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Legacy perfluoroalkyl acids and their oxidizable precursors in plasma samples of Norwegian women

Ana Carolina M F Coêlho [a,*], Lara Cioni [a,b], Wendy Van Dreunen [a], Vivian Berg [c,d], Charlotta Rylander [a], Ilona Urbarova [a], Dorte Herzke [b,e], Torkjel M Sandanger [a,b]

[a] Department of Community Medicine, Faculty of Health Sciences, UiT - The Arctic University of Norway, Tromsø, Norway
[b] Norwegian Institute for Air Research, Fram Centre, Tromsø, Norway
[c] Department of Medical Biology, Faculty of Health Sciences, UiT - The Arctic University of Norway, Tromsø, Norway
[d] Department of Laboratory Medicine, Division of Diagnostic Services, University Hospital of North-Norway, Tromsø, Norway
[e] Department for Arctic and Marine Biology, UiT – The Arctic University of Norway, Tromsø, Norway

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ABSTRACT

Humans are exposed to perfluoroalkyl acids (PFAA) mainly through direct pathways, such as diet and drinking water, but indirect exposure also occurs when PFAA precursors break down to form legacy PFAA. Exposure to PFAA precursors raises particular concern, as neither the exposure nor the precursors themselves have been well described. In the present study, we aimed to assess the indirect contribution of oxidizable PFAA precursors to the total per- and polyfluoroalkyl substances (PFAS) burden in human plasma following the voluntary phase-out of production of long-chain PFAS. In addition, multiple logistic regression was used to explore associations between selected lifestyle and dietary factors and the oxidizable PFAA precursors fraction. This study included 302 cancer-free participants of the Norwegian Women and Cancer postgenome cohort. PFAS analyses were performed in plasma samples to determine PFAS concentrations before and after oxidation with the Total Oxidizable Precursor (TOP) assay. In pre-TOP analyses, perfluorooctane sulfonic acid (PFOS) was the dominant compound, followed by perfluorooctanoic acid (PFOA).The vast majority (98%) of the study population had increased post-TOP concentrations for at least one PFAA. The formation of PFAA accounted for 12% of the total PFAS burden, with seven PFAA observed post-TOP in at least 30% of study participants. PFHpA, br- PFOA, and PFDA were only detected in post-TOP analyses and showed the highest increase in concentrations. Of the PFAA with increased concentrations, we noted significant associations for year of birth, parity, BMI, and some dietary factors, although they were not consistent between the different PFAA. These results indicate that while the TOP assay might not provide a complete assessment of total PFAS burden in humans, it offers comprehensive assessment of unknown PFAA precursors that might be present in plasma, and it could therefore be implemented as an auxiliary tool in this regard.

1. Introduction

Humans are exposed to a wide range of environmental contaminants in their everyday life, including dioxins, organochlorines, pesticides, and heavy metals. A specific class of environmental contaminants linked to numerous adverse health effects are per- and polyfluoroalkyl substances (PFAS). PFAS represent a broad group of synthetic chemicals that are used in a variety of industrial and consumer products due to their distinctive physicochemical properties [Nair et al. 2021; Göckener et al. 2021; Buck et al., 2011]. One fundamental property is thermal and chemical stability due to the robust carbon–fluorine bonds, which make PFAS highly resistant to degradation [Sivaram et al. 2022; Sunderland et al. 2018]. Perfluoroalkyl acids (PFAA), which include legacy perfluorocarboxylic acids (PFCA) and legacy perfluorosulfonic acids (PFSA), are the most studied PFAS and have been frequently detected in abiotic and biotic matrices [Buck et al. 2011]. Extensive exposure to PFAS might pose health risks to both wildlife and humans, including disruption of the endocrine, immune, and reproductive systems [Sunderland et al. 2018; Seshasayee et al. 2021; McDermett et al. 2022].

Human exposure to PFAS occurs directly through the consumption of

* Corresponding author.
E-mail address: ana.carolina.coelho@uit.no (A.C. M F Coêlho).

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food and drinking water, as well as through air, indoor environments, and skin contact [Seshasayee et al. 2021; Domingo and Nadal, 2019]. Blood PFAS concentrations are commonly used as markers of the total PFAS burden in humans [Nyström et al 2022], as these concentrations reflect cumulative PFAS exposure, mostly from diet and contact with consumer products. PFAS concentrations may be influenced by socio-demographic, lifestyle, and reproductive factors, including age, number of childbirths, cumulative duration of breastfeeding, and geographic location [Manzano-Salgado et al. 2016; Sagiv et al. 2015; Wise et al. 2022; Nyström et al 2022].

Humans are also exposed through the biotransformation of PFAA precursors [Poothong et al 2020]. These precursors comprise a variety of less persistent PFAS [Göckener et al. 2021; Lewis et al. 2022] and can be converted into highly stable, persistent terminal legacy PFAA within the human body or in the environment [Lewis et al. 2022; Rodgers et al. 2022]. Currently, there are very limited data on human exposure to PFAA precursors [Gebbink et al. 2015; McDonough et al. 2022]. A major challenge is that targeted measurement of PFAA precursor concentrations is not always possible due to the lack of structural information and analytical limitations [Göckener et al. 2021]. One promising approach is the Total Oxidizable Precursor (TOP) assay [Houtz and Sedlak, 2012], in which oxidant agents are used to degrade PFAS precursors under basic conditions into stable, quantifiable PFAA [Göckener et al. 2021]. The comparison of PFAA concentrations before and after the TOP assay provides an estimate of the oxidizable PFAA precursors that are present in a sample [Göckener et al. 2022; Lewis et al. 2022], including those that are already known and identified as well as those that are currently unknown or not fully understood. In the present study, we aimed to assess the indirect contribution of oxidizable PFAA precursors to the total PFAS burden in plasma samples from healthy controls of the Norwegian Women and Cancer (NOWAC) postgenome cohort, using the TOP assay. The samples were obtained in 2003–2006, a period shortly following the initial phase out efforts of PFAS. Furthermore, we investigated whether selected lifestyle and dietary factors are associated with PFAA precursors by analyzing the changes in PFAA concentrations post-TOP assay.

2. Methods

2.1. Study population from the Norwegian women and cancer postgenome cohort

Approximately 50,000 NOWAC participants constitute the NOWAC postgenome cohort, which is a subset of the NOWAC study [details on the NOWAC study are available in Lund et al. 2008]. In the years 2003–2006, women included in the NOWAC postgenome cohort had donated a blood sample [Dumeaux et al. 2008] and completed two self-administered questionnaires: one on their lifestyle and use of medications at the time of blood sample collection, and a validated, semi-quantitative food frequency questionnaire (FFQ) [Parr, et al. 2006; Hjartåker, et al. 2007], which covered the frequency and quantity of consumption of more than 90 common food items over the previous year.

The present study is a secondary analysis of cross-sectional survey that includes questionnaire data and plasma samples from a subset of the NOWAC postgenome cohort who served as age-matched controls in case-control studies of breast [Dumeaux et al., 2015] and lung cancer [Sandanger et al. 2018]. The recruitment period enabled us to explore changes that occurred during this time. This subset originally included 316 women who were healthy controls at enrollment and at the time of blood sample collection. We excluded women without complete information on the selected lifestyle and dietary factors, resulting in a final study sample of 302 women.

2.2. Laboratory methods

Plasma samples were stored at −80 °C after blood sample collection and prior to analysis. The samples were stored in the freezer between 15 and 18 years. All PFAS analyses, including target analyses and an optimized TOP assay for small volumes, were carried out at the Norwegian Institute for Air Research (NILU, Tromsø, Norway). The PFAS analysis included a total of 16 legacy PFAA (seven PFSA (C4-C10) and nine PFCA (C4, C7-C13)), and four known PFAA precursors (including perfluorooctane sulfonamide [FOSA] and three fluorotelomers sulfonic acids [FTSA]: 4:2 FTS, 6:2 FTS, and 8:2 FTS) (Table S1). Linear and branched isomers were quantified separately. Only linear isomer standards were included in the analysis; thus, the branched isomers were quantified using the corresponding linear isomer standards.

A detailed description of the PFAS analysis and the optimized TOP assay is provided in Cioni et al. (2022), including information regarding chemicals, standards, and quality control procedures. Briefly, 250 μL of plasma were taken from each sample and spiked with 20 μL of an internal standard mixture (0.5 ng/μL) containing isotopically labeled PFSA (C6, C8) and PFCA (C4-C12, C14). Next, the targeted PFAA and PFAA precursors were extracted by adding methanol, and samples were sonicated 3 times and centrifuged for 10 min at 10,000 rpm. All supernatants were then collected and divided into two parts: 50 μL were spiked with 20 μL of recovery standard and used for the PFAS analysis without any additional clean-up step, and 450 μL were used for the TOP assay.

Prior to the TOP assay, the supernatants were evaporated to dryness. The dried material was resuspended with 500 μL of a 0.8 M solution of sodium persulfate (7.6 g of $Na_2S_2O_8$ in 40 mL of MilliQ water) and 120 μL of a 10 M solution of sodium hydroxide (20 g in 50 mL MilliQ water), and then heated at 85 °C for 24 h. Next, samples were neutralized with concentrated HCl and extracted with methyl-*tert*-butylether. All PFAS analyses were performed by using an ultra-high pressure liquid chromatography coupled to triple-quadrupole mass-spectrometry (UHPLC-MS/MS), as previously described by Hanssen et al. (2013). LC Quan software (v.2.6, Thermo Fisher Scientific Inc., Waltham, Massachusetts, USA) was used for quantification. PFAS analyses were carried out before and after TOP assay (pre- and post-TOP).

2.3. QA/QC

Details on the validation of the method are reported elsewhere [Cioni et al. 2022]. For each batch of samples (n = 18), a method blank and a reference serum sample were included. The blanks were analyzed pre- and post-TOP assay to evaluate possible background contamination. Limits of detection (LOD) were calculated as the average concentration in the blanks plus 3 times the standard deviation of the blanks. In case of no detection in the blanks, LOD was calculated by multiplying the noise of the blanks by 3. The reference serum was obtained from the Arctic Monitoring and Assessment Programme (AMAP) Ring Test for Persistent Organic Pollutants. Measured PFAA concentrations pre-TOP assay in the AMAP serum were within +/- 20% of the reference values. Recoveries of target PFAA were evaluated using labelled standards, and the median recoveries ranged from 62 to 77% pre-TOP and from 45 to 93% post-TOP PFAS analysis (Table S2).

2.4. Lifestyle and dietary factors

Information regarding year of birth, parity, duration of previous breastfeeding, time since last childbirth, smoking status, weight and height was taken from the questionnaire that participants completed at the time of blood sample collection. Body mass index (BMI) was calculated as weight (kilograms) divided by height (meters) squared.

Information from the FFQ that participants completed at the time of blood sample collection was used to calculate the daily intake of included food items, using statistical syntax in SAS (SAS Institute Inc.,

Cary, NC, USA) at the Department of Community Medicine, UiT The Arctic University of Norway [Parr et al., 2006]. The analyses focused either on individual food items or food groups that have been previously identified as potential sources of legacy PFAS exposure [Rylander et al. 2010], including lean fish, fatty fish, shellfish, fish products, fish spread, meats, egg, vegetables, fruits, dairy products (milk, yoghurt, and cheese), saturated fats (butter and margarine), cereals (rice, pasta, and bread), chocolate, pastries, ice cream, salty snacks, and beverages.

2.5. Statistical analysis

All data processing and statistical analyses were conducted using R version 4.0.2 (R Core Team). Concentrations of PFAA and PFAA precursors below the LOD were substituted by LOD/$\sqrt{2}$. Statistical analyses were performed only for PFAA and PFAA precursors with a detection frequency above 70% in either the pre- or post-TOP PFAS analyses, and statistical significance was set at $p < 0.05$.

The ratio of pre- and post-TOP PFAA concentrations ($PFAA_{post\text{-}TOP}/PFAA_{pre\text{-}TOP}$) was used as an indicator of which PFAS had increased concentrations after oxidation. Changes in pre- and post-TOP PFAA concentrations (ΔPFAA, calculated as $PFAA_{post\text{-}TOP} - PFAA_{pre\text{-}TOP}$) was used as an indicator of the amount of oxidizable PFAA precursors in the sample. $PFAA_{post\text{-}TOP}/PFAA_{pre\text{-}TOP}$ and ΔPFAA were calculated for each individual sample. In cases where post-TOP PFAA concentrations were below the corresponding pre-TOP concentrations, $PFAA_{post\text{-}TOP}/PFAA_{pre\text{-}TOP}$ and ΔPFAA were shown as 1 and 0, respectively. Whereas, in cases where pre-TOP PFAA concentrations were below LOD, $PFAA_{post\text{-}TOP}/PFAA_{pre\text{-}TOP}$ and ΔPFAA were calculated based on the substituted value, as mentioned above. As PFAA precursor concentrations were determined by the difference between two measurements with their own uncertainties, a conservative approach was chosen to avoid overestimating the number of individuals with increased PFAA concentrations. Thus, concentrations were only considered to have increased when $PFAS_{pre\text{-}TOP}/PFAS_{post\text{-}TOP}$ was ≥ 1.2.

Considering that increased post-TOP concentrations were not observed in all samples, we dichotomized the samples into two groups based on the established threshold (1.2): those that showed positive post-TOP changes and those that showed no changes. Associations between lifestyle and dietary factors and post-TOP PFAA concentration changes were investigated by multiple logistic regression models. We selected factors previously associated with legacy PFAA concentrations, including year of birth, parity, cumulative duration of breastfeeding, BMI, and smoking status [Rylander et al. 2010; Berg et al. 2014]. After calculating the Spearman's rank correlation coefficient between continuous factors (year of birth, parity, cumulative duration of breastfeeding, and BMI), only parity and cumulative duration of breastfeeding were moderately correlated ($s = 0.6$, $p < 0.05$) (Table S3), but cumulative duration of breastfeeding was removed, as this information was missing for 31 women. Therefore, the following factors were included in the models: year of birth, parity, BMI, and smoking status. Considering that use of legacy PFAA was expected to decline after their production phase-out in the early 2000 s [Manzano-Salgado et al. 2016], we also included the blood sampling period (May 2003-November 2006, divided into 180-day periods) in the models.

3. Results

3.1. General characteristics of the study population

All participants were aged between 43 and 62 years old, with mean year of birth of 1949. The mean BMI was 25.0 kg/m^2. A total of 276 study participants (91.4%) had at least one child prior blood sample collection. A total of 119 study participants (39.4%) were non-smokers. The full blood sample collection period lasted 1259 days, with the period between November 2005 and May 2006 having the highest number of blood sample collections (a total of 70 study participants donating during this interval) (Table 1). The dietary characteristics varied among the study participants (Table S4).

3.2. Pre-TOP PFAS analysis

A total of seven PFAA were detected in more than 70% of the study participants in the pre-TOP PFAS analysis, including four PFCA (lin-PFOA, PFNA, PFUnDA, and PFDoDA) and three PFSA (PFHxS, PFHpS, lin-PFOS, and br-PFOS). Lin-PFOS (hereafter referred as PFOS) showed the highest median concentration of 11.41 ng/mL, followed by br-PFOS (8.02 ng/mL), lin-PFOA (2.88 ng/mL, hereafter referred as PFOA), and PFHxS (1.02 ng/mL). Amongst the other PFAA detected, median concentrations were < 1 ng/mL (Table S5).

3.3. Post-TOP PFAS analysis

Of the PFAA detected in the pre-TOP analysis, four PFAA, including C9, C11 PFCA and C7-C8 PFSA, were observed to increase concentrations in at least 30% of samples in the post-TOP PFAS analysis (Table 2). In addition, three other PFCA (C7, C8, C10) were detected in more than 70% of the study participants only in the post-TOP PFAS analysis (Fig. 1). Even if the changes in concentration for those newly formed PFCA were low on an absolute scale (ranged from < LOD to 0.30 ng/ml), the changes in their detection frequency are substantial. The ΔPFAA ranged from 0.41 to 27.19 ng/mL, and the sum ΔPFAA accounted for approximately 12% of the total PFAS burden. PFHpA showed the highest proportion of study participants with increased post-TOP concentrations (83.1%), followed by 73 % for br-PFOA, 53% for PFDA, 49.3% PFHpS, and 48.3% br-PFOS. A minority of study participants showed increased concentrations of PFHxS (9.3%), lin-PFOA (6.6%), and lin-PFOS (2%), and only two study participants (0.7%) did not show increased concentrations of any of the targeted PFAA.

Among individuals with increased PFAA concentrations, mixtures of newly formed PFAA were observed more frequently than single compounds. For instance, a total of 92 samples showed simultaneous increases in concentrations of PFHpA, br-PFOA, and PFDA (Fig. 2). The largest median $PFAA_{post\text{-}TOP}/PFAA_{pre\text{-}TOP}$ were 24.0, 2.7, and 1.7 for PFDA, br-PFOA, and PFHpA, respectively (Figure S1). Looking at the individual PFAA with increased concentrations in at least 30% of the study participants, the largest ΔPFAA median was 2.40 ng/mL for br-PFOS, followed by 0.23 ng/mL for PFDA, and 0.10 ng/mL for PFHpS.

Table 1
Characteristics of the study population (n = 302).

Characteristic	Mean or N	SD or P (%)	Range (min – max)
Year of birth	1949	4.2	1943 – 1957
BMI (kg/m^2)	25.0	3.6	17.0 – 36.3
Parity			
Nulliparous	26	8.6%	
Primiparous	24	7.9%	
Duoparous	140	46.4%	
Multiparous	112	37.1%	
Smoking status			
Never	119	39.4%	
Former	94	31.1%	
Smoker	89	29.5%	
Blood sampling period (from first day of the study)	669.8	343.1	0 – 1259
May 2003 – Nov 2003	45	14.9%	
Nov 2003 – May 2004	26	8.6%	
May 2004 – Nov 2004	34	11.3%	
Nov 2004 – May 2005	61	20.2%	
May 2005 – Nov 2005	36	11.9%	
Nov 2005 – May 2006	70	23.2%	
May 2006 – Nov 2006	30	9.9%	

Abbreviations: N: number of study participants; SD: standard deviation; P: percentage; BMI: body mass index.

Table 2
Plasma PFAA concentrations (ng/mL) in pre-TOP and post-TOP PFAS analyses. NOWAC postgenome cohort (n = 302). Bold data indicates PFAA with increases in post-TOP concentrations in greater than 30% samples.

	Pre-TOP			post-TOP					
PFAA	**Median**	**LOD**	**% DF[1]**	**Median**	**LOD**	**% DF[1]**	**% (n) samples with detected increase[2]**	**$PFAA_{post\text{-}TOP}/PFAA_{pre\text{-}TOP}$[3]**	**ΔPFAA[4]**
PFCA									
PFHpA	<LOD	0.06	37.1	0.09	0.04	99.3	**83.1 (251)**	1.7	0.04
PFOA	2.88	0.13	100	2.92	0.21	100	6.6 (20)	1.3	0.55
br-PFOA	<LOD	0.06	0	0.09	0.07	72.5	**72.5 (219)**	2.7	0.07
PFNA	0.63	0.24	98.7	0.68	0.09	100	**32.4 (98)**	1.3	0.18
PFDA	0.22	0.02	53.6	0.30	0.04	99.7	**53.0 (160)**	24.0	0.23
PFUnDA	0.36	0.02	99.7	0.38	0.07	98.7	**33.4 (101)**	1.4	0.11
PFSA									
PFHxS	1.02	0.11	100	1.02	0.10	100	9.3 (28)	1.2	0.20
PFHpS	0.27	0.03	97.7	0.31	0.03	99.7	**49.3 (149)**	1.5	0.10
PFOS	11.41	0.02	100	11.29	0.18	100	2 (6)	2.1	5.6
br-PFOS	8.02	0.26	100	9.49	0.41	100	**48.3 (146)**	1.3	2.4

Abbreviations: LOD: limit of detection; DF: Detection frequency; br-PFOA: sum of branched isomers of PFOA; br-PFOS: sum of branched isomers of PFOS.
[1] DF: Detection frequency; only compounds with detection frequency ≥ 70% in either PFAS analyses or TOP assay were included for further assessment.
[2] Proportion of study participants with increased post-TOP PFAA concentration ($PFAA_{post\text{-}TOP}/PFAA_{pre\text{-}TOP} \geq 1.2$). The number in the parentheses indicates the number of samples with increased post-TOP PFAA concentration.
[3] Median ratio of pre- and post-TOP PFAS concentrations ($PFAA_{post\text{-}TOP}/PFAA_{pre\text{-}TOP}$). Included only samples with detected increase ($PFAA_{post\text{-}TOP}/PFAA_{pre\text{-}TOP} \geq 1.2$).
[4] Median change in pre- and post-TOP PFAA concentrations ($\Delta PFAA = PFAA_{post\text{-}TOP} - PFAA_{pre\text{-}TOP}$). Included only samples with detected increase ($PFAA_{post\text{-}TOP}/PFAA_{pre\text{-}TOP} \geq 1.2$).

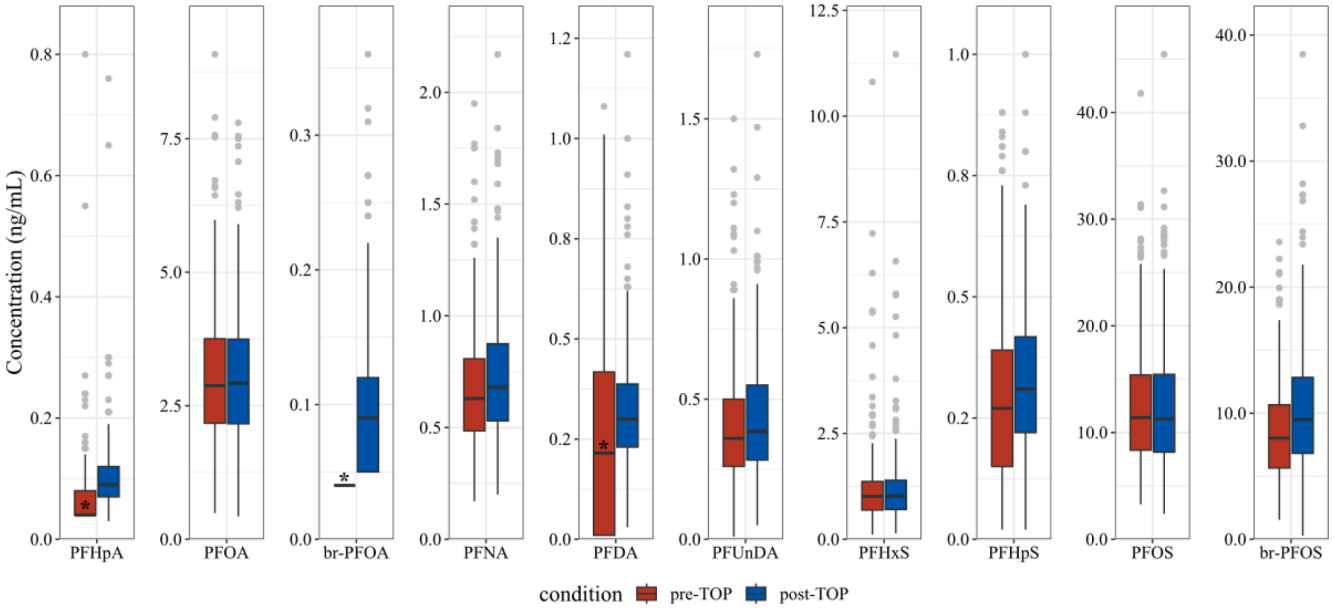

Fig. 1. Boxplot illustrating the concentrations (ng/mL) of individual PFAA in plasma in pre-TOP and post-TOP PFAS analyses. Only PFAA with detection frequency ≥ 70% in either pre-TOP or post-TOP analyses are displayed. The asterisk (*) indicates PFAA below the limit of detection frequency. Abbreviations: br-PFOA: sum of branched isomers of PFOA; br-PFOS: sum of branched isomers of PFOS.

Median concentrations of PFOA exhibited only a slight increase of 0.04 ng/mL, while the median of PFHxS remained constant. Concentration of PFOS exhibited a median decrease of 0.12 ng/mL. Increased concentrations of these PFAA were observed in<10% of individuals in the post-TOP PFAS analysis.

3.4. Association between post-TOP PFAA concentration changes and selected lifestyle and dietary factors

We observed positive changes (Δ) in the post-TOP concentrations of PFHpA, br-PFOA, PFNA, PFDA, PFUnDA, PFHpS, and br-PFOS in more than 30% of participants, and therefore, included in the multiple logistic regression analyses. After adjusting for the selected lifestyle and dietary factors, the estimated odds of observing a positive change in Δbr-PFOA decreased by 9% for each 1-unit increase in year of birth (odds ratio [OR] = 0.91, 95% confidence interval [CI]: 0.85–0.97), whereas the estimated odds for positive change in Δbr-PFOS increased by 32% with each 1-unit increase in parity (OR = 1.32, CI: 1.06–1.66). Furthermore, the estimated odds of observing a positive change in ΔPFUnDA decreased by 8% for each 1-kg/m^2 increase in BMI (OR = 0.92, CI: 0.85–0.99). Regarding the dietary factors, the estimated odds of observing a positive change in ΔPFHpS increased 5% (OR = 1.05, CI: 1.01–1.10) and 30% (OR = 1.30, CI: 1.06–1.61) for each 10gr/day increase in intake of breads and cereals and fat on bread, respectively. The estimated odds of observing a positive change in ΔPFHpA increased 17% (OR = 1.17, CI: 1.03–1.35) for each 10gr/day increase in intake of pastries. No other clear associations were observed between other lifestyle or dietary factors and ΔPFAA (Table S6).

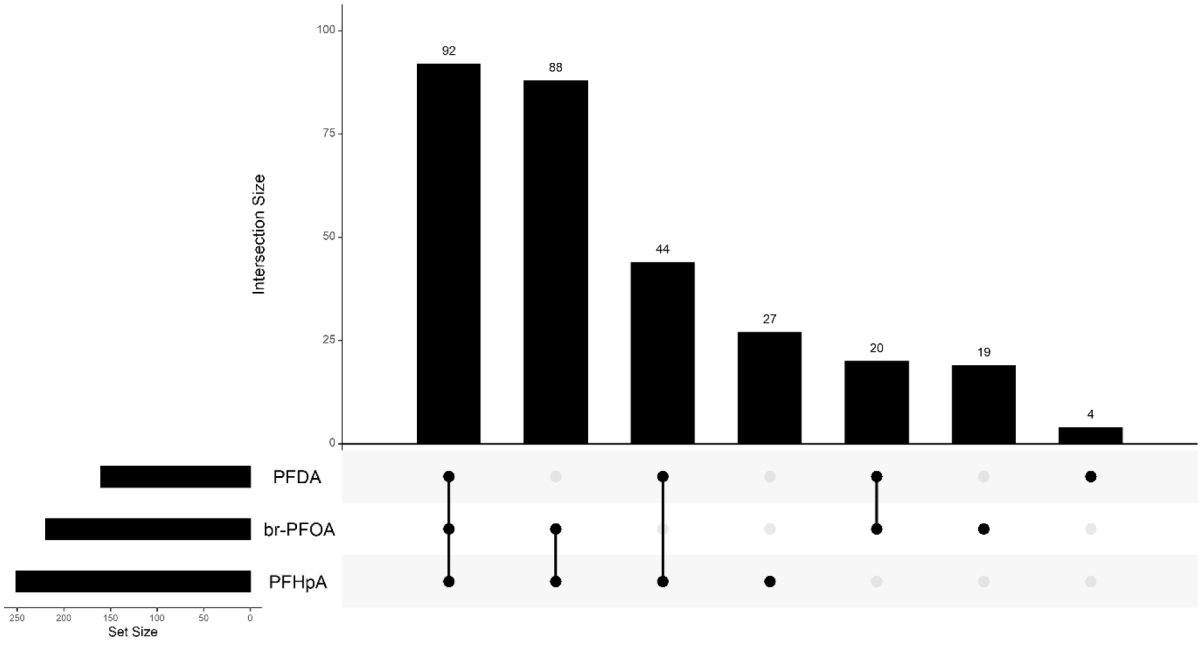

Fig. 2. UpSet plot showing the intersection of PFAA with increased post-TOP concentrations in more than 50% of participants. The bar chart shows the number of participants with post-TOP increases in concentrations of a combination of PFAA. The graphical table underneath indicates the PFAA combinations (black dots and lines). The frequency count of each PFAA across all subsets is shown as a smaller bar chart on the left side of the graphical table. Abbreviations: br-PFOA: sum of branched isomers of PFOA.

4. Discussion

To the best of our knowledge, this is the first study to (1) use the TOP assay to assess the indirect contribution of unknown oxidizable PFAA precursors to the total PFAS burden in plasma samples from Norwegian women, and (2) investigate the association between changes in post-TOP PFAA concentrations and lifestyle and dietary factors. More than 50% of participants showed increased post-TOP concentrations mainly in three PFCA: PFHpA, br-PFOA, and PFDA, which might indicate that there are one or more precursors present that can be degraded into these compounds. Formation of two other PFCA (PFNA and PFUnDA) was also observed, but only in approximately 30% of the individuals. In addition, we observed increased post-TOP concentrations of br-PFOS and PFHpS in more than 40% of study participants. In spite of the assumption that concentrations of PFSA are unlike to change in post-TOP analyses [Houtz and Sedlak, 2012], PFSA are likely formed only by sulfonamide-based precursors [Zhang et al. 2021; Cioni et al. 2022]. Thus, ignoring the formation of PFSA might cause underestimation of the total PFAS burden [Cook et al. 2022].

Median $PFAA_{post\text{-}TOP}/PFAA_{pre\text{-}TOP}$ ratio ranged from 1.2 to 24.0 among individuals with increased post-TOP PFAA concentrations. These medians were overall similar among the detected legacy PFAA, except for PFDA, which deviated substantially from the rest of the compounds. This was likely due to the low concentrations and detection frequency observed pre-TOP. This might also explain the larger median $PFAA_{post\text{-}TOP}/PFAA_{pre\text{-}TOP}$ of PFHpA and br-PFOA, as both were detected in more than 70% of study participants only in post-TOP analyses. Compared to pre-TOP, PFHpA was detected only in 37.1% of the study participants, while br-PFOA was below LOD. Looking at the actual concentrations, we observed relatively small post-TOP increases in the targeted legacy PFAA, suggesting that oxidizable precursors only contribute in a minor way to the total PFAS burden. Nevertheless, accounting for precursors is essential for a better understanding of the contribution of PFAA precursors to total PFAS burden [McDonough et al. 2022; De Silva et al. 2021], and for better describe the human exposure to potentially harmful PFAA [Cioni et al. 2022]. Moreover, investigating the contribution of PFAA precursors is also important for the biomonitoring of PFAS [Ao et al. 2023].

Although the pattern of post-TOP PFAA differed across participants, it is important to point out that more than 90% showed increased concentrations of more than one PFAA. For instance, approximately one-third of the study participants showed simultaneous increases in post-TOP concentrations of a combination of PFAA, particularly PFHpA, br-PFOA, and PFDA. Combinations of PFAA have also been observed after applying the TOP assay to consumer products [Rodgers et al. 2022]. Previous studies have suggested the pattern of end products after oxidation originated from known precursors during the TOP assay. For FTS and fluorotelomer carboxylic acids (FTCA), a mixture of shorter chain PFCA compounds is commonly observed [Houtz and Sedlak, 2012; Martin et al. 2019; Cioni et al. 2022]. In human serum, the oxidation of two sulfonamidoacetic acids (Me-FOSAA and Et-FOSAA) also resulted in a combination of PFCA and PFOS compounds [Cioni et al. 2022]. These previously published results could explain the observed pattern in the increased post-TOP concentrations in the legacy PFCA in our study, as the known precursors here investigated (FTS and FOSA) were below LOD in post-TOP analyses. Nevertheless, the concentrations of these known precursors were generally below LOD in the pre-TOP PFAS analysis as well, indicating the presence of other PFAA precursors in human blood and might have gone unrecognized in our PFAS analyses. This has already been suggested by Aro et al. (2021), who measured extractable organofluorine (EOF). However, in contrast to EOF analysis, some structural information is still preserved after oxidation in the TOP assay. Therefore, this assay yields more valuable information on the contribution of known and unknown oxidizable PFAA precursors to the total PFAS burden [Juhasz et al. 2022; Göckener et al. 2022]. Another interesting finding was the increase in post-TOP concentrations of br-PFOA and br-PFOS, but not in their corresponding linear isomers. This might be explained by the preferential oxidation of branched isomers among certain PFAA precursors, since the isomer oxidation rates can differ greatly and might be influenced by several factors [McDonough et al. 2022]. Further, a higher presence of branched PFAA precursors compared to the linear isomers could be the of cause our observations.

Previous studies have described important sources of exposure to legacy PFAA [Hu et al. 2018], but little is known about the sources of

PFAA precursors. Moreover, there might be no conclusive evidence to support that the rate of biotransformation is directly related to lifestyle and dietary factors, therefore our study focused on investigating whether lifestyle and dietary factors are sources of human exposure to PFAA precursors. Our findings revealed that year of birth, parity, and BMI were significant predictors of changes in post-TOP concentrations of br-PFOA, br-PFOS, and PFUnDA, respectively. Nevertheless, the observed associations were not consistent among the compounds. For instance, we observed that having positive changes in post-TOP concentrations of br-PFOA was likely lower in younger participants, suggesting that younger participants have been less exposed to precursors of this compound compared to older participants, which might be explained by the shift in production with electrochemical fluorination to more advanced methods of telomerization. Compared to post-TOP concentrations of br-PFOS, this pattern was not observed. Thus, these associations were not sufficient to accurately draw conclusions about the importance of lifestyle and dietary factors with regard to concentrations of PFAA precursors, and the relatively small increase in post-TOP PFAA concentrations, observed in a limited number of samples, may have limited the statistical power of our analyses.

The present study targeted a limited number of known PFAA precursors, and only the analysis of specific PFAA precursors could comprehensively describe the extent of contamination and narrow down the source of exposure [Göckener et al 2022]. Furthermore, alternative PFAA possibly formed under oxidation, as for example very short chained PFCA, were not included in our post-TOP analyses, potentially representing an important contribution to post-TOP concentrations. Another probable reason is that other sources or routes of exposure to PFAA precursors were not accounted for in this analysis, and it is worth mentioning that alternative PFAS are being emitted from a broader range of sources, rather than just the food chain. In addition, plasma might not be the most suitable blood matrix for detecting these PFAA precursors. For instance, whole blood appears to be the most appropriate matrix for the detection of FOSA [Poothong et al. 2017], since it appears to be more predominant in the cellular fraction of the blood, rather than serum or plasma [Yang et al. 2016].

There are additional limitations of this study that should be mentioned. The study population was limited to women of a certain age, who have menstruation, parity, and breastfeeding as critical PFAS elimination routes. Therefore, our findings might be representative of this group only. Another limitation is the lack of available information regarding menopause status, which could potentially impact the elimination of PFAS. Furthermore, the blood samples were collected between 2003 and 2006, which covers the period following the phase-out of PFOS and PFOS-based substances but does not reflect the present scenario of human PFAS exposure. In addition, although the blood samples were frozen at −70 °C to minimize potential breakdown of PFAA precursors, the long-term storage might be a limitation of the study. Moreover, given the timeframe of this study, concentrations of ultra-short-chain and alternative PFAS were not assessed, because these compounds only started to be used more frequently in the last decade [Zhang et al. 2022]. Another limitation is the TOP assay itself. First, the oxidative process in the TOP assay does not represent human metabolism pathways, as the transformation of PFAA precursors occurs under extreme conditions [Göckener et al. 2021]. Second, the TOP assay is not optimized for all PFAS [Göckener et al. 2022], and a 100% oxidation yield cannot be assumed for all precursors [Cioni et al. 2022]. Third, numerous reaction parameters, including insufficient amount of NaOH, can affect oxidation and the corresponding end products [Al Amin et al. 2021; Shojaei et al. 2022]. Therefore, the TOP assay can characterize only a fraction of many PFAA precursors not yet covered by targeted analyses.

5. Conclusions

Our results indicated that more than 90% of study participants had detectable amounts of PFAA precursors. Therefore, when analyzing only targeted PFAA, the total PFAS burden is most likely be underestimated. Although the limitations of the TOP assay, our results advocate the need of complementary approaches to the comprehensive assessment of total PFAS burden in humans. Additional analytical tools are still required to characterize the yet unidentified oxidizable PFAA precursors. In addition, our study identified few associations between selected lifestyle or dietary factors and the unknown oxidizable PFAA precursors, although not consistent across the different PFAA. Future studies should therefore further investigate these associations, along with expanding the compound spectrum to widen the range of PFAA, PFAA precursors, and other PFAS. Furthermore, including recent data might help to understand and monitor the extent of PFAS exposure one decade after the voluntary phase-out.

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Ethics approval and consent to participate

Participation in the NOWAC Study was voluntarily, and a signed consent form was obtained from all participants. The study was approved by the Regional Committee for Medical Research Ethics (REK, case number: 2015/1780).

CRediT authorship contribution statement

Ana Carolina M F Coêlho: Data curation, Formal analysis, Software, Investigation, Writing – original draft. **Lara Cioni:** Methodology, Writing – review & editing. **Wendy Van Dreunen:** Investigation, Writing – review & editing. **Vivian Berg:** Validation, Writing – review & editing. **Charlotta Rylander:** Validation, Writing – review & editing. **Ilona Urbarova:** Validation, Supervision, Writing – review & editing. **Dorte Herzke:** Conceptualization, Resources, Supervision, Writing – review & editing. **Torkjel M Sandanger:** Conceptualization, Funding acquisition, Writing – review & editing, Supervision.

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

Data will be made available on request.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envint.2023.108026.

References

Al Amin, M., Luo, Y., Nolan, A., Robinson, F., Niu, J., Warner, S., et al., 2021. Total oxidizable precursor assay towards selective detection of PFAS in AFFF. J. Clean. Prod. 328, 129568.

Ao, Y., Nian, M., Tang, W., Zhang, J., Zhang, Q., Ao, J., 2023. A sensitive and robust method for the simultaneous determination of thirty-three legacy and emerging per- and polyfluoroalkyl substances in human plasma and serum. Anal. Bioanal. Chem. 415 (3), 457–470.

Aro, R., Eriksson, U., Kärrman, A., Yeung, L., 2021. Organofluorine mass balance analysis of whole blood samples in relation to gender and age. Environ. Sci. Tech.

Berg, V., Nøst, T.H., Huber, S., Rylander, C., Hansen, S., Veyhe, A.S., Fuskevåg, O.M., Odland, J.Ø., Sandanger, T.M., 2014. Maternal serum concentrations of per- and polyfluoroalkyl substances and their predictors in years with reduced production and use. Environ. Int. 69, 58–66.

Buck, R.C., Franklin, J., Berger, U., Conder, J.M., Cousins, I.T., de Voogt, P., Jensen, A.A., Kannan, K., Mabury, S.A., van Leeuwen, S.P.J., 2011. Perfluoroalkyl and polyfluoroalkyl substances in the environment: terminology, classification, and origins. Integr. Environ. Assess. Manag. 7 (4), 513–541.

Cioni, L., Nikiforov, V., Coêlho, A.C.M.F., Sandanger, T.M., Herzke, D., 2022. Total oxidizable precursors assay for PFAS in human serum. Environ. Int. 170, 107656.

Cook, E.K., Olivares, C.I., Antell, E.H., Yi, S., Nickerson, A., Choi, Y.J., Higgins, C.P., Sedlak, D.L., Alvarez-Cohen, L., 2022. Biological and chemical transformation of the six-carbon polyfluoroalkyl substance *n*-dimethyl ammonio propyl perfluorohexane sulfonamide (AMPR-FHXSA). Environ. Sci. Tech. 56 (22), 15478–15488.

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 (3), 631–657.

Domingo, J.L., Nadal, M., 2019. Human exposure to per- and polyfluoroalkyl substances (PFAS) through drinking water: a review of the recent scientific literature. Environ. Res. 177, 108648.

Dumeaux, V., Børresen-Dale, A., Frantzen, J., Kumle, M., Kristensen, V., Lund, E., 2008. Gene expression analyses in breast cancer epidemiology: the Norwegian Women and Cancer postgenome cohort study. Breast Cancer Res. 10 (1).

Dumeaux, V., Ursini-Siegel, J., Flatberg, A., Fjosne, H.E., Frantzen, J.-O., Holmen, M.M., Rodegerdts, E., Schlichting, E., Lund, E., 2015. Peripheral blood cells inform on the presence of breast cancer: a population-based case–control study. Int. J. Cancer 136 (3), 656–667.

Gebbink, W., Glynn, A., Berger, U., 2015. Temporal changes (1997–2012) of perfluoroalkyl acids and selected precursors (including isomers) in Swedish human serum. Environ. Pollut. 199, 166–173.

Göckener, B., Fliedner, A., Rüdel, H., Badry, A., Koschorreck, J., 2021. Long-Term Trends of Per- and Polyfluoroalkyl Substances (PFAS) in suspended particular matter from german rivers using the direct total oxidizable precursor (dTOP) Assay. Environ. Sci. Tech. 56 (1), 208–217.

Göckener, B., Lange, F.T., Lesmeister, L., Gökçe, E., Dahme, H.U., Bandow, N., Biegel-Engler, A., 2022. Digging deep—implementation, standardisation and interpretation of a total oxidisable precursor (TOP) assay within the regulatory context of per- and polyfluoroalkyl substances (PFASs) in soil. Environmental Sciences. Europe 34 (1).

Hanssen, L., Dudarev, A., Huber, S., Odland, J., Nieboer, E., Sandanger, T., 2013. Partition of perfluoroalkyl substances (PFASs) in whole blood and plasma, assessed in maternal and umbilical cord samples from inhabitants of arctic Russia and Uzbekistan. Sci. Total Environ. 447, 430–437.

Hjartåker, A., Andersen, L., Lund, E., 2007. Comparison of diet measures from a food-frequency questionnaire with measures from repeated 24-hour dietary recalls. the norwegian women and cancer study. Public Health Nutr. 10 (10), 1094–1103.

Houtz, E., Sedlak, D., 2012. Oxidative conversion as a means of detecting precursors to perfluoroalkyl acids in urban runoff. Environ. Sci. Tech. 46 (17), 9342–9349.

Hu, X.C., Dassuncao, C., Zhang, X., Grandjean, P., Weihe, P., Webster, G.M., Nielsen, F., Sunderland, E.M., 2018. Can profiles of poly- and perfluoroalkyl substances (pfass) in human serum provide information on major exposure sources? Environ. Health 17 (1).

Juhasz, A.L., Kastury, F., Herde, C., Tang, W., 2022. Application of soil amendments for reducing PFAS leachability and bioavailability. Environ. Pollut. 307, 119498.

Lewis, A.J., Yun, X., Spooner, D.E., Kurz, M.J., McKenzie, E.R., Sales, C.M., 2022. Exposure pathways and bioaccumulation of per- and polyfluoroalkyl substances in freshwater aquatic ecosystems: key considerations. Sci. Total Environ. 822, 153561.

Lund, E., Dumeaux, V., Braaten, T., Hjartaker, A., Engeset, D., Skeie, G., Kumle, M., 2008. Cohort profile: the norwegian women and cancer study–NOWAC–Kvinner og kreft. Int. J. Epidemiol. 37 (1), 36–41.

Manzano-Salgado, C.B., Casas, M., Lopez-Espinosa, M.-J., Ballester, F., Martinez, D., Ibarluzea, J., Santa-Marina, L., Schettgen, T., Vioque, J., Sunyer, J., Vrijheid, M., 2016. Variability of perfluoroalkyl substance concentrations in pregnant women by socio-demographic and dietary factors in a Spanish birth cohort. Environ. Int. 92-93, 357–365.

Martin, D., Munoz, G., Mejia-Avendaño, S., Duy, S.V., Yao, Y., Volchek, K., Brown, C.E., Liu, J., Sauvé, S., 2019. Zwitterionic, cationic, and anionic perfluoroalkyl and polyfluoroalkyl substances integrated into total oxidizable precursor assay of contaminated groundwater. Talanta 195, 533–542.

McDermett, K.S., Guelfo, J., Anderson, T.A., Reible, D., Jackson, A.W., 2022. The development of diffusive equilibrium, high-resolution passive samplers to measure perfluoroalkyl substances (PFAS) in groundwater. Chemosphere 303, 134686.

McDonough, C.A., Li, W., Bischel, H., De Silva, A., DeWitt, J., 2022. Widening the lens on PFASs: direct human exposure to perfluoroalkyl acid precursors (pre-PFAAs). Environ. Sci. Tech. 56 (10), 6004–6013.

Nair, A.S., Ma, Z.-Q., Watkins, S.M., Wood, S.S., 2021. Demographic and exposure characteristics as predictors of serum per- and polyfluoroalkyl substances (PFASs) levels – a community-level biomonitoring project in Pennsylvania. Int. J. Hyg. Environ. Health 231, 113631.

Nyström, J., Benskin, J.P., Plassmann, M., Sandblom, O., Glynn, A., Lampa, E., Gyllenhammar, I., Moraeus, L., Lignell, S., 2022. Demographic, life-style and physiological determinants of serum per- and polyfluoroalkyl substance (PFAS) concentrations in a national cross-sectional survey of Swedish adolescents. Environ. Res. 208, 112674.

Parr, C., Veierød, M., Laake, P., Lund, E., Hjartåker, A., 2006. Test-retest reproducibility of a food frequency questionnaire (FFQ) and estimated effects on disease risk in the Norwegian Women and Cancer Study (NOWAC). Nutr. J. 5 (1).

Poothong, S., Thomsen, C., Padilla-Sanchez, J.A., Papadopoulou, E., Haug, L.S., 2017. Distribution of novel and well-known poly- and perfluoroalkyl substances (PFASs) in human serum, plasma, and whole blood. Environ. Sci. Tech. 51 (22), 13388–13396.

Poothong, S., Papadopoulou, E., Padilla-Sánchez, J.A., Thomsen, C., Haug, L.S., 2020. Multiple pathways of human exposure to poly- and perfluoroalkyl substances (PFASs): from external exposure to human blood. Environ. Int. 134, 105244.

Rodgers, K., Swartz, C., Occhialini, J., Bassignani, P., McCurdy, M., Schaider, L., 2022. How well do product labels indicate the presence of PFAS in consumer items used by children and adolescents? Environ. Sci. Tech. 56 (10), 6294–6304.

Rylander, C., Sandanger, T., Frøyland, L., Lund, E., 2010. Dietary patterns and plasma concentrations of perfluorinated compounds in 315 norwegian women: the NOWAC Postgenome study. Environ. Sci. Tech. 44 (13), 5225–5232.

Sagiv, S.K., Rifas-Shiman, S.L., Webster, T.F., Mora, A.M., Harris, M.H., Calafat, A.M., Ye, X., Gillman, M.W., Oken, E., 2015. Sociodemographic and perinatal predictors of early pregnancy per- and polyfluoroalkyl substance (PFAS) Concentrations. Environ. Sci. Tech. 49 (19), 11849–11858.

Sandanger, T.M., Nøst, T.H., Guida, F., Rylander, C., Campanella, G., Muller, D.C., van Dongen, J., Boomsma, D.I., Johansson, M., Vineis, P., Vermeulen, R., Lund, E., Chadeau-Hyam, M., 2018. DNA methylation and associated gene expression in blood prior to lung cancer diagnosis in the Norwegian Women and Cancer cohort. Sci. Rep. 8 (1).

Seshasayee, S.M., Rifas-Shiman, S.L., Chavarro, J.E., Carwile, J.L., Lin, P.-I D., Calafat, A. M., Sagiv, S.K., Oken, E., Fleisch, A.F., 2021. Dietary patterns and PFAS plasma concentrations in childhood: Project Viva, USA. Environ. Int. 151, 106415.

Shojaei, M., Kumar, N., Guelfo, J.L., 2022. An integrated approach for determination of total per- and polyfluoroalkyl substances (PFAS). Environ. Sci. Tech. 56 (20), 14517–14527.

Sivaram, A.K., Panneerselvan, L., Surapaneni, A., Lee, E., Kannan, K., Megharaj, M., 2022. Per- and polyfluoroalkyl substances (PFAS) in commercial composts, garden soils, and potting mixes of Australia. Environ. Adv. 7, 100174.

Sunderland, E., Hu, X., Dassuncao, C., Tokranov, A., Wagner, C., Allen, J., 2018. A review of the pathways of human exposure to poly- and perfluoroalkyl substances (PFASs) and present understanding of health effects. J. Eposure Sci. Environ. Epidemiol. 29 (2), 131–147.

Wise, L.A., Wesselink, A.K., Schildroth, S., Calafat, A.M., Bethea, T.N., Geller, R.J., Coleman, C.M., Fruh, V., Claus Henn, B., Botelho, J.C., Harmon, Q.E., Thirkill, M., Wegienka, G.R., Baird, D.D., 2022. Correlates of plasma concentrations of per- and poly-fluoroalkyl substances among reproductive-aged Black women. Environ. Res. 203, 111860.

Yang, L, Wang, Z, Shi, Y, Li, J, Wang, Y, Zhao, Y, et al., 2016. Human placental transfer of perfluoroalkyl acid precursors: Levels and profiles in paired maternal and Cord Serum. Chemosphere 144, 1631–1638. https://doi.org/10.1016/j.chemosphere.2015.10.063.

Zhang, W., Pang, S., Lin, Z., Mishra, S., Bhatt, P., Chen, S., 2021. Biotransformation of perfluoroalkyl acid precursors from various environmental systems: advances and perspectives. Environ. Pollut. 272, 115908 https://doi.org/10.1016/j.envpol.2020.115908.

Zhang, M., Yu, C., Wang, G., Buckley, J., Hong, X., Pearson, C., et al., 2022. Longitudinal trajectories and determinants of plasma per- and polyfluoroalkyl substance (PFAS) levels from birth to early childhood and metabolomic associations: a pilot study in the Boston Birth Cohort. Precision. Nutrition 1 (1).