**Perfluoroalkyl acids (PFAAs) in serum from 2-4-month-old infants – influence of maternal serum concentrations, gestational age, breastfeeding and contaminated drinking water**

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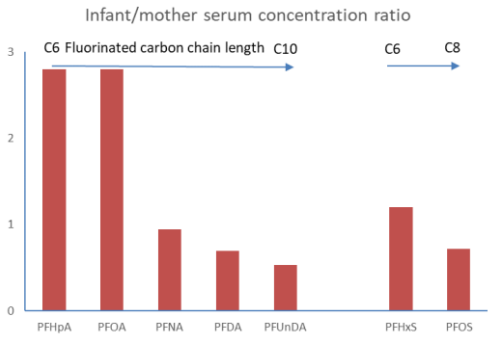
## Abstract

Little is known about factors influencing infant perfluorinated alkyl acid (PFAA) concentrations. Associations between serum PFAA concentrations in 2-4-month-old infants (*n*=101) and determinants were investigated by multiple linear regression and General Linear Model (GLM) analysis. In exclusively breastfed infants, maternal serum PFAA concentrations 3 weeks after delivery explained 13% (perfluoroundecanoic acid, PFUnDA) to 73% (perfluorohexane sulfonate, PFHxS) of infant PFAA concentration variation. Median infant/maternal ratios decreased with increasing PFAA carbon chain length from 2.8 for perfluoroheptanoic acid (PFHpA) and perfluorooctanoic acid (PFOA) to 0.53 for PFUnDA, and from 1.2 to 0.69 for PFHxS and perfluorooctane sulfonate (PFOS). Infant PFOA, perfluorononanoic acid (PFNA) and PFOS increased 0.7-1.2% per day of gestational age. Bottle-fed infants had 2 times lower mean concentrations of PFAAs, and a higher mean percentage of branched (%br) PFOS isomers, than exclusively breastfed infants. PFOA, PFNA and PFHxS increased 8-11% per week of exclusive breastfeeding. Infants living in an area receiving PFAA-contaminated drinking water had 3-fold higher mean perfluorobutane sulfonate (PFBS) and PFHxS concentrations, and higher mean %br PFHxS. Pre- and post-natal PFAA exposure significantly contribute to infant PFAA serum concentrations, depending on PFAA carbon-chain length. Moderately PFBS- and PFHxS-contaminated drinking water is an important indirect exposure source.

## TOC Graphic


Infant/mother serum concentration ratio
C6 Fluorinated carbon chain length
C10
C6
C8
3
2
1
0
PFHpA
PFOA
PFNA
PFDA
PFUnDA
PFHxS
PFOS

## Introduction

Per- and polyfluoroalkyl substances (PFASs) are synthetic fluorinated substances used in numerous commercial applications and occurring ubiquitously in humans and the environment. Sources of human exposure include food, drinking water, dust, air and the use of PFAS-containing consumer products.[1-4] Among the ~3000 known PFASs,[5] perfluoroalkyl acids (PFAAs) are of greatest concern due to their persistence and chain length-dependent bioaccumulation potential.[6] PFAAs may be formed from degradation of other PFASs (commonly referred to as PFAA precursors).[7,8]

PFAAs could cross the placenta and are excreted in mother´s milk.[9-11] Modeling of infant exposure to some PFAAs suggests that *in utero* exposure and breastfeeding are important determinants of blood concentrations.[12,13] Food, drinking water, dust and air contribute to exposure when the child gets older.[4,14-17] High early life exposure to some PFAAs is related to lower birth weight and immune toxicity, [18-20] and knowledge of determinants of PFAS concentrations in infant blood is urgently needed for identification of infant exposure sources. Moreover, as pointed out by Loccisano et al.[12] more data on PFAA concentrations in infants is crucial for development/refinement of infant physiologically-based pharmacokinetic (PBPK) models, which are important for risk assessment. To our knowledge only one study has reported PFAA concentrations in infants (6 months old),[21] showing higher median concentrations of perfluorohexane sulfonate (PFHxS), perfluorooctane sulfonate (PFOS), perfluorooctanoic acid (PFOA) and perfluorononanoic acid (PFNA) in infant serum than in cord serum from the same study participants. The increase was most likely due to exposure during breastfeeding.[21]

The aim of the present study was to investigate maternal to infant transfer of PFHxS (linear and branched isomers), PFOS (linear and branched isomers), perfluoroheptanoic acid (PFHpA), PFOA, PFNA, perfluorodecanoic acid (PFDA), and perfluoroundecanoic acid (PFUnDA), as well as influence of potential determining factors of infant serum concentrations, such as maternal PFAA concentrations close to delivery, duration of *in utero* exposure (days of gestational age at delivery), duration of breastfeeding, infant weight gain, and maternal exposure to PFAA-contaminated drinking water.

## Methods

### Study Population

The study is based on samples and data collected within the POPUP cohort (Persistent Organic Pollutants in Uppsala Primiparas), an on-going investigation of POPs in first-time mothers and their children that started in 1996.[22] The present study is based on recruitment of mother/child pairs 1996-1999. From early fall 1996 to May 1999, 395 randomly selected primipara women in late pregnancy were asked to participate in the study.[23] The Swedish speaking women had been recruited in early pregnancy as controls in a case-control study of caffeine as a risk factor for miscarriage.[24] In total 325 women (82%) agreed to participate in the POPUP study.

The participating women were asked if a maternal blood sample could be taken 3 weeks after delivery and an infant sample about 3 months after delivery. The mothers were Swedish-born and had singleton births with no birth defects. Almost all women had a full-term pregnancy (37–42 weeks), except in a few cases with the shortest gestational age being 35 weeks and the longest 43 weeks. Women in the POPUP cohort are homogenous regarding ethnic background. In 2006, only about 4% of the Swedish population had one or two foreign-

born parents.[25] Maternal sampling 3 weeks after delivery was accepted by 211 participants. Infant sampling was accepted by 138 participants. Due to limited blood volumes 101 maternal and 107 infant samples were available for PFAA analyses (Table 1). In total 101 matched mother/infant samples were available. Informed consent was obtained from all participating women. The study was approved by the Ethics Committee of the Medical Faculty at Uppsala University (2004:M-177).

**Interviews, Questionnaires and Blood sampling**

In-person interviews, using a structured questionnaire, were conducted at 6-12 and 32-34 completed gestational weeks.[24] Height of the women was measured and women were weighed on both interview occasions. Data on maternal characteristics included age, pre-pregnancy body mass index (BMI), years of education, place of residence, and smoking habits. At the infant sampling, the mothers answered a self-administered questionnaire including questions about delivery, and personal characteristics not covered by the interviews, such as data on breast-feeding. Vacutainer® or Vacuette® serum tubes were used for blood sampling, and after separation serum was stored at -20°C until analysis.

**Chemical Analyses**

The target analytes included $C_4$, $C_6$ and $C_8$ perfluoroalkane sulfonic acids (PFSAs; PFBS, PFHxS, PFOS) and $C_6$-$C_{15}$ perfluoroalkyl carboxylic acids (PFCAs; PFHxA, PFHpA, PFOA, PFNA, PFDA, PFUnDA, PFDoDA, PFTrDA, PFTeDA, PFPeDA; for details see Supporting Information, Table A1). The serum samples were analyzed as described previously.[26] In short, serum (0.1 g for infants and 0.5 g for adults) was spiked with internal standards (Supporting Information, Table A2) and extracted with acetonitrile (Honeywell CHROMASOLV™, ≥99.95% purity) in an ultrasonic bath. The concentrated extract underwent dispersive clean-up

with graphitized carbon (Supelclean™ ENVI-Carb™, Sigma-Aldrich). Aqueous ammonium acetate (2mM; EMSURE®, Sigma-Aldrich) and volumetric standards ($^{13}C_8$-PFOS and $^{13}C_8$-PFOA; both from Wellington Labs) were added before instrumental analysis on an Acquity ultra performance liquid chromatography system (UPLC) coupled to a Xevo TQ-S tandem mass spectrometer (both Waters Corp., Milford, MA, U.S.) operated in negative electrospray ionization, multiple reaction monitoring mode. The mobile phase consisted of water and acetonitrile (ACN; both containing 2mM ammonium acetate), with the relative proportions varied under gradient conditions. ACN-based mobile phases such as that used here are highly effective at removing bile acid interferences which may affect PFOS and PFHxS analysis.[27-29] Further, instrumental parameters are provided in Supporting Information (Table A2). Quantification was performed by isotope dilution using a 5-point external 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, the linear isomer and the sum of all branched isomers (as one signal) were quantified separately using a linear isomer calibration curve. For PFOS, concentrations obtained from the *m/z* 499/80 and 499/99 ions were averaged, which provides a reasonable estimate of the weight % branched (%br) content in the absence of isomer-specific quantification.[30] PFHxS was also monitored using *m/z* 399/80 and 399/99 ions; however, since the branched isomer peak in the latter transition was often below method detection limits (MDLs), concentrations were only reported from the *m/z* 399/80 transition.

Both a procedural blank (0.1 or 0.5 mL Milli-Q water) and QC sample (pooled human serum analyzed repeatedly in-house) were included with every batch of samples (see Supporting Information, Table A3, for QC performance metrics). In addition, concentrations determined in $n = 3$ replicates of NIST SRM 1957 were compared to reference values to

assess the accuracy of the method (results provided in Supporting Information Tables A4 and A5). Measured concentrations in SRM 1957 were consistent with reference values for all targets, while coefficients of variation (CVs) in in-house reference serum ranged from 12 - 36% (median 19%; $n$ = 20; Table A3). For targets observable in method blanks, the MDL was based on the mean blank + 3× the standard deviation of the blanks. For those targets with no observable blank contamination, detection limits were based on the concentration producing a signal-to-noise ratio of 3 in sample chromatograms. Analyses of PFAAs in infants and their mothers were accomplished within three different analytical batches in 2013, 2014, and 2016. Batch-specific MDLs are provided in Table A6 of the Supporting Information. Further method validation parameters are provided in Glynn et al. 2012.[26]

**Statistical Analyses**

MINITAB 15® Statistical Software for Windows was used for all statistical analyses. When PFAA concentrations were below the method detection limit (MDL), MDL/√2 was taken as an estimated value in the statistical analyses. The PFAA concentrations in maternal serum were measured in different analytical batches and for PFHpA and PFBS the MDL differed between the batches (Supporting Information Table A6). In the statistical analysis including maternal PFAA data, all PFHpA and PFBS data below MDL in the batch with the highest MDL was omitted (batch 3, $n$=17). The proportions of branched and linear isomers for PFHxS and PFOS were expressed as a percentage of the total concentration. Correlations between infant and mother serum PFAA concentrations were investigated using the Pearson´s correlation test, and only pairs with data >MDL for both the mother and the infant were included in the analyses.

For PFAAs with over 75% of infant data >MDL multiple linear regressions were used to analyze if infant serum concentrations are influenced by maternal PFAA concentrations 3

weeks after delivery (ng $g^{-1}$), gestational age at delivery (days), infant weight gain (% of birth weight) and duration of breastfeeding (weeks) (Table 1). The inclusion of the potential determinants were based on the assumption that they may influence fetal/infant exposure (maternal PFAA concentrations, gestational age, duration of breastfeeding) or infant PFAA distribution volume (infant weight gain). In univariate analyses infant PFAA concentrations did not differ between sexes (Mann-Whitney U test, $p>0.05$), and infant sex was not included as a covariate in the regression models. In this analysis, only data from infants that were exclusively breastfed during the whole study period from birth to infant blood sampling were included. Stepwise regression analysis was used to estimate how much of the variation in PFAA concentrations that could be explained by the variation of each individual determinant (partial coefficient of determination, $R^2$). Criteria for determinant inclusion in the regression model was $\alpha<0.15$, but in the analyses determinants with $\alpha\geq0.15$ were forced to be included in order to assure that the same model was used in all analyses. Partial $R^2$ for each significant determinant ($p\leq0.05$) was calculated by subtraction of the $R^2$ of the model not including the determinant in question from the $R^2$ of the model including the determinant in question.

In separate general linear model (GLM) analyses, using all participating mother/infant pairs, the influence of breastfeeding on infant PFAA concentration was investigated. Maternal PFAA concentration was used as a covariate in the GLM model, and infants were divided into a group that were exclusively bottle-fed from birth to sampling, a group with mixed breast-/bottle-feeding, and infants that were exclusively breastfed.

As only three of the formula-fed infants were living in a city district that received contaminated drinking water, it was not possible to statistically analyze the influence of direct drinking water exposure from formula feeding. Instead, the influence of maternal drinking water PFAA exposure on infant PFAA concentrations was investigated by grouping participants according to home address in city districts receiving drinking water with different

contributions from PFAA-contaminated production well water. Uppsala County and the City of Uppsala can be divided into different drinking water districts depending on proportion of contaminated drinking water distributed to the districts.[31] There were two districts that did not receive any water from the contaminated wells from 1996 to 1999, comprised of i) areas outside the City of Uppsala and ii) District 1 within the city. District 2 in the City of Uppsala received drinking water containing <10% water from contaminated wells, District 3 received 10-90% contaminated water, and District 4 received >90% water from contaminated wells.[31] GLM analysis was used to calculate adjusted means of PFAA concentrations for the different city districts, with "days of gestational age", "infant weight gain after birth" and "duration of breastfeeding" included as covariates in the GLM model. Maternal PFAA concentration was not included as covariate since this would result in over-adjustment of the association between infant city district and infant PFAA concentrations. Maternal PFAA concentrations are on the casual pathway between city district and infant PFAA. Exclusively bottle-fed infants were not included in the analyses (N=5).

Logarithmically-transformed infant PFAA concentrations were used in the regression and GLM analyses, since the distribution of data closely followed a log-normal distribution. Observations with standardized residuals ≥3 were excluded from regression and GLM analysis due to their large influence on the results. The statistical significance level was set to $p \leq 0.05$.

## Results and Discussion

### Serum Concentrations in Infants and Their Mothers

PFOS showed the highest median concentration followed by PFOA, PFHxS, PFNA, PFDA and PFUnDA in both infants and mothers (Table 2). Concentrations of short-chain PFBS, PFHxA and PFHpA were in many cases <MDL. Among the long-chain PFCAs (≥7

fluorinated carbon atoms) median concentrations decreased with increasing chain length in both infants and mothers, with >50% of concentrations below MDL for PFDoDA, PFTrDA, PFTeDA and PFPeDA (Table 2). The observed concentrations differ from those reported for 6-month-old infants from Germany,[21] the only previously published infant study we could find in the literature. Different sampling periods (present study:1996-99, German study:2007-2009) and ages of the infants (present study:2-4 months, German study: 6 months) are most likely important determinants of the observed differences.

To our knowledge, no other study has reported isomer-specific data on PFHxS and PFOS for infants. We found similar %br PFOS and PFHxS in mothers and infants (Table 2), suggesting that infant isomer patterns to a large extent are dependent on maternal patterns. In Canadian mothers, median %br PFOS (36%) was similar to that of Swedish mothers, although Canadian mothers were sampled almost 10 years after the Swedish mothers.[32] In maternal serum from China, sampled 2015-2016, branched PFHxS accounted for 14% (median) of the total PFHxS and branched PFOS 17% of total PFOS.[33] A comparison of maternal %br PFHxS and PFOS between studies is hampered by differences in how branched content was determined in each study (e.g. isomer-specific versus sum branched isomer quantification using linear and/or individual branched isomer standards). Nevertheless, differences due to ongoing maternal exposure to for instance PFHxS and PFOS precursors in China are likely since perfluorooctane sulfonyl fluoride-based chemistry is still used in China.[33]

Maternal serum/plasma concentrations of PFHxS, PFOS, PFOA, PFNA, PFDA, and PFUnDA during pregnancy and a few weeks after delivery both reflect placental and mother´s milk PFAA transfer, as shown by strong correlations between PFAA concentrations in maternal and cord blood, and between concentrations in maternal blood and mother´s milk.[9,10,26,34,35] We found positive correlations between maternal and infant PFAA

concentrations, with weakening strength as chain length increased of both long-chain PFCAs and PFSAs (Table 3). This suggests that factors other than maternal concentrations become more important as determinants for infant PFAA concentrations as perfluoroalkyl chain length increases.

This is further supported by the decreased strength of association between maternal and infant concentrations with increased PFAA carbon chain length among exclusively breastfed infants, as determined in multiple log-linear regression analyses (Table 4). Variation in maternal PFOA concentrations explained 53% of the infant concentration variation, whereas only 13% of the variation in infant PFUnDA was explained by maternal variation. For PFHxS, 73% of the infant variation was explained by maternal variation, and the corresponding figure for PFOS was 36%. The short-chain PFHpA deviated from this pattern of chain-length dependency, showing a weaker infant/mother correlation than PFOA did (Table 3) and a weaker strength of association (Table 4). For PFAA homologues with less strong infant/mother correlations and associations, contribution of infant exposure sources not studied by us may be important, including ingestion of dust, suction on materials containing PFASs, inhalation of fine particles/air, and skin exposure through use of personal care products.[4] To what degree these different exposure pathways contribute to total infant exposure has not been fully characterized, but the contribution of each component most probably varies with infant age.[4]

Our results suggest that PFAA transfer efficiency from mother to infant decreases with increasing perfluoroalkyl chain length, as indicated by the infant/maternal serum concentration ratios (Table 3). On average, PFHpA and PFOA showed higher concentrations in infants than in mothers, PFHxS concentrations were similar, and concentrations for PFNA, PFOS, PFDA and PFUnDA were lower in infants. This is in agreement with the previously observed chain length-dependent decrease in transfer efficiency over the placenta and from

maternal blood to mother´s milk.[9,32,36,37] It has been proposed that decreases in PFAA placental transfer with increased chain length may be due to decreases in water solubility and increases in PFAA affinity to serum albumin.[4,37] It has also been hypothesized that there are chain length differences in active PFAA transport in the placenta,[4,11] although this hypothesis has to our knowledge not been tested. Similarly, it is not known to what degree mammary gland active PFAA transport is involved in the carbon chain-dependency of PFAA transfer to mother´s milk.

In contrast to Fromme et al.[21], our study does not include matched cord blood data. We have previously published cord whole blood data for PFOS, PFOA and PFNA in the Uppsala study 1996-99.[26] Assuming a distribution factor of 2 between serum and whole blood, average cord serum concentrations were 2-3 times lower than those in maternal serum sampled in late pregnancy,[26] which is in accordance with data from Fromme et al.[21] A comparison of published cord/maternal serum or plasma PFAA ratios with the infant/maternal serum PFAA ratios among Uppsala infants (Table 3) and German infants (plasma) show that the infant/maternal ratios generally are higher than the cord/maternal rations.[21,32,36] This strongly suggests that breastfeeding gives a significant contribution to the PFAA exposure of the infants.

The similar median ratios of infant/maternal linear and branched PFHxS, as well as of PFOS isomers (Table 3), show that linear and branched isomers were retained in the infants to a similar degree as in their mothers. A few studies have reported less efficient placental transfer of linear PFOS (relative to branched), but found no isomer specific differences in transfer via mother´s milk.[10,32,35] To our knowledge, only one study has reported placental transfer efficiencies of PFHxS isomers showing, contrary to PFOS, a more efficient transfer of the linear isomer than of the branched isomers.[33] Our results suggest that the more efficient trans-placental transfer of branched PFOS, and less efficient transfer of branched PFHxS, are

"compensated for" after birth, presumably from mother´s milk, resulting in similar %br isomers in mothers and infants.

**Impact of Duration of Breastfeeding, Gestational Age and Infant Weight Gain**

Concentrations of PFOA, PFNA, PFDA, PFUnDA, PFHxS and PFOS were lower in exclusively bottle-fed infants than in exclusively breastfed infants, strongly suggesting that mother´s milk is an important source of infant exposure to all these PFAAs (Fig. 1). The results are however uncertain since we only had 5 exclusively bottle-fed infants, but the importance of breastfeeding as an exposure source of all these PFAAs is supported by our findings of lower serum PFAA concentrations also among mixed breast-/bottle-fed infants (Fig. 1). The lower PFAA concentrations in bottle-fed infants are most probably a net result of infant excretion and growth dilution of PFAAs accumulated *in utero*, and much lower PFAA exposures from infant formula than from mother´s milk.[38,39] No exclusively bottle-fed infants were living in a district receiving contaminated drinking water, so it was not possible to determine the contribution of this potential exposure route.

The higher %br PFOS in the few exclusively bottle-fed infants than in exclusively breastfed infants (Fig. 1) is most probably a result of the higher placental transfer of branched PFOS isomers than of the linear isomer, and a "mother´s milk compensation" with the linear isomer among exclusively breastfed infants.[10,32,35] Branched PFOS isomers are preferentially excreted in urine in adults compared with the linear isomer,[40,41] but excretion of different PFOS isomers in infants apparently was too slow to cause preferential retention of the linear isomer in bottle-fed infants. The reported lower trans-placental transfer efficiency of branched PFHxS isomers than of the linear isomer[33] did not result in lower %br PFHxS concentration in the exclusively bottle-fed infants (Fig. 1). Assuming that the excretion of PFHxS was

negligible during infancy, the results suggests that the transfer of branched PFHxS isomers to mother´s milk is not higher than transfer of the linear isomer.

In the multiple regression analyses of PFAA concentrations in exclusively breastfed infants, duration of breastfeeding explained <10% of the variation in infant PFAA concentrations, with estimated increases in serum concentrations of ≤10% per week of breastfeeding as the infant age at sampling increased from 11 to 18 weeks (Table 4). Associations with breastfeeding were statistically significant for PFOA, PFNA and PFHxS. The low variation in duration of breastfeeding (7 weeks) among the exclusively breastfed infants may have contributed to the relatively weak associations. Only exclusively breastfed infants were included in the analyses, and duration of breastfeeding was therefore the same as infant age. Physiological changes during infancy may influence associations between PFAAs and breastfeeding duration, including for instance possible age-dependent differences in efficiency of infant intestinal PFAA absorption and excretion, as well as in body PFAA distribution.

In our study, PFOA concentrations increased on average 8% per week of exclusive breastfeeding, corresponding to about 30% per month. The increase for PFNA was estimated to on average 45% per month and for PFHxS 40% per month. Similar results were observed for 1.5-year-old children from the Faroe Islands, with PFOA increasing 30% per month of exclusive breastfeeding and PFNA 20% per month.[42] Among 3-4 year-old children from Norway and USA, every month of breastfeeding was associated with a 3-6% increase in PFHxS, PFOS and PFOA in child serum.[16,17] The lower contribution of breastfeeding in the older children is most probably an effect of excretion of maternally transferred PFAA and contribution of non-maternal sources of PFAA exposure during childhood.

Our results suggests that duration of *in utero* exposure influences infant concentrations of PFOA, PFNA and PFOS up to 2-4 months after delivery, since days of gestational age in

general was positively associated with infant PFAA concentrations (Table 4). However, the associations were fairly weak, explaining only 2-10% of the variation in infant concentrations of PFOA, PFNA and PFOS. An earlier study found strong positive correlations between fetal age and fraction of maternal PFAAs present in fetal tissue.[43] Residual confounding of the gestational age-PFAA associations cannot be excluded. For instance a study from Canada reported that increases in maternal gestational weight gain (GWG), which increases with gestational age, were associated with elevated cord blood PFOS and PFOA concentrations[44] Inclusion of maternal weight gain as a covariate in the regression models did however not change the associations between gestational age and PFOA, PFNA and PFOS serum concentrations in exclusively nursed infants (Supporting Information, Table A8). Birth weight, which is associated with gestational age, may be another potential confounder through influence on distribution volume of infant PFAAs. For PFNA and PFOS inclusion of birth weight as a covariate did not influence the associations with gestational age, but for PFOA the association became weaker (Supporting Information, Table A8).

Branched PFOS isomers increased on average with 1% per day of gestational age, whereas the linear isomer increased 0.75% per day of gestational age (Table 4), supporting a higher placental transfer of branched as opposed to linear isomers.[32,33] No significant change in branched and linear PFHxS per day of gestational age was observed, further supporting the low influence of gestational age on serum PFHxS concentrations in infants at 2-4 months of age (Table 4).

It may be hypothesized that infant weight gain affects PFAA concentrations due to growth dilution of PFAA accumulated pre- and post-natally. However, the inverse association was only significant ($p=0.03$) for PFHpA (Table 4), explaining about 10% of variation in infant concentrations (Table 4). Infant PFHpA concentrations decreased on average about 1% with each percentage of weight gain during the study period (Table 4). The infant weight gain

effect on concentrations of other PFAAs than PFHpA was not large enough to be detected in the multiple regression analyses.

**Impact of Contaminated Drinking Water**.

In 2012, a PFAA contamination was discovered in Uppsala drinking water, with distribution of contaminated drinking water to some city districts dating back to at least 1996-1999.[31] The affected production wells were quickly taken out of production after the discovery of the contamination. In 2012-2014, the median PFHxS concentration was 80 ng $L^{-1}$ in contaminated drinking water production wells, followed by PFOS (50 ng $L^{-1}$), and PFBS and PFHxA (both 10 ng $L^{-1}$). Our results suggest that maternal ingestion of the moderately PFHxS- and PFBS-contaminated drinking water strongly influence serum concentrations of the two PFAAs in infants (Fig 2). The adjusted mean infant serum concentrations of PFBS and PFHxS were about 3-fold higher among infants living in District 4, receiving >90% PFAA-contaminated drinking water between 1996 and 1999, than among infants living in the districts not receiving contaminated water (Fig. 2).

Similarly as among Uppsala mothers,[31] no influence of contaminated drinking water on infant PFOS concentrations was observed (Supporting Information, Table A7), although PFOS concentrations in the contaminated drinking water were only slightly lower than those of PFHxS and much higher than PFBS concentrations. Taken together the results suggest that, in contrast to PFBS and PFHxS, maternal PFOS exposure sources other than drinking water were more important indirect determinants of infant PFAA concentrations.

%br PFHxS was significantly higher in infants living in Uppsala districts receiving 10-90% and >90% contaminated water than in infants living in the district receiving uncontaminated water (Fig. 2). We have previously proposed that increased %br PFHxS in adult serum, compared to background in a population, may be an indicator for drinking water

exposure to PFHxS originating from fire-fighting foams.[31] Our infant results suggest that enrichment of branched PFHxS persists also in infants of mothers exposed to drinking water PFHxS.

**Strengths and Limitations**

Our study includes data on PFBS, PFHxA, PFHpA, PFUnDA, PFDoDA, PFTrDA, PFTeDA and PFPeDA which were lacking in Fromme et al. (2011).[21] For many of these PFAA, however, concentrations were generally below LOQ, making it impossible to analyze associations with potential determinants. We have data on branched and linear isomers of PFHxS and PFOS in infants, thus enabling a first look at possible isomer-specific differences in mother/infant dynamics of PFHxS and PFOS. We only had PFAA concentration data from 5 infants that were exclusively bottle-fed, making the comparisons with PFAA concentrations in exclusively breastfed infants uncertain. However, with supporting data from partially bottle-fed infants we can nevertheless conclude that breast-feeding is a significant source of infant exposure to PFHxS, PFOS, PFOA, PFNA, PFDA and PFUnDA. Moreover our study includes data on several potential determinants not studied before, such as gestational age (duration of *in utero* exposure), infant body weight gain (growth dilution), and home address in drinking water districts with differences in drinking water PFAA contamination (indirect drinking water exposure). A weakness is that we did not have data on PFAA concentrations in drinking water ingested by the mothers and maternal drinking water consumption, which would have improved the analyses of contribution of maternal PFAA drinking water exposure to infant serum PFAA concentrations.

The Swedish infants were only sampled once after birth and more extensive serial sampling of neonates and infants will give better information about factors influencing serum/plasma PFAA concentrations during early childhood. As shown by the decrease in

strength of determination with increasing PFAA chain length, there are unknown determinants, not studied by us, that have an important influence on infant PFAA concentration.

# Figures

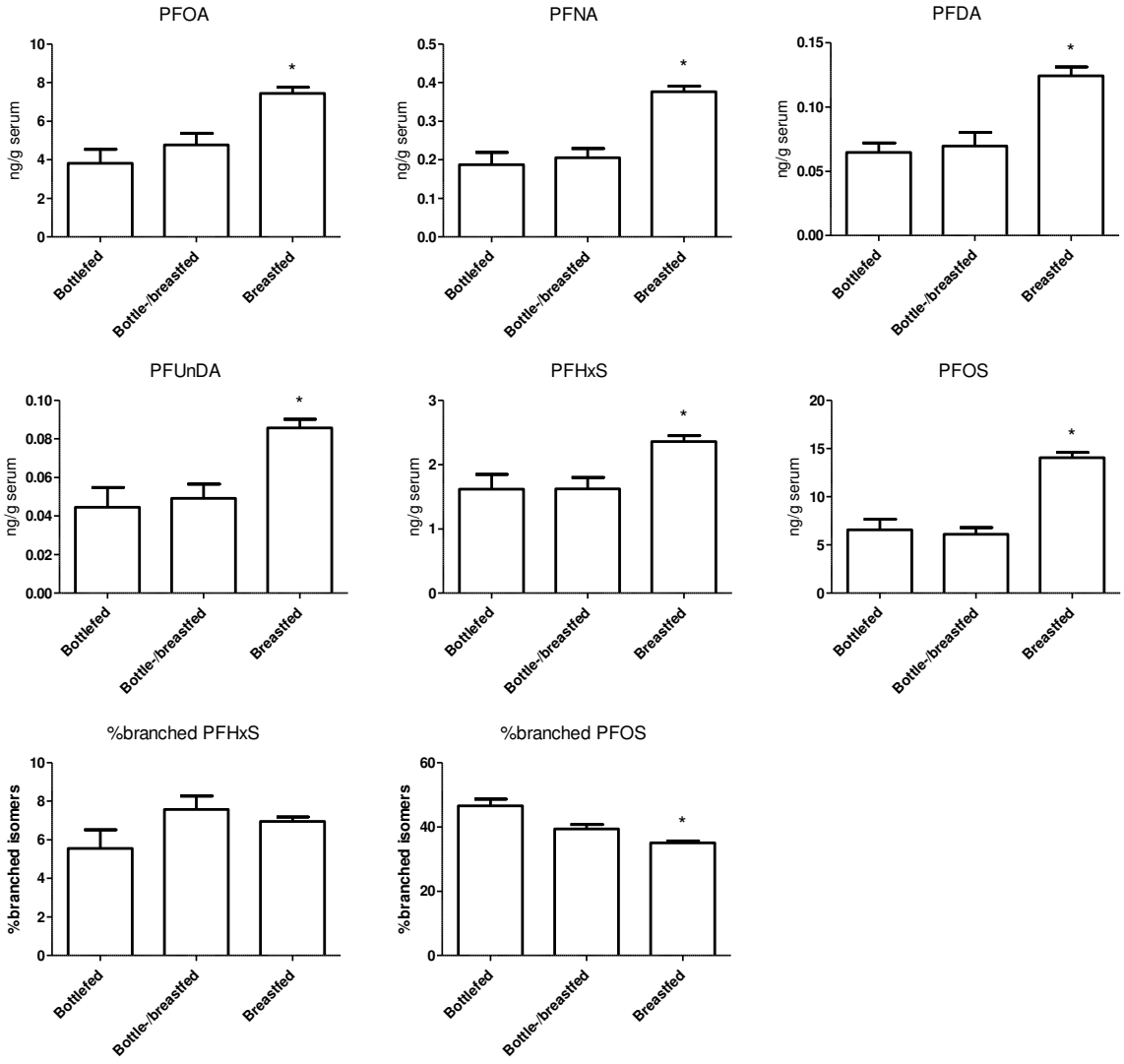



**Figure 1.** Concentrations (ng $g^{-1}$ serum) of selected PFAAs in serum from 2-4-month-old infants sampled 1996-1999 with different degrees of breastfeeding during the study period. 'Bottle-fed' (N=5) represents infants which were not breastfed at all prior to sampling. 'Bottle-/breastfed' (N=11) corresponds to infants that were exclusively breastfed followed by partial/exclusive bottle-feeding before sampling. 'Breastfed' (N=83) represents infants exclusively breastfed from birth until sampling. PFAA concentrations <MDL were replaced with MDL/√2. Concentrations are shown as adjusted mean and SE determined by general linear model (GLM) analysis. In the GLM analyses the covariate "maternal PFAA serum concentration" was included. Asterisk (*) represents a significant difference ($p<0.05$) relative to infants with exclusive bottle-feeding determined in multiple regression analysis.

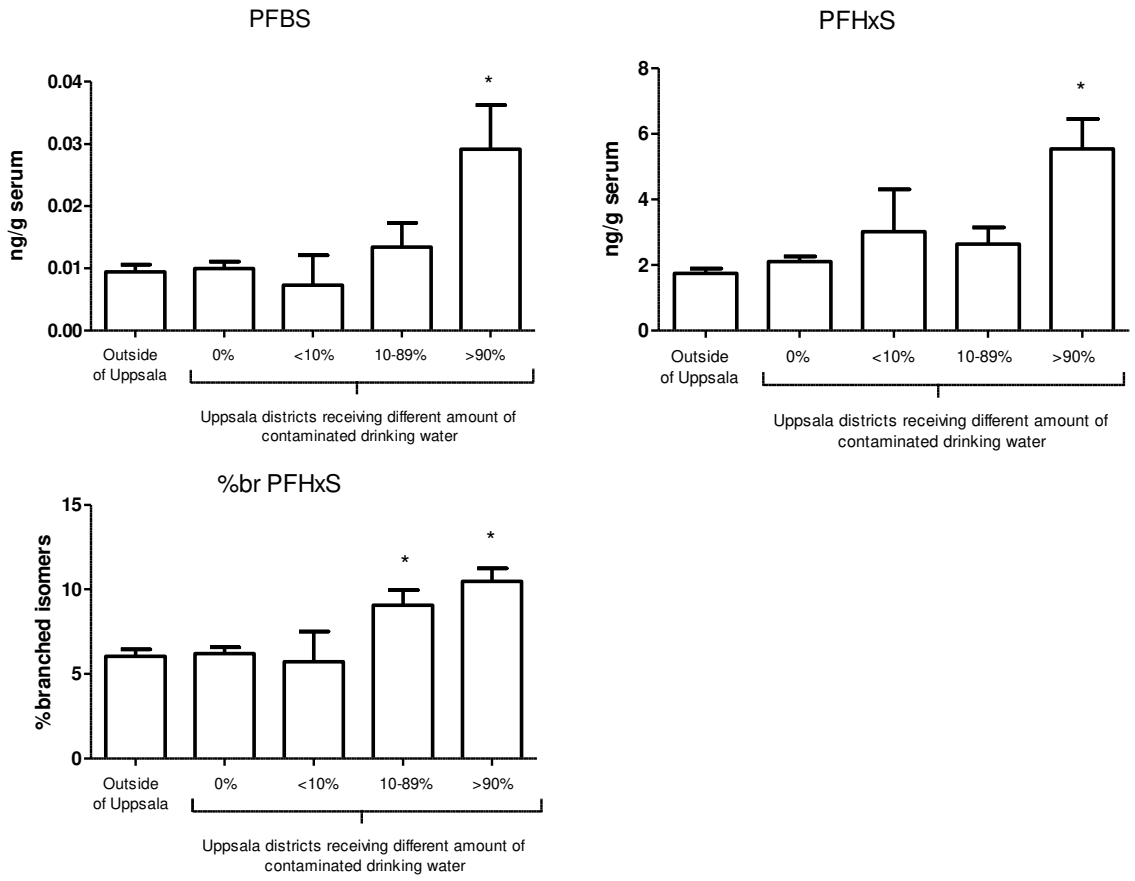



**Figure 2.** Concentrations (ng $g^{-1}$ serum) of PFBS (N=88) and PFHxS (N=91), as well as %branched PFHxS (N=91) in serum from breastfed 2-4-month-old infants sampled 1996-1999, living in different drinking water districts in Uppsala County receiving different percentages of drinking water from PFAA-contaminated wells. The district "Outside Uppsala" did not receive contaminated water. PFBS concentrations <MDL were replaced with MDL/√2. Concentrations are shown as adjusted mean and SE determined by general linear model (GLM) analysis. In the GLM analyses the covariates "gestational age", "infant weight gain after birth" and "duration of breastfeeding" were included. *Significantly different from district "Outside of Uppsala" in multiple linear regression analyses ($p<0.05$). PFBS: District "Outside Uppsala" N=30, 0% N=40, <10% N=2, 10-90% N=7, >90% N=9. PFHxS: District "Outside Uppsala" N=31, 0% N=41, <10% N=2, 10-90% N=7, >90% N=10.

**Table 1. Characteristics of the mothers and infants investigated in the present study**

| Characteristics | n[a] | Mean | Range | %[b] |
|---|---|---|---|---|
| Mothers | | | | |
| Age (year) | 101 | 29 | 22-41 | |
| Pre-pregnancy BMI[c] | 101 | 24 | 18-36 | |
| Years of education | 98 | | | 100 |
| ≤4 years of high school | 41 | | | 42 |
| 1-3 years of higher education | 27 | | | 28 |
| >3 years of higher education | 30 | | | 31 |
| Infants | | | | |
| Age at sampling (weeks) | 105 | 13 | 11-18 | |
| Gestational age (days) | 100 | 280 | 250-300 | |
| Weight (kg) | 103 | 6.1 | 4.4-8.6 | |
| Weight gain (% of birth weight) | 103 | 75 | 30-150 | |
| Exclusive breastfeeding (weeks) | 105 | 12 | 0-18 | |
| Sampling year | 107 | | | 100 |
| 1996-1997 | 48 | | | 44 |
| 1998-1999 | 59 | | | 66 |
| Sex | 107 | | | 100 |
| Girls | 58 | | | 54 |
| Boys | 49 | | | 46 |
| PFAA drinking water districts | 107 | | | |
| Outside of Uppsala city | 38 | | | 36 |
| Uppsala 1, no PFAA contamination | 47 | | | 44 |
| Uppsala 2, <10% contaminated water | 2 | | | 2.0 |
| Uppsala 3, 10-89% | 9 | | | 8.0 |
| Uppsala 4, ≥ 90% | 11 | | | 10 |

[a]Of the original 101 mothers 3 mothers had missing data for education. The different total N values for infants variables are also due to missing data. [b]Percent of total N. [c]Body mass index (kg/m$^2$).

**Table 2. PFAA serum concentrations in infants at 2-4 months of age and their mothers sampled 3 weeks after delivery**

| | Infants | | | | | Mothers | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| PFAA (ng $g^{-1}$) | Mean[a] | SD | Median | Range | DF[b] (%) | Mean[a] | SD | Median | Range | DF[b] (%) |
| PFHxA | | | <0.10 | <0.10-0.27 | 37 | | | <0.08 | <0.08-0.11 | 1 |
| PFHpA | 0.23 | 0.22 | 0.19 | <0.10-2.1 | 88 | | | 0.055 | <0.04-0.40 | 66[c] |
| PFOA | 7.7 | 3.7 | 7.2 | 1.3-20 | 100 | 2.8 | 0.96 | 2.7 | 1.2-6.7 | 100 |
| PFNA | 0.38 | 0.21 | 0.34 | 0.10-1.5 | 100 | 0.41 | 0.20 | 0.37 | 0.08-1.4 | 100 |
| PFDA | 0.12 | 0.07 | 0.11 | <0.07-0.47 | 86 | 0.19 | 0.11 | 0.17 | <0.05-0.72 | 94 |
| PFUnDA | 0.09 | 0.05 | 0.08 | <0.05-0.23 | 79 | 0.12 | 0.09 | 0.11 | <0.05-0.37 | 64 |
| PFDoDA | | | <0.05 | <0.05-0.06 | 3 | | | <0.05 | <0.05-0.22 | 13 |
| PFTrDA | | | <0.05 | <0.05-0.15 | 37 | | | <0.05 | <0.05-0.19 | 11 |
| PFTeDA | | | <0.05 | <0.05-0.13 | 14 | | | <0.07 | <0.07-0.38 | 4 |
| PFPeDA | | | | | 0 | | | | | 0 |
| PFBS | | | <0.01 | <0.01-0.14 | 30 | | | <0.01 | <0.01-0.15 | 57[c] |
| Linear PFHxS | 2.6 | 2.2 | 1.9 | 0.41-19 | 100 | 2.2 | 1.4 | 1.7 | 0.36-8.9 | 100 |
| Branched PFHxS | 0.21 | 0.24 | 0.13 | <0.01-1.8 | 99 | 0.14 | 0.13 | 0.10 | <0.01-0.76 | 96 |
| %branched PFHxS | 6.9 | 3.0 | 6.4 | 1.8-18 | | 5.7 | 2.9 | 5.3 | 0.41-14 | |
| Total PFHxS | 2.8 | 2.5 | 2.0 | 0.43-21 | 100 | 2.3 | 1.5 | 1.8 | 0.37-9.5 | 100 |
| Linear PFOS | 8.6 | 4.0 | 8.4 | 1.3-26 | 100 | 14 | 5.6 | 12 | 5.0-41 | 100 |
| Branched PFOS | 5.0 | 2.9 | 4.4 | 0.87-21 | 100 | 7.0 | 3.5 | 5.8 | 2.6-20 | 100 |
| %branched PFOS | 36 | 6.2 | 36 | 26-62 | | 33 | 4.1 | 34 | 24-45 | |
| Total PFOS | 14 | 6.7 | 13 | 2.2-44 | 100 | 20 | 8.9 | 18 | 7.7-61 | 100 |

[a]Concentration below MDL were set to MDL/√2 in calculations of mean and SD. Infants: N=107; Mothers: N=101. [b]Detection frequency. [c]N=84. MDL differed between the 3 analytical batches. Those with concentrations below MDL in the batch with the highest MDL deleted from the analyses.

**Table 3. Relationship between PFAA concentrations in matched infant serum (IS) and maternal serum (MS)[a]**

| PFAA | N | No. of carbons in fluorinated chain | Ratio IS:MS | Spearman´s correlation[b] | |
|---|---|---|---|---|---|
| | | | Median (range) | r | p |
| PFCAs | | | | | |
| PFHpA | 51 | 6 | 2.8 (0.56-8.2) | 0.31 | 0.005 |
| PFOA | 101 | 7 | 2.8 (0.43-5.7) | 0.59 | <0.001 |
| PFNA | 101 | 8 | 0.94 (0.28-6.4) | 0.42 | <0.001 |
| PFDA | 54 | 9 | 0.69 (0.095-4.0) | 0.34 | 0.001 |
| PFUnDA | 49 | 10 | 0.53 (0.21-1.5) | 0.18 | 0.069 |
| PFSAs | | | | | |
| Linear PFHxS | 101 | 6 | 1.2 (0.22-2.2) | 0.84 | <0.001 |
| Branched PFHxS | 101 | 6 | 1.3 (0.22-17) | 0.80 | <0.001 |
| Total PFHxS | 101 | 6 | 1.2 (0.22-2.3) | 0.85 | <0.001 |
| Linear PFOS | 100 | 8 | 0.66 (0.095-1.4) | 0.37 | <0.001 |
| Branched PFOS | 100 | 8 | 0.72 (0.19-1.7) | 0.46 | <0.001 |
| Total PFOS | 100 | 8 | 0.69 (0.14-1.5) | 0.39 | <0.001 |

[a]Only infant/mother pairs with concentrations ≥MDL included in the analyses. Infants sampled 2-4 months after delivery, mothers 3 weeks after delivery. [b]Correlation between infant and maternal PFAA concentrations.

**Table 4. Percent change in serum PFAA concentrations in exclusively breastfed infants per unit change of maternal PFAAs, gestational age, and infant weight gain[a]**

| PFAA | No. of carbons | Maternal PFAA (ng $g^{-1}$) | | Gestational age (days) | | Breastfeeding (weeks) | | Weight gain (% of birth weight) | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | β (% change) | P (partial $R^2$) | β (% change) | P (partial $R^2$) | β (% change) | P (partial $R^2$) | β (% change) | P (partial $R^2$) | Full $R^2$ % |
| PFHpA (N=62)[b] | 6 | 380 (123) | 0.003 (4%) | 1.1 (0.78) | 0.178 | 7.5 (7.5) | 0.32 | -0.87 (0.38) | 0.03 (12%) | 27 |
| PFOA (N=70) | 7 | 26 (2.6) | <0.001 (53%) | 0.67 (0.29) | 0.028 (2%) | 8.0 (2.8) | 0.006 (6%) | 0.010 (0.014) | 0.92 | 63 |
| PFNA (N=75) | 8 | 150 (20) | <0.001 (33%) | 1.2 (0.42) | 0.004 (10%) | 11 (4.1) | 0.007 (6%) | 0.0070 (0.18) | 0.97 | 48 |
| PFDA (N=75) | 9 | 200 (40) | <0.001 (23%) | 0.91 (0.47) | 0.058 | 8.3 (4.5) | 0.068 | -0.21 (0.20) | 0.31 | 33 |
| PFUnDA (N=75) | 10 | 220 (66) | 0.001 (13%) | 0.02 (0.6) | 0.980 | 5.6 (5.9) | 0.34 | 0.21 (0.27) | 0.45 | 14 |
| Linear PFHxS (N=75) | 6 | 35 (2.4) | <0.001 (72%) | 0.45 (0.41) | 0.271 | 10 (3.8) | 0.01 (2%) | -0.09 (0.18) | 0.63 | 76 |
| Branched PFHxS (N=75) | 6 | 600 (47) | <0.001 (67%) | -0.07 (0.63) | 0.913 | 9.6 (5.9) | 0.11 | 0.10 (0.28) | 0.71 | 71 |
| PFHxS (N=75) | 6 | 32 (2.2) | <0.001 (73%) | 0.44 (0.41) | 0.294 | 10 (3.9) | 0.01 (2%) | -0.080 (0.2) | 0.64 | 76 |
| Linear PFOS (N=73) | 8 | 4.2 (0.56) | <0.001 (36%) | 0.75 (0.34) | 0.031 (3%) | 2.9 (3.1) | 0.29 | 0.25 (0.15) | 0.10 | 50 |
| Branched PFOS (N=74) | 8 | 8.2 (1.2) | <0.001 (37%) | 1.0 (0.47) | 0.036 (4%) | 2.9 (4.4) | 0.51 | 0.29 (0.21) | 0.16 | 45 |
| PFOS (N=73) | 8 | 2.9 (0.38) | <0.001 (36%) | 0.84 (0.36) | 0.025 (4%) | 3.1 (3.4) | 0.36 | 0.22 (0.16) | 0.19 | 46 |

[a]Adjusted mean (standard error). Mutually adjusted for each other in the multiple regression model. Outliers with standardized residual ≥3 excluded in the regression analyses. Partial $R^2$ was estimated by stepwise regression analyses. [b]MDL differed between analytical batches. Mother/infant pairs with concentrations below MDL in the batch with the the highest MDL deleted from the analyses.

## 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). Method detection limits for PFASs analyzed in each of the three batches (Table A6). Concentrations of PFAAs in serum from 2-4-month-old children sampled 1996-1999, living in different drinking water districts in the Uppsala County (Table A7). The influence of maternal weight gain during pregnancy and birth weight on percent change in serum PFAA concentrations per unit change of gestational age in exclusively breastfed infants (Table A8). References.

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## Disclosures

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

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Maternal and neonatal levels of perfluorinated substances in relation to gestational weight gain. *Int. J. Environ. Res. Publ. Health* **2016**, *13*, 146.

# Supporting Information

## Perfluoroalkyl acids (PFAAs) in serum from 2-4-month-old infants – influence of maternal serum concentrations, gestational age, breastfeeding and contaminated drinking water

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**Table A1.** PFAAs included in this study.

| Substance | No. of carbons in fluorinated chain | Abbreviation |
|---|---|---|
| **Perfluoroalkyl carboxylic acids (PFCAs)** | | |
| Perfluorohexanoic acid | 5 | PFHxA |
| Perfluoroheptanoic acid | 6 | PFHpA |
| Perfluorooctanoic acid | 7 | PFOA |
| Perfluorononanoic acid | 8 | PFNA |
| Perfluorodecanoic acid | 9 | PFDA |
| Perfluoroundecanoic acid | 10 | PFUnDA |
| Perfluorododecanoic acid | 11 | PFDoDA |
| Perfluorotridecanoic acid | 12 | PFTrDA |
| Perfluorotetradecanoic acid | 13 | PFTeDA |
| Perfluoropentadecanoic acid | 14 | PFPeDA |
| **Perfluoroalkyl sulfonic acids (PFSAs)** | | |
| Perfluorobutane sulfonic acid | 4 | PFBS |
| Perfluorohexane sulfonic acid[a] | 6 | PFHxS |
| Perfluorooctane sulfonic acid[a] | 8 | PFOS |

[a]Branched and linear isomers

**Table A2.** Target compounds and selected instrumental parameters for quantification of each compound by UPLC/ESI-MS/MS.

| Target Analytes[a, c] | Precursor / product ion (qualitative product ion)[b] | Cone voltage (V) | Collision energy (eV) | Internal standards[c] |
|---|---|---|---|---|
| PFBS | 299 / 80 (99) | 45 | 30 | $^{13}C_2$-PFHxS |
| PFHxS | 399 / 80 (99) | 55 | 36 | $^{18}O_2$-PFHxS |
| PFOS | 499 / 80 (99) | 65 | 40 | $^{13}C_4$-PFOS |
| PFHxA | 313 / 269 (119) | 20 | 10 | $^{13}C_2$-PFHxA |
| PFHpA | 363 / 319 (169) | 21 | 11 | $^{13}C_4$-PFHpA |
| PFOA | 413 / 369 (169) | 22 | 11 | $^{13}C_4$-PFOA |
| PFNA | 463 / 419 (219) | 24 | 11 | $^{13}C_5$-PFNA |
| PFDA | 513 / 469 (269) | 26 | 11 | $^{13}C_2$-PFDA |
| PFUnDA | 563 / 519 (269) | 28 | 11 | $^{13}C_2$-PFUnDA |
| PFDoDA | 613 / 569 (169) | 30 | 12 | $^{13}C_2$-PFDoDA |
| PFTrDA | 663 / 619 (169) | 32 | 12 | $^{13}C_2$-PFDoDA |
| PFTeDA | 713 / 669 (169) | 35 | 12 | $^{13}C_2$-PFDoDA |
| PFPeDA | 763 / 719 (169) | 38 | 13 | $^{13}C_2$-PFDoDA |
| | | | | |
| Recovery Standards | | | | |
| $^{13}C_8$-PFOA | 421 / 376 | 22 | 11 | |
| $^{13}C_8$-PFOS | 507 / 80 | 65 | 42 | |

[a]Acronyms are according to (Buck et al., 2011).[1]

[b]Product ions in brackets were used as confirmation ions.

[c]All standards were purchased from Wellington Laboratories (Guelph, ON, Canada).

**Table A3.** Summary of PFAS concentrations (ng/mL) in-house reference material (pooled human serum) analyzed together with real samples to assess inter-batch precision (i.e. reproducibility). Two different QC samples were included: Control A (n = 17) and Control B (n = 3). Non-detects (ND) were not included in determination of averages or coefficient of variations (CVs).

| Reference Material | PFHxA | PFHpA | PFOA | PFNA | PFDA | PFUnDA | PFDoDA | PFTrDA | PFTeDA | PFPeDA | PFBS | ∑br-PFHxS | lin-PFHxS | ∑br-PFOS (Avg. 80+99) | lin-PFOS-80 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A-1 | ND | ND | 1.09 | 0.54 | 0.19 | 0.31 | ND | 0.12 | ND | ND | ND | 0.03 | 0.73 | 0.95 | 2.36 |
| A-2 | ND | ND | 1.30 | 0.65 | 0.25 | 0.29 | ND | 0.08 | ND | ND | ND | 0.06 | 0.68 | 0.95 | 2.42 |
| A-3 | ND | ND | 1.60 | 0.47 | 0.25 | 0.30 | ND | 0.12 | ND | ND | ND | 0.05 | 0.71 | 0.98 | 2.39 |
| A-4 | ND | ND | 1.53 | 0.59 | 0.25 | 0.27 | ND | 0.11 | ND | ND | ND | 0.05 | 0.75 | 1.10 | 2.55 |
| A-5 | ND | ND | 1.45 | 0.65 | 0.33 | 0.41 | 0.05 | 0.08 | ND | ND | 0.07 | 0.03 | 0.83 | 2.07 | 2.87 |
| A-6 | ND | ND | 1.18 | 0.61 | 0.31 | 0.30 | ND | 0.09 | ND | ND | ND | 0.02 | 0.88 | 1.53 | 2.11 |
| A-7 | ND | ND | 1.47 | 0.68 | 0.27 | 0.50 | 0.06 | 0.14 | ND | ND | 0.04 | 0.04 | 0.77 | 1.84 | 2.25 |
| A-8 | ND | ND | 1.80 | 0.59 | 0.30 | 0.44 | 0.08 | 0.10 | ND | ND | 0.06 | 0.04 | 0.85 | 2.12 | 3.02 |
| A-9 | ND | ND | 1.52 | 0.69 | 0.47 | 0.35 | 0.09 | 0.08 | ND | ND | ND | 0.06 | 0.91 | 3.13 | 3.64 |
| A-10 | ND | ND | 1.69 | 0.82 | 0.31 | 0.42 | 0.04 | 0.05 | ND | ND | 0.05 | 0.04 | 0.96 | 2.41 | 2.72 |
| A-11 | ND | ND | 1.48 | 0.70 | 0.31 | 0.42 | ND | 0.08 | ND | ND | ND | 0.04 | 0.79 | 2.53 | 3.46 |
| A-12 | ND | ND | 1.31 | 0.64 | 0.25 | 0.36 | 0.05 | ND | ND | ND | ND | 0.04 | 0.78 | 2.18 | 2.75 |
| A-13 | ND | ND | 1.41 | 0.59 | 0.28 | 0.35 | 0.05 | 0.04 | ND | ND | ND | 0.02 | 0.91 | 2.24 | 3.58 |
| A-14 | ND | ND | 1.36 | 0.66 | 0.24 | 0.35 | 0.05 | 0.05 | ND | ND | 0.06 | 0.02 | 0.91 | 1.62 | 2.83 |
| A-15 | ND | ND | 1.34 | 0.71 | 0.29 | 0.34 | ND | ND | ND | ND | ND | 0.02 | 0.61 | 1.91 | 3.05 |
| A-16 | ND | ND | 1.22 | 0.67 | 0.22 | 0.36 | ND | 0.06 | ND | ND | ND | 0.03 | 0.90 | 1.79 | 1.93 |
| A-17 | ND | ND | 1.35 | 0.54 | 0.23 | 0.44 | 0.06 | 0.04 | ND | ND | ND | 0.05 | 0.70 | 1.58 | 2.33 |
| **Avg Ref Material A** | **ND** | **ND** | **1.42** | **0.64** | **0.28** | **0.36** | **0.06** | **0.08** | **ND** | **ND** | **0.06** | **0.04** | **0.80** | **1.82** | **2.72** |
| **CV Ref Material A** | **ND** | **ND** | **12.9** | **12.6** | **22.8** | **17.6** | **30.1** | **36.4** | **ND** | **ND** | **18.9** | **34.9** | **12.4** | **33.6** | **18.6** |
| B-1 | ND | ND | 2.99 | 0.23 | 0.14 | 0.15 | ND | ND | ND | ND | 0.01 | 0.12 | 2.23 | 3.67 | 8.59 |
| B-2 | ND | ND | 2.03 | 0.48 | 0.21 | 0.23 | ND | ND | ND | ND | 0.02 | 0.13 | 2.03 | 3.31 | 8.94 |
| B-3 | ND | ND | 2.45 | 0.32 | 0.30 | 0.17 | ND | ND | ND | ND | ND | 0.18 | 2.31 | 2.87 | 6.34 |
| **Avg Ref Material B** | **ND** | **ND** | **2.49** | **0.34** | **0.22** | **0.18** | **ND** | **ND** | **ND** | **ND** | **0.01** | **0.15** | **2.19** | **3.28** | **7.96** |
| **CV Ref Material B** | **ND** | **ND** | **19** | **37** | **38** | **21** | **ND** | **ND** | **ND** | **ND** | **34** | **23** | **7** | **12** | **18** |
| **Pooled CV (A+B material)** | **ND** | **ND** | **13** | **17** | **24** | **18** | **30** | **36** | **ND** | **ND** | **23** | **33** | **12** | **32** | **18** |

**Table A4.** PFCA concentrations (mean ± 95% confidence intervals) measured in n = 3 replicates of NIST SRM 1957 compared to reference values. ND = non-detect. NA = not available.

| | PFHxA | PFHpA | PFOA | PFNA | PFDA | PFUnDA | PFDoDA | PFTrDA | PFTeDA | PFPeDA |
|---|---|---|---|---|---|---|---|---|---|---|
| **Mean (this study; ng $g^{-1}$)** | <0.160 | 0.278 ± 0.091 | 4.04 ± 1.77 | 0.818 ± 0.135 | 0.24 ± 0.13 | 0.121 ± 0.052 | <0.030 | <0.020 | <0.060 | <0.010 |
| **Reference values (ng $g^{-1}$)** | <0.093 | 0.305 ± 0.051 | 5.00 ± 0.44 | 0.878 ± 0.077 | 0.39 ± 0.12 | 0.172 ± 0.036 | <0.025 - 0.203 | <0.131 | <0.448 | NA |

**Table A5.** PFSA concentrations (mean ± 95% confidence intervals) measured in n = 3 replicates of NIST SRM 1957 compared to reference values. ND = non-detect. NA = not available.

| | **PFBS** | **br-PFHxS** | **l-PFHxS** | **Total PFHxS** | **br-PFOS** | **l-PFOS** | **Total PFOS** |
|---|---|---|---|---|---|---|---|
| **Mean (n $g^{-1}$)** | <0.01 | 0.11 ± 0.071 | 4.02 ± 2.28 | 4.13 ± 2.35 | 8.12 ± 2.06 | 12.6 ± 3.64 | 20.7 ± 5.41 |
| **Reference values (ng $g^{-1}$)** | <0.25 | NA | NA | 4.00 ± 0.83 | NA | NA | 21.1 ± 1.3 |

**Table A6.** Method detection limits (ng $g^{-1}$) for PFASs analyzed in each of the three batches.

| Target Analytes[a, c] | Batch 1 | Batch 2 | Batch 3 |
|---|---|---|---|
| PFBS | 0.01 | 0.01 | 0.09 |
| PFHxS | 0.01 | 0.01 | 0.02 |
| PFOS | 0.01 | 0.01 | 0.04 |
| PFHxA | 0.3 | 0.1 | 0.08 |
| PFHpA | 0.04 | 0.1 | 0.14 |
| PFOA | 0.2 | 0.25 | 0.03 |
| PFNA | 0.05 | 0.1 | 0.03 |
| PFDA | 0.05 | 0.07 | 0.07 |
| PFUnDA | 0.05 | 0.05 | 0.02 |
| PFDoDA | 0.05 | 0.05 | 0.03 |
| PFTrDA | 0.05 | 0.05 | 0.02 |
| PFTeDA | 0.05 | 0.05 | 0.07 |
| PFPeDA | 0.05 | 0.03 | n.a. |

n.a. not available.

**Table A7.** Concentrations of PFAAs in serum from 2-4-month-old children sampled 1996-1999, living in different drinking water districts in Uppsala County[a]

| PFAA (ng $g^{-1}$) | Outside of Uppsala | Uppsala districts receiving different percentages of PFAA-contaminated drinking water | | | |
|---|---|---|---|---|---|
| | | 0% | <10% | 10-90% | >90% |
| PFHxA | 0.093 (0.006) | 0.094 (0.0060) | 0.14 (0.043) | 0.087 (0.013) | 0.080 (0.010) |
| PFOA | 7.4 (0.58) | 6.4 (0.45) | 9.4 (3.7) | 6.4 (1.1) | 7.3 (1.1) |
| PFBS | 0.0090 (0.0010) | 0.010 (0.0010) | 0.0070 (0.0050) | 0.013 (0.0040) | 0.029 (0.0070)[a] |
| PFHxS | 1.7 (0.15) | 2.1 (0.16) | 3.0 (1.3) | 2.6 (0.52) | 5.5 (0.91)[a] |
| %br PFHxS | 6 (0.40) | 6.0 (0.40) | 6.0 (2.0) | 9.0 (0.90)[a] | 10 (0.8)[a] |
| PFOS | 13 (0.87) | 12 (0.74) | 17 (5.9) | 9.9 (1.6) | 14 (1.8) |
| %br PFOS | 37 (0.90) | 36 (0.80) | 36 (4) | 40 (2) | 34 (2) |

[a]Least square means and standard error. In the GLM analyses the covariates "gestational length", "infant weight gain after birth", and "duration of nursing" were included. a = significantly different ($p<0.05$) from drinking water district "Outside of Uppsala", as determined in multiple regression analyses.

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**Table A8**. The influence of maternal weight gain during pregnancy and birth weight on percent change in serum PFAA concentrations per unit change of gestational age in exclusively breastfed infants.[a]

| PFAA | +maternal weight gain<br>β (% change) (p) | +birth weight<br>β (% change) (p) |
|---|---|---|
| PFOA | 0.66 (0.30) (0.030) | 0.46 (0.33) (0.165) |
| PFNA | 1.3 (0.42) (0.003) | 1.2 (0.47) (0.015) |
| Linear PFOS | 0.77 (0.35) (0.029) | 0.87 (0.38) (0.027) |
| PFOS | 0.86 (0.37) (0.021) | 0.91 (0.41) (0.030) |

[a]Adjusted mean (standard error). Covariates 'maternal PFAA', 'breastfeeding' and 'infant weight gain' was included in the multiple regression model. Outliers with standardized residual ≥3 excluded in the regression analyses.

## References

(1) Buck, R.C., Franklin, J., Berger, U., Conder, J.M., Cousins, I.T., de Voogt, P., Jensen, A.A., Kannan, K., Mabury, S.A., and van Leeuwen, S.P. 2011. Perfluoroalkyl and polyfluoralkyl substances (PFASs) in the environment: terminology, classification, and origins. Integr Environ Assess Manag.