PFAS AND FLUORINE MASS BALANCE ANALYSIS OF WHOLE BLOOD SAMPLES FROM SWEDEN

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Introduction

Per- and polyfluoroalkyl substances (PFAS) are a group anthropogenic compounds containing the perfluoroalkyl moiety C_nF_{2n+1}-. Nearly 5000 PFAS are commercially available as of 2018[1], excluding production intermediates, impurities and degradation products[2]. Due to their unique properties, PFAS have a wide range of uses; from cosmetics to chrome plating[3,4]. The same properties (e.g. thermal and chemical stability) result in PFAS being persistent in the environment, leading to their ubiquitous presence. Whilst perfluorooctane sulfonate (PFOS) was phased out since 2000[5] due to displaying detrimental effects on living organisms (e.g. immunotoxicity in humans[6]), information is scarce regarding the majority of the remaining PFASs. Usually, only a fraction of the PFAS are commonly monitored, as the number of mass-labelled standards is limited and often the structure of the "novel PFAS" is unknown. Without knowing the identities of the novel PFAS, it is not possible to assess their toxicity and if they pose a threat to human health. Fluorine mass balance (F-MaB) analysis combines target PFAS and extractable organofluorine (EOF) analysis, to elucidate the fraction of organofluorine compounds that remains to be identified. Due to the relatively high limit of detection (LoD) of the EOF measurement, it is necessary to extract a large sample amount – necessitating a standalone sampling campaign for sufficiently large blood samples.

The present study compiles the preliminary results of a larger investigation, for which whole blood samples have been collected from 6 municipalities in Sweden, each city contributing samples from approximately 25 individuals for a total of 180 samples. This investigation strives for a comprehensive PFAS exposure assessment[7] by monitoring the levels of precursors[8], intermediates[9], novel PFAS[10] and stable degradation products[11]. The unknown organofluorine (UOF) burden, that people are exposed to, will be elucidated using the F-MaB approach. The work at hand presents the initial results, which encompasses the levels of 19 target analytes and the EOF levels in a selection of sample; C3-C11 perfluoroalkyl carboxylic acids (PFCAs) and C2-C10 and C12 perfluoroalkyl sulfonic acids (PFSAs). The PFCA and PFSA groups were chosen for quantification as they have shown high concentrations in human blood in recent studies[12], quantifying their contribution to the F-MaB provides a glance of the overall situation.

Whole blood was chosen as the matrix because it is more representative of the whole body burden. While serum and plasma are the most common matrices for PFAS analysis, due to practical considerations, the partitioning of PFAS is not equal between these matrices and whole blood[13] due PFAS having different properties[14]. While the partitioning of more analytes between these matrices have been studied[12], it is not feasible to quantify them for all commercial PFAS. This is further complicated when performing F-MaB analysis as the structure of the organofluorines (OF) is not elucidated.

Materials and methods

The samples in this study were collected from four municipalities in Sweden: Stockholm (*n=6*), Uppsala (*n=6*), Malmö (*n=6*) and Örebro (*n=6*). The samples were collected from healthy volunteers with their age (ranging from 19 to 84 years) and gender (17 female and 7 male) logged, the whole blood was taken into vacutainers and stored at +4 °C until sample preparation. The samples were vigorously shaken before taking aliquots for extraction, to ensure their homogeneity. The ion-pair method was adapted from[15]. All samples were extracted in duplicate; one (fraction A) spiked with internal standards before the extraction and used for target analysis; the other (fraction B) duplicate was extracted without spiking any internal standard and analyzed for the EOF content (see Figure 2 for more details).

For F-MaB the PFAS concentrations in the fraction B sample extract were inferred by multiplying the concentration of each compound in the fraction A sample by the respective recovery. The latter was calculated from a spike recovery experiment, in which whole blood samples were spiked before and after extraction with the target analytes. Thereafter, the amount of fluorine originating from each analyte was calculated, summed up and compared to the measured EOF concentration.

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:water mixture, both with 2 mmol/L ammonium acetate and 5 mmol/L 1-methylpiperidine as additives[16]. 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. The UPCC was coupled to the Waters XEVO TQ-S detector[17]. The EOF content of the samples was determined using a combustion ion chromatography system (CIC) from Metrohm AG[18].

Method detection limit (MDL), for EOF and target PFAS found in procedural blanks, was determined as the average of procedural blanks plus three times their standard deviation. If the compound was not found in the procedural blanks, the lowest point of calibration was used instead. Each extraction batch included a procedural blank to monitor and quantify any possible contamination of the samples during extraction and/or analysis. A quality control (QC) sample (SRM 1957, organic contaminants in non-fortified human serum, NIST) was included in every batch to monitor the performance of the target analysis. To ensure the reliability of the EOF measurement, a standard solution with a known concentration of PFOS was analyzed at regular intervals during CIC analysis.

Results and discussion:
The concentration of the 19 target PFASs (∑19PFAS) of individual samples ranged from 0.4 ng/g (nr. 23, Örebro) to 12.5 ng/g (nr. 17, Malmö), see Figure 1 for more details. However, the differences were smaller when comparing municipalities, the highest mean concentration of ∑19PFAS was in the samples from Malmö – 5.2 ng/g (range of 1.5 – 12.5 ng/g), followed by Stockholm – 3.8 ng/g (2.3 – 15.5 ng/g), Uppsala – 3.5 ng/g (0.7 – 16.2 ng/g) and Örebro – 2.6 ng/g (0.4 – 15.0 ng/g).

Overall 16 of the 19 target compounds were detected and C8-C10 PFCAs and C6-C8 PFSAs were present in all of the samples analyzed. Perfluoroheptane carboxylate (PFHpA) was observed in nearly 80 % of the samples. The detection frequencies of C4, C5 PFSAs and C11 PFCA (perfluoroundecane carboxylate, PFUnDA) were around 40 %. The shorter PFCAs (C3-C6) and PFSAs (C2-C3) were found in approximately 20 % of the samples.

The PFSAs accounted for almost 70 % of the ∑19PFAS in all of the samples and the remaining coming from PFCAs. The largest single contributors to the PFAS budget were L-PFOS, perfluorohexane sulfonate (L-PFHxS) and perfluorooctane carboxylate (PFOA), with each accounting for 45 %, 16 % and 15 % of ∑19PFAS respectively.

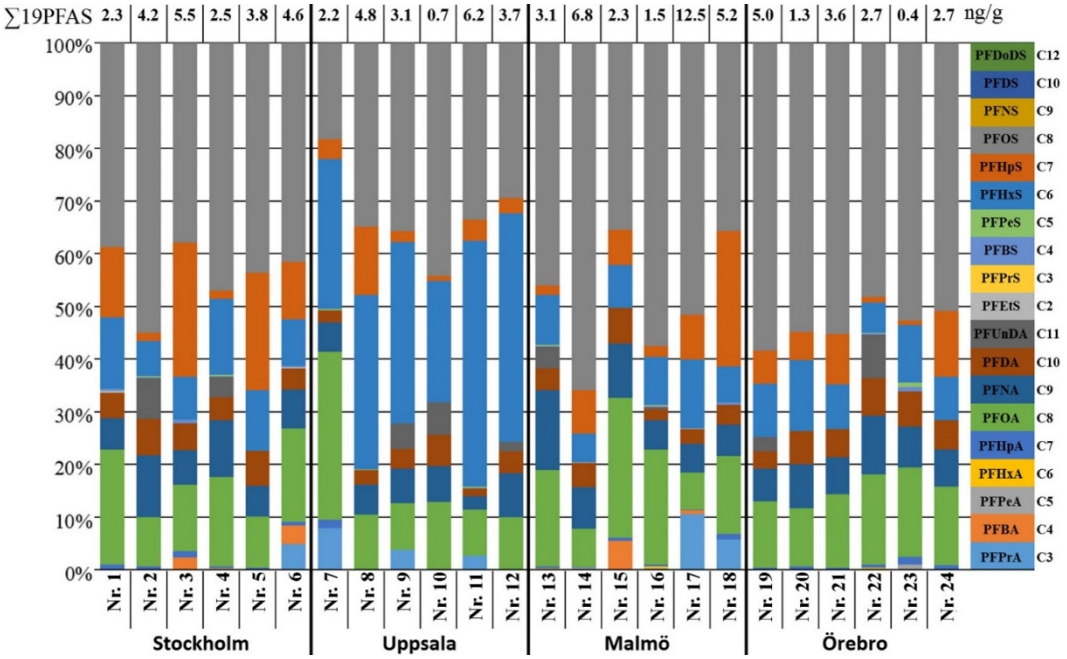

Figure 1. PFAS homologue profiles in blood samples from municipalities in Sweden.

While the overall contribution of PFSAs towards the ∑19PFAS was similar in all samples, the ones from Uppsala showed a different ratio between L-PFOS and L-PFHxS. In other municipalities L-PFHxS accounted for 10 % of ∑19PFAS and L-PFOS for around 50 %. In Uppsala the numbers were 35 % and 33 % respectively, which is in good agreement with a study from 2015[19]. Additionally, the samples from Stockholm stood out with an elevated fraction of perfluoroheptane sulfonate (PFHpS).

All samples from Stockholm and Malmö showed quantifiable EOF, while only two samples from both Örebro and Uppsala had detectable levels of EOF. The highest mean concentration EOF concentration was observed in the samples from Örebro 28.3 ng F/g (range: 25.1 – 31.6 ng F/g), followed by Stockholm 20.1 ng F/g (11.5 – 42.3 ng F/g), Malmö 17.6 ng F/g (10.3 – 35.8 ng F/g) and Uppsala 7.9 ng F/g.

The fraction of fluorine, the origin of which can be traced to the target compounds (iPFAS) was the highest in samples from Uppsala – mean: 37 % (range: 32 – 41 %), followed by Malmö – 19 % (3.2 – 42 %), Stockholm – 13 % (3.0–26 %) and Örebro – 3.0 % (0.6 – 5.5 %).

Although Uppsala and Örebro had both only two samples with quantifiable EOF levels, they indicate different trends. The two samples from Uppsala had the lowest EOF levels out of all the samples, which had quantifiable EOF levels, suggesting that the OF exposure is comparatively low in that region. This is further supported by the relatively high fraction of iPFAS. These results are in stark contrast to the samples from Örebro. While only a third of the samples had EOF levels exceeding the MDL, the EOF levels in those samples were some of the highest in this study. This points towards very different exposure routes of the individuals enlisted from Örebro.

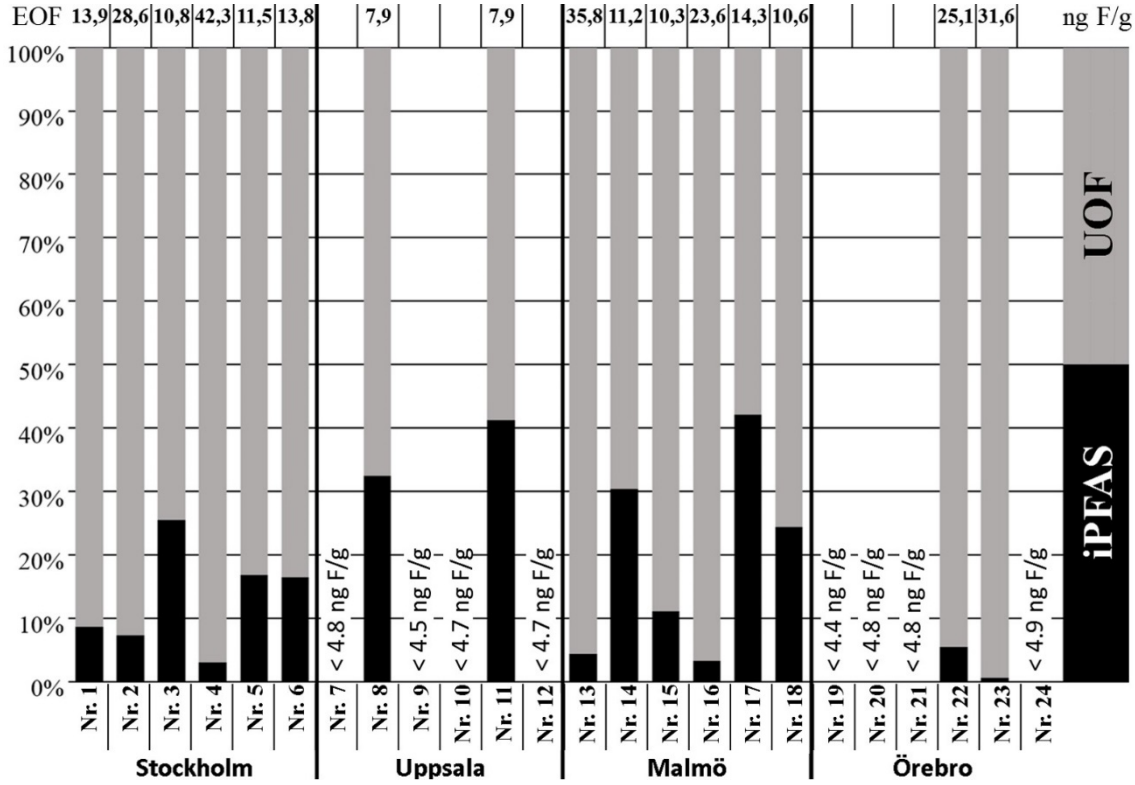

Figure 2. Fractions of unidentified extractable organofluorine (UOF) and identified PFAS (iPFAS).

While the fraction of the OF extracted (EOF) from the sample depends on the extraction method, resulting in a systematic bias[20], it provides an insight to human OF exposure levels. The preliminary results presented in this work underline that our current knowledge regarding human PFAS exposure is still insufficient, as we were only able to identify only a small portion of the overall OF burden. The fraction of iPFAS will increase as more target compounds are analyzed, for which work is already underway, but it is unlikely that the overall picture would significantly change. However, given more samples from each municipality, it might be possible to better discern regional differences and track down point sources[21]. Given the availability of both target PFAS and EOF data, it presents an opportunity to look for any links between stable degradation product (PFAA) and UOF levels. Additional research is also required to elucidate the origins of UOF– whether it is composed of yet to be identified PFAS, fluorinated pesticides or pharmaceuticals.

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