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PERFLUOROALKYL SUBSTANCES; LEVELS AND PROFILES IN HUMAN SERUM, PLASMA, AND WHOLE BLOOD IN A NORWEGIAN COHORT

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Introduction

Poly- and perfluoroalkyl substances (PFASs) include a wide range of organofluorine compounds that have been applied to numerous consumer products the last 60 years. Applications of PFASs include surface coating for paper products approved for food contact, fabrics, carpets, wetting agents in waxes, fire-fighting foams, and insecticide formulations. The extensive use of PFASs is due to their oil and water repellent properties and surface activity. These unique physicochemical properties also contributed to their widespread distribution in the environment, wildlife, and humans. In 2000, the 3M Company, a major producer of these compounds phased out the production of perfluorooctane sulfonate (PFOS) and related compounds while providing shorter chain PFASs as replacements [1]. PFOS and its salt were included in the list of restricted chemicals in the Stockholm Convention in 2009 [2]. While the perfluorooctanoate (PFOA) stewardship programme was committed to phase out PFOA and longer chain perfluoroalkyl carboxylates (PFCAs) by 2015 [3]. Moreover, in 2015, the European Union submitted a proposal to list PFOA, its salts and PFOA-related substances in the Stockholm Convention [4], and substitutes for PFOA, PFOS, and other long chain PFASs are under review by the United States Environmental Protection Agency [5].

However, the human exposure to PFASs is still of concern. Adverse health effects have been observed in animal studies and associations between PFAS levels and a range of health outcomes have been observed in epidemiological studies. Assessment of human exposure pathways and exploring relative importance of different human exposure pathways for PFASs are still challenging. In addition to direct exposure to perfluoroalkyl substances, exposure may also occure through indirect exposure to precursors, such as perfluoroalkyl sulfonamides (FOSAs) polyfluoroalkyl phosphate esters (PAPs) and perfluorophosphonates (PFPA) [6-8].

Blood is a favorable matrix for determining the internal dose of PFASs, and usually plasma or serum is used due to practical reasons. Concentrations of PFASs in blood have been reported in individuals all over the world, but the data often comprise only a limited number of compounds. Due to restrictions and measures taken, the levels of some PFASs have been declining the last decade. However, in the same time period increasing concentrations have been observed for other PFASs [9]. Little is known about levels of emerging PFASs, such as PAPs and PFPAs, in humans. Morover, the concentration rations between serum, plasma, and whole blood for many PFASs is still unknown.

The aim of this study was to assess the presence, concentration, and the profile of a broad range of PFASs in whole blood, plasma and serum from a Norwegian cohort. To characterize the potential differences of PFASs in various blood matrices the same analytical method, online-solid phase extraction (SPE) and column switching on ultra-high performance liquid chromatography coupled with tandem mass spectrometry (UHPLC-MS/MS), was used. Paired human serum, plasma, and whole blood samples from adults (n=61) in Norway were analysed. Apart from measuring the traditional PFASs such as perfluoroalkyl sulfonates (PFSAs; C4, C6, C7, C8, and C10), perfluoroalkyl carboxylates (PFCAs; C5 - C14), and perfluoroalkyl sulfonamides (FOSAs; C8, N-methyl, and N-ethyl), this study also included the emerging PFASs such as PAPs (6:2, 8:2, 6:2/6:2, and 8:2/8:2) , and PFPAs (C6, C8, and C10).

Materials and methods

Study population and sample collection

Serum, plasma, and whole blood were collected from 61 participants in the Advanced Tools for Exposure Assessment and Biomonitoring (A-TEAM) project [10]. The samples were obtained from 45 female and 16 male volunteers between November 2013 and April 2014. All participants were between 20 and 66 years old, with a mean age of 42 years. The A-TEAM sampling campaign was approved by the Regional Committees for Medical and Health Research Ethics in Norway (2013/1269), and all participants completed a written consent form before participating. Blood samples were drawn from a

single venipuncture site. Whole blood and plasma samples were collected in EDTA vacutainer tubes. After a fraction of the whole blood was transferred to a 2 mL polypropylene tube, the remaining blood in the EDTA vacutainer was subjected to plasma preparation by centrifuging for 15 min at 2500 rpm. To obtain a serum sample, a vacutainer without anticoagulant was used to collect blood from the participants. The sample in the vacutainer without anticoagulant was centrifuged for 15 min at 2500 rpm, obtaining serum as the supernatant. All the blood samples were stored in polypropylene tubes and kept at -20°C until analysis.

Chemicals

The twenty-five native PFASs and eleven mass-labeled PFASs were purchased from Wellington Laboratories (Guelph, Ontario, Canada) as 50 µg mL-1 solutions in methanol. The native and mass-labeled PFASs included 6:2 polyfluoroalkyl phosphoric acid monoesters (6:2PAP), 8:2 polyfluoroalkyl phosphoric acid monoesters (8:2PAP), 6:2 polyfluoroalkyl phosphoric acid diesters (6:2diPAP), 8:2 polyfluoroalkyl phosphoric acid diesters (8:2diPAP), perfluorohexylphosphonate (PFHxPA), perfluorooctylphosphonate (PFOPA), perfluorodecylphosphonate (PFDPA), perfluorobutane sulfonate (PFBS), perfluorohexane sulfonate (PFHxS), perfluoroheptane sulfonate(PFHpS), PFOS, perfluorodecane sulfonate (PFDS), perfluoropentanoate (PFPeA), perfluorohexanoate (PFHxA), perfluoroheptanoate (PFHpA), PFOA, perfluorononanoate (PFNA), perfluorodecanoate (PFDA), perfluoroundecanoate (PFUnDA), perfluorododecanoate (PFDoDA), perfluorotridecanoate (PFTrDA), perfluorotetradecanoate (PFTeDA), perfluorooctane sulfonamide (PFOSA), N-methyl perfluorooctane sulfonamide (MeFOSA), N-ethyl perfluorooctane sulfonamide (EtFOSA), sodium bis(1H,1H,2H,2H-[1,2-13C2] perfluorooctyl) phosphate (13C4-6:2 diPAP), sodium bis(1H,1H,2H,2H-[1,2-13C2]perfluorodecyl) phosphate (13C4-8:2 diPAP), sodium perfluoro-1-hexane [18O2]sulfonate (18O2-PFHxS), sodium perfluoro-1-[1,2,3,4-13C4] octanesulfonate (13C4-PFOS), perfluoro-n-[1,2-13C2] hexanoic acid (13C2-PFHxA), perfluoro-n-[1,2,3,4-13C4] octanoic acid (13C4-PFOA), perfluoro-n-[1,2,3,4,5-13C5] nonanoic acid (13C5-PFNA), perfluoro-n-[1,2-13C2] decanoic acid (13C2-PFDA), perfluoro-n-[1,2-13C2] undecanoic acid (13C2-PFUnDA), perfluoro-n-[1,2-13C2] dodecanoic acid (13C2-PFDoDA), and N-methyl-d3-perfluoro-1-octanesulfonamide (D3-N-MeFOSA). Formic acid (eluent additive for LC-MS, ~98%) and ammonium hydroxide (≥25% NH3 in H2O) were obtained from Sigma Aldrich (Steinheim, Germany). HPLC-grade acetonitrile (≥99.9% purity), methanol (≥99.99% purity), and water were obtained from J.T. Baker (Deventer, The Netherlands).

Matrix matched calibrations

Matrix matched standards were prepared using calf serum (Invitrogen, Oslo, Norway), calf plasma (Lampire Biological labs, Pipersville, USA), and calf whole blood (Lampire Biological labs, Pipersville, USA). Duplicate matrix matched calibration standard were prepared in twelve different concentration levels at 0.006, 0.012, 0.03, 0.06, 0.15, 0.3, 0.6, 1.2, 3, 6, 15, and 45 ng mL-1 blood.

Sample preparation

PFAS concentrations in serum, plasma, and whole blood were determined using an established method which is described elsewhere [11]. In short, 50 µL of blood was added to a 2 mL centrifuge tube, and then 90 µL of a 20 pg µL-1 internal standard solution and 90 µL of methanol were added. The tube was subjected to a whirl mixing, and centrifuged for 40 min at 14000 rpm and 20°C to precipitate the proteins. After centrifugation, the supernatant was transferred to a 250 µL polypropylene vial for injection in the online-SPE UHPLC-MS/MS system.

Quantification

All analyses were performed using an online-SPE UHPLC-MS/MS on an Agilent 1290 UHPLC interfaced to an Agilent 6490 Triple Quadrupole mass spectrometer (MS/MS) (Agilent Technologies, Palo Alto, CA, USA). The column switching system contained two columns; a Betasil C8, 10 mm × 3 mm, 5 µm particle size SPE column (Thermo scientific, CA, USA), and an Agilent ZORBAX Eclipse Plus C18, UHPLC, 50mm × 2.1 mm, 1.8 µm particle size (Agilent Technologies, California, USA) analytical column. The injection volume was 80 µL. More details are described elsewhere [11].

Estimated method detection limits (MDLs) and limits of quantification (LOQs) were obtained using the established online-SPE UHPLC-MS/MS method. The MDLs and LOQs were extrapolated from the matrix matched calibration curves and defined as signal to noise ratios (S/N) of 3 and 10, respectively. The MDLs ranged from 0.0018 to 0.09 ng mL-1, depending on the compound and the blood matrix.

Results and discussion

Overview on the detection of PFASs in serum, plasma, and whole blood

Twenty-five PFASs were determined in paired samples of serum, plasma, and whole blood. Concentrations above the MDLs were used for calculation of detection frequency, and median, mean, and range of PFAS levels. In brief, PFHxS, PFHpS, PFOS, PFOA, PFNA, and PFDA had 100% detection frequency in all blood matrices. No PFDPA, PFPeA, MeFOSA, EtFOSA were detected in any kind of blood matrices. Among PAPs, 6:2diPAP had the highest detection frequency (49 - 98%) in all blood matrices, followed by 6:2PAP (3 - 73%), and 8:2diPAP (21 - 30%), while 8:2PAP was detected only 5% in whole blood samples, and was not detected in serum and plasma. PFHxPA was the only compound in the PFPA group that was detected in all types of blood samples, and had a 100% detection frequency in plasma. PFDPA was not detected in all samples, and PFOPA was not found in plasma and whole blood, and had only 15% detection frequency in serum (concentations between MDL and LOQ). This is in contrast to the reported environmental occurrence of PFPAs, where PFOPA was the predominant compound in surface water and wastewater [7]. For PFSAs, PFHxS, PFHpS, and PFOS were found in all samples in all blood matrices, while PFBS was detected in all plasma samples. The highest mean concentration was observed for PFOS followed by PFHxS, PFHpS, PFBS, and PFDS. This pattern was observed in all blood matrices. For PFCAs, PFOA, PFNA, PFDA, PFUnDA, and PFDoDA were detected 91 - 100% of the samples in all blood matrices, with the highest concentration of PFOA followed by PFNA, PFDA, and PFUnDA. PFHxA had a 100% detection frequency in whole blood, and the same was seen for PFOSA.

Concentrations and profiles of PFASs in serum, plasma, and whole blood

The sumPFAS concentrations in serum, plasma, and whole blood for the 61 participants were in the range 4.0 - 34.8, 3.8 - 37.8, and 2.7 – 20 ng mL-1 in serum, plasma, and whole blood, respectively. The mean sumPFAS concentrations were 13.1, 12.4, and 6.8 ng mL-1 in serum, plasma, and whole blood, respectively. The total amount of PFASs in whole blood was approximately half of that in serum or plasma. The mean concentrations of each PFAS in the three matrices are shown in Figure 1. The highest mean concentration was observed for PFOS, with concentrations of 7.0, 6.3, and 3.6 ng mL-1 in serum, plasma, and whole blood, respectively. PFOA was the second most prominent PFAS in all matrices. The concentrations of the various PFASs differed between whole blood and serum or plasma. PFHxA was only detected in whole blood and the concentration of PFOSA in whole blood (0.14 ng mL-1) was 5 to 7 fold higher than in serum and plasma. The sum concentrations of PAPs and PFPAs in whole blood were also higher than in serum and plasma. Higher concentrations of sumPFSAs, and sumPFCAs were seen in serum than in plasma and whole blood.

When considering only the PFASs with 100% detection frequency in all blood matrices, PFOS was the predominant PFAS followed by PFOA, PFHxS, and PFNA. The relative composition of these four PFASs, and the sum the remaining PFASs in human serum, plasma, and whole blood is shown in Figure 2. The profiles of the three blood matrices were slightly different. The concentrations of PFOS in serum, plasma, and whole blood contributed to 53%, 51%, and 45%, of the total PFAS concentrations respectively, while PFOA contributed to 18%, 17%, and 13%. The total concentration of other PFASs (excluding PFOS, PFOA, PFHxS and PFNA) was higher in whole blood (29%) than in plasma (19%), and serum (13%).

Correlations between total PFASs in different blood matrices

The relation between PFASs in the different blood matrices was evaluated using linear regression. The concentration of sumPFASs in each of the paired samples was compared (i.e. plasma versus serum, whole blood versus serum, and whole blood versus plasma). Excellent correlations of total PFASs between blood matrices were observed The correlations were linear with R2 equal to 0.945, 0.922, and 0.932 for plasma versus serum, whole blood versus serum, and whole blood versus plasma, respectively. Linear regression test also indicated that the total PFASs in plasma appeared to be in accordance with that in serum, but the total PFASs distribution in whole blood was only ½ of that in serum and plasma. This result is in accordance with previous studies on PFOS, PFOA, and PFHxS in serum, plasma, and whole blood [12]. However, the presence of some of the compounds such as PFHxA, PFOSA, PAPs and PFPAs needs to be further studied as these compounds appear to have a different binding in human blood when compared to the rest of the PFASs.

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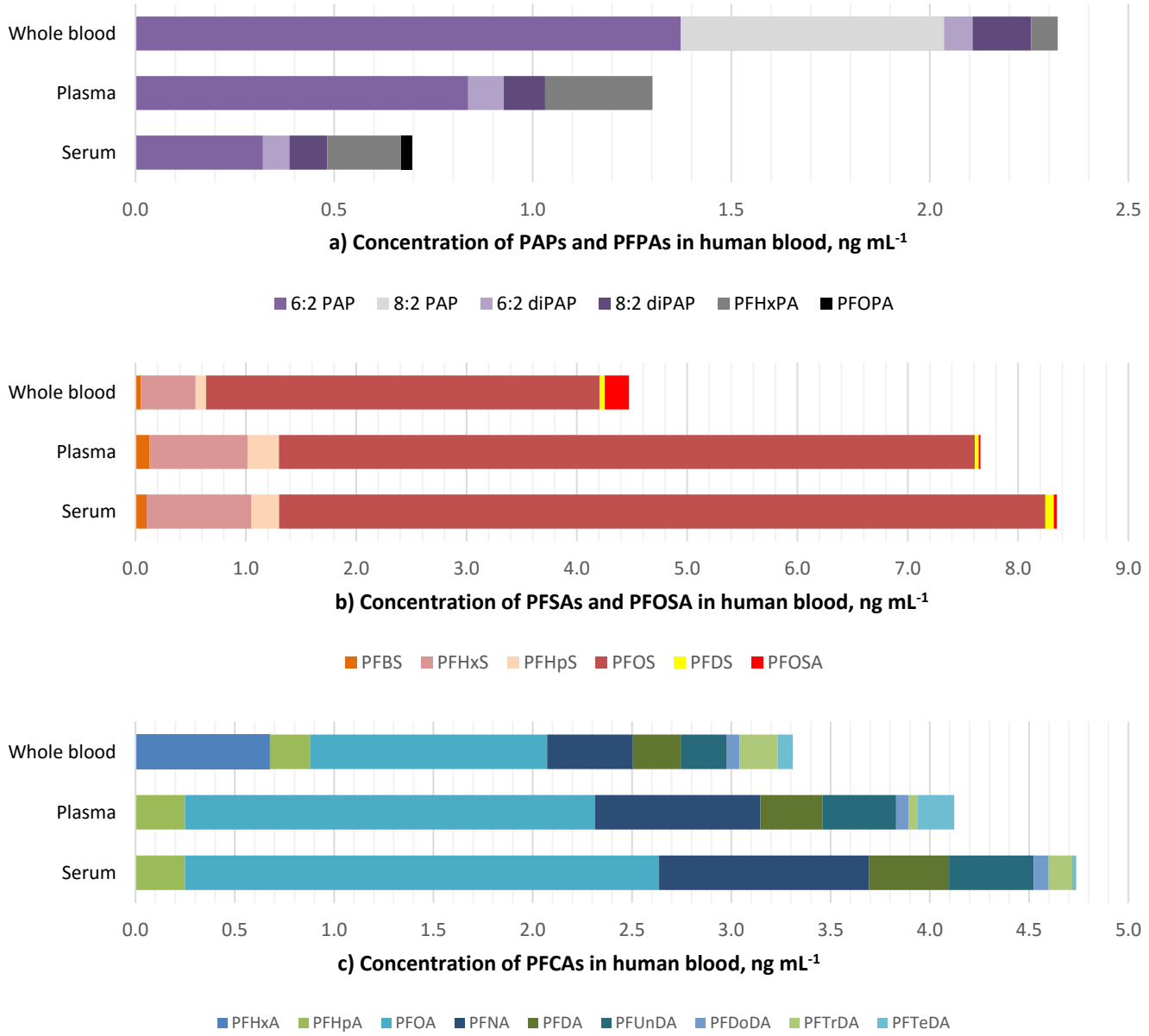

Figure 1: Concentrations (ng mL^{-1}) of a) PAPs and PFPAs , b) PFSAs and PFOSA, c) PFCAs in human serum, plasma, and whole blood

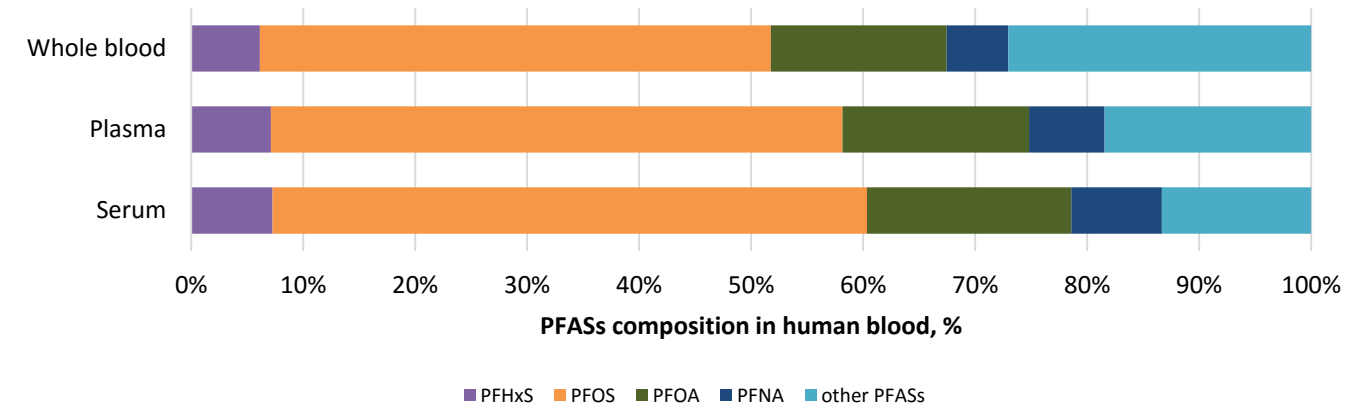

Figure 2: Mean relative compositions (%) of PFASs in human serum, plasma, and whole blood (other PFASs is represented by the sum of concentration of all PFASs in this study, except PFHxS, PFOS, PFOA, and PFNA)