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Partition of perfluoroalkyl substances (PFASs) in whole blood and plasma, assessed in maternal and umbilical cord samples from inhabitants of arctic Russia and Uzbekistan

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HIGHLIGHTS

- PFSA blood concentrations elevated in the Russian Arctic.
- FOSA prefers the blood cell fraction.
- A priori predictions of PFAS plasma/whole blood ratios.

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ABSTRACT

Perfluoroalkyl substances (PFASs) are ubiquitous in the environment world-wide. Our overall objective was to assess the exposure to PFASs experienced by delivering women and their new-borns in the industrial city of Norilsk (arctic Russia) and the rural Aral Sea region of Uzbekistan, with the secondary objective of evaluating the distribution of PFASs between blood cell and plasma fractions. Six PFASs were detected in every sample from Norilsk city with the plasma concentration sequence of: PFOS ≫ PFOA > PFNA > FOSA > PFHxS > PFUnDA. In the Uzbekistani samples, only PFOS was reported above the MDL (0.08 ng/mL). The median plasma concentrations of PFOS of 11.0 ng/mL for the Norilsk mothers was comparable to that reported for western countries, while that for Uzbekistan was considerably lower (0.23 ng/mL). Apparent increases in the maternal-cord concentration ratios for both whole blood and plasma were evident with the length of the carbon chain for both the carboxylate and the sulfonate PFASs. The median value of this ratio for FOSA in plasma was the lowest, while that for whole blood was the highest. Other than for FOSA, the observed plasma–whole blood concentration ratios for maternal and umbilical cord blood were consistent with a priori calculations using appropriate packed cell and plasma volumes for neonates and pregnant women at term. Clearly FOSA favored whole blood, and acid–base equilibrium calculations suggested that the resonance-stabilized sulfonamidate ion resides in the blood cell fraction. Thus for PFASs and related compounds with pK_a values with magnitudes comparable to physiological pH, it is pertinent to measure the cell-associated fraction (separately or as whole blood). Our study illustrates that consideration of both the physico-chemical properties of the contaminants and the physiological attributes of blood matrices were helpful in the interpretation of our findings.

1. Introduction

Perfluoroalkyl substances (PFASs) have been commercially available since the 1950s. They consist of a carbon backbone (normally with 4–14 carbons) that is completely fluorinated and can have various functional groups (Lau et al., 2007). The two main compounds reported in studies are perfluorooctane sulfonic acid (PFOS) and perfluorooctanoic acid (PFOA). PFASs can be synthesized in two ways, electrochemical fluorination (ECF) and telomerization. In the ECF process, perfluorooctane sulfonyl fluoride (POSF) was produced from octane sulfonyl fluoride, resulting in a mixture of linear and branched isomers with odd and even number carbon chains (Butt et al., 2010; Buck et al., 2011). It is

Abbreviations: AMAP, Arctic Monitoring Assessment Programme; ECF, Electrochemical fluorination; EtFOSA, *N*-Ethyl perfluorooctane sulfonamide; FOSA, Perfluorooctane sulfonamide; LRT, Long range transport; MQL, Method quantification limit; NHANES, National Health and Nutrition Examination Survey; PCV, Packed cell volume; PFAS, Perfluoroalkyl substance; PFCAs, Perfluoroalkyl carboxylic acids; PFHxS, Perfluorohexane sulfonic acid; PFNA, Perfluorononanoic acid; PFOA, Perfluorooctanoic acid; PFOS, Perfluorooctane sulfonic acid; PFUnDA, Perfluoroundecanoic acid; POPs, Persistent organic pollutants; POSF, Perfluorooctane sulfonyl fluoride; SRM, Standard reference material; TTE, Transplacental transfer efficiency; UHPLC-MS/MS, Ultrahigh pressure liquid chromatography triple–quadrupole mass-spectrometry.

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generally assumed that the ratio between linear and branched isomers was 70:30 (Martin et al., 2010; Vyas et al., 2007). ECF generated precursors to both PFOS and PFOA. In the telomerization process, only linear isomers with even number carbon chains were produced including fluorotelomer alcohols (FTOHs), fluorotelomer olefins, fluorotelomer acrylates, and fluorotelomer iodides — which could act as "precursors" to PFOA (Butt et al., 2010; Buck et al., 2011). Both abiotic and biotic degradations of these compounds give PFOS and PFOA as the final products (Butt et al., 2010; Martin et al., 2010). Long-range transport by air (volatile precursors) and ocean currents (perfluorinated carboxylates sulfonates) are suspected to be major source pathways for the world's Arctic regions (Butt et al., 2010). In 2002 the main POSF manufacturer, the 3M Company, voluntarily phased-out their production of it in the USA and Belgium (Prevedouros et al., 2006). However, ongoing PFOS production in China might well influence global emission patterns (Butt et al., 2010). PFOS was included in Annex B of the Stockholm Convention in 2009 (http://chm.pops.int/Convention/ConventionText/tabid/2232/Default.aspx).

Due to manufacturing and numerous applications (including industrial and consumer applications as surfactants, inert protective coatings and in fire-fighting foams among others (ATSDR, 2009)), PFASs have been found world-wide in environmental matrices (e.g., air, surface water, sludge, soil, sediments, snow and ice caps), wildlife and humans (Giesy and Kannan, 2001; Kannan et al., 2004; Houde et al., 2006, 2011; Saez et al., 2008; Lau et al., 2007; Butt et al., 2010). Although food constitutes a major exposure pathway for PFASs (including food packing materials), drinking water, indoor air and house dust also appear to contribute (Domingo, 2011).

The persistence and toxicological properties of some of these compounds have been studied in several animal models (Lau et al., 2007), and evidence for potential adverse health effects in animals have raised concerns about human exposure and its consequence. Exposure by way of contaminated drinking water has been reported for residents living in a mid-Ohio Valley U.S. community surrounding a chemical plant (Steenland et al., 2009). The findings of this study (referred to as the C8 Health Project) included significant positive associations between serum concentrations of PFOS and PFOA in children and adolescents and total cholesterol, low-density lipoprotein cholesterol and thyroxine (total T4); a significant reduction in calculated thyroid hormone (T3) uptake was also observed (Frisbee et al., 2010; Knox et al., 2011). It should be noted that these exposures were atypical and thus their relevance to more normal exposure is not clear. In the National Health and Nutrition Examination Survey (NHANES), elevated serum levels of PFASs were associated with chronic kidney disease ($p<0.0001$) (Shankar et al., 2011). Several studies have documented placental transfer of PFASs, and concerns regarding human developmental outcomes have been raised (Apelberg et al., 2007; Fei et al., 2007; Washino et al., 2009). Hamm et al. (2010) suggested that the overall picture that emerges from the literature shows that PFAS exposure is not associated with any clinically relevant birth outcomes, such as birth weight. More recently, a negative correlation between maternal PFOS concentrations and fetal plasma T3 levels was reported ($r=-0.41$, $p<0.05$ after adjustment; Kim et al., 2011).

Generally speaking, in human biomonitoring studies, serum (or plasma) is a preferred matrix from the analytical perspective and is commonly used in hospital clinical chemistry tests (e.g., Burtis et al., 2006). The most prevalent PFASs occur in the environment as well as in human blood as negatively charged compounds. In vitro studies indicate that PFASs bind to a liver fatty-acid binding protein (Luebker et al., 2002). When comparing concentrations between blood components, Ehresman et al. (2007) concluded that anionic PFASs are found primarily in the human serum (or plasma) fraction. A value of 2 is often assigned to the PFOA/PFOS serum (or plasma)/whole blood concentration ratio (e.g., Ehresman et al., 2007), although a value around 1.25 has also been reported (Kärrman et al., 2006). The latter authors also showed that perfluorooctane sulfonamide (FOSA) distributed differently between plasma and blood cells than PFOS and PFOA, and that FOSA concentrations were higher in whole blood compared to plasma.

Our overall objective was to assess the exposure to PFASs experienced by delivering women and their new-borns in countries for which this information was lacking. We now report our findings for the analysis of maternal whole blood and plasma and umbilical cord blood collected under the auspices of the Arctic Monitoring and Assessment Programme (AMAP) from delivering women and their new-borns residing in Norilsk (Russia) and the Aral region of Uzbekistan (AMAP, 2004). A secondary objective was to evaluate the distribution of PFASs between blood cell and plasma fractions.

2. Material and methods

2.1. Study population and community

Sampling of human blood was undertaken in parallel with dietary and lifestyle surveys as part of the GEF/UNEP/AMAP/RAIPON project *"Persistent Toxic Substances (PTS), Food Security and Indigenous Peoples of the Russian North"* 2001–2004 (AMAP, 2004). The sampling period in Norilsk was from October to December 2001, and April to June 2002 in Uzbekistan. Mothers from Norilsk city (Taimyr okrug of Krasnoyarsk kraj; $n=7$) in the Russian Arctic were non-indigenous. Mothers from Urgench ($n=6$) and Khazarasp cities ($n=4$) (Khorezm oblast, Uzbekistan; about 200 km from the Aral Sea) were indigenous Uzbeks (see Fig. 1 for the geographical areas mentioned).

All delivering women were invited to participate in hospital delivery departments when giving birth. Mothers were interviewed three to five days after delivery. Maternal and umbilical cord blood samples were collected onsite (60 in Norilsk and 30 in Uzbekistan). Both maternal whole blood and plasma samples were available for only a minority of the participants, and this limited the sample size for the current investigation. Norilsk participants mostly consumed store bought foods (primarily non-local). Plant cultivation in Norilsk area was minimal. Because of regional reindeer breeding, fishing and hunting activities, there was some consumption of tundra reindeer, ptarmigan, and fish (mostly Salmonidae and Coregonidae species). By contrast, the diet among the Uzbekistan women featured a variety of items produced locally (e.g., meat, poultry, fish, cereal, fruits, and vegetables). All study communities had urban water supplies. Household use of insecticides in both areas was similar [all women from Uzbekistan (but one) and all women from Norilsk (except two) reported regular use of domestic insecticides (indoor sprays, chalks and pastes)].

The maternal median age at delivery was 24 (range 24–28) and 25 (range 21–41), respectively for the Norilsk and Uzbekistan study groups. Of the Uzbekistan mothers 60% had 2–8 children, while 71% of the Norilsk mothers had one child and 29% had two (means of 2.7 and 1.3 respectively). In Norilsk, the women lived in hostels (29%) or family owned flats (71%); all lived in family owned houses in Uzbekistan. The number of people per household in Norilsk and Uzbekistan was 3–4 and 5–16 respectively. Eight out of ten Uzbekistani mothers were unemployed, and one in Norilsk.

2.2. Blood sampling

Blood was collected from mothers during the first three days after delivery. Cord blood was sampled immediately after tying and cutting off the umbilical cord. For whole-blood sampling, Becton Dickinson Vacutainer System (USA) with K_2-EDTA was used (BD 366457). An aliquot of whole blood was centrifuged at 3000 rpm to separate blood cells from plasma. Cord blood was handled in the same manner (Butler Walker et al., 2006). Samples were stored in a freezer at −20 °C and transported to the laboratory in special thermally insulated containers

Fig. 1. Map of the Russian Arctic and Uzbekistan with the regions involved in this study highlighted: Red, Taimyr AO Region of Russia; dark tan, Uzbekistan. Reproduced with permission from AMAP.

and subsequently again stored at −20 °C until analyzed. To minimize inadvertent contamination of blood samples in the analytical procedures, all pipettes and storage vials were known not to be contaminated; procedural blanks identified any contamination during sample clean-up.

2.3. Ethical clearance and consent

The study protocol, training of personnel and the sample collection strategy concurred with those adopted by the AMAP Human Health Assessment Group (AMAP, 1998). The study protocol was also approved by the Ethical Committee at the Pasteur Institute, St Petersburg (international reference # T5096). Written informed consent was obtained from the participating mothers.

2.4. Chemicals and reagents used

All solvents used in this work were of Lichrosolv® grade, and were purchased from Merck-Schuchardt (Hohenbrunn, Germany). Native PFASs were obtained from Wellington Laboratories Inc. (Guelph, Ontario, Canada), and all were of >98% purity: perfluorobutane sulfonic acid (PFBS), perfluorohexane sulfonic acid (PFHxS), perfluoroheptane sulfonic acid (PFHpS), PFOS, perfluorodecane sulfonic acid (PFDS), FOSA, perfluorobutanoic acid (PFBA), perfluoropentanoic acid (PFPeA), perfluorohexanoic acid (PFHxA), perfluoroheptanoic acid (PFHpA), PFOA, pefluorononanoic acid (PFNA), perfluorodecanoic acid (PFDA), perfluoroundecanoic acid (PFUnDA), perfluorododecanoic acid (PFDoDA), perfluorotridecanoic acid (PFTrDA) and perfluorotetradecanoic acid (PFTeDA). Mass-labeled compounds used as internal standard were of >98% purity and were also obtained from Wellington Laboratories Inc., and included: MPFHxS (PFHxS$^{18}O_2$), MPFOS ($^{13}C_4$PFOS), MFOSA ($^{13}C_8$FOSA), MPFBA ($^{13}C_4$PFBA), MPFHxA ($^{13}C_2$PFHxA), MPFOA ($^{13}C_4$PFOA), MPFNA ($^{13}C_5$PFNA), MPFDA ($^{13}C_2$PFDA), MPFUnDA ($^{13}C_2$PFUnDA) and MPFDoDA ($^{13}C_2$PFDoDA). Similarly, 3,7-dimethyl-branched perfluorodecanoic acid (bPFDA) of 97% purity was obtained from ABCR (Karlsruhe, Germany), and used as recovery standard (RSTD). All concentration calculations took into account the atomic mass of the sulfonate anions.

2.5. Analytical methodology

The analytical method was slightly modified from that reported by Rylander et al. (2009). The main differences were the amounts of plasma/whole blood and methanol used in the extraction. In short, 0.5 to 1.0 mL plasma/whole blood was transferred to a 15 mL polypropylene centrifugation tube (VWR Collection, VWR, Radnor, PA, USA), and 25 μL of an 0.1 ng/μL internal standard mixture was added before adding of 4 mL methanol. After extraction, the supernatant was transferred to a new tube and the volume reduced to approximately 1 mL using a RapidVap (Rapid Vap; Labconco Corp., Kansas City, MO, USA). The sample was vortexed with acidified ENVI-Carb 120/400 (Supelco, PN, USA) (Powley et al., 2005). Finally, 5 ng of bPFDA was added as the recovery standard. Prior to analysis, an aliquot of 100 μL was transferred to a vial and mixed with an equal amount of 2 mM aqueous ammonium acetate (NH_4OAc, ≥99%, Sigma-Aldrich, St. Louis, MO, USA).

PFASs were analyzed by ultrahigh pressure liquid chromatography triple–quadrupole mass-spectrometry (UHPLC-MS/MS). Analysis was

performed on a Thermo Scientific quaternary Accela 1250 pump (Thermo Fisher Scientific Inc., Waltham, MA, USA) with a PAL Sample Manager (Thermo Fisher Scientific Inc., Waltham, MA, USA) coupled to a Thermo Scientific Vantage MS/MS (Vantage TSQ) (Thermo Fisher Scientific Inc., Waltham, MA, USA); 10 μL was injected on a Waters Acquity UPLC HSS 3 T column (2.1×100 mm, 1,8 μm) (Waters Corporation, Milford, MA, USA) equipped with a Waters Van guard HSS T3 guard column (2.1×5 mm, 1.8 μm) (Waters Corporation, Milford, MA, USA). Separation was achieved using 2 mM NH_4OAc in 90:10 methanol/water (A) and 2 mM NH_4OAc in methanol (B) as the mobile phases. In order to distinguish the perfluoroalkyl carboxylic acids (PFCAs) leaching from the pump and the degasser from that originating from a sample, a Waters XBridge C_{18} column (2.1×50 mm, 5 μm) (Waters Corporation, Milford, MA, USA) was installed after the pump and before the injector. Details about the analytical conditions, the parent ions, monitored transitions, collision energies and S-lens settings are provided in Table S1 (Supplementary data). Quantification was conducted using the LCQuan software from Thermo Scientific (Version 2.6) (Thermo Fisher Scientific Inc., Waltham, MA, USA).

2.6. Quality control

Quantification was achieved by the internal-standard method with isotope-labeled PFASs. An eight point calibration curve with a concentration range from 0.02 pg/μL to 10 pg/μL was used for their quantification. Calibration curves exhibited good linearity with correlation coefficients ≥0.99 for each analyte. Concentrations of PFASs in all samples were within both the linear range of the instrument and the calibration curve. The corresponding labeled compound was used as the internal standard (IS) for ^{12}C native compounds, with the following exceptions: MPFHxS for PFBS; MPFOS for PFHpS and PFDS; MPFBA for PFPeA; MPFOA for PFHpA; and MPFDoDA for PFTeDA and PFTrDA. The recovery of the mass-labeled internal standards ranged 44% to 110%; for details see Table S2. The quality of the analysis was verified by regular analysis of a certified reference material (SRM 1957, NIST, Gaithersburg, MD, USA) and samples with known PFAS concentrations from previous round-robbins of international calibrations. For each batch of 20 samples, one blank sample (Milli-Q water) and one SRM/reference sample were employed. In addition, our laboratory participates three times a year in an interlaboratory comparison program, namely the AMAP Ring Test for Persistent Organic Pollutants in Human Serum (organized by the Laboratoire de toxicologie, Institut National de Sante Publique du Quebec, Canada; AMAP, 2009). Results from the interlaboratory comparison program and analysis of SRM 1957 indicate that any uncertainties in our analysis were well within 20% of the assigned values.

A matrix-matched bovine serum calibration curve, spiked with ^{12}C-PFAS and ^{13}C-PFAS ISTD, was used for calculating the method detection limit (MDL), controlling the range of linearity and the method performance. Concentrations of ^{12}C-PFAS ranged 0.025 pg/μL to 50 pg/μL. Two bovine serum samples were also treated as blanks to control for possible inadvertent contamination of the bovine serum used in the matrix-matched calibration curve. MDLs are reported in footnote of Table 1. The MDL was set as the concentration in the matrix-matched calibration curve where the peak had S/N>3. For both PFOS and FOSA, separate peaks were observed which allowed the calculation of percentage of the sum of branched and linear isomers. The branched isomers eluted earlier than the linear isomers as indicated in Figures S1A (FOSA) and S1B (PFOS) of the Supplementary data. PFOS and FOSA results are presented in the text as the sum of the linear and branched compound, unless specified otherwise. As a quality control for the calculation of branched PFOS, we used a non-commercial Wellington standard (available through our laboratory's participation in an EU project; Perfood), containing a defined amount of linear and branched PFOS (78.8% and 21.2% respectively as confirmed by NMR).

Percentage linear FOSA and PFOS was calculated only for the maternal samples from Norilsk. For both PFOS and FOSA, the linear isomer was used for calculating the relative response factors for the branched compounds. No branched PFHxS was observed. In some samples branched PFOA was present, but was not investigated further.

2.7. Statistical analyses

Statistical analysis was conducted using the STATA package, version 12 (StataCorp LP, College Station, TX, USA). The non-parametric Wilcoxon signed-rank test for dependent variables was used to test for significant differences between blood and plasma and maternal and cord samples, while the non-parametric Mann–Whitney *U*-test for independent variables was employed for differences between study sites. Pearson correlation coefficients are reported for the observed associations. The median was only calculated and reported for compounds with detection frequency above 80%.

3. Results

PFOS was the most prominent compound among the seventeen PFASs investigated. The results (median, arithmetic mean and range) for those with a detection frequency exceeding 80% are presented in Table 1. The observed concentrations for all of the detected compounds in each sample are provided in Table S3.

The maternal PFOS concentration was significantly higher ($p<0.001$) in Norilsk compared to Uzbekistan (see Tables 1 and S3). Only maternal PFOS concentrations are reported in Table 1 for the Uzbekistani maternal study group, as only 50% of the cord blood samples had PFOS concentrations above the MDL (range 0.09–0.69 ng/mL; Table S3); for other PFASs, they were mostly below the MDLs in both plasma and whole blood. As indicated in Table 1, the percentage of

Table 1
PFAS concentrations (ng/mL) in whole blood (wb) and plasma (pl) in maternal and umbilical cord blood samples with detection frequency ≥80% for delivering women and their new-born from Norilsk (n=7); only maternal PFOS is shown for Uzbekistan (n=10) The complete data set is provided in Table S3. Maternal whole blood and plasma concentrations were significantly different ($p<0.05$) from the corresponding umbilical cord samples with the exception of those highlighted.

		Maternal			Umbilical cord		
Russia		Median	AM	Range	Median	AM	Range
PFOA	wb	0.89	0.89	0.33–1.40	0.49	0.58	0.15–1.12
	pl	1.61	1.50	0.63–2.48	1.00	1.26	0.36–2.32
PFNA	wb	0.35	0.49	0.23–1.43	0.15	0.22	0.08–0.78
	pl	0.60	0.89	0.38–2.75	0.29	0.50	0.21– 1..85
PFUnDA	wb	0.16	0.24	0.10–0.70	–	–	<0.05–0.23
	pl	0.22	0.33	0.13–0.96	0.10	0.16	0.08–0.43
PFHxS	wb	0.16	0.15	0.08–0.23	0.07	0.07	0.03–0.14
	pl	0.26	0.26	0.15–0.46	0.14	0.17	0.08–0.33
PFOS	wb	5.79	6.11	3.61–8.38	1.88	1.92	0.49–3.89
	pl	11.0	10.7	5.56–14.5	4.11	3.93	1.75–6.27
FOSA	wb	2.05	2.97	1.42–8.42	0.38	0.55	0.20–1.37
	pl	0.33	0.41	0.19–1.17	0.31	0.45	0.12–1.31
% lin PFOS	wb	46.6	46.6	40.5–50.5	52.7	52.2	45.1–57.5
	pl	50.6	50.2	44.8–54.9	57.5	57.2	51.3–59.7
% lin FOSA	wb	44.6	42.6	26.6–54.5	–	–	–
	Pl	40.8	43.3	22.9–64.3	–	–	–
Uzbekistan							
PFOS[a)]	wb	0.24	0.40	0.11–1.20	–	–	–
	pl	0.23	0.33	<0.08–0.89	–	–	–

AM: Arithmetic mean; [a]this is the only compound with a detection frequency above 80% for the Uzbekistan samples; MDL (method detection limit, ng/mL): PFOA (0.05), PFNA (0.08), PFUnDA (0.05), PFHxS (0.03), PFOS (0.08), FOSA (0.10).

linear PFOS was somewhat higher ($p<0.05$) in cord whole blood and plasma compared to the maternal samples.

Abundances in whole blood relative to PFOS for Norilsk are presented in Fig. 2. Clearly, PFOS levels were considerably higher than all the other PFASs. The same trend was observed for both maternal and cord plasma samples (data not shown), although for maternal plasma the FOSA/PFOS ratio was considerably lower (median≈0.03) than seen for whole blood in Fig. 2.

In maternal whole blood, most PFAS concentrations were significantly higher ($p<0.018$–0.043) than in cord blood for complete data sets in Table 1; this was also true for the plasma comparison, with PFOA and FOSA as exceptions (because of the wide range). This is illustrated in Fig. 3A and B, where maternal-cord concentration ratios for both plasma and whole blood are shown respectively. The general trend exhibited is that the ratio medians are higher for whole blood than for plasma samples (of special note is the different positioning of FOSA in these figures).

The relative distribution of PFASs between plasma and whole blood in the samples are depicted in Fig. 4. For anionic PFASs, the median ratios for cord samples were near 2; for the corresponding maternal samples they were below 2. On comparing the ratios for maternal and cord blood, significance differences ($p<0.001$–0.04) were evident for PFOA, PFNA and FOSA.

Associations between pairs of PFASs were observed for the maternal plasma data: PFNA/PFUnDA ($r=0.95$; $p\leq0.0013$, $n=7$); PFNA/PFHxS ($r=0.86$; $p\leq0.012$, $n=7$); and for whole blood the correlations were comparable (respectively, $r=0.94$ and 0.80, $n=7$). Robust associations ($r=0.80$–0.98; $p\leq0.02$, $n=7$) were also observed when exploring the relationships between cord and maternal plasma concentrations, as well as for the whole blood comparisons (see Table S3). The only exceptions were for PFOS in both compartments ($r=0.18$–0.35, $p>0.05$, $n=7$) and PFUnDA in whole blood ($r=0.91$, $p=0.09$, $n=4$).

4. Discussion

The observed PFAS concentration sequence PFOS ≫ PFOA > PFNA > FOSA > PFHxS > PFUnDA is consistent with previous reports for delivering mothers (e.g., Gützkow et al., 2012; Liu et al., 2011; Needham et al., 2011), even though some of the samples in the referenced studies were from a later time period. Although reported in other studies (Beesson et al., 2011; Gützkow et al., 2012), in the present study PFDcA was below the detection limit. This could be due to differences in sampling year. After the phase-out of POSF production in 2002, declining PFAS concentrations in the general population have been noted (Haug et al., 2009; Olsen et al., 2012). This trend has been most prominent for PFOS, PFOA, FOSA and PFHxS, while for PFNA it is not so clear.

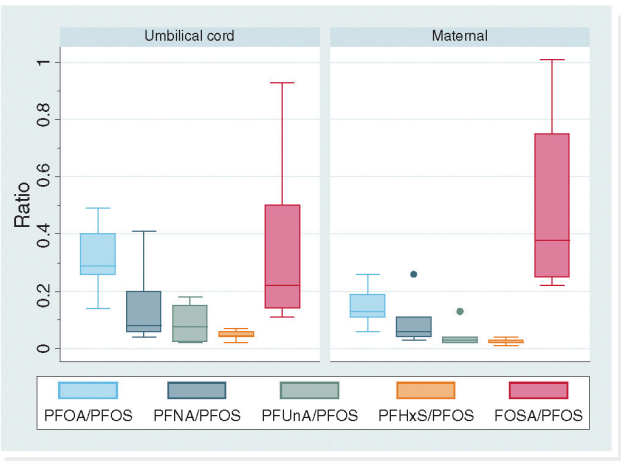

Fig. 2. Abundances of PFASs relative to PFOS in whole blood (maternal and umbilical cord; Norilsk data). The center line of the box represents the median and its top (Q3) and bottom (Q1) the 75th and 25th percentiles, respectively; Q3–Q1 is the interquartile range (IQR); the top and bottom whiskers represent ± 1.5 IQR; and solid circles denote outliers.

The low abundances of anionic PFASs found in Uzbekistan are similar to our South African observations (Hanssen et al., 2010), and reflect low exposure. By comparison, Norilsk is a heavily industrialized area located in the Russian Arctic. Our observed Norilsk PFOS plasma concentrations are comparable to those in samples collected in 2001–2002 from indigenous delivering women from remote areas of Taimyr ($n=12$) and Naryan Mar ($n=12$), respectively 9.3 and 16.0 ng/mL (Odland et al., 2006). Similar levels were also observed for Inuit adults of Nunavik in the Canadian Arctic (Dallaire et al., 2009). Saez et al. (2008) reported PFAS concentrations in snow, and concluded that long-range transport (LRT) by air was the mode of transport to remote areas in the Russian Arctic, as no local sources were evident. This was also supported by the presence of high concentrations of the volatile precursor FOSA in the snow samples. Although Norilsk is above the Arctic Circle (see Fig. 1), it is much more industrialized and prone to local pollution than many other Arctic and Siberian cities, and certainly considerably more so than the Aral rural region. Consequently, local/regional sources cannot be discounted. We should also take into account that local food and drinking water could be polluted from the Yenisei River — one of two biggest and longest rivers (4000 km) in Russia that likely accumulates pollutants from different up-river cities and settlements. Without additional source information, it is not possible to assign relative weightings to these two potential sources.

The high percentage of branched PFOS relative to the total sum of PFOS observed is consistent with other reports (Beesoon et al., 2011; Hanssen et al., 2010; Kärrman et al., 2007). The increased proportion of branched relative to that reported for ECF production of PFOS and what is currently seen in house dust constitutes somewhat of a riddle. Animal studies suggest that bioaccumulation of branched and linear PFOS are comparable, and that branched isomers are predicted to be more hydrophilic (Beesoon et al., 2011). Our semi-quantitative determination of the percentage of linear FOSA indicated a similar distribution percentage as seen for PFOS, and thus a higher proportion of branched forms than in original technical mixtures. Interestingly, Benskin et al. (2009) reported that the branched isomer of Et-FOSA metabolized more rapidly to FOSA than the linear one. Unfortunately, Et-FOSA was not quantified in our study.

In this study, we reported a higher percentage of linear PFOS in umbilical cord than in maternal samples. This is not consistent with our previous reports from South African study (Hanssen et al., 2010), nor with the reports by Beesoon et al. (2011) and Gützkow et al. (2012) findings. In the South African study, we used a high resolution instrument (LC-QToF) and the difference in response for branched and linear PFOS was not a problem as it can be for MS–MS instruments (Berger et al., 2011). Beesoon et al. (2011) used an MS–MS instrument and reported several congeners of the branched isomers with individual response factors, not a sum as in this paper. Information about the quantification of the amount of linear PFOS in by Gützkow et al. (2012) seems similar to the approach in the present study. An important difference between our work and that by Beesoon et al. (2011) and Gützkow et al. (2012) is time of sampling during pregnancy (respectively at delivery, week 15 and week 37).

To date serum (or plasma) has been the body fluid of choice for FOSA measurements. The reported concentrations have been low, with corresponding low detection frequencies. The rationale for the focus on whole blood in our paper was to illustrate the prominence of FOSA in the blood cell fraction. Clearly the concentrations in both the plasma and blood cell fractions need to be considered to fully account for exposure to this contaminant. This was first suggested by Kärrman et al. (2006) and further discussed by Martin et al. (2010). Few studies have emphasized this aspect.

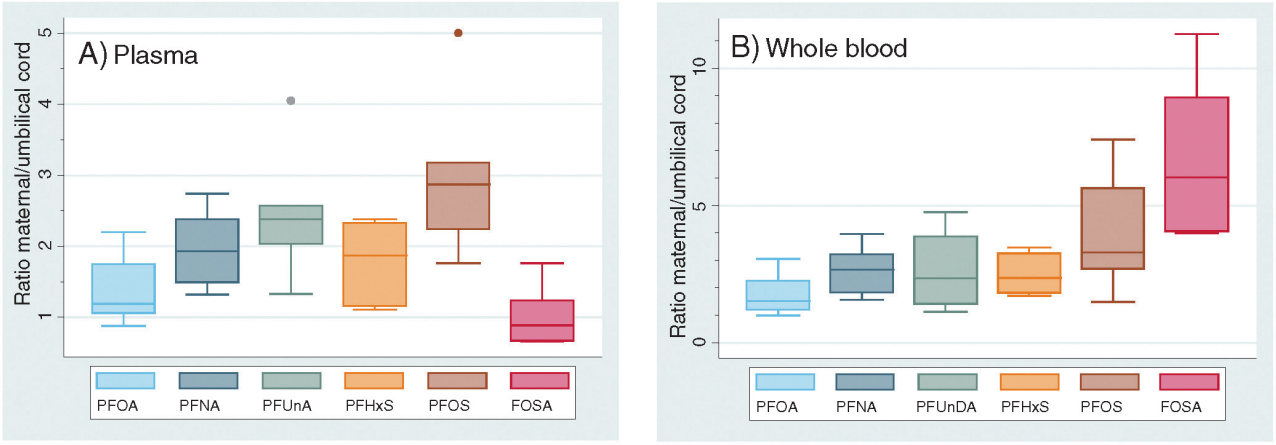

Fig. 3. Maternal-cord concentration ratios for PFASs (Norilsk data) in plasma (A) and whole blood (B). See legend to Fig. 2 for box plot explanation.

The maternal-cord concentration plasma ratio has been suggested as a measure of transplacental transfer efficiency (TTE) (Beesoon et al., 2011). The maternal-cord ratio for both whole blood and plasma increases with the length of the carbon chain for carboxylate PFASs (from 8 to 11 carbons; see Fig. 3). A similar trend is reflected for the sulfonates (6 to 8 carbons). The median ratio values for plasma for these anionic PFASs fall between 1.2 and 2.9 (Fig. 3A); those for whole blood for the same compounds are somewhat higher (1.5–3.3; note that the scale in Fig. 3B is more compressed). While the plasma ratio for FOSA was the lowest (median, 0.9), the same ratio for whole blood was the highest (6.0). A similar trend in the magnitude of this ratio in plasma samples has been noted by Beesoon et al. (2011) and Gützkow et al. (2012). Consideration of the plasma–whole blood concentration ratios plotted in Fig. 4 provides insight into these patterns. As indicated in the introduction, PFOS plasma/whole-blood concentration ratios of 1.25 (2 men and 3 non-pregnant women; Kärrman et al., 2006) to around 2.0 (adults; Ehresman et al., 2007) have been reported. This has not been examined for umbilical cord prior to the current work. With reference to the data in Fig. 4, expected plasma–whole blood concentrations ratios were calculated for both maternal and umbilical cord samples using well established estimates of packed cell and plasma volumes. Assuming that the contaminant resides in the plasma, dilution factors (plasma-to-whole blood) correspond to the reciprocal of the plasma volume, which is 1 − PCV, with PCV being the packed cell volume. During pregnancy, the median PCV value is 0.38 and thus the plasma volume (PV) = 0.62 L/L (Blackburn, 2007). A comparable value is obtained by adjusting the PCV for non-pregnant women of 0.41 ± 0.05 L/L (Lewis et al., 2001) for the 18% expansion of PCV and 30% of PV during pregnancy (evaluated at delivery; Blackburn, 2007). The maternal dilution factor for plasma-to-whole blood is therefore estimated at 1/0.62 = 1.6. This is in agreement with that indicated in Fig. 4 for PFOA, PFNA, PFHxS and PFOS. The observed ratio for PFUnDA is somewhat lower (1.44) than predicted, and FOSA had an even lower value (0.14). The latter is consistent with a substantial fraction of FOSA residing in the cell fraction of blood (see below). A similar approach is possible for cord blood. At birth, the newborn PCV is 0.60 ± 0.15 (Lewis et al., 2001) yielding PV = 0.40 and 2.5 for the plasma-to-whole blood dilution factor. Again the predicted value is close to that observed for the same anionic PFASs mentioned for the maternal ratio. This observation indicates that the at-birth blood PCV value applied to the cord blood samples was appropriate, suggesting that the latter were drawn from the fetal/baby blood circulation. The ratio of near unity for FOSA may indicate a more even distribution between the two compartments. Relative to the four anionic PFASs, the liphophilicity of PFUnDA is likely enhanced by its longer hydrocarbon chain, presumably thereby increasing its preference for the cell fraction.

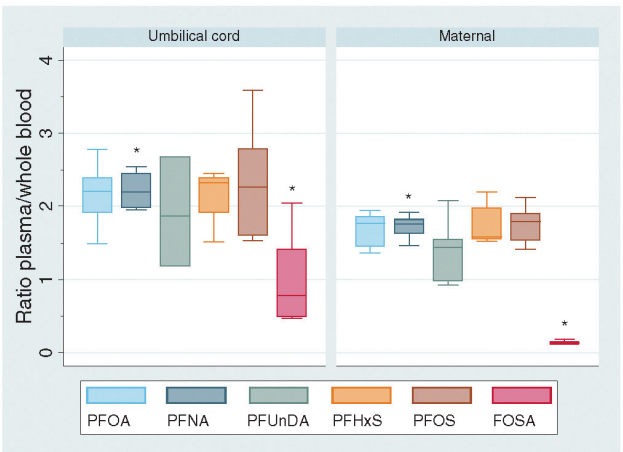

Fig. 4. Plasma–whole blood concentration ratios for PFASs in maternal and umbilical cord samples (Norilsk data). The asterisk (*) signifies a statistically significant difference (Wilcoxon signed rank test) between cord and maternal samples. See legend to Fig. 2 for box plot explanation.

Using the pK_a of 6.27 (ATSDR, 2009) and the pH of blood as 7.37 (Burtis et al., 2006), we calculated (without considerations of activity coefficients and ionic strength) that about 7% of FOSA in maternal blood would be uncharged or in a neutral form, and this fraction is close to what we observed to be in plasma. This suggests that most of the FOSA occurs in the blood cell fraction as the sulfonamidate ion, which concurs with the whole blood/plasma concentration ratio observed. It has been pointed out that the negative charge of the sulfonamidate ion is stabilized by resonance and is thus thereby reduced (i.e., the negative point charge on the deprotonated amide functional group is spread over the nitrogen, sulfur and oxygen atoms of the sulfonamidate moiety; DeRuiter, 2005, see Figure S2 in the Supplementary data). Resonance renders sulfonic acids more acidic than carboxylic acids because their extra oxygen atom results in stabilization of the conjugate anion. The replacement of one oxygen atom of the sulfonic acid group by nitrogen explains the reduced acidity of sulfonamides relative to sulfonic acids, since nitrogen is less electronegative than oxygen (DeRuiter, 2005).

There are some limitations to this study. It is unfortunate that the concentrations for the Uzbekistani samples were so low. This pre-empted a comparable analysis of the whole blood/plasma distribution analysis as done for the Norilsk study subjects. The low sample size for Norilsk further limited the external validity. However, we are

encouraged by the good agreement of our findings with those reported by others, the minimal number or absence of serious outliers in the Norilsk maternal-cord concentration ratios (Fig. 3) and the plasma/whole-blood ratios (Fig. 4), the robust correlations coefficients (paired PFASs in plasma and whole blood; and between concentrations in these two media), as well as the good agreement with a priori calculated predictions. The cord data display more uncertainty as observed by others (Needham et al., 2011). The basis for this is likely multifactorial. Sampling issues such as the possibility of concurrent sampling of fetal and maternal blood constitute inherent limitations but, as pointed out above, we have some evidence that the sampling integrity was satisfactory. The variation seen in Fig. 4A could also be biological in origin, such as that implied by the large standard deviation for the umbilical cord PCV. With respect to possible analytical bias, we are aware of the limitations inherent in the quantification of branched PFOS (Berger et al., 2011). However we are confident that the NMR verified standard used provided suitable quality assurance for the reported results. Another issue is the co-elution of PFOS and bile acid (Keller et al., 2010). A difference of 0.3 min in retention time of linear PFOS and bile acid was achieved, and the latter did not co-elute with branched PFOS.

5. Concluding remarks

Delivering women from the Arctic community of Norilsk were considerably more exposed to PFASs than the women from Uzbekistan, supporting previous limited findings of elevated concentrations in the Arctic. Both LRT and local sources could contribute for Norilsk. In the absence of more detailed information about the local sources there, it is difficult to conclude which dominated.

The observation that a large fraction of FOSA is associated with the cell fraction indicates that to date most of this compound has remained undetected. A singular focus on serum/plasma likely has contributed to this underestimation of exposure. As previously reported for PFOS and confirmed in the present study, we also observed the presence of significant amounts of both linear and branched forms for its precursor FOSA. The implication of this is that FOSA as a PFOS precursor may well account for the increased percentage of branched PFOS observed.

Our comparison of the plasma/whole blood concentration ratios for PFASs in maternal and cord blood and its interpretation constitutes a novel feature. For PFASs and related compounds with pK_a values with magnitudes comparable to physiological pH, it seems prudent to measure the cell-associated fraction (separately or as whole blood). This also applies to emerging contaminants with acid/base properties. The observed concentration differences for plasma and whole blood indicate that it is not only the chemical differences between the compounds that should be considered. The physiological attributes of these two matrices are also of importance. Additional studies with ample sample size of fluorinated substances and well-defined (but varied) exposure patterns are needed to affirm the novel insights reported.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.scitotenv.2013.01.029.

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