Environmental Science & Technology

Article

pubs.acs.org/est

Isomer–Specific Distribution of Perfluoroalkyl Substances in Blood

Hangbiao Jin,[†,⊥,∥] Yifeng Zhang,[†,⊥,∥] Weiwei Jiang,[§] Lingyan Zhu,[*,†] and Jonathan W. Martin[*,‡]

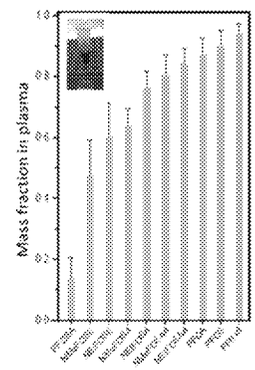
[†]Key Laboratory of Pollution Processes and Environmental Criteria, Ministry of Education, Tianjin Key Laboratory of Environmental Remediation and Pollution Control, College of Environmental Science and Engineering, Nankai University, Tianjin, P.R. China

[‡]Division of Analytical and Environmental Toxicology, Department of Laboratory Medicine and Pathology, University of Alberta, Edmonton, Alberta Canada T6G 2G3;

[§]South China Institute of Environmental Science, Ministry of Environmental Protection, Guangzhou, P.R. China

Supporting Information

ABSTRACT: Perfluoroalkyl substances (PFASs) such as perfluorohexanesulfonate (PFHxS), perfluorooctanoate (PFOA), perfluorooctanesulfonate (PFOS) and PFOS–precursors are routinely measured in human plasma and serum, but their relative abundance in the blood cell fraction has not been carefully examined, particularly at the isomer–specific level. Human plasma and whole blood were collected and partitioning behaviors of PFASs and their isomers between plasma and blood cells were investigated. In human samples, mass fraction in plasma (F_p) for PFASs increased among perfluoroalkyl carboxylates as the carbon chain length increased from C6 (mean 0.24) to C11 (0.87), indicating preference for the plasma fraction with increasing chain length. However, among perfluoroalkyl sulfonates, PFHxS (mean 0.87) had a slightly higher F_p than PFOS (0.85). In vitro assays with spiked Sprague–Dawley rat blood were also conducted, and the results showed that PFOS–precursors had lower F_p values than perfluoroalkyl acids, with perfluoroctanesulfonamide having the lowest F_p (mean 0.24). Consistently, linear isomers of PFOS and PFOS–precursors had lower mean F_p than their corresponding total branched isomers. Multiplying by a factor of 2 is not a reasonable method to convert from whole blood to plasma PFAS concentrations, and current ratios could be used as more accurate conversion factors.



INTRODUCTION

Perfluoroalkyl substances (PFASs) are a broad group of chemicals that are distinguished by a fully fluorinated carbon chain moiety of various length and structure.[1] Many PFASs have a polar headgroup such as a carboxylate, sulfonate, alcohol, or phosphate and have been used extensively in consumer products over the last five decades.[2] PFASs have been synthesized by at least two major manufacturing methods: electrochemical fluorination (ECF) and telomerization.[3] The ECF process yields a wide variation of structural isomers,[4] with historic commercial ECF–derived products usually consisting of ~70% linear isomer and ~30% of various branched isomers.[5] Conversely, telomerization results in products with a high isomeric purity, usually straight or isopropyl branched chain, but with a distribution of homologue chain–lengths.[6]

Perfluorooctanoate (PFOA), perfluorohexanesulfonate (PFHxS), perfluorooctanesulfonate (PFOS), and perfluoroctane sulfonamide (FOSA) have been reported in human serum and plasma from around the world.[7,8] Health concerns have been raised due to associations between PFOA, PFHxS, and PFOS concentrations in serum and various effects in humans including reduced birth weight,[9] thyroid abnormalities,[10] humoral immune response,[11] and low–density lipoprotein cholesterol.[12]

Human blood consists of plasma (~55% v/v), red blood cells (RBC, ~45% v/v), and leukocytes and platelets (~0.4% v/v, isolated as buffy coat).[13] To date, biomonitoring of PFASs in human has predominantly been conducted in serum or plasma (which have been shown to be equivalent),[14] including in large–scale epidemiology studies such as for the United State's National Health and Nutrition Examination Survey,[15] C8 Health Project,[16] the American Red Cross study,[17] as well as for the Canadian Health Measures Survey.[12] This strong tradition of serum or plasma analysis for PFAS biomonitoring is based on the assumption that PFASs are predominantly or exclusively in the plasma or serum fraction. Based on this, in some studies whole blood PFAS concentrations have been converted to plasma or serum concentrations by multiplying each concentration by a generic and rather arbitrary factor of 2.0.[18–20]

Only four small studies have directly measured the distribution of PFASs between plasma and whole blood in humans, and none of these studies examined the individual isomers. In the earliest report, and based on a small sample size of adult samples (n = 3–5), Kärrman et al.[21] reported mean plasma/whole blood concentration ratios of 1.1 for perfluorononanoate (PFNA, n = 4), 1.2 for both PFOS and PFHxS (n = 5), and 1.4 for PFOA (n = 5), and a considerably lower ratio for FOSA (0.2, n = 3). For the perfluoroalkyl acids, these results are at odds with those of Ehresman et al.[14] and Hanssen et al.[22]

Received: April 9, 2016
Revised: June 2, 2016
Accepted: June 13, 2016
Published: June 13, 2016

ACS Publications © 2016 American Chemical Society

DOI: 10.1021/acs.est.6b01698
Environ. Sci. Technol. 2016, 50, 7808–7815

ED_002330_00120349-00001

For example, in 3 M Company employees (n = 18), Ehresman et al. reported ratios of 2.0−2.5 for PFOS, PFHxS, and PFOA, and in fact reported that concentrations in the RBC fraction were below limits of detection (1 ng/mL) when analyzed directly, suggesting that these analytes were predominantly in the serum/plasma fraction. In a small set of samples (n = 7) from pregnant women in Arctic Russia, Hanssen et al. came to similar conclusions as Ehresman et al., but nevertheless did find a similar result to Kärrman et al. for FOSA which was strongly associated with the blood cell fraction.

Therefore, the overall understanding of PFAS blood partitioning is not strong and very inconsistent, even for the major analytes routinely monitored. Furthermore, the blood partitioning behaviors of individual PFOS and PFOA isomers have never been studied. Moreover, for PFOS precursors detected occasionally in humans, such as *N*-methyl perfluorooctane sulfonamide (NMeFOSA), *N*-methyl perfluorooctane sulfonamidoethanol (NMeFOSE), and *N*-ethyl perfluorooctane sulfonamidoacetic acid (NMeFOSAA), we are unaware of any information about their partitioning behaviors in blood. Previous studies demonstrated that the toxicities of PFOA and PFOS are isomer specific.[23,24] A better understanding of the plasma−RBC partitioning behaviors on an isomer level would lead to better interpretation and comparison of biomonitoring data from around the world, allow better predictions of the total internal dose, and possibly a better understanding of PFAS toxicokinetics.

In the present study we collected 60 whole blood samples from a Chinese population, and three whole blood samples from a highly exposed Canadian family to investigate the distribution of PFASs and their major isomers in human blood. Fresh Sprague−Dawley (SD) rat blood was also spiked and incubated to explore the whole blood distribution of seven PFOS precursors.

MATERIALS AND METHODS

Nomenclature. For PFAS isomers, we adopt the nomenclature and acronyms defined in a previous study.[25] Taking PFOS as an example, we denote linear PFOS as *n*-PFOS, perfluoroisopropyl as *iso*-PFOS, 5-perfluoromethyl as 5*m*-PFOS, 3-perfluoromethyl as 3*m*-PFOS, 1-perfluoromethyl as 1*m*-PFOS, sum of all diperfluoromethyl isomers as m_2−PFOS, and total branched isomers as *Br*-PFOS. For PFHxS, FOSA, and NMeFOSA, we assign their chromatographically separated branched isomers of unknown structure as B_x, (where x = 1, 2, 3, etc.) in decreasing order of retention time (e.g., B_1-PFHxS is the latest eluting branched isomer of PFHxS, and B_2-PFHxS elutes earlier) (for detail, see Supporting Information (SI), Figure S1).

Chemical Standards. 3 M Co. provided ECF PFOA (~22% total branched isomers by ^{19}F nuclear magnetic resonance), PFOS (~30% total branched isomers), and FOSA (~25% total branched isomers).[26] Mass-labeled internal standards ($^{18}O_2$-PFHxS, $^{13}C_4$-PFOS, $^{13}C_4$-PFOA, $^{13}C_8$-FOSA, $^{13}C_3$-NMeFOSA and $^{13}C_3$-NMeFOSE; exclusively linear) and all native PFAS standards (including PFOS, PFOA, and PFHxS isomer standards) were from Wellington Laboratories (Guelph, ON, Canada).

Rat Blood Collection and in Vitro Experiments. In vitro assays with spiked rat blood have been reliably used to predict drug distribution in human blood.[27−29] Animal work was conducted in accordance with a protocol approved by the Animal Care and Use Committee, University of Alberta. Sprague−Dawley rats were obtained from Biosciences Animal Service (University of Alberta, Edmonton, AB, Canada), raised in plastic cages and kept under conditions of controlled temperature (25 ± 2 °C) and lighting (12 hr dark:light cycle), and given standard rodent food and tap water ad libitum. Blood samples from both male and female rats were collected from the saphenous vein into BD-Vacutainer blood collection tubes (Becton Dickinson, NJ) embedded with ethylene diaminetetraacetic acid (EDTA) as an anticoagulant.

Mixed whole rat blood (6.0 mL), half from male and half from female rats, was spiked with target analytes at 10 and 100 ng/mL in triplicate in capped Labcon SuperClear 8 mL polypropylene (PP) tubes (Cyagen Biosciences, CA). The tubes were capped and incubated at 37 °C, and gently rotated at 10 rpm to mimic in vivo conditions. Whole rat blood with no spike, as well as phosphate buffer saline (PBS) solution (pH 7.3−7.5) spiked with the same level of analytes as whole blood served as separate controls. Blood samples (500 μL) were withdrawn from each tube at 10, 30, 60, 120, and 200 min into centrifuge tubes that were immediately spun at 3500 g for 5 min to separate plasma from blood cells. Hematocrit of sampled rat blood was measured volumetrically. The isolated plasma and cell (erythrocytes, leukocytes, and platelets combined) fractions were stored frozen (−20 °C) prior to analysis.

Human Blood Collection. Physical examinations of 30 healthy couples from Shijiazhuang, Hebei Province, in northern China were conducted in May, 2013. The age of recruited couples was in the range of 23−71. Ethical clearance was obtained from the research ethics boards of the University of Alberta and Nankai University, and all participants provided informed consent prior to blood sampling. About 6−10 mL of whole blood was collected from each person into two vacuum blood tubes containing EDTA. One tube of whole blood (ca. 3−5 mL) was instantly transferred into solvent−rinsed 15 mL PP tubes (Fisher Scientific, ON, Canada). The other tube of whole blood (ca. 3−5 mL) was centrifuged at 3500 g for 15 min, and plasma was transferred to solvent−rinsed PP vials. Paired plasma and whole blood samples were stored at −20 °C. Participants provided data including age, parity, body mass index, and blood type by answering questionnaires (for detail, see SI, Table S1).

Three additional blood samples (1 female and 2 males) were collected from a Canadian family (Edmonton, AB, Canada) which had previously been identified to have high PFAS exposure.[30] These blood samples were collected into vacutainers containing EDTA as anticoagulant (as for Chinese participants) but were never frozen prior to processing, and were processed within 30 min by centrifugation (3500 rpm for 5 min). The separated cell fraction was gently washed three times using PBS to minimize residual plasma and to remove the buffy coat (leukocytes and platelets). We stored the prepared plasma and RBC samples at −20 °C before PFAS analysis.

Sample Preparation. Prior to extraction, all samples were spiked with mass−labeled PFASs. Rat plasma and blood cell samples were extracted individually with methanol using a method adapted from previous studies,[31,32] but with additional clean−up by passing extracts through ENVI-Carb cartridges for cleanup (100 mg, 1 cc, Supelclean, Sigma Aldrich). Washed human RBC samples were treated similarly. Human whole blood samples were extracted by the ion pairing method.[22] Human plasma samples were extracted by solid phase extraction on Oasis HLB SPE cartridges (150 mg, 6 CC,

DOI: 10.1021/acs.est.6b01698
Environ. Sci. Technol. 2016, 50, 7808−7815

ED_002330_00120349-00002

Waters Co.). For full sample preparation details, see SI. Matrix spike and recovery experiments were performed in triplicate to evaluate the effect of each extraction technique (see SI, Table S2 and S3). Isomer signatures were all conserved during extraction, and different matrixes did not influence isomer profiles, as was previously shown.[33]

Isomer Separation and PFAS Analysis. NMeFOSE and *N*-ethyl perfluorooctane sulfonamidoethanol (NEtFOSE) were analyzed in the extracts by gas chromatography mass spectrometry equipped with an electron ionization source, and chromatograms were recorded by selected ion monitoring. The analytes were separated on a DB-5MS column (Agilent Technologies, CA) (30 m × 0.25 mm i.d., 0.25 μm film thickness). All other analytes were determined by high performance liquid chromatography tandem mass spectrometry (HPLC−MS/MS) with an electrospray ionization source (negative mode). Isomer separations were performed on a FluoroSep RP Octyl (ES Industries, NJ, USA) or an Ascentis Express F5 HPLC column (Sigma Aldrich, ON). At least two transitions were selected for each compound. For detail, see SI. The interference free transition for PFHxS (m/z 399/119) was always chosen for quantification.[34] Quantification was by relative response to the respective internal standard, using at least six points per curve.

Mass Fraction in Plasma Calculation. In both the in vitro and human blood biomonitoring assays, we calculated mass fraction in plasma (F_p) to evaluate the partitioning potential of PFASs to the plasma as follows:

$$F_p = \frac{C_p(1 - f_{cell})}{C_{blood}}$$

where C_p and C_{blood} refer to the concentrations of individual PFASs in plasma (ng/mL) and whole blood (ng/mL), respectively, and f_{cell} is the whole blood volume fraction of blood cells (i.e., hematocrit). Sex−specific hematocrit data (40% for female and 45% for male) was used for F_p calculation.[13] Consequently, an F_p value approaching 1.0 indicates majority of the analytes in plasma, an F_p value of 0.5 indicates equal mass of PFASs in plasma and cells, whereas as F_p approaching zero indicates stronger partitioning to the blood cell fraction.

RESULTS AND DISCUSSION

Total PFAS in Human Plasma and Whole Blood. In the samples from Chinese participants, the major total PFASs in human plasma (n = 60) and whole blood (n = 60) were PFOS, PFOA, PFHxS, and PFHxA (see SI, Table S4 and S5), similar to previous findings.[17,35,36] The target PFOS precursor compounds (e.g., FOSA, NMeFOSA, and NEtFOSA) were not detected in these human samples. Mean concentrations of PFOS, PFOA, PFHxS, and PFHxA in plasma (whole blood) were 9.8 (6.4), 1.2 (1.0), 1.1 (0.63), and 0.46 (1.4) ng/mL, respectively. Concentrations of most individual PFASs in plasma were positively correlated with those in the whole blood (Spearman correlation coefficients ranging from 0.49−0.87, $p < 0.01$). PFHxA and PFNA also showed significant yet weaker correlations, having Spearman correlation coefficients of 0.27 ($p < 0.05$) and 0.29 ($p < 0.05$), respectively.

In the three blood samples from the Canadian family, PFOA, PFHxS, PFOS, and NMeFOSAA were detectable and their mean concentrations in plasma (whole blood) were 0.28 (0.19), 1.4 (0.68), 0.13 (0.09), and 0.18 (0.10) ng/mL, respectively (see SI, Table S6).

The 60 Chinese samples were from 30 married couples, and men had significantly higher concentrations of PFHxS (paired t-test, p = 0.004) and PFOS ($p < 0.01$) than women. However, no such difference was observed for the perfluoroalkyl carboxylates (PFCAs). Wong et al.[37] reported that the half−life of PFOS was shorter in females than in males, and Zhang et al.[38] predicted that female menstrual blood loss was an important loss mechanism for women, particularly for PFHxS and PFOS which are most strongly bound to proteins in the plasma.

Total PFAS Distribution in Human Blood. In the 30 female and 30 male Chinese blood samples, no significant (p > 0.05) sex, parity, body mass index or blood−type difference was observed with respect to F_p. Among the perfluoroalkyl acids, PFHxS had the highest mean F_p, followed by PFUnA, total PFOS, PFDA, PFNA, total PFOA, and PFHxA (Figure 1). For

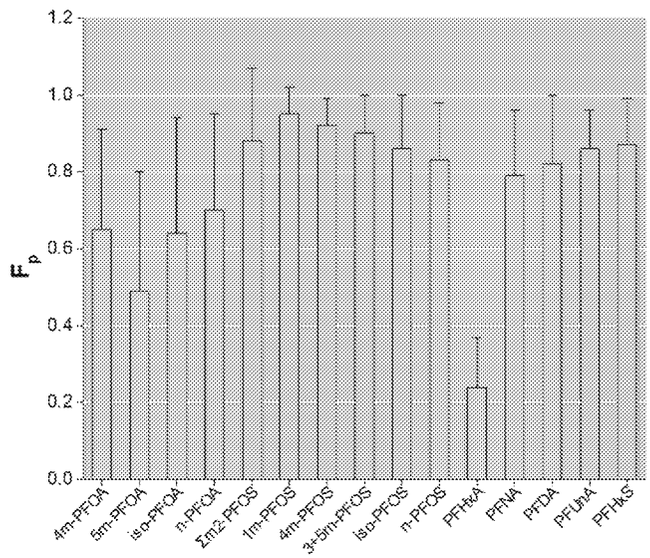


Figure 1. Calculated mass fraction in plasma (F_p) of PFASs in 60 Chinese blood samples.

PFCAs, F_p increased as the perfluoroalkyl chain length increased, indicating a preference for longer PFCAs to partition in the plasma fraction. This is likely due to the higher affinity to human serum albumin (HSA) with increasing perfluoroalkyl chain length, as previously described.[39]

Although PFOS ($C_8F_{17}SO_3^-$) and PFNA ($C_8F_{17}CO_2^-$) have identical perfluorocarbon chain lengths, total PFOS displayed a significantly higher (t−test, $p < 0.05$) F_p than PFNA. This may be attributable to the larger size of a sulfonate moiety relative to a carboxylate, leading to greater hydrophobic and electrostatic attractive interactions of PFOS with HSA, compared to PFNA.[40] Total PFOS also had a greater F_p than total PFOA. This is consistent with findings of Beesoon and Martin[41] whom reported a higher binding affinity of PFOS to HSA than PFOA.

It is interesting that total PFHxS had a higher F_p than total PFOS, because PFHxS has a shorter perfluoroalkyl chain (six carbon) than PFOS (eight carbon). Nevertheless, these F_p measurements are consistent with Bischel et al.[40] who reported that PFHxS had a greater binding affinity with HSA than PFOS. This is possibly because PFHxS has a less rigid perfluoroalkyl

DOI: 10.1021/acs.est.6b01698
Environ. Sci. Technol. 2016, 50, 7808−7815

ED_002330_00120349-00003

Table 1. Summary of Existing Studies on Serum−whole Blood Distribution of Total PFOA, PFHxS, and PFOS

study	sampling year	location	population	sample size	mean serum:whole blood concentration		
					PFOA	PFHxS	PFOS
Taniyasu et al.[38]	2002	Japan	general	3	N/A[a]	2.5	N/A
Kärrman et al.[21]	2004	Swedish	general	5	1.4	1.2	1.2
Ehresman et al.[14]	2004−2005	America	occupational	18	2.0−2.1[b]	2.5[b]	2.2−2.3[b]
Hanssen et al.[22]	2001	Russia	maternal	7	1.7	1.8	1.8
			umbilical cord	7	2.2	2.2	2.3
present study	2012	China	general	60	1.2 ± 0.43[c]	1.6 ± 0.21[c]	1.5 ± 0.42[c]

[a]N/A = not available. [b]Plasma concentrations were used. Meanwhile, either EDTA or heparin was used as anticoagulation. All other studies used serum concentrations for calculation. [c]Mean ± SD.

chain than PFOS and PFHxS may additionally adopt a zig−zag geometry that more closely resembles endogenous fatty acids.[42]

A stronger protein binding affinity for PFHxS than either PFOS and PFOA in plasma can also explain the consistent observations of the relatively longer serum elimination half−life of PFHxS (30 days, 100 days, and 7.3 years) relative to PFOS (25 days, 45 days, and 4.8 years) and PFOA (<5 days, 14−42 days, and 3.5 year) in rodent species, monkeys, and humans, respectively.[43] The rank order of F_p values for PFHxS, PFOS, and the PFCAs are furthermore consistent with the urinary elimination half-lives for humans reported by Zhang et al.[38] Overall evidence is strong that the persistence of perfluoroalkyl acids in blood is strongly influenced by the relative affinity for plasma.

In the three fresh blood samples collected from a Canadian family, F_p was calculated for *n*-PFOA, PFHxS, total PFOS, and NEtFOSAA, and the mean F_p (±SD) was 0.73 ± 0.02, 0.91 ± 0.02, 0.80 ± 0.04, and 0.87 ± 0.02, respectively, similar to the values calculated for the 60 Chinese participants. For these three samples the blood cell fraction was also analyzed directly (rather than whole blood), and only as much as 5% of residual total PFOS and PFOA in the blood cells could be removed by rinsing the cells with PBS solution. This demonstrated that detection in the cell fraction was not only due to contamination from residual plasma for these major analytes. However, all detectable residual PFHxS in the blood cell fraction was removed by washing, with the mass−balance recovered in the PBS washing solution (see SI, Figure S2). This further demonstrates that PFHxS was almost exclusively distributed in plasma phase of human blood, as reflected by its mean F_p of 0.91.

The literature shows large inter study variation in plasma to whole blood concentration ratios for total PFOA, PFHxS, and PFOS (Table 1). Among them, Ehresman et al. reported the highest ratio for total PFHxS (mean, 2.5) and PFOS (2.2−2.3), nearly twice as high as ratios reported by Kärrman et al.[21] This may be because the target population, where the blood were sampled, have different exposure sources. Ratios of total PFOA, PFHxS, and PFOS calculated from seven umbilical cord samples are comparable to that reported by Ehresman et al.,[22] all higher than 2.0. The partitioning ratios of total PFHxS and PFOS in the current study are low and most similar to Kärrman et al., and all less than 2.0. As noted, in previous studies the concentrations of PFASs (mainly C_6−C_{11} PFCAs, PFHxS, and PFOS) detected in human whole blood were multiplied by a factor of 2.0 to convert those in equivalent serum concentrations.[18−20] Results here suggest that this greatly overestimates their serum concentrations, and the overestimation would be greatest for C_6−C_8 PFCAs (Figure 1).

PFAS Isomer Profiles in Plasma and Whole Blood. In the 60 Chinese whole blood samples, both PFOA and PFOS were detected with various branched isomers. PFOA isomers were always detected in the Chinese human plasma and whole blood samples, except for 3*m*-PFOA. There was no significant difference (paired *t*-test, $p = 0.09$) in the percentage of *n*-PFOA between the paired plasma (mean ± SD, 92% linear ±6%) and whole blood (91% linear ±9%) samples, nor between males and females. Overall, *n*-PFOS contributed 46 ± 14% (mean ± SD) of total PFOS in the 60 Chinese plasma and 49 ± 16% in whole blood samples, a similar result to the three samples of highly exposed Canadians. Like for *n*-PFOA, a paired *t*-test indicated there was no statistical difference in the percentage of *n*-PFOS in the plasma and whole blood ($p = 0.32$).Interestingly, however, the females (mean, 53%) displayed significantly higher (paired *t*-test, $p < 0.001$) percentage of *n*-PFOS than the males (mean, 39%) in plasma samples. This may be because females preferentially excreted branched PFOS isomers through special routes, such as through menstruation, through child birth and lactation.[24,44] PFOS was the only PFAS to have detectable branched isomers in the three blood samples from the Canadian family. The mean *n*-PFOS percentage was 49% in plasma and 50% in whole blood, similar to results in Chinese participants whereby %*n*-PFOS was not different in plasma and whole blood.

The proportions of *n*-PFOA in both human plasma and whole blood were much higher than that in historical 3 M Company ECF PFOA (78.9%; 95% confidence interval (CI): 78.6, 79.2%) or in commercial PFOA products (range, 77−78%) in China.[45] However, the percentage of *n*-PFOS in Chinese plasma and whole blood samples was distinctly lower than in the historical 3 M Company ECF PFOS (70%; 95% CI: 69.3, 70.7%) or in ECF PFOS products (range, 66−72%) manufactured in China,[26] consistent with previous biomonitoring studies globally.[44,46−48] Possible explanations for these results have been discussed.[38,44]

As discussed above, no statistical differences were observed among the % *n*−PFOS or % *n*-PFOA in the plasma compared to whole blood. This is consistent with few statistical differences detected among F_p of the linear and branched isomers of PFOS or PFOA ($p > 0.05$), however some relative patterns were notable. For example, among PFOS isomers F_p of the branched isomers was greater than for *n*-PFOS. F_p for individual PFOS isomers followed the rank order of $1m > 4m > 3 + 5m > \sum m_2 > iso > n$. Among PFOA isomers, a different relative pattern emerged whereby *n*-PFOA had the highest mean F_p value, followed by $4m \approx iso > 5m$. The 4*m*- and *iso*-PFOA branched isomers had lower F_p values than *n*-PFOA, but not significantly. 5*m*-PFOA had a significantly lower F_p than *n*-PFOA, but the concentration contribution of 5*m*-PFOA to total

DOI: 10.1021/acs.est.6b01698
Environ. Sci. Technol. 2016, 50, 7808−7815

ED_002330_00120349-00004

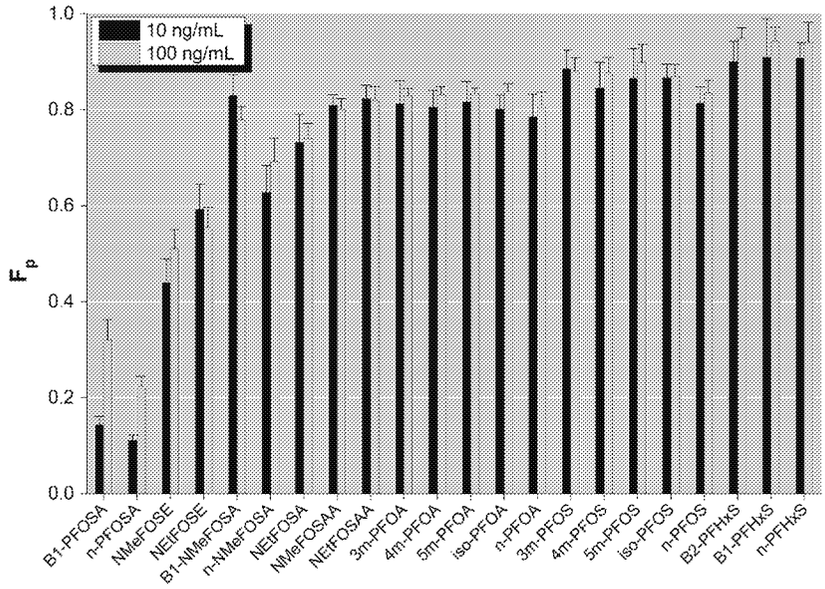


Figure 2. F_p values (mean ± SD) of spiked PFASs in rat blood during in vitro incubation at 37 °C.

PFOA was less than 5% in blood. Therefore, the percentage of *n*-PFOA in plasma was somewhat higher than that in whole blood, but the difference was not statistically significant.

In vitro PFAS Distribution. Low concentrations of *n*-PFOA (~0.1 ng/mL) and *n*-PFOS (~0.07 ng/mL) were occasionally detected in unspiked rat blood samples, but the low levels had negligible effect on the results. Attention to the experimental conditions was made to ensure no obvious clotting of whole blood, nor hemolysis of RBCs during 200 min incubations. Control water incubations showed negligible loss of analytes from the aqueous phase, and PFASs spiked to whole rat blood were stable and did not significantly adsorb to the inner walls of the PP tubes, in line with previous results.[49] Furthermore, spiked PFOS−precursors showed no evidence of degradation during the 200 min incubation. The in vitro distribution of PFASs at both 10 and 100 ng/mL (nominal concentrations) in rat blood generally reached equilibrium within 60 min (for kinetics, see SI, Figure S1). The mean F_p values of spiked PFASs at steady−state (i.e., based on the data at 60, 120, and 200 min) were always >0.5, (except for FOSA) (Figure 2), indicating preferential partitioning of most target analytes to rat plasma, relative to blood cells (all $p < 0.05$).

Among target PFASs, total PFHxS, PFOS, and PFOA had comparatively higher F_p, with mean F_p (±SD) of 0.91 ± 0.02, 0.83 ± 0.02, and 0.75 ± 0.03, respectively, agreeing well with the values calculated for human samples. Previous in vitro and vivo assays demonstrated these compounds had strong binding affinities to serum albumin of rat, monkey, and humans.[50,51]

The lowest F_p in spiked rat blood was for total FOSA (mean, 0.24 ± 0.03), indicating preferential accumulation of FOSA in the cell fraction. This observation is in line with the results reported by Hanssen et al.[22] and Kärrman et al.[21] in humans. For the other spiked PFOS precursors, F_p followed the order of NEtFOSAA (mean, 0.83 ± 0.03) > NMeFOSAA (0.80 ± 0.05) > NEtFOSA (0.74 ± 0.04) > NMeFOSA (0.70 ± 0.03) > NEtFOSE (0.57 ± 0.07) > NMeFOSE (0.47 ± 0.08). Thus, in general for the compounds with the same functional groups (i.e., − SAA, − SA, or − SE), those with longer alkyl chains (i.e., *N*-ethyl) displayed stronger binding affinity to the proteins than shorter ones (i.e., N−methyl). This is likely attributed to the greater hydrophobicity of longer alkyl chain compounds. On the other hand, F_p declined in the order R−CH_2COOH > R−H > R−CH_2CH_2OH, whereby R represents $C_8F_{17}SO_2(CH_3)N$ or $C_8F_{17}SO_2(CH_2CH_3)N$. Interestingly, this decreasing order is opposite to the increasing order of acid dissociation constants (pK_a) for these compounds, whereby R−CH_2COOH (pK_a = 3.9 for both NMeFOSAA and NEtFOSAA) < R−H (pK_a = 6.5 and 6.8 for NMeFOSA and NEtFOSA, respectively) < R−CH_2CH_2OH (pK_a = 14.9 for both NMeFOSE and NEtFOSE).[52−55] The physiological pH of rat plasma is 7.3−7.5, at which R−CH_2COOH is >99% ionized, R−H is mostly (80−89%) ionized, whereas R−CH_2CH_2OH will be near exclusively present in neutral form. Thus, we speculate that the negatively charged head groups of PFASs interact with proteins in rat plasma by an electrostatic mechanism, as observed between PFOS and serum albumin.[56] Nevertheless, it is notable that FOSA with a pK_a = 6.3, similar to the pK_a of NMeFOSA and NEtFOSA, has a much lower F_p. This may be because the electron of the sulfonamidate anion (SO_2NH^-) is stabilized by resonance,[22] thereby reducing its electrostatic interactions with proteins in the rat plasma. Among all spiked PFOS precursors, resonance is only possible for FOSA.[57]

Although branched PFHxS isomers were not detectable in human blood samples, the current experiment demonstrated that the various linear and branched PFHxS isomers (B_1-, B_2-, and *n*-PFHxS) had nearly equal F_p values in spiked rat blood (Figure 2). For isomers of PFOA, PFOS, FOSA, and NMeFOSA, spiked rat blood revealed that the percentage of linear isomer, relative to total isomers, was consistently lower in plasma than in whole blood; for PFOA isomers the difference was not statistically significant (see SI, Table S7). Consistent with these finding, individual branched isomers had higher F_p than their respective linear isomer for FOSA, NMeFOSA, and PFOS (Figure 2). For instance, among PFOS isomers, 3*m*- and 4*m*-PFOS always had greater F_p, followed by *iso*-, 5*m*-, and *n*-PFOS.

Overall, partitioning distinctions observed for PFASs and their isomers in human and rat blood demonstrate that multiplying whole blood concentrations by a factor of 2 is

DOI: 10.1021/acs.est.6b01698
Environ. Sci. Technol. 2016, 50, 7808−7815

ED_002330_00120349-00005

overly simplistic and can lead to overestimation of serum or plasma concentrations.

Study Limitations. Although this is the largest study to examine the blood partitioning of PFASs, one limitation of this study is the wide variation in F_p values observed in the human blood for PFOA isomers. The very low F_p value (<0.3) implies PFASs are predominantly accumulated in the blood cell fraction of blood in some individuals, which is opposite to our overall conclusion. Second, all targeted PFOS precursors were not detected in collected human blood samples, rendering it hard to validate the distribution outcomes of these compounds in the in vitro experiment, although this study was not designed for this purpose.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.est.6b01698.

Details of standards used for LC-MS/MS and GC/MS, spike and recovery results, concentrations of PFASs in human blood samples, and distribution kinetics of PFASs in rat blood (PDF)

AUTHOR INFORMATION

Corresponding Authors

*(L.Z.) Phone: +86-22-23500791; fax:+86-22-23503722; e-mail: zhuly@nankai.edu.cn;.

*(J.W.M.) Phone: +1-780-492-1190; fax: +1-780-492-7800; e-mail: jon.martin@ualberta.ca.

Author Contributions

‖H.J. and Y.Z. contributed equally to this work.

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

We thank the participants for their contributions to the study. H.J. acknowledges support from the China Scholarship Council to support his doctoral research at the University of Alberta. L.Z. acknowledges financial support from the Natural Science Foundation of China (NSFC 21325730, 21577067, 21277077), Ministry of Education (20130031130005) and the Ministry of Education innovation team (IRT 13024). J.W.M. acknowledges financial support for the research through the Natural Sciences and Engineering Research Council of Canada (NSERC Discovery Grant), and from Alberta Health for support of daily laboratory operations.

REFERENCES

(1) Buck, R. C.; Franklin, J.; Berger, U.; Conder, J. M.; Cousins, I. T.; de Voogt, P.; Jensen, A. A.; Kannan, K.; Mabury, S. A.; van Leeuwen, S. P. J. Perfluoroalkyl and polyfluoroalkyl substances in the environment: terminology, classification, and origins. *Integr. Environ. Assess. Manage.* **2011**, *7*, 513−541.

(2) Prevedouros, K.; Cousins, I. T.; Buck, R. C.; Korzeniowski, S. H. Sources, fate and transport of perfluorocarboxylates. *Environ. Sci. Technol.* **2006**, *40*, 32−44.

(3) Kissa, E. *Fluorinated Surfactants and Repellents*, 2 ed.; Marcel Dekker: New York, 2001.

(4) Paul, A. G.; Jones, K. C.; Sweetman, A. J. A first global production, emission, and environmental inventory for perfluorooctane sulfonate. *Environ. Sci. Technol.* **2009**, *43*, 386−392.

(5) Giesy, J. P.; Kannan, K. Perfluorochemical surfactants in the environment. *Environ. Sci. Technol.* **2002**, *36*, 146A−152A.

(6) D'eon, J. C.; Mabury, S. A. Is indirect exposure a significant contributor to the burden of perfluorinated acids observed in humans? *Environ. Sci. Technol.* **2011**, *45*, 7974−7984.

(7) Kannan, K.; Corsolini, S.; Falandysz, J.; Fillmann, G.; Kumar, K. S.; Loganathan, B. G.; Mohd, M. A.; Olivero, J.; Van Wouwe, N.; Yang, J. H.; Aldous, K. M. Perfluorooctanesulfonate and related fluorochemicals in human blood from several countries. *Environ. Sci. Technol.* **2004**, *38*, 4489−4495.

(8) Fromme, H.; Tittlemier, S. A.; Voelkel, W.; Wilhelm, M.; Twardella, D. Perfluorinated compounds - exposure assessment for the general population in western countries. *Int. J. Hyg. Environ. Health* **2009**, *212*, 239−270.

(9) Fei, C.; McLaughlin, J. K.; Tarone, R. E.; Olsen, J. Perfluorinated chemicals and fetal growth: a study within the Danish national birth cohort. *Environmental Health Perspectives.* **2007**, *115*, 1677−1682.

(10) Lopez-Espinosa, M.-J.; Mondal, D.; Armstrong, B.; Bloom, M. S.; Fletcher, T. Thyroid function and perfluoroalkyl acids in children living near a chemical plant. *Environmental Health Perspectives.* **2012**, *120*, 1036−1041.

(11) Grandjean, P.; Andersen, E. W.; Budtz-Jorgensen, E.; Nielsen, F.; Molbak, K.; Weihe, P.; Heilmann, C. Serum vaccine antibody concentrations in children exposed to perfluorinated compounds. *Jama-Journal of the American Medical Association.* **2012**, *307*, 391−397.

(12) Fisher, M.; Arbuckle, T. E.; Wade, M.; Haines, D. A. Do perfluoroalkyl substances affect metabolic function and plasma lipids?-analysis of the 2007−2009, Canadian Health Measures Survey (CHMS) cycle 1. *Environ. Res.* **2013**, *121*, 95−103.

(13) Miao, G.; Yan, Y.; Chuanmin, Z.; Naiying, L. Discussion on the relationship between normal hematocrit and geographical factors in China. *Biorheology* **1997**, *17*, 459−65.

(14) Ehresman, D. J.; Froehlich, J. W.; Olsen, G. W.; Chang, S.-C.; Butenhoff, J. L. Comparison of human whole blood, plasma, and serum matrices for the determination of perfluorooctanesulfonate (PFOS), perfluorooctanoate (PFOA), and other fluorochemicals. *Environ. Res.* **2007**, *103*, 176−184.

(15) Kato, K.; Wong, L. Y.; Jia, L. T.; Kuklenyik, Z.; Calafat, A. M. Trends in exposure to polyfluoroalkyl chemicals in the US population: 1999−2008. *Environ. Sci. Technol.* **2011**, *45*, 8037−8045.

(16) Frisbee, S. J.; Brooks, A. P., Jr.; Maher, A.; Flensborg, P.; Arnold, S.; Fletcher, T.; Steenland, K.; Shankar, A.; Knox, S. S.; Pollard, C.; Halverson, J. A.; Vieira, V. M.; Jin, C.; Leyden, K. M.; Ducatman, A. M. The C8 Health Project: design, methods, and participants. *Environmental Health Perspectives.* **2009**, *117*, 1873−1882.

(17) Olsen, G. W.; Lange, C. C.; Ellefson, M. E.; Mair, D. C.; Church, T. R.; Goldberg, C. L.; Herron, R. M.; Medhdizadehkashi, Z.; Nobiletti, J. B.; Rios, J. A.; Reagen, W. K.; Zobel, L. R. Temporal trends of perfluoroalkyl concentrations in American Red Cross adult blood donors, 2000−2010. *Environ. Sci. Technol.* **2012**, *46*, 6330−6338.

(18) Pan, Y.; Shi, Y.; Wang, J.; Cai, Y.; Wu, Y. Concentrations of perfluorinated compounds in human blood from twelve cities in China. *Environ. Toxicol. Chem.* **2010**, *29*, 2695−2701.

(19) Yeung, L. W. Y.; So, M. K.; Jiang, G. B.; Taniyasu, S.; Yamashita, N.; Song, M. Y.; Wu, Y. N.; Li, J. G.; Giesy, J. P.; Guruge, K. S.; Lam, P. K. S. Perfluorooctanesulfonate and related fluorochemicals in human blood samples from China. *Environ. Sci. Technol.* **2006**, *40*, 715−720.

(20) Zhang, T.; Wu, Q.; Sun, H. W.; Zhang, X. Z.; Yun, S. H.; Kannan, K. Perfluorinated compounds in whole blood samples from infants, children, and adults in China. *Environ. Sci. Technol.* **2010**, *44*, 4341−4347.

(21) Kärrman, A.; van Bavel, B.; Jarnberg, U.; Hardell, L.; Lindstrom, G. Perfluorinated chemicals in relation to other persistent organic pollutants in human blood. *Chemosphere* **2006**, *64*, 1582−1591.

(22) Hanssen, L.; Dudarev, A. A.; Huber, S.; Odland, J. O.; Nieboer, E.; Sandanger, T. M. Partition of perfluoroalkyl substances (PFASs) in whole blood and plasma, assessed in maternal and umbilical cord samples from inhabitants of Arctic Russia and Uzbekistan. *Sci. Total Environ.* **2013**, *447*, 430−437.

ED_002330_00120349-00006

(23) Loveless, S. E.; Finlay, C.; Everds, N. E.; Frame, S. R.; Gillies, P. J.; O'connor, J. C.; Powley, C. R.; Kennedy, G. L. Comparative responses of rats and mice exposed to linear/branched, linear, or branched ammonium perfluorooctanoate (APFO). *Toxicology* **2006**, *220*, 203−217.

(24) O'Brien, J. M.; Kennedy, S. W.; Chu, S. G.; Letcher, R. J. Isomer-specific accumulation of perfluorooctane sulfonate in the liver of chicken embryos exposed in ovo to a technical mixture. *Environ. Toxicol. Chem.* **2011**, *30*, 226−231.

(25) Benskin, J. P.; Bataineh, M.; Martin, J. W. Simultaneous characterization of perfluoroalkyl carboxylate, sulfonate, and sulfonamide isomers by liquid chromatography-tandem mass spectrometry. *Anal. Chem.* **2007**, *79*, 6455−6464.

(26) Beesoon, S.; Webster, G. M.; Shoeib, M.; Harner, T.; Benskin, J. P.; Martin, J. W. Isomer profiles of perfluorochemicals in matched maternal, cord, and house dust samples: manufacturing sources and transplacental transfer. *Environmental Health Perspectives.* **2011**, *119*, 1659−1664.

(27) Lemaire, M.; Tillement, J. P. Role of lipoproteins and erythrocytes in the *in vitro* binding and distribution of cyclosporin a in the blood. *J. Pharm. Pharmacol.* **1982**, *34*, 715−8.

(28) Gaver, R. C.; George, A. M.; Deeb, G. *In vitro* stability, plasma protein binding and blood cell partitioning of ^{14}C-carboplatin. *Cancer Chemother. Pharmacol.* **1987**, *20*, 271−6.

(29) Pang, K. S.; Barker, F.; Simard, A.; Schwab, A. J.; Goresky, C. A. Sulfation of acetaminophen by the perfused rat liver: The effect of red blood cell carriage. *Hepatology (Hoboken, NJ, U. S.)* **1995**, *22*, 267−82.

(30) Beesoon, S.; Genuis, S. J.; Benskin, J. P.; Martin, J. W. Exceptionally high serum concentrations of perfluorohexanesulfonate in a canadian family are linked to home carpet treatment applications. *Environ. Sci. Technol.* **2012**, *46*, 12960−12967.

(31) Reiner, J. L.; Phinney, K. W.; Keller, J. M. Determination of perfluorinated compounds in human plasma and serum standard reference materials using independent analytical methods. *Anal. Bioanal. Chem.* **2011**, *401*, 2899−2907.

(32) Yeung, L. W. Y.; Taniyasu, S.; Kannan, K.; Xu, D. Z. Y.; Guruge, K. S.; Lam, P. K. S.; Yamashita, N. An analytical method for the determination of perfluorinated compounds in whole blood using acetonitrile and solid phase extraction methods. *Journal of Chromatography A* **2009**, *1216*, 4950−4956.

(33) Ross, M. S.; Wong, C. S.; Martin, J. W. Isomer-specific biotransformation of perfluorooctane sulfonamide in Sprague-Dawley rats. *Environ. Sci. Technol.* **2012**, *46*, 3196−3203.

(34) Chan, E.; Sandhu, M.; Benskin, J. P.; Ralitsch, M.; Thibault, N.; Birkholz, D.; Martin, J. W. Endogenous high-performance liquid chromatography/tandem mass spectrometry interferences and the case of perfluorohexane sulfonate (PFHxS) in human serum; are we overestimating exposure? *Rapid Commun. Mass Spectrom.* **2009**, *23*, 1405−1410.

(35) Olsen, G. W.; Huang, H. Y.; Helzlsouer, K. J.; Hansen, K. J.; Butenhoff, J. L.; Mandel, J. H. Historical comparison of perfluorooctanesulfonate, perfluorooctanoate, and other fluorochemicals in human blood. *Environmental Health Perspectives.* **2005**, *113*, 539−545.

(36) Zhang, Y.; Jiang, W.; Fang, S.; Zhu, L.; Deng, J. Perfluoroalkyl acids and the isomers of perfluorooctanesulfonate and perfluorooctanoate in the sera of 50 new couples in Tianjin, China. *Environ. Int.* **2014**, *68*, 185−191.

(37) Wong, F.; MacLeod, M.; Mueller, J. F.; Cousins, I. T. Enhanced elimination of perfluorooctane sulfonic acid by menstruating women: evidence from population-based pharmacokinetic modeling. *Environ. Sci. Technol.* **2014**, *48*, 8807−8814.

(38) Zhang, Y.; Beesoon, S.; Zhu, L.; Martin, J. W. Biomonitoring of perfluoroalkyl acids in human urine and estimates of biological half-life. *Environ. Sci. Technol.* **2013**, *47*, 10619−10627.

(39) Hebert, P. C.; MacManus-Spencer, L. A. Development of a fluorescence model for the binding of medium- to long-chain perfluoroalkyl acids to human serum albumin through a mechanistic evaluation of spectroscopic evidence. *Anal. Chem.* **2010**, *82*, 6463−6471.

(40) Bischel, H. N.; MacManus-Spencer, L. A.; Zhang, C.; Luthy, R. G. Strong associations of short-chain perfluoroalkyl acids with serum albumin and investigation of binding mechanisms. *Environ. Toxicol. Chem.* **2011**, *30*, 2423−2430.

(41) Beesoon, S.; Martin, J. W. Isomer-specific binding affinity of perfluorooctanesulfonate (PFOS) and perfluorooctanoate (PFOA) to serum proteins. *Environ. Sci. Technol.* **2015**, *49*, 5722−5731.

(42) Ellis, D. A.; Denkenberger, K. A.; Burrow, T. E.; Mabury, S. A. The use of F-19 NMR to interpret the structural properties of perfluorocarboxylate acids: a possible correlation with their environmental disposition. *J. Phys. Chem. A* **2004**, *108*, 10099−10106.

(43) Kowalczyk, J.; Ehlers, S.; Oberhausen, A.; Tischer, M.; Fuerst, P.; Schafft, H.; Lahrssen-Wiederholt, M. Absorption, distribution, and milk secretion of the perfluoroalkyl acids PFBS, PFHxS, PFOS, and PFOA by dairy cows fed naturally contaminated feed. *J. Agric. Food Chem.* **2013**, *61*, 2903−2912.

(44) Zhang, Y.; Beesoon, S.; Zhu, L.; Martin, J. W. Isomers of perfluorooctanesulfonate and perfluorooctanoate and total perfluoroalkyl acids in human serum from two cities in North China. *Environ. Int.* **2013**, *53*, 9−17.

(45) Jiang, W.; Zhang, Y.; Yang, L.; Chu, X.; Zhu, L. Perfluoroalkyl acids (PFAAs) with isomer analysis in the commercial PFOS and PFOA products in China. *Chemosphere* **2015**, *127*, 180−187.

(46) Hanssen, L.; Roellin, H.; Odland, J. O.; Moe, M. K.; Sandanger, T. M. Perfluorinated compounds in maternal serum and cord blood from selected areas of South Africa: results of a pilot study. *J. Environ. Monit.* **2010**, *12*, 1355−1361.

(47) Liu, Y.; Pereira, A. S.; Beesoon, S.; Vestergren, R.; Berger, U.; Olsen, G. W.; Glynn, A.; Martin, J. W. Temporal trends of perfluorooctanesulfonate isomer and enantiomer patterns in archived Swedish and American serum samples. *Environ. Int.* **2015**, *75*, 215−222.

(48) Kärrman, A.; Langlois, I.; Bavel, B.; Lindstrom, G.; Oehme, M. Identification and pattern of perfluorooctane sulfonate (PFOS) isomers in human serum and plasma. *Environ. Int.* **2007**, *33*, 782−788.

(49) van Leeuwen, S. P. J.; de Boer, J. Extraction and clean-up strategies for the analysis of poly- and perfluoroalkyl substances in environmental and human matrices. *Journal of Chromatography A* **2007**, *1153*, 172−185.

(50) Han, X.; Snow, T. A.; Kemper, R. A.; Jepson, G. W. Binding of perfluorooctanoic acid to rat and human plasma proteins. *Chem. Res. Toxicol.* **2003**, *16*, 775−781.

(51) Olsen, G. W.; Burris, J. M.; Ehresman, D. J.; Froehlich, J. W.; Seacat, A. M.; Butenhoff, J. L.; Zobel, L. R. Half-life of serum elimination of perfluorooctanesulfonate, perfluorohexanesulfonate, and perfluorooctanoate in retired fluorochemical production workers. *Environmental Health Perspectives.* **2007**, *115*, 1298−1305.

(52) Wang, Z.; Xie, Z.; Moeller, A.; Mi, W.; Wolschke, H.; Ebinghaus, R. Atmospheric concentrations and gas/particle partitioning of neutral poly- and perfluoroalkyl substances in Northern German coast. *Atmos. Environ.* **2014**, *95*, 207−213.

(53) Ahrens, L.; Harner, T.; Shoeib, M.; Lane, D. A.; Murphy, J. G. Improved characterization of gas-particle partitioning for per- and polyfluoroalkyl substances in the atmosphere using annular diffusion denuder samplers. *Environ. Sci. Technol.* **2012**, *46*, 7199−7206.

(54) Brooke, D., Footitt, A.; Nwaogu, T. A. Environmental risk evaluation report: perfluorooctanesulphonate (PFOS). Environment Agency. UK., 2004.

(55) ATSDR. *Draft Toxicological Profile for Perfluoroalkyls*; United States Dept of Health and Human Services: Washington, DC, 2015; Http://www.Atsdr.Cdc.Gov/toxprofiles/tp.Asp?Id=1117&tid=237.

(56) Salvalaglio, M.; Muscionico, I.; Cavallotti, C. Determination of energies and sites of binding of PFOA and PFOS to human serum albumin. *J. Phys. Chem. B* **2010**, *114*, 14860−14874.

(57) Deruiter, J.*Carboxylic Acid Structure and Chemistry: Part 2*; Auburn university, 2005. Http://www.Auburn.Edu/~deruija/pda1_acids2.Pdf.

(58) Taniyasu, S.; Kannan, K.; Horii, Y.; Hanari, N.; Yamashita, N. A survey of perfluorooctane sulfonate and related perfluorinated organic

DOI: 10.1021/acs.est.6b01698
Environ. Sci. Technol. 2016, 50, 7808−7815

ED_002330_00120349-00007

compounds in water, fish, birds, and humans from Japan. *Environ. Sci. Technol.* **2003**, *37*, 2634−2639.

DOI: 10.1021/acs.est.6b01698
Environ. Sci. Technol. 2016, 50, 7808−7815

ED_002330_00120349-00008