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Perfluorooctanesulfonate and Related Fluorochemicals in Human Blood from Several Countries

KURUNTHACHALAM KANNAN,*,† SIMONETTA CORSOLINI,‡ JERZY FALANDYSZ,§ GILBERTO FILLMANN,|| KURUNTHACHALAM SENTHIL KUMAR,⊥ BOMMANNA G. LOGANATHAN,# MUSTAFA ALI MOHD,▽ JESUS OLIVERO,○ NATHALIE VAN WOUWE,☆ JAE HO YANG,@ AND KENNETH M. ALDOUS†

Wadsworth Center, New York State Department of Health, and Department of Environmental Toxicology and Health, State University of New York, Empire State Plaza, P.O. Box 509, Albany, New York 12201-0509, Dipartimento di Scienze Ambientali, Università di Siena, I-53100 Siena, Italy, Department of Environmental Chemistry and Ecotoxicology, University of Gdańsk, Gdańsk, Poland, Departemento de Oceanografia, Fundação Universidade Federal do Rio Grande, C.P. 474, Rio Grande RS 96201-900, Brazil, Shimadzu Techno-Research Inc., 1 Nishinokyo-Shimoaicho, Nakagyo-ku, Kyoto 604-8436, Japan, Department of Chemistry and Center for Reservoir Research, Murray State University, Murray, Kentucky 42071, Department of Pharmacology, Faculty of Medicine, University of Malaya, 50603 Kuala Lumpur, Malaysia, Environmental and Computational Chemistry Group, University of Cartagena, Cartagena, Colombia, Scientific Institute of Public Health, J. Wytsmanstreet, 14, 1050 Brussels, Belgium, and School of Medicine, Catholic University of Daegu, 3056-6 Daemyong-4-dong, Namgu, Daegu 705-718, Korea

Perfluorooctanesulfonyl fluoride based compounds have been used in a wide variety of consumer products, such as carpets, upholstery, and textiles. These compounds degrade to perfluorooctanesulfonate (PFOS), a persistent metabolite that accumulates in tissues of humans and wildlife. Previous studies have reported the occurrence of PFOS, perfluorohexanesulfonate (PFHxS), perfluorooctanoate (PFOA), and perfluorooctanesulfonamide (PFOSA) in human sera collected from the United States. In this study, concentrations of PFOS, PFHxS, PFOA, and PFOSA were measured in 473 human blood/serum/plasma samples collected from the United States, Colombia, Brazil, Belgium, Italy, Poland, India, Malaysia, and Korea. Among the four perfluorochemicals measured, PFOS was the predominant compound found in blood. Concentrations of PFOS were the highest in the samples collected from the United States and Poland (>30 ng/mL); moderate in Korea, Belgium, Malaysia, Brazil, Italy, and Colombia (3 to 29 ng/mL); and lowest in India (<3 ng/mL). PFOA was the next most abundant perfluorochemical in blood samples, although the frequency of occurrence of this compound was relatively low. No age- or gender-related differences in the concentrations of PFOS and PFOA were found in serum samples. The degree of association between the concentrations of four perfluorochemicals varied, depending on the origin of the samples. These results suggested the existence of sources with varying levels and compositions of perfluorochemicals, and differences in exposure patterns to these chemicals, in various countries. In addition to the four target fluorochemicals measured, qualitative analysis of selected blood samples showed the presence of other perfluorochemicals such as perfluorodecanesulfonate (PFDS), perfluoroheptanoic acid (PFHpA), perfluorononanoic acid (PFNA), perfluorodecanoic acid (PFDA), perfluorododecanoic acid (PFDoA), and perfluoroundecanoic acid (PFUnDA) in serum samples, at concentrations approximately 5- to 10-fold lower than the concentration of PFOS. Further studies should focus on identifying sources and pathways of human exposure to perfluorochemicals.

Introduction

Perfluorooctanesulfonate (PFOS) is known to be the end-stage metabolite of fluorochemicals produced using perfluorooctanesulfonylfluoride (POSF) as a precursor (*1*). POSF-based fluorochemicals have been used in a wide variety of industrial and consumer products, including protective coatings for carpets and apparel, paper coatings, insecticide formulations, and surfactants (*2*). Besides being formed as an end product of metabolism of several POSF-based fluorochemicals, PFOS is also used as a surfactant in fire-fighting foams (*3*). The presence of PFOS has been found to be widespread in the environment, wildlife, and humans (*4*−*11*).

While studies investigating the occurrence of organofluorine compounds in human tissues date back to the late 1960s (*12*), only recently has compound-specific analysis been performed on sera from employees in the fluorochemical manufacturing industry; PFOS and perfluorooctanoic acid (PFOA) were identified at concentrations of up to 12.8 and 114 μg/mL, respectively (*1*). Similarly, concentrations of PFOS and related fluorochemicals in the general population in the United States have been reported (*4*, *13*). Of particular concern is the occurrence of perfluorochemicals in the blood of children (*14*). The occurrence of PFOS and related fluorochemicals in humans in countries other than the United States is not well studied. The exposure levels and pathways leading to the presence of perfluorochemicals in humans can be better characterized by monitoring these compounds in human blood. In particular, analysis of blood from diverse countries will provide information on the geographical extent of human exposures to perfluorochemicals. In the present study, concentrations of PFOS, perfluorohexane sulfonate (PFHxS), PFOA, and perfluorooctanesulfonamide (PFOSA) were measured in blood of the general population from the United States, Colombia, Brazil, Italy, Belgium, Poland, India, Malaysia, and Korea. Our earlier data reporting the concentrations of perfluorochemicals in human blood from Japan

* Corresponding author phone: (518)474-0015; fax: (518)473-2895; e-mail: Kkannan@wadsworth.org.
† New York State Department of Health and State University of New York.
‡ Università di Siena.
§ University of Gdańsk.
|| Fundação Universidade Federal do Rio Grande.
⊥ Shimadzu Techno-Research Inc.
Murray State University.
▽ University of Malaya.
○ University of Cartagena.
☆ Scientific Institute of Public Health.
@ Catholic University of Daegu.

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TABLE 1. Details of Blood Samples Analyzed

country	location	no. of samples	date of collection	age (yrs): mean (min−max)	gender distribution	remarks
United States	Central Michigan	75 (sera)	Jun 2000	42 (17−72)	29 M, 46 F	suburban
	Murray, Kentucky	30 (whole blood)	Nov 2002	34 (20−68)	19 M, 11 F	suburban
	New York City	70 (plasma)	2002	NA[a]	NA	urban
Colombia	Cartagena	56 (whole blood)	May 2003	24 (20−29)	31 M, 25 F	industrial and port city, population ∼726,000
Brazil	Rio Grande	29 (whole blood)	Dec 2003	45 (18−74)	11 M, 17 F, 1 UK[a]	coastal and port city, southern Brazil, population ∼200,000
Italy	Siena	50 (sera)	Jan 2001	39 (20−59)	42 M, 8 F	population ∼60,000
Poland	Gdańsk	25 (whole blood)	Apr 2003	35−58	10 M, 15 F	port city, population ∼465,000
Belgium	Flanders, Wallonia	20 (plasma)	1998 and 2000	43 (19−63)	16 M, 4 F	several cities; suburban
India	Coimbatore	45 (sera)	Jan 2000	21 (17−48)	34 M, 11 F	agricultural and industrial area, population ∼1 million
Malaysia	Kuala Lumpur	23 (whole blood)	Mar2004	24 (21−26)	16 M, 7 F	urban
Korea	Daegu	50 (whole blood)	Jul 2003	47 (15−95)	25 M, 25 F	industrial area, population ∼3.5 million
Japan[b]	Yokohama and Tsukuba	38 (sera)	Mar and Jun 2002	33 (23−66)	25 M, 13 F	urban industrial area

[a] NA = Not available; UK = Unknown. [b] Data for Japan are from refs 15 and 16.

were also included for comparison (*15*, *16*). The purpose of this study was to characterize the geographical distribution of PFOS and related chemicals in human blood collected from both developed and developing countries. The influence of age and gender on fluorochemical concentrations in the blood has also been examined.

Materials and Methods

Blood samples were collected from the Red Cross or local hospitals or universities from volunteer donations. Samples were void of personal identifiers. The only known demographic factors were age, gender, sampling location, date of collection, smoking habits, and alcohol consumption of donor. Institutional Review Board (IRB) approvals were obtained for the analysis of blood samples. Details regarding donor's city of residence, sampling date, age, and gender are provided in Table 1. Serum was obtained from whole blood after clotting. Sera were analyzed for the samples from Michigan (U.S.), Italy, and India. However, since samples from Kentucky (U.S.), Colombia, Brazil, Poland, Malaysia, Korea, and Japan were treated with an anticoagulant, EDTA (∼1 mg/mL) or heparin, whole blood samples were analyzed for these sites. Only blood plasma was available from New York (U.S.) and Belgium. Samples were stored in polypropylene containers or vacutainers. All of the samples were kept at −20 °C until analysis.

For the purpose of comparison, whole-blood data were converted to a serum basis by multiplying by a factor of 2, based on the information obtained elsewhere (*15*, *16*). All data are reported on a serum basis except for Belgium and New York (U.S.), for which only plasma samples were analyzed.

PFOS, PFHxS, PFOA, and PFOSA were extracted using an ion-pairing extraction procedure and were determined by use of a high-performance liquid chromatograph (HPLC) with an electrospray tandem mass spectrometer (ES-MS/MS) (*13*). Both HPLC-MS/MS and HPLC-MS analyses were performed for some samples to compare and confirm the results. Approximately 0.5−1.0 mL of serum/whole blood, 1 mL of 0.5 M tetrabutylammonium hydrogen sulfate solution (adjusted to pH 10), and 2 mL of 0.25 M sodium carbonate buffer were added to a 15-mL polypropylene tube for extraction. After thorough mixing, 5 mL of methyl-*tert*-butyl ether (MTBE) was added, and the mixture was shaken for 20 min. The organic and aqueous layers were separated by centrifugation, and an exact volume of MTBE (4 mL) was removed from the solution. The aqueous mixture was rinsed with MTBE and separated twice; all rinses were combined in a second polypropylene tube. The solvent was allowed to evaporate under nitrogen before being reconstituted in 0.5−1.0 mL of methanol. The sample was vortexed for 30 s and passed through a 0.2-μm nylon mesh filter into an autosampler vial.

Analyte separation was performed using a Hewlett-Packard HP1100 HPLC. A 10 μL aliquot of the extract was injected onto a 50 × 2 mm (5 μm) Keystone Betasil C_{18} column with a 2 mM ammonium acetate/methanol mobile phase starting at 10% methanol. At a flow rate of 300 μL/min, the gradient increased to 100% methanol at 10 min before reverting to original conditions at 12 min. Column temperature was maintained at 20 °C. For quantitative determination, the HPLC system was interfaced to a Micromass (Beverly, MA) Quattro II atmospheric pressure ionization tandem mass spectrometer operated in the electrospray negative mode. Instrumental parameters were optimized to transmit the $[M - K]^-$ ion before fragmentation to one or more product ions. Cone voltage and collision energies were optimized for each analyte, and ranged from 35 to 90 V and 10 to 35 eV, respectively. Data were acquired by tandem mass spectrometry using multiple reaction monitoring for the transitions 499 > 99, 498 > 78, 399 > 80, and 413 > 369, for PFOS, PFOSA, PFHxS, and PFOA, respectively. When possible, multiple daughter ions were monitored for confirmation, but quantitation was based on a single product ion. In all cases, the capillary was held at 1 kV. Desolvation temperature and gas (nitrogen) flow were kept at 400 °C and 685 L/hr, respectively.

Potassium salts of PFOS (86.4%), PFHxS (99.9%), PFOA (98%), and PFOSA (95%) were provided by the 3M Company, St. Paul, MN. 1H,1H,2H,2H-perfluorooctanesulfonate (THPFOS; ICN, Costa Mesa, CA) and/or perfluorobutanesulfonate (PFBS; 99% purity, The 3M Company, St. Paul, MN) were used as internal standards and were spiked into blood samples prior to the addition of reagents for extraction. Recoveries of PFBS ranged from 75 to 120%. Reported concentrations were not corrected for the recoveries. Solvents, blood collection tubes, and method and matrix blanks were checked for the presence of the perfluorinated compounds analyzed in this study. Blanks contained PFOA and PFOS at concentrations less than 1 pg/mL. Attempts were

TABLE 2. Recoveries (%; mean ± SD) of Target Perfluorochemicals Spiked into Human Blood through the Analytical Procedure

source of blood	no. of samples	PFOS	PFHxS	PFOA	PFOSA
Michigan, U.S. (sera)	6	92 ± 10	87 ± 9	99 ± 6	92 ± 2
Italy (sera)	6	72 ± 24	82 ± 27	84 ± 27	44 ± 14
Brazil (whole blood)	6	115 ± 9	117 ± 10	NA[a]	55 ± 10
Korea (whole blood)	6	115 ± 7	86 ± 12	97 ± 26	80 ± 28
India (sera)	6	117 ± 14	106 ± 12	110 ± 20	110 ± 10

[a] NA: Not analyzed.

made to reduce the background levels of contamination in procedural blanks (discussed below). The limit of quantitation (LOQ) was determined based on the linear range of the calibration curve prepared at a concentration range of 0.5−100 ng/mL. Concentrations in samples that were at least 3-fold greater than the lowest acceptable standard concentration were considered to be valid. A curve point was deemed acceptable if (i) it was back-calculated to be within 30% of the theoretical value when evaluated versus the $^1/_x$ weighted curve, and (ii) the peak area of the standard was at least 3 times greater than that in the blank. Concentration/dilution factors are included in the calculation of the LOQ. The LOQs for PFOS, PFHxS, PFOA, and PFOSA varied from 1 to 1.3, from 1 to 1.3, from 3 to 20, and from 1.3 to 6 ng/mL, respectively. Extensive matrix spike studies were performed to evaluate the precision and accuracy of the analysis; the results are shown in Table 2. Thirty matrix spikes were performed in serum, whole blood, or plasma. The spike concentrations were prepared at ∼10−20 ng/mL levels of target analytes in the samples.

Results and Discussion

Quality Assurance/Quality Control. Mean (±standard deviation) recoveries of PFOS, PFHxS, PFOA, and PFOSA spiked into serum or whole blood are shown in Table 2. All of the target analytes were spiked into serum/blood prior to extraction. The recoveries of PFOS, PFHxS, and PFOA spiked into serum/blood were in the range of 72−117%. Mean recoveries of PFOSA spiked into serum/blood samples from Italy and Brazil were between 44 and 55%. PFOSA was not analyzed in plasma samples from New York (U.S.). However, mean recoveries of PFOSA spiked into serum samples from Michigan (U.S.), Korea, and India were in the range of 80−110%. The matrix-spike studies suggest that the data can be considered to be precise to within one standard deviation of the average fortified sample recovery.

THPFOS was used as a surrogate standard for samples collected from Michigan (U.S.), India, and Italy. However, we found a background level of ∼10 ng/mL of this compound in serum samples when they were monitored at an LC-MS/MS transition of 427 > 407. Procedural blanks did contain low levels of this compound, although the concentrations in serum samples were greater than the levels found in procedural blanks. Therefore, PFBS was used as a surrogate standard for the samples collected from Kentucky (U.S.), New York (U.S.), Brazil, Belgium, Poland, India, Malaysia, and Korea. PFBS was spiked at 15.5 ng/mL. PFBS, which has the same functional group as PFOS, was not found in solvent or procedural blanks. It has not been produced commercially until recently, and was not found in biological samples in an earlier study (*16*). Nevertheless, PFBS is being considered as a replacement for PFOS (*17*).

Trace levels of PFOS and PFOA have been reported to occur in procedural blanks (*4, 7, 8, 13, 18*). In this study, sources of contamination such as poly(tetrafluoroethylene) present in internal fluoropolymer parts of the HPLC and autosampler vial caps were replaced with polyethylene material. Methanol was injected between samples to avoid potential injection-port carryover. Further details of the analytical improvements to eliminate sources of contamination have been discussed in detail elsewhere (*19*).

Concentrations. Mean, median, and range concentrations of PFOS, PFHxS, PFOA, and PFOSA in serum samples from several countries are shown in Table 3. Mean and median concentrations have been presented on a serum basis by multiplying whole blood concentrations by a factor of 2 for the samples from Kentucky (U.S.), Colombia, Brazil, Poland, Malaysia, Korea, and Japan. Mean and median were calculated for those samples that contained concentrations above the LOQ. Therefore, the reported mean and median values will overestimate the actual values, particularly for those compounds that had a low frequency of occurrence in blood samples. The frequency of occurrence of target analytes was dependent on the LOQ for the particular set of analyses. Detection frequencies increased with a decrease in a compound's LOQ.

Among the four fluorochemicals, PFOS was the most frequently detected in samples from all countries investigated. The PFOS concentration in serum samples from the United States donors ranged from <1.3 to 164 ng/mL. Earlier studies have reported the occurrence of PFOS in United States serum samples at concentrations ranging from <4.3 to 1660 ng/mL (*4*). The highest PFOS concentration of 1660 ng/mL reported in that study was 10-fold greater than what was measured in our study. The median PFOS concentration of 35.8 ng/mL reported by Olsen and co-workers (*4*) for sera from six cities in the United States was comparable to what was found in our study (31.2 ng/mL). In addition, the narrow range of geometric means for PFOS concentration in sera from 645 donors from six cities in the United States (*4*) is consistent with what was found in our study. These results suggest that the exposure levels to PFOS among the general population of the United States are relatively uniform.

There was considerable variation by country in the concentrations of PFOS measured in sera. PFOS was found in all of the samples collected from Colombia, Brazil, Belgium, Italy, Poland, Malaysia, and Korea. However, only 51% of the samples from India contained PFOS at concentrations greater than 1 ng/mL. The mean PFOS concentration in serum samples collected from the United States and Poland was greater than 30 ng/mL, the highest among the samples analyzed in this study. The mean concentrations of PFOS in serum/plasma from Japan, Korea, Malaysia, Belgium, and Brazil were between 10 and 25 ng/mL, and the means from Italy and Colombia were between 4 and 10 ng/mL. Serum samples from India contained the lowest mean concentrations of PFOS (<3 ng/mL). When the values below the LOQ were treated as zeroes, the mean PFOS concentration in Indian sera was 1 ng/mL, which was approximately 20−30 times lower than mean levels in sera from the United States. Concentrations of PFOS in sera from Italy, Brazil, and Colombia were approximately 5−10 times lower than levels found in the sera of donors in the United States. These results suggest that the magnitude of exposure to PFOS is variable among the countries. Prolonged use of perfluorochemicals for a wide variety of applications, such as paper and packing products, residential and mill-applied carpet spraying, stain-resistant textiles, and cleaners, may be a major source of

TABLE 3. Perfluorochemical Concentrations in Sera (ng/mL) from Various Countries Stratified by Donor Gender[a]

gender (no. of samples)		PFOS	PFHxS	PFOA	PFOSA
		Michigan (U.S.)			
female	mean[b]	32.5	3.6	4.7	3.7
($n = 46$)	median	28.9	2.8	4.4	2.2
	range	<1.3−91.7	<1.3−13.2	<3−7.3	<1.3−23.5
	% positive	91	85	46	44
male	mean	32.9	4.3	5.7	3.0
($n = 29$)	median	26.2	3.3	4.4	2.9
	range	<1.3−124	<1.3−13.6	<3−14.7	<1.3−6.1
	% positive	93	76	45	52
		Kentucky (U.S.)			
female	mean	66	4.2	23	4.7
($n = 11$)	median	81	1.1	20	3.1
	range	11−130	<1−32	15−39	1.3−20
	% positive	100	55	100	100
male	mean	73.2	4.0	41.6	6.6
($n = 19$)	median	72.0	2.2	38.1	4.0
	range	19−164	<1−20	11−88	1.5−26
	% positive	100	95	100	100
		New York City (U.S.)			
UK[c]	mean	42.8	4.1	27.5	NA[c]
($n = 70$)	median	42	2.9	25.2	NA
	range	16−83	0.2−23	14−56	NA
	% positive	100	100	100	NA
		Colombia			
female	mean	8.0	0.2	6.1	1.4
($n = 25$)	median	7.3	0.2	5.6	0.9
	range	4.6−13	<0.4	3.7−9.2	<0.4−3.8
	% positive	100	0	100	96
male	mean	8.5	0.2	6.2	1.7
($n = 31$)	median	8.1	0.2	5.9	1.4
	range	6.2−14	<0.4−0.9	3.9−12.2	0.4−5.6
	% positive	100	10	100	100
		Brazil			
female	mean	10.7	5.4	<20	0.7
($n = 17$)	median	8.4	2.2	<20	0.7
	range	4.3−35	<0.6−15.3	<20	<0.4−1
	% positive	100	52.9	0	17.6
male	mean	13.5	1.0	<20	1.5
($n = 10$)	median	12.7	0.8	<20	1.7
	range	6.8−24	0.6−1.9	<20	<0.4−2.3
	% positive	100	100	0	30
		Italy			
female	mean	4.4	1.3	<3	1.7
($n = 8$)	median	3.5	1.3	<3	1.7
	range	<1−8	<1−1.4	<3	<1.3−1.7
	% positive	87.5	37.5	0	12.5
male	mean	4.3	1.7	<3	1.8
($n = 42$)	median	4.2	1.7	<3	1.6
	range	<1−10.3	<1−2.1	<3	<1.3−2.3
	% positive	90.5	33.0	0	9.5
		Poland			
female	mean	33.3	1.3	21.9	2.3
($n = 15$)	median	33.8	1.2	23.2	1.6
	range	16−60	0.5−2.6	9.7−34	0.4−7.7
	% positive	100	100	100	100
male	mean	55.4	1.3	20.5	1.7
($n = 10$)	median	40.9	1.2	18.4	1.0
	range	21−116	<0.4−1.8	11−40	<0.4−4.4
	% positive	100	90	100	90

gender (no. of samples)		PFOS	PFHxS	PFOA	PFOSA
		Belgium			
female	mean	11.1	<1	4.1	<3
($n = 4$)	median	10.4	<1	2.4	<3
	range	4.9−19	<1	<1−7.6	<3
	% positive	100	0	75	0
male	mean	16.8	1.3	5.0	<3
($n = 16$)	median	17.6	1.2	4.3	<3
	range	4.5−27	<1−1.4	1.1−13	<3
	% positive	100	50	100	0
		India			
female	mean	2.3	1.6	<3	<3
($n = 11$)	median	2.5	1.6	<3	<3
	range	<1−3	<1−1.8	<3	<3
	% positive	55	36	0	0
male	mean	1.7	1.6	3.5	<3
($n = 34$)	median	1.3	1.5	3.5	<3
	range	<1−3.1	<1−2.9	<3−3.5	<3
	% positive	50	41	3	0
		Malaysia			
female	mean	11.7	2.4	<10	3.8
($n = 7$)	median	12.7	2.3	<10	4.1
	range	7.6−17	1.2−4.2	<10	1.3−6.0
	% positive	100	100	0	100
male	mean	13.2	1.8	<10	4.9
($n = 16$)	median	13.1	1.4	<10	3.9
	range	6.2−18.8	1.2−6.8	<10	1.4−11
	% positive	100	100	0	100
		Korea			
female	mean	15.1	3.8	88.1	1.1
($n = 25$)	median	11.3	2.9	30.9	1.1
	range	3.0−61.3	0.9−20	<15−256	<0.1−2.1
	% positive	100	100	19	96
male	mean	27.1	4.1	35.5	1.5
($n = 25$)	median	21.7	3.4	26.8	1.3
	range	6.6−92	1.3−9.6	<15−71.4	0.4−7.2
	% positive	100	100	25	100
		Japan[d]			
female	mean	20.1	3.3	12.3	3.3
($n = 13$)	median	18.3	3.3	12.3	5.8
	range	6.3−40.3	<2.6−4.7	<6.8−12.3	<2.6−7.1
	% positive	100	23	8	44
male	mean	14.1	4.2	<6.8	6.2
($n = 25$)	median	12.4	3.7	<6.8	5.8
	range	4.1−38	<2.6−7.6	<6.8	<2.6−9.5
	% positive	100	27	0	38

[a] Whole-blood data were converted to a serum basis by multiplying by a factor of 2. [b] Samples with values below the limits of quantitation were excluded in the calculation of mean and median. [c] NA = Not analyzed. UK = Unknown. [d] Values for Japan are from refs 15 and 16.

human exposure to these compounds. For instance, occurrence of sulfonated perfluorochemicals in indoor air of carpeted homes in Canada and in a vacuum-cleaner dust from Japan has been reported recently (*20*, *21*), suggesting the occurrence of sources of PFOS and its precursor molecules in the indoor environment. Use of carpets and specialty paper products (e.g., folding cartons for snack foods) is widespread in developed nations such as the United States, whereas it is minimal in India.

The mean concentration of PFOS measured in plasma from Belgium (15.7 ng/mL) was comparable to the concentration reported earlier for five pooled samples of sera from a blood bank in Belgium (17 ng/mL) (*2*). Concentrations of PFOS in pooled samples of sera from The Netherlands and Germany were 53 and 37 ng/mL, respectively (*2*). The concentrations that were reported for The Netherlands and Germany are similar to those from the samples from the United States and Poland analyzed in our study.

It should be noted that the samples collected in our study were from selected cities, and thus the data may not represent the entire population of a country. Nevertheless, the samples were collected randomly from a diverse donor age group,

and they represent diverse occupational and/or exposure histories. We believe that, given the general consistency of PFOS concentrations found for the samples from the United States (where the largest number of samples thus far has been analyzed), the data obtained in our study for other countries should provide reasonable representation of the respective populations. Our study was not intended as an epidemiological investigation, but rather its purpose was to use human blood as a matrix to evaluate the geographical extent of perfluorochemicals exposures in humans.

Concentrations and frequency of occurrence of PFHxS, PFOA, and PFOSA in serum were relatively lower than those of PFOS. Several serum samples from Korea, however, contained relatively higher concentrations of PFOA than PFOS. This suggests the presence of specific sources of exposure to PFOA in Korea. In particular, sera from two of the female donors from Korea showed PFOA concentrations greater than 100 ng/mL. This value is greater than the highest concentration that was reported for serum samples from the general population of the United States (*4*). Concentrations of PFOA were greater than those of PFHxS and PFOSA in sera from the United States, Colombia, Poland, Belgium, and Japan. In general, concentrations of PFOA in serum were 2- to 7-fold lower than the PFOS concentrations. Serum samples collected from Siena, Italy, did not contain PFOA, at a detection limit of 3 ng/mL. Similarly, only one of the 45 samples from India contained PFOA at 3.5 ng/mL. The median concentration of PFOA in sera collected during 2000−2001 in six cities in the United States was 4.4 ng/mL (*4*), a concentration 2- to 3-fold lower than the concentration measured in our study (11 ng/mL). Concentrations of PFOA found for whole blood samples collected from Kentucky (U.S.) and for blood plasma from New York (U.S.) were higher than the concentrations reported earlier (*4*). The difference could be due to the differing methods of deriving mean concentrations in the two studies. Olsen and co-workers (*4*) used the midpoint value between zero and the LOQ to replace nondetects, whereas we excluded nondetects from the calculation of means. However, all of the samples from Kentucky and New York showed concentrations above the LOQ for PFOA. Differences in the matrices analyzed (whole blood/plasma in our study versus serum in Olsen's study) or exposure histories may also have contributed to the variations in concentrations. The median concentrations of PFOA in Polish and Korean serum samples were 21 and 28 ng/mL, respectively; these concentrations are approximately 2-fold greater than the concentrations found for the plasma samples from the United States.

Concentrations of PFHxS were approximately 10- to 20-fold lower than those of PFOS in sera from the United States and Japan. Serum samples from Colombia and Poland showed much lower concentrations of PFHxS, relative to PFOS concentrations. In contrast, the ratios of PFHxS to PFOS concentrations in serum samples from India and Italy were higher (1:0.3−0.9) than those found for other countries. The differences in the relative concentrations of PFHxS and PFOS may be due to exposure to sources that contain greater proportions of PFHxS. PFHxS is an impurity in POSF-based products. In addition, perfluorohexanesulfonyl fluoride (PHSF)-based compounds are used in fire-fighting foams and post-market carpet treatment applications (*4*). PFHxS is a breakdown product of compounds that are based on PHSF. Median concentrations of PFHxS in sera from the United States, Korea, and Japan were in the range of 1.5−3 ng/mL, higher than concentrations found in other countries. The median concentration of PFHxS in sera from six cities in the United States was reported to be 1.5 ng/mL (*4*), comparable to the concentration found in our study.

PFOSA was found in sera from Kentucky (U.S.), Colombia, and Poland. A PFOSA concentration as high as 26 ng/mL was found in a serum sample from Kentucky. Few earlier studies have reported the occurrence of PFOSA in human blood. PFOSA had not been found in any of the 645 serum samples collected in the United States (*4*) at a detection limit of 3.2 ng/mL. In our study, however, a lower detection limit (1.3 ng/mL) allowed the identification and quantification of PFOSA in serum. PFOSA is a metabolic product of *N*-methyl- and *N*-ethyl- perfluorooctanesulfonamidoethanol, which are used primarily in surface treatment applications on textiles or papers. PFOSA can be further metabolized to PFOS in mammals and fish (*22*). PFOSA can also be formed as a metabolic product of *N*-ethyl perfluorooctanesulfonamide, commonly known as Sulfluramid, an insecticide used to control cockroaches, termites, and ants. Occurrence of PFOSA in certain serum samples may indicate recent exposure to *N*-methyl- and/or *N*-ethyl- perfluorooctanesulfonamidoethanol used in consumer products.

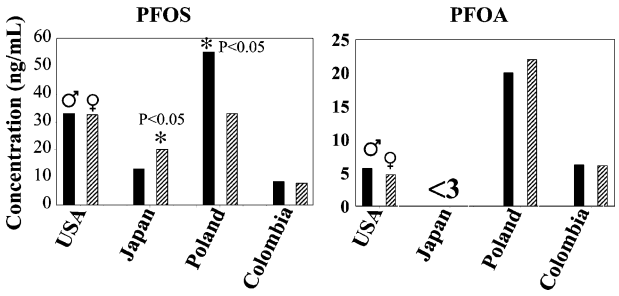

FIGURE 1. Mean concentrations of PFOS and PFOA (ng/mL) in male and female donors from Michigan (U.S.), Japan, Poland, and Colombia. Whole-blood values from Japan, Poland, and Colombia were converted to serum basis by 2×. Values below the LOQs are not included in the calculation of mean.

Gender- and Age-Related Accumulation. In general, no significant difference ($p > 0.05$) in the concentration of either PFOS or PFOA was found between the sexes (Figure 1). However, serum samples from Japan contained significantly higher concentrations of PFOS in female donors, whereas samples from Poland contained higher concentrations in male donors. This difference is probably not genuinely gender-related, but rather may be due to other confounding factors, such as the number of samples analyzed and the frequency of analyte detection. Overall our results are similar to those of an earlier study, which reported no gender-related differences in the concentrations of PFOS or PFOA (*4*). Earlier, no apparent gender difference in the concentrations of PFOS and PFOA was found in birds or marine mammals (*7*−*11*).

Influence of age of donors on the concentrations of PFOS and PFOA was examined. A lack of association was found for serum samples from all of the countries investigated, as exemplified by PFOS or PFOA concentrations and age of donors from Michigan (U.S.) in Figure 2. A similar lack of age-related increase was found for PFOSA and PFHxS concentrations in sera. This is different from the trend observed for neutral, lipophilic contaminants such as polychlorinated biphenyls (PCBs), for which concentrations in adult females are significantly lower than those in adult males, due to the transfer of these contaminants to offspring via parturition and lactation. Lack of gender- and age-related accumulation patterns of perfluorochemicals in humans may be related to these compounds' affinity for lipoproteins rather than neutral lipids (*23*).

Relationships Among Perfluorochemicals. A significant relationship ($p < 0.05$) was found between PFOS and PFHxS concentrations in sera from donors in Michigan (U.S.) (Figure 3) and Poland. These results suggest that the sources of exposures may be correlated. However, such a relationship was not statistically significant ($p > 0.05$) for samples from Kentucky (U.S.), Colombia, and Japan. Similarly, a significant

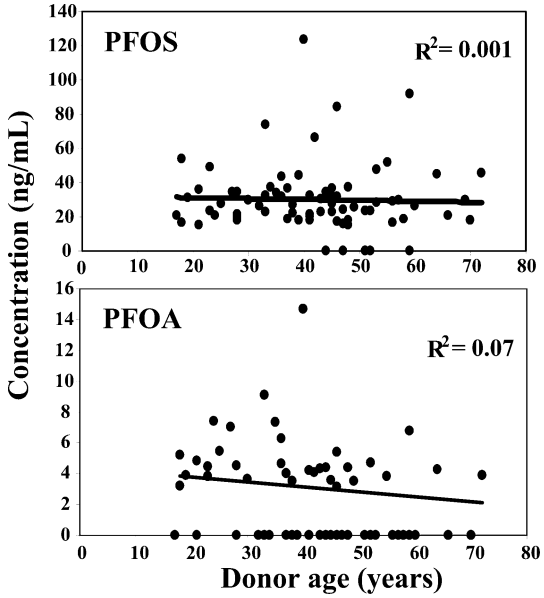

FIGURE 2. Relationship of PFOS and PFOA concentrations (ng/mL) in sera with age of donors from Michigan (U.S.) (values below the LOQ were treated as zero).

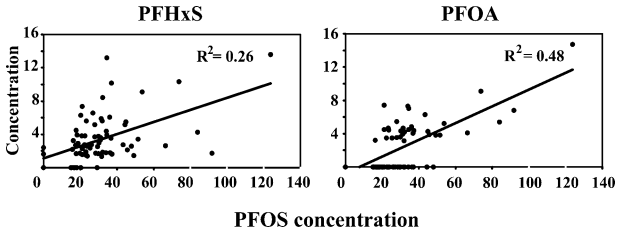

FIGURE 3. Relationship between PFOS and PFHS or PFOA concentrations (ng/mL) in sera of donors from Michigan (U.S.) (values below the LOQs were assigned zero).

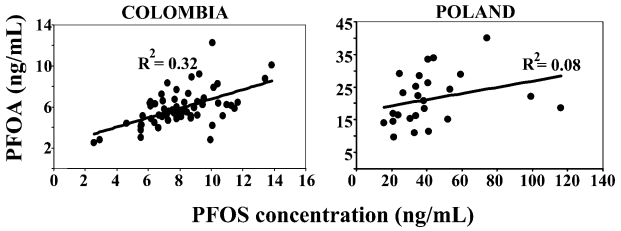

FIGURE 4. Relationship between PFOS and PFOA concentrations (ng/mL) in sera of Colombian and Polish donors (values below the LOQs were assigned zero; concentrations are presented on a serum basis).

correlation between PFOS and PFOA concentrations was found for sera from Michigan (U.S.) donors (Figure 3). However, when values below the LOQ were included in the analysis, the significant correlation was no longer found. Moreover, a significant relationship between PFOS and PFOA concentrations was found for sera from Colombian donors, but not for Polish donors (Figure 4). These results suggest varying and inconsistent degrees of association between different perfluorochemicals in human sera. The existence of a multitude of sources, with varying levels and compositions of perfluorochemicals, and varying exposure patterns in different countries, are causes for such variations in relationships. The association between PFOS and PFOA is of interest, given that we wish to be able to identify potential sources of exposures. Perfluorooctanesulfonamides and PFOS cannot convert directly into PFOA, or vice versa (*22*). The apparent associations may therefore be due to co-exposures to these compounds present in various products.

Other Perfluorochemicals. Selected samples were screened for the presence of other perfluorinated compounds in addition to the four target fluorochemicals analyzed in this study. Perfluorodecanesulfonate (PFDS; LC-MS/MS transition of 599 > 99) was found in serum samples collected from the United States, Poland, and Belgium. However, an analytical standard for PFDS was not available for quantification. A semiquantitative estimate of PFDS concentrations in serum samples suggested that the levels were comparable to those of PFHxS. PFDS is an impurity in POSF-based products. Perfluoroheptanoic acid (PFHpA; 363 > 319), perfluorononanoic acid (PFNA; 463 > 419), and perfluorodecanoic acid (PFDA; 513 > 469) were commonly detected, each at a concentration in the range of 0.1−2 ng/mL, in the plasma samples collected from New York (U.S.). Some of these long-chain perfluorocarboxylates have been reported to be found in wildlife tissues (*18*). In addition to these, earlier studies have reported the occurrence of *N*-ethyl perfluorooctanesulfonamidoacetate (PFOSAA; $C_8F_{17}SO_2N$-$(CH_2CH_3)CH_2COO^-$), *N*-methyl perfluorooctanesulfonamidoacetate (M570; $C_8F_{17}SO_2N$ $(CH_3)CH_2COO^-$), and perfluorooctanesulfonamidoacetate (M556; $C_8F_{17}SO_2NHCH_2COO^-$), which are oxidation products of perfluorochemical mixtures used in paper-protectant and surface treatment applications (*4*). Concentrations of these three oxidation products were, on average, 10−20 times lower than the concentrations of PFOS. These results suggest the coexistence of several perfluorochemicals in human blood. Hazard assessment for perfluorochemicals in humans should take into consideration all of these compounds and their co-occurrence. Further studies should focus on sources and pathways of human exposure to these compounds.

Acknowledgments

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