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Citation for the published paper:

Irina Gyllenhammar, Jonathan P. Benskin, Oskar Sandblom, Urs Berger,
Lutz Ahrens, Sanna Lignell, Karin Wiberg, Anders Glynn. (2019)
Perfluoroalkyl Acids (PFAAs) in Children's Serum and Contribution from
PFAA-Contaminated Drinking Water. *Environmental Science & technology*.
Volume: 53, Number: 19, pp 11447-11457.

<https://doi.org/10.1021/acs.est.9b01746>

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**Perfluoroalkyl acids (PFAAs) in children's serum and contribution from PFAA
contaminated drinking water**

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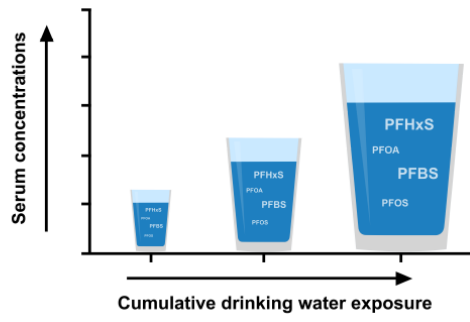
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Abstract

We investigated associations between serum perfluoroalkyl acid (PFAA) concentrations in children aged 4, 8, and 12 years (sampled in 2008-2015; $n=57$, 55, and 119, respectively) and exposure via placental transfer, breast-feeding, and ingestion of PFAA-contaminated drinking water. Sampling took place in Uppsala County, Sweden, where the drinking water has been historically contaminated with perfluorobutanesulfonate (PFBS), perfluorohexanesulfonate (PFHxS), perfluorooctanesulfonate (PFOS), perfluoroheptanoate (PFHpA), and perfluorooctanoate (PFOA). PFOS showed the highest median concentrations in serum (3.8-5.3 ng g⁻¹ serum) followed by PFHxS (1.6-5.0 ng g⁻¹ serum), PFOA (2.0-2.5 ng g⁻¹ serum), and perfluorononanoate (PFNA) (0.59-0.69 ng g⁻¹ serum) in children. Including all children, serum PFOA, PFHxS, and PFOS concentrations in children increased 10%, 10%, and 1.3% (adjusted mean), respectively, per unit (ng g⁻¹ serum) of increase in maternal serum level (at delivery), the associations being strongest for 4-year-old children. PFHxS and PFOS significantly increased 3.9% and 3.8%, respectively, per month of nursing, with the highest increase for 4-year-olds. PFOA, PFBS, PFHxS, and PFOS increased 1.2%, 207%, 7.4%, and 0.93%, respectively, per month of cumulative drinking water exposure. Early life exposure to PFOA, PFHxS, and PFOS is an important determinant of serum concentrations in children, with the strongest influence on younger ages. Drinking water with low to moderate PFBS, PFHxS, PFOS, and PFOA contamination is an important source of exposure for children with background exposure from other sources.

TOC Graphic



Introduction

Per- and polyfluoroalkyl substances (PFASs) are synthetic highly fluorinated substances that have been produced in large volumes and which have broad commercial applications. PFASs are ubiquitous in humans and the environment. Human exposure media include food, drinking water, dust, air and products containing PFASs.^{1 2 3} Perfluoroalkyl acids (PFAAs) are a class of PFASs which are intentionally manufactured, but which may also occur from degradation of other PFASs (i.e. PFAA-precursors).^{4 5} PFAAs display extreme environmental persistence and chain length-dependent bioaccumulation in humans.^{6, 7}

For the general population, exposure to PFAAs via placental transfer⁸⁻¹¹ and ingestion of mother's milk¹²⁻¹⁴ are major determinants of blood PFAAs concentrations in infants.¹⁵⁻²⁰ In fact, exposure to certain PFAAs via breast milk as an infant represents a significant fraction of a child's overall exposure up to 3-5 years of age, most probably due to the long half-lives of these PFAAs in the body.^{21, 22} Other exposure media like diet, drinking water, dust and air contribute to a greater extent as the child gets older.²²⁻²⁶ Early life exposure to some PFAAs during pregnancy has been associated with lower birth weight²⁷⁻²⁹ and increased childhood adiposity.³⁰⁻³³ Positive associations between maternal PFAA levels during pregnancy and children's weight or body mass index (BMI) have also been reported^{29, 31, 34} along with relations to immune toxicity in children.^{35, 36} Improved knowledge of the determinants of blood PFAA concentrations in infants/children, in particular in scenarios involving point source contamination (e.g. contaminated drinking water) is needed for understanding the exposure sources responsible for observed relationships between blood PFAA concentrations and health outcomes.

Drinking water in the City of Uppsala, Sweden, was contaminated with PFAAs for at least 20 years³⁷ before the contamination was discovered in 2012 and affected production wells were

closed or severely restricted. Perfluorohexane sulfonate (PFHxS) was the most prevalent PFAA in the contaminated production wells at the time of well closure (mean 80 ng L⁻¹) followed by perfluorooctane sulfonate (PFOS; 50 ng L⁻¹) and perfluorobutane sulfonate (PFBS; 10 ng L⁻¹)³⁷. Uppsala is thus a good setting for studies investigating different sources of PFAA exposure (e.g. trans-placental transfer, mother's milk, drinking water) as determinants of blood PFAA concentrations during childhood.

In a previous study of 2-4-month-old infants from Uppsala participating in the POPUP cohort (Persistent Organic Pollutants in Uppsala Primiparas) it was shown that prenatal and postnatal PFAA exposure significantly contributed to the serum concentrations in infants and that maternal PFHxS and PFBS exposure from drinking water was an important indirect infant exposure source.³⁸

The aim of the present study was to investigate determinants of PFAA serum concentrations in older children at ages 4, 8, and 12 years, from the POPUP cohort, focussing on maternal PFAA concentrations at the time of delivery, nursing history of the child, and history of drinking water exposure of the child. Specific research objectives addressed here include: a) determining the contribution of PFAA exposure *in utero* and during nursing at different ages of children and b) to assess the extent to which PFAA exposure via medium grade contaminated drinking water (10-100 ng/l of single PFAAs) is a determinant of PFAA serum concentrations during childhood in a population with background exposure from other sources.

Materials and methods

Sampling

All mother/child pairs included in the present paper are participants in the POPUP study, an on-going investigation of POPs in first-time mothers and their children in Uppsala County, Sweden. Mothers were randomly recruited during pregnancy (1996-1999) or shortly after delivery (2000-2011).^{39, 40} The mothers answered a self-administered questionnaire about life-style factors and health of the mother and child. Information about nursing was given by the mother, answering for each month after birth up to 13 months, if the child had been only-, part time-, or not breastfeeding. Blood samples from the mothers were collected 3 weeks after delivery. Following up on this, serum samples were collected from the children when they were 4-, 8-, and 12 years of age between 2008 and 2015 ($n=57$, $n=55$, and $n=119$, respectively; Fig. 1). None of the children were sampled at all ages, in total 33 children were sampled twice ($n=13$ at age 4 and 8 and $n=20$ at age 8 and 12). Detailed characteristics of the children are provided in Table 1. Plastic Vacutainer[®] or Vacuette[®] serum tubes were used for blood sampling and serum was stored at -20°C until analysis. The study was approved by the local ethics committee in Uppsala, Sweden (dnr 2004/177 and 2007/147/1), and the participating women and children gave informed consent.

Chemical analyses

A total of 13 PFAAs were targeted in the present work, including C_4 , C_6 and C_8 perfluoroalkane sulfonic acids (PFSA; i.e. PFBS, PFHxS, PFOS) and C_6 - C_{15} perfluoroalkyl carboxylic acids (PFCA; i.e. PFHxA, PFHpA, PFOA, PFNA, PFDA, PFUnDA, PFDoDA, PFTTrDA, PFTeDA, PFPeDA; for details see Supporting Information, Table A1). The serum samples were analysed as described previously.³⁷ In short, 0.5 g serum was spiked with internal standards and extracted with acetonitrile in an ultrasonic bath. The concentrated

extract underwent dispersive clean-up with graphitized carbon. Aqueous ammonium acetate and volumetric standards were added before analysis on an Acquity ultra performance liquid chromatography system (UPLC) coupled to a Xevo TQ-S tandem mass spectrometer (MS/MS; both Waters Corp., Milford, MA, U.S.) operated in negative electrospray ionization, multiple reaction monitoring mode. Instrumental parameters are provided in Supporting Information, Table A2.

Quantification was performed by isotope dilution using a 5-point calibration curve (linear, 1/x weighting), which was run before and after samples. For most targets, analogous isotopically labelled internal standards were available. For PFBS, PFTrDA, PFTeDA, and PFPeDA, a structurally similar internal standard was used (Supporting Information, Table A2). For PFHxS and PFOS, Σ branched (br) and linear (lin) isomers were quantified separately using the calibration curve for the lin isomer and the concentrations for the m/z 499/80 and 499/99 product ions were averaged, as described in Riddell et al.⁴¹

A procedural blank and a quality control (QC) sample (pooled human serum analyzed repeatedly in-house) were included with every batch of samples to assess background contamination and reproducibility, respectively (see Supporting Information Table A3 for QC performance metrics). In addition, three replicates of standardized and certified reference material from NIST (SRM 1957) were analyzed, and quantified concentrations were compared to reference values to assess method accuracy (results provided in Supporting Information Tables A4 and A5). Measured concentrations in SRM 1957 were consistent with reference values for all targets, while CVs in control serum ($n=8$) ranged from 11-30%, with the exception of PFBS (41%), which was close to detection limits and only intermittently detected in control serum. For targets observable in method blanks, the detection limit was based on the mean blank + 3 \times the standard deviation of the blanks. For targets absent in blanks, detection limits were based on a signal to noise ratio of 3. The method quantification

limits (MQL) were 0.16 ng g⁻¹ serum for PFHxA, 0.08 ng g⁻¹ serum for PFHpA, 0.8 ng g⁻¹ serum for PFOA, 0.08 ng g⁻¹ serum for PFNA, 0.10 ng g⁻¹ serum for PFDA and PFUnDA, 0.08 ng g⁻¹ serum for PFDoDA, 0.02 ng g⁻¹ serum for PFTrDA, 0.06 ng g⁻¹ serum for PFTeDA, 0.01 ng g⁻¹ serum for PFPeDA, 0.01 ng g⁻¹ serum for PFBS, PFHxS, and PFOS.

Exposure via drinking water

Data on the occurrence of PFAAs in drinking water were only available for a few samples ($n=9$) collected at the tap in different parts of Uppsala County in 2012, when PFAA contamination was first discovered.⁴² A study of PFAA concentrations in maternal serum from 1996 to 2011 revealed that the drinking water was already contaminated during the initial study period (1996-1999).³⁷ Modeling the distribution of contaminated well water from 1996 to 2012 made it possible to estimate the extent of exposure to PFAA-contaminated water depending on location of residence within Uppsala County.³⁷

The cumulative number of months with PFAA exposure from drinking water (DW_{cumexp}) were calculated for the five PFAAs that were detected in the drinking water: PFHpA, PFOA, PFBS, PFHxS, and PFOS. Details of the distribution patterns of PFAA-contaminated drinking water in Uppsala City from 1996 to 2012 were collected, and an overview of the distribution was obtained by modeling.³⁷ This information revealed that residential addresses of the children over the duration of the study (data obtained from the Swedish Population Register) could be divided into four different PFAA drinking water districts (up to July 2012; thereafter contamination was mitigated), with District 1 not receiving a contribution from PFAA-contaminated wells, and Districts 2, 3, and 4 receiving contributions of <10%, 10-89% and $\geq 90\%$, respectively, from the contaminated wells. In the calculations of the DW_{cumexp} each child was assigned to a district based on home address for each month of life until blood

sampling. Children assigned to District 1 were estimated to have been exposed to 0% of the contaminated water on a monthly basis ($DW_{exp}=0$), while children in Districts 2, 3, and 4 were estimated to have been exposed to 5%, 50%, and 95%, respectively, of contaminated water ($DW_{exp}=0.05, 0.50, \text{ and } 0.95$). After July 2012, it was assumed that no district received contaminated water (i.e. $DW_{exp} = 0$).

In the next step, each DW_{exp} was half-life-adjusted based on the number of months between the month in question and blood sampling. The half-lives ($T^{1/2}$) used were 70 days (2.3 months) for PFHpA,⁴³ 26 days (0.87 months) for PFBS,⁴⁴ 2.7 years (32 months) for PFOA, 5.3 years (64 months) for PFHxS, and 3.4 years (41 months) for PFOS.⁴⁵

Each participant's cumulative number of months with exposure to a given PFAA from drinking water (DW_{cumexp}) could thus be estimated by the formula: (see Supporting Information Table A6 for example calculations).

$$DW_{cumexp} = \sum_{i=1}^n DW_{exp_i} * \frac{1^{(n-i)/T}}{2}$$

DW_{exp_i} = proportion of contaminated water in the drinking water during month i (0, 0.05, 0.5, or 0.95)

$T^{1/2}$ = half-life of the PFAA

n = number of months from birth to blood sampling, i.e. $(n-i)$ = number of months from month i to blood sampling)

Statistical analyses

MINITAB 15[®] Statistical Software for Windows was used for all statistical analyses. When PFAA concentrations were below the MQL, $MQL/\sqrt{2}$ was used in the statistical analyses.

The proportions of *br* and *lin* isomers for PFHxS and PFOS were expressed as a percentage of the total concentration. Correlations among serum PFAAs were investigated using average linkage cluster analysis, which is a hierarchical analysis clustering method based on the average distance between all pairs of objects. Kruskal-Wallis test was used to evaluate possible differences in serum PFAA concentrations among children aged 4, 8, and 12 years. General linear model (GLM) analysis was used to investigate differences in serum PFAA concentrations between age groups, adjusted for sampling year and drinking water exposure. Multiple linear regressions (MLR) were used to analyze associations between PFAA concentrations in child serum and maternal PFAA level at delivery, duration of breastfeeding during infancy, and childhood drinking water exposure. When analyzing %*br* PFHxS or PFOS in children, the maternal serum %*br* PFHxS or PFOS was included instead of maternal concentrations of PFHxS or PFOS. These MLR analyses were not performed for PFAAs where >25% of the reported concentrations were below MQL, except for PFHpA and PFBS when analyzing the influence of drinking water exposure on serum concentrations. PFHpA and PFBS have relatively short serum half-lives (70 and 26 days, respectively^{43, 44}); consequently, maternal PFAA levels at delivery and duration of breastfeeding are not expected to make a significant contribution to serum PFAA levels in children and were therefore not included as exposure sources. The associations between child PFAA concentrations and other determinants (i.e. age at sampling, sampling year, body weight, and sex) were first analyzed in univariate linear analyses and those associated with PFAA concentrations at $p \leq 0.1$ significance levels were included in the MLR model.

In addition, stepwise regression was used to estimate how much of the variation in PFAA concentrations was explained by the variation of the determining factors. Logarithmically-transformed PFAA concentrations were used in the statistical analyses, since the distribution of data closely followed a log-normal distribution. As a consequence, partial regression

coefficients (β) of the independent variables may be interpreted as % change in serum concentrations of PFAA per unit of change in the independent variable, calculated as $\% \text{change} = (1 - \exp(\beta)) * 100$. In the analyses of all children (aged 4, 8, and 12) together, only results from one sampling age were used for children that were sampled more than once (n=33). For children sampled both at 8 and 12 years of age, the results from age 8 were used, due to a smaller sample size than among 12-year-old children. Children sampled both at 4 and 8 years of age were allocated equally into the two age groups, as the sample sizes were similar. A sensitivity test was performed when observations with standardized residuals ≥ 3 were excluded from analysis due to their large influence on the regression results. The statistical significance was set to $p \leq 0.05$.

Results and discussion

PFAA serum concentrations

PFAA serum concentrations in children at different ages are presented in Table 2 (PFCAs) and Table 3 (PFSAs). For the investigated sampling years (i.e. 2008-2015), total PFOS, total PFHxS, and PFOA displayed the highest median concentrations in children's serum, in all age groups. Significant differences were observed between PFAA concentrations in 4-, 8-, and 12-year-olds ($p < 0.05$; Kruskal-Wallis test) for all detected targets, except PFUnDA, PFTrDA, and PFBS. For PFHpA, PFOA, and PFHxS the highest concentrations were observed in 4-year-olds, while PFOS concentrations increased with increasing age. No general age-dependent pattern was observed for PFNA and PFDA. However, due to differences in timing of sampling between age groups (Fig. 1) and possible differences in drinking water exposure, it is more relevant to compare age-dependent differences in concentrations after adjustment of concentrations for sampling year and half-life-adjusted

months of contaminated drinking water. In this case (using GLM), PFHpA, PFOA, and PFHxS serum concentrations were significantly higher among 4-year-old children compared to 8- and 12-year-olds (Fig. 2), and PFHpA, PFNA and PFDA were significantly higher in 8- compared to 12-year-olds.

For comparison, blood PFAA concentrations in children from other studies during the same time period are provided in the Supporting Information, Table A7. Studies reporting age-dependent differences in PFAA concentrations among children have observed diverging results.^{46, 47 36, 48 49 50 51-53} The comparisons are, however, hampered by differences among studies with respect to study design, location, child age, nursing history, and sampling year. Taking these uncertainties into account, there are few marked differences in PFAA concentrations between children from Uppsala examined in the present study, and those with background exposure from Denmark, the Faroe Islands, Germany, and the U.S.^{46, 47 36, 48 49 50}⁵¹ The few exceptions include PFNA, where higher serum levels were reported in two studies from the U.S.,^{46, 47} and PFHxS, where concentrations in children's serum in the present study are elevated, most likely due to drinking water exposure.³⁷ Moreover, 3-6-fold higher concentrations of PFOS, PFDA, PFUnDA, and PFTTrDA, and 30-fold higher concentrations of PFBS were reported in serum from children in South Korea and Taiwan compared to the present study.^{52, 53}

Historical production of PFOS, its salts and derivatives by the major global manufacturer (the 3M Company) resulted in a technical mixture of about 70% lin and 30% Σ br isomers.⁵ The major technical PFOS mixture (3M) contained impurities of PFHxS consisting of about 82% lin and 18% br PFHxS isomers.⁵⁴ Previous studies in adults have reported a slightly higher percentage of br PFOS isomers in human serum than in the historical technical mixture.^{37, 55} This is supported by our finding of 37% br PFOS isomers (median) in POPUP children (Table 3). The %br PFHxS and PFOS are in agreement with the values observed in 3-month-

old POPUP infants and their mothers sampled in 1996-1999.³⁸ The differences in %br PFHxS and PFOS in children in relation to technical mixtures may for instance be due to differences in historical PFHxS and PFOS exposure patterns and sources or how the content of br isomers has been determined analytically. The difference may also be explained by different toxicokinetics of lin and br isomers in humans^{9 56} or that the children in the present study have been exposed to PFAS-contaminated drinking water (see discussion below). Studies of PFAA isomers in children are scarce, and to our knowledge this is the first study of serum concentrations of br and lin PFHxS in children. In Danish children aged 6-11 years sampled in 2011, the median br PFOS content in serum was 32%⁴⁸ and 29% in children aged 6-10 years sampled 2007-2010 in the U.S.⁴⁶ We did not observe any age differences in %br PFOS and PFHxS isomers in the Uppsala children (Table 3), suggesting that differences in elimination rates between br and lin isomers⁵⁷ are not significant determinants of the proportions of br and lin isomers in serum during childhood.

Cluster analysis of PFAA based on correlations between serum concentrations in 4-, 8-, and 12-year-old children are shown in Figure 3. PFBS and PFHxS clustered together in the children in the present study, which may be due to drinking water being a common source of exposure in the Uppsala children, as shown in their mothers.³⁷ Long-chain PFCAs and PFOS clustered separately from PFBS and PFHxS as well as from PFOA and PFHpA (Fig. 3).

Apart from drinking water exposure as a possible explanation to the separate clustering of PFBS and PFHxS, differences in dietary sources could explain separate clustering of long-chain PFCAs/PFOS and PFOA. A study of PFASs in food on the Swedish market showed that in 2010 fish consumption contributed with more than 80% of total per capita exposure of long-chain PFCAs and PFOS from food, whereas PFOA intake from fish consumption was estimated to be $\leq 10\%$ of total per capita exposure.³ Sub-clustering of PFUnDA and PFTrDA separately from PFNA, PFDA, and PFOS within the same hierarchy (Fig. 3) points to fish

consumption as a common source of exposure to these PFASs, but more so for PFUnDA and PFTTrDA compared to PFNA, PFDA, and PFOS.³

Determinants for PFAA in children sampled 2008-2015

All 4-, 8-, and 12-year-old children were first analyzed together in order to increase statistical power (n=198), and the results are given in table 4. In the next step, the different age groups were analyzed separately to determine age-related difference regarding associations between maternal concentrations at delivery and breastfeeding duration and child concentrations (Table 5). In Supporting Information, the results from all analyzed PFAA at the different ages are presented in table A10.

In the MLR analyses, including all children, age-dependent differences in adjusted mean PFAA concentrations were less obvious (Supporting Information, Table A8) than in the GLM-analyses adjusting only for sampling year and drinking water exposure (Fig. 2). Consequently, the age differences observed in the GLM-analyses after adjustment for only sampling year and drinking water exposure were to some extent due to the influence of the other determinants of serum PFAA concentrations investigated in the present work, such as maternal serum concentration, breastfeeding, weight, and sex (Table 4).

The influence of fetal and postnatal lactation exposure on child serum PFAA levels was investigated by including the variables “maternal serum concentrations at delivery” and “breastfeeding duration” in the MLR model, except for PFHpA and PFBS, which have relatively short half-lives in serum. When including all children in the MLR analyses, increased maternal serum concentrations (at delivery) were associated with increased child serum concentration for PFHxS (coefficient of determination (R^2) =0.11), PFOA (R^2 =0.04), and PFOS (R^2 =0.03).

Maternal PFAA concentrations at birth most probably reflect both *in utero* and lactational exposure of the children, since maternal serum/plasma concentrations of PFHxS, PFOS, PFOA, PFNA, PFDA, and PFUnDA during pregnancy and close to delivery are strongly correlated with PFAA concentrations both in cord blood and mother's milk.^{12, 18 58 8 19 59} For PFOA, PFHxS and PFOS, the impact of early exposure was greater in 4-year-old children compared to the older age groups (Table 5). For example, PFOA serum concentrations in the 4-year-olds increased 29% per unit (ng g⁻¹ serum) of increase in maternal serum PFOA level ($R^2=0.24$), whereas in 12-year-olds the increase was 8.4% ($R^2=0.04$).

The strong association between levels of PFOA, PFHxS, and PFOS in serum of mothers at the time of delivery and 4-year-olds but not in the older age groups may be due to a combination of growth dilution of PFAAs accumulated *in utero* and during nursing, a longer period of excretion of PFAAs that were accumulated early in life among the older children, and an increased contribution of PFAAs accumulated for instance from food among older children. For the long-chain PFCAs, PFNA and PFDA, we observed no associations between early life exposure and serum concentrations in the children, suggesting that early life exposure to these PFAAs have little influence on concentrations later in childhood. Similarly, in 3-month-old POPUP infants the influence of maternal PFAA concentrations at delivery decreased with increasing perfluoroalkyl chain length.³⁸ Factors other than early life exposure are apparently more important in determining concentrations of PFDA and PFNA than for PFOA, PFHxS, and PFOS.

Percent of br PFOS in children increased with increasing %br in maternal serum at delivery, whereas no such association was found for PFHxS (Tables 4 and 5). When stratifying for child age, the associations for %br PFOS were positive in all three age groups but only significant for 8- and 12-year-olds. The positive association between %br PFOS in mothers at delivery and %br PFOS in the children suggest significant maternal influence on PFOS

isomer patterns in children for many years after birth. This could, apart from the remaining influence from *in utero* and breastfeeding exposure, mirror similar food habits and exposure sources between mothers and their children.

PFOA (only in 4-year-olds), PFHxS, and PFOS were associated with breastfeeding duration, showing an increase with increased breastfeeding duration with partial $R^2=0.01$ and 0.04 , for PFHxS and PFOS respectively and 0.05 for PFOA in 4-year-olds (Table 4 and 5). As shown by the R^2 s, only a small percentage of variation of child PFAA concentrations were explained by the *in utero* and breastfeeding exposure, most likely due to greater contribution of PFAA exposure during the years after cessation of breastfeeding. Moreover, since the mothers gave the information on breastfeeding several years after the breastfeeding period, recall bias may also have contributed to the low R^2 s.

Duration of breastfeeding showed a similar age-dependent influence on child PFOS and PFOA concentrations as maternal PFOS and PFOA serum concentrations at delivery. Serum PFOA concentrations increased 5.1% per month of breastfeeding ($R^2=0.05$) in 4-year-olds, but at 8 and 12 years of age no associations were found. Serum concentrations of PFOS in 4-year-olds increased 7.9% per month of breastfeeding ($R^2=0.10$), 5.3% per month for 8-year-olds ($R^2=0.08$), and for 12-year-olds the association was not significant. For PFHxS the association was not significant when the children were divided into the three age groups, although the 4-year-olds showed an increase of 7.6% in serum levels per month of breastfeeding with $p=0.053$ (Table 5). As with maternal PFAA concentrations, growth dilution, excretion and exposure from sources other than breastfeeding most likely contributed to this decrease in importance of early life exposure.

Studies of 3-year-old children from Norway and children <3.5 years old from the U.S. have found similar results as among our 4-year-old children, with PFOS and PFOA concentrations

increasing 3-6% per month of breastfeeding.^{20 60} A study from the Faroe Islands showed much stronger associations between breastfeeding duration and child PFAA concentrations, with concentrations of PFOS and PFOA increasing with 30% per month of breastfeeding in fully nursed children at ages 1.5 and 5 years.²¹ In children from the Faroe Islands concentrations of PFNA and PFDA increased about 20% per month of breastfeeding,²¹ whereas Norwegian²⁰ and U.S. children⁶⁰ showed similar results to the present study with no associations between time of breastfeeding and increase in serum concentrations for PFNA and PFDA.

We tested the hypothesis that body weight could influence serum PFAA concentrations, through an effect on volume of distribution. This was only indicated for PFOA, for which concentrations significantly decreased with increased body weight ($R^2=0.05$), giving some support to this hypothesis. Among 3-month-old POPUP infants sampled 1996-1999 no association between PFOA serum concentrations and weight gain from birth was observed.³⁸ Instead, PFHpA concentrations were negatively associated with weight gain. Although, both studies give some support to an influence of growth dilution on child PFAA concentrations, more controlled studies are needed in order to draw firm conclusions about the influence of growth dilution on serum PFAA concentrations during childhood. The fact that PFAA do not appreciably partition into fat may have contributed to the weak or non-significant associations with weight of the children.⁶¹

Overall, divergent associations were observed between year of sampling, 2008-2015, and PFAS concentrations over the 7 year sampling period. For example, PFOA displayed an inverse associations with sampling year (-7.6% per year) whereas PFHxS displayed a positive association (7.5% per year) (Table 4). PFOS also displayed an inverse association (-3.4% per year), but this was not statistically significant. Similar trends were previously reported in POPUP mothers between 1996 and 2012,^{42 55} and are attributed to a) decreased exposure to

PFOS and PFOA, and their precursors, due to the phase out of production of these PFASs, and b) cumulative long-term drinking water PFHxS exposure in the Uppsala area.³⁷

The basic regression models used by us only included covariates significantly associated with PFAA concentrations at the $p \leq 0.1$ significance levels in univariate analyses. An analysis using a regression model with all possible covariates (full model) was done in order to determine if the partial regression coefficients changed significantly compared with the basic models (Supporting Information, Table A9). When comparing the results of the two MRL analyses including children of all ages (Supporting Information, Tables A8 and A9) only slight differences in mean percent changes or significance levels were found. As expected the R^2 of the full models were in many cases slightly higher, but they did not differ markedly from those of the basic model. This shows that the covariates with significance levels $p > 0.1$ in univariate analyses only explained a small fraction of the variation in serum PFAA concentrations. Only two statistically significant association became non-significant when using the full model, i.e. PFBS and age, and PFOA and body weight. A few marginally non-significant associations in the basic model became significant in the full model. Similarly as for 8-year-olds, the adjusted mean PFOA concentration among 12-year-olds was lower than that of 4-year-old. %br PFHxS decreased as number of months of breastfeeding increased. Most importantly, however, among the covariates not included in the basic models for PFHpA and PFDA, sampling year was significantly associated (negative) in the full model. This may indicate that PFHpA and PFDA exposure of Swedish children decreased between 2008 and 2015.

Drinking water exposure

When including all children in the MLR models, serum concentrations in children increased with increasing drinking water exposure for PFOA, PFBS, PFHxS, and PFOS (Table 4). The

strongest associations were observed for PFBS and PFHxS, for which drinking water exposure explained 20% and 41%, respectively, of the variation in serum concentrations. Serum concentrations increased by 207% and 7.4% per month of cumulative PFBS and PFHxS drinking water exposure, respectively. This shows that drinking water is an important exposure medium for PFBS and PFHxS for children even in cases when drinking water contamination is moderate to low as in the Uppsala case. In the contaminated production wells in Uppsala, the median PFHxS concentration was 80 ng L⁻¹, followed by PFOS (50 ng L⁻¹) and PFBS (10 ng L⁻¹) in samples collected 2012-2014.³⁷ PFOA was detected in one fifth of the samples in these production wells, at a detection limit of 10 ng L⁻¹. Concentrations in drinking water before 2012 are not known, but the PFBS and PFHxS concentrations in serum from POPUP mothers living in areas receiving potentially contaminated drinking water were elevated already between 1996 and 1999 and only slightly lower than concentrations in mothers living in the same areas from 2008 to 2011.³⁷ This suggests that contamination of the drinking water may not have been markedly lower between 1996 and 1999 compared to 2012. The (on average) 1.2% increase in PFOA serum concentration per month of cumulative PFOA drinking water exposure among the children (Table 4) suggests that even low PFOA contamination may be enough to significantly influence total PFOA exposure in children with background exposure from other sources. PFOS serum concentrations in the children increased on average 0.9% per month of cumulative PFOS drinking water exposure (Table 4). These results differ from mothers to the children in the present study, where drinking water exposure was not associated with increased PFOS levels, although PFOS concentrations in the production wells were clearly elevated from background.³⁷ The much higher exposure to PFOS from food than of PFBS and PFHxS³ may mask contributions of drinking water exposure to serum PFOS concentrations.

The %br PFHxS and %br PFOS in children's serum were positively associated with cumulative drinking water exposure, with a stronger association for %br PFHxS ($R^2=0.15$) than for %br PFOS ($R^2=0.02$) (Table 4). The results support earlier findings that enrichment of br PFHxS isomers in serum samples relative to proportions observed in the general population could possibly be used as marker of exposure to PFAA polluted drinking water caused by contamination from fire-fighting training areas.³⁷ We hypothesize that higher percentages of br PFOS isomers in children with higher cumulative exposure to contaminated drinking water also was caused by enrichment of br PFOS isomers in contaminated drinking water. The proportion of br PFOS isomers in Uppsala drinking water has been determined to be on average 53%,³⁷ which is considerably higher than the 30% contribution in the major commercial product.⁵⁴ It is possible that the enrichment of br isomers in drinking water is due to the preferential leaching of br PFOS into the drinking water supply⁶² and/or preferential biodegradation of br PFOS-precursors during water treatment.⁵ The elevated exposure to drinking water PFOS in this study may also have contributed to the higher %br PFOS (37%) in the Uppsala children compared to percentages reported in other studies of children from Denmark and the U.S (32% and 29% respectively), which most likely are exposed from other, generally dominating pathways (i.e. ingestion of food, dust, etc.).^{46, 48}

In conclusion, early life exposure to PFOA, PFHxS, and PFOS is an important determinant of serum concentrations in children, with the strongest influence on younger ages. Drinking water with low to moderate PFBS, PFHxS, PFOS, and PFOA contamination is an important source of exposure for children with background exposure from other sources.

Table 1. Characteristics of the children

Age	Characteristics	<i>n</i>	Mean	Range	%	
4 years	Age (years)	57	3.9	3.3-5.1		
	Sampling year 2008-2009	6			11	
	2010-2012	24			42	
	2013-2015	27			47	
	Weight (kg)	57	17	13-23		
	Time of breastfeeding (months)	57	6.8	1- >13		
	Girls	21			37	
	Boys	36			63	
	DW exposure ^a	PFHpA	57	0.38	0-1.9	
	(cumulative PFAA months)	PFOA	57	4.1	0-17	
		PFBS	57	0.17	0-0.91	
		PFHxS	57	5.1	0-23	
		PFOS	57	4.5	0-19	
	8 years	Age (years)	55	8.4	7.3-9.6	
Sampling year 2008-2009		16			29	
2010-2012		20			36	
2014-2015		19			35	
Weight (kg)		54	29	20-44		
Time of breastfeeding (months)		55	7.1	0.5->13		
Girls		21			38	
Boys		34			62	
DW exposure ^a		PFHpA	55	0.29	0-2.0	
(cumulative PFAA months)		PFOA	55	3.2	0-32	
		PFBS	55	0.13	0-0.91	
		PFHxS	55	4.6	0-48	
		PFOS	55	3.7	0-38	
12 years		Age (years)	119	12.2	11.1-13.2	
	Sampling year 2008-2009	31			26	
	2010-2012	76			64	
	2014	12			10	
	Weight (kg)	113	44	28-67		
	Time of breastfeeding (months)	117	6.3	0- >13		
	Girls	56			47	
	Boys	63			53	
	DW exposure ^a	PFHpA	119	0.44	0-1.9	
	(cumulative PFAA months)	PFOA	119	5.0	0-31	
		PFBS	119	0.20	0-0.91	
		PFHxS	119	8.0	0-54	
		PFOS	119	6.0	0-38	

^aCumulative exposure to PFAA from drinking water during the whole life-time until sampling.

Table 2. Perfluoroalkyl carboxylic acid (PFCA) serum concentrations in children at 4 (*n*=57), 8 (*n*=55), and 12 (*n*=119) years of age (ng g⁻¹ serum)

Children	Age	Mean	SD	Median	Range	DF ^a (%)
PFHpA	4	0.18	0.18	0.12	<0.08-1.0	79
	8	0.12	0.11	0.08	<0.08-0.75	60
	12	0.09	0.06	0.08	<0.08-0.52	51
PFOA	4	2.7	1.3	2.5	0.86-8.3	100
	8	2.1	0.81	2.0	<0.80-4.0	98
	12	2.1	0.70	2.0	0.86-4.0	100
PFNA	4	0.85	0.79	0.67	0.26-5.5	100
	8	0.76	0.33	0.69	0.34-2.1	100
	12	0.67	0.46	0.59	<0.08-3.9	99
PFDA	4	0.26	0.11	0.25	<0.10-0.54	98
	8	0.30	0.11	0.29	<0.10-0.67	96
	12	0.25	0.092	0.23	<0.10-0.52	99
PFUnDA	4	0.21	0.12	0.18	<0.10-0.77	74
	8	0.20	0.079	0.18	<0.10-0.46	78
	12	0.17	0.071	0.16	<0.10-0.51	65
PFDoDA	4			<0.08	<0.08-0.21	12
	8			<0.08	<0.08-0.07	2
	12			<0.08	<0.08-0.06	1
PFTrDA	4	0.03	0.05	<0.02	<0.02-0.35	35
	8	0.03	0.02	0.02	<0.02-0.13	51
	12	0.02	0.02	<0.02	<0.02-0.10	29
PFTeDA	4			<0.06	<0.06-0.43	11
	8			<0.06		0
	12			<0.06	<0.06-0.10	3
PFPeDA	4			<0.01	<0.01-0.06	4
	8			<0.01	<0.01-0.04	2
	12			<0.01	<0.01-0.02	1

^aDetection frequency.

Table 3. Perfluoroalkane sulfonic acid (PFSA) serum concentrations in children at 4 (n=57), 8 (n=55), and 12 (n=119) years of age (ng g⁻¹ serum)

Children	Age	Mean	SD	Median	Range	DF ^a (%)
PFBS	4	0.03	0.03	0.02	<0.01-0.11	65
	8	0.02	0.02	<0.01	<0.01-0.09	44
	12	0.03	0.03	0.02	<0.01-0.23	60
lin PFHxS	4	6.5	6.3	4.6	0.55-35	100
	8	3.6	5.0	1.5	0.41-29	100
	12	3.5	5.0	1.5	0.41-23	100
br PFHxS	4	0.39	0.42	0.20	<0.01-1.6	98
	8	0.23	0.35	0.07	<0.01-1.6	96
	12	0.27	0.43	0.08	<0.01-2.6	90
%br PFHxS	4	5.1	2.1	5.0	0.25-9.5	100
	8	4.8	2.3	4.5	0.53-11	100
	12	5.7	3.1	5.3	0.23-12	100
Total PFHxS	4	6.9	6.7	5.0	0.60-37	100
	8	3.9	5.4	1.6	0.43-30	100
	12	3.8	5.4	1.6	0.43-26	100
lin PFOS	4	2.9	1.6	2.4	0.87-7.1	100
	8	3.8	2.7	3.2	1.3-19	100
	12	3.7	1.8	3.4	1.2-9.7	100
br PFOS	4	1.8	0.93	1.5	0.51-4.2	100
	8	2.1	1.0	1.8	0.76-4.7	100
	12	2.1	0.95	1.9	0.80-5.1	100
%br PFOS	4	38	6.4	38	26-56	100
	8	37	7.2	38	18-50	100
	12	37	6.9	37	17-54	100
Total PFOS	4	4.7	2.4	3.8	1.4-11	100
	8	5.9	3.5	4.9	2.5-23	100
	12	5.9	2.6	5.3	2.1-14	100

^aDetection frequency.

1 **Table 4. Mean percent changes (standard error) [partial R^2]^a of serum concentrations of PF_{AA} in children, ($n=198$, aged 4, 8, and 12 years),**
 2 **per unit change of each variable, assessed via multiple linear regression analysis^b**

	Drinking water exposure (Cumulative PF _{AA} months)	Maternal serum conc (ng g ⁻¹ serum)	Time of breastfeeding (Months)	Sampling year (Years)	Weight (kg)	Sex	R^{2c}	n
PFCA								
PFHpA ^d	1.5 (5.0) p=0.76	e	e	-	-0.47 (0.60) p=0.43	-	0.14	188
PFOA	1.2 (0.41) [0.06] p=0.004	10 (3.4) [0.04] p=0.001	1.4 (1.2) p=0.25	-7.6 (1.5) [0.12] p<0.001	-1.0 (0.46) [0.05] p=0.039	-	0.30	148
PFNA	^f	15 (19) p=0.36	-0.31 (1.5) p=0.83	-	-	-	0.02	149
PFDA	^f	12 (35) p=0.67	2.2 (1.3) p=0.090	-	-	-8.1 (5.8) p=0.17	0.06	151
PFSA								
PFBS ^d	207 (52) [0.20] p<0.001	e	e	-	-	-	0.22	198
Total PFHxS	7.4 (0.53) [0.41] p<0.001	10 (1.6) [0.11] p<0.001	3.9 (2.0) [0.01] p=0.046	7.5 (3.0) [0.01] p=0.009	-1.1 (0.75) p=0.16	-	0.75	147
%br PFHxS	0.11 (0.02) [0.15] p<0.001	-0.06 (0.08) p=0.48	-0.16 (0.08) p=0.054	-0.32 (0.12) [0.03] p=0.009	-	-	0.20	153
Total PFOS	0.93 (0.44) [0.02] p=0.034	1.3 (0.55) [0.03] p=0.015	3.8 (1.5) [0.04] p=0.010	-3.4 (2.1) p=0.10	-0.32 (0.57) p=0.58	-11 (6.2) p=0.081	0.16	148
%br PFOS	0.14 (0.06) [0.02] p=0.033	0.28 (0.10) [0.04] p=0.004	-0.006 (0.22) p=0.98	-	-	-	0.07	151

3 - = the covariate was not significantly associated in the univariate linear regression ($p \geq 0.1$) and was therefore not included in the total model.

4 ^aPartial coefficient of determination from stepwise regression analyses.

5 ^bThe categories age 4, 8, and 12 years, with age 4 as the reference category, were also adjusted for in the multiple linear regression analyses.

6 ^cCoefficient of determination for the whole regression model.

7 ^d>25% below MQL.

8 ^eNot included in the regression model due to short half-life.

9 ^fNot detected in the drinking water.

Table 5. Percent change (standard error) [partial R^2]^a in PFAA serum concentrations per unit change of maternal PFAA serum concentration (ng g⁻¹ serum) and nursing duration (months) in children at 4 ($n=57$), 8 ($n=55$), and 12 ($n=119$) years of age, assessed via multiple linear regression analysis^b

	Age	Maternal serum conc (ng g ⁻¹ serum)	Time of breastfeeding (months)	R^{2c}	n
PFOA	4	29 (7.8) [0.24] p<0.001	5.1 (2.4) [0.05] p=0.034	0.42	52
	8	3.1 (7.1) p=0.66	1.7 (2.2) p=0.42	0.42	40
	12	8.4 (3.9) [0.04] p=0.026	-1.9 (1.3) p=0.15	0.21	87
Total PFHxS	4	11 (1.7) [0.29] p<0.001	7.6 (4.1) p=0.053	0.69	52
	8	9.7 (5.6) p=0.071	-0.18 (3.0) p=0.95	0.73	40
	12	7.2 (5.6) p=0.18	2.7 (2.5) p=0.28	0.68	85
Total PFOS	4	5.6 (1.5) [0.24] p=0.001	7.9 (2.8) [0.10] p=0.005	0.39	51
	8	-0.86 (1.2) p=0.49	5.3 (2.6) [0.08] p=0.038	0.27	40
	12	1.1 (0.59) p=0.054	1.7 (1.7) p=0.34	0.13	86
%br PFOS	4	0.20 (0.14) p=0.14	0.33 (0.41) p=0.43	0	51
	8	0.45 (0.20) [0.09] p=0.031	0.20 (0.41) p=0.64	0.09	40
	12	0.33 (0.15) [0.04] p=0.038	-0.15 (0.27) p=0.58	0.15	86

^aPartial coefficient of determination from stepwise regression analyses.

^bDrinking water exposure (Cumulative PFAA months) were also adjusted for in the models and the covariates sampling year, age, and sex if they were significantly associated in the univariate linear regression ($p \geq 0.1$).

^cCoefficient of determination for the whole regression model.

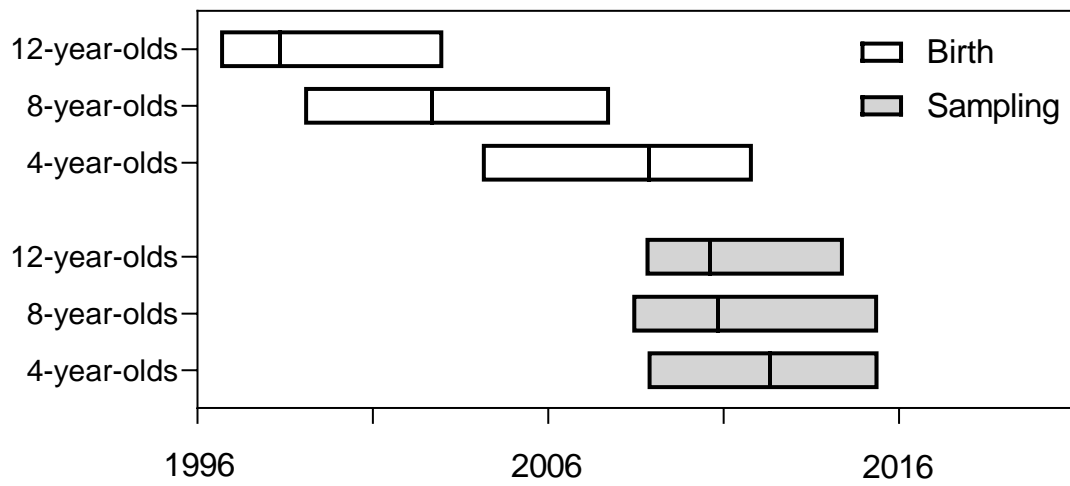


Figure 1. Years of birth and sampling period with median, for the children in the present study at 4 (n=57), 8 (n=55), and 12 (n=119) years of age.

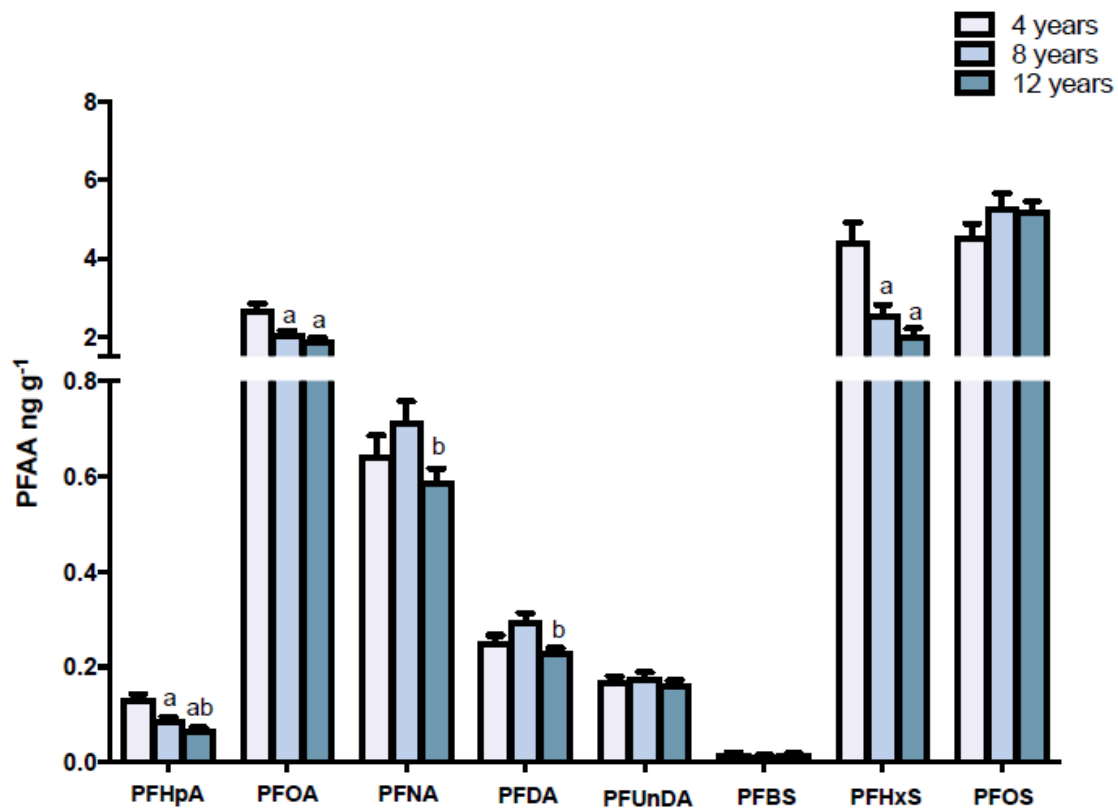


Figure 2. Concentrations of PFAA in children at 4 ($n=50$), 8 ($n=49$), and 12 ($n=99$) years of age, sampled 2008-2015. Concentrations are shown as least square means and standard error (SE) determined by general linear model (GLM) analysis adjusted for sampling year and drinking water exposure (cumulative PFAA months). a = significantly different from 4-year-olds and b = significantly different from 8-year-olds ($p<0.05$).

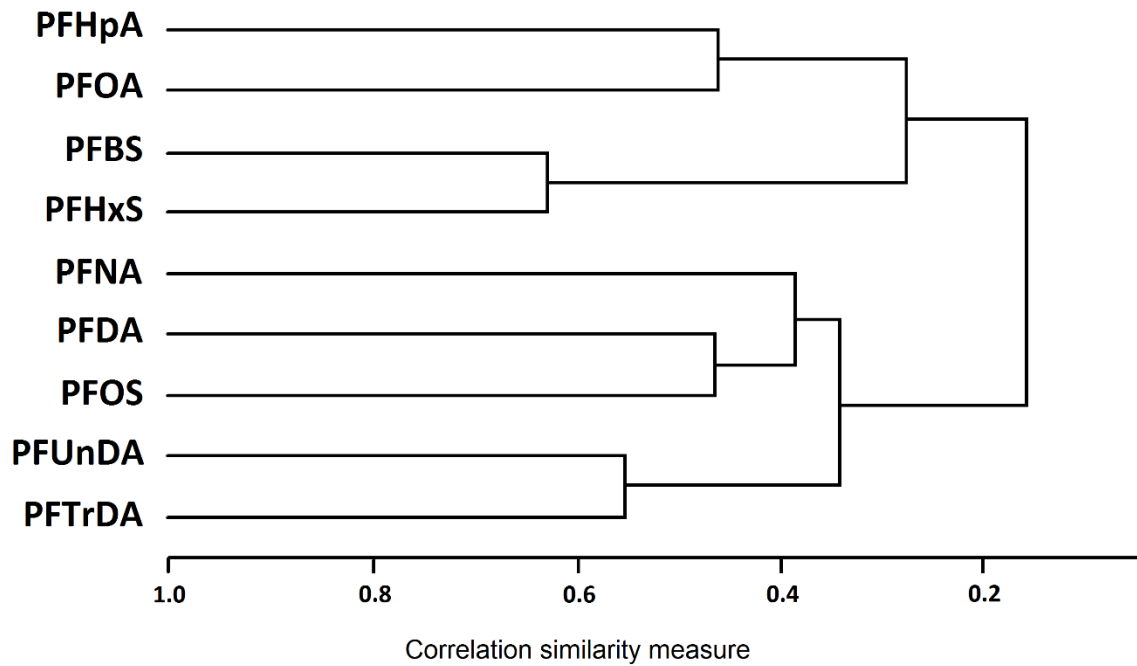


Figure 3. Cluster analysis of PFAS based on correlations between serum concentration in children at 4, 8, and 12 years of age ($n=198$), sampled 2008-2015, using average linkage cluster analysis, which is a hierarchical analysis clustering method based on the average distance between all pairs of objects.

Supporting Information

PFAAs included in the study (Table A1). Target compounds and selected instrumental parameters for quantification of each compound by UPLC/ESI-MS/MS (Table A2).

Summary of analysis of in-house reference material (pooled human serum) analyzed together with real samples to assess inter-batch precision (i.e. reproducibility) (Table A3). PFCA concentrations measured in 3 replicates of NIST SRM 1957 compared to reference values (Table A4). PFSA concentrations measured in 3 replicates of NIST SRM 1957 compared to reference values (Table A5). Calculation example of cumulative exposure to PFAA from drinking water (Table A6). Blood PFAA concentrations in children from other studies (Table A7). Mean percent changes of serum PFAA concentrations including all children and results from the age categories, with age 4 as the reference category (Table A8). Mean percent changes of serum PFAA concentrations including all children and all variables in all multiple linear regression analyses (Table A9). Mean percent changes of serum PFAA concentrations for the three age groups (4, 8, and 12) separately Table A10).

Acknowledgements

This research was financially supported by the Swedish Research Council Formas (SafeDrink, grant no 222-2012-2124), and the Swedish Environmental Protection Agency (contract no 2215-15-001). Participating mothers and children are thanked for their dedication to the study. Ingalill Gadhasson, Anne-Sofie Kärsrud, and Tomas Alsberg are thanked for excellent laboratory and instrument support. We are also very grateful to midwife Marianne Leimar and nurse Christine Strömbom responsible for recruitment and blood sampling of mothers and children for doing an excellent job.

Disclosures

The authors declare that they have no actual or competing financial interest.

References

1. D'Hollander, W.; de Voogt, P.; De Coen, W.; Bervoets, L., Perfluorinated substances in human food and other sources of human exposure. *Rev Environ Contam Toxicol* **2010**, *208*, 179-215.
2. Gebbink, W. A.; Berger, U.; Cousins, I. T., Estimating human exposure to PFOS isomers and PFCA homologues: the relative importance of direct and indirect (precursor) exposure. *Environ Int* **2015**, *74*, 160-9.
3. Vestergren, R.; Berger, U.; Glynn, A.; Cousins, I. T., Dietary exposure to perfluoroalkyl acids for the Swedish population in 1999, 2005 and 2010. *Environ Int* **2012**, *49*, 120-7.
4. D'Eon J, C.; Mabury, S. A., Is indirect exposure a significant contributor to the burden of perfluorinated acids observed in humans? *Environ Sci Technol* **2011**, *45*, (19), 7974-84.
5. Martin, J. W.; Asher, B. J.; Beesoon, S.; Benskin, J. P.; Ross, M. S., PFOS or PreFOS? Are perfluorooctane sulfonate precursors (PreFOS) important determinants of human and environmental perfluorooctane sulfonate (PFOS) exposure? *J Environ Monit* **2010**, *12*, (11), 1979-2004.
6. Giesy, J. P.; Kannan, K., Global distribution of perfluorooctane sulfonate in wildlife. *Environ Sci Technol* **2001**, *35*, (7), 1339-42.
7. Kannan, K.; Corsolini, S.; Falandysz, J.; Fillmann, G.; Kumar, K. S.; Loganathan, B. G.; Mohd, M. A.; Olivero, J.; Van Wouwe, N.; Yang, J. H.; Aldoust, K. M., Perfluorooctanesulfonate and related fluorochemicals in human blood from several countries. *Environ Sci Technol* **2004**, *38*, (17), 4489-95.
8. Gutzkow, K. B.; Haug, L. S.; Thomsen, C.; Sabaredzovic, A.; Becher, G.; Brunborg, G., Placental transfer of perfluorinated compounds is selective--a Norwegian Mother and Child sub-cohort study. *Int J Hyg Environ Health* **2012**, *215*, (2), 216-9.
9. Beesoon, S.; Webster, G. M.; Shoeib, M.; Harner, T.; Benskin, J. P.; Martin, J. W., Isomer profiles of perfluorochemicals in matched maternal, cord, and house dust samples: manufacturing sources and transplacental transfer. *Environ Health Perspect* **2011**, *119*, (11), 1659-64.
10. Chen, F.; Yin, S.; Kelly, B. C.; Liu, W., Isomer-Specific Transplacental Transfer of Perfluoroalkyl Acids: Results from a Survey of Paired Maternal, Cord Sera, and Placentas. *Environ Sci Technol* **2017**, *51*, (10), 5756-5763.
11. Yang, L.; Wang, Z.; Shi, Y.; Li, J.; Wang, Y.; Zhao, Y.; Wu, Y.; Cai, Z., Human placental transfer of perfluoroalkyl acid precursors: Levels and profiles in paired maternal and cord serum. *Chemosphere* **2016**, *144*, 1631-8.
12. Kärrman, A.; Ericson, I.; van Bavel, B.; Darnerud, P. O.; Aune, M.; Glynn, A.; Lignell, S.; Lindstrom, G., Exposure of perfluorinated chemicals through lactation: levels of matched human milk and serum and a temporal trend, 1996-2004, in Sweden. *Environ Health Perspect* **2007**, *115*, (2), 226-30.
13. Kubwabo, C.; Kosarac, I.; Lalonde, K., Determination of selected perfluorinated compounds and polyfluoroalkyl phosphate surfactants in human milk. *Chemosphere* **2013**, *91*, (6), 771-7.

14. Sundström, M.; Ehresman, D. J.; Bignert, A.; Butenhoff, J. L.; Olsen, G. W.; Chang, S. C.; Bergman, A., A temporal trend study (1972-2008) of perfluorooctanesulfonate, perfluorohexanesulfonate, and perfluorooctanoate in pooled human milk samples from Stockholm, Sweden. *Environ Int* **2011**, *37*, (1), 178-83.
15. Apelberg, B. J.; Goldman, L. R.; Calafat, A. M.; Herbstman, J. B.; Kuklenyik, Z.; Heidler, J.; Needham, L. L.; Halden, R. U.; Witter, F. R., Determinants of fetal exposure to polyfluoroalkyl compounds in Baltimore, Maryland. *Environ Sci Technol* **2007**, *41*, (11), 3891-7.
16. Fei, C.; McLaughlin, J. K.; Tarone, R. E.; Olsen, J., Perfluorinated chemicals and fetal growth: a study within the Danish National Birth Cohort. *Environ Health Perspect* **2007**, *115*, (11), 1677-82.
17. Fromme, H.; Mosch, C.; Morovitz, M.; Alba-Alejandre, I.; Boehmer, S.; Kiranoglu, M.; Faber, F.; Hannibal, I.; Genzel-Boroviczeny, O.; Koletzko, B.; Volkel, W., Pre- and postnatal exposure to perfluorinated compounds (PFCs). *Environ Sci Technol* **2010**, *44*, (18), 7123-9.
18. Liu, J.; Li, J.; Liu, Y.; Chan, H. M.; Zhao, Y.; Cai, Z.; Wu, Y., Comparison on gestation and lactation exposure of perfluorinated compounds for newborns. *Environ Int* **2011**, *37*, (7), 1206-12.
19. Cariou, R.; Veyrand, B.; Yamada, A.; Berrebi, A.; Zalko, D.; Durand, S.; Pollono, C.; Marchand, P.; Leblanc, J. C.; Antignac, J. P.; Le Bizec, B., Perfluoroalkyl acid (PFAA) levels and profiles in breast milk, maternal and cord serum of French women and their newborns. *Environ Int* **2015**, *84*, 71-81.
20. Verner, M. A.; Ngueta, G.; Jensen, E. T.; Fromme, H.; Volkel, W.; Nygaard, U. C.; Granum, B.; Longnecker, M. P., A Simple Pharmacokinetic Model of Prenatal and Postnatal Exposure to Perfluoroalkyl Substances (PFASs). *Environ Sci Technol* **2016**, *50*, (2), 978-86.
21. Mogensen, U. B.; Grandjean, P.; Nielsen, F.; Weihe, P.; Budtz-Jorgensen, E., Breastfeeding as an Exposure Pathway for Perfluorinated Alkylates. *Environ Sci Technol* **2015**, *49*, (17), 10466-73.
22. Papadopoulou, E.; Sabaredzovic, A.; Namork, E.; Nygaard, U. C.; Granum, B.; Haug, L. S., Exposure of Norwegian toddlers to perfluoroalkyl substances (PFAS): The association with breastfeeding and maternal PFAS concentrations. *Environ Int* **2016**, *94*, 687-94.
23. Haug, L. S.; Huber, S.; Becher, G.; Thomsen, C., Characterisation of human exposure pathways to perfluorinated compounds--comparing exposure estimates with biomarkers of exposure. *Environ Int* **2011**, *37*, (4), 687-93.
24. Mondal, D.; Lopez-Espinosa, M. J.; Armstrong, B.; Stein, C. R.; Fletcher, T., Relationships of perfluorooctanoate and perfluorooctane sulfonate serum concentrations between mother-child pairs in a population with perfluorooctanoate exposure from drinking water. *Environ Health Perspect* **2012**, *120*, (5), 752-7.
25. Holzer, J.; Midasch, O.; Rauchfuss, K.; Kraft, M.; Reupert, R.; Angerer, J.; Kleeschulte, P.; Marschall, N.; Wilhelm, M., Biomonitoring of perfluorinated compounds in children and adults exposed to perfluorooctanoate-contaminated drinking water. *Environ Health Perspect* **2008**, *116*, (5), 651-7.
26. Winkens, K.; Vestergren, R.; Berger, U.; Cousins, I. T., Early life exposure to per- and polyfluoroalkyl substances (PFASs): A critical review. *Emerging Contaminants* **2017**, *3*, (2), 55-68.
27. Bach, C. C.; Bech, B. H.; Nohr, E. A.; Olsen, J.; Matthiesen, N. B.; Bonefeld-Jorgensen, E. C.; Bossi, R.; Henriksen, T. B., Perfluoroalkyl Acids in Maternal Serum and Indices of Fetal Growth: The Aarhus Birth Cohort. *Environ Health Perspect* **2016**, *124*, (6), 848-54.
28. Johnson, P. I.; Sutton, P.; Atchley, D. S.; Koustas, E.; Lam, J.; Sen, S.; Robinson, K. A.; Axelrad, D. A.; Woodruff, T. J., The Navigation Guide - evidence-based medicine meets environmental health: systematic review of human evidence for PFOA effects on fetal growth. *Environ Health Perspect* **2014**, *122*, (10), 1028-39.
29. Gyllenhammar, I.; Diderholm, B.; Gustafsson, J.; Berger, U.; Ridefelt, P.; Benskin, J. P.; Lignell, S.; Lampa, E.; Glynn, A., Perfluoroalkyl acid levels in first-time mothers in relation to offspring weight gain and growth. *Environ Int* **2018**, *111*, 191-199.

30. Braun, J. M., Early-life exposure to EDCs: role in childhood obesity and neurodevelopment. *Nat Rev Endocrinol* **2017**, *13*, (3), 161-173.
31. Halldorsson, T. I.; Rytter, D.; Haug, L. S.; Bech, B. H.; Danielsen, I.; Becher, G.; Henriksen, T. B.; Olsen, S. F., Prenatal exposure to perfluorooctanoate and risk of overweight at 20 years of age: a prospective cohort study. *Environ Health Perspect* **2012**, *120*, (5), 668-73.
32. Braun, J. M.; Chen, A.; Romano, M. E.; Calafat, A. M.; Webster, G. M.; Yolton, K.; Lanphear, B. P., Prenatal perfluoroalkyl substance exposure and child adiposity at 8 years of age: The HOME study. *Obesity (Silver Spring)* **2016**, *24*, (1), 231-7.
33. Mora, A. M.; Oken, E.; Rifas-Shiman, S. L.; Webster, T. F.; Gillman, M. W.; Calafat, A. M.; Ye, X.; Sagiv, S. K., Prenatal Exposure to Perfluoroalkyl Substances and Adiposity in Early and Mid-Childhood. *Environ Health Perspect* **2017**, *125*, (3), 467-473.
34. Maisonet, M.; Terrell, M. L.; McGeehin, M. A.; Christensen, K. Y.; Holmes, A.; Calafat, A. M.; Marcus, M., Maternal concentrations of polyfluoroalkyl compounds during pregnancy and fetal and postnatal growth in British girls. *Environ Health Perspect* **2012**, *120*, (10), 1432-7.
35. NTP, Immunotoxicity Associated with Exposure to Perfluorooctanoic Acid or Perfluorooctane Sulfonate. In Services, U. S. D. o. H. a. H., Ed. National Toxicology Program: 2016.
36. Grandjean, P.; Heilmann, C.; Weihe, P.; Nielsen, F.; Mogensen, U. B.; Budtz-Jorgensen, E., Serum Vaccine Antibody Concentrations in Adolescents Exposed to Perfluorinated Compounds. *Environ Health Perspect* **2017**, *125*, (7), 077018.
37. Gyllenhammar, I.; Berger, U.; Sundstrom, M.; McCleaf, P.; Euren, K.; Eriksson, S.; Ahlgren, S.; Lignell, S.; Aune, M.; Kotova, N.; Glynn, A., Influence of contaminated drinking water on perfluoroalkyl acid levels in human serum--A case study from Uppsala, Sweden. *Environ Res* **2015**, *140*, 673-83.
38. Gyllenhammar, I.; Benskin, J. P.; Sandblom, O.; Berger, U.; Ahrens, L.; Lignell, S.; Wiberg, K.; Glynn, A., Perfluoroalkyl Acids (PFAAs) in Serum from 2-4-Month-Old Infants: Influence of Maternal Serum Concentration, Gestational Age, Breast-Feeding, and Contaminated Drinking Water. *Environ Sci Technol* **2018**, *52*, (12), 7101-7110.
39. Glynn, A.; Aune, M.; Darnerud, P. O.; Cnattingius, S.; Bjerselius, R.; Becker, W.; Lignell, S., Determinants of serum concentrations of organochlorine compounds in Swedish pregnant women: a cross-sectional study. *Environ Health* **2007**, *6*, 2.
40. Lignell, S.; Aune, M.; Darnerud, P. O.; Cnattingius, S.; Glynn, A., Persistent organochlorine and organobromine compounds in mother's milk from Sweden 1996-2006: compound-specific temporal trends. *Environ Res* **2009**, *109*, (6), 760-7.
41. Riddell, N.; Arsenault, G.; Benskin, J. P.; Chittim, B.; Martin, J. W.; McAlees, A.; McCrindle, R., Branched perfluorooctane sulfonate isomer quantification and characterization in blood serum samples by HPLC/ESI-MS(/MS). *Environ Sci Technol* **2009**, *43*, (20), 7902-8.
42. Glynn, A., Perfluorerede alkylysyror (PFAA) i Uppsala dricksvatten. In *Riskvärdering*, Division, R. B. A., Ed. Swedish National Food Agency: 2012; Vol. dnr 1192/2012.
43. Russell, M. H.; Himmelstein, M. W.; Buck, R. C., Inhalation and oral toxicokinetics of 6:2 FTOH and its metabolites in mammals. *Chemosphere* **2015**, *120*, 328-35.
44. Olsen, G. W.; Chang, S. C.; Noker, P. E.; Gorman, G. S.; Ehresman, D. J.; Lieder, P. H.; Butenhoff, J. L., A comparison of the pharmacokinetics of perfluorobutanesulfonate (PFBS) in rats, monkeys, and humans. *Toxicology* **2009**, *256*, (1-2), 65-74.
45. Li, Y.; Fletcher, T.; Mucs, D.; Scott, K.; Lindh, C. H.; Tallving, P.; Jakobsson, K., Half-lives of PFOS, PFHxS and PFOA after end of exposure to contaminated drinking water. *Occup Environ Med* **2018**, *75*, (1), 46-51.
46. Harris, M. H.; Rifas-Shiman, S. L.; Calafat, A. M.; Ye, X.; Mora, A. M.; Webster, T. F.; Oken, E.; Sagiv, S. K., Predictors of Per- and Polyfluoroalkyl Substance (PFAS) Plasma Concentrations in 6-10 Year Old American Children. *Environ Sci Technol* **2017**, *51*, (9), 5193-5204.

47. Wu, X. M.; Bennett, D. H.; Calafat, A. M.; Kato, K.; Strynar, M.; Andersen, E.; Moran, R. E.; Tancredi, D. J.; Tulse, N. S.; Hertz-Picciotto, I., Serum concentrations of perfluorinated compounds (PFC) among selected populations of children and adults in California. *Environ Res* **2015**, *136*, 264-73.
48. Morck, T. A.; Nielsen, F.; Nielsen, J. K.; Siersma, V. D.; Grandjean, P.; Knudsen, L. E., PFAS concentrations in plasma samples from Danish school children and their mothers. *Chemosphere* **2015**, *129*, 203-9.
49. Schechter, A.; Malik-Bass, N.; Calafat, A. M.; Kato, K.; Colacino, J. A.; Gent, T. L.; Hynan, L. S.; Harris, T. R.; Malla, S.; Birnbaum, L., Polyfluoroalkyl compounds in Texas children from birth through 12 years of age. *Environ Health Perspect* **2012**, *120*, (4), 590-4.
50. Wilhelm, M.; Wittsiepe, J.; Volkel, W.; Fromme, H.; Kasper-Sonnenberg, M., Perfluoroalkyl acids in children and their mothers: Association with drinking water and time trends of inner exposures--Results of the Duisburg birth cohort and Bochum cohort studies. *Int J Hyg Environ Health* **2015**, *218*, (7), 645-55.
51. Trasande, L.; Koshy, T. T.; Gilbert, J.; Burdine, L. K.; Attina, T. M.; Ghassabian, A.; Honda, M.; Marmor, M.; Chu, D. B.; Han, X.; Shao, Y.; Kannan, K., Serum perfluoroalkyl substances in children exposed to the world trade center disaster. *Environ Res* **2017**, *154*, 212-221.
52. Kim, D. H.; Lee, M. Y.; Oh, J. E., Perfluorinated compounds in serum and urine samples from children aged 5-13 years in South Korea. *Environ Pollut* **2014**, *192*, 171-8.
53. Zeng, X. W.; Qian, Z.; Emo, B.; Vaughn, M.; Bao, J.; Qin, X. D.; Zhu, Y.; Li, J.; Lee, Y. L.; Dong, G. H., Association of polyfluoroalkyl chemical exposure with serum lipids in children. *Sci Total Environ* **2015**, *512-513*, 364-70.
54. Benskin, J. P.; De Silva, A. O.; Martin, J. W., Isomer profiling of perfluorinated substances as a tool for source tracking: a review of early findings and future applications. *Rev Environ Contam Toxicol* **2010**, *208*, 111-60.
55. Gebbink, W. A.; Glynn, A.; Berger, U., Temporal changes (1997-2012) of perfluoroalkyl acids and selected precursors (including isomers) in Swedish human serum. *Environ Pollut* **2015**, *199C*, 166-173.
56. Zhang, Y.; Beesoon, S.; Zhu, L.; Martin, J. W., Biomonitoring of perfluoroalkyl acids in human urine and estimates of biological half-life. *Environ Sci Technol* **2013**, *47*, (18), 10619-27.
57. Gao, Y.; Fu, J.; Cao, H.; Wang, Y.; Zhang, A.; Liang, Y.; Wang, T.; Zhao, C.; Jiang, G., Differential accumulation and elimination behavior of perfluoroalkyl Acid isomers in occupational workers in a manufactory in China. *Environ Sci Technol* **2015**, *49*, (11), 6953-62.
58. Kim, S. K.; Lee, K. T.; Kang, C. S.; Tao, L.; Kannan, K.; Kim, K. R.; Kim, C. K.; Lee, J. S.; Park, P. S.; Yoo, Y. W.; Ha, J. Y.; Shin, Y. S.; Lee, J. H., Distribution of perfluorochemicals between sera and milk from the same mothers and implications for prenatal and postnatal exposures. *Environ Pollut* **2011**, *159*, (1), 169-174.
59. Pan, Y.; Zhu, Y.; Zheng, T.; Cui, Q.; Buka, S. L.; Zhang, B.; Guo, Y.; Xia, W.; Yeung, L. W.; Li, Y.; Zhou, A.; Qiu, L.; Liu, H.; Jiang, M.; Wu, C.; Xu, S.; Dai, J., Novel Chlorinated Polyfluorinated Ether Sulfonates and Legacy Per-/Polyfluoroalkyl Substances: Placental Transfer and Relationship with Serum Albumin and Glomerular Filtration Rate. *Environ Sci Technol* **2017**, *51*, (1), 634-644.
60. Mondal, D.; Weldon, R. H.; Armstrong, B. G.; Gibson, L. J.; Lopez-Espinosa, M. J.; Shin, H. M.; Fletcher, T., Breastfeeding: a potential excretion route for mothers and implications for infant exposure to perfluoroalkyl acids. *Environ Health Perspect* **2014**, *122*, (2), 187-92.
61. Loccisano, A. E.; Longnecker, M. P.; Campbell, J. L., Jr.; Andersen, M. E.; Clewell, H. J., 3rd, Development of PBPK models for PFOA and PFOS for human pregnancy and lactation life stages. *J Toxicol Environ Health A* **2013**, *76*, (1), 25-57.
62. Kärrman, A.; Elgh-Dalgren, K.; Lafossas, C.; Mørskeland, T., Environmental levels and distribution of structural isomers of perfluoroalkyl acids after aqueous fire-fighting foam (AFFF) contamination. *Environmental Chemistry* **2011**, *8*, (4), 372-380.

