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3M Company
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Principal Investigators: Geary Olsen, D.V.M., Ph.D.[1]
David Mair, M.D.[2]

Co-Investigators: William Reagen, Ph.D[3]
Mark E. Ellefson, B.S.[3]
David J. Ehresman, M.S.[1]
John L. Butenhoff, Ph.D.

Study Director: Larry Zobel, M.D.[1]

1. 3M Company, Medical Department, Mail Stop 220-6W-08, St. Paul, MN 55144
2. American Red Cross, North Central Blood Services, 100 S. Robert St., St. Paul, MN 55107
3. 3M Company, Environmental Laboratory, Mail Stop 2-2E-02, St. Paul, MN 55144

ABSTRACT

The purpose of this pilot study was to determine whether general population PFOS and PFOA concentrations, as measured in 2005 in a small sample of American Red Cross blood donors, may represent a decline from prior general populations measurements made in 2000. Although different matrices were used in 2000 (serum) than 2005 (plasma), both were considered equivalent matrices for the analysis of PFOS and PFOA based on a prior evaluation study. Regardless of year and matrix, all donor samples were obtained from the American Red Cross North Central Blood Services (St. Paul, Minnesota). A total of 40 plasma samples, equally distributed into 4 groups by age (< 40 years and ≥ 40 years) and sex (male, female), were obtained. PFOS and PFOA were extracted from the plasma of the 2005 samples by protein precipitation in acetonitrile. Calibration standards, quality control spikes, and blanks were extracted in control rabbit plasma. Additionally, ^{13}C-PFOA was spiked into all samples, control human plasma, and the calibration curve prior to extraction and was used as a surrogate to monitor extraction efficiency. Quantification was accomplished by high performance liquid chromatography tandem mass spectrometry. The geometric mean for PFOS in the 100 samples collected in 2000 was 31.7 ng/mL (95% CI 29.8 – 36.7) compared to 15.1 ng/mL (95% CI 13.3-17.1) in 2005 ($p < .0001$). Likewise, the geometric mean for PFOA in 2000 was 4.5 ng/mL (95% CI 4.1 – 5.0) compared to 2.2 ng/mL (95% CI 1.9-2.6) in 2005 ($p < .0001$). The approximate 50 percent decline was also consistently observed across sex and two age categories (< 40 years, ≥ 40 years) for both PFOS and PFOA. This finding is consistent with the pilot study's hypothesis that the phase-out of POSF-based material production by 3M, the primary global manufacturer, that occurred several

years ago, in conjunction with the approximate 5 year average half-life of elimination of PFOS, may have resulted in such a decline. The reason for the observed decline in PFOA concentrations is less obvious. The average elimination half-life of 4 years together with an approximate 50 percent reduction in concentration suggests that the source of PFOA exposure has also been reduced. Whether this is a result of cessation of POSF production, which PFOA was a minor-by product, or industry efforts to control other sources of PFOA, remain to be determined. Further research of other temporal/geographical locations is highly warranted.

INTRODUCTION

For approximately 50 years, the 3M Company produced perfluorooctanesulfonyl fluoride (POSF, $C_8F_{17}SO_2F$)-based materials that had a variety of applications including surfactants, paper and packaging treatments, and surface protectants (e.g., for carpet, upholstery, textile). Depending on the specific functional derivatization or the degree of polymerization, such POSF-based products may have degraded or metabolized, to an undetermined degree, to perfluorooctanesulfonate (PFOS, $C_8F_{17}SO_3^-$), a stable and persistent end-product that has the potential to bioaccumulate. In May 2000, the 3M Company (3M) announced that it would voluntarily cease manufacturing materials based on POSF after a metabolite of this compound, PFOS, was found to be widespread in human populations and wildlife (3M Company 2003). The mechanisms and pathways leading to the presence of PFOS in human blood are not well characterized but likely involved environmental exposure to PFOS or to precursor molecules and residual levels of PFOS or PFOS precursors in industrial and commercial products. Following 3M's announcement, the U.S. Environmental Protection Agency (EPA) finalized a significant new use rule to regulate PFOS and related chemicals (U.S. EPA 2002). As part of an extensive research effort to characterize PFOS concentrations in the environment, Olsen et al. (2003a) analyzed 645 American Red Cross blood donor samples, collected in 2000, for perfluorooctanesulfonate (PFOS) as well as perfluorooctanoate (PFOA, $C_7F_{15}CO_2^-$). Geometric mean (95% confidence intervals in parentheses) were 34.9 (33.3 – 36.5) and 4.6 (4.3-4.8), respectively. No substantial differences were noted by age, sex or geographic location. PFOA is not known to be a metabolite of POSF or PFOS.

The ammonium salt of PFOA has been an important processing aid in fluoropolymer production. PFOA was also a minor by-product of POSF production. It is also a degradation product of telomer alcohols used in numerous commercial and consumer applications. Whereas PFOS production in the United States has ceased because 3M was the sole manufacturer, production of PFOA has continued worldwide by several other companies. Based on a 5-year follow-up study of 26 3M retirees (Olsen et al. 2005) both of these fluorochemicals have long serum half-lives of elimination in humans approximating 5 years for PFOS and 4 years for PFOA.

The primary purpose of this pilot study was to determine whether PFOS and PFOA concentrations, measured in 2005, have declined since 2000 in the general population in a small sample of American Red Cross blood donors from the North Central Blood Services (Minneapolis-St. Paul). Although different matrices were used in 2000 (serum) than 2005 (plasma), both were considered equivalent matrices for the analysis of PFOS and PFOA based on a blood-matrix comparison study performed by Ehresman et al. (2005). Upon statistical analysis of the present study data leading to a conclusion that there was a suggestive pattern of a decline in serum PFOS and PFOA concentrations between 2000 and 2005, a more in-depth study is warranted, designed in the similar manner as Olsen et al. 2003a with its six geographical locations, in order to further test the hypothesis of a decline in PFOS and PFOA concentrations in American Red Cross blood donors across geographical locations, age and sex.

METHODS

All donor samples were obtained from the American Red Cross North Central Blood Services (St. Paul, Minnesota). A total of 40 plasma samples equally distributed into 4 groups by age (< 40 years and ≥ 40 years) and sex (male, female) were obtained at the time of blood donation. No personal identifiers for these 40 donor samples were maintained by the American Red Cross North Central Blood Services or provided to 3M. Per the past policy of the American Red Cross North Central Blood Services (Olsen et al. 2003a), a signed consent form specific for analysis of PFOS and PFOA was not required. The study was approved by the American Red Cross Institutional Review Board (IRB).

Details of the laboratory methods used to analyze the 40 plasma samples for PFOS and PFOA are provided in Appendix A. Briefly, PFOS and PFOA were extracted from plasma by protein precipitation in acetonitrile. Calibration standards, quality control spikes, and blanks were extracted in control rabbit plasma. Additionally, ^{13}C PFOA was spiked into all samples, control human plasma, and the calibration curve prior to extraction and was used as a surrogate to monitor extraction efficiency. Quantification was accomplished by high performance liquid chromatography tandem mass spectrometry.

Statistical analysis of the data, including both parametric and nonparametric methods, was used to evaluate and compare 100 samples collected in 2000 to the 40 plasma samples collected in 2005 from the same American Red Cross location (North Central Blood Services, St. Paul, MN). Statistical analysis included calculation and comparison (e.g., one way analysis of variance, student t test) of the arithmetic mean and

geometric mean between the two comparison years. Any sample that was lower than the limit of quantitation (LLOQ) was imputed to be the LLOQ divided by the square root of two. This method should not bias the variance when LLOQ samples are few, as was the situation in the present study. Non-parametric statistics included the Wilcoxon rank sum test. All statistical analyses were calculated using JMP (Cary, NC).

RESULTS

All 40 donor plasma samples were greater than the LLOQ for PFOS and the range was 6.6 ng/mL to 36.9 ng/mL. Thirty-eight (95 percent) of the 40 plasma samples were above the LLOQ for PFOA. The range was LLOQ (1.01 ng/mL) to 4.7 ng/mL. Measures of central tendency (arithmetic mean, geometric mean and median) are presented in Table 1 for the 2005 data and the corresponding 2000 data for North Central Blood Services. For example, the geometric mean for PFOS in 2000 was 31.7 ng/mL (95% CI 29.8 – 36.7) compared to 15.1 ng/mL (95% CI 13.3-17.1) in 2005 ($p < .0001$). Likewise, the geometric mean for PFOA in 2000 was 4.5 ng/mL (95% CI 4.1 – 5.0) compared to 2.2 ng/mL (95% CI 1.9-2.6) in 2005 ($p < .0001$). The difference between years was also statistically significant based on the nonparametric Wilcoxon rank sum test. The approximate 50 percent decline was also consistently observed across sex and the two age categories for both PFOS (Table 2) and PFOA (Table 3). The distribution of PFOS and PFOA by the two comparison years is provided in Figures 1 and 2, respectively. Despite the approximate 50 percent decline in concentrations for PFOS and PFOA between 2000 and 2005, the positive correlation observed between PFOS and

PFOA serum concentrations in the 2000 samples (r = .70) was not as evident (r = .49) in the 2005 plasma samples as shown in the scatterplot in Figure 3.

DISCUSSION

The approximate 50 percent decline in PFOS concentrations observed between 2000 and 2005 is consistent with the pilot study's hypothesis that the phase-out of POSF-based material production by the primary global manufacturer, 3M, that occurred several years ago, in conjunction with the approximate 5 year average half-life of elimination of PFOS, could result in a substantial decline in the serum (or plasma) concentration of PFOS. Our pilot study results strongly suggest further analyses are warranted and it is our intention to obtain 2006 American Red Cross blood donor data from the same six locations that were collected in 2000-2001 and reported by Olsen et al. (2003a) in order to test whether this approximate 50 percent decline is seen in these other geographical locations.

Furthermore, since Olsen et al. (2004) reported a historical trend analysis for one particular location, the area surrounding Hagerstown, Maryland, the analysis for this location will have four separate years (1974, 1989, 2001 and 2006) to examine. A hypothesized 50 percent decline in PFOS in 2005 from this location would be substantially different than the reported increase in serum PFOS concentrations between 1974 and 1989 and the plateau between 1989 and 2001 (Olsen et al. 2005).

The similar percentage decline in PFOS and PFOA concentrations is of significant interest, because PFOA cannot convert directly from POSF or PFOS (or vice versa). The reduction in PFOA concentration suggests a substantial reduction in

exposure sources. The reason for the observed decline in PFOA concentrations is less obvious. The average elimination half-life of 4 years together with an approximate 50 percent reduction in concentration suggests that the source of PFOA exposure has also been reduced. Whether this is a result of cessation of POSF production, which PFOA was a minor-by product, or industry efforts to control other sources of PFOA, remain to be determined. PFOA was a minor by-product of POSF production. It is also a degradation product of telomer alcohols used in numerous commercial and consumer applications. The ammonium salt of PFOA is also used in fluoropolymer production.

It is unlikely that adverse health outcomes would be causally associated with the measurements of central tendency of PFOS or PFOA in these American Red Cross blood donors in 2000 or in 2005 as such associations have not been reported in epidemiologic studies of 3M fluorochemical production workers who have historically had serum levels approximately 1 to 2 orders of magnitude higher than what has been observed in the general population (Alexander 2001; 2004; Alexander et al. 2003; Olsen et al. 1998;1999; 2000; 2003b; 2003c). The approximate 50 percent decline in the concentration of PFOS or PFOA in these limited number of general population samples, if repeatedly observed in subsequent biomonitoring efforts, would thereby result in higher Margins of Exposure (MOE), as calculated based on the 2000 data for PFOS (3M Company 2003) and PFOA (Butenhoff et al. 2004).

In conclusion, an approximate 50 percent decline in concentrations of PFOS and PFOA have been observed in samples of American Red Cross blood donors collected in 2000 and 2005. It is unlikely that any of these were paired samples, although certainty can not be shown as donor identity was not maintained in either study. The results from

this pilot study strongly suggest further examination by expanding the analysis to the other American Red Cross geographic locations (Portland, OR; Los Angeles, CA; Charlotte, NC; Hagerstown, MD; and Boston, MA) that participated in 2000-2001 (Olsen et al. 2003a).

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Table 1. Measures of Central Tendency for PFOS and PFOA Concentrations (ng/mL) in American Red Cross Blood Donor Samples (North Central Region; St. Paul, MN) in 2000 (serum) and 2005 (plasma)

	2000						2005					
	Arithmetic		Geometric				Arithmetic		Geometric			
	Mean	95% CI	Mean	95% CI	Median	Range	Mean	95% CI	Mean	95% CI	Median	Range
PFOS	38.6	33.3-43.8	33.1	29.8-36.7	31.7	7.7-207.0	16.1[1]	14.0-18.2	14.9[2]	13.2-16.9	15.8	6.7-36.4
PFOA	5.2	4.5-5.9	4.5	4.1-5.0	4.4	1.4-20.0	2.4[1]	2.0-2.8	2.2[2]	1.9-2.6	2.3	0.7-4.7

[1]statistically significantly lower ($p < .0001$) than corresponding arithmetic mean in 2000

[2]statistically significantly lower ($p < .0001$) than corresponding geometric mean in 2000

Table 2. Measures of Central Tendency for PFOS Concentrations (ng/mL) in American Red Cross Blood Donor Samples (North Central Region, St. Paul, MN) in 2000 (serum) and 2005 (plasma) by Gender and Age Category (< 40 yrs and >= 40 yrs)

	2000					2005				
Gender	N	Arithmetic Mean	95% CI	Median	Range	N	Arithmetic Mean	95% CI	Median	Range
Female	50	39.2	29.8-48.6	28.8	7.7-207.0	20	13.3[2#]	10.6-16.1	11.6	6.7-29.9
Male	50	37.9	32.8-43.0	34.5	11.7-100.0	20	19.2[1]	16.3-22.0	18.6	10.0-36.4

	2000					2005				
Gender/Age	N	Arithmetic Mean	95% CI	Median	Range	N	Arithmetic Mean	95% CI	Median	Range
Female										
Age < 40	20	30.4	25.1-35.8	29.0	14.8-55.5	10	12.6[1]	10.1-15.1	12.0	8.1-19.9
Age >= 40	30	45.1	29.8-60.4	28.3	7.7-207.0	10	14.0[4]	8.6-19.5	10.7	6.6-30.2
Male										
Age < 40	20	42.7	33.3-52.4	36.4	17.7-88.8	10	18.2[2]	15.9-20.4	18.4	12.1-22.7
Age >= 40	30	34.7	28.9-40.5	32.9	11.7-100.0	10	20.2[3]	14.3-26.0	19.9	11.6-36.9

[1] statistically significantly lower ($p < .0001$) than corresponding arithmetic mean in 2000
[2] statistically significantly lower ($p < .001$) than corresponding arithmetic mean in 2000
[3] statistically significantly lower ($p < .01$) than corresponding arithmetic mean in 2000
[4] statistically significantly lower ($p < .05$) than corresponding arithmetic mean in 2000
[#] statistically significantly lower ($p < .01$) than male arithmetic mean

Table 3. Measures of Central Tendency for PFOA Concentrations (ng/mL) in American Red Cross Blood Donor Samples (North Central Region, St. Paul, MN) in 2000 (serum) and 2005 (plasma) by Gender and Age Category (< 40 yrs and >= 40 yrs)

	2000					2005				
Gender	N	Arithmetic Mean	95% CI	Median	Range	N	Arithmetic Mean	95% CI	Median	Range
Female	50	5.1	4.2-6.1	4.1	1.4-20.0	20	2.3[2]	1.7-2.9	2.3	0.7-4.7
Male	50	5.3	4.5-6.1	4.9	2.2-18.7	20	2.6[1]	2.1-3.1	2.4	0.7-4.2

Gender/Age	N	Arithmetic Mean	95% CI	Median	Range	N	Arithmetic Mean	95% CI	Median	Range
Female										
Age < 40	20	4.7	2.8-6.6	3.9	1.4-20.0	10	2.4	1.7-3.1	2.1	1.2-3.7
Age >= 40	30	5.4	4.1-6.8	4.2	1.4-15.8	10	2.2[3]	1.2-3.3	1.5	0.7-4.7
Male										
Age < 40	20	6.0	4.6-7.4	5.0	2.2-13.8	10	2.7[2]	2.0-3.4	2.8	1.4-4.2
Age >= 40	30	4.8	3.8-5.9	4.5	2.4-18.7	10	2.5[2]	1.7-3.3	2.4	0.7-4.2

[1] statistically significantly lower ($p < .001$) than corresponding arithmetic mean in 2000
[2] statistically significantly lower ($p < .01$) than corresponding arithmetic mean in 2000
[3] statistically significantly lower ($p < .05$) than corresponding arithmetic mean in 2000

Figure 1. Distribution of American Red Cross Blood Donor PFOS Concentrations, by Year, North Central Region (Minneapolis-St. Paul, Minnesota)

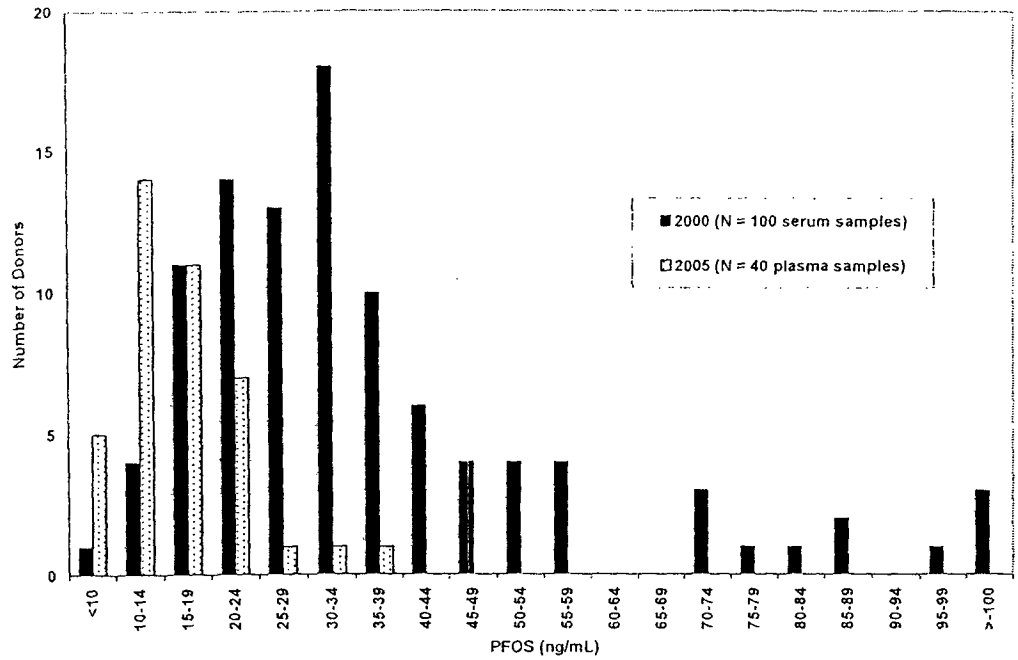

Figure 2. Distribution of American Red Cross Blood Donor PFOA Concentrations, by Year, North Central Region (Minneapolis-St. Paul, Minnesota)

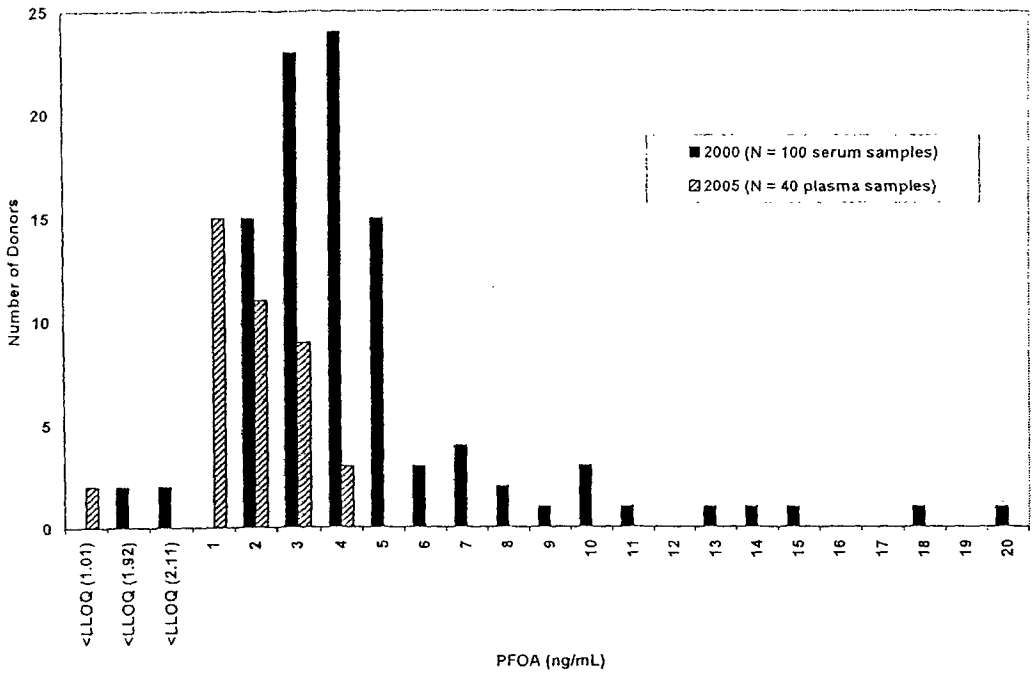

Figure 3. Scatterplot (log scale) of Associations Between PFOS and PFOA, by Year, American Red Cross North Central Blood Services (Minneapolis-St. Paul)