Livsmedelsverket
Swedish Food Agency

SWEDISH ENVIRONMENTAL PROTECTION AGENCY

NATIONAL ENVIRONMENTAL MONITORING COMMISSIONED BY THE SWEDISH EPA

*Report to the Swedish EPA (the Health-Related Environmental Monitoring Program)*

*Contract no. 215-21-003*

# Fluorine mass balance and suspect screening in first-time mothers from Uppsala, Sweden: Results for years 2018-2021

Majid Mustafa,[a] Merle M. Plassmann,[a] Pernilla Hedvall Kallerman,[b] Irina Gyllenhammar[b] and Jonathan P. Benskin[a]

[a]Department of Environmental Science, Stockholm University, Stockholm, Sweden.

[b]Division for Risk and Benefit Assessment, Swedish Food Agency, P.O. Box 622, SE-751 26 Uppsala, Sweden

2024-02-29

NATIONELL
MILJÖÖVERVAKNING
PÅ UPPDRAG AV
NATURVÅRDSVERKET

ÄRENDENNUMMER NV-00791-21
AVTALSNUMMER 215-21-003
PROGRAMOMRÅDE Hälsorelaterad MÖ
DELPROGRAM Biologiska mätdata – organiska ämnen

# Fluorine mass balance and suspect screening in first-time mothers from Uppsala, Sweden: Results for years 2018-2021


**Rapportförfattare**
Majid Mustafa, Stockholm University
Merle M. Plassmann, Stockholms universitet
Pernilla Hedvall Kallerman, Livsmedelsverket
Irina Gyllenhammar, Livsmedelsverket
Jonathan P. Benskin, Stockholms universitet



**Utgivare**
Livsmedelsverket

**Postadress**
Box 622, 751 26 Uppsala

**Telefon**
018-175500


**Rapporttitel**
Fluorine mass balance and suspect screening in first-time mothers from Uppsala, Sweden: Results for years 2018-2021


**Beställare**
Naturvårdsverket
106 48 Stockholm

**Finansiering**
Nationell hälsorelaterad miljöövervakning

**Nyckelord för plats**
Uppsala, Sverige

**Nyckelord för ämne**
Perfluorerade alkylsyror, PFCA, PFSA, serum, kvinnor

**Tidpunkt för insamling av underlagsdata**
2018-2021



**Sammanfattning**

Sedan 1996 har Livsmedelsverket regelbundet samlat in blodprover från förstföderskor i Uppsala för analys av persistenta organiska miljöföroreningar (POP). Poly- och perfluorerade alkylsyror (PFAS) är en sådan substansgrupp. Huvudsyftet med denna studie var att fortsätta de tidigare trenderna för fluormassbalans i serum från förstföderskor i POPUP-kohorten (1996–2017), med ytterligare fyra år (2018-2021). Dessutom gjordes suspect screening med särskild fokus på fluorerade läkemedel och bekämpningsmedel med hjälp av högupplöst masspektrometri. Resultaten visar att halterna för PFAS och totalt fluor (TF) fortsätter att minska under de senaste åren. Även om det inte observerades någon trend för extraherbart organiskt fluor (EOF) visade en jämförelse mellan halterna av summaPFAS och EOF-nivåer en ökning av den procentuella andelen oidentifierad EOF under de senaste åren. En pool år 2019 visade särskilt höga TF och EOF-nivåer, vilket troligtvis kan tillskrivas läkemedlet Escitalopram som utifrån enkätsvar används av en deltagare i denna pool. Suspect screening-analysen identifierade preliminärt 5 nya organofluorämnen som tidigare har rapporterats i fisk och humant serum från Great Lakes-regionen i USA, vilka föreslogs vara av naturligt ursprung. Dessa strukturer kräver bekräftelse med autentiska standarder. Sammantaget visar dessa data att trots minskande PFAS-koncentrationer är den övergripande exponeringen för PFAS sannolikt underskattad på grund av förekomsten av nya, tidigare oidentifierade ämnen.

## TABLE OF CONTENTS

## INTRODUCTION

Per- and polyfluoroalkyl substances (PFAS) constitute a large (>4700 substances) and diverse class of chemicals which are used throughout society (OECD, 2018). It is widely acknowledged that nearly all PFAS are persistent or have the potential to transform to persistent transformation products. This property, combined with evidence of toxicity, bioaccumulation, and long range transport potential for some substances, has contributed to increased regulation of PFAS over the past 20 years (United Nations, 2020). For example, several PFAS are now included in the United Nations Stockholm Convention on Persistent Organic Pollutants and most recently, a broad restriction proposal on PFAS was submitted to the European Chemicals Agency in 2023, which is expected to go into force as early as 2025.

In the general adult population, diet and to a lesser extent dust/indoor air are considered the major sources of exposure. Drinking water has also been shown to contribute in communities with a contaminated water supply (Johanson et al., 2023). For example, drinking water in Uppsala, Sweden was contaminated with PFAS from ~1996-2012 due to the use of aqueous film forming foams (AFFF) at a nearby military airport (Glynn, 2012). Human biomonitoring in Uppsala as part of the Persistent Organic Pollutants in Uppsala Primiparas (POPUP) study has revealed elevated levels of perfluorohexane sulfonate (PFHxS) and perfluorobutane sulfonic acid (PFBS) in individuals that were consuming contaminated drinking water (Gyllenhammar et al., 2015). While an investigation into pooled serum from this population (1996-2017) revealed declining trends for all target PFAS starting as early as the year 2000 (Miaz et al., 2020), the same study also showed an increasing trend in the percentage of unidentified extractable organic fluorine (UEOF) in more recent years.

Efforts to identify UEOF in human and wildlife samples have primarily focused on conventional structures with C4-C14 perfluoroalkyl chains, since chain lengths shorter than C4 are generally considered non-bioaccumulative. However, fluorinated pharmaceuticals and pesticides (some of which meet the OECD definition of PFAS), may also contribute to the UEOF. Indeed, recent work on municipal wastewater treatment plant sludge revealed up to 20% of UEOF attributed to pharmaceutical and pesticide substances (Spaan et al., 2023), while self-reported use of organofluorine pharmaceuticals in US serum was associated with a 0.36 ng F/mL (95% CL: −1.26 to 1.97) increase in UEOF (Pennoyer et al., 2023). These data point to the importance of fluorinated pharmaceuticals and pesticides for closing the EOF mass balance. Nevertheless, the identities of these substances remain unclear.

The main objective of this study was to extend the previous trends (1996 to 2017) in fluorine mass balance for the POPUP cohort reported in Miaz et al. (2020), with four additional years (2018 to 2021). Moreover, in an effort to close the fluorine mass balance, samples were subjected to high resolution mass spectrometry-based suspect screening, with a particular emphasis on fluorinated pharmaceuticals and pesticides.

## MATERIALS AND METHODS

### Recruitment and sampling

First-time mothers were recruited from the general population living in Uppsala County, Sweden as described by Glynn et al. (2012). Blood samples were collected 3 weeks after delivery using 10 ml Vacutainer® or Vacuette® serum tubes, and serum was stored at the Swedish Food Agency at -20°C. Trends in PFAS and fluorine mass balance in pooled human serum were previously reported up to and including 2017 (Miaz et al. 2020). To extend these trends, the present work included 12 pooled samples for the years 2018-2021 (3 pooled samples/year) with serum from 10 individuals in each pool. The study was approved by the local ethics committee of Uppsala University, and the participating women gave informed consent prior to inclusion in the study.

### *Sample Extraction*

Each serum sample (0.5 mL) was transferred to a polypropylene centrifuge tube. The extraction involved addition of 4 mL acetonitrile, mixing using a vortex-mixer, placing the sample in an ultra-sonic bath followed by centrifuging. The supernatant was transferred to a new vial and the procedure was repeated, the two supernatants were combined, and concentrated down to 1 mL under a stream of nitrogen and a water bath at 40 °C. Extracts were subsequently purified with ~25 mg graphitized non-porous carbon (Supelclean™ ENVI-Carb™ SPE Bulk packing purchased from Sigma-Aldrich) and 50 µL glacial acetic acid. Thereafter, each sample was vortex-mixed and centrifuged. The resulting supernatant was split: 700 µL was transferred to a new vial and concentrated to 200µL for analysis of EOF by CIC, while 90 µL was fortified with 10 µL of an internal standard mixture (10 pg/µL; see Table 1) and 50 µL of 4 mM ammonium acetate in water, prior to analysis by LC-high resolution mass spectrometry.

**Instrumental Analysis**

Both target and suspect screening were carried out using a Dionex UltiMate 3000 Ultrahigh performance liquid chromatograph (Thermo Scientific) coupled to a Q Exactive HF hybrid Quadrupole-Orbitrap mass spectrometer (Thermo Scientific). Chromatographic separation of analytes was achieved on a BEH C18 column (2.1 × 50 mm, 1.7 µm particle size; Waters) connected in series to a BEH C18 guard column (2.1 × 5 mm, 1.7 µm; Waters). In addition, an extra C18 "isolator" column (2.1 × 50 mm; Waters) was mounted before the injector to trap possible PFAS contamination from the pump. The injection volume was 5 µL. All samples were run in negative ionization, full scan mode (200–1200 Da) at a resolution of 120 000 full width at half maximum (fwhm) with data-dependant MS2 fragmentation using an inclusion list.

Targeted data processing was carried out using Trace Finder version 4.1 (Thermo Scientific version). An 8-point calibration curve was used to quantify the target PFASs listed in Table 1. The lowest concentration calibration standard for which a well-shaped peak was observed was used as the limit of quantification (LOQ), with exception where a signal was observable in the blanks, in which case the LOQ was defined as the mean concentration detected in the blanks plus three times the standard deviation of the blanks. For calculation of averages, concentrations below LOQ were replaced with zero.

**Fluorine Mass Balance**

Total- and extractable organic fluorine (TF and EOF, respectively) determination was carried out by combustion ion chromatography (CIC) using a previously developed method (Schultes et al., 2018). For EOF, sample extracts (ca 200 µL) were placed in a ceramic sample boat containing glass wool, while for TF measurements, 200 µL of serum was weighed directly into the sample boat. The samples were combusted at 1100˚C under a flow of oxygen (400 L/min) and argon mixed with water vapor (200 L/min) for ~5 minutes. Combustion gases were collected in 10 mL water in an absorber unit (GA-210, Mitsubishi), after which an aliquot of the absorption solution (200 µL) was injected onto the IC (Dionex Integrion HPIC, Thermo Fisher Scientific) which was equipped with an anion exchange column (Dionex IonPac AS19 2 × 50 mm guard column and 2 × 250 mm analytical column, 7.5 µm particle size) operated at 30˚C. The mobile phase (hydroxide) was ramped from 8 mM to 60 mM at a flow rate of 0.25

mL/min over the course of the run and measurement of fluoride was achieved by conductivity detection.

Samples for EOF analysis were prepared together with blanks and spiked samples (150 ng F total of PFOS/PFOA; n=2). EOF concentrations were recovery-corrected based on PFOS/PFOA recoveries (average 85.3%). For assessment of accuracy and precision of TF data, replicate certified reference material (BCR®-461, fluorine in clay) was analysed (86.3% recovery; RSD = 3.8%).

For comparison to EOF and TF data, individual PFAS concentrations were converted to fluorine equivalents using equation 1.

*Eqn 1.* $C_{F\_PFAS} = n_F \times MW_F / MW_{PFAS} \times C_{PFAS}$

$C_{F\_PFAS}$ (ng F/g) is the concentration of the PFAS of interest in equivalents of fluorine, $n_F$ is the number of fluorine atoms on the molecule, $MW_F$ is the weight of 1 mol of fluorine, $MW_{PFAS}$ is the molecular weight of the PFAS of interest, and $C_{PFAS}$ is the concentration of the PFAS determined using LC-MS/MS.

The total known EOF concentration ($\Sigma C_{F\_PFAS}$; ng F/g) was obtained by summing the fluorine concentrations from all individual PFASs. Thereafter, the concentration of unidentified, extractable organic fluorine ($C_{F\_extr.unknown}$; ng F/g) was determined by subtracting $\Sigma C_{F\_PFAS}$ from the total extractable organic fluorine concentration ($C_{F\_EOF}$; ng F/g), according to equation 2.

*Eqn 2.* $C_{F\_EOF} = \Sigma C_{F\_PFAS} + C_{F\_extr.unknown}$

The TF concentration ($C_{F\_TF}$; ng F/g) is the sum of $C_{F\_EOF}$ and the total non-extractable fluorine concentration ($C_{F\_non\ extr.}$; ng F/g), as shown in equation 3.

*Eqn 3.* $C_{F\_TF} = C_{F\_EOF} + C_{F\_non\ extr.}$

**Suspect Screening**

Suspect screening was carried out using Compound Discoverer (CD) 3.3 (Thermo Scientific) on 1215 organofluorine substances (not all of which met the OECD definition of PFAS), including 333 fluorinated pharmaceuticals and 418 pesticides. An overview of the workflow is

provided in Figure 3. The inclusion list for the data-dependent MS2 analysis was the same as the suspect list used in CD to search for suspects. CD handles peak alignment, peak picking, blank subtraction and comparison with databases (suspect list of exact masses and mzCloud). For the peaks matching a molecular mass in the suspect list (within 5 ppm), the MS2 spectra were further inspected. The correspondence of the MS2 data from CD with the suspect compound was assessed in silico using MetFrag.

***Table 1.*** *List of target PFAS included in the study.*

| **Compound** | **Abbreviation** | **Internal standard** | **LOQ (ng/g)** |
|---|---|---|---|
| Perfluorobutanoic acid | PFBA | M4PFBA | 0.164 |
| Perfluoropentanoic acid | PFPeA | M5PFPeA | 0.164 |
| Perfluorohexanoate | PFHxA | M4PFHpA | 0.164 |
| Perfluoroheptanoate | PFHpA | M4PFHpA | 0.164 |
| Perfluorooctanoate | PFOA | M4PFOA | 0.164 |
| Perfluorononanoate | PFNA | M5PFNA | 0.164 |
| Perfluorodecanoate | PFDA | M2PFDA | 0.164 |
| Perfluoroundecanoate | PFUnDA | M2PFUnDA | 0.164 |
| Perfluorododecanoate | PFDoDA | M2PFDoDA | 0.164 |
| Perfluorotridecanoate | PFTriDA | M2PFDoDA | 0.164 |
| Perfluorotetradecanoate | PFTeDA | M2PFDoDA | 0.164 |
| Perfluoropentadecanoate | PFPeDA | M2PFDoDA | 0.164 |
| Perfluorohexadecanoic acid | PFHxDA | M2PFDoDA | 0.164 |
| Perfluorooctadecanoic acid | PFOcDA | M2PFDoDA | 0.164 |
| Perfluorobutanesulfonate | PFBS | 18O2-PFHxS | 0.145 |
| Perfluoropentanesulfonate | PFPeS | 18O2-PFHxS | 0.155 |
| Perfluorohexanesulfonate lin. | lin-PFHxS | 18O2-PFHxS | 0.155 |
| Perfluorohexanesulfonate br. | br-PFHxS | 18O2-PFHxS | 0.155 |
| Perfluoroheptanesulfonic acid br. | br-PFHpS | M4PFOS | 0.157 |
| Perfluoroheptanesulfonate | PFHpS | M4PFOS | 0.157 |
| Perfluorooctanesulfonate lin. | lin-PFOS | M4PFOS | 0.157 |
| Perfluorooctanesulfonate br. | br-PFOS | M4PFOS | 0.157 |
| Perfluorononanesulfonate | PFNS | M4PFOS | 0.157 |
| Perfluorodecane sulfonic acid br. | br-PFDS | M4PFOS | 0.158 |
| Perfluorodecanesulfonate | PFDS | M4PFOS | 0.158 |
| Perfluoroundecanesulfonic acid | PFUnDS | M4PFOS | 0.158 |
| Perfluorooctanesulfonamide br. | br-FOSA | M8FOSA | 0.167 |
| Perfluorooctane sulfonamide | FOSA | M8FOSA | 0.167 |
| Perfluorooctane sulfonamidoacetate | FOSAA | d3-MeFOSAA | 0.584 |
| Methylperfluorooctanesulfonamide | MeFOSA | d3-MeFOSAA | 0.164 |
| Ethylperfluorooctanesulfonamide | EtFOSA | d5-EtFOSAA | 0.166 |
| Ethyl perfluorooctane sulfonamidoacetate lin. | lin-EtFOSAA | d5-EtFOSAA | 0.165 |
| Ethyl perfluorooctane sulfonamidoacetate br. | br-EtFOSAA | d5-EtFOSAA | 0.165 |
| Methyl perfluorooctane sulfonamidoacetate lin. | lin-MeFOSAA | d3-MeFOSAA | 0.168 |

| | | | |
|---|---|---|---|
| Methyl perfluorooctane sulfonamidoacetate br. | br-MeFOSAA | d3-MeFOSAA | 0.168 |
| 3:3 Fluorotelomer carboxylic acid | 3:3 FTA (FPrPA) | M2PFHxA | 0.163 |
| 5:3 Fluorotelomer carboxylic acid | 5:3 FTA (FPePA) | M4PFOA | 0.164 |
| 7:3 Fluorotelomer carboxylic acid | 7:3 FTA (FHpPA) | M2PFDA | 0.165 |
| 4:2 Fluorotelomer sulfonate | 4:2 FTS | M2 6:2 FTS | 0.165 |
| 6:2 Fluorotelomer sulfonate | 6:2 FTS | M2 6:2 FTS | 0.164 |
| 8:2 Fluorotelomer sulfonate | 8:2 FTS | M2 6:2 FTS | 0.163 |
| Potassium 9-chlorohexadecafluoro-3-oxanonanoe-1-sulfonate | 9Cl-PF3ONS | M2PFDA | 0.164 |
| Potassium 11-chloroeicosafluoro-3-oxaundecane-1-sulfonate | 11Cl-PF3OUdS | M2PFDA | 0.164 |
| 4:2 Fluorotelomer phosphate diester | 4:2 diPAP | M4 6:2/6:2 diPAP | 0.241 |
| 6:2 Fluorotelomer phosphate diester | 6:2 diPAP | M4 6:2/6:2 diPAP | 0.169 |
| 6:2/8:2 Fluorotelomer phosphate diester | 6:2/8:2 diPAP | M4 8:2/8:2 diPAP | 0.166 |
| 8:2 Fluorotelomer phosphate diester | 8:2 diPAP | M4 8:2/8:2 diPAP | 0.165 |
| 10:2 Fluorotelomer phosphate diester | 10:2 diPAP | M4 8:2/8:2 diPAP | 24.376 |
| Sodium dodecafluoro-3H-4,8-dioxanonanoate | NaDONA | M4PFOA | 0.168 |

## RESULTS AND DISCUSSION

### Target PFAS

In total, 5 PFAS (PFOA, PFOS, PFHxS, PFNA and PFDA) were detected in the pools from 2018-2021 (Figure 1). All other targets were below the LOQ. With the exception of PFDA, which was detected only in two pooled serum samples, the 4 remaining PFAS were detected at 100% frequency. Our prior analysis of individual women from POPUP during this time period revealed additional PFAS at low detection frequency (e.g. PFUnDA, PFTriDA, PFPeS, PFHpS), suggesting that these targets may have been diluted to below LOQs as a result of pooling (Gyllenhammar et al. 2022). PFOS was observed at the highest mean concentration (3.17 ng/g; range 2.13-5.11 ng/g) followed by PFHxS (1.81 ng/g; range 1.19-2.82 ng/g), PFOA (1.06 ng/g; 0.69-2.09 ng/g), br-PFOS (0.83 ng/g; range 0.55-1.59 ng/g), PFNA (0.35 ng/g; range 0.27-0.61 ng/g) and PFDA (0.25 ng/g; range 0.22-0.29 ng/g).

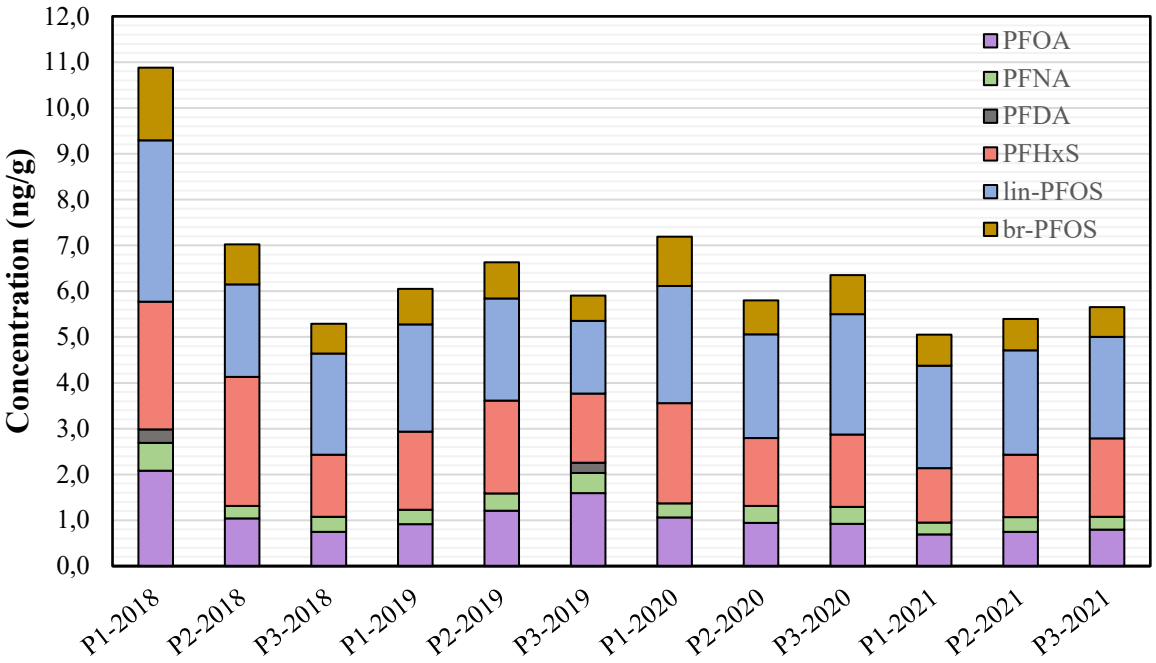


***Figure 1.*** *∑PFAS and individual PFAS concentrations (ng/g) in pooled serum samples of first-time mothers during 2018-2021.*

The highest concentration of target ∑PFAS was observed for P1-2018 (10.99 ng/g) while for other pools, concentrations ranged from 5.05-7.19 ng/g. These PFAS levels are substantially lower than what was reported for the same cohort between 1996-2017, i.e., 8.1 to 32 ng/g (Miaz et al., 2020). According to the European Food Safety Authority (EFSA), a serum level of ≤6.9 ng/mL for the sum of four PFAS (PFOA, PFNA, PFHxS, and PFOS) is considered safe for a mother, without elevating levels in her child, after pregnancy and one year of breastfeeding (Gyllenhammar et al., 2022). Our data indicate that 3 pools (P1-2018, P2-2018, and P1-2020) exceeded this guideline level, while the remainder were below.

**Fluorine Mass Balance**

EOF concentrations were above MDLs for all pooled serum samples from 2018-2021, compared to a detection rate of 33% in our previous analyses for the time period 1996-2017. This is likely due to the use of a larger volume of sample extract for combustion on the most recent samples (as opposed to higher concentrations), which served to lower MDLs. TF, EOF and ΣPFAS concentrations ranged 37-300 ng F $mL^{-1}$, 8-296 ng F $g^{-1}$ and 2.8-6.0 ng F $g^{-1}$, respectively (Figure 2). Notably, both TF and EOF in pool P2-2019 were substantially higher than the other pooled samples, despite having a similar (and relatively low) contributions from target PFAS. An investigation into the questionnaires provided by POPUP-mothers revealed

that an individual in this pool was taking the fluorinated antidepressant drug Escitalopram ($C_{20}H_{21}FN_2O$), which may explain the high levels of TF and EOF in this pool. This result remains tentative and will be confirmed in future HRMS experiments.

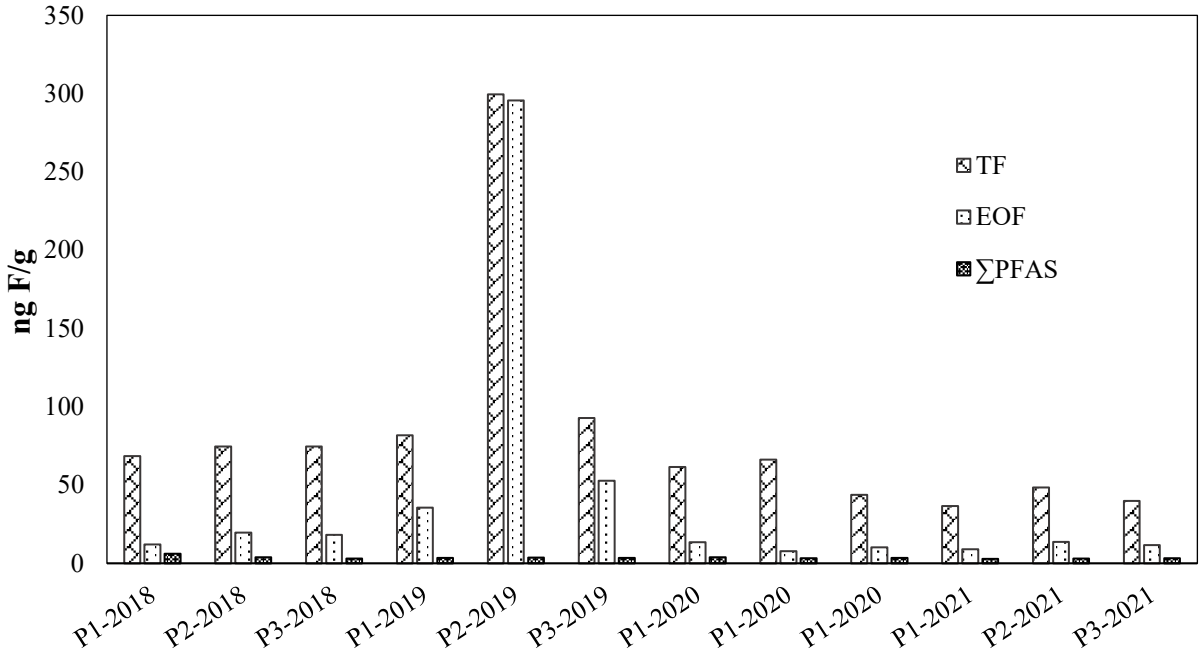


***Figure 2.*** *TF, EOF and ∑PFAS (ng F/ g blood serum) in individual pooled serum samples.*

Overall, ∑PFAS in pools from 2018-2021 accounted for 1-51% of EOF (1-9% of TF; Table 3), which is lower than reported previously (11-75%) for 1996-2017 in the same population (Miaz et al., 2020). This result appears to follow the same trend of increasing UEOF in more recent years as reported previously (Miaz et al., 2020). In comparison, two other studies from Sweden and the U.S. showed that the concentration of $\sum_{61}$PFAS and $\sum_{44}$PFAS accounted for 30-74% (Aro et al., 2021) and 14-85% (Pennoyer et al., 2023) of EOF, respectively. Likewise, a fluorine mass balance study in Norwegian serum revealed an increase in unidentified EOF from 2007 (10%) to 2015 (37%), consistent with observations in the present work (Cioni et al., 2023).

***Table 2.*** *Temporal trend of %EOF accounted for by ΣPFAS, %of TF accounted for by ΣPFAS, % of TF accounted for by EOF, and % of unidentified EOF in pooled serum samples.*

| **Pool** | % EOF accounted for by **Σ**PFAS | % TF accounted for by **Σ**PFAS | % TF accounted for by EOF | Unidentified EOF (%) |
|---|---|---|---|---|
| *P1-2018* | 51 | 9 | 17 | 49 |
| *P2-2018* | 20 | 5 | 26 | 80 |
| *P3-2018* | 17 | 4 | 24 | 83 |
| *P1-2019* | 10 | 4 | 43 | 90 |
| *P2-2019* | 1 | 1 | 99 | 99 |
| *P3-2019* | 7 | 4 | 57 | 93 |
| *P1-2020* | 29 | 6 | 22 | 71 |
| *P1-2020* | 42 | 5 | 12 | 58 |
| *P1-2020* | 35 | 8 | 23 | 65 |
| *P1-2021* | 32 | 8 | 25 | 68 |
| *P2-2021* | 22 | 6 | 28 | 78 |
| *P3-2021* | 28 | 8 | 29 | 72 |

**Suspect Screening**

Out of 1215 substances on our suspect list, 156 matched exact masses observed in serum within 5 ppm mass error, and only 13 substances triggered an MS2 scan. Of these 13, five substances were already included in the target list, leaving a total of 8 suspects for investigation. Based on exact mass alone, 5 were matched to monofluoroalkyl ether carboxylic acids (MFECAs), while 3 were tentatively matched to tetrafluoroalkyl carboxylic acids (TeFCAs). While inspection of MS2 spectra revealed some matches to predictions in MetFrag, these assignments remain highly tentative, and require further confirmation with authentic standards. Nevertheless, we note that two prior studies in fish from the Great Lakes reported the occurrence of MFECAs and TeFCAs (Fakouri Baygi et al., 2016) (Baygi et al., 2021). Moreover, in the latter study, these substances were also observed in human serum from local fish consumers, at concentrations ranging from 0.12-2.43 ng/g for MFECAs and 0.03-1.30 ng/g TeFCAs.

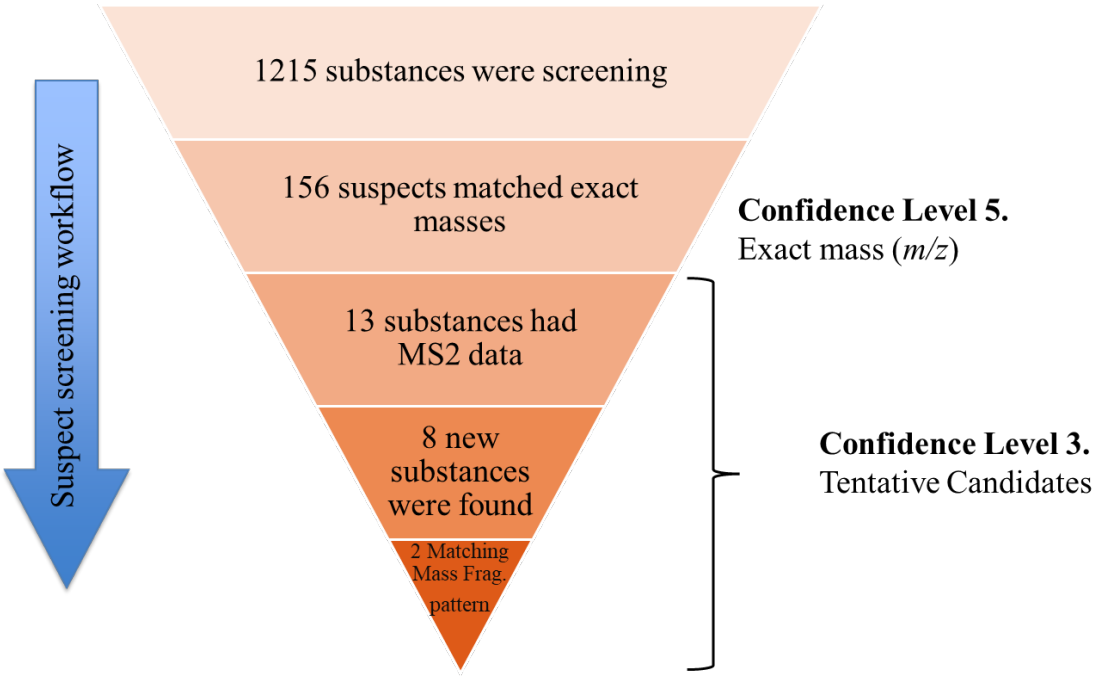


***Figure 3.*** *Workflow of Suspect Screening*

**Extended temporal trends of target ∑PFAS and TF during 1996-2021**

A complete temporal trend for the period 1996-2021 for TF and ∑PFAS is shown in Figure 5. The decline in ∑PFAS reported previously from 1996-2017 (Miaz et al., 2020), appeared to continue in the most recent years. Among individual target PFAS, the highest decreasing trend was observed for PFOA followed by PFHxS, PFNA and PFOS. PFDA was only detected in two pooled serum samples during 2018-2019. With the exception of the outlier observed in 2019 (discussed above), TF levels also appeared to be steadily decreasing over the entire time period, with the lowest levels of TF observed in the most recent years. No specific temporal trend was observed for EOF concentrations (data not shown), despite what appears to be an increase in the percentage of UEOF in more recent years.

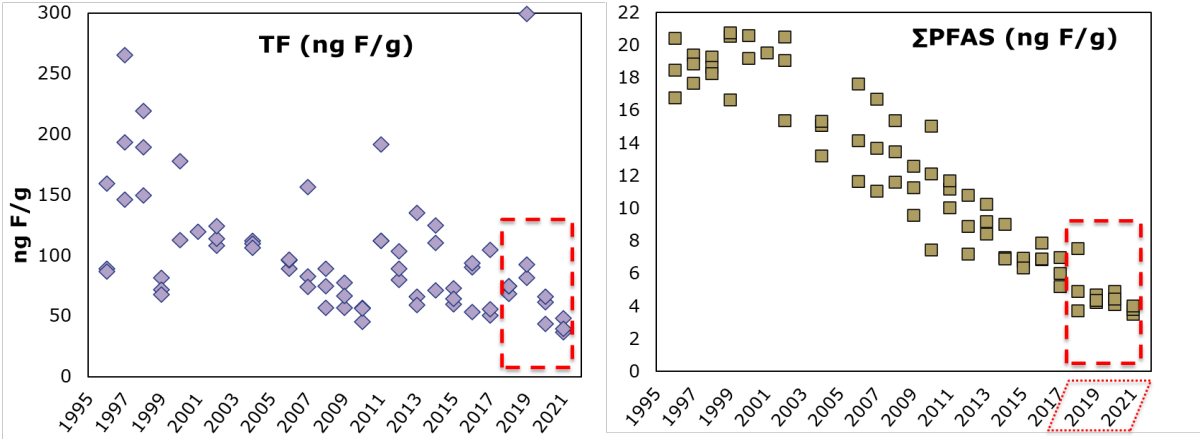


***Figure 5.*** *Temporal trends of TF and ∑PFAS (ng F $g^{-1}$ blood serum). The red dotted rectangles indicate results from current study with their respective years while other years (i.e., 1996-2017) were reported by Miaz et al (2020).*


## ACKNOWLEDGEMENT

We thank the Swedish Environmental Protection Agency for financial support. Appreciation is expressed to the participating women and to Marianne Leimar who assisted in recruitment, interviewing, and sample collection. The laboratory technicians at Livsmedelsverket are acknowledged for their assistance.


## REFERENCES


Aro, R., Eriksson, U., Kärrman, A., Yeung, L.W.Y., 2021. Organofluorine Mass Balance Analysis of Whole Blood Samples in Relation to Gender and Age. Environ. Sci. Technol. 55, 13142–13151. https://doi.org/10.1021/acs.est.1c04031

Baygi, S.F., Fernando, S., Hopke, P.K., Holsen, T.M., Crimmins, B.S., 2021. Nontargeted Discovery of Novel Contaminants in the Great Lakes Region: A Comparison of Fish Fillets and Fish Consumers. Environ. Sci. Technol. 55, 3765–3774. https://doi.org/10.1021/acs.est.0c08507

Cioni, L., Plassmann, M., Benskin, J.P., Coêlho, A.C.M.F., Nøst, T.H., Rylander, C., Nikiforov, V., Sandanger, T.M., Herzke, D., 2023. Fluorine Mass Balance, including Total Fluorine, Extractable Organic Fluorine, Oxidizable Precursors, and Target Per- and Polyfluoroalkyl Substances, in Pooled Human Serum from the Tromsø Population in 1986, 2007, and 2015. Environ. Sci. Technol. 57, 14849–14860. https://doi.org/10.1021/acs.est.3c03655

Fakouri Baygi, S., Crimmins, B.S., Hopke, P.K., Holsen, T.M., 2016. Comprehensive emerging chemical discovery: Novel polyfluorinated compounds in lake Michigan trout. Environ. Sci. Technol. 50, 9460–9468. https://doi.org/10.1021/acs.est.6b01349

Glynn, A., 2012. Perfluorerade alkylsyror (PFAA) i uppsala dricksvatten. Swedish National Food Agency, Uppsala.

Gyllenhammar, I., Berger, U., Sundström, M., McCleaf, P., Eurén, K., Eriksson, S., Ahlgren, S., Lignell, S., Aune, M., Kotova, N., Glynn, A., 2015. Influence of contaminated drinking water on perfluoroalkyl acid levels in human serum - A case study from Uppsala, Sweden.

Environ. Res. 140, 673–683. https://doi.org/10.1016/j.envres.2015.05.019
Gyllenhammar, I., Benskin, J.P., Plassmann, M., Kruså, M., Kallerman, P.H., Lampa, E., 2022. Levels of poly- and perfluoroalkyl substances (PFAS) in individual serum samples from first-time mothers in Uppsala, Sweden: results from year 2020-2022, and temporal trends for the time period 1996-2022. Report to the Swedish EPA, the Health-Related Environmental Monitoring Program.
Johanson, G., Gyllenhammar, I., Ekstrand, C., Pyko, A., Xu, Y., Li, Y., Norström, K., Lilja, K., Lindh, C., Benskin, J.P., Georgelis, A., Forsell, K., Jakobsson, K., Glynn, A., Vogs, C., 2023. Quantitative relationships of perfluoroalkyl acids in drinking water associated with serum concentrations above background in adults living near contamination hotspots in Sweden. Environ. Res. 219. https://doi.org/10.1016/j.envres.2022.115024
Miaz, L.T., Plassmann, M.M., Gyllenhammar, I., Bignert, A., Sandblom, O., Lignell, S., Glynn, A., Benskin, J.P., 2020. Temporal trends of suspect-and target-per/polyfluoroalkyl substances (PFAS), extractable organic fluorine (EOF) and total fluorine (TF) in pooled serum from first-time mothers in Uppsala, Sweden, 1996-2017. Environ. Sci. Process. Impacts 22, 1071–1083. https://doi.org/10.1039/c9em00502a
OECD, 2018. Organisation for Economic Co-operation and Development. Toward a new comprehensive global database of per- and polyfluoroalkyl substances (PFASs): summary report on updating the OECD 2007 list of per- and polyfluoroalkyl sub- stances (PFASs); 2018.
Pennoyer, E.H., Heiger-Bernays, W., Aro, R., Yeung, L.W.Y., Schlezinger, J.J., Webster, T.F., 2023. Unknown Organofluorine Mixtures in U.S. Adult Serum:Contribution from Pharmaceuticals? Toxics 11. https://doi.org/10.3390/toxics11050416
Schultes, L., Vestergren, R., Volkova, K., Westberg, E., Jacobson, T., Benskin, J.P., 2018. Per- and polyfluoroalkyl substances and fluorine mass balance in cosmetic products from the Swedish market: Implications for environmental emissions and human exposure. Environ. Sci. Process. Impacts 20, 1680–1690. https://doi.org/10.1039/c8em00368h
Spaan, K.M., Seilitz, F., Plassmann, M.M., de Wit, C.A., Benskin, J.P., 2023. Pharmaceuticals Account for a Significant Proportion of the Extractable Organic Fluorine in Municipal Wastewater Treatment Plant Sludge. Environ. Sci. Technol. Lett. 10, 328–336. https://doi.org/10.1021/acs.estlett.3c00108
United Nations, 2020. Stockholm Convention, United Nations Environment Programme. Stockholm Convention on Persistent Organic Pollutants.