Check for
updates

Changes in perfluoroalkyl substances (PFAS) concentrations in human milk over the course of lactation: A study in Ronneby mother-child cohort

Annelise J. Blomberg [a,b,*], Line S. Haug [c], Christian Lindh [a], Azemira Sabaredzovic [c], Daniela Pineda [a], Kristina Jakobsson [d,e], Christel Nielsen [a,f]

[a] *Division of Occupational and Environmental Medicine, Department of Laboratory Medicine, Lund University, Lund, Sweden*
[b] *Department of Environmental Health, Harvard T.H. Chan School of Public Health, Boston, MA, United States*
[c] *Department of Food Safety, Norwegian Institute of Public Health, Oslo, Norway*
[d] *School of Public Health and Community Medicine, Institute of Medicine, Sahlgrenska Academy, University of Gothenburg, Gothenburg, Sweden*
[e] *Department of Occupational and Environmental Medicine, Sahlgrenska University Hospital, Gothenburg, Sweden*
[f] *Clinical Pharmacology, Pharmacy and Environmental Medicine, Department of Public Health, University of Southern Denmark, Odense, Denmark*

A R T I C L E   I N F O

A B S T R A C T

Background: Little is known about how PFAS concentrations in human milk change over the course of lactation, although this is an important determinant of cumulative infant exposure from breastfeeding.
Objective: To estimate changes in PFAS concentrations in human milk over the course of lactation in a population with a wide range of exposure from background-to high-exposed.
Methods: We measured PFAS concentrations in colostrum and mature milk samples from women in the Ronneby Mother-Child Cohort. For each PFAS, we estimated the change in concentration from colostrum collected 3–4 days postpartum to mature milk collected 4–12 weeks postpartum using linear mixed-effects models. We evaluated whether this estimated change varied by quartiles of colostrum concentrations. In a subset of mothers with at least three mature milk samples, we estimated the change in concentration per month over the first eight months of lactation.
Results: Our study included 77 mother-child pairs, of whom 74 had colostrum and initial mature milk samples and 11 had three or more repeated samples. The concentration change from colostrum to mature milk varied by PFAS. While PFOS increased by 21% (95% CI: 8.9, 35), PFOA decreased by 17% (95% CI: −28, −3.5) and PFHxS decreased by 12% (95% CI: −24, 3.3). In addition, PFAS concentrations tended to increase in women with lower colostrum levels, but decreased or remained the same in women with high colostrum concentrations. When we estimated changes over the course of lactation, we found that PFOA concentrations decreased the most (−12% per month; 95% CI: −22, −1.5), whereas PFHxS and PFOS showed small nonsignificant decreases.
Conclusions: Models for cumulative infancy exposure from breastfeeding need to account for differences in concentration trajectories by PFAS and possibly by maternal exposure level. Additional research is needed to evaluate the relative exposure from breastfeeding vs prenatal exposure, especially in highly exposed communities where breastfeeding guidance is urgently needed.

1. Introduction

Per- and polyfluoroalkyl substances (PFAS) are a class of synthetic chemicals that are widely used in industrial and consumer applications because of their desirable properties, including chemical and thermal stability and water and oil repellency (Sunderland et al., 2019). However, these same properties also make PFAS extremely persistent and mobile in the environment, leading to widespread human exposures and growing concern over potential health effects (Fenton et al., 2021; Kato et al., 2011).

Infants and young children may be especially vulnerable to PFAS exposures. Early development is sensitive to hormonal disruption by chemicals (Barouki et al., 2012; Dietert and Piepenbrink, 2006), and it is increasingly recognized that exposure during these sensitive periods may have adverse consequences for health later in life (Barouki et al., 2012; Dietert et al., 2010). Epidemiological studies have linked prenatal and childhood PFAS exposures to adverse health outcomes including dyslipidemia, changes in fetal and postnatal growth and impaired immunity (Liew et al., 2018; Rappazzo et al., 2017).

Breastfeeding is one of two important sources of early-life PFAS exposure, the other being prenatal exposure from transplacental transfer during gestation. PFAS are transferred from maternal serum into human milk, although concentrations in human milk are substantially lower than in maternal serum (Blomberg et al., 2022; EFSA Panel on Contaminants in the Food Chain, 2020). Breastfeeding has been associated with increased child serum levels and a corresponding decrease in maternal serum levels in background-exposed populations (Gyllenhammar et al., 2019; Mogensen et al., 2015; Mondal et al., 2014; Papadopoulou et al., 2016).

Exposure to PFAS from breastfeeding is of particular concern in communities impacted by PFAS contamination from point source(s), including industries that have produced or used PFAS, airports and/or military bases that have used aqueous film forming foam (AFFF), and landfills (De Silva et al., 2021). Many highly exposed communities have been identified around the globe and new communities continue to be discovered (De Silva et al., 2021; Hu et al., 2016; Salvatore et al., 2022). There is an urgent need for scientifically-informed breastfeeding guidelines that balances benefits of breastfeeding with the potential negative health effects of additional PFAS exposure (LaKind et al., 2022). To properly account for the cumulative PFAS dose, the guidelines must consider not only the PFAS concentration in milk at initiation, but also changes in milk PFAS concentrations over the course of lactation as well as changes in the volume of milk consumed over time.

Only three existing studies, all at background exposure levels, have attempted to characterize changes in PFAS concentrations over lactation. The first study used repeated measurements of PFAS concentrations in human milk, collected from nine Norwegian women who breastfed for at least six months, to estimate changes in PFOA and PFOS concentrations over the course of lactation and found a linear reduction of -7.7% per month for PFOA and -3.1% per month for PFOS (Thomsen et al., 2010). A second study, which did not use repeated measurements but instead pooled human milk measurements from a cohort of 128 mothers in Korea, found that concentrations of PFOS, PFNA and PFOA increased in milk collected 30 days after delivery compared to colostrum (Lee et al., 2018). In contrast, a third study from Korea found that PFOA levels decreased over lactation, while concentrations of PFPeA and PFHxA increased (Kang et al., 2016). However, this study used different populations for each lactation period, so changes over time may reflect population differences in underlying exposure profiles or participant characteristics. In light of this limited and conflicting evidence, more studies are needed to estimate changes in PFAS concentrations in human milk over lactation.

Importantly, there are no existing studies of PFAS trajectories in the milk of highly exposed women, which may vary from trajectories in background-exposed women for several reasons. First, the mechanisms of transfer have not been clarified and they may behave differently at high and low levels of exposure. In addition, differences in the source of exposure in high-vs background-exposed women may cause apparent differences in PFAS concentration trajectories. While background-exposed populations are typically exposed to continuous low levels of several PFAS, women in highly exposed communities are often exposed to one (or few) dominating PFAS sources that are eliminated or reduced after identification. This difference in exposure characteristics (low and continuous vs high and time-limited) may cause differences in the relative change of PFAS concentrations in milk over the course of lactation.

To address this gap, we measured PFAS concentrations in colostrum and mature milk samples from a cohort of women with a wide range of PFAS exposures, from background to extremely high. Our primary aim was to estimate the relative change in PFAS concentrations 1) from colostrum to an initial mature milk sample collected 4–12 weeks after delivery, and 2) over the course of lactation up to eight months. We hypothesized that the magnitude of these changes would depend on the exposure level of the mother.

2. Materials and methods

2.1. The Ronneby Mother-Child cohort

In 2013, high levels of PFAS were measured in a municipal water supply in Ronneby, Sweden. The contamination was linked to AFFF runoff from a military airport located within the water supply area. Based on the airport's purchase records, it is likely that the use of AFFFs began in the mid-1980s. The time at which the contamination reached the drinking water is unknown. Of the two municipal waterworks in Ronneby, one had extremely high levels of PFAS (sum of 12 PFAS measured in 2013: 10,380 ng/L). The second waterworks had a slightly elevated concentration of PFAS (sum of 12 PFAS in 2013: 47.6 ng/L). In contrast, the sum of 12 PFAS in the water supply of the nearby municipality of Karlshamn was <5 ng/L. Following identification of the PFAS contamination, the highly contaminated waterworks was immediately closed and water supply for the entire Ronneby municipality was provided by the second waterworks (Xu et al., 2021).

The prospective Ronneby Mother-Child cohort was initiated after the contamination was discovered to investigate the transfer of PFAS from mother to child. Between 2015 and 2020, all pregnant women in Ronneby municipality were invited to enroll at their antenatal clinic. Enrollment was also conducted in the nearby municipality of Karlshamn starting in 2018, so that the cohort would include women with a wide range of PFAS exposures (Blomberg et al., 2022). The final cohort included 263 women (225 mothers from Ronneby, 35 mothers from Karlshamn, and three missing recruitment location information).

A maternal blood sample (2 × 5 mL) was collected at a routine maternal care visit, usually during the second trimester, and transported to the Department of Clinical Chemistry in Karlskrona on the same day. Each sample was centrifuged at 3000 rpm for 10 min within 12 h from the collection and the serum was transferred to three cryotubes (Sarstedt, Nümbrecht, Germany). Serum samples were frozen to $-70\ ^\circ$C before cold chain transport to the Department of Occupational and Environmental Medicine at Lund University, where they were stored at $-80\ ^\circ$C until analysis.

Participants collected a colostrum sample 3–4 days postpartum and a mature milk sample 4–12 weeks postpartum in 50 mL screw cap polypropylene tubes (Sarstedt, Nümbrecht, Germany). Both samples were collected at home, and could be collected in one feeding or over the course of several feedings in one day using hand expression, a pump, or by collecting excess milk while nursing. A subset of mothers was requested to provide milk samples monthly over the course of lactation. Milk samples were stored in the participants' domestic freezers until sampling was completed and then shipped to the Department of Occupational and Environmental Medicine in Lund where they were stored at $-80\ ^\circ$C until analysis at the Norwegian Institute of Public Health in Oslo. Samples were shipped on dry ice.

The study was approved by the Regional Ethical Review Board in Lund, Sweden (no. 2017/437, with amendments). Written informed consent was provided by all participants.

2.2. PFAS assessment

Seven PFAS were measured in colostrum, mature milk and maternal serum samples (perfluorooctanoic acid, PFOA; perfluorononanoic acid, PFNA; perfluorodecanoic acid, PFDA; perfluoroundecanoic acid,

PFUnDA; perfluorohexane sulfonic acid, PFHxS; perfluoroheptane sulfonic acid, PFHpS, and perfluorooctane sulfonic acid, PFOS). Serum samples were analyzed at the Division of Occupational and Environmental Medicine at Lund University using ultra high-performance liquid chromatography–mass spectrometry (UHPLC-MS/MS; QTRAP 5500, AB Sciex, Framingham, MA, USA). The method is described by Norén et al. (2021). The level of quantification (LOQ) for each PFAS in maternal serum was 0.1 ng/mL.

Colostrum and mature milk samples were analyzed at the Department of Food Safety, Norwegian Institute of Public Health, following the method detailed in Thomsen et al. (2010). These samples were also analyzed using UHPLC-MS/MS (Agilent, Santa Clara, CA, USA). The concentration of PFOS was measured as the integration of the total area of the linear and branched isomers. Two in-house quality control samples were analyzed to confirm measurement quality (n = 6 each; mean concentrations between 10 and 160 pg/mL). The relative standard deviations for the two sets of replications were 28% and 11.1% for PFOA, 28.0% and 33.8% for PFNA, 48.2% and 28.8% for PFDA, 64.2% and 15.9% for PFHxS, and 20.2% and 8.8% for PFOS. The LOQ for each PFAS in colostrum and mature milk was 10 pg/mL.

2.3. Statistical analysis

We limited our statistical analysis to PFAS that were detectable above the LOQ in at least 70% in both colostrum and mature milk samples. When samples were measured below the LOQ, we used the measured concentration when available, and otherwise used $LOQ/\sqrt{2}$.

For each PFAS, we estimated the percent change in concentration from the colostrum sample to the initial mature milk sample using a linear mixed-effects model with a random intercept by mother-child pair and an unstructured covariance matrix. Each PFAS was modeled separately, and PFAS concentrations were log-transformed to ensure residuals were normally distributed. Our primary model can be written as:

$$Y_{i,s} = \alpha + \beta(I_{s=M}) + b_i + \varepsilon_{i,s}$$

where $Y_{i,s}$ is the natural logarithm of the PFAS concentration for woman i in sample s, $I_{s=M}$ is an indicator variable for the mature milk sample (vs. colostrum), β is the estimated change in concentration between colostrum and mature milk, b_i is a subject-specific (i.e., mother-child pair) intercept, and $\varepsilon_{i,s}$ is the within-subject error.

To evaluate whether the relative change in PFAS concentration varied by the colostrum concentration (i.e., by maternal exposure level), we categorized each participant by whether the colostrum concentration was high (top quartile), low (bottom quartile) or medium for each PFAS. We then included this exposure category as an effect-modifier of the concentration change from colostrum to mature milk. The fit of the exposure-interaction models were compared to the corresponding model without the interaction term using likelihood ratio tests. As a secondary analysis, we also categorized mothers as low, medium, or highly exposed based on quartiles of PFHxS concentrations measured in serum collected in pregnancy. PFHxS levels are a good indicator of exposure to AFFF-contaminated water in Ronneby (Xu et al., 2021) and unlike colostrum concentrations, PFAS concentrations in pregnancy serum samples can be measured before delivery and therefore be used to inform future breastfeeding decisions. We then re-ran our models with this new exposure categorization.

We evaluated trends in PFAS concentration over the course of lactation in mothers with at least three colostrum and mature milk samples. For each PFAS, we used a linear mixed-effects model with a random intercept by mother-child pair and an unstructured variance-covariance matrix to model repeated milk concentrations as a function of months of lactation. We estimated the adjusted intraclass correlation coefficient (ICC) for each mixed-effects model by dividing the random effect variance by the total variance (i.e., the sum of random effect variance and residual variance) (Lüdecke et al., 2021; Nakagawa et al.,

2017). Because PFAS concentrations in colostrum may behave differently than concentrations in mature milk, we also ran a second set of models that excluded colostrum samples and thus provided estimates of the concentration change over the lactation in relation to early mature milk.

All statistical analyses were conducted in R version 4.2.0 (2022-04-22) (R Core Team, 2021) using the package 'tidyverse' version 1.3.1 (Wickham et al., 2019); mixed effects models were run using the package 'lme4' version 1.1.29 (Bates et al., 2015) and ICC values were calculated using the package 'performance' version 0.9.0 (Lüdecke et al., 2021).

3. Results

The Ronneby Mother-Child cohort included 263 mother-child pairs. After excluding two sets of twins, there were 139 mother-child pairs with at least one colostrum or mature milk sample. Of these, 74 had both a colostrum and initial mature milk sample and were included in our paired analyses of colostrum and mature milk. There were 11 mother-child pairs with at least three samples over the course of lactation (Fig. 1). A detailed breakdown of the final sample and participant counts by sample availability is included in the Supplemental Material as Table S1. Two mothers were enrolled repeatedly in the final study, as they each had two children in the cohort and provided milk samples in
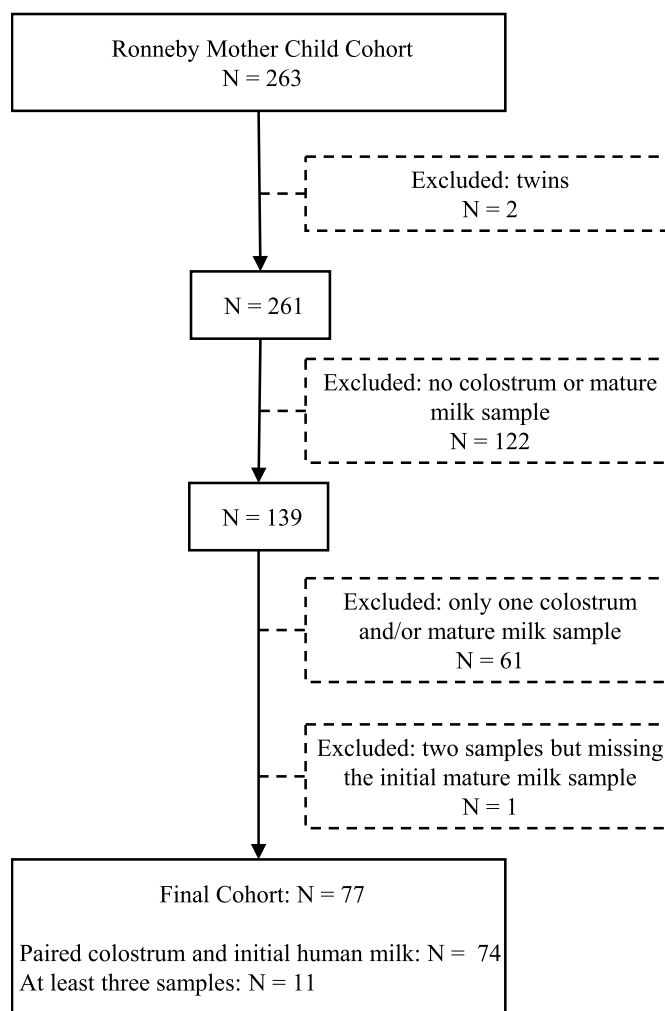

Fig. 1. Study selection flow chart, where N refers to the number of mother-child pairs included or excluded. In the case of twins, one mother and her two twins are considered to be one pair.

both instances.

Characteristics of the study population are included in Table 1. The majority of participants were multiparous and non-smokers. All women who provided at least three milk samples received maternal care in Ronneby. Mothers who provided at least one colostrum and/or mature milk sample were more likely to have never smoked and attended university compared to mothers who did not provide a milk sample (Table S2).

Concentrations of AFFF-associated PFAS (PFOS, PFHxS, PFOA and PFHpS) were strongly correlated within each sample type (maternal serum during pregnancy, colostrum, and initial mature milk) (Fig. S1). Concentrations of each AFFF-associated PFAS were also highly correlated across the three samples. Other PFAS showed weaker correlations within and across samples (Fig. S1).

PFOS and PFHxS had the highest median concentrations across all colostrum and mature milk samples (Table 2). PFAS concentrations in colostrum and mature milk samples were generally higher in women receiving maternal health care in Ronneby than women receiving care in Karlshamn (Table S3). For example, the median concentration of PFOS in the colostrum of women receiving healthcare in Ronneby was 130 pg/mL (n = 65; IQR = 50–360) while the median concentration in women receiving healthcare in Karlshamn was 10 pg/mL (n = 9; IQR = 10–20). Women receiving maternal health care in Ronneby also had higher serum PFAS concentrations during pregnancy (e.g., a median PFHxS concentration of 14 ng/mL vs 0.45 ng/mL) (Table S3).

Of the seven PFAS measured in this study, three (PFOS, PFHxS, and PFOA) were above the LOQ in at least 70% of both colostrum and mature milk samples and were included in subsequent analyses. Exposure category cutoffs for these three PFAS and concentrations by primary and secondary exposure categorizations are included in Tables S4 and S5.

The estimated change in PFAS concentration from colostrum to the initial mature milk sample varied by PFAS. While the PFOS concentration increased by 21% (95% CI: 8.9, 35), PFOA decreased by 17% (95% CI: −28, −3.5) and PFHxS decreased by 12% (95% CI: −24, 3.3) (Table 3 and Fig. 2). The three models had ICC values ranging from 0.75 (PFOA) to 0.94 (PFOS), indicating that most of the variability was due to between-subject variation.

Including an interaction term between exposure category and sample type significantly improved model fit for models of all three PFAS (p-value <0.001). Results from the interaction models indicated that the change in concentration varied by maternal exposure level (Table 3 and Fig. 2). In the low-exposure groups, PFOS, PFOA and PFHxS concentrations increased from colostrum to the first mature milk sample. In contrast, concentrations of PFOA and PFHxS decreased in the medium-

Table 1

Characteristics of the 77[a] mother-child pairs, displayed as N (%) or mean ± SD.

Variable	Paired colostrum and initial mature milk sample	At least 3 samples
$N_{mother-child\ pairs}$	74	11
Maternal age at delivery	30.6 ± 4.5	31.6 ± 5.9
Parity = Primiparous	24 (32)	5 (46)
Smoking Status		
Never Smoker	44 (59)	8 (73)
Current Smoker	2 (3)	0 (0)
Past Smoker	23 (31)	1 (9)
Missing	5 (7)	2 (18)
Education Status		
Less than high school	3 (4)	0 (0)
High school	28 (38)	3 (27)
University (3 or more years)	36 (49)	7 (64)
Other	3 (4)	0 (0)
Missing	4 (5)	1 (9)
Location of Maternal Care = Ronneby	65 (88)	11 (100)

[a] A detailed breakdown of the final participant and sample counts by sample availability is presented in Table S1.

Table 2

PFAS concentrations (pg/mL) in colostrum and mature milk samples included in the study.

	Sample 1 Colostrum	Sample 2 Initial mature milk	Samples 3-9 Repeated mature milk
$N_{samples}$	74	76	34
PFOA			
% > LOQ[a]	97%	95%	97%
5th – 95th perc.	10–190	<10-135	10–53.5
Median (IQR)	30 (20, 68)	30 (20, 50)	20 (13, 40)
PFNA			
% > LOQ	46%	70%	74%
5th – 95th perc.	<10-20	<10-10	<10–13.5
Median (IQR)	<10 (<10, 10)	10 (<10, 10)	10 (<10, 10)
PFDA			
% > LOQ	3%	0%	0%
5th – 95th perc.	<10-<10	<10-<10	<10-<10
Median (IQR)	<10 (<10, <10)	10 (<10, <10)	<10 (<10, <10)
PFUnDA			
% > LOQ	8%	3%	3%
5th – 95th perc.	<10-10	<10-<10	<10-<10
Median (IQR)	<10 (<10, <10)	<10 (<10, <10)	<10 (<10, <10)
PFHxS			
% > LOQ	72%	79%	74%
5th – 95th perc.	<10-1320.5	<10–882.5	<10-797
Median (IQR)	110 (<10, 355)	90 (10, 323)	160 (<10, 348)
PFHpS			
% > LOQ	34%	38%	38%
5th – 95th perc.	<10-84	<10-50	<10-40
Median (IQR)	<10 (<10, 20)	<10 (<10, 20)	<10 (<10, 20)
PFOS			
% > LOQ	97%	100%	100%
5th – 95th perc.	10–890	20–867.5	10–784
Median (IQR)	120 (33, 300)	160 (40, 390)	150 (30, 408)

[a] LOQ = 10 pg/mL for each PFAS.

and high-exposure groups, while concentrations of PFOS still increased in the medium-exposure group but at a lower relative magnitude. For example, while concentrations of PFHxS in mature milk increased by 62% in low-exposed women (95% CI: 24, 110), concentrations decreased by 23% in highly exposed women (95% CI: −41, −0.35).

Our secondary analysis used exposure categories based on PFHxS serum concentrations in pregnancy, as high PFHxS concentrations are a valid indicator of AFFF-exposure in Ronneby (Xu et al., 2021). Although the inclusion of an interaction term between serum exposure category and sample type significantly improved model fit for all three PFAS compared to models that did not include an interaction (p-value <0.001), the estimated change from colostrum to mature milk was not significantly different in high-exposed participants compared to low-exposed participants for PFOA or PFHxS. Model results for PFOS were more similar to our primary interaction models, where women in the low and medium exposure groups had an estimated increase in PFOS concentration while women in the high exposure group had no change in PFOS concentrations (Table S6 and Fig. S2).

Repeated measurements of PFAS concentrations in women with at least three samples are illustrated in Fig. 3. While PFOA decreased by 12% per month (95% CI: −22, −1.5), PFHxS and PFOS concentrations showed small nonsignificant monthly decreases (PFHxS: −2.5, 95% CI: −9.7%, 5.4%; PFOS: −3.2%, 95% CI: −8.4%, 2.3%) (Table 4). When we limited our analysis to only mature milk samples by excluding the colostrum sample, PFOS showed a greater decrease over time (−5.8% per month; 95% CI: −12, 0.32).

Table 3

Estimated mean change (%) in PFAS concentration between the colostrum sample (collected 3–4 days after delivery) and the first mature milk sample (collected 4–12 weeks after delivery).

Exposure Level[a]	Colostrum Cutoff Concentration (pg/mL)	$N_{samples}$	% Change (95% CI)
PFOA			
Overall		74	−16.7 (−28, −3.5)
Low	$C_{PFOA} \leq 20$	22	13.8 (−11.8, 46.7)
Medium	$20 < C_{PFOA} \leq 67.5$	33	−23.7 (−38, −6.1)
High	$67.5 < C_{PFOA}$	19	−32.3 (−48.5, −11.1)
PFHxS			
Overall		74	−11.6 (−24.4, 3.3)
Low	$C_{PFHxS} \leq 7.4$	19	61.7 (24.4, 110.1)
Medium	$7.4 < C_{PFHxS} \leq 35.5$	36	−30.7 (−42.7, −16.2)
High	$355 < C_{PFHxS}$	19	−23.3 (−41, −0.4)
PFOS			
Overall		74	21.1 (8.9, 34.7)
Low	$C_{PFOS} \leq 32.5$	19	64.2 (35.7, 98.7)
Medium	$32.5 < C_{PFOS} \leq 300$	37	15 (0.4, 31.9)
High	$300 < C_{PFOS}$	18	−2.3 (−19.7, 18.8)

[a] Effects by exposure category (low, medium, and high; based on colostrum concentrations) were calculated for each PFAS from a model with an interaction term between sample and exposure group.

4. Discussion

In this study, we analyzed changes in PFAS concentrations in paired colostrum and mature milk samples collected from women in the Ronneby Mother-Child cohort. This cohort was initiated in 2015 after the end of exposure to high levels of PFAS contamination in the drinking water of Ronneby, Sweden. When we analyzed changes in PFAS concentration from colostrum to mature milk collected 4–12 weeks postpartum, we found that the direction and the relative magnitude of change varied by PFAS. Concentrations of PFOA decreased, while concentrations of PFOS increased. Similarly, when we examined changes in PFAS concentration over eight months of lactation, PFOA showed the greatest estimated decrease (12% per month), while concentrations of PFOS and PFHxS showed smaller nonsignificant monthly decreases.

Differences in trajectories by PFAS may be due to differences in the PFAS-specific transfer efficiency (the estimated ratio between PFAS measured in human milk to PFAS measured in maternal serum), as a higher transfer efficiency may lead to a faster decline in maternal body burden and therefore a faster decrease in PFAS concentrations in milk over the course of lactation. Several studies have found that PFOA has a higher transfer efficiency than PFOS (Blomberg et al., 2022; Haug et al., 2011; Liu et al., 2011). Variations in transfer efficiency by PFAS arise from the biological process (es) regulating PFAS transfer into human milk. PFAS transfer is different from other more well-studied lipophilic persistent organic pollutants (e.g., polychlorinated biphenyls) because PFAS do not accumulate in lipids (Zheng et al., 2021). Instead, PFAS are transported in the body bound to proteins in the blood, primarily albumin (Forsthuber et al., 2020). The binding affinity of each PFAS to albumin partially determines its unbound concentration and its availability for transfer into human milk by passive diffusion (Anderson, 2018). Previous studies have consistently found that PFOS has a higher binding affinity to albumin than PFOA (Beesoon and Martin, 2015; Chi et al., 2018; Forsthuber et al., 2020; Gao et al., 2019; Jackson et al., 2021), which is consistent with the lower transfer efficiency seen for PFOS. However, active transfer may also play a role in the transport of PFAS into human milk and additional research is needed.

The protein content of human milk is dynamic over the course of lactation in terms of both composition and relative amount (Andreas et al., 2015). In relation to mature milk, colostrum is rich in the soluble whey proteins while the content of casein micelles is low. Additionally, the protein content of human milk gradually decreases over the course of lactation (Lönnerdal et al., 2017). Although the extent to which PFAS bind to milk proteins has rarely been investigated, binding of PFOA to β-lactoglobulin, the major whey protein component, has been confirmed (Schwieger and Ropers, 2013). If different PFAS bind to milk proteins with varying affinity, this may have contributed to the observed differences in PFAS concentrations between colostrum and mature milk and over time.

Our results illustrate the importance of considering PFAS-specific behavior when estimating cumulative infancy exposures. They also have important implications for pharmacokinetic modeling of infancy PFAS exposures from breastfeeding. For example, the European Food Safety Authority (EFSA) report, "Risk to human health related to the presence of perfluoroalkyl substances in food," used a PBPK model to estimate serum PFOA and PFOS concentrations in age-one infants (2020). This model assumed an estimated decline in human milk concentrations of 3.1% per month for PFOS and 7.7% per month for PFOA. While this is similar to our PFOS estimate, it is much lower than our
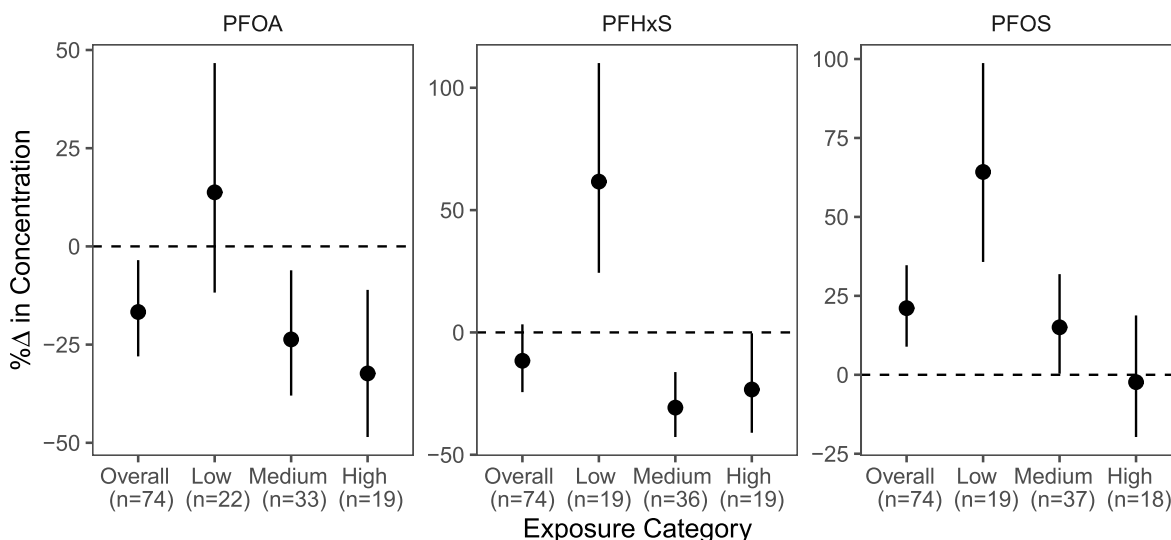

Fig. 2. Estimated change (%) in PFAS concentration from colostrum (collected 3–4 days after delivery) to mature milk collected 4–12 weeks after delivery.
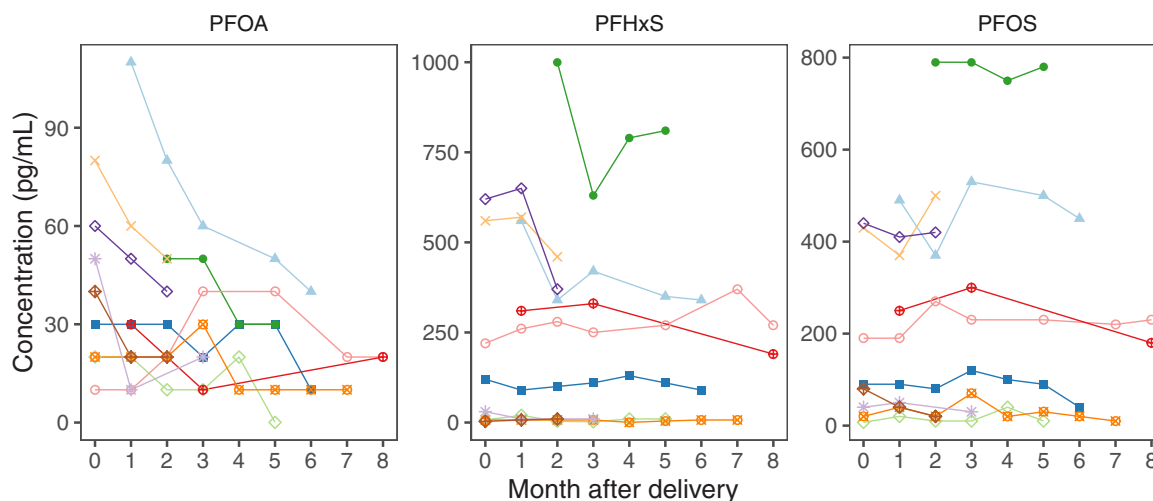
Fig. 3. Concentrations of PFAS over eight months of lactation in 11 women who provided at least 3 colostrum and/or mature milk samples.

Table 4
Estimated change (%) in PFAS concentration per month estimated over 8 months of lactation from 11 women with at least 3 colostrum and/or mature milk samples.

PFAS	Including colostrum samples $N_{samples} = 52$		Excluding colostrum samples $N_{samples} = 44$	
	Effect Estimate (95% CI)	p-value	Effect Estimate	p-value
PFOA	−12 (−22, −1.5)	0.03	−13 (−25, 0.43)	0.06
PFHxS	−2.5 (−9.7, 5.4)	0.53	−2.1 (−11, 7.6)	0.66
PFOS	−3.2 (−8.4, 2.3)	0.24	−5.8 (−12, 0.32)	0.07

estimate for PFOA. This large discrepancy also contradicts EFSA's assumption of a low level of uncertainty in the estimated decline values.

We hypothesized that highly exposed women from Ronneby would have a larger relative decrease in PFAS concentrations from colostrum to mature milk, as the primary source in the population (contaminated drinking water) had been eliminated before initiation of the study. In contrast, continuous and ongoing exposures in low-exposed women represent a higher fraction of their total exposure, leading to a lower relative decrease in concentration. To test this, we evaluated whether the change in PFAS concentration between colostrum and the initial mature milk sample varied by maternal exposure, operationalized as the PFAS concentration in the initial colostrum sample. While PFAS concentrations in low-exposed women tended to increase from colostrum to the initial mature milk sample, concentrations on average decreased or remained constant for high-exposed women. However, in our secondary analysis using serum PFHxS concentrations measured during pregnancy as an indication of exposure level, we identified this pattern for PFOS but not PFOA or PFHxS. There are several possible explanations for these different results. High colostrum levels in some women may be due to higher transfer efficiencies of PFAS from serum to colostrum and/or mature milk, instead of reflecting high initial exposure levels as we assumed. This possibility is underscored by the wide range in transfer efficiencies we recently identified in this cohort (Blomberg et al., 2022). Measurement error may also induce bias in our models, as there is likely more measurement error in relative terms in measurements of low colostrum and human milk concentrations compared to higher concentrations. Finally, colostrum levels are also dependent on maternal parity, as multiparous women typically have lower PFAS concentrations in serum and human milk compared to primiparous women (Rawn et al., 2022). Regardless, the differences in results between these two analyses highlight how PFAS transfer and excretion in human milk is a complex process dependent on many different factors.

This study has several important strengths. First, it is the first study of PFAS concentrations in human milk using a population with a wide range of exposures, varying from low-to high-exposed. For example, while a recent study of milk samples collected 0–90 days after delivery had a median PFOS concentration of 48 pg/mL (IQR: 35, 68) (Lee et al., 2018), the median concentration in samples collected over the same period in our study was 130 pg/mL (IQR: 40, 365). This allowed us to assess whether exposure levels modified estimated changes in PFAS concentrations over lactation. In addition, the high overall concentrations of PFAS in milk in this cohort likely reduced the uncertainty in the measured concentrations. Second, this is the largest study that we are aware of that uses repeated milk samples from the same cohort of mothers, rather than pooling milk samples collected at different times from different women. Using repeated measures from the same mothers provides more reliable estimates of per-person changes than a cross-sectional design (Fitzmaurice et al., 2012; Hooper et al., 2007) and ensures that estimated changes over time are not actually due to underlying differences in exposure. Finally, this was the first study of PFHxS trajectories, a PFAS that is often elevated in communities impacted by AFFF-exposure (Rotander et al., 2015; Xu et al., 2021).

Our study also has some limitations. Our initial mature milk sample had a wide sampling period (between 4 and 12 weeks in lactation), and the specific sampling date was not known. The method of milk collection was also not standardized. However, all mothers received the same sampling instructions, so variation in sample methods or dates is most likely non-systematic. It is possible that highly-exposed women from Ronneby were more interested in the study and eager to participate, which may have led to these mothers collecting their samples earlier in the sampling window (i.e., closer to 4 weeks than 12 weeks postpartum). This sampling bias would make the estimated decrease in PFAS concentration from the colostrum to the initial human milk sample smaller in the highly-exposed group than the low-exposed group, acting opposite to our observed patterns in the two groups. In addition, previous research has linked PFAS exposures to decreased initiation and duration of breastfeeding (Nielsen et al., 2022; Timmermann et al., 2022); therefore, it is possible that some highly exposed women from the cohort did not contribute samples because they were unable to initiate breastfeeding, or that some women did not provide repeat milk samples because they stopped breastfeeding due to limited milk supply. However, the distribution of pregnancy serum PFHxS concentrations in women who provided a paired colostrum and initial human mature milk sample is similar to those who did not (Fig. S3) and the limited number of women with repeated milk samples also reflect the wide range of pregnancy serum PFHxS concentrations in the cohort (Fig. S4),

indicating that participation in this study was representative of the underlying PFAS exposures.

5. Conclusions

This study estimated changes in PFAS concentration between paired colostrum and mature milk samples from mothers with a wide range of PFAS exposures, including women who had been highly exposed to PFAS from AFFF-contaminated drinking water. The estimated changes varied by PFAS and by initial maternal colostrum concentration, suggesting a possible effect of maternal exposure level. These results illustrate the importance of studying PFAS exposures in highly exposed populations. The cumulative breastfeeding exposures in children of highly exposed mothers will depend both on the specific mixture of PFAS contamination as well as the nature of the exposure source (i.e., ongoing or stopped). Additional research should evaluate the relative contribution of breastfeeding to cumulative prenatal and infancy exposures, especially in exposed populations where potential developmental exposures are highest and evidence-based breastfeeding recommendations are needed.

Credit author statement

Annelise J. Blomberg: conceptualization, Methodology, Formal analysis, Data curation, Writing – original draft, writing-review & editing, Visualization; Line S. Haug: methodology, Investigation, Resources, Writing – review & editing, Supervision; Christian Lindh: methodology, Validation, Investigation, Resources, Writing – review & editing, Supervision; Azemira Sabaredzovic: investigation; Daniela Pineda: investigation, Writing – review & editing; Kristina Jakobsson: writing – review & editing, Project administration, Funding acquisition; Christel Nielsen: conceptualization, Writing – review & editing, Supervision, Project administration, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

The data that has been used is confidential.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envres.2022.115096.

References

Anderson, P.O., 2018. Drugs in lactation. Pharm. Res. (N. Y.) 35, 45. https://doi.org/10.1007/s11095-017-2287-z.

Andreas, N.J., Kampmann, B., Mehring Le-Doare, K., 2015. Human breast milk: a review on its composition and bioactivity. Early Human Development, Special Issue: Neonatal Update 91, 629–635. https://doi.org/10.1016/j.earlhumdev.2015.08.013, 2015.

Barouki, R., Gluckman, P.D., Grandjean, P., Hanson, M., Heindel, J.J., 2012. Developmental origins of non-communicable disease: implications for research and public health. Environ. Health 11, 42. https://doi.org/10.1186/1476-069X-11-42.

Bates, D., Mächler, M., Bolker, B., Walker, S., 2015. Fitting linear mixed-effects models using lme4. J. Stat. Software 67, 1–48. https://doi.org/10.18637/jss.v067.i01.

Beesoon, S., Martin, J.W., 2015. Isomer-specific binding affinity of perfluorooctanesulfonate (PFOS) and perfluorooctanoate (PFOA) to serum proteins. Environ. Sci. Technol. 49, 5722–5731. https://doi.org/10.1021/es505399w.

Blomberg, A., Norén, E., Haug, L.S., Lindh, C., Sabaredzovic, A., Pineda, D., Jakobsson, K., Nielsen, C., 2022. Estimated transfer of perfluoroalkyl substances (PFAS) from maternal serum to breastmilk in women highly exposed from contaminated drinking water: a study in Ronneby Mother-Child Cohort. Environ. Health Perspect. https://doi.org/10.1289/EHP11292 (in press).

Chi, Q., Li, Z., Huang, J., Ma, J., Wang, X., 2018. Interactions of perfluorooctanoic acid and perfluorooctanesulfonic acid with serum albumins by native mass spectrometry, fluorescence and molecular docking. Chemosphere 198, 442–449. https://doi.org/10.1016/j.chemosphere.2018.01.152.

De Silva, A.O., Armitage, J.M., Bruton, T.A., Dassuncao, C., Heiger-Bernays, W., Hu, X.C., Kärrman, A., Kelly, B., Ng, C., Robuck, A., Sun, M., Webster, T.F., Sunderland, E.M., 2021. PFAS exposure pathways for humans and wildlife: a synthesis of current knowledge and key gaps in understanding. Environ. Toxicol. Chem. 40, 631–657. https://doi.org/10.1002/etc.4935.

Dietert, R.R., DeWitt, J.C., Germolec, D.R., Zelikoff, J.T., 2010. Breaking patterns of environmentally influenced disease for health risk reduction: immune perspectives. Environ. Health Perspect. 118, 1091–1099. https://doi.org/10.1289/ehp.1001971.

Dietert, R.R., Piepenbrink, M.S., 2006. Perinatal immunotoxicity: why adult exposure assessment fails to predict risk. Environ. Health Perspect. 114, 477–483. https://doi.org/10.1289/ehp.8566.

EFSA Panel on Contaminants in the Food Chain, 2020. Risk to human health related to the presence of perfluoroalkyl substances in food. EFSA J. 18, e06223 https://doi.org/10.2903/j.efsa.2020.6223.

Fenton, S.E., Ducatman, A., Boobis, A., DeWitt, J.C., Lau, C., Ng, C., Smith, J.S., Roberts, S., 2021. Per- and polyfluoroalkyl substance toxicity and human health review: current state of knowledge and strategies for informing future research. Environ. Toxicol. Chem. 40, 606–630. https://doi.org/10.1002/etc.4890.

Fitzmaurice, G.M., Laird, N.M., Ware, J.H., 2012. Applied Longitudinal Analysis. John Wiley & Sons.

Forsthuber, M., Kaiser, A.M., Granitzer, S., Hassl, I., Hengstschläger, M., Stangl, H., Gundacker, C., 2020. Albumin is the major carrier protein for PFOS, PFOA, PFHxS, PFNA and PFDA in human plasma. Environ. Int. 137, 105324 https://doi.org/10.1016/j.envint.2019.105324.

Gao, K., Zhuang, T., Liu, X., Fu, Jianjie, Zhang, J., Fu, Jie, Wang, L., Zhang, A., Liang, Y., Song, M., Jiang, G., 2019. Prenatal exposure to per- and polyfluoroalkyl substances (PFASs) and association between the placental transfer efficiencies and dissociation constant of serum proteins–PFAS complexes. Environ. Sci. Technol. 53, 6529–6538. https://doi.org/10.1021/acs.est.9b00715.

Gyllenhammar, I., Benskin, J.P., Sandblom, O., Berger, U., Ahrens, L., Lignell, S., Wiberg, K., Glynn, A., 2019. Perfluoroalkyl acids (PFAAs) in children's serum and contribution from PFAA-contaminated drinking water. Environ. Sci. Technol. 53, 11447–11457. https://doi.org/10.1021/acs.est.9b01746.

Haug, L.S., Huber, S., Becher, G., Thomsen, C., 2011. Characterisation of human exposure pathways to perfluorinated compounds — comparing exposure estimates with biomarkers of exposure. Environ. Int. 37, 687–693. https://doi.org/10.1016/j.envint.2011.01.011.

Hooper, K., She, J., Sharp, M., Chow, J., Jewell, N., Gephart, R., Holden, A., 2007. Depuration of polybrominated diphenyl ethers (PBDEs) and polychlorinated biphenyls (PCBs) in breast milk from California first-time mothers (primiparae). Environ. Health Perspect. 115, 1271–1275. https://doi.org/10.1289/ehp.10166.

Hu, X.C., Andrews, D.Q., Lindstrom, A.B., Bruton, T.A., Schaider, L.A., Grandjean, P., Lohmann, R., Carignan, C.C., Blum, A., Balan, S.A., Higgins, C.P., Sunderland, E.M., 2016. Detection of poly- and perfluoroalkyl substances (PFASs) in U.S. Drinking water linked to industrial sites, military fire training areas, and wastewater treatment plants. Environ. Sci. Technol. Lett. 3, 344–350. https://doi.org/10.1021/acs.estlett.6b00260.

Jackson, T.W., Scheibly, C.M., Polera, M.E., Belcher, S.M., 2021. Rapid characterization of human serum albumin binding for per- and polyfluoroalkyl substances using differential scanning fluorimetry. Environ. Sci. Technol. 55, 12291–12301. https://doi.org/10.1021/acs.est.1c01200.

Kang, H., Choi, K., Lee, H.-S., Kim, D.-H., Park, N.-Y., Kim, S., Kho, Y., 2016. Elevated levels of short carbon-chain PFCAs in breast milk among Korean women: current

status and potential challenges. Environ. Res. 148, 351–359. https://doi.org/10.1016/j.envres.2016.04.017.

Kato, K., Wong, L.-Y., Jia, L.T., Kuklenyik, Z., Calafat, A.M., 2011. Trends in exposure to polyfluoroalkyl chemicals in the U.S. Population: 1999−2008. Environ. Sci. Technol. 45, 8037–8045. https://doi.org/10.1021/es1043613.

LaKind, J.S., Verner, M.-A., Rogers, R.D., Goeden, H., Naiman, D.Q., Marchitti, S.A., Lehmann, G.M., Hines, E.P., Fenton, S.E., 2022. Current breast milk PFAS levels in the United States and Canada: after all this time, why don't we know more? Environ. Health Perspect. 130, 025002 https://doi.org/10.1289/EHP10359.

Lee, S., Kim, Sunmi, Park, J., Kim, H.-J., Choi, G., Choi, S., Kim, Sungjoo, Kim, S.Y., Kim, Sungkyoon, Choi, K., Moon, H.-B., 2018. Perfluoroalkyl substances (PFASs) in breast milk from Korea: time-course trends, influencing factors, and infant exposure. Sci. Total Environ. 612, 286–292. https://doi.org/10.1016/j.scitotenv.2017.08.094.

Liew, Z., Goudarzi, H., Oulhote, Y., 2018. Developmental exposures to perfluoroalkyl substances (PFASs): an update of associated health outcomes. Current environmental health reports 5, 1–19. https://doi.org/10.1007/s40572-018-0173-4.

Liu, J., Li, J., Liu, Y., Chan, H.M., Zhao, Y., Cai, Z., Wu, Y., 2011. Comparison on gestation and lactation exposure of perfluorinated compounds for newborns. Environ. Int. 37, 1206–1212. https://doi.org/10.1016/j.envint.2011.05.001.

Lönnerdal, B., Erdmann, P., Thakkar, S.K., Sauser, J., Destaillats, F., 2017. Longitudinal evolution of true protein, amino acids and bioactive proteins in breast milk: a developmental perspective. J. Nutr. Biochem. 41, 1–11. https://doi.org/10.1016/j.jnutbio.2016.06.001.

Lüdecke, D., Ben-Shachar, M., Patil, I., Waggoner, P., Makowski, D., 2021. Performance: an R package for assessment, comparison and testing of statistical models. JOSS 6, 3139. https://doi.org/10.21105/joss.03139.

Mogensen, U.B., Grandjean, P., Nielsen, F., Weihe, P., Budtz-Jørgensen, E., 2015. Breastfeeding as an exposure pathway for perfluorinated alkylates. Environ. Sci. Technol. 49, 10466–10473. https://doi.org/10.1021/acs.est.5b02237.

Mondal, D., Weldon, R.H., Armstrong, B.G., Gibson, L.J., Lopez-Espinosa, M.-J., Shin, H.-M., Fletcher, T., 2014. Breastfeeding: a potential excretion route for mothers and implications for infant exposure to perfluoroalkyl acids. Environ. Health Perspect. 122, 187–192. https://doi.org/10.1289/ehp.1306613.

Nakagawa, S., Johnson, P.C.D., Schielzeth, H., 2017. The coefficient of determination R2 and intra-class correlation coefficient from generalized linear mixed-effects models revisited and expanded. J. R. Soc. Interface 14, 20170213. https://doi.org/10.1098/rsif.2017.0213.

Nielsen, C., Li, Y., Lewandowski, M., Fletcher, T., Jakobsson, K., 2022. Breastfeeding initiation and duration after high exposure to perfluoroalkyl substances through contaminated drinking water: a cohort study from Ronneby, Sweden. Environ. Res. 207, 112206 https://doi.org/10.1016/j.envres.2021.112206.

Norén, E., Lindh, C., Glynn, A., Rylander, L., Pineda, D., Nielsen, C., 2021. Temporal trends, 2000–2017, of perfluoroalkyl acid (PFAA) concentrations in serum of Swedish adolescents. Environ. Int. 155, 106716 https://doi.org/10.1016/j.envint.2021.106716.

Papadopoulou, E., Sabaredzovic, A., Namork, E., Nygaard, U.C., Granum, B., Haug, L.S., 2016. Exposure of Norwegian toddlers to perfluoroalkyl substances (PFAS): the association with breastfeeding and maternal PFAS concentrations. Environ. Int. 94, 687–694. https://doi.org/10.1016/j.envint.2016.07.006.

R Core Team, 2021. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria.

Rappazzo, K.M., Coffman, E., Hines, E.P., 2017. Exposure to perfluorinated alkyl substances and health outcomes in children: a systematic review of the epidemiologic literature. Int. J. Environ. Res. Publ. Health 14, 691. https://doi.org/10.3390/ijerph14070691.

Rawn, D.F.K., Dufresne, G., Clément, G., Fraser, W.D., Arbuckle, T.E., 2022. Perfluorinated alkyl substances in Canadian human milk as part of the Maternal-Infant Research on Environmental Chemicals (MIREC) study. Sci. Total Environ. 831, 154888 https://doi.org/10.1016/j.scitotenv.2022.154888.

Rotander, A., Toms, L.-M.L., Aylward, L., Kay, M., Mueller, J.F., 2015. Elevated levels of PFOS and PFHxS in firefighters exposed to aqueous film forming foam (AFFF). Environ. Int. 82, 28–34. https://doi.org/10.1016/j.envint.2015.05.005.

Salvatore, D., Mok, K., Garrett, K.K., Poudrier, G., Brown, P., Birnbaum, L.S., Goldenman, G., Miller, M.F., Patton, S., Poehlein, M., Varshavsky, J., Cordner, A., 2022. Presumptive contamination: a new approach to PFAS contamination based on likely sources. Environ. Sci. Technol. Lett. https://doi.org/10.1021/acs.estlett.2c00502.

Schwieger, C., Ropers, M.-H., 2013. Binding of a perfluorinated surfactant to β-lactoglobulin in aqueous solutions. Food Hydrocolloids 30, 241–248. https://doi.org/10.1016/j.foodhyd.2012.05.021.

Sunderland, E.M., Hu, X.C., Dassuncao, C., Tokranov, A.K., Wagner, C.C., Allen, J.G., 2019. A review of the pathways of human exposure to poly- and perfluoroalkyl substances (PFASs) and present understanding of health effects. J. Expo. Sci. Environ. Epidemiol. 29, 131–147. https://doi.org/10.1038/s41370-018-0094-1.

Thomsen, C., Haug, L.S., Stigum, H., Frøshaug, M., Broadwell, S.L., Becher, G., 2010. Changes in concentrations of perfluorinated compounds, polybrominated diphenyl ethers, and polychlorinated biphenyls in Norwegian breast-milk during twelve months of lactation. Environ. Sci. Technol. 44, 9550–9556. https://doi.org/10.1021/es1021922.

Timmermann, C.A.G., Andersen, M.S., Budtz-Jørgensen, E., Boye, H., Nielsen, F., Jensen, R.C., Bruun, S., Husby, S., Grandjean, P., Jensen, T.K., 2022. Pregnancy exposure to perfluoroalkyl substances and associations with prolactin concentrations and breastfeeding in the odense child cohort. J. Clin. Endocrinol. Metab. 107, e631–e642. https://doi.org/10.1210/clinem/dgab638.

Wickham, H., Averick, M., Bryan, J., Chang, W., McGowan, L.D., François, R., Grolemund, G., Hayes, A., Henry, L., Hester, J., Kuhn, M., Pedersen, T.L., Miller, E., Bache, S.M., Müller, K., Ooms, J., Robinson, D., Seidel, D.P., Spinu, V., Takahashi, K., Vaughan, D., Wilke, C., Woo, K., Yutani, H., 2019. Welcome to the tidyverse. Journal of Open Source Software 4, 1686. https://doi.org/10.21105/joss.01686.

Xu, Y., Nielsen, C., Li, Y., Hammarstrand, S., Andersson, E.M., Li, H., Olsson, D.S., Engström, K., Pineda, D., Lindh, C.H., Fletcher, T., Jakobsson, K., 2021. Serum perfluoroalkyl substances in residents following long-term drinking water contamination from firefighting foam in Ronneby, Sweden. Environ. Int. 147, 106333 https://doi.org/10.1016/j.envint.2020.106333.

Zheng, G., Schreder, E., Dempsey, J.C., Uding, N., Chu, V., Andres, G., Sathyanarayana, S., Salamova, A., 2021. Per- and polyfluoroalkyl substances (PFAS) in breast milk: concerning trends for current-use PFAS. Environ. Sci. Technol. 55, 7510–7520. https://doi.org/10.1021/acs.est.0c06978.