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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 2023-2025 and temporal trends for the time period 2008-2025

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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 2023-2025 and temporal trends for the time period 2008-2025

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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 dessa mor–barn-par tillfrågats att delta i en uppföljningsstudie när barnen uppnått 4, 8 respektive 12 års ålder. I följande rapport redovisas halterna av 10 per- och polyfluorerade alkylsubstanser (PFAS) i serum från 4-, 8- och 12-åriga barn som provtagits under perioden 2023–2025.

På grund av byte av laboratorium för PFAS-analyser av serumprover från mammor och barn i POPUP-studien genomfördes en kalibreringsstudie. Tidigare analyser utfördes vid Stockholms universitet (mammor 1996–2022, barn 2008–2022), medan analyserna sedan 2023 utförs vid Lunds universitet. PFAS-koncentrationerna som uppmättes vid Lunds universitet var generellt jämförbara med dem från Stockholms universitet, även om viss variation förekom. Jämförelser av regressionsanalyser visade liknande samband, vilket tyder på att laboratoriebytet inte nämnvärt påverkar trendanalyserna och att någon justering av koncentrationerna därför inte bedömdes vara nödvändig.

Under perioden 2023–2025 uppmättes de högsta serumhalterna för tot-PFOS, med medianhalter mellan 2,7 och 3,3 ng/mL, följt av PFHxS och PFNA. För att studera tidstrender kombinerades de nya resultaten med tidigare insamlade data från barn i samma studie, vilket möjliggjorde analyser för tidsperioden 2008–2025. Analysen visade signifikanta minskningar i medelhalter för flera PFAS-föreningar: 6 % för PFOA, 5 % för PFNA, 3 % för PFDA, 2% för PFHxS, 3 % för tot-PFOS och 4 % för summan av PFAS4, när alla barn inkluderades. Mot bakgrund av den tidigare PFAS-föroreningen av Uppsalas dricksvatten analyserades även tidstrenden för PFHxS efter att åtgärder för att minska exponeringen infördes 2012. Resultaten visade att PFHxS-halterna minskat signifikant med 6 % per år under perioden 2013–2025. Resultaten tyder på att de genomförda åtgärderna har varit effektiva för att minska PFAS-exponeringen bland barn i Uppsala. Vid jämförelse mellan åldersgrupperna hade 4-åringar signifikant högre halter av PFOA än 8-åringar, medan inga åldersrelaterade skillnader kunde observeras för övriga PFAS.

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1. Introduction

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

PFAS comprise a large and diverse group of thousands of compounds with varying physicochemical 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 are via food and drinking water, as well as transplacental transfer and breastfeeding during early life (Efsa 2020; Gyllenhammar et al. 2018). In Uppsala, contamination of the drinking water has resulted in elevated serum PFAS levels among mothers and children participating in the POPUP-study (Gyllenhammar et al. 2015, 2019 and 2025).

Following the phase-out of perfluorooctane sulfonic acid (PFOS) and perfluorooctanoic acid (PFOA) in the early 21st century, declining levels of these PFAS have been observed among first-time mothers in Uppsala (1996-2022), and in their first-born children (2008-2022) within the POPUP-study (Lindfeldt et al. 2023, Gyllenhammar et al. 2025). In addition, declining temporal trends have been observed for perfluoroheptanoic acid (PFHpA), perfluorononanoic acid (PFNA) and perfluorodecanoic acid (PFDA) in the children during the same time period, which is coherent with the decline of PFNA and PFDA in the mothers (Gyllenhammar et al. 2025). 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 followed by declining trends thereafter (Gyllenhammar et al. 2020). No significant temporal trends in PFHxS concentrations were detected in children between 2008 and 2022. However, a significant decline was observed during the period 2013–2022 (Lindfeldt et al., 2023). The delayed decline in PFHxS concentrations is likely attributable to a local contamination source, affecting the drinking water supply, for which measures to reduce exposure were implemented starting in 2012.

The present report presents results of PFAS analyses in individual serum samples collected from children at 4, 8 and 12 years of age during 2023-2025 (according to agreement 215-24 005). Temporal trends for children during the period 2008-2022 have been reported previously (Gyllenhammar et al. 2016, Hedvall Kallerman et al. 2020, Lindfeldt et al. 2023), and the newly generated data in the present report were used to update the temporal trends for the period 2008-2025.

Due to the change in laboratory for the PFAS analyses of serum samples from mothers and children in the POPUP-study, a calibration study was performed. Previous PFAS analyses of serum samples from mothers (1996–2022) and children (2008–2022) were performed at Stockholm University, whereas from 2023 onwards the analyses for both mothers and children have been performed at Lund University.

2. Calibration study

2.1 Participants and sampling

In the present calibration study, serum samples from first-time mothers in the POPUP-study were used. Over 1100 first-time mothers from the general population living in Uppsala County were recruited between 1996 and 2025. Blood sampling was performed three weeks after delivery using 9 ml Vacutainer® or Vacuette® serum tubes and serum was stored at -20°C. The POPUP-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.

2.2 Sample selection, data preparation and chemical analyses

Twenty serum samples collected from the first-time mothers between 1997 and 2022 were randomly selected, including samples with both high and low concentrations. In addition, all 30 serum samples from mothers from 2022 were included.

Concentrations reported by Stockholm University were expressed in ng/g, whereas concentrations reported by Lund University were expressed in ng/mL. However, density-adjusted concentrations could be calculated based on the recorded sample weights, allowing direct comparison between the laboratories. Unfortunately, two sample weights were missing and 48 out of 50 possible concentrations could be density-adjusted.

PFAS concentrations were reanalysed in 2026 at Lund University and compared with results from Stockholm University. The method used at Lund University is described in the present report whereas the method used at Stockholm University is described in detail by Gyllenhammar and colleagues (Gyllenhammar et al. 2015 and 2025).

2.3 Statistical analyses

Statistical analyses were conducted using STATA version 19.0. Linear regression models were used to evaluate associations between ln-transformed PFAS serum concentrations analysed at Stockholm and Lund University. In total, six PFAS had sufficient numbers of concentrations above limit of quantification (LOQ) or limit of detection (LOD) to allow comparison: PFOA, PFNA, PFDA, PFUnDA, PFHxS and tot-PFOS. At Stockholm University, LOQ was used, and branched and linear PFHxS were analysed separately. At Lund University, these isomers were not analysed separately, and therefore their sum (PFHxS) was used for the comparison. Both laboratories analysed branched and linear PFOS; however, the sum (tot-PFOS) was used in the analyses.

2.4 Results and discussion

Median, minimum and maximum concentrations from the two laboratories together with intercepts, beta (β)-coefficients, and coefficients of determination (R^2) from the linear regressions are shown in Table 1.

Comparisons between linear regressions with (ng/mL) and without (ng/g) density adjusted concentrations from the previous laboratory and concentrations in ng/mL from the new laboratory showed no difference in associations (data not presented). Therefore, linear regressions were performed with log transformed concentrations in ng/g from the previous laboratory as the dependent variable and log transformed concentrations in ng/mL from the new laboratory as the independent variable to include all available data from the 50 individuals.
Results from the linear regressions are presented in Table 1 and Figure 1.
The calibration study showed generally comparable PFAS concentrations between the two laboratories, with regression coefficients ranging from 0.81 to 1.06 and R^2 values ranging from 0.35 to 0.79. Although some variability was observed between the laboratories, the results support the continued use of data from the new laboratory in long-term PFAS trend analyses.

Table 1. Comparisons of PFAS median concentrations (minimum and maximum in brackets) in serum samples from first-time mothers between previous and new laboratories. Intercepts, β– coefficients, and coefficients of determination (R^2) from the linear regressions are also presented.

PFAS	N	Stockholm University Median (min-max) ng/g	Lund University Median (min-max) ng/mL	Intercept	β - coefficient	R^2
PFOA	50	1.1 (0.24-5.3)	0.79 (0.29-5.1)	0.12	0.90	0.78
PFNA	50	0.48 (0.08-1.6)	0.34 (0.09-1.7)	0.18	0.96	0.54
PFDA	50	0.22 (0.07-1.1)	0.19 (0.05-0.95)	-0.29	0.81	0.35
PFUnDA	50	0.18 (0.01-0.99)	0.17 (0.05-0.58)	0.03	1.06	0.40
PFHxS[a]	50	1.8 (0.36-16)	1.5 (0.32-17)	0.27	0.84	0.79
PFOS[a]	50	3.8 (0.21-42)	3.4 (0.83-34)	0.22	0.94	0.60

[a]Sum of branched and linear isomers.

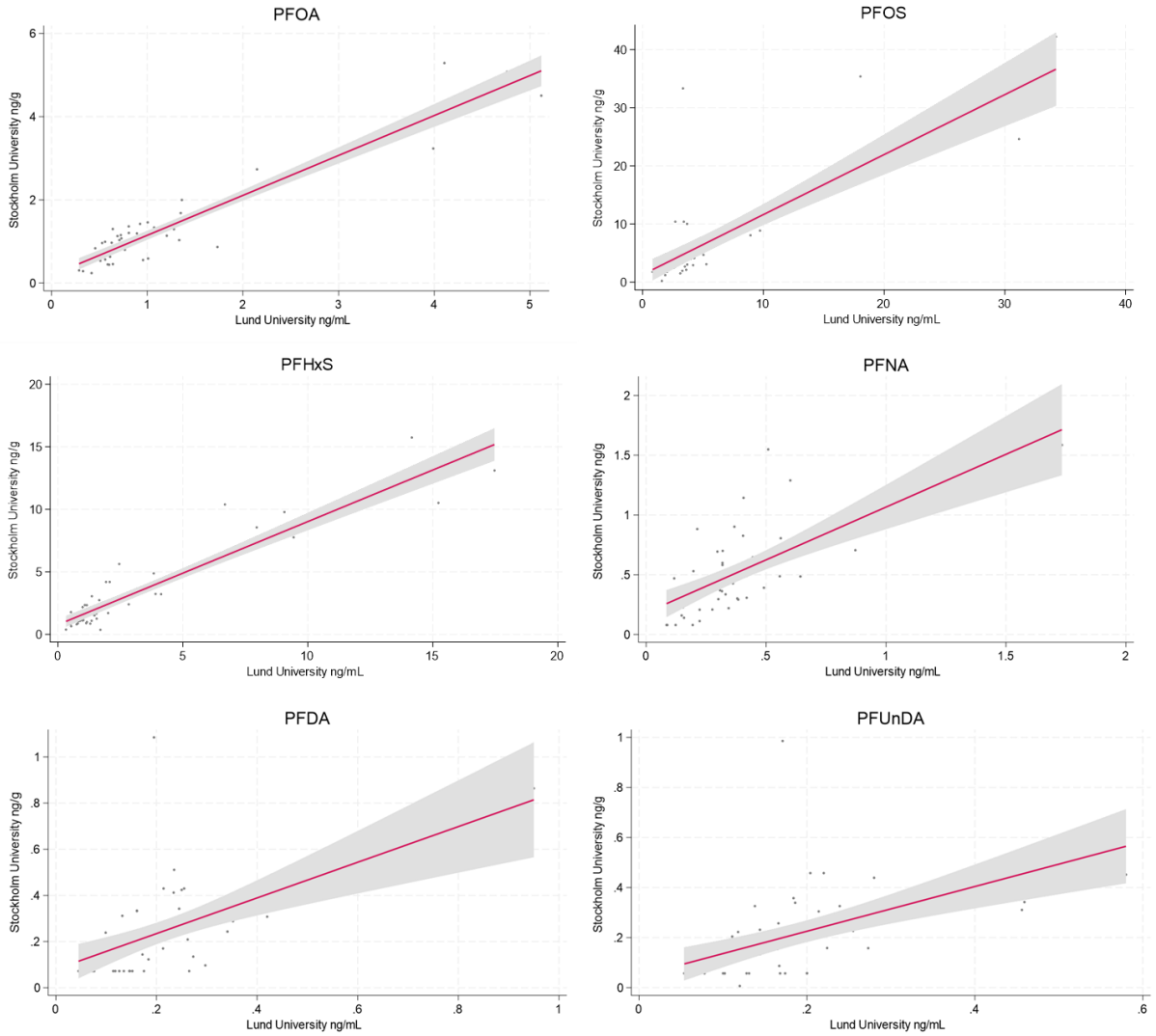

Figure 1. Associations between concentrations analysed in serum samples from first-time mothers in the POPUP-study at Stockholm University and Lund University. Grey areas represent the 95% confidence interval, and the red lines are the regression lines.

2.5 Conclusion

Overall, PFAS concentrations measured at Lund University were comparable to those measured at Stockholm University, although some variability between laboratories was observed. Comparisons of linear regression analyses using concentrations from the two laboratories showed similar associations, suggesting that the change in laboratory is unlikely to substantially affect the long-term trend analyses. Therefore, no adjustments of the concentrations were considered necessary.

3. Material and methods

3.1 Recruitment and sampling

In the POPUP-study, first-time mothers from the general population of Uppsala County were recruited between 1996 and 2025 as described in Glynn et al. (2007) and Lignell et al. (2009). All mothers delivered at Uppsala University hospital and the vast majority of mothers were born in Sweden. In 2008, a follow-up study including the mothers and their first-born children was initiated. At 4, 8 and 12 years of age, parents completed a self-administered questionnaire, and a study nurse collected blood samples from the children during home visits (Gyllenhammar et al. 2019). Blood samples were drawn into 9 mL Vacutainer® or Vacuette® serum tubes and serum was separated by centrifugation and stored at -20°C until analysis. The study was approved by the Regional Ethics Committee in Uppsala, Sweden, and written consent was obtained from the parents prior to the inclusion of the children in the study.

In the present report, serum PFAS levels in children from the POPUP-study were updated with data from 2023-2025, extending the time series to cover the period 2008-2025. Descriptive characteristics for the participating children enrolled between 2023-2025 are presented in Table 2.

Table 2. Descriptive characteristics of the participating children 2023-2025.

Age category	Variable		n	Mean	±SD	Median	Range
4	Age (year)		26	3.7	0.3	3.6	3.4 – 4.6
	Weight (kg)		26	17	1.9	16	13 – 21
	Height (cm)		26	101	3.6	100	95 – 110
	Variable		**n**	**%**			
	Sex	Female	14	54			
		Male	12	46			
Age category	**Variable**		**n**	**Mean**	**±SD**	**Median**	**Range**
8	Age (year)		23	7.8	0.3	7.8	7.4 – 8.3
	Weight (kg)		23	26	3.3	25	18 – 35

	Height (cm)		23	129	4.8	129	120 – 135
	Variable		**n**	**%**			
	Sex	Female	13	57			
		Male	10	43			
Age category	**Variable**		**n**	**Mean**	**±SD**	**Median**	**Range**
12	Age (year)		18	12.0	0.4	12.0	11.4 – 12.6
	Weight (kg)		18	43	12	40	32 – 84
	Height (cm)		18	155	7.3	154	143 – 173
	Variable		**n**	**%**			
	Sex	Female	9	50			
		Male	9	50			

3.2 PFAS analyses

In the present study, the samples were prepared using a method previously described by Norén et al. (Norén et al., 2021). An overview of the PFAS analytes and internal standards with abbreviations is given in Table 3. All native (MPFAC-MXA, and MP4HpA) and isotopically labeled standards (MPFAC-MXA and M4 PFHpA) were purchased from Wellington Laboratories (Guelph, Ontario, Canada) as diluted reference standards. For the calibration standards, a blank matrix, Fetal Bovine Serum (FBS, Gibco, Thermo Fisher scientific, Waltham, MA, USA) was used and prepared in the same way as the samples, Briefly, an aliquot of 0.1 mL of sample was prepared in a 96-well plate and de-conjugated using β-glucuronidase. Isotope-labelled internal standards of PFAS were added and proteins were precipitated with acetonitrile.

The quantitative analysis was conducted using triple quadrupole linear ion trap mass spectrometers equipped with TurboIonSpray sources (QTRAP 5500, AB Sciex, Framingham, MA, USA) coupled to a liquid chromatography system (UFLCXR, Shimadzu Corporation, Kyoto, Japan (LC/MS/MS). An aliquot of 4µl of the supernatant was analysed on the analytical column Acquity UPLC BEH C18 1.7 µm, 100*2.1mm (Waters, Wilmslow, UK). A C18 column (2.1 mm i.d. x 50 mm, Genesis Lightn; Hichrom, Reading, UK) was used before the injector to filter the mobile phases from contaminating PFAS. The mobile phases were A) 5mM ammonium acetate in water and B) methanol. The MS analyses were carried out using selected reaction monitoring (SRM). Two reference samples were prepared for quality control (QC1 and QC2) by pooling serum samples containing different concentrations of the biomarkers. The two QC samples, four chemical blanks (water), and calibration standards were included in each sample batch (96-well plate).

The concentrations of PFAS were determined by peak area ratios between analyte and IS. In the quantitative analysis of PFHxS, three transitions were evaluated, using the transitions m/z 399/80, 399/99, and 399/119. In the analysis of PFOS, the chromatography separated branched PFOS as one peak and separated from linear PFOS. The quantitative analysis of PFOS (linear and branched) was evaluated using the transitions m/z 499/99. The LOD, determined as the concentrations corresponding to three times the standard deviation of the responses in chemical blanks and, are shown in Table 4. The laboratory also participates in analysis G-EQUAS quality control program between analytical laboratories coordinated by University of Erlangen-Nuremberg, Germany. The analysis of PFHpA, PFOA, PFNA, PFDA, PFUnDA, PFBS, PFHxS, PFHpS, PFOS is approved by the Partnership for the Assessment of Risks from Chemicals (PARC).

Table 3. PFAS included in the study.

Compound	Abbreviation	Internal standard	Abbreviation
Perfluoroheptanoic acid	PFHpA	Perfluoro-n-[1,2,3,4-$^{13}C_4$] heptanoic acid	$^{13}C_4$-PFHpA
Perfluorooctanoic acid	PFOA	Perfluoro-n-[1,2,3,4-$^{13}C_4$] octanoic acid	$^{13}C_4$-PFOA
Perfluorononanoic acid	PFNA	Perfluoro-n-[1,2,3,4,5-$^{13}C_5$] nonanoic acid	$^{13}C_5$-PFNA
Perfluorodecanoic acid	PFDA	Perfluoro-n-[1,2-$^{13}C_2$] decanoic acid	$^{13}C_2$-PFDA
Perfluoroundecanoic acid	PFUnDA	Perfluoro-n-[1,2-$^{13}C_2$] undecanoic acid	$^{13}C_2$-PFUnDA
perfluorobutane sulfonate	PFBS	perfluoro-1-(2,3,4-$^{13}C_3$) butanesulfonate	$^{13}C_3$- PFBS
perfluoropentane sulfonate	PFPeS	Perfluoro-1-[$^{18}O_2$ hexanesulfonate	$^{18}O_2$-PFHxS
Perfluorohexanesulfonate	PFHxS	Perfluoro-1-[$^{18}O_2$] hexanesulfonate	$^{18}O_2$-PFHxS
perfluoroheptane sulfonate	PFHpS	Perfluoro-1-[$^{18}O_2$] hexanesulfonate	$^{18}O_2$-PFHxS
Perfluorooctanesulfonate-lin	PFOS	Perfluoro-1-[1,2,3,4-$^{13}C_4$] octanesulfonate	$^{13}C_4$-PFOS
Perfluorooctanesulfonate-br	PFOS	Perfluoro-1-[1,2,3,4-$^{13}C_4$] octanesulfonate	$^{13}C_4$-PFOS

Table 4. Limit of quantification (LOQ) or Limit of detection (LOD) (ng/mL) for the different analytical batches from year 2014, 2015, 2019, 2022 and 2026.

PFAS	Year of analysis				
	2026	2022	2019	2015	2014
	LOD	LOQ	LOQ	LOQ	LOQ
PFHpA	0.04	0.164	0.08	0.08	0.03
PFOA	0.06	0.164	0.08	0.8	0.25
PFNA	0.03	0.164	0.08	0.08	0.2
PFDA	0.1	0.164	0.08	0.1	0.1
PFUnDA	0.09	0.164	0.08	0.1	0.15
PFBS	0.08	0.145	0.25	0.01	0.01
PFPeS	0.03	0.155	0.08		
PFHxS	0.05	0.155[a]	0.08[a]	0.01[a]	0.15[a]
PFHpS	0.02	0.157	0.08	-	-
PFOS-linear	0.1	0.157	0.14	0.01	0.25
PFOS-branched	0.1	0.157	0.08	0.01	0.05

[a]The former laboratory quantified linear and branched PFHxS isomers separately but with the same LOQs, except for 2014 (branched PFHxS LOQ = 0.01 and linear PFHxS LOQ = 0.15).

3.3 Statistical analyses

Statistical analyses were conducted using STATA version 19.0. For the period 2008–2022, PFAS concentrations below LOQ were assigned a value of LOQ/√2 prior to analysis. From 2023 onwards, samples were analysed by a new laboratory and concentrations are reported relative to the LOD. As instrument-generated values were available for observations below the LOD during 2023–2025, these values were retained and used in the statistical analyses.

Multiple linear regression models were used to evaluate associations between ln-transformed PFAS serum concentrations and sampling year over the study period 2008–2025, adjusting for age, sex, weight, and height at sampling. For PFHxS, an additional analysis restricted to the period 2013–2025 was performed, as PFAS contamination of the drinking water supply in Uppsala was identified in July 2012 and mitigation measures were subsequently implemented. Temporal trends were analysed both for all children combined (n=452) and separately for the three age groups: 4 years (n=132), 8 years (n=129), and 12 years (n=191). The associations between age categories and PFAS concentrations were also evaluated using multiple linear regression models, adjusted for the same covariates (except for age) included in the temporal trend analyses. Because PFAS concentrations were ln-transformed prior to analysis, temporal trends are presented as the annual percentage change in concentration rather than as absolute changes in concentration.

4. Results and discussion

4.1 PFAS levels in children

Among perfluoroalkyl carboxylic acids (PFCAs), the median level across all three age groups was highest for PFOA (0.75-1.31 ng/mL serum) and declined in the order PFOA>PFNA>PFHpA>PFDA~PFUnDA during 2023-2025 (Table 5). Across all three age groups, some individuals had PFHpA and PFUnDA concentrations below the LOD, ranging from 4% to 30%. All children had levels above the LOD for the sulfonic acids; PFHxS and tot-PFOS, with median levels ranging from 1.7 to 3.3 ng/mL (Table 5). The proportion of individuals with PFBS and PFPeS concentrations below the LOD ranged from 4% to 89% and for PFHpS, concentrations below the LOD were observed only in the 12-year-old age group (17%). Median levels of PFPeS and PFHpS ranged from 0.04 to 0.10 ng/mL and for PFBS the levels were <LOD. The median sum level of PFAS4 (i.e. sum of PFOA, PFNA, PFHxS and tot-PFOS), ranged between 6.1 to 7.5 ng/mL.

Table 5. Concentrations of PFAS (ng/mL) in serum samples from children at 4, 8, and 12 years of age from Uppsala, Sweden 2023-2025.

Substance	Age	n	<LOD (%)	Mean	±SD	Median	Range
PFHpA	4	26	26	0.11	0.08	0.09	0.02[c] – 0.35
	8	23	30	0.08	0.06	0.08	0.009[c] – 0.28
	12	18	17	0.07	0.03	0.07	0.01[c] – 0.13
PFOA	4	26	0	1.47	0.72	1.31	0.37 – 3.37
	8	23	0	0.86	0.28	0.78	0.49 – 1.81
	12	18	0	0.75	0.23	0.75	0.40 – 1.34
PFNA	4	26	0	0.44	0.28	0.36	0.15 – 1.31
	8	23	0	0.36	0.13	0.35	0.13 – 0.74
	12	18	0	0.31	0.16	0.28	0.15 – 0.83
PFDA	4	26	0	0.22	0.08	0.21	0.10 – 0.39
	8	23	0	0.20	0.05	0.21	0.11 – 0.29
	12	18	6	0.19	0.09	0.17	0.09[c] – 0.41
PFUnDA	4	26	8	0.17	0.06	0.16	0.07[c] – 0.34
	8	23	4	0.19	0.08	0.18	0.09 – 0.39
	12	18	11	0.16	0.07	0.14	0.04[c] – 0.32
PFBS	4	26	58	0.07	0.04	0.06[c]	0.01[c] – 0.16
	8	23	74	0.06	0.03	0.05[c]	0.02[c] – 0.14
	12	18	89	0.06	0.02	0.05[c]	0.02[c] – 0.11
PFPeS	4	26	4	0.06	0.04	0.06	0.02[c] – 0.20
	8	23	13	0.06	0.04	0.06	0.02[c] – 0.20
	12	18	56	0.05	0.04	0.04	0.01[c] – 0.14
PFHxS	4	26	0	2.52	1.40	2.25	0.72 – 5.47
	8	23	0	2.26	1.51	1.72	0.36 – 6.43
	12	18	0	1.90	0.99	1.73	0.41 – 3.68
PFHpS	4	26	0	0.10	0.04	0.10	0.04 – 0.19
	8	23	0	0.10	0.04	0.10	0.04 – 0.19
	12	18	17	0.09	0.04	0.09	0.03 – 0.17
PFOS-linear	4	26	0	2.77	1.72	2.15	0.95 – 7.88
	8	23	0	2.46	0.92	2.68	1.17 – 4.83
	12	18	0	2.56	1.48	2.17	0.83 – 5.71
PFOS-branched	4	26	0	0.85	0.36	0.79	0.30 – 1.95
	8	23	0	0.72	0.28	0.67	0.30 – 1.41
	12	18	0	0.58	0.19	0.59	0.31 – 0.98
tot-PFOS[a]	4	26	0	3.63	1.92	3.09	1.41 – 9.24
	8	23	0	3.17	1.14	3.30	1.51 – 6.24
	12	18	0	3.14	1.61	2.69	1.14 – 6.62
PFAS4[b]	4	26	0	8.06	3.70	7.46	2.88 – 16.64
	8	23	0	6.66	2.43	6.56	3.31 – 12.99
	12	18	0	6.10	2.42	6.05	2.22 – 10.80

[a]Sum of linear and branched isomers.
[b]Sum of PFOA, PFNA, tot-PFHxS and tot-PFOS.
[c]Reported concentration <LOD.

PFAS concentrations for the period 2008–2025 are shown in Figure 2 for the three age groups. Tot-PFOS showed the highest median concentrations in the present study, consistent with

findings from Swedish adolescents and children in the Swedish national dietary survey Riksmaten (RMS) (Livsmedelsverket 2020 and 2025), Australian children (Toms et al. 2019), and German children (Duffek et al. 2020), where PFOS was also the predominant PFAS compound. An exception was a Finnish birth cohort study, in which PFOA concentrations exceeded those of PFOS (Koponen et al. 2018). PFAS levels in 4-year-olds in the present study were compared with those reported for 4-year-olds in RMS, sampled 2021–2023 (Livsmedelsverket 2025). For most PFAS the median and mean levels were either comparable (PFHpA and PFPeS), or slightly higher in the POPUP-children (PFOA, PFDA, PFUnDA, PFNA, PFBS, PFHpS, and branched PFOS). In addition, median and mean levels of linear PFOS and total PFOS were 1.3–1.5 times higher in the POPUP children, and PFHxS levels were 3-4 times higher than the 4-year-olds in RMS (Livsmedelsverket 2025).

Parts of Uppsala have historically been affected by PFAS-contaminated drinking water, primarily PFHxS and, to a lesser extent, PFOS, PFOA, and PFBS (Gyllenhammar et al. 2015, 2019, 2025). Although the contamination was identified in 2012, the long biological half-lives of PFAS (EFSA 2020) and the continued presence of PFAS in drinking water, albeit at lower concentrations (Livsmedelsverket 2021), may still influence exposure among children in Uppsala. Consequently, PFHxS levels and possibly also PFOS may not be directly comparable with those observed in Swedish children with lower background exposure.

The tolerable weekly intake (TWI) established by EFSA in 2020 (4.4 ng PFAS4/kg body weight) is based on evidence of reduced vaccine antibody responses in 1-year-old children (EFSA 2020). EFSA identified a PFAS4 serum concentration of 17.5 ng/mL in 1 year old children as the critical effect level and estimated that a maternal serum concentration of 6.9 ng/mL would be protective against exceeding this level in the child. Median PFAS4 concentrations in POPUP- children sampled during 2023–2025 (6.1–7.5 ng/mL serum) were below the critical effect level for 1-year-olds of 17.5 ng/mL but for 4-year-olds it was above the maternal reference level of 6.9 ng/mL. None of the children exceeded 17.5 ng/mL, whereas 62% of the 4-year-olds, 52% of the 8-year-olds, and 28% of the 12-year-olds exceeded 6.9 ng/mL. For the entire study period (2008–2025), 17%, 11%, and 10% of the 4-, 8-, and 12-year-olds, respectively, exceeded 17.5 ng/mL, while 76%, 70%, and 73% exceeded 6.9 ng/mL.

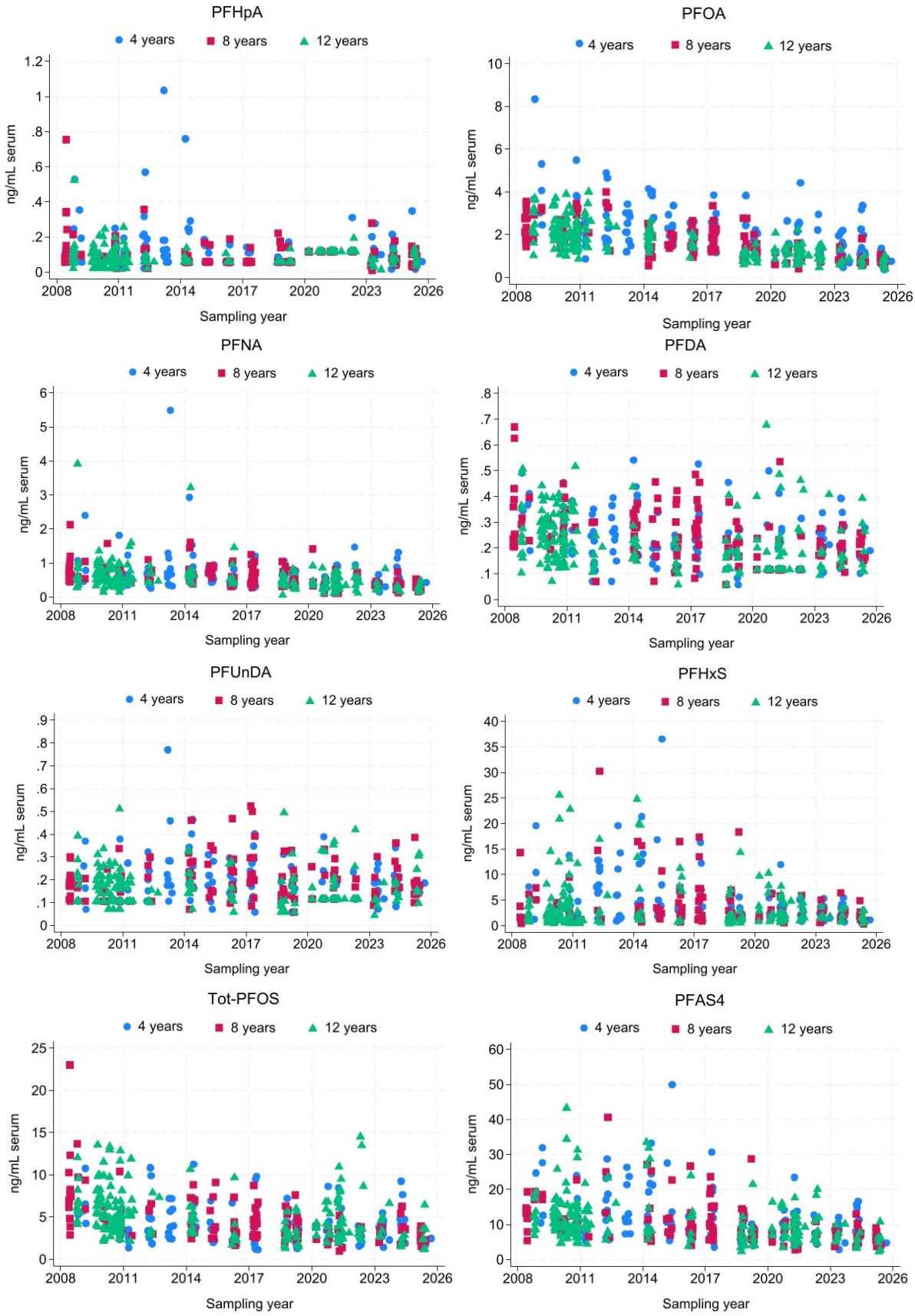

Figure 2. PFAS concentrations in serum from the POPUP-children during the time period 2008-2025 (n=452 for all PFAS except for PFAS4 where n=449).

4.2 Temporal trends

Significant decreases over time were observed for PFOA, PFNA, PFDA, tot-PFOS and PFAS4 concentrations in POPUP-children during 2008–2025, corresponding to annual declines of 2–7% (Table 6). The trends were evident both in the cohort as a whole and within the individual age groups, except for tot-PFOS, for which no significant trend (p=0.057) was observed among the 4-year-old children (Table 6). In the previous study of temporal trends (2008–2022), similar declining trends were observed for PFOA, PFNA, PFDA, and tot-PFOS (Lindfeldt et al. 2023). Consistent with these findings, decreasing concentrations of PFOA and PFOS have also been reported in Australian children between 2007 and 2017, both in the 0–4-year and 5–15-year age groups (Toms et al. 2019).

PFUnDA did not show any statistically significant temporal trends in POPUP-children between 2008 and 2025, consistent with the findings reported previously (Lindfeldt et al., 2023). Previous studies have demonstrated that PFAS contamination of the drinking water in Uppsala contributed to elevated serum concentrations of PFHxS among mothers and children participating in the POPUP-study (Gyllenhammar et al. 2015, 2019 and 2025). Significant declining temporal trends in PFHxS concentrations between 2013 and 2022 have previously been reported both for the overall cohort and for the 4-year-old children (Lindfeldt et al., 2023). These findings are supported by the present analysis, which includes additional measurements from 2023–2025. Furthermore, a significant decline was also observed among the 12-year-old children (Table 6), a trend that was not evident in the previous analysis (Lindfeldt et al. 2023). Together, these results suggest that the measures implemented to reduce PFAS exposure have been effective in lowering serum PFHxS concentrations.

PFHpA showed a declining trend during 2008–2019 but was excluded from both the present and previous assessment (Lindfeldt et al. 2023) due to the high proportion of measurements below the LOQ throughout the whole study period. The proportion of values below the LOQ increased from 62% during 2008–2020 to 80–100% during 2016–2022, which may partly be explained by increasing LOQs over time (Table 4). From 2023–2025, analyses were conducted by a new laboratory using the LOD, with 26, 30 and 17 % of the values below the LOD in the three different age groups, respectively, while the previous laboratory reported results relative to the LOQ.

Table 6. Percent change in concentrations of PFAS per year in serum from children in Uppsala 2008-2025, at 4, 8, and 12 years of age. Adjusted for exact age, weight, height and sex. Significant results are marked in bold.

Compound	Age	n	Change/year (%)		R^2	p	<LOD/LOQ[d] (%)
			Mean	95 % CI			
PFOA	**4**	**132**	**-5.3**	**-3.8/-6.8**	**27**	**<0.001**	**0**
	8	**129**	**-7.4**	**-6.1/-8.7**	**54**	**<0.001**	**0.7**
	12	**191**	**-6.7**	**-5.9/-7.5**	**58**	**<0.001**	**0**
	All	**452**	**-6.3**	**-5.7/-7.0**	**48**	**<0.001**	**0.2**
PFNA	**4**	**132**	**-3.8**	**-1.9/-5.7**	**20**	**<0.001**	**1.5**
	8	**129**	**-5.4**	**-3.6/-7.1**	**28**	**<0.001**	**3.0**
	12	**191**	**-4.9**	**-3.5/-6.3**	**22**	**<0.001**	**4.4**
	All	**452**	**-4.7**	**-3.8/-5.5**	**22**	**<0.001**	**3.2**
PFDA	**4**	**132**	**-1.9**	**-0.3/-3.5**	**6**	**0.024**	**9.0**
	8	**129**	**-4.2**	**-2.7/-5.7**	**22**	**<0.001**	**9.6**
	12	**191**	**-2.6**	**-1.3/-3.8**	**11**	**<0.001**	**14**
	All	**452**	**-2.8**	**-2.0/-3.6**	**11**	**<0.001**	**12**
PFUnDA	4	132	-0.7	1.1/-2.4	-0.5	0.46	24
	8	129	-0.2	1.5/-2.0	1	0.80	14
	12	191	-0.4	0.8/-1.7	-2	0.48	39
	All	452	-0.5	0.4/-1.3	2	0.28	28
PFHxS	**4**	**132**	**-5.5**	**-2.6/-8.3**	**9**	**<0.001**	**0**
	8	129	-2.5	0.9/-5.7	7	0.14	0
	12	191	-1.2	1.5/-3.8	5	0.37	0
	All	**452**	**-1.6**	**0.0/-3.2**	**7**	**0.048**	**0**
2013-2025[a]	**4**	**102**	**-7.6**	**-3.9/-11.2**	**13**	**<0.001**	**-**
	8	94	-3.6	0.8/-7.8	9	0.11	**-**
	12	**92**	**-6.7**	**-0.8/-12.2**	**5**	**0.027**	**-**
	All	**288**	**-6.1**	**-3.6/-8.5**	**12**	**<0.001**	**-**
Tot-PFOS[b]	4	132	-1.8	0.1/-3.7	7	0.057	0
	8	**129**	**-4.4**	**-2.8/-5.9**	**34**	**<0.001**	**0**
	12	**191**	**-3.5**	**-2.2/-4.8**	**18**	**<0.001**	**0**
	All	**452**	**-3.4**	**-2.6/-4.2**	**20**	**<0.001**	**0**
PFAS4[c]	**4**	**132**	**-4.4**	**-2.6/-6.1**	**17**	**<0.001**	**-**
	8	**126**	**-4.7**	**-3.0/-6.4**	**23**	**<0.001**	**-**
	12	**191**	**-3.9**	**-2.6/-5.2**	**18**	**<0.001**	**-**
	All	**449**	**-3.9**	**-3.1/-4.7**	**19**	**<0.001**	**-**

[a]Percent change in concentrations of PFHxS during 2013-2025.
[b]The sum of branched and linear isomers.
[c]PFAS4: sum of PFOA, PFNA, tot-PFHxS and tot-PFOS.
[d]LOD: Limit of detection, LOQ: Limit of quantification.

4.3 Differences in PFAS levels between age groups

When comparing adjusted mean PFAS serum levels between the three age groups, 4-, 8-, and 12-year-olds during the time period 2008-2025, significantly higher levels of PFOA were observed in 4-year-old children (mean: 2.25, SD: ± 0.10) compared to 8-year-olds (mean: 1.66, SD: ± 0.07) (Figure 3). Consistent with our findings several previous studies have reported decreasing serum concentrations of PFAS with increasing age in children (Koponen et al. 2018, Toms et al. 2019, Forthun et al., 2023). For example, higher PFOA concentrations have been observed in younger children compared with older age groups in Australian cohorts (Toms et al., 2019), and significant age-related decreases in serum concentrations of PFOA, PFOS, PFNA and PFHxS have been reported in Finnish children sampled at 1, 6 and 10.5 years of age (Koponen et al. 2018). In the present study, however, no significant age-related differences were observed for any PFAS other than PFOA.

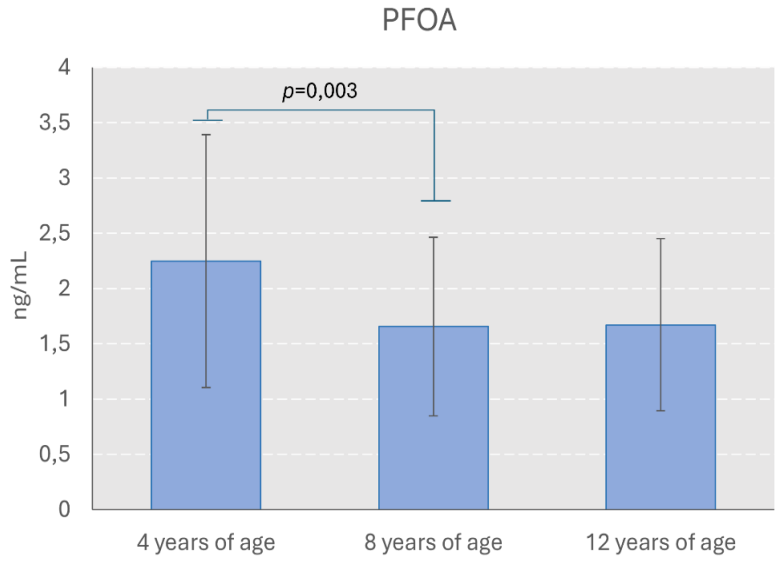

Figure 3. Mean concentrations and standard deviation of PFOA (ng/mL) in 4-, 8- and 12-year-old children from the POPUP-study during the time period 2008-2025.

5. Conclusion

Among the POPUP-children sampled during 2023–2025, the highest serum concentrations were observed for tot-PFOS, followed by tot-PFHxS and PFNA. Significant decreasing temporal trends between 2008 and 2025 were identified for PFOA, PFNA, PFDA, tot-PFOS and PFAS4. In addition, the significant decline in PFHxS serum concentrations between 2013-2025 indicates that the measures implemented to reduce PFAS contamination in Uppsala's drinking water have been effective in decreasing exposure among children. Age-group

comparisons further revealed significantly higher PFOA concentrations in 4-year-olds than in 8-year-olds.

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