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Screening of Poly- and Perfluoroalkyl Substances (PFASs) and Extractable Organic Fluorine (EOF) in the Blood of Highly Exposed People

Analyser av PFAS-prekursorer o extraherbart organiskt fluor (EOF) från individer boende i Ronneby med hög PFAS-exponering

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Summary

This report summarises the findings of an investigation into the occurrence of poly- and perfluoroalkyl substances (PFASs) in the whole blood of people living in the municipality of Ronneby. A total of 20 whole blood samples from individuals who have known to been expose to PFASs via consumption of PFAS contaminated drinking water were analysed in this study. Using both liquid and supercritical chromatography coupled with tandem mass spectrometers a total of 63 PFASs were analysed. These results were then compared with extractable organofluorine (EOF) levels measured with combustion ion chromatography. The data from both target PFAS analysis and EOF was used to perform fluorine mass balance analysis.

In general, the PFAS profile was dominated by long-chain perfluoroalkyl sulfonates (PFSAs with more than 6 fluorinated carbons), on average accounting for 97% of the total PFAS budget. The second most prominent PFAS class were long-chain perfluoroalkyl carboxylates (PFCAs with more than 7 fluorinated carbons), accounting for an additional 2.6% of the PFAS exposure. The average sum PFAS concentrations was 346 ng/g (from 74.1 ng/g to 715 ng/g). The average EOF concentration was 186 ng F/g and 79% of the EOF was explained by the target analytes.

Sammanfattning

Denna rapport redovisar resultaten från en studie angående förekomsten av poly- och perfluoroalkylsubstanser (PFAS) i helblodsprover hos människor som bor i Ronneby kommun. Totalt har 20 helblodsprover analyserats i studien. Totalt analyserades 63 PFAS-ämnen genom att använda både vätske- och superkritisk vätskekromatografi kopplad till masspektrometrisk detektion. Dessa resultat jämfördes sedan med de detekterade halterna av extraherbart organisk fluor (EOF) i en massbalans analys av fluor.

PFAS profilen i blodproverna dominerades generellt av långkedjiga perfluoralkyl-sulfonsyror (PFSA med fler än sex fluorerade kol), vilket i genomsnitt stod för 97% av den totala PFAS-halten. Den näst mest framträdande PFAS klassen var långkedjiga perfluoroalkyl-karboxylsyror (PFCA med fler än sju fluorerade kol) som utgjorde ytterligare 2.6% av PFAS exponeringen. Den genomsnittliga summan av PFAS koncentrationerna var 346 ng/g (74.1 – 715 ng/g). Den genomsnittliga summan av EOF halten var 186 ng F/g, vilket innebar att 79% av EOF var identifierade ämnen.

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1. Frame of the study

The objective of this investigation was to screen for legacy as well as novel PFASs [1], and to perform fluorine mass balance analysis on a selection of people who are known to have been exposed to elevated levels of PFASs through their drinking water supply. Fluorine mass balance analysis would help to estimate the levels of unknown organofluorine compounds that these people are exposed to. The results from this study can be used to guide further allocation of resources in subsequent cases of high PFAS exposure.

The target analysis of individual PFASs provided homologue profiles for each sample. Those results, combined with values obtained from the local drinking water supply [1], could be used to monitor the bioaccumulation and biotransformation of these compounds.

The fluorine mass balance analysis could be used as a gauge to estimate conceivable future health risks and possible degradation products that are not included in the list of target analytes. High levels of unidentified organofluorine compounds would warrant further investigations.

A total of 63 individual PFASs were monitored in this study and divided into the following groups:

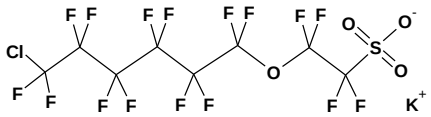
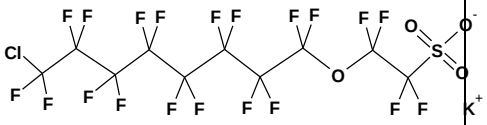
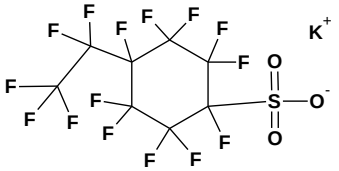
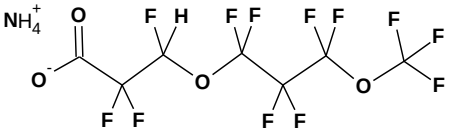
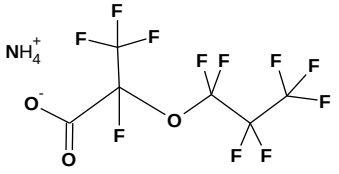
1. Ultra-short PFASs
2. Perfluoroalkyl carboxylic acids and sulfonic acids (PFCAs and PFSAs)
3. Precursor PFASs
4. Perfluoroalkyl phosphonic and phosphinic acids (PFPAs/PFPiAs)
5. Novel PFASs

Many of these groups do not meet the criteria of a persistent organic pollutant set by the Stockholm Convention [2]. Ultra-short chain PFASs (PFASs having between 1 to 3 fluorinated carbons) are not bioaccumulative, but they are persistent and high levels of them have been reported [3]. Some of the novel PFASs have been detected in water samples from Sweden, but there is little information regarding their levels in humans.

2. Background

Highly fluorinated chemicals, also known as poly- and perfluoroalkyl substances (PFASs), have been produced and used over the past six decades in various industrial and commercial applications [4]. Some of these man-made highly fluorinated chemicals are persistent organic pollutants (POPs) under Stockholm Convention. Several highly fluorinated chemicals (i.e., perfluorooctane sulfonate (PFOS) and perfluorooctanoate (PFOA)) have been banned (some are still in-use with exemption) or voluntarily phase-out by industries [5]–[8]). Their levels in the environment, including humans, have shown to decrease [9]. However, commercial fluorinated replacement products with the same desirable properties are introduced and a recent investigation reported more than 4 700 of highly fluorinated compounds registered on the global market [10]. The chemical identities for many of these replacement products are not known because they are proprietary. Scientists are working towards identifying novel highly fluorinated compounds using various techniques such as suspect screening or non-target screening [11]–[13] and more and more novel chemicals have been identified and reported [14]–[17]. Below are some examples of the replacement products (novel PFASs).

Table 2-1. Novel PFAS included in this study.

Name	Abbreviation	CAS nr.	Replace-ment for	Structure
6:2 chlorinated polyfluorinated ether sulfonate	6:2 Cl-PFESA F-53B (major)	73606-19-6	PFOS	
8:2 chlorinated polyfluorinated ether sulfonate	8:2 Cl-PFESA F-53B (minor)		PFOS	
Perfluoro-4-ethylcyclohexane-sulfonate	PFECHS	335-24-0		
3H-perfluoro-3-[(3-methoxy-propoxy) propanoic acid]	ADONA	958445-44-8	PFOA	
Hexafluoropropylene oxide dimer acid	HFPO-DA (also known as GenX)	62037-80-3	PFOA	

In addition to the thousands of PFASs being produced, there are precursor [18] and intermediate [19] compounds.

Drinking water has been identified as an important source of exposure for many populations, especially to those living near contaminated sites or the drinking water sources have been contaminated with these chemicals [1], [20], [21]. In Ronneby, Sweden, municipal drinking water was contaminated with PFASs and affected one-third of the households. The source of PFAS was believed to be the firefighting foam used in a nearby airfield since the mid-1980s. Clean water was provided from 16 December 2013. Therefore, some individuals living in Ronneby might have been exposed to high levels of PFASs present in the firefighting foam, which may contain several precursors of PFOS and PFCAs. A report on chemical analysis of selected fire-fighting foams on the Swedish market in 2014 indicated that perfluorohexanoate (PFHxA) and 6:2 fluorotelomer sulfoante (6:2 FTSA) were found at the highest concentrations in the foams [22] and some 6:2 fluorotelomer-based products were also identified in most of the foam samples. Inhabitants might have exposed to these fluorotelomer-based products.

As there are 4700 PFAS registered and current analytical methods can quantitatively measure less than 100 of them, the current investigation used the concept of mass balance analysis of organofluorine to estimate human exposure to organofluorine and the amounts of unknown organofluorine that cannot be accounted by target PFASs. The total fluorine (TF) content of a sample is made up of both inorganic fluorine (IF) and organic fluorine (OF, dark blue in Figure 2-1). As the IF levels can be an order of magnitude higher than that of OF [23] and the combustion ion chromatography (CIC) method (current method) does not distinguish between IF and OF, it is important to separate these two types of fluorine. The OF present in a sample is further divided into non-extractable organic fluorine (NEOF) and extractable organic fluorine (EOF). Depending on the chosen extraction method, some organofluorine compounds might not be extracted from the sample – forming the NEOF fraction. The organofluorine compounds that are extracted constitute the EOF (light blue in Figure 2-1) – which is analysed for both target PFAS and fluorine content for fluorine mass balance analysis. The amount of identified organic fluorine (blue pattern in Figure 2-1) is calculated from the levels of target PFASs, using the formula presented by Figure 2-2. This conversion has to be done for each target compound separately as the degrees of fluorination and molecular weights are different. This value is substracted from the measured EOF content to find the fraction that remains unidentified – the unidentified organic fluorine (UOF). The fluorine mass balance approach has been applied to various matrices: blood [23]–[25], water [17], [26] and various biota samples [17].

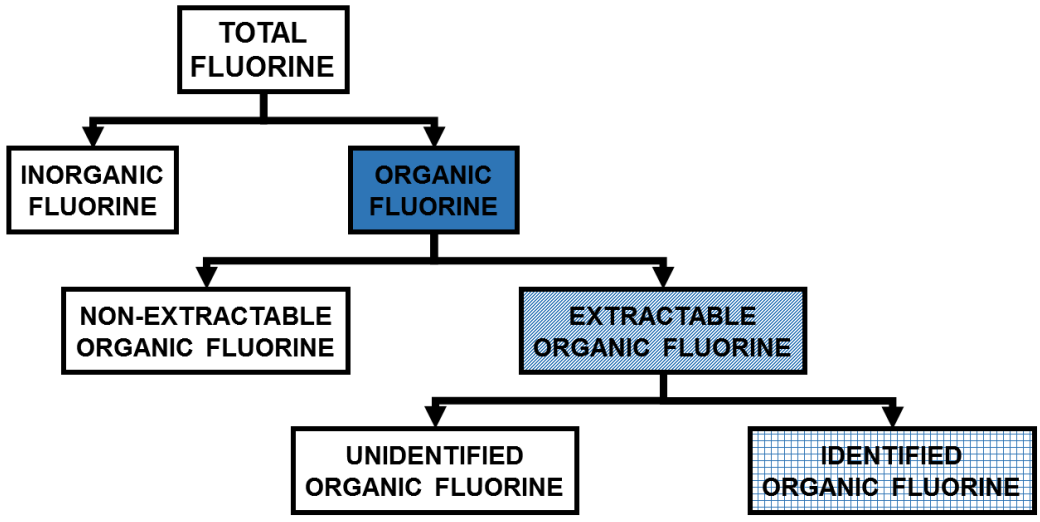


Figure 2-1. Scheme showing the different types of fluorine present in a samples.

$$C_F = n_F \times \frac{MW_F}{MW_{PFAS}} \times C_{PFAS}$$

Figure 2-2. Formula for converting from PFASs to fluorine. C_F: the corresponding fluoride concentration ($ng \times F \times g^{-1}$); n_F: the number of fluorine atoms in the PFAS molecule; MW_F: the molecular weight of fluorine; MW_{PFAS}: the molecular weight of the individual target PFAS; C_{PFAS}: the concentration of the target PFAS from LC-MS/MS.

Human blood is an important matrix to assess human exposure to PFASs, as these compounds have been shown to preferentially accumulate in protein-rich sites such as blood and liver [27]–[29]. In this investigation, blood samples collected from individuals with a known "high" exposure of PFAS in living in Ronneby were analyzed for EOF and a total number of 63 PFASs including ultrashort, intermediates, precursors and novel PFAS. Composition of PFAS in these highly exposed individuals may be different from the "background" or "general" population; a number of intermediate/transformation products may be present in their blood. The results were compared with those from our parallel investigation [32] which measured EOF and the same suite of PFASs in human blood samples from general Swedish population.

3. Samples for PFASs and EOF screening

A recent study showed that some compounds are preferentially found in only one sample matrix (e.g. PFHxA in whole blood) [51], probably due to preferential binding of some PFASs to blood cellular materials. The partitioning of EOF between whole blood, serum and plasma has not been studied in detail. There is a risk that some organofluorine compounds may be left behind when separating serum or plasma from whole blood. Thus whole blood was the matrix of choice for this investigation to evaluate human exposure to PFAS and EOF.

In December 2013 it was discovered that one out of two municipal waterworks in Ronneby, a municipality with 28 000 inhabitants in southern Sweden, was contaminated by high levels of PFAS from firefighting foams used at a nearby military airport. About one third of the households had been supplied by the contaminated waterworks for decades. Clean water was immediately supplied from the other waterworks. Large-scale biomonitoring started in June 2014, i.e. six months later. All residents in the municipality were invited to free-of-charge blood samplings, approximately 30% of the population in the contaminated area, and 5% from the uncontaminated area participated; in all 3297 subjects who also consented to participate in subsequent scientific studies (Ethical permission, Lund dnr 2014-267). Among them, a panel study group of 107 individuals have regularly donated blood samples for i.e. determination of half-lives of PFAS [1]. For the present study 1-2 mL whole blood was obtained from 20 randomly selected adults participants in the panel study, 7 women and 13 men. Their age ranged from 20 to 42 years with a median of 39 years. The samples were from October 2014.

Venous blood was collected for serum and whole blood biobanking, and stored at +4 °C before transportation in unbroken cold chain to Örebro.

4. Analysis and quantification

The analytical method was the same as used in the report "Screening of Poly- and Perfluoroalkyl Substances (PFASs) and Extractable Organic Fluorine (EOF) in Swedish Blood Samples". All samples were extracted in duplicate (see Figure 4-1), the first one (Replicate 1) was spiked with internal standards (IS) before the extraction and used for target analysis (more details in Section 4.1 and Figure 4-2). The second replicate (Replicate 2) was extracted without spiking any IS and analyzed for EOF content (more details in Section 4.1 and Figure 4-2).

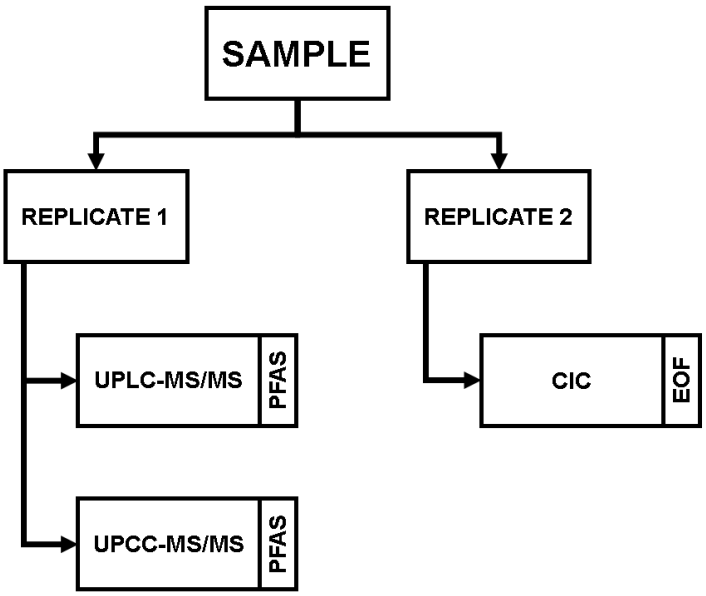


Figure 4-1. Overview of the sample analysis scheme.

4.1. Extraction procedure

Prior to sample extraction, individual whole blood samples were vigorously shaken and/or vortexed to mix the contents of each vacutainer. Two aliquots of the whole blood were taken into pre-cleaned 15 mL polypropylene (PP) tubes, the mass of each sub-sample was recorded. The subsample for target analysis (Replicate 1) was spiked with an IS mixture; the second subsample, for EOF analysis (Replicate 2), was extracted without any IS. The omission of IS for Replicate 2 was necessary as this would interfere with the EOF analysis, because the CIC system cannot differentiate between different sources of fluorine. These duplicate samples were extracted in the same batch to minimize the variability between them.

Samples were extracted in duplicates using the ion pair method [30]. In brief, 2 mL of 0.5 M tetrabutyl-ammonium (TBA) solution in water was added to the extract. Then, 5 mL of methyl tert-butyl ether (MTBE) was added to the tube. The mixture was shaken horizontally for 15 minutes at 250 rpm and centrifuged for 10 minutes at 8000 g to separate the organic and aqueous phases. The top layer (MTBE) was transferred to a new pre-cleaned PP tube and the extraction was repeated twice with 3 mL of MTBE. The extracts were combined and evaporated to 200 µL using an evaporation system. The combined extracts were reconstituted to 1.0 mL with MeOH and evaporated 0.5 mL with the evaporation system and the supernatants were transferred to LC vials.

The sample extracts were then split for different instrumental analyses as shown in Figures 4-1 and 4-2-1. Most of the analytes were quantified in the sample with 40% organic solvent content. The sample with 80% organic solvent content was used for PAPs and ultra-short chain PFAS analyses.

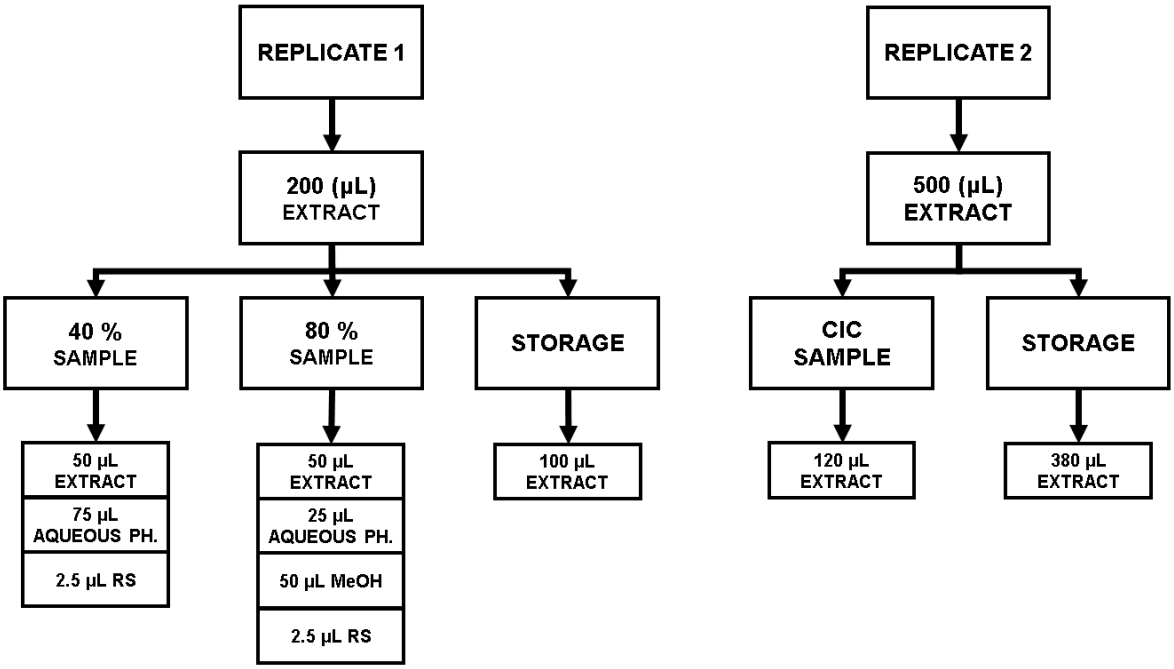


Figure 4-2-1. Detailed scheme of how the samples were divided for different instrumental analysis. Replicate 1 was analyzed with two different methanol compositions, 40% and 80% to improve chromatography. Replicate 2 was analyzed for the EOF content with CIC. RS – mass labelled recovery standard; aqueous phase – 2 mmol/L ammonia acetate in MilliQ water; all extracts were in methanol (MeOH).

4.2. Quantification of target analytes

4.2.1. Instrumentation

Analytes with four or more fluorinated carbons were quantified by ultra performance liquid chromatography electrospray ionization tandem mass spectrometry (UPLC-ESI-MS/MS) in negative mode. The analytes were separated on a Waters Acquity UPLC with a BEH column (2.1 × 100 mm, 1.7 µm) coupled to a Waters XEVO TQ-S MS/MS. The mobile phases were methanol (MeOH) and 30:70 MeOH:MilliQ water mixture, both with 2 mmol/L ammonium acetate and 5 mmol/L 1-methylpiperidine as additives [31]. Ultra-short chain compounds (C2-C3) were separated by a supercritical fluid chromatographic system (Waters Ultra Performance Convergence Chromatograph, UPCC), using CO_2 and MeOH with 0.1% ammonia as mobile phases with a Torus DIOL analytical column (3.0 × 100 mm, 1.7 µm). The UPCC was coupled to the Waters XEVO TQ-S detector [3]. Levels of two novel compounds (HFPO-DA and ADONA) were monitored using a Waters Acquity UPLC system coupled with a XEVO TQ-S micro MS/MS, the mobile phases and column were as described above. The list of analytes and their abbreviations are in Appendix 1 and Appendix 2 gives the parameters of the mass spectrometer.

4.2.2. Standards and calibration

Quantification of the analytes was done using native and isotope labelled internal standards purchased from Wellington Laboratories (Guelph, Canada), except for 10:2 monoPAP and 10:2 diPAP, which were purchased from Chiron (Trondheim, Norway). The PFOS isomers

were reported as a sum of individual isomers, 1-*m*-PFOS, 6/2-*m*-PFOS, 3/4/5-*m*-PFOS, 4.4/4.5/5.5-m_2-PFOS. Concentrations of all analytes were recovery-corrected using labelled internal standards. For those homologues of PFCAs, PFSAs, PAPs, fluorotelomer sulfonates (FTSAs), fluorotelomer carboxylates/fluorotelomer unsaturated carboxylates (FTCA/FTUCAs), and perfluoroalkyl sulfonamidoacetates (FOSAAs) where no isotope labelled standard were available, the internal standard closest in retention time within the same compound class was used for quantification. For Cl-PFESAs, PFECHS, polyfluorinated ether carboxylates (PFECAs), perfluoroalkyl phosphonates/ perfluoroalkyl phosphinates (PFPA/PFPiAs), and ADONA, the IS closest in retention time of the compound classes PFCAs and PFSAs was used for quantification. Multiple reaction monitoring (MRM) was used and at least two transitions were monitored for all analytes, except for TFA, perfluoropropanoate (PFPrA), perfluorobutanoate (PFBA) and perfluoropentanoate (PFPeA) where one transition was monitored. Due to poor recoveries of PFPrA and TFA in the blood samples, their concentrations were not reported but their detection are indicated.

In total, the levels of 63 PFASs were monitored in this study. The choice of analytes was based on previous studies and aimed to cover the most commonly found PFASs – PFAAs, to which people have had historical exposure and which are the stable degradation products of different precursors compounds. While PFAAs have accounted for most of the known PFASs exposure, several classes of PFCA and PFSA precursors were included in an attempt to elucidate possible exposure pathways to PFASs. Several intermediates (e.g., FTCAs and FTUCAs) were also monitored to assess human exposure to precursors. Besides, some ultrashort and novel PFASs (e.g., ADONA, GenX,) were also included to assess human exposure, as their information is limited.

The concentrations of each analyte were calculated using relative response factors (RRF, see Figure 4-2-2-1). The RRF was determined by analyzing calibration samples containing both the native (^{12}C) and isotope labelled (^{13}C) compounds. The calibration range was from approximately 0.005 to 30 ng/mL, the limit of detection (LOD) of each analyte is given in Appendix 3.

$$C_x = \frac{A_x}{A_{IS}} \times C_{IS} \times \frac{1}{RRF_x}$$

Figure 4-2-2-1. Calculation of analyte concentration in a sample; C_x - analyte concentration, C_{IS} - internal standard concentration, A_x - peak area of analyte, A_{IS} - peak area of internal standard, RRF - relative response factor determined separately.

4.2.3. Limit of detection and quantification

The limit of detection (LOD) for target analytes was determined separately for each sample preparation batch, it was calculated as the sum of the procedural blank and three times the pooled standard deviation of the analyte. The limit of quantification (LOQ) was determined as the procedural blank plus 10 times the pooled standard deviation. If a compound was not found in any of the procedural blanks, the lowest point of the calibration curve was used as the LOQ instead.

4.2.4. Recoveries, precision and accuracy

Samples with recoveries between 20 and 150 % were considered acceptable and the analyte concentrations were calculated using internal standards. The recoveries for different internal standards are given in Table 4-2-4-1.Samples with IS recoveries below 20% or great than 150%

were not reported and were denoted as not quantified (n.q.) in the results. Results for PFHxA and PFBS had abnormally high recoveries, thus the samples were further cleaned up using solid phase extraction (SPE) (Appendix 5) and results for those compounds are reported after the extra cleanup.

Each extraction batch included a quality control (QC) samples to monitor both accuracy and reproducibility. The QC sample was the Standard Reference Material (SRM) 1957, organic contaminants in non-fortified human serum (National Institute of Standards and Technology (NIST); Maryland, United States). The observed relative standard deviations (RSD) of L-PFOS (linear PFOS) and L-PFOA (linear PFOA) concentrations in QC samples were below 20%.

Additional spike-recovery experiments were done with pooled whole blood, a selection of the results (for novel PFASs) is presented in Table 4-2-4-2.

Table 4-2-4-1. Results of internal standard recovery in whole blood from the study. The relative standard deviation (RSD) and the number of samples where the recovery was within the acceptable range (20-150 %).

Analyte	Average recovery	RSD	n
^{13}C-PFBA	46%	10%	20
^{13}C-PFPeA	53%	9%	20
^{13}C-PFHxA*	49%	10%	20
^{13}C-PFHpA	30%	55%	20
^{13}C-PFOA	49%	11%	20
^{13}C-PFNA	46%	14%	20
^{13}C-PFDA	42%	18%	20
^{13}C-PFUnDA	42%	19%	20
^{13}C-PFBS*	92%	10%	20
^{18}O-PFHxS	74%	13%	20
^{13}C-PFOS	61%	18%	20
^{13}C-6:2 monoPAP	53%	9%	20
^{13}C-8:2 monoPAP	40%	25%	20
^{13}C-6:2 diPAP	21%	39%	20
^{13}C-8:2 diPAP	20%	38%	20
^{2}H EtFOSAA	28%	22%	20
^{13}C-HFPO-DA	27%	40%	20

* Recoveries after SPE cleanup.

Table 4-2-4-2. Results of spike-recovery experiments (%) for novel PFASs (4 ng) in blood.

Analyte	Average recovery	n
6:2 Cl-PFESA	86%.	2
8:2 Cl-PFESA	103%	2
PFECHS	87%	2

ADONA	61%	2
HFPO-DA	58%	2

4.3. Quantification of EOF content

4.3.1. Instrumentation

Extractable organofluorine (EOF) content was measured using a combustion ion chromatography (CIC) system. The CIC consists of a combustion module (Analytik Jena, Germany), a 920 Absorber Module and a 930 Compact IC Flex ion chromatograph (both from Metrohm, Switzerland). Separation of anions was performed on an ion exchange column (Metrosep A Supp 5 – 150/4.0) using carbonate buffer (64 mmol/L sodium carbonate and 20 mmol/L sodium bicarbonate) as eluent for isocratic elution. In brief, the sample extract (0.1 mL) was injected on to a quartz boat, which was pushed into the furnace by the autosampler. The furnace was kept at 1000-1050 °C for combustion, during which, all organofluorine compounds were converted into hydrogen fluoride (HF). A carrier gas (argon) was constantly pumped through the combustion tube, the gas carries all formed HF into the absorber module where MilliQ water is used to capture the HF. A 2 mL aliquot of the absorber solution is then injected on a pre-concentration column and then injected on the ion chromatograph. The concentration of F^- ions in the solution was measured using ion chromatography.

4.3.2. Standards and calibration

Standard solutions from a solid PFOS potassium salt (Fluka, part of Fisher Scientific, Hampton, United States) were prepared in methanol. These solutions were used to created injection standards to monitor the performance of the CIC system. Quantification of samples was based on an external calibration curve. For both calibration and project samples the peak area of the preceding combustion blank was subtracted from peak area of the sample to correct for the background contamination. A five point calibration curve (50, 100, 200, 500 and 1000 ng F/mL) was constructed, with each level analyzed in triplicates.

Fluoride signal was observed in combustion blank even when no sample was analyzed. Prior to sample analysis, multiple combustion blanks were performed until stable fluoride signals were reached; the RSD of the three most recent combustion blanks lower than 5 %.

4.3.3. Limit of detection

The limit of detection (LOD) was determined separately for each sample preparation batch, the procedural blank of the batch plus three times the pooled standard deviation of the procedural blanks. The reported values were not corrected for extraction blanks.

4.3.4. Precision and accuracy

Combustion blanks (CIC analysis cycle without a sample) were made between sample injections to evaluate the presence of carryover between samples and to obtain a reliable estimate of the background fluorine levels. The repeatability of the instrument was tested by triplicate analysis of dilutions made from an anion SRM solution (product code 89886, Sigma-Aldrich). The five dilutions were in the range of 60 ng F/g to 1200 ng F/g and the relative standard deviation at all five dilution levels was below 25%. The calibration curve, which was made from a PFOS salt by a series of dilutions by weight, was compared to an older calibration

curve. The difference of the slope of these two separate calibrations was below 10%. This was in the same range as the relative standard deviation of the calibration point replicates.

4.4. Data treatment

When concentrations of analytes were below LOQ, zero was assigned for them for any further data treatment; this approach was used for both target PFAS and EOF data. Thus, when calculating the sum concentration of the 63 PFASs ($\sum_{63}$PFAS) in a sample, the concentrations of individual analytes were added up with those below LOQ were kept as zero. When calculating detection frequencies for analytes, all samples with levels above LOD were counted.

5. Results

A total of 20 whole blood samples from Ronneby were analysed for their PFAS and EOF contents (Table 5-1). All samples showed quantifiable levels of PFASs and 19 had EOF levels above the LOD. The average sum concentration of the 63 PFASs monitored in this study ($\sum_{63}$PFAS) for the 20 samples was 346 ng/g, ranging from 74.1 to 715 ng/g (Figure 5-1-1). A total of 21 different PFAS showed detectable concentrations at least once; the maximum number of different PFASs in one sample was 9 (Appendix 4); average molar concentrations of analytes are provided in Appendix 5.

The PFAS homologue profiles of the samples were dominated by long-chain PFSAs, on average accounting for 97% of $\sum_{63}$PFAS (Figure 5-1-1). The most abundant long-chain PFSAs were PFOS (L-PFOS - 29% of $\sum_{63}$PFAS, 3/4/5-*m*-PFOS – 8.8%, 6/2-*m*-PFOS - 4.8%, 1-*m*-PFOS – 4.0%), followed by perfluorohexane sulfonate (PFHxS - 44%) and perfluoroheptane sulfonate (PFHpS - 4.6%). Of the long-chain PFSAs, PFHxS, PFHpS and PFOS were detected in all samples. Short-chain PFSAs (perfluorobutane sulfonate - PFBS and perfluoropentane sulfonate - PFPeS) made up only 1.5 % of the $\sum_{63}$PFAS. Of the short-chain PFSAs, PFBS was found in one sample, while PFPeS was detected in all samples. Perfluoroethane sulfonate (PFEtS) was the only detected ultra-short chain PFSAs, with a maximum concentration of 0.14 ng/g, which was found in 15% of the samples and on average in made up 0.01% of the $\sum_{63}$PFAS.

The most abundant long-chain PFCAs were PFOA and perfluorononanoate (PFNA), which contributed 2.6% and 0.1% to the $\sum_{63}$PFAS respectively. Smaller contributions also came from perfluorodecanoate (PFDA - 0.04% of $\sum_{63}$PFAS) and perfluoroundecanoate (PFUnDA - 0.06%). PFOA and PFNA were found in all samples; PFCAs with longer perfluorinated carbon backbones showed lower detection frequencies; PFDA was detected in 35% and PFUnDA in 85% of the samples. Of the short-chain PFCAs perfluorobutanoate (PFBA) was not detected in any samples, while perfluoropentanoate (PFPeA) was found at trace levels in 25% of the samples. Of the ultra-short chain PFCAs (trifluoroacetate - TFA and perfluoropropanoate – PFPrA), only TFA was detected in one sample.

The PFCA and PFSA precursors had a negligible contribution to the $\sum_{63}$PFAS. One of the PFCA precursors – 6:2 FTSA was detected in 20% of the samples. Perfluorooctane sulfonamidoacetate (FOSAA), a PFSA precursor, was detected once, in sample nr. 13; perfluorobutane sulfonamide (FBSA) was detected at trace levels in 15% of the samples. The only novel PFAS that was detected was PFECHS, which was found in 15% of the samples.

Out of the 20 samples analysed, 19 had EOF levels above the LOD (Figure 5-1-2). Their average EOF concentration was 147 ng F/g and on average 86% of it was accounted for by the 63 PFASs monitored in this study (identified PFAS, iPFAS). Of the 19 samples that had

quantifiable levels of EOF, 4 had all of their EOF explained by the identified PFAS (samples nr. 2, 5, 17, 18).

Table 5-1. a) Average sum PFAS concentrations (ng/g whole blood) of different classes and their respective b) contribution (%) to the average sum PFAS in Ronneby and the general population [32].

a)	**Ronneby**	**General population**
	n=20	*n*=148
	Concentration (ng/g)	
∑ultrashort	0.09	0.01
∑PFCA	9.05	1.09
∑PFSA	336	4.45
∑FTSA	0.00	0.00
∑FTCA	0.00	0.00
∑FTUCA	0.00	0.00
∑FASA/FASE	0.00	0.00
∑FOSAA	0.02	0.02
∑PAP	0.00	0.00
∑SamPAP	0.00	0.00
∑PFPA	0.00	0.00
∑PFPiA	0.00	0.00
∑Novel	0.01	0.03
Total	346	5.61

b)	**Ronneby**	**General population**
	Composition (%)	
∑ultrashort	0.0%	0.2%
∑PFCA	2.6%	19.5%
∑PFSA	97.3%	79.3%
∑FTSA	0.0%	0.1%
∑FTCA	0.0%	0.0%
∑FTUCA	0.0%	0.0%
∑FASA	0.0%	0.1%
∑FOSAA	0.0%	0.4%
∑PAP	0.0%	0.0%
∑SamPAP	0.0%	0.0%
∑PFPA	0.0%	0.0%
∑PFPiA	0.0%	0.0%
∑Novel	0.0%	0.5%

∑*ultrashort - PFEtS, PFPrS; TFA and PFPrA were not quantified*

∑*PFCA - PFBA, PFPeA, PFHxA, PFHpA, PFOA, PFNA, PFDA, PFUnDA, PFDoDA, PFTrDA, PFTDA, PFHxDA, PFOcDA*

∑*PFSA - PFBS, PFPeS, PFHxS, PFHpS, PFOS, PFNS, PFDS, PFDoDS*

∑*FTSA - 4:2 FTSA, 6:2 FTSA, 8:2 FTSA, 10:2 FTSA*

∑*FTCA - 3:3 FTCA, 5:3 FTCA, 7:3 FTCA*

∑*FTUCA - 6:2 FTUCA, 8:2 FTUCA, 10:2 FTUCA*

∑*FASA - FBSA, MeFBSA, FHxSA, MeFHxSA, FOSA*

∑*FOSAA - FOSAA, MeFOSAA, EtFOSAA*

∑*PAP - 6:2 mPAP, 8:2 mPAP, 10:2 mPAP, 6:2 diPAP, 6:2/8:2 diPAP, 8:2 diPAP, 10:2 diPAP*

∑*SamPAP - SAmPAP, diSAmPAP*

∑*PFPA - PFHxPA, PFOPA, PFDPA*

∑*PFPiA- 6:6 PFPiA, 6:8 PFPiA, 8:8 PFPiA*

∑*Novel - PFECHS, 8:2 Cl-PFESA, 6:2 Cl-PFESA, ADONA, HFPO-DA*

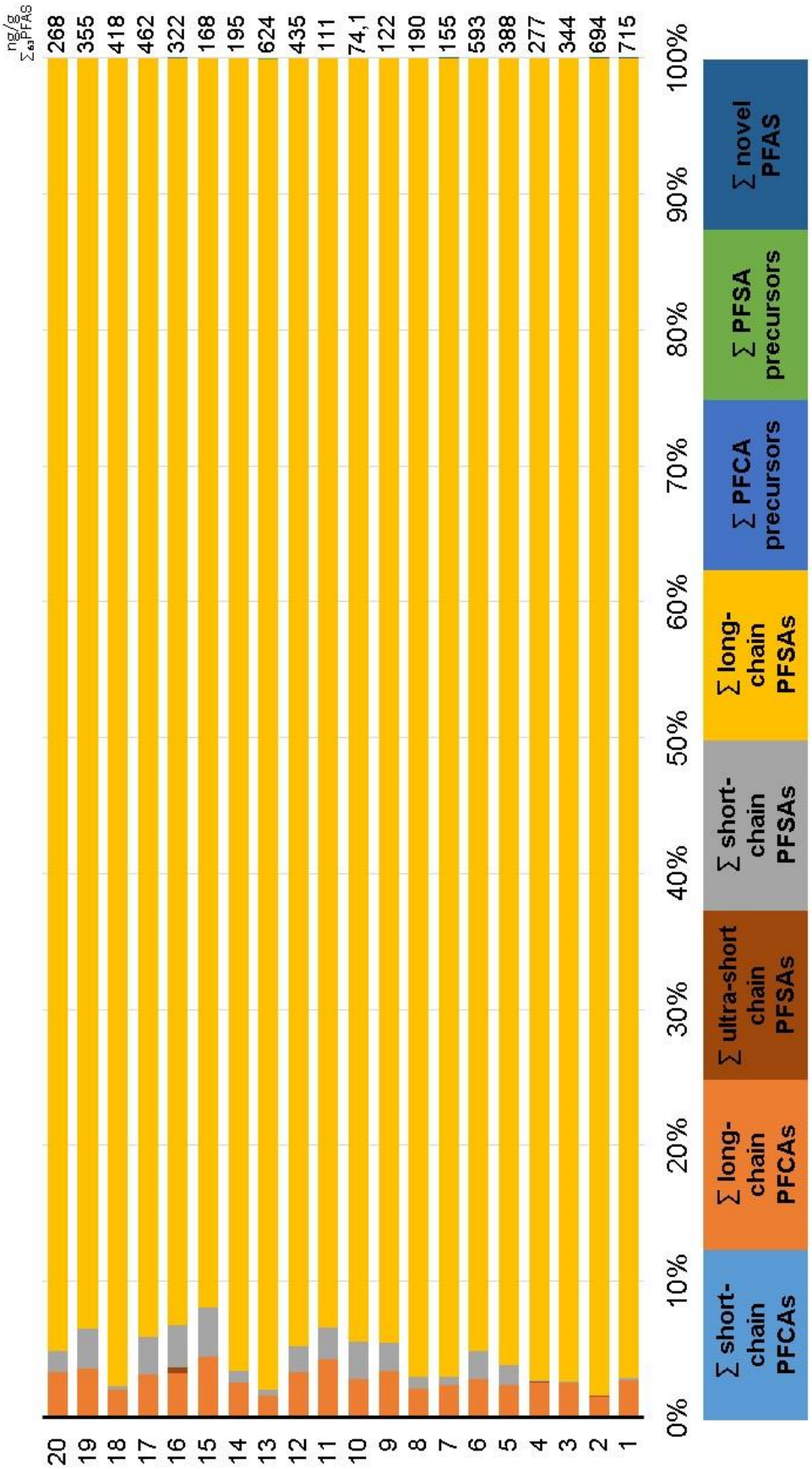


Figure 5-1-1. PFAS homologue profiles of the highly exposed people from Ronneby.

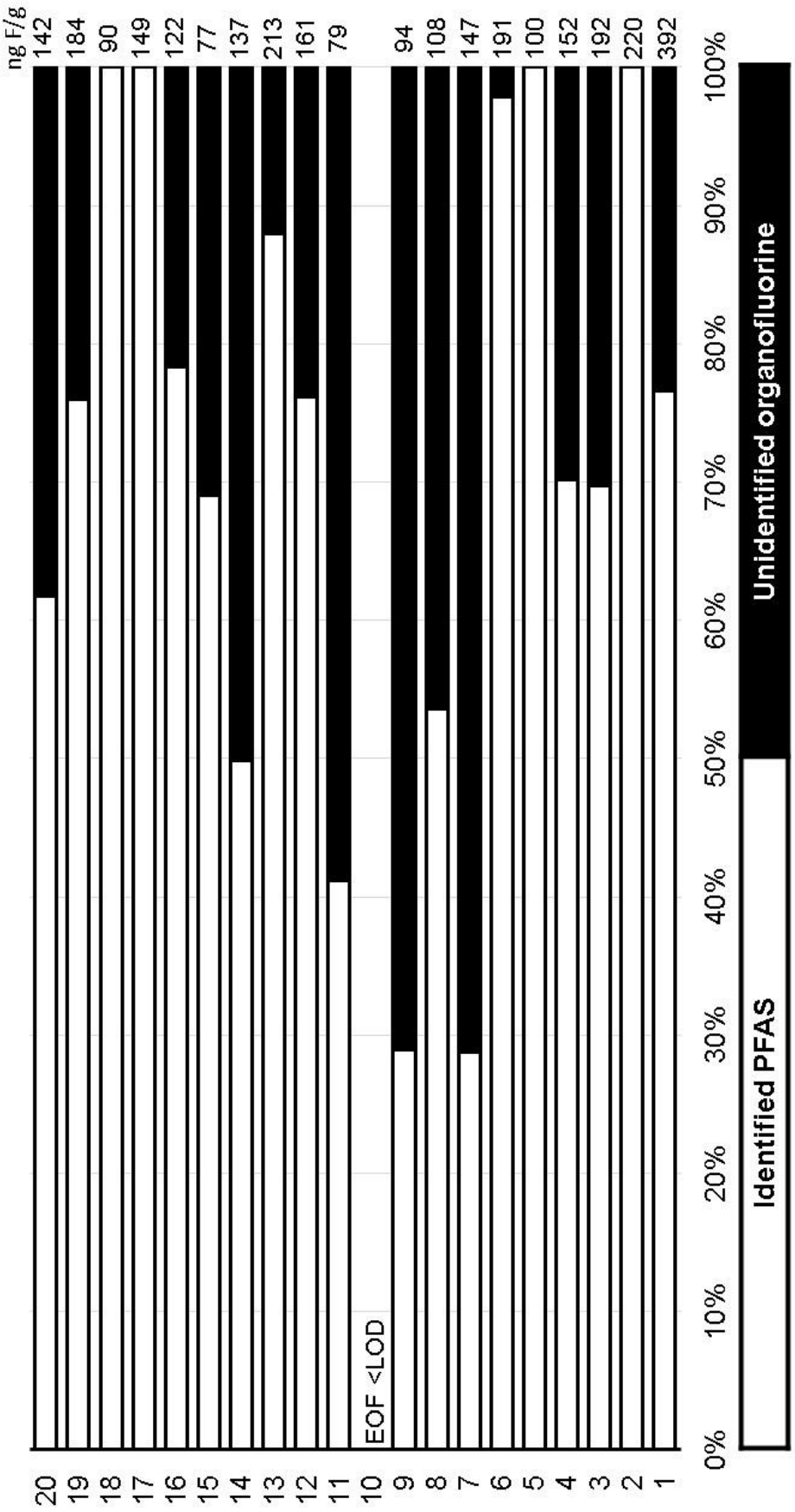


Figure 5-1-2. Fluorine mass balance of the samples collected in Ronneby.

6. Discussion

6.1. Target PFASs

The main contributors to PFAS exposure were the legacy compounds (PFOA, PFHxS and PFOS). On average those compounds (including branched PFOS isomers) accounted for 94% of the $\sum_{63}$PFAS (Figure 6-1-1). The average concentrations for these analytes were as follows: PFOA - 8.4 ng/g (ranging from 1.9 to 18.4 ng/g), PFHxS - 151 ng/g (from 28.0 to 327 ng/g), PFOS (branched + linear) - 165 ng/g (from 39.5 to 388 ng/g). These levels are comparable to those reported previously by Li et al. [1] (PFOA - 9 ng/g, PFHxS - 139 ng/g and PFOS - 173 ng/g; values have been converted for whole blood comparison).

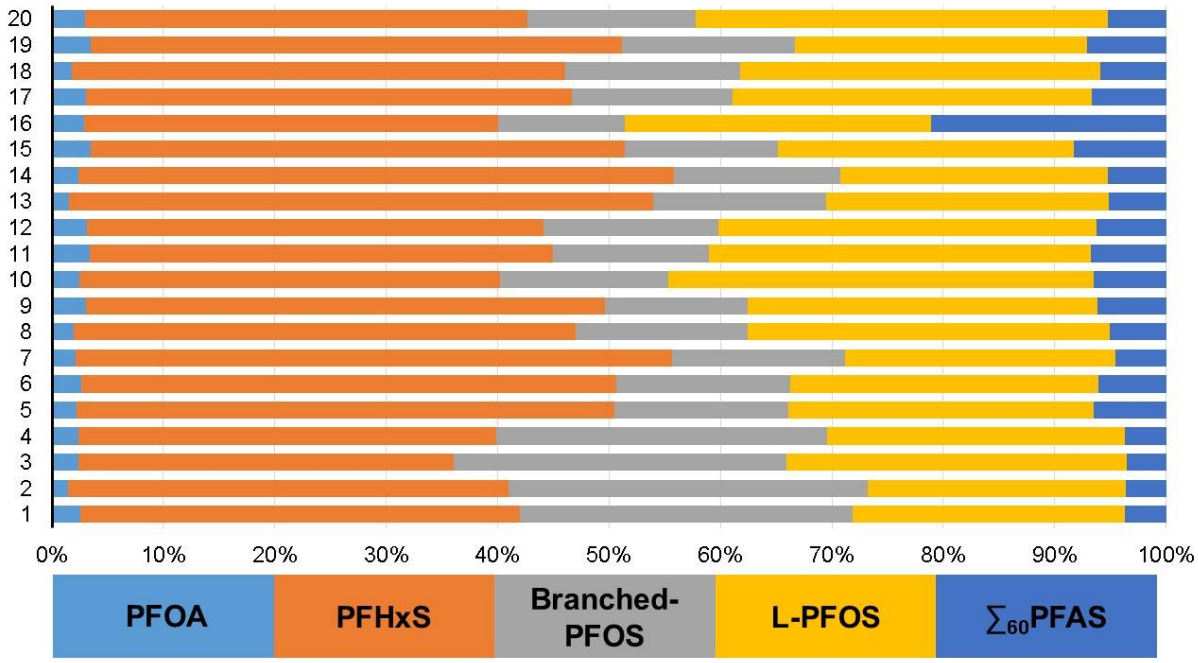


Figure 6-1-1. Relative contributions of the key PFASs to the $\sum_{63}$PFAS, where $\sum_{60}$PFAS is the combined contribution of the remaining 60 PFAS monitored in this study.

The levels of the most commonly found PFCAs (C9-C11) were similar between the people from Ronneby (later referred to as Ronneby group) and the Swedish general population (Figure 6-1-2) [32]. While the highest levels in both cases was found for PFOA – 8.4 ng/g in Ronneby and 0.56 ng/g in the general population. The profile of C7-C11 PFCA distribution was different, in the Ronneby group PFOA accounted for 93% of the C7-C11 PFCAs, in the general population it accounted for 54% of C7-C11 PFCAs. The pattern was markedly different for compounds with longer perfluorinated backbones – in the Ronneby group PFNA, PFDA and PFUnDA accounted for 4.2%, 1.2% and 1.5% of C7-C11 PFCAs respectively; in the general population they made up 26.0%, 13.0% and 7.9% of C7-C11 PFCAs.

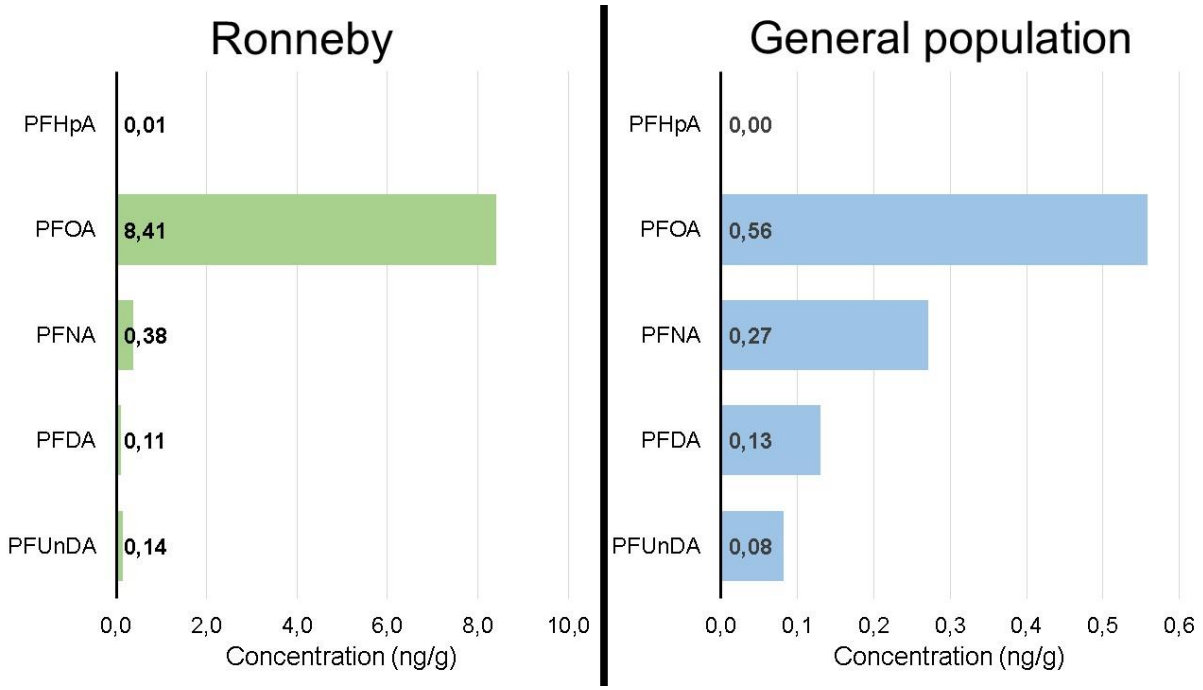


Figure 6-1-2. The average concentrations of selected PFCAs in whole blood from the people from Ronneby and in the Swedish general population.

The levels of the most commonly found PFSAs (C5-C8) were approximately 2 orders of magnitude higher in the Ronneby group than in the Swedish general population (Figure 6-1-3) [32]. Similar to the results from the the general population of Sweden, the highest level among PFSAs in the participants from Ronneby was found for PFOS, followed by PFHxS. The branched and linear PFOS together accounted for 80% of C5-C8 PFSAs in the general population and 49% in the Ronneby group. PFPeS detection frequency in the Ronneby group was higher than in the general population – 100% and 51% respectively; PFPeS accounted for a higher fraction of the C5-C8 PFSAs in the Ronneby group as well (1.1% and 0.3% of C5-C8 PFSAs).

The levels of target PFASs found in the Ronneby group are in line with values reported earlier by Li et al. as was briefly mentioned at the beginning of this section; however, in their study levels for only PFOA, PFHxS and PFOS were reported. Although these analytes accounted for the majority of the PFAS exposure, the additional information regarding a wider range of PFASs can contribute to further work.

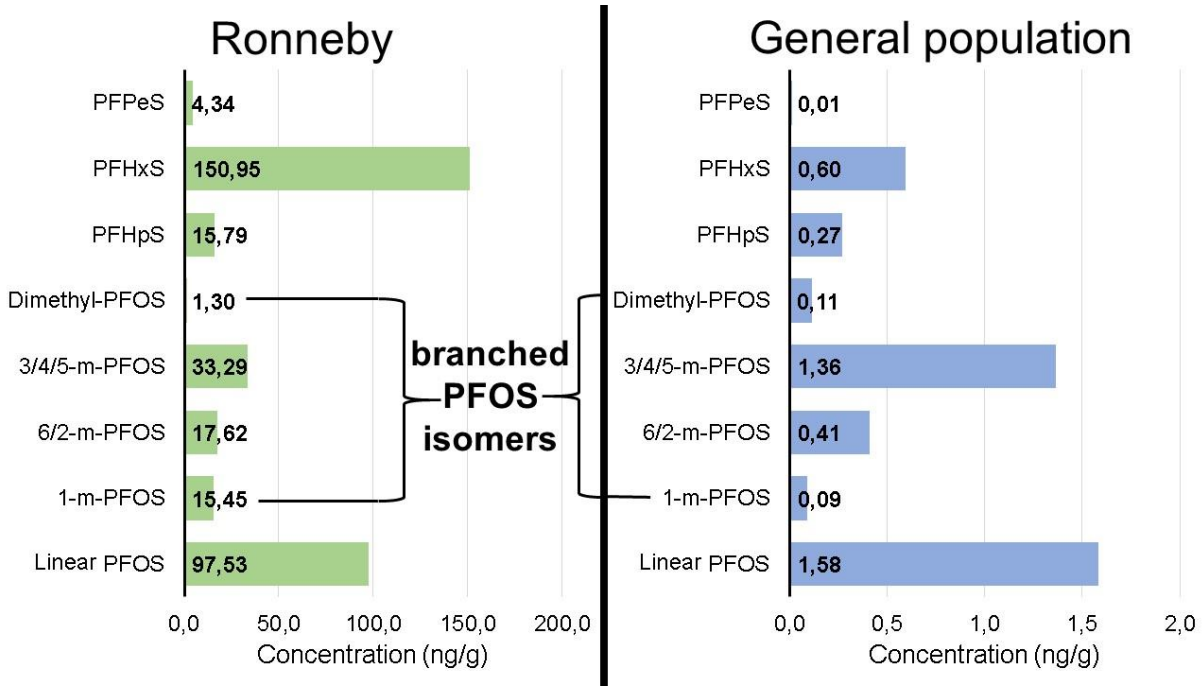


Figure 6-1-3. The average concentrations of selected PFSAs in whole blood from the people from Ronneby and in the Swedish general population.

6.2. Fluorine mass balance

Fluorine mass balance analysis is a useful tool to estimate the overall levels of organofluorine compounds. In the current study, EOF data from from the combustion ion chromatograph was compared with the levels of individual PFAS from target analysis. Of the 20 samples collected from Ronneby, 19 had EOF levels that could be quantified and on average the 63 target analytes could explain 79 % of the EOF – leaving 21 % of the EOF unidentified. As PFOA, PFHxS and PFOS (branched and linear) were the dominant PFASs, they also accounted for majority of the EOF – on average these three analytes explained 75% of the EOF (Figure 6-2-1).

The levels of EOF were much higher in the Ronneby group than in the general population [32] – 147 ng F/g and 7.8 ng F/g, respectively. However, in the general population 71% of that EOF remained unidentified, compared to 21% in the Ronneby group. Although higher proportion of EOF was explained by quantifiable PFAS, the amount of unidentified organfluorine in the exposed people (40.3 ng F/g) was several times higher than those of the general population (4.9 ng F/g, [32]) in Sweden.

The levels of EOF observed in this study (Ronneby group) a closer to those observed in Japan in the blood of fluorochemical plant employees – 465 ng F/g (n = 2)[23], than the levels observed in the general population – 7.8 ng F/g [32].

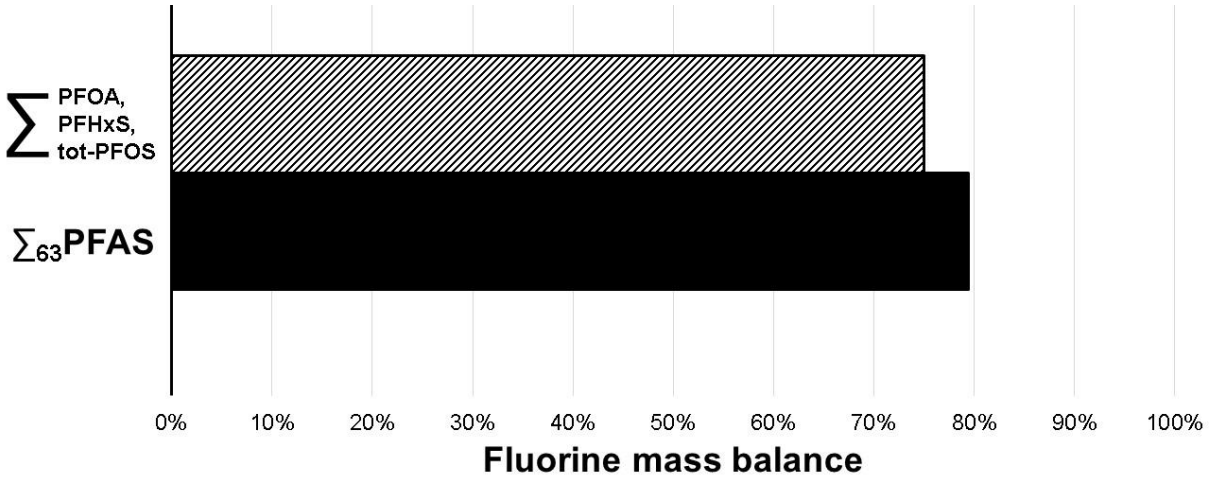


Figure 6-2-1. Average concentration of fluorine accounted for by PFOA, PFHxS and PFOS (branched+linear) and fluorine from all analytes included in this study ($\sum_{63}$PFAS) in the whole blood samples from Ronneby.

7. Findings

- The average sum concentration of the $\sum_{63}$PFAS in the whole blood samples from Ronneby was 346 ng/g (ranging from 74.1 to 715 ng/g)
- People from Ronneby were exposed to 21 different PFAS and low levels of unidentified organofluorine compounds
- Long-chain PFSAs (mainly PFOS and PFHxS) and long-chain PFCAs (mainly PFOA) accounted for 97% and 2.6% of the $\sum_{63}$PFAS respectively
- Short-chain PFCAs and PFSAs, ultra-short chain PFCAs and PFSAs, PFCA and PFSA precursors and novel PFASs were detected in only a few samples
- 19 samples out of 20 had detectable levels of EOF, with an average EOF concentration of 147 ng F/g (ranging from below LOD to 364 ng F/g)
- 79% of the EOF was explained by the identified PFAS on average
- Levels of unidentified organofluorine in the highly exposed group (40.3 ng F/g) were several times higher than those of the general population (4.9 ng F/g) from Sweden

8. Conclusions and Future Work

This screening study has shown that monitoring only a few compounds (e.g. PFOS, PFOA and PFHxS) can account for 79% of the $\sum_{63}$PFAS and these three PFAS accounted for 75% of EOF. With the inclusion of further 60 PFAS, an additional 4% of the EOF was explained. Although higher proportion of EOF was explained by quantifiable PFAS, the amount of unidentified organfluorine in the exposed people (40.3 ng F/g) was several times higher than that of the general population (4.9 ng F/g, [32]) in Sweden, suggesting that contaminated drinking water also includes presently unidentified organofluorine.

The EOF levels in the current investigation showed much higher levels than those of the general population, which may suggest EOF measurement to be a useful tool to detect human exposure to a PFAS source. For example, some samples from Umeå [32] showed low levels (1.77 – 12.5 ng/g) of $\sum_{63}$PFAS; however, the EOF ranged 32.5 – 48.7 ng F/g when compared to other individuals from the same area ranged below LOD up tp 13.9 ng F/g. These individuals may have been exposed to a source of unknown organofluorines that warrant further investigation.

Apart from PFOS, PFOA and PFHxS, other detected PFASs showed similar levels to the general population [32] indicating that monitoring only PFOS, PFOA and PFHxS cannot represent a complete human exposure to PFAS in highly exposed group. Another observation is that one of the novel PFASs the PFECHS was detected in 15% of the samples and this compound was also detected in the general Swedish population. It is known that the chemical has a specific use in aircraft hydraulic fluids. The ubiquitous occurrence of this chemical, even though found at low concentration, suggest other use in our daily life.

Different PFAS composition profiles and EOF levels were observed from different municipalities in Sweden [32]. Sources of exposure may vary among municipalities. Further investigation should also compare with a reference group, a population that inhabit in Ronneby without history of consuming PFAS contaminated drinking water, to understand human exposure of PFAS there. Furthermore, another highly exposed people via consumption of PFAS contaminated water should be analysed for the same suite of PFASs and EOF to compare and contrast the results of current investigation to further understand human exposure to known source of PFAS.

Population halving times have been estimated for some PFASs (e.g., PFOS, PFHxS, PFOA). Information about the population halving time on EOF, including those unidentified organofluorines, remains unknown. It is important to conduct a longitudinal study on EOF to the same study group to understand the population halving time of those unidentified organofluorine compounds.

Besides, plasma and sera samples were commonly used for different biomonitoring studies. A recent study showed different preferential binding of some PFAS (FOSA and PFHxA) to whole blood [33]. Further work should also compare the PFAS and EOF levels among plasma, sera and wholeblood samples to understand the representation of results from different matrices.

Furthermore, inclusion of total oxidizable precursor assay to the fluorine mass balance approach may help convert possible precursor compounds or intermediates into more readily measurable PFAAs to complete the fluorine mass balance.

8. Acknowledgements

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Appendix 1. Full list of target PFASs and their abbreviations

Table A1-1. List of abbreviations of target PFASs in this study.

Class	Subgroup	Acronym	Name
PFSA	Ultra-short chain	PFEtS	Perfluoroethane sulfonic acid
	Ultra-short chain	PFPrS	Perfluoropropane sulfonic acid
	Short-chain	PFBS	Perfluorobutane sulfonic acid
	Short-chain	PFPeS	Perfluoropentane sulfonic acid
	Long-chain	PFHxS	Perflurohexane sulfonic acid
	Long-chain	PFHpS	Perfluoroheptane sulfonic acid
	Long-chain	PFOS	Perfluorooctane sulfonic acid
	Long-chain	PFNS	Perfluorononane sulfonic acid
	Long-chain	PFDS	Perfluorodecane sulfonic acid
	Long-chain	PFDoDS	Perfluorododecane sulfonic acid
PFCA	Ultra-short chain	TFA	Trifluoroacetic acid
	Ultra-short chain	PFPrA	Perfluoropropanoic acid
	Short-chain	PFBA	Perfluorobutanoic acid
	Short-chain	PFPeA	Perfluoropentanoic acid
	Short-chain	PFHxA	Perfluorohexanoic acid
	Short-chain	PFHpA	Perfluoroheptanoic acid
	Long-chain	PFOA	Perfluorooctanoic acid
	Long-chain	PFNA	Perfluorononanoic acid
	Long-chain	PFDA	Perfluorodecanoic acid
	Long-chain	PFUnDA	Perfluoroundecanoic acid
	Long-chain	PFDoDA	Perfluorododecanoic acid
	Long-chain	PFTrDA	Perfluorotridecanoic acid
	Long-chain	PFTDA	Perfluorotetradecanoic acid
	Long-chain	PFHxDA	Perfluorohexadecanoic acid
	Long-chain	PFOcDA	Perfluorooctadecanoic acid
FTCA	Precursor	3:3 FTCA	3:3 Fluorotelomer carboxylic acid
	Precursor	5:3 FTCA	5:3 Fluorotelomer carboxylic acid
	Precursor	7:3 FTCA	7:3 Fluorotelomer carboxylic acid
FTUCA	Precursor	6:2 FTUCA	6:2 Fluorotelomer unsaturated carboxylic acid
	Precursor	8:2 FTUCA	8:2 Fluorotelomer unsaturated carboxylic acid
	Precursor	10:2 FTUCA	10:2 Fluorotelomer unsaturated carboxylic acid
FTSA	Precursor	4:2 FTSA	4:2 Fluorotelomer sulfonic acid
	Precursor	6:2 FTSA	6:2 Fluorotelomer sulfonic acid
	Precursor	8:2 FTSA	8:2 Fluorotelomer sulfonic acid
	Precursor	10:2 FTSA	10:2 Fluorotelomer sulfonic acid

monoPAP	Precursor	6:2 monoPAP	6:2 Fluorotelomer phosphate monoester
	Precursor	8:2 monoPAP	8:2 Fluorotelomer phosphate monoester
	Precursor	10:2 monoPAP	10:2 Fluorotelomer phosphate monoester
diPAP	Precursor	6:2 diPAP	6:2 Fluorotelomer phosphate diester
	Precursor	8:2 diPAP	8:2 Fluorotelomer phosphate diester
	Precursor	6:2/8:2 diPAP	6:2/8:2 Fluorotelomer phosphate diester
	Precursor	10:2 diPAP	10:2 Fluorotelomer phosphate diester
PFPA		PFHxPA	Perfluorohexyl phosphonic acid
		PFOPA	Perfluorooctyl phosphonic acid
		PFDPA	Perfluorodecyl phosphonic acid
PFPiA	Potential precursors	C6/C6 PFPiA	Bis (perfluorohexyl) phosphinic acid
		C6/C8 PFPiA	Perfluoro (hexyloctyl) phosphinic acid
		C8/C8 PFPiA	Bis (perfluorooctyl) phosphinic acid
FASA	Precursor	FBSA	Perfluorobutane sulfonamide
		MeFBSA	Methyl perfluorobutane sulfonamide
		FHxSA	Perfluorohexane sulfonamide
		MeFHxSA	Methyl perfluorohexane sulfonamide
		FOSA	Perfluorooctane sulfonamide
FASAA	Precursor	FOSAA	Perfluorooctane sulfonamidoacetic acid
	Precursor	MeFOSAA	Methyl perfluorooctane sulfonamidoacetic acid
	Precursor	EtFOSAA	Ethyl perfluorooctane sulfonamidoacetic acid
PFCHS	Novel	PFECHS	Perfluoroethylcyclohexane sulfonic acid
PFECA	Novel	ADONA	3H-perfluoro-3-[(3-methoxy-propoxy)propanoic acid]
	Novel	HFPO-DA (GenX)	Hexafluoropropylene oxide dimer acid
PFESA	Novel	6:2 Cl-PFESA (F-53B)	6:2 chlorinated polyfluorinated ether sulfonate
	Novel	8:2 Cl-PFESA	8:2 chlorinated polyfluorinated ether sulfonate

Appendix 2. Instrumental parameters for LC-MS/MS

Table A2-1. List of analytes, MRM transitions, cone voltage, and collision energy used for quantification and qualification of PFAS.

Analyte	Precursor/ product ions quantification (m/z)	Cone (V)	Coll (eV)	Precursor/ product ions qualification (m/z)	Cone (V)	Coll (eV)	Internal standard
TFA	112.9/68.96	26	10				^{13}C-PFBA
PFPrA	162.97/118.9	20	10				^{13}C-PFBA
PFBA	212.97/169	20	11				^{13}C-PFBA
PFPeA	262.97/219	20	8				^{13}C-PFPeA
PFHxA	312.97/269	20	9	312.97/118.95	20	26	^{13}C-PFHxA
PFHpA	362.97/319	20	10	362.97/168.97	20	16	^{13}C-PFHpA
PFOA	412.97/369	20	10	412.97/168.97	20	18	^{13}C-PFOA
PFNA	462.99/419	20	12	462.99/219	20	18	^{13}C-PFNA
PFDA	512.97/469	20	11	512.97/219	20	18	^{13}C-PFDA
PFUnDA	562.97/519	20	12	562.97/268.99	20	18	^{13}C-PFUnDA
PFDoDA	612.97/569	34	14	612.97/168.96	40	22	^{13}C-PFUnDA
PFTrDA	662.9/619	20	14	662.9/168.96	20	26	^{13}C-PFUnDA
PFTDA	712.9/669	20	14	712.9/168.97	20	28	^{13}C-PFUnDA
PFHxDA	812.9/769	30	15	812.9/168.96	42	32	^{13}C-PFUnDA
PFOcDA	912.9/869	36	15	912.9/168.96	36	36	^{13}C-PFUnDA
PFEtS	198.8/79.8	65	20				^{13}C-PFBS
PFPrS	248.9/80.0	70	25				^{13}C-PFBS
PFBS	298.9/98.9	20	26	298.9/79.96	20	26	^{13}C-PFBS
PFPeS	348.90/98.96	20	26	348.90/79.96	20	30	^{18}O-PFHxS
PFHxS	398.9/98.9	20	30	398.9/79.96	20	34	^{18}O-PFHxS
PFHpS	448.97/98.90	20	30	448.97/79.96	20	35	^{13}C-PFOS
PFOS	498.97/98.96	20	38	498.97/79.96, 498.97/169.03	20	44, 34	^{13}C-PFOS
PFNS	548.90/98.96	20	38	548.90/79.96	20	44	^{13}C-PFOS
PFDS	598.97/98.9	20	42	598.97/79.96	20	58	^{13}C-PFOS
PFDoDS	698.90/98.90	20	40	698.90/79.96	20	45	^{13}C-PFOS
3:3 FTCA	240.9/136.97	10	16	240.9/116.93	10	22	^{13}C-PFPeA
5:3 FTCA	340.9/236.97	10	16	340.9/216.93	10	22	^{13}C-PFHpA
6:2 FTUCA	356.9/292.91	10	18	356.9/242.95	10	36	^{13}C-PFHpA
7:3 FTCA	440.9/336.89	12	14	440.9/316.93	12	20	^{13}C-PFNA
8:2 FTUCA	456.9/392.84	10	18	456.9/392.84	10	38	^{13}C-PFNA
10:2 FTUCA	556.84/492.82	8	16	556.84/242.94	8	38	^{13}C-PFUnDA
FBSA	297.9/77.92	20	20	297.9/118.94	20	15	^{13}C-PFHxA

MeFBSA	311.97/111.93	14	20	397.9/168.94	14	16	^{13}C-PFOA
FHxSA	397.9/77.92	30	26	411.97/318.96	30	28	^{13}C-PFOS
MeFHxSA	411.97/168.93	24	24	411.97/318.96	24	20	^{13}C-PFOA
FOSA	497.9/78	82	30	497.9/168.96	82	29	^{13}C-PFOA
FOSAA				555.8/418.85			^{2}H -Et-FOSAA
MeFOSAA				569.78/482.76			^{2}H -Et-FOSAA
EtFOSAA				583.84/482.8			^{2}H -Et-FOSAA
4:2 FTSA	327/307	20	20	327/81	20	28	^{13}C-PFHxA
6:2 FTSA	427/407	20	20	427/81	20	28	^{13}C-PFOA
8:2 FTSA	527/507	20	20	527/80	20	28	^{13}C-PFDA
10:2 FTSA	627/607	20	20	627/80	20	28	^{13}C-PFUnDA
6:2 Cl-PFESA	530.9/351	58	24	530.9/83.0	58	24	^{13}C-PFOS
8:2 Cl-PFESA	630.9/451	58	24	630.9/83.0	58	24	^{13}C-PFOS
PFECHS	460.84/380.9	2	24	460.84/98.88	2	26	^{13}C-PFOA
6:2 mPAP	442.9/96.95			442.9/79			^{13}C-6:2mPAP
8:2 mPAP	542.9/97			542.9/79			^{13}C-8:2 mPAP
10:2 mPAP	642.968/97			642.968/79			^{13}C-8:2 mPAP
6:2 diPAP	788.9/97	64	28	788.9/442.91	64	18	^{13}C-6:2 diPAP
6:2/8:2 diPAP	888.78/96.94	66	34	888.78/442.81, 888.78/542.81	66	26	^{13}C-6:2 diPAP
8:2 diPAP	988.78/96.9	68	34	988.78/542.81	68	26	^{13}C-8:2 diPAP
10:2 diPAP	1188.78/96.9	68	34	1188.78/642.81	68	26	^{13}C-8:2 diPAP
SAmPAP	649.78/525.8			649.78/96.9			^{13}C-8:2 mPAP
diSAmPAP	1202.6/525.8			1202.6/168.9			^{13}C-8:2 diPAP
PFHxPA	398.97/79	62	26				^{13}C-PFOA
PFOPA	499/79	62	30				^{13}C-PFOA
PFDPA	599.03/79	62	30				^{13}C-PFNA
C6/C6 PFPiA	701/401	62	28				^{13}C-PFUnDA
C6/C8 PFPiA	801/401	24	28	801/501	24	28	^{13}C-PFUnDA
C8/C8 PFPiA	901/501	24	28				^{13}C-PFUnDA
HFPO-DA (GenX)	284.92/168.72	20	7	328.95/284.86	20	17	^{13}C-HFPO-DA
ADONA	376.97/250.8	30	37	376.97/84.69	15	29	^{13}C-HFPO-DA

Appendix 3. LOD range for LC-MS/MS

Table A3-1. List of analytes and their minimum and maximum LODs, as it was estimated for each sample preparation batch separately.

Analyte	LOD min (pg/mL)	LOD max (pg/mL)	Analysis type
PFBA	52	222	Quantitative
PFPeA	23	38	Quantitative
PFHxA	52	78	Semi-Quantitative
PFHpA	38	65	Quantitative
PFOA	22	297	Quantitative
PFNA	11	21	Quantitative
PFDA	65	162	Quantitative
PFUnDA	22	26	Quantitative
PFDoA	13	56	Semi-Quantitative
PFTrDA	11	18	Semi-Quantitative
PFTeDA	11	33	Semi-Quantitative
PFHxDA	22	23	Semi-Quantitative
PFODA	9834	9834	Semi-Quantitative
PFBS	10	19	Semi-Quantitative
PFPeS	10	10	Quantitative
PFHxS	26	80	Quantitative
PFHpS	10	10	Quantitative
PFOS	139	661	Quantitative
PFNS	21	21	Semi-Quantitative
PFDS	10	10	Semi-Quantitative
PFDoDS	21	21	Semi-Quantitative
PFECHS	10	10	Semi-Quantitative
FBSA	15	29	Semi-Quantitative
MeFBSA	210	210	Semi-Quantitative
FHxSA	22	22	Semi-Quantitative
MeFHxSA	51	69	Semi-Quantitative
FOSA	11	11	Semi-Quantitative
FPrPA (3:3 FTCA)	52	52	Semi-Quantitative
FPePA (5:3 FTCA)	22	22	Semi-Quantitative
FHpPA (7:3 FTCA)	22	22	Semi-Quantitative
FHUEA (6:2 FTUCA)	22	22	Semi-Quantitative
FOUEA (8:2 FTUCA)	11	11	Semi-Quantitative
FDUEA (10:2 FTUCA)	11	19	Semi-Quantitative
4:2FTSA	5	5	Semi-Quantitative
6:2FTSA	5	23	Semi-Quantitative
8:2FTSA	11	11	Semi-Quantitative
10:2FTSA	212	212	Semi-Quantitative
PFHxPA	52	52	Semi-Quantitative
PFOPA	212	212	Semi-Quantitative
PFDPA	11	11	Semi-Quantitative
6:6 PFPi	50	50	Semi-Quantitative
6:8 PFPi	3945	3945	Semi-Quantitative
8:8 PFPi	9598	9598	Semi-Quantitative
11ClPF3OUdS (8:2 Cl-PFESA)	10	10	Semi-Quantitative
9ClPF3ONS (6:2 Cl-PFESA)	10	10	Semi-Quantitative
FOSAA	273	273	Semi-Quantitative

MeFOSAA	50	50	Semi-Quantitative
EtFOSAA	204	204	Semi-Quantitative
SAmPAP	255	255	Semi-Quantitative
diSAmPAP	268	268	Semi-Quantitative
6:2 mPAP	587	766	Semi-Quantitative
8:2 mPAP	504	504	Semi-Quantitative
10:2 mPAP	547	547	Semi-Quantitative
6:2 diPAP	182	241	Semi-Quantitative
8:2 diPAP	70	100	Semi-Quantitative
6:2/8:2 diPAP	390	518	Semi-Quantitative
10:2 diPAP	988	988	Semi-Quantitative
ADONA	7	20	Semi-Quantitative
HFPO-DA	22	22	Quantitative

Appendix 4. Average concentration (ng/g) of different PFASs

Table A4-1. Average concentration (ng/g) of different PFASs, number of sample above LOQ and number of samples between LOD and LOQ

		Ronneby (*n*=20)			General population (n=148)		
		C (ng/g)	n>LOQ	LOQ>n>LOD	C (ng/g)	n>LOQ	LOQ>n>LOD
Ultra-short chain	TFA		0	1		0	92
Ultra-short chain	PFPrA		0	0		0	33
Short-chain	PFBA	0.00	0	0	0.00	4	1
Short-chain	PFPeA	0.00	0	5	0.00	3	11
Short-chain	PFHxA	0.00	0	0	0.00	2	0
Short-chain	PFHpA	0.01	1	3	0.00	5	42
Long-chain	PFOA	8.41	20	0	0.56	147	0
Long-chain	PFNA	0.38	20	0	0.27	146	0
Long-chain	PFDA	0.11	7	0	0.13	103	32
Long-chain	PFUnDA	0.14	17	0	0.08	68	0
Long-chain	PFDoDA	0.00	1	0	0.02	20	7
Long-chain	PFTrDA	0.00	0	0	0.00	21	0
Long-chain	PFTDA	0.00	0	0	0.01	9	0
Long-chain	PFHxDA	0.00	1	0	0.01	14	0
Long-chain	PFOcDA	0.00	0	0	0.00	2	0
Ultra-short chain	PFEtS	0.06	3	0	0.01	73	0
Ultra-short chain	PFPrS	0.00	0	0	0.00	2	0
Short-chain	PFBS	0.01	1	0	0.00	45	0
Short-chain	PFPeS	4.34	20	0	0.01	76	0
Long-chain	PFHxS	150.95	20	0	0.60	148	0
Long-chain	PFHpS	15.79	20	0	0.27	147	0
Long-chain	Dimethyl-PFOS	4.55	20	0	0.11	135	0
Long-chain	3/4/5-m-PFOS	79.08	20	0	1.36	148	0
Long-chain	6/2-m-PFOS	34.95	20	0	0.41	148	0
Long-chain	1-m-PFOS	20.11	20	0	0.09	101	0
Long-chain	PFOS	102.39	20	0	1.58	148	0
Long-chain	PFNS	0.00	2	0	0.00	0	0
Long-chain	PFDS	0.00	0	0	0.00	0	0
Long-chain	PFDoDS	0.00	0	0	0.00	0	0
Novel	PFECHS	0.01	3	0	0.02	119	0
Precursor	FBSA	0.00	0	3	0.00	5	12
Precursor	MeFBSA	0.00	0	0	0.00	1	0
Precursor	FHxSA	0.00	0	0	0.00	9	0
Precursor	MeFHxSA	0.00	0	0	0.00	0	2
Precursor	FOSA	0.00	0	0	0.00	22	0

Precursor	3:3 FTCA	0.00	0	0	0.00	0	0
Precursor	5:3 FTCA	0.00	0	0	0.00	0	0
Precursor	7:3 FTCA	0.00	0	0	0.00	0	0
Precursor	6:2 FTUCA	0.00	0	0	0.00	0	0
Precursor	8:2 FTUCA	0.00	0	0	0.00	1	1
Precursor	10:2 FTUCA	0.00	0	0	0.00	6	1
Precursor	4:2 FTSA	0.00	0	0	0.00	0	0
Precursor	6:2 FTSA	0.00	1	3	0.00	15	8
Precursor	8:2 FTSA	0.00	0	0	0.00	36	0
Precursor	10:2 FTSA	0.00	0	0	0.00	0	0
	PFHxPA	0.00	0	0	0.00	2	0
	PFOPA	0.00	0	0	0.00	0	0
	PFDPA	0.00	0	0	0.00	0	0
Potential precursor	6:6 PFPiA	0.00	0	1	0.00	0	0
Potential precursor	6:8 PFPiA	0.00	0	0	0.00	0	0
Potential precursor	8:8 PFPiA	0.00	0	0	0.00	0	0
Novel	8:2 Cl-PFESA	0.00	0	0	0.00	0	0
Novel	6:2 Cl-PFESA	0.00	0	0	0.00	18	0
Precursor	FOSAA	0.03	1	0	0.01	8	0
Precursor	MeFOSAA	0.00	0	0	0.01	24	0
Precursor	EtFOSAA	0.00	0	0	0.00	0	0
Precursor	SAmPAP	0.00	0	0	0.00	0	0
Precursor	diSAmPAP	0.00	0	0	0.00	0	0
Precursor	6:2 mPAP	0.00	0	0	0.00	0	2
Precursor	8:2 mPAP	0.00	0	0	0.00	0	0
Precursor	10:2 mPAP	0.00	0	0	0.00	0	0
Precursor	6:2 diPAP	0.00	0	0	0.00	0	3
Precursor	8:2 diPAP	0.00	0	0	0.00	0	0
Precursor	6:2/8:2 diPAP	0.00	0	0	0.00	0	2
Precursor	10:2 diPAP	0.00	0	0	0.00	0	0
Novel	ADONA	0.00	0	0	0.01	12	11
Novel	HFPO-DA	0.00	0	0	0.00	0	0

Appendix 5. Additional Solid Phase Extraction Method

SPE, Oasis WAX 150 mg, 6cc	
Conditioning	4 mL 0.1% NH_4OH in MeOH 4 mL MeOH 4 mL MilliQ water
Load sample (100 µL diluted to 4 mL with MilliQ water)	
Wash	4 mL MilliQ water 4 mL 25 mmol/L ammonium acetate buffer (pH 4) 4 ml 20% MeOH in MilliQ water
Centrifuge SPE cartridge at 3000 rpm for 2 min.	
Elute sample with 4 mL 0.1% NH4OH in MeOH	
Evaporate to 0.5 mL	
Prepare sample for LC-MS analysis	

Appendix 5. Average Molar Concentration of Analytes (*n* = 20)

Compound	Average concentration (nM)	Compound	Average concentration (nM)
PFBA	0.00	PFHxPA	0.00
PFPeA	0.00	PFOPA	0.00
PFHxA	0.00	PFDPA	0.00
PFHpA	0.02	6:6 PFPiA	0.00
PFOA	20.32	6:8 PFPiA	0.00
PFNA	0.82	8:8 PFPiA	0.00
PFDA	0.21	8:2 Cl-PFESA	0.00
PFUnDA	0.24	6:2 Cl-PFESA	0.00
PFDoDA	0.00	FOSAA	0.05
PFTrDA	0.00	MeFOSAA	0.00
PFTDA	0.00	EtFOSAA	0.00
PFHxDA	0.00	SAmPAP	0.00
PFOcDA	0.00	diSAmPAP	0.00
PFEtS	0.46	6:2 mPAP	0.00
PFPrS	0.00	8:2 mPAP	0.00
PFBS	0.00	10:2 mPAP	0.00
PFPeS	12.41	6:2 diPAP	0.00
PFHxS	377.37	8:2 diPAP	0.00
PFHpS	35.09	6:2/8:2 diPAP	0.00
Br-PFOS	135.33	10:2 diPAP	0.00
PFOS	195.06	ADONA	0.00
PFNS	0.01	HFPO-DA	0.00
PFDS	0.00		
PFDoDS	0.00		
PFECHS	0.01		
FBSA	0.00		
MeFBSA	0.00		
FHxSA	0.00		
MeFHxSA	0.00		
FOSA	0.00		
3:3 FTCA	0.00		
5:3 FTCA	0.00		
7:3 FTCA	0.00		
6:2 FTUCA	0,00		
8:2 FTUCA	0.00		
10:2 FTUCA	0.00		
4:2 FTSA	0.00		
6:2 FTSA	0.00		
8:2 FTSA	0.00		
10:2 FTSA	0.00		