Decline in Perfluorooctanesulfonate and Other Polyfluoroalkyl Chemicals in American Red Cross Adult Blood Donors, 2000−2006

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In 2000, 3M Company, the primary global manufacturer, announced a phase-out of perfluorooctanesulfonyl fluoride (POSF, $C_8F_{17}SO_2F$)-based materials after perfluorooctanesulfonate (PFOS, $C_8F_{17}SO_3^-$) was reported in human populations and wildlife. The purpose of this study was to determine whether PFOS and other polyfluoroalkyl concentrations in plasma samples, collected in 2006 from six American Red Cross adult blood donor centers, have declined compared to nonpaired serum samples from the same locations in 2000−2001. For each location, 100 samples were obtained evenly distributed by age (20−69 years) and sex. Analytes measured, using tandem mass spectrometry, were PFOS, perfluorooctanoate (PFOA), perfluorohexanesulfonate (PFHxS), perfluorobutanesulfonate (PFBS), *N*-methyl perfluorooctanesulfonamidoacetate (Me-PFOSA-AcOH), and *N*-ethyl perfluorooctanesulfonamidoacetate (Et-PFOSA-AcOH). The geometric mean plasma concentrations were for PFOS 14.5 ng/mL (95% CI 13.9−15.2), PFOA 3.4 ng/mL (95% CI 3.3−3.6), and PFHxS 1.5 ng/mL (95% CI 1.4−1.6). The majority of PFBS, Me-PFOSA-AcOH, and Et-PFOSA-AcOH concentrations were less than the lower limit of quantitation. Age- and sex-adjusted geometric means were lower in 2006 (approximately 60% for PFOS, 25% for PFOA, and 30% for PFHxS) than those in 2000−2001. The declines for PFOS and PFHxS are consistent with their serum elimination half-lives and the time since the phase-out of POSF-based materials. The shorter serum elimination half-life for PFOA and its smaller percentage decline than PFOS suggests PFOA concentrations measured in the general population are unlikely to be solely attributed to POSF-based materials. Direct and indirect exposure sources of PFOA could include historic and ongoing electrochemical cell fluorination (ECF) of PFOA, telomer production of PFOA, fluorotelomer-based precursors, and other fluoropolymer production.

Introduction

In 2000, the 3M Company (3M) announced that, as the primary global manufacturer, it would phase-out perfluorooctanesulfonyl fluoride (POSF, $C_8F_{17}SO_2F$)-based materials after perfluorooctanesulfonate (PFOS, $C_8F_{17}SO_3^-$) was reported to be found in human populations and wildlife (*1*, *2*). At the time of the phase-out, 3M reported approximately 8 million pounds of annual production of POSF-based equivalents. By the end of 2000, there was a 98% reduction in the electrochemical cell production of POSF and complete cessation occurred by the end of 2002. Commercial uses of these POSF-based materials included surface treatments for soil- and stain-resistant coatings on fabrics, carpets, and leather, coatings on paper and packaging products for grease and oil resistance, including food contact papers, and performance chemicals for a variety of applications (*1*).

Many human biomonitoring studies of polyfluoroalkyl chemicals have examined primarily blood samples collected prior to and/or during the phase-out time period (*1*). Several years have elapsed since the phase-out of POSF-based materials and at question is whether a decline has occurred in general population blood levels for PFOS and related polyfluoroalkyl chemicals. A pilot study showed an approximate 50% decline in PFOS in 40 serum samples collected in 2005 from American Red Cross adult blood donors from Minneapolis-St. Paul compared to 2000−2001 data (*3*). Calafat et al. (*4*) reported 35%, 25%, and 10% declines in the geometric means of PFOS, PFOA, and perfluorohexanesulfonate (PFHxS, $C_6F_{13}SO_3^-$) from United States Centers for Disease Control and Prevention (CDC) Nutritional Health and Examination Survey (NHANES) participants (n = 2,094), whose blood samples were collected in 2003−2004, compared to 1,562 nonpaired samples collected in 1999−2000.

The purpose of the present study was to compare age-, sex-, and location-specific PFOS and other polyfluoroalkyl concentrations in 600 plasma samples collected in 2006 from six American Red Cross adult blood donor centers to 645 nonpaired serum samples analyzed from the same locations in 2000−2001 (*5*). Plasma and serum have been shown to be comparable matrices for these analyses (*6*).

Materials and Methods

Sample Collection. Through collaboration with six American Red Cross blood donor centers between April and July 2006, 600 plasma samples (301 male, 299 female) were obtained from donors aged 20 to 69 years. Each center provided 100 plasma samples: 10 samples per every 10-year age interval (20−29 through 60−69) for each sex. Each 1 mL plasma

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sample was stored at −20 °C in a plastic tube until laboratory analysis. The six blood donor centers represented the following areas: Boston, Massachusetts; Charlotte, North Carolina; Hagerstown, Maryland; Los Angeles, California; Minneapolis-St. Paul, Minnesota; and Portland, Oregon. Approval for this study was obtained from the American Red Cross Biomedical Services Institutional Review Board. Samples were void of personal identifiers. The only available demographic factors were age, sex, and location.

Reference Materials. The six target analytes quantified in this study were PFOS, PFOA, PFHxS, perfluorobutanesulfonate (PFBS, $C_4F_9SO_3^-$), *N*-methyl perfluorooctanesulfonamidoacetate (Me-PFOSA-AcOH, $C_8F_{17}SO_2N(CH_3)$-CH_2COO^-), and *N*-ethyl perfluorooctanesulfonamidoacetate (Et-PFOSA-AcOH, $C_8F_{17}SO_2N(CH_2CH_3)CH_2COO^-$). The well-characterized reference materials used to make the calibration standards were of 86.4%, 95.0%, 98.6%, 96.7%, 98.9%, and 98.6% purity, respectively and were characterized for purity and degree of isomer branching using ^{1}H NMR, ^{19}F-NMR, and LC-MS/MS. All reference materials were produced by 3M. Isotopically labeled reference standards were not available for all of the target analytes. Prior to the study, six lots of human plasma were purchased (Lampire Biological Laboratories, Pipersville, PA) and subsequently screened to determine which lot contained the lowest levels of endogenous target analytes. To account for matrix effects, differences in extraction efficiency, and to eliminate response factor variation introduced by using an eight-carbon internal standard to quantify target analytes ranging in chain length from four to eight carbons, matrix-matched external calibration curves were constructed using nonlabeled reference standards and the prescreened lot of purchased human plasma. As an additional quality control element, $^{18}O_2$-ammonium perfluorobutanesulfonate (^{18}O-PFBS, $C_4F_9S[^{18}O_2]O^-NH_4^+$) (RTI International, Research Triangle Park, NC) was spiked into each sample as well as the matrix-matched human plasma calibration curve prior to extraction. The ^{18}O-PFBS was not used to quantify the samples, rather it was used as a surrogate to monitor extraction efficiency and method performance for individual samples. If the percent recovery of the individual sample surrogate spike fell outside of the 100% ± 15% acceptance criterion, the sample was re-extracted and reanalyzed.

Sample Preparation. The 3M Environmental Laboratory prepared and analyzed the plasma samples. Target analytes were extracted from plasma or serum by protein precipitation in acetonitrile (*8*) via a MultiPROBE II HT EX robotic liquid handling system (PerkinElmer, Wellesley, MA) utilizing 96-well plates. Additional details are found in the Supporting Information.

HPLC/MS/MS. Quantitation of sample extracts was accomplished by high-performance liquid chromatography tandem mass spectrometry (HPLC/MS/MS) using an Applied Biosystems MDS Sciex API 5000 triple-quadrupole mass spectrometer equipped with a TurboIon Spray Source (PE Sciex, Concord, Ontario, Canada) maintained at 450 °C in the negative ion mode. All six target analytes were monitored in a single run recording multiple transitions for each ion. Benskin et al. reported that PFHxS analyses of human blood could suffer interference from endogenous steroid sulfates (*9*). However, by changing LC solvents from methanol to acetonitrile, we were able to separate the endogenous interferents from the PFHxS peak(s), allowing for accurate quantitation. Additional details, including the ions monitored, are provided in the Supporting Information.

Calibration, Accuracy, and Precision. External calibration with control human plasma was used for quantitation of the human plasma samples. The ^{18}O-PFBS internal standard was spiked into all samples and the human plasma calibration curve prior to extraction, and was used as a surrogate to monitor extraction efficiency. As a cross validation, PFOS, PFOA, PFHxS, PFBS, Me-PFOSA-AcOH, and Et-PFOSA-AcOH were matrix spiked into control human plasma and control rabbit plasma to demonstrate analytical method accuracy and precision (USFDA, 2001). Data accuracy and precision of 99.2% ± 11.7% (for PFOS), 101% ± 16.6% (for PFOA), 98.3% ± 9.7% (for PFHxS), 97.8% ± 9.9% (for PFBS), 103% ± 23.7% (for Me-PFOSA-AcOH), and 111% ± 33.4% (for Et-PFOSA-AcOH) were determined by analyzing two levels of QC samples in replicates of three in control human plasma for each analytical batch, and were demonstrated intrabatch and between batch over a 25−80 ng/mL range. Practical lower limits of quantitation (LLOQ) were established for the plasma samples as the lowest standard on the calibration curve where the LLOQ was at least two times the method blank. Analytical method LLOQs were established via prior method validations (*5, 7*).

Quality Assurance. For quality assurance purposes, 60 samples were randomly chosen to be reanalyzed from the 600 American Red Cross samples collected in 2006. A total of 78 American Red Cross samples, collected and analyzed in 2000−2001 (*5*), were reanalyzed using the 2006 methods. The highest and lowest 10 concentrations for PFOS and PFOA samples, as originally measured, were included. The remaining selected for reanalysis was a random sample. Likewise, among the 356 Hagerstown (Washington County), Maryland samples, collected in 1974 and 1989 and originally analyzed by Olsen et al. (*8*), 70 samples were reanalyzed using the 2006 analytical methods. Again, the 10 highest and lowest concentrations for PFOS and PFOA samples, as originally measured, were included. The remainder was randomly sampled. Evaluation of quality control samples injected during the analytical runs of the original studies had indicated that the reported quantitative results may have varied ±10% for precision and accuracy (*5, 8*). The samples collected and analyzed earlier had been stored in plastic vials at −80 °C until their reanalysis in 2006.

Data Analysis. Parametric (Student *t* test) and nonparametric (Wilcoxon matched-pair signed-rank test) methods were used to compare mean fluorochemical concentrations measured in 60 samples divided into two (i.e., split samples) collected in 2006. Similar quality assurance analyses were performed with a reanalysis of 78 American Red Cross samples collected in 2000−2001 (*5*) using the 2006 analytical methods as well as with the 1974 and 1989 Hagerstown samples ($n = 70$) (*8*).

For the 600 American Red Cross plasma samples collected in 2006, measures of central tendency applicable to log-normally distributed data (e.g., geometric mean) were used in the descriptive analysis. Any measured sample reported as <LLOQ was calculated to be the LLOQ divided by the square root of two. This method should not bias the variance when LLOQ samples are few (*10*), which was the situation for PFOS, PFOA, and PFHxS but not for Me-PFOSA-AcOH, Et-PFOSA-AcOH, and PFBS. For comparison purposes, the 2000−2001 American Red Cross and 1974 and 1989 Hagerstown data when reported as <LLOQ by Olsen et al. (*5, 8*), were also recalculated to be the LLOQ divided by the square root of two.

To compare the means overall and within location between 2000 and 2006, while adjusting for age and sex, the general linear model with a logarithmic link function and normal error was specified as follows:

$$\ln[\mathrm{PFC}] = \mathbf{Location} \times \beta_c + \mathrm{Year} \times \beta_y + \mathbf{Age} \times \beta_a + \mathrm{Sex} \times \beta_s + \mathrm{Sex} \times \mathbf{Age} \times \beta_{sa} + \epsilon$$

where PFC is the metabolite of interest, **Location** and **Age** are vectors indicating location and age category, respectively, Year and Sex are indicator variables, and ϵ is a normally distributed error term with mean zero. The model was fit to

1000 bootstrap samples for each metabolite, and the maximum likelihood estimates of means were contrasted using two-sided 95% confidence intervals that were constructed using the bias-corrected, accelerated bootstrap method (*11*). All the generalized linear models were fit using maximum likelihood in the S-Plus (S-Plus 7, Insightful Corporation, 2005) program "lm" and the bootstrap estimates were generated using the S-Plus program "bootstrap".

To minimize parametric assumptions in the estimation of extreme percentiles of the population, the bias-corrected, accelerated bootstrap method (*11*) with 3000 replications was used to generate confidence intervals around the empirical percentiles for serum concentrations of the analyzed metabolites. The bias-correction factor is derived by comparing empirical percentiles to bootstrap percentiles and acceleration is accomplished by partial jackknifing, a method of systematically resampling the data. Bootstrap analyses were conducted to provide upper tolerance limits confidence bounds that represent the concentration of each fluorochemical below which the stated proportion of population is expected to be found.

Results and Discussion

Quality Assurance. Provided in Table S2 in the Supporting Information are the analyses of the quality assurance results of the 60 split samples from 2006, the reanalysis of 78 samples from the original American Red Cross study (*5*), and the reanalysis of 70 samples from Hagerstown originally collected in 1974 and 1989 (*8*). These quality assurance analyses provided reasonable confidence in the results obtained over time using different analytical methods (e.g., ion-pairing vs protein precipitation extraction methods). For example, reanalysis of the 78 samples from the original American Red Cross study (*5*) was highly correlated with the original samples for PFOS (Spearman Rho = 0.97), PFOA (Spearman Rho = 0.86), and PFHxS (Spearman Rho = 0.91) although the mean differences were statistically significantly different for PFOS (12.5% lower) and PFOA (8.9% higher) when comparing the reanalysis to the original samples (Table S2). Mean PFHxS concentrations were identical between the reanalyzed and original samples. Me-PFOSA-AcOH and Et-PFOSA-AcOH were not as highly correlated (Spearman Rho = 0.78 for each) due to the different values for these two analytes reported at <LLOQ.

2006 American Red Cross. Provided in Table 1 for the overall 2006 analyses are measures of central tendency and distribution for the six analytes. The percentages of samples < LLOQ were PFOS, 0.3%; PFOA, 0.5%; PFHxS, 0.5%, Me-PFOSA-AcOH 31%; Et-PFOSA-AcOH, 98%; and PFBS, 99%. The geometric mean plasma concentration for PFOS was 14.5 ng/mL (95% CI 13.9−15.2) and statistically significantly higher ($p < 0.05$) for males (17.1 ng/mL) than females (12.3 ng/mL). The geometric mean plasma concentration for PFOA was 3.4 ng/mL (95% CI 3.3−3.6) and significantly higher for males (3.9 ng/mL) than females (3.0 ng/mL). These sex-related differences were observed across the six age ranges, but age, itself, was not associated with the concentrations (Table 2). Analyzed by location, Charlotte had significantly higher PFOS and PFOA plasma concentrations than the other areas (see Table S3 in Supporting Information).

Table 3 provides the bias-corrected estimates for the 90th, 95th, and 99th percentile upper tolerance limits along with the upper limit (bound) from the 95% CI. For example, the 90th, 95th, and 99th percentiles for PFOS were 29.6, 35.2, and 49.7 ng/mL with the upper confidence limit calculated at 31.5, 36.8, and 62.4 ng/mL, respectively.

Log concentrations of PFOS and PFOA were moderately correlated ($r = 0.67$) with lower correlations between PFOS and either PFHxS ($r = 0.38$), Me-PFOSA-AcOH ($r = 0.25$), or Et-PFOSA-AcOH ($r = 0.05$). Lower correlations were also observed between the log concentrations of PFOA and PFHxS ($r = 0.28$).

Comparison of 2000−2001 to 2006 American Red Cross Data. Provided in Figure S5 in the Supporting Information is the comparison of the overall distribution of the measured concentrations for PFOS, PFOA, PFHxS, Me-PFOSA-AcOH, and Et-PFOSA-AcOH between adult blood donors collected in 2000−2001 (*5*) and the present study in 2006. PFOS has a more pronounced downward shift in its distribution than either PFOA or PFHxS. As shown in Figures 1 and 2 for each location, the age- and sex-adjusted measures of central tendency (geometric mean) and distributions were consistently lower in 2006 than 2000−2001 for each compound. The age- and sex-adjusted geometric mean estimate comparisons from 2000 to 2006 were the following: PFOS 34.3 vs 14.5 ng/mL; PFOA 4.7 vs 3.4 ng/mL; PFHxS 2.2 vs 1.5 ng/mL; Me-PFOSA-AcOH 1.6 vs 0.5 ng/mL; and Et-PFOSA-AcOH 2.5 vs 0.5 ng/mL. This represents an approximate geometric mean decline of 60% for PFOS, 25% for PFOA, and 30% for PFHxS in the elapsed time period since the phase-out. The majority of these percentage declines can not be attributable to the different analytical methods between the two American Red Cross studies because, as presented above, the 78 quality assurance samples had reanalyzed mean concentrations within 12.5%, 8.9%, and 0% for PFOS, PFOA, and PFHxS, respectively, compared to the original analyses.

Comparison of Hagerstown, MD Area, 1974−2006. The decline in PFOS concentrations in 2006, and to a lesser extent PFOA and Et-PFOSA-AcOH, is shown in Figure 2 for the Hagerstown, Maryland summary data when compared with prior measurements from this region in 1974, 1989, and 2001, as previously reported (*8*).

Comparison of American Red Cross and CDC NHANES, 1999−2006. Figure 3 provides the time trend comparisons of the geometric means and 95% confidence limits for the American Red Cross and NHANES data (Figure 3a). Declines in the geometric means that approached the LLOQ in 2006 were observed for Me-PFOSA-AcOH and Et-PFOSA-AcOH. Figure 3b compares the tolerance limits for the 95th percentile and their upper bound estimates for the two American Red Cross studies, and the geometric means for the 95th percentiles, and associated 95% confidence intervals, as reported from the two CDC NHANES analyses (*4*). The American Red Cross studies showed a definitive downward trend for all compounds in both the geometric mean and the 95th percentile tolerance limit (and its upper bound). Both the American Red Cross and the NHANES databases have consistently observed PFOS and PFOA concentrations to be higher in males than females, by approximately 10−20%, but with minimum differences observed by age.

The approximate 60% decline in PFOS concentrations from 2000−2001 to 2006 in the American Red Cross studies, uniform across age, sex, and location, is consistent with the time period since phase-out of POSF-based materials and the geometric mean serum elimination half-life of PFOS reported at 4.8 years (95% CI 4.0−5.8) (*12*). This half-life in humans may be the result of a saturable renal resorption process (*13*).

Besides declines in polyfluoroalkyl concentrations in general population studies conducted in the United States, a decline in serum PFOS concentrations has been reported in Germany from 40 ng/mL in 1985 to 15 ng/mL in 2004 (*14*). Other data from China, however, where production of POSF continues, has shown an increase in PFOS concentrations from 0.03 ng/mL in 1987, to 3.2 ng/mL in 1999, and 22.4 ng/mL in 2002 (*15*).

The pathway leading to the presence of PFOS in human blood likely involves environmental exposure to PFOS or to precursor molecules, and to residual levels of PFOS precursors

TABLE 1. Measures of Central Tendency and Distribution for Six Polyfluoroalkyl Plasma Concentrations (ng/mL), American Red Cross Adult Blood Donors, 2006

	PFOS	PFOA	PFHxS	Me-PFOSA-AcOH	Et-PFOSA-AcOH	PFBS
			All Subjects ($n = 600$)			
range	<LLOQ(2.5)−77.9	<LLOQ(1.0)−28.1	<LLOQ(0.5)−56.5	<LLOQ(0.3)−0.3	<LLOQ(0.3)−3.3	<LLOQ(0.3)−2.9
<LLOQ (number)	<2.5(2)	<1.0(3)	<0.5(26)	<0.3(101) <0.6(4) <0.8(34) <1.3 (47)	<0.3(48) <0.5(159) <1.0(383)	<0.3(480) <0.5(113)
interquartile range	10.2−21.3	2.4−4.8	0.9−2.4	LLOQ(0.8)−LLOQ(1.3)	LLOQ(0.5)−LLOQ(1.0)	LLOQ(0.3)−LLOQ(0.5)
90th percentile	29.7	6.4	3.7	1.4	LLOQ(1.0)	LLOQ(0.5)
median	14.2	3.6	1.5	0.6	LLOQ(1.0)	LLOQ(0.3)
geometric mean	14.5	3.4	1.5	0.6	0.5	0.2
95% CI geometric mean	13.9−15.2	3.3−3.6	1.4−1.6	0.6−0.6	N/A	-
arithmetic mean	16.9	3.9	2.2	0.8	0.6	0.2
95% CI arithmetic mean	16.1−17.7	3.8−4.1	1.9−2.5	0.7−0.8	N/A	-
			Males ($n = 301$)			
range	<LLOQ(2.5)−62.4	0.8−28.1	<LLOQ(0.5)−56.5	<LLOQ(0.3)−4.7	<LLOQ(0.3)−3.3	LLOQ(0.3)−0.3
<LLOQ (number)	<2.5(1)	-	<0.5 (4)	<0.3(41) <0.6(3) <0.8(16) <1.3(22)	<0.3(25) <0.5(85) <1.0(185)	<0.3(252) <0.5(45)
interquartile range	12.3−24.1	3.0−5.5	1.2−2.8	0.4−0.9	LLOQs(0.5−1.0)	LLOQs(0.3−0.5)
90th percentile	32.6	7.3	4.6	0.6	LLOQ(1.0)	LLOQ(0.5)
median	16.8	4.0	1.8	0.6	LLOQ(1.0)	LLOQ(0.3)
geometric mean	17.1	3.9	1.9	0.6	0.5	0.2
95% CI geometric mean	16.2−18.1	3.7−4.2	1.8−2.1	0.6−0.7	N/A	N/A
arithmetic mean	19.3	4.4	2.9	0.8	0.6	0.2
95% CI arithmetic mean	18.2−20.5	4.2−4.6	2.3−3.4	0.7−0.9	N/A	N/A
			Females ($n = 299$)			
range	<LLOQ(2.5)−77.9	<LLOQ(1.0)−11.9	<LLOQ(0.5)−14.7	<LLOQ(0.3)−5.3	<LLOQ(0.3)−1.9	<LLOQ(0.3)−2.9
LLOQ(number)	<2.5(1)	<1.0(3)	<0.5(22)	<0.3(60) <0.6(1) <1.3(25)	<0.3(23) <0.5(74) <1.0(198)	<0.3(226) <0.5(68)
interquartile range	8.5−18.4	2.1−4.1	0.7−1.8	0.3−0.9	LLOQs(0.5−1.0)	LLOQs(0.3−0.5)
90th percentile	25.6	5.6	3.1	1.4	LLOQ(1.0)	LLOQ(0.3)
median	11.9	3.1	1.2	0.6	LLOQ(1.0)	LLOQ(0.3)
geometric mean	12.3	3.0	1.2	0.6	0.5	0.2
95% CI geometric mean	11.5−13.1	2.8−3.2	1.1−1.3	0.5−0.6	N/A	N/A
arithmetic mean	14.5	3.5	1.6	0.7	0.6	0.2
95% CI arithmetic mean	13.4−15.6	3.2−3.8	1.4−1.8	0.7−0.8	N/A	N/A

TABLE 2. Geometric Means (95% Confidence Intervals) for PFOS, PFOA, and PFHxS Plasma Concentrations (ng/mL) by Age and Sex, American Red Cross Blood Donors, 2006

	Age (years)				
Fluorochemical	**20−29**	**30−39**	**40−49**	**50−59**	**60−69**
PFOS					
all	14.9 (13.8−16.4)	14.6 (13.3−16.1)	13.3 (12.1−14.8)	14.9 (13.3−16.7)	14.7 (13.2−16.4)
males	18.6 (16.8−20.5)	17.8 (15.9−20.0)	15.6 (13.6−17.9)	16.8 (14.6−19.5)	16.9 (14.6−19.6)
females	12.2 (10.7−13.8)	12.0 (10.6−13.7)	11.4 (9.9−13.1)	13.1 (11.0−15.7)	12.8 (11.0−14.9)
PFOA					
all	3.8 (3.5−4.1)	3.8 (3.4−4.2)	3.2 (2.9−3.5)	3.5 (3.1−3.8)	3.1 (2.8−3.4)
males	4.4 (4.0−4.8)	4.7 (4.2−5.3)	3.7 (3.2−4.2)	3.7 (3.2−4.2)	3.4 (3.0−3.9)
females	3.2 (2.8−3.6)	3.0 (2.6−3.4)	3.9 (2.5−3.2)	3.3 (2.8−3.8)	2.8 (2.4−3.1)
PFHxS					
all	1.8 (1.6−2.1)	1.5 (1.3−1.7)	1.3 (1.2−1.5)	1.5 (1.3−1.7)	1.5 (1.3−1.7)
males	2.5 (2.0−3.1)	2.1 (1.8−2.5)	1.7 (1.4−2.1)	1.8 (1.5−2.1)	1.7 (1.4−2.1)
females	1.3 (1.1−1.6)	1.1 (0.9−1.3)	1.0 (0.9−1.3)	1.2 (1.0−1.5)	1.3 (1.1−1.6)

in industrial and commercial products. Which source(s) lead to exposure, however, remains debatable. There are several candidates including industrial emissions, both from primary manufacturer(s) and downstream users for production applications, and the environmental degradation of precursor compounds from consumer products.

Prime examples of precursor compounds that have been detected in ambient air include *N*-methyl-*N*-(2-hydroxy-

TABLE 3. Estimates of Plasma Concentrations (ng/mL) for the Upper Tolerance Limits and Their Upper 95% Confidence Limits for Five Polyfluoroalkyl Chemicals

	upper tolerance limit	estimate	upper 95% confidence limit
PFOS	90%	29.6	31.5
	95%	35.2	36.8
	99%	49.7	62.4
PFOA	90%	6.4	6.8
	95%	7.7	8.3
	99%	10.9	11.9
PFHxS	90%	3.7	4.1
	95%	5.6	7.0
	99%	14.4	31.2
Me-PFOSA-AcOH	90%	1.4	1.5
	95%	1.8	2.0
	99%	3.5	4.6
Et-PFOSA-AcOH	90%	0.7	1.0
	95%	0.7	1.0
	99%	1.0	1.4

ethyl)perfluorooctanesulfonamido alcohol (N-MeFOSE) and *N*-ethyl-*N*-(2-hydroxyethyl)perfluorooctanesulfonamido alcohol (N-EtFOSE) that could degrade to their respective sulfonamides (*16*). In the present study, Et-PFOSA-AcOH and Me-PFOSA-AcOH declined appreciably compared to American Red Cross samples collected in 2000−2001.

Because C2−C4 perfluorinated carboxylates were determined from atmospheric degradation of *N*-methyl-perfluorobutanesulfonamidoethanol and *N*-ethylperfluorobutanesulfonamide, it is also conceivable that exposure to their perfluorooctane equivalents might be exposure sources for PFOA (*17, 18*).

Other possible pathways for human exposure may have occurred through volatility or cutaneous contact of precursors present in POSF-based consumer products and/or absorption via ingestion from migration into food from coatings on food contact paper. Markers for these exposure pathways would include Me-PFOSA-AcOH (carpets, textiles) and Et-PFOSA-AcOH (food contact paper), and both have been shown to metabolize to PFOS (*19*). Coatings on paper for food contact applications have been discussed as a possible major source of exposure for the PFOS concentrations (*20*). POSF-based materials, however, were not sold for coatings on food contact paper protectant applications for human use until 1974, yet a limited number of serum samples collected prior to that time period found PFOS concentrations in the 20−30 ng/mL range (*8, 19*). This observation would therefore not support the hypothesis that coatings on food contact paper protectant applications would be the primary source of human exposure for PFOS in the general population.

The approximate 60% decline in PFOS was consistent with its serum elimination half-life and the time period since phase-out of POSF-based materials. PFOA, with its shorter serum elimination half-life of approximately 3.5 years (95% CI 3.0−4.1) (*12*), should have declined at a percentage similar to that of PFOS, if POSF-based materials were the sole source of exposure. However, only a 25% decline was observed with PFOA. This 25% decline in PFOA was reported with NHANES data collected in 2003−2004 (*4*), suggesting a possible moderation in decline since that time period. The basis for the differences in the rates of decline of PFOS and PFOA in general population serum/plasma may relate to continued exposure to PFOA and/or the elimination rate of PFOA from human serum lessens with decreasing serum concentration. The data from Olsen et al. (*12*) do not offer support for the latter because there was not an association between longer serum elimination half-lives and lower initial and final PFOA serum concentrations for the 5-year study time period (*12*).

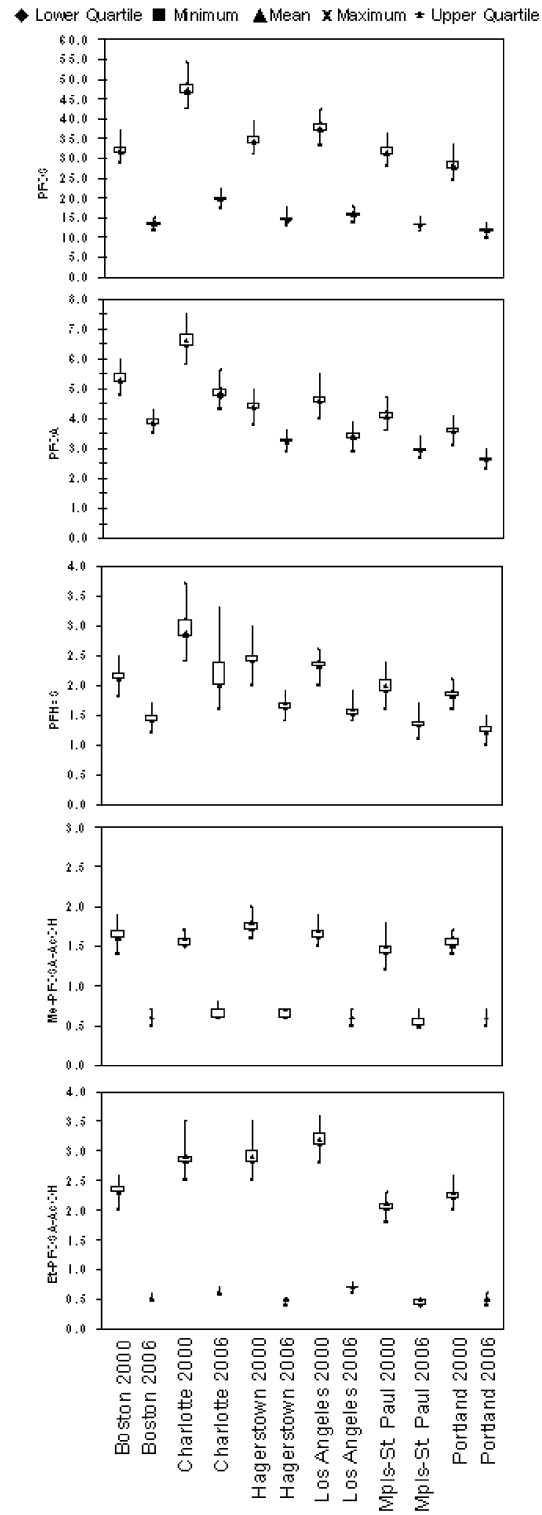

FIGURE 1. Comparison of age- and sex-adjusted distributions (geometric mean, lower and upper quartiles, minimum and maximum) for the American Red Cross 2000−2001 (labeled 2000 in graph) (serum) and 2006 (plasma) concentrations (ng/mL) of PFOS, PFOA, PFHxS, Me-PFOSA-AcOH, and Et-PFOSA-AcOH.

However, this study was conducted on retired fluorochemical production workers with the lowest one-third of their final PFOA concentrations ranging between 37 and 84 ng/mL which are 5 to 17 times higher than the average PFOA concentrations in the general population. A larger sample size with lower concentrations might have increased the possibility of detecting an influence of serum concentration

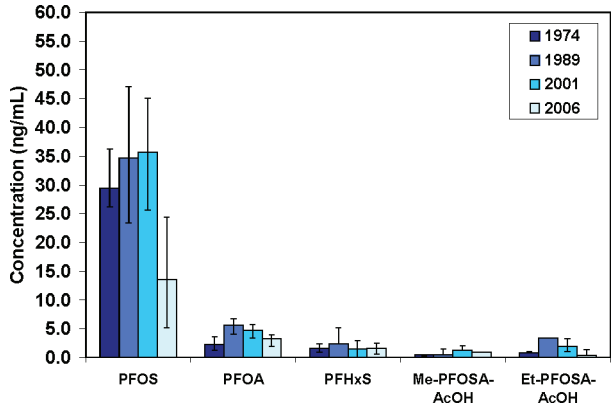

FIGURE 2. Median and interquartile range of serum or plasma concentrations (ng/mL) by year (1974, 1989, 2001, and 2006), Hagerstown, Maryland, for PFOS, PFOA, PFHxS, Me-PFOSA-AcOH, and Et-PFOSA-AcOH.

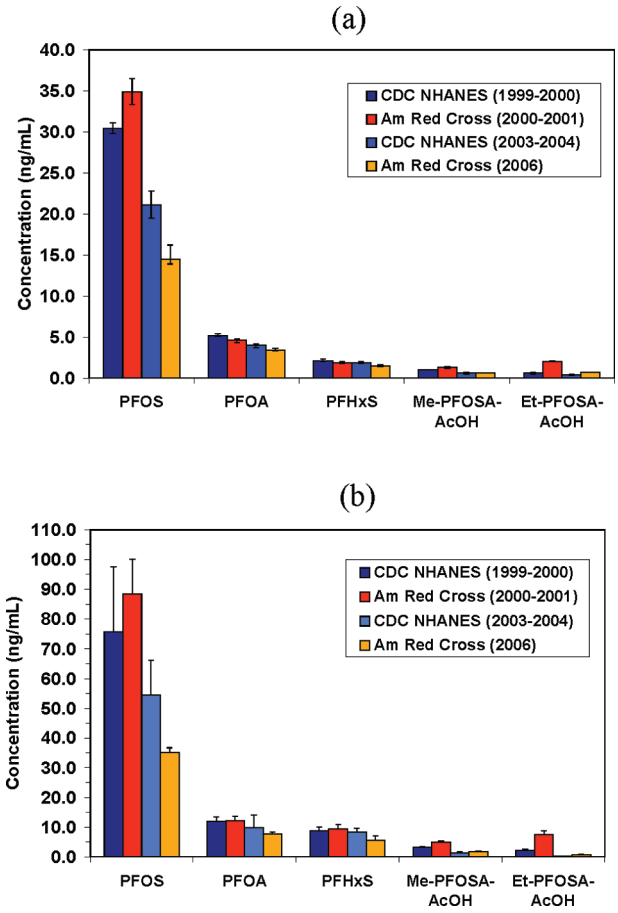

FIGURE 3. Temporal trends for five polyfluoroalkyl concentrations (ng/mL) from the CDC NHANES and American Red Cross study populations for (a) the population geometric means (95% confidence intervals), and (b) the geometric mean (CDC NHANES) or estimated 95th percentile tolerance limit (American Red Cross) with their upper 95th percent confidence limits.

on the elimination rate, but this would be complicated by potential for low-level exposure to influence apparent elimination kinetics. A more likely explanation for the percentage decline difference between PFOS and PFOA is that PFOA concentrations in the general population are unlikely to be solely attributed to POSF-based production activities, and other exposure sources of PFOA remain. Although 3M phased-out its ECF (electrochemical cell fluorination) production of PFOA at the same time it did POSF-based materials (beginning in 2000), PFOA continued to be produced by others and used in fluoropolymer production. ECF production of PFOA, telomer-based production of PFOA, fluorotelomer-based precursors of PFOA, and other fluoropolymer production need to be considered as either historic and/or ongoing sources of direct and indirect potential human exposure to PFOA (*20*). Exposure models indicate PFOA and other perfluorinated carboxylates may decline in the future as a result of reduction of manufacturing emissions and residual fluorotelomer raw materials (*21*). Besides general population exposure, PFOA contamination of community drinking water sources has also been reported (*23–25*).

In the NHANES data, a 10% decrease in PFHxS concentrations occurred between 1999−2000 and 2003−2004 (*4*), whereas in the present study an approximate 30% decline in PFHxS was observed in adult blood donor samples collected in 2000−2001 and 2006. Both of these time-dependent percentage declines are consistent with the longer half-life for PFHxS (geometric mean 7.3 years (95% CI 5.8−9.2) (*12*). It might also reflect more persistent exposures for PFHxS from residential sources, including household dust (*26*).

Less than 1% of the PFBS concentrations were >LLOQ (0.25 ng/mL). Materials derived from perfluorobutanesulfonyl fluoride (PBSF, $C_4F_9SO_2F$) have been introduced as replacements for the eight-carbon homologue products that were manufactured from POSF. PFBS would be expected to be formed from PBSF and the metabolism of *N*-alkyl derivatives of perfluorobutanesulfonamides. *N*-alkyl derivatives of perfluorobutanesulfonamides are used in various polymeric applications including fabric, carpet, and upholstery protectants, and as surfactants. PFBS may have not been quantifiable because of reduced production volume, compared to POSF-based materials, as well as the much shorter serum elimination half-life in humans of approximately 30 days for PFBS (*27*) compared to that of PFOS (*12*).

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Supporting Information Available

Additional details of sample preparation, ions monitored, quality assurance, and study results. This information is available free of charge via the Internet at http://pubs.acs.org.

Literature Cited

(1) Butenhoff, J. L.; Olsen, G. W.; Pfahles-Hutchens, A. The applicability of biomonitoring data for perfluorooctanesulfonate (PFOS) to the environmental public health continuum. *Environ. Health Perspect.* **2006**, *114*, 1776–1782.

(2) Houde, M.; Martin, J. W.; Letcher, R. J.; Solomon, K. R.; Muir, D. C. G. Biological monitoring of polyfluoroalkyl substances: a review. *Environ. Sci. Technol.* **2006**, *40*, 3463–3473.

(3) Olsen, G. W.; Mair, D. C.; Reagen, W. K.; Ellefson, M. E.; Ehresman, D. J.; Butenhoff, J. L.; Zobel, L. R. Preliminary evidence of a decline in perfluorooctanesulfonate (PFOS) and perfluorooctanoate (PFOA) in American Red Cross blood donors. *Chemosphere* **2007**, *68*, 105–111.

(4) Calafat, A. M.; Wong, L. Y.; Kuklenyik, Z.; Reidy, J. A.; Needham, L. L. Polyfluoroalkyl chemicals in the U.S. population: Data from the National Health and Nutrition Examination Survey (NHANES) 2003−2004 and comparisons to NHANES 1999−2000. *Environ. Health Perspect* **2007**, *115*, 1596–1602.

(5) Olsen, G. W.; Church, T. R.; Miller, J. P.; Burris, J. M.; Hansen, K. J.; Lundberg, J. K.; Armitage, J. B.; Herron, R. M.; Medhdizadehkashi, Z.; Nobiletti, J. B.; O'Neill, E. M.; Mandel, J. H.; Zobel, L. R. Perfluorooctanesulfonate and other fluorochemicals

in the serum of American Red Cross adult blood donors. *Environ. Health Perspect.* **2003**, *111*, 1892–1901.
(6) Ehresman, D. J.; Froehlich, J. W.; Olsen, G. W.; Tanaka, S. C.; Butenhoff, J. L. Evaluation of whole blood, plasma, and serum for the determination of perfluorooctanesulfonate (PFOS) and other fluorochemicals. *Environ. Res.* **2007**, *103*, 176–184.
(7) Flaherty, J. M.; Connolly, P. D.; Decker, E. R.; Kennedy, S. M.; Ellefson, M. E.; Reagen, W. K.; Szostek, B. Quantitative determination of perfluorooctanoic acid in serum and plasma by liquid chromatography tandem mass spectrometry. *J. Chromatogr., B. Anal. Technol. Biomed. Life. Sci.* **2005**, *819*, 329−338.
(8) 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. *Environ. Health Perspect.* **2005**, *113*, 539–545.
(9) 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.
(10) Lubin, J. H.; Colt, J. S.; Camann, D.; Davis, S.; Cerhan, J. R.; Severson, R. K.; Bernstein, L.; Hartge, P. Epidemiologic evaluation of measurement data in the presence of detection limits. *Environ. Health Perspect* **2004**, *112*, 1691–1696.
(11) Efron, B.; Tibshirani. R. J. An introduction to the bootstrap. In *Monographs on Statistics and Applied Probability, Vol 57*; Cox, D. R., Hinkley, D. V., Reid, N., Rubin, D. B., Silverman, B. W., Eds.; Chapman Hall: New York, 1993; pp 1−177.
(12) 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 workers. *Environ. Health Perspect.* **2007**, *115*, 1298–1305.
(13) Andersen, M. E.; Clewell, H. J.; Tan, Y. M.; Butenhoff, J. L.; Olsen, G. W. Pharmacokinetic modeling of saturable, renal resorption of perfluoroalkylacids in monkeys - probing the determinants of long plasma half-lives. *Toxicology* **2006**, *227*, 156–164.
(14) Wiesmüller, G A.; Eckard, R.; Dobler, L.; Günsel, A.; Oganowski, M.; Schröter-Kermani, C.; Schlüter, C.; Gies, A.; Kemper, F. H. The Environmental Specimen Bank for Human Tissues as part of the German Environmental Specimen Bank. *Int. J. Hyg. Environ. Health* **2007**, *210*, 299–305.
(15) Jin, Y.; Saito, N.; Harada, K.; Inoue, K.; Koizumi, A. Historical trends in human serum levels of perfluorooctanoate and perfluorooctane sulfonate in Shenyang, China. *Tohoku J. Exp. Med.* **2007**, *212*, 63–70.
(16) Martin, J. W.; Muir, D. C. G.; Moody, C. S.; Ellis, D. A.; Kwan, W. C.; Solomon, K. R.; Mabury, S. A. Collection of airborne fluorinated organics and analysis by gas chromatography/chemical ionization mass spectrometry. *Anal. Chem.* **2002**, *74*, 584–590.
(17) Martin, J. W.; Mabury, S. A.; O'Brien, P. J. Atmospheric chemistry of perfluoroalkanesulfonamides:kinetic and product studies of the OH radical and Cl atom initiated oxidation of *N*-ethyl perfluorobutanesulfonamide. *Environ. Sci. Technol.* **2006**, *40*, 864–872.
(18) D'eon, J. C.; Hurley, D. H.; Wallington, T. J.; Mabury, S. A. Atmospheric chemistry of *N*- methyl perfluorobutane sulfonamidoethanol $C_4F_9SO_2N(CH_3)CH_2CH_2OH$: Kinetics and mechanism of reaction with OH. *Environ. Sci. Technol.* **2006**, *40*, 1862–1868.
(19) 3M Company. *Health and Environmental Assessment of Perfluorooctane Sulfonic Acid and Its Salts*; U.S. EPA docket AR-226-1486; Washington, DC, 2003.
(20) Tittlemier, S. A.; Pepper, K.; Seymour, C.; Moisey, J.; Bronson, R.; Cao, X. L.; Dabeka, R. W. Dietary exposure of Canadians to perfluorinated carboxylates and perfluorooctane sulfonate via consumption of meat, fish, fast foods, and food items prepared in their packaging. *J. Agric. Food Chem.* **2007**, *55*, 3203–3210.
(21) De Silva, A. O.; Mabury, S. A. Isomer distribution of perfluorocarboxylates in human blood: potential correlation to source. *Environ. Sci. Technol.* **2006**, *40*, 2903–2909.
(22) Yarwood, G.; Kemball-Cook, S.; Keinath, M.; Waterland, R. L.; Korzeniowski, S. H.; Buck, R. C.; Russell, M. H.; Washburn, S. T. High-resolution modeling of fluorotelomer alcohols and perfluorocarboxylic acids in the North American troposphere. *Environ. Sci. Technol.* **2007**, *15*, 5756–5762.
(23) Emmett, E. A.; Shofer, F. S.; Zhang, H.; Freeman, D.; Desai, C.; Shaw, L. M. Community exposure to perfluorooctanoate: relationships between serum concentrations and exposure sources. *J. Occup. Environ. Med.* **2006**, *48*, 759–770.
(24) Minnesota Department of Health. *Ground-water sampling for PFOA near the Washington Co. landfill*; http://www.health.state.mn.us/divs/eh/hazardous/sites/washington/pfoa0408.
(25) Skutlarek, D.; Exner, M.; Färber, H. Perfluorinated surfactants in surface and drinking waters. *Environ. Sci. Pollut. Res. Int.* **2006**, *13*, 299–307.
(26) Kubwabo, C.; Stewart, B.; Zhu, J.; Marro, L. Occurrence of perfluorosulfonates and other perfluorochemicals in dust from selected homes in the city of Ottawa, Canada. *J. Environ. Monit.* **2005**, *11*, 1074–1078.
(27) Olsen, G.; Lieder, P.; Noker, P.; Gorman, G.; Butenhoff, J. The pharmacokinetics of perfluorobutanesulfonate (PFBS) in monkeys and humans (abstract). *Toxicologist* **2006**, *90*, 124.

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