has made yearly measurements of persistent organic pollutants (POP) in human samples from primiparous women in Uppsala since 1996, in the study "POPUP" (Persistent Organic Pollutants in Uppsala Primiparas). Since 2008, these mothers and their first-born children have been recruited to a follow-up study at the age of 4, 8 and 12 years. The mothers and children provided blood samples and answered a questionnaire. The following report presents results of analyses of per- and polyfluorinated substances (PFAS) in serum samples from the children.

PFAS are a large group with more than 10,000 different compounds with varying properties. All PFAS are considered environmentally persistent, or degrade to persistent end products (e.g. perfluorinated alkyl acids (PFAAs)). The major routes of human exposure to PFAS are via food and drinking water, as well as transfer from the placenta and into breastmilk early in life (Efsa 2020; Gyllenhammar et al. 2018). In Uppsala, contamination of the drinking water has resulted in elevated PFAS serum levels in mothers and children in the POPUP study (Gyllenhammar et al. 2015, Gyllenhammar et al. 2019). Due to the phase-out of perfluorooctane sulfonic acid (PFOS) and perfluorooctanoic acid (PFOA) since the start of the 21st century, declining levels of these PFAS have been observed among first-time mothers from Uppsala (1996-2019), and in their first born children (2008-2019) in the POPUP study (Gyllenhammar et al. 2020; Hedvall Kallerman et al. 2020). Declining temporal trends were also observed for perfluoroheptanoic acid (PFHpA), perfluorononanoic acid (PFNA) and perfluorodecanoic acid (PFDA) in the children during the same time period, but not for the mothers. For perfluorohexane sulfonic acid (PFHxS), increasing levels were observed in the POPUP-mothers in the beginning of the study, with a change point around 2010-11, and decreasing trends after that (Gyllenhammar et al. 2020). No temporal trends were observed for PFHxS in the children 2008 to 2019 (Hedvall Kallerman et al. 2020). The delay in declining PFHxS concentrations is likely due to a local source of PFHxS, which contaminated the drinking water until 2012.

The following report presents results of PFAS analyses in individual serum samples from children at 4, 8 and 12 years of age sampled in 2020-2022 (according to agreement 215-21-003). Temporal trends for the period 2008-2019 have been published earlier (Gyllenhammar et

al. 2016, Hedvall Kallerman et al. 2020) and the new data were used to establish updated temporal trends for the period 2008-2022.

Material and methods

Recruitment and sampling

In the POPUP study, first-time mothers from the general population living in Uppsala County were recruited between 1996 and 2022 as described in Glynn et al. (2007) and Lignell et al. (2009). The mothers delivered at the Uppsala University hospital and all mothers, except of a few, were born in Sweden. In 2008, a follow-up study of the mothers and their first-born children was initiated. The parents answered a self-administered questionnaire and a nurse took blood samples from the children at home, when the child was at 4, 8 and 12 years of age (Gyllenhammar et al. 2019). Blood samples were taken using 9 ml Vacutainer® or Vacuette® serum tubes and after centrifugation serum was stored at -20°C. The study was approved by the local ethics committee in Uppsala, Sweden, and the parents of the participating children gave informed consent prior to the inclusion of the children in the study.

In the present report, PFAS concentrations in serum from children in the POPUP study were updated with data from 2020-2022, so that the entire time series spans from 2008-2022. Personal characteristics for the children participating from 2020-2022 are shown in Table 1.

Table 1. *Personal characteristics of the participating children 2020-2022.*

Age category	**Variable**		**n**	**Mean**	**±SD**	**Median**	**Range**
4	Age (year)		19	4.0	0.4	4.2	3.5 – 4.6
	Weight (kg)		19	17	2.2	16	14 – 22
	Length (cm)		19	103	4.7	104	95 – 110
	Variable		**n**	**%**			
	Sex	Female	10	53			
		Male	9	47			
Age category	**Variable**		**n**	**Mean**	**±SD**	**Median**	**Range**
8	Age (year)		16	8.1	0.4	8.1	7.4 – 8.8
	Weight (kg)		15	28	5.2	27	21 – 42
	Length (cm)		15	130	5.4	128	120 – 140
	Variable		**n**	**%**			
	Sex	Female	10	63			
		Male	6	38			
Age category	**Variable**		**n**	**Mean**	**±SD**	**Median**	**Range**
12	Age (year)		44	11.9	0.3	11.9	11.4 – 12.5
	Weight (kg)		41	43	8.3	42	31 – 80
	Length (cm)		43	155	7.2	154	142 – 173
	Variable		**n**	**%**			
	Sex	Female	21	48			
		Male	23	52			

PFAS analyses

PFAS (Table 2) were analyzed in 19 samples from 4-year-old children, 16 samples from 8-year-old children and 44 samples from 12-year-old children as described in Gyllenhammar et al. (2015). In short, 0.5 g serum was spiked with internal standards and extracted with acetonitrile in an ultrasonicating bath. The concentrated extract underwent dispersive clean-up with graphitized carbon. Aqueous ammonium acetate and volumetric standards were added before instrument analysis on a Thermo Dionex Ultima 3000+ ultra performance liquid chromatography system (UPLC) coupled to a Thermo TSQ Quantiva tandem mass spectrometer (MS/MS) operated in negative electrospray ionization, multiple reaction monitoring mode. The instrumental method is described in detail in Miaz et al. (2020). Quantification was performed by isotope dilution using a 7-point calibration curve (linear, 1/x weighting) which was run before and after samples. For most targets, exactly matched isotopically labelled internal standards were available. For PFBS, PFTriDA, PFTeDA, and PFPeDA, a structurally similar internal standard was used (Table 2). For PFHxS and PFOS, branched and linear isomers were quantified separately. The limits of quantification (LOQ) for the different PFAS can be seen in Table 3, together with the LOQs for the previous years of analyses.

Table 2. *PFAS included in the study.*

Compound	Abbreviation	IS
Perfluorohexanoate	PFHxA	M4PFHpA
Perfluoroheptanoate	PFHpA	M4PFHpA
Perfluorooctanoate	PFOA	M4PFOA
Perfluorononanoate	PFNA	M5PFNA
Perfluorodecanoate	PFDA	M2PFDA
Perfluoroundecanoate	PFUnDA	M2PFUnDA
Perfluorododecanoate	PFDoDA	M2PFDoDA
Perfluorotridecanoate	PFTriDA	M2PFDoDA
Perfluorotetradecanoate	PFTeDA	M2PFDoDA
Perfluoropentadecanoate	PFPeDA	M2PFDoDA
Perfluorobutanesulfonate	PFBS	18O2-PFHxS
Perfluoropentanesulfonate	PFPeS	18O2-PFHxS
Perfluorohexanesulfonate lin.	PFHxS	18O2-PFHxS
Perfluorohexanesulfonate br.	PFHxS-br	18O2-PFHxS
Perfluoroheptanesulfonate	PFHpS	M4PFOS
Perfluorooctanesulfonate lin.	PFOS	M4PFOS
Perfluorooctanesulfonate br.	PFOS-br	M4PFOS
Perfluorononanesulfonate	PFNS	M4PFOS
Perfluorodecanesulfonate	PFDS	M4PFOS
Dodecafluoro-3H-4,8,-dioxanonanoate	ADONA	M4PFOA
Potassium 9-chlorohexadecafluoro-3-oxanonanoe-1-sulfonate	9Cl-PF3ONS	M2PFDA
Potassium 11-chloroeicosafluoro-3-oxaundecane-1-sulfonate	11Cl-PF3OUdS	M2PFDA
3:3 Fluorotelomer carboxylic acid	3:3 FTA (FPrPA)	M2PFHxA
5:3 Fluorotelomer carboxylic acid	5:3 FTA (FPePA)	M4PFOA
7:3 Fluorotelomer carboxylic acid	7:3 FTA (FHpPA)	M2PFDA
4:2 Fluorotelomer sulfonate	4:2 FTS	M2 6:2 FTS
6:2 Fluorotelomer sulfonate	6:2 FTS	M2 6:2 FTS
8:2 Fluorotelomer sulfonate	8:2 FTS	M2 6:2 FTS
Perfluorooctane sulfonamide	FOSA	M8FOSA
Methyl perfluorooctane sulfonamidoacetate lin.	MeFOSAA	d3-MeFOSAA
Methyl perfluorooctane sulfonamidoacetate br.	MeFOSAA-br	d3-MeFOSAA
Ethyl perfluorooctane sulfonamidoacetate lin.	EtFOSAA	d5-EtFOSAA
Ethyl perfluorooctane sulfonamidoacetate br.	EtFOSAA-br	d5-EtFOSAA
6:2 Fluorotelomer phosphate diester	6:2 diPAP	M4 6:2/6:2 diPAP
6:2/8:2 Fluorotelomer phosphate diester	6:2/8:2 diPAP	M4 8:2/8:2 diPAP
8:2 Fluorotelomer phosphate diester	8:2 diPAP	M4 8:2/8:2 diPAP

***Table 3**. Limit of quantification (LOQ) for the different analytical batches from year 2014, 2015, 2019 and 2022.*

PFAS	**Year of analysis**			
	2022	**2019**	**2015**	**2014**
PFBA		0.29		
PFPeA		0.08		
PFHxA	0.164	0.08	0.16	
PFHpA	0.164	0.08	0.08	0.03
PFOA	0.164	0.08	0.8	0.25
PFNA	0.164	0.08	0.08	0.2
PFDA	0.164	0.08	0.1	0.1
PFUnDA	0.164	0.08	0.1	0.15
PFDoDA	0.164	0.08	0.08	0.05
PFTriDA	0.193	0.08	0.02	0.15
PFTeDA	0.575	0.29	0.06	0.05
PFPeDA	0.575	0.29	0.1	0.01
PFHxDA		0.44		
PFOcDA		1.52		
PFBS	0.145	0.25	0.01	0.01
PFPeS	0.155	0.08		
PFHxS-lin	0.155	0.08	0.01	0.15
PFHxS-br	0.155	0.08	0.01	0.01
PFHpS	0.157	0.08		
PFOS-lin	0.157	0.14	0.01	0.25
PFOS-br	0.157	0.08	0.01	0.05
PFNS	0.157	0.08		
PFDS	0.173	0.08		0.01
PFUnDS		0.08		
FOSA	0.167	0.15 (*lin)* 0.08 (*br)*		0.01
FOSAA-lin		0.29		
FOSAA-br		0.29		
EtFOSAA-lin	0.579	0.29		
EtFOSAA-br	0.579	0.29		
MeFOSAA-lin	0.168	0.10		
MeFOSAA-br	0.168	0.08		
FPrPA	2.006	12.50		
FPePA	0.164	0.29		
FHpPA	0.165	0.29		
4_2FTS	0.577	0.08		
6_2FTS	0.458	0.92		
8_2FTS	0.573	0.08		
9Cl-PF3ONS	0.575	0.13		
11Cl-PF3OUdS	0.164	0.23		
ADONA	0.168	0.29		
6_2diPAP	0.169	0.30		
6_2_8_2diPAP	0.166	0.29		
8_2diPAP	0.165	0.29		
HFPO-DA		1.04		

A procedural blank and QC serum sample was included with every batch of samples. For targets observable in method blanks, LOQs were based on 3× standard deviation of the blanks. For those with no observable blank contamination, LOQs were calculated based on the lowest calibration point, showing at least a signal to noise ratio of 3. Further method validation parameters are provided in Glynn et al. (2012).

Statistical analyses

Statistical analyses were performed using the software package STATA version 15.1. When PFAS concentrations were below the LOQ, LOQ/√2 was used as an estimated value in the statistical analyses. Multiple linear regressions were used to analyze associations between ln-transformed PFAS concentrations in serum and sampling year for the whole period (2008-2022) including the covariates age, sex, weight and height at sampling. For PFHxS, the time period 2013-2022 was also evaluated because PFAS drinking water contamination was discovered in Uppsala in July 2012 and after that measures were taken to mitigate the levels. Temporal trends were analyzed for all children together (n=384), and for the three age groups 4, 8 and 12 years, separately (n=104 for 4-year-olds, n=106 for 8-year-olds, and n=174 for 12-year-olds). As a consequence of the logarithmic transformation, the associations between sampling year and PFAS concentrations are presented as percent change of concentrations per year, and not as change in absolute levels. Adjusted means of PFAS serum concentration in the categories sex and age were calculated and compared using ANCOVA and a linear model. The means and 95% confidence interval were adjusted for weight and height at sampling, sampling year, sex (for comparisons between age categories) and age (for comparisons between the sexes).

Results and discussion

PFAS levels in children

Among perfluoroalkyl carboxylic acids (PFCAs), the median level across all three age groups was highest for PFOA (0.95-1.59 ng/g serum) and declined in the order PFOA>PFNA>PFDA~PFUnDA during 2020-2022 (Table 4). All children had levels above the LOQ for the sulfonic acids, PFOS and PFHxS, with median levels ranging from 1.8 to 3.8 ng/g (Table 4). The median sum level of PFAS4 (i.e. sum of PFOA, PFNA, tot-PFHxS and tot-PFOS), ranged between 7.1 to 9.1 ng/g serum. Only a few individuals had levels above LOQ of PFHpA, PFPeS, PFHpS, MeFOSAA, FHpPA and 6:2 FTS, in total 2, 8, 7, 3, 1 and 1 out of 79 children, respectively (Table 4). In all children, 4, 8 and 12 years old, the levels of

PFHxA, PFDoDA, PFTriDA, PFTeDA, PFPeDA, PFBS, PFNS, PFDS, FOSA, lin-Et-FOSAA, br-Et-FOSAA, br-Me-FOSAA, FPrPA, FPePA, 4:2 FTS, 8:2 FTS, 9Cl-PF3ONS, 11Cl-PF3OUdS, ADONA, 6:2 diPAP, 8:2 diPAP and 6:2/8:2 diPAP were below the LOQ.

Table 4. *Concentrations of PFAS (ng/g) in serum samples from children at 4, 8, and 12 years of age from Uppsala, Sweden 2020-2022.*

Substance	Age	n	<LOQ (%)	Mean[a]	±SD[a]	Median[a]	Range
PFHpA	4	19	95				<LOQ – 0.31
	8	16	100				
	12	44	98				<LOQ – 0.19
PFOA	4	19	0	1.95	0.88	1.59	1.01 – 4.42
	8	16	0	0.99	0.36	0.95	0.42 – 1.60
	12	44	0	1.14	0.37	1.15	0.46 – 2.22
PFNA	4	19	11	0.55	0.33	0.45	<LOQ – 1.47
	8	16	25	0.47	0.33	0.45	<LOQ – 1.41
	12	44	16	0.40	0.22	0.34	<LOQ – 0.91
PFDA	4	19	53	0.20	0.12	0.12	<LOQ – 0.50
	8	16	63	0.18	0.11	0.12	<LOQ – 0.54
	12	44	59	0.19	0.13	0.12	<LOQ – 0.68
PFUnDA	4	19	58	0.16	0.07	0.12	<LOQ – 0.39
	8	16	38	0.19	0.07	0.19	<LOQ – 0.33
	12	44	75	0.15	0.08	0.12	<LOQ – 0.42
PFPeS	4	19	89				<LOQ – 0.24
	8	16	94				<LOQ – 0.21
	12	44	89				<LOQ – 0.19
lin-PFHxS	4	19	0	3.26	2.37	2.34	0.73 – 10.90
	8	16	0	2.38	1.87	1.67	0.45 – 6.16
	12	44	0	2.88	2.38	1.92	0.50 – 9.47
br-PFHxS	4	19	79				<LOQ – 1.08
	8	16	94				<LOQ – 0.18
	12	44	77				<LOQ – 0.46
tot-PFHxS[b]	4	19		3.44	2.56	2.45	0.84 – 11.98
	8	16		2.49	1.88	1.78	0.56 – 6.27
	12	44		3.05	2.49	2.03	0.61 - 10.27
PFHpS	4	19	89				<LOQ – 0.27
	8	16	100				
	12	44	89				<LOQ – 0.24
lin-PFOS	4	19	0	2.74	1.47	2.21	0.77 – 6.03
	8	16	0	1.94	0.79	1.96	0.63 – 3.61
	12	44	0	3.37	2.15	2.60	1.03 - 10.59
br-PFOS	4	19	0	1.33	0.68	1.22	0.42 – 3.05
	8	16	0	0.99	0.35	1.06	0.34 – 1.55
	12	44	0	1.56	0.91	1.20	0.69 – 4.77
tot-PFOS[b]	4	19		4.07	2.10	3.39	1.57 – 8.62
	8	16		2.93	1.11	2.98	0.97 – 5.16
	12	44		4.93	3.00	3.82	1.95 - 14.51

MeFOSAA-lin	4	19	95				<LOQ-0.45
	8	16	100				
	12	44	95				<LOQ – 0.36
FHpPA	4	19	100				
	8	16	94				<LOQ – 0.17
	12	44	100				
6:2 FTS	4	19	100				
	8	16	94				<LOQ – 4.47
	12	44	100				
PFAS4[c]	4	19		10.0	4.40	8.40	4.44 – 23.47
	8	16		6.89	2.77	7.07	2.78 – 10.92
	12	44		9.51	4.16	9.05	3.45 – 20.04

[a]Levels below LOQ were replaced with LOQ/√2.
[b]Sum of linear (lin) and branched (br) isomers. Levels below LOQ were replaced with LOQ/√2.
[c]Sum of PFOA, PFNA, tot-PFHxS and tot-PFOS.

PFAS levels for the whole time period, 2008-2022, are shown in Figure 1 for the three age groups. Similar to the present study, the highest median level was seen for tot-PFOS in the Swedish study Riksmaten adolescents (3.3 ng/g in 11-12 year-olds sampled 2016-2017) (Livsmedelsverket 2020). Further, the highest levels in pooled serum samples from Australian children, sampled 2016-2017, in the age groups 1-4 years and 5-15 years, were observed for tot-PFOS, with mean levels within the same range (3.0-3.1 ng/mL) (Toms et al. 2019). Also, in a study including German children aged 3-17 years, sampled 2014-2017, the highest mean serum levels were observed for PFOS (2.49 ng/mL) (Duffek et al. 2020). In a Finish cohort-study, where the same children (born 2004-2005) were sampled at 1-, 6- and 10.5 years, the highest median levels were instead observed for PFOA, followed closely by PFOS (Koponen et al. 2018).

The tolerable weekly intake (TWI) published by EFSA in 2020 at 4.4 ng PFAS4/kg bodyweight is based on a study with serum levels in 1-year-old children and a reduced antibody response after vaccination (EFSA 2020). A critical serum level of PFAS4 in the children was calculated into 17.5 ng PFAS4/mL, corresponding to a reduced antibody response of 10%. The critical serum level was used to calculate a safe serum level for a mother, at 6.9 ng PFAS4/mL, which would not lead to an exceedance of 17.5 ng/mL in her child. The median PFAS4-levels in the POPUP-children sampled 2020-2022 (7.1-9.1 ng/g) were below the critical serum level for 1-year-olds but exceeding the critical serum level for mothers. In total, 5% of the 4-year-olds, none of the 8-year-olds and 7% of the 12-year-olds had levels above 17.5 ng/mL and 79%, 56% and 7% had levels above 6.9 ng/mL,

respectively. The corresponding numbers for all POPUP-children, from 2008 to 2022, were 22% of the 4-year-olds, 14% of the 8-year-olds and 11% of the 12-year-olds that exceeded the critical serum level of 17.5 ng/mL, and 79%, 76% and 77% exceeded 6.9 ng/mL.

In some parts of Uppsala, the drinking water has historically been contaminated with PFHxS, and to a lesser degree with PFOS, PFOA and PFBS (Gyllenhammar et al. 2015). The contamination was discovered in 2012, however as the PFAS4 are known to have long half-lives (EFSA 2020) and as the drinking water still contains PFAS (albeit at lower levels) (Livsmedelsverket 2021), the Uppsala children may still be affected by the contamination. Therefore, the levels of PFHxS, and possibly also PFOS and PFOA, are probably not comparable to children in Sweden with normal/lower background exposure.

Figure 1. *PFAS concentrations in serum from the POPUP children 2008-2022 (n=384).*

Temporal trends

Declining temporal trends of PFOA, PFNA, PFDA, PFOS and PFAS4 were observed in POPUP children during 2008-2022, ranging from 3.2 to 7.0% per year (Table 5). The trends were seen both when considering the whole cohort as well as when the children were separated into the different age groups. The exception was for PFOS, where the trend was non-significant for the 4-year-old children (Table 5). In the previous study of temporal trends (2008-2019), similar decreasing trends were shown for PFOA, PFNA, PFDA and PFOS (Hedvall Kallerman et al 2020). Declining concentrations have also been observed for PFOA and PFOS during the time period 2007-2017 in Australian children, in the age groups 0-4 years and 5-15 years (Toms et al. 2019).

PFUnDA and PFHxS did not show any statistically significant trends in the POPUP-children during the time period 2008 to 2022, which is in line with the results in the previous report (Hedvall Kallerman et al 2020). For PFHxS it is known that the previous PFAS contamination of the drinking water in Uppsala has contributed to elevated serum levels in mothers and children in the POPUP study (Gyllenhammar et al 2015, Gyllenhammar et al 2016). As the contamination was mitigated in 2012, it was expected that the serum levels would decrease after that. Consequently, additional temporal trend analyses were performed between the years 2013 to 2022. The results showed declining temporal trends in the whole cohort as well as for the 4-year-old children (Table 5) indicating that the measures taken to reduce the exposure were effective at reducing serum PFHxS levels.

PFHpA showed a declining trend for 2008-2019 but was excluded in this report because the majority of measurements were below the LOQ. For the time period 2008-2020, 62% of the levels were below the LOQ, and in the later years (2016-2022) 80-100% of the levels were below the LOQ. This might partly be explained by the increasing LOQ by time, from 0.03 to 0.08 ng/g 2014-2019, and further into 0.164 ng/g in the latest analysis (Table 3) together with a likely continuation of the declining trend, as seen in the POPUP-mothers (Miaz et al. 2020).

Table 5. *Percent change in concentrations of PFAS per year in serum from children in Uppsala 2008-2022, at 4, 8, and 12 years of age. Adjusted for exact age, weight, length and sex. Significant results are marked in bold.*

Compound	Age	n	Change/year (%) Mean	95 % CI	R^2	p	<LOQ/MDL (%)
PFOA	**4**	**104**	**-4.4**	**-2.4/-6.3**	**15**	**<0.001**	**0**
	8	**106**	**-7.0**	**-5.2/-8.8**	**43**	**<0.001**	**0.9**
	12	**174**	**-6.3**	**-5.3/-7.3**	**46**	**<0.001**	**0**
	All	**384**	**-5.8**	**-5.0/-6.6**	**38**	**<0.001**	**0.3**
PFNA	**4**	**104**	**-3.2**	**-0.5/-5.8**	**16**	**0.021**	**1.9**
	8	**106**	**-4.3**	**-1.8/-6.7**	**16**	**0.001**	**3.6**
	12	**174**	**-4.9**	**-3.1/-5.4**	**15**	**<0.001**	**4.8**
	All	**384**	**-4.3**	**-3.1/-5.4**	**14**	**<0.001**	**3.7**
PFDA	**4**	**104**	**-3.5**	**-1.2/-5.6**	**12**	**0.003**	**12**
	8	**106**	**-5.5**	**-3.4/-7.5**	**23**	**<0.001**	**12**
	12	**174**	**-3.5**	**-2.0/-4.9**	**13**	**<0.001**	**15**
	All	**384**	**-3.9**	**-2.9/-4.9**	**13**	**<0.001**	**14**
PFUnDA	4	104	-1.1	1.5/-3.6	4	0.42	29
	8	106	-0.2	2.3/-2.6	2	0.88	17
	12	174	-0.6	0.9/-2.0	-1	0.45	43
	All	384	-0.6	0.5/-1.7	2	0.27	32
PFHxS[a]	4	104	-4.1	0.2/-8.1	4	0.06	32[c]/0[d]
	8	106	-1.0	3.9/-5.6	8	0.69	37[c]/0[d]
	12	174	-0.6	2.8/-3.9	5	0.73	33[c]/0[d]
	All	384	0.0	2.2/-2.1	7	0.97	34[c]/0[d]
2013-2022[b]	**4**	**74**	**-7.9**	**-1.7/-13.7**	**7**	**0.016**	**-**
	8	70	0.8	10/-7.7	0	0.86	-
	12	73	-6.6	1.7/-14.2	2	0.12	-
	All	**217**	**-5.6**	**-1.4/-9.5**	**7**	**0.010**	**-**
PFOS[a]	4	104	-2.1	0.4/-4.6	6	0.11	0[c]/0[d]
	8	**106**	**-5.5**	**-3.3/.7.7**	**31**	**<0.001**	**0[c]/0[d]**
	12	**174**	**-2.9**	**-1.3/-4.4**	**11**	**<0.001**	**0[c]/0[d]**
	All	**384**	**-3.4**	**-2.3/-4.5**	**16**	**<0.001**	**0[c]/0[d]**
PFAS4	**4**	**104**	**-3.7**	**-1.3/-6.1**	**11**	**0.004**	**-**
	8	**106**	**-4.8**	**-2.3/-7.2**	**13**	**<0.001**	**-**
	12	**174**	**-3.2**	**-1.6/-4.7**	**10**	**<0.001**	**-**
	All	**384**	**-3.2**	**-2.1/-4.3**	**10**	**<0.001**	**-**

[a]Sum of branched and linear isomers
[b]Percent change in concentrations of PFHxS during 2013-2022
[c]Branched isomers
[d]Linear isomers

Differences in PFAS levels between genders and age

Adjusted mean serum levels of PFOA, PFNA, PFDA, PFOS and PFAS4 were significantly higher in 12-year-old boys compared to girls (Figure 2). Among 4- and 8-year-olds, no significant differences between the genders were observed. When considering the whole dataset, with all age groups put together, the serum levels of PFNA, PFOS and PFAS4 were significantly higher in boys compared to girls. Similarly, in a study involving German children (3-17 years), lower concentrations of PFOS, PFOA and PFHxS, were observed in girls (Duffek et al. 2020). In contrast, no difference between genders were observed in Finish children, aged 1-, 6- and 10.5 years, except for PFOA where 10.5-year-old boys had higher levels compared to girls (Koponen et al. 2018). For PFUnDA and PFHxS no gender differences were observed in the POPUP-children.

When comparing adjusted mean PFAS serum levels between the three age groups, 4-, 8-, and 12-year-olds, significantly higher levels of PFOA were observed in 4-year-olds compared to 8-year-olds (Figure 3). A similar pattern were observed in Australian children, with higher levels of PFOA in the youngest age group (0-4 years) compared to the next age group (5-15 years) (Toms et al. 2019). In a Finish cohort, significant decreases in serum concentrations of PFOA, as well as PFOS, PFNA and PFHxS, were observed with increasing age in children sampled at 1, 6 and 10.5 years of age (Koponen et al. 2018). For PFDA and PFUnDA, the 8-year old POPUP-children had significantly higher serum levels than the 12-year-olds (Figure 3). No difference in serum levels of PFNA, PFHxS, PFOS and PFAS4 were seen between the age groups.

Differences in PFAS levels between gender and age could possibly be due to physiological differences and dilution of levels with an increase in body mass and blood volume during growth. In a study from the US it was shown that serum concentrations of PFOA, PFNA, PFHxS and PFOS are almost always higher in males compared to females from the age of 12 (Jain et al. 2022).

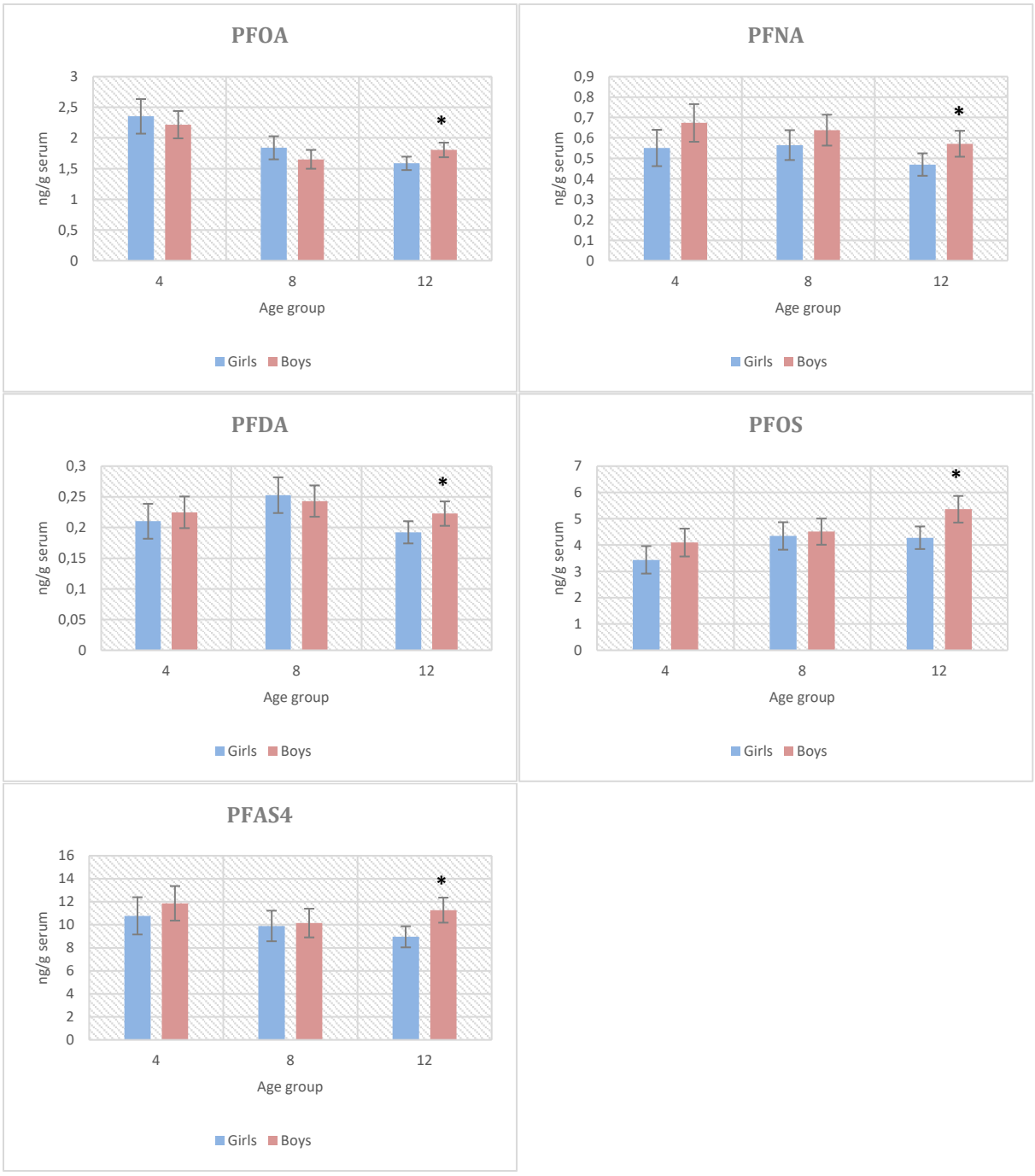

Figure 2. *PFAS concentrations in serum from the POPUP children 2008-2022 in the three different age groups, 4 (n=104), 8 (n=106) and 12 years old (n=174). Back-transformed means and 95% confidence intervals, calculated by ANCOVA, adjusted for exact age, weight, length and sampling year. *=significantly different from girls.*

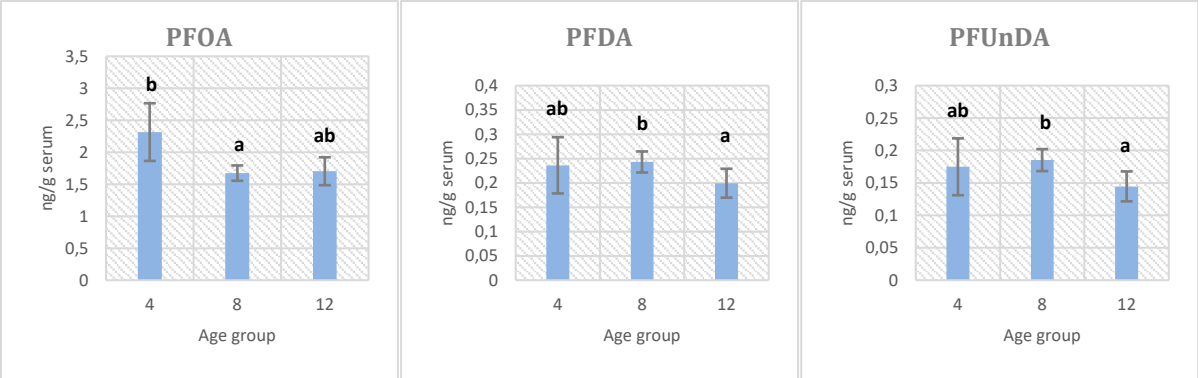

Figure 3. *PFAS concentrations in serum from the POPUP children 2008-2022 in the three different age groups, 4 (n=104), 8 (n=106) and 12 years old (n=174). Back-transformed means and 95% confidence intervals, calculated by ANCOVA, adjusted for sex, weight, length and sampling year. Bars with different letters (a and b) differ significantly from each other (p=0.05).*

Conclusion

The highest serum levels in the POPUP-children were observed for tot-PFOS followed by tot-PFHxS, PFOA and PFNA. The significant declining temporal trends observed for PFOA, PFNA, PFDA and PFOS suggests that there has been a decrease in exposure of Uppsala children to these PFAS during 2008 to 2022. The decreasing serum levels of PFHxS in the children after 2012 indicates that the measures to reduce the levels of PFAS in Uppsala's drinking water have been effective at reducing exposure. No gender differences were shown for PFAS serum levels in the younger children (4- and 8-year-olds) however, in the older children (12-year-olds), boys had higher levels of PFOA, PFNA, PFDA, PFOS and PFAS4 compared to girls. In the comparison between age groups, higher levels of PFOA were seen in 4-year-olds compared to 8-year-olds and of PFDA and PFUnDA in 8-year-olds compared to 12-year-olds.

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