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Epidemiology
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Title: Identification of Fluorochemicals in Human Sera. I. American Red Cross Adult Blood Donors

Study

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ABSTRACT

Through cooperation with six American Red Cross blood banks, 645 serum samples from adult donors (ages 20-69, equally represented of both sexes) were obtained for fluorochemical analyses. Blood bank locations were Los Angeles (CA), Portland (OR), Minneapolis-St. Paul (MN), Charlotte (NC), Hagerstown (MD) and Boston (MA). Samples were void of personal identifiers. Age, gender and location were the only known demographic factors.

Sera samples were extracted and quantitatively analyzed for seven fluorochemicals using high-pressure liquid chromatography/electrospray tandem mass spectrometry and evaluated versus an extracted curve from a human plasma matrix. The seven fluorochemicals were perfluorooctanesulfonate (PFOS, $C_8F_{17}SO_3^-$); N-ethyl perfluorooctanesulfonamidoacetate (PFOSAA, $C_8F_{17}SO_2N(CH_2CH_3)CH_2COO^-$); N-methyl perfluorooctanesulfonamidoacetate (M570, $C_8F_{17}SO_2N(CH_3)CH_2COO^-$); perfluorooctanesulfonamidoacetate (M556, $C_8F_{17}SO_2N(CH)CH_2COO^-$); perfluorooctanesulfonylamide (PFOSA, $C_8F_{17}SO_2NH_2$); perfluorooctanoate (PFOA, $C_7F_{13}COO^-$); and perfluorohexanesulfonate (PFHS, $C_6F_{13}SO_3^-$).

Overall, the geometric mean measured concentration of PFOS was 34.9 ppb (95% CI 33.3-36.5). The measured PFOS concentration ranged from less than the lower limit of quantitation (LLOQ) of 4.1 ppb to 1656.0 ppb. The geometric mean for PFOS was significantly higher among males (37.8 ppb; 95% CI 35.5-40.3) than females (31.3 ppb; 95% CI 30.0-34.3). No significant difference was observed with age. Charlotte (NC) had the highest geometric mean serum PFOS concentration (51.5 ppb) and Boston (MA) the lowest (29.5 ppb). Bootstrap analyses were used to calculate a 95% tolerance limit for

PFOS of 88.5 ppb with an upper 95% confidence limit of 100.0 ppb. Additional geometric mean and tolerance limit data are reported for PFOA, PFHS, PFOSAA and M570. The geometric mean and 95% tolerance limits of these fluorochemicals were, on average, an order of magnitude (or more) lower than PFOS. PFOS and PFOA were highly correlated ($r = .63$). PFOS had lower correlations with PFOSAA ($r = .42$), PFHS ($r = .38$) and M570 ($r = .20$). The number of samples with measured PFOSA and M556 concentrations below the LLOQ prohibited meaningful statistical analyses for these compounds.

The findings from this analysis of serum PFOS concentrations are consistent with those previously reported. The human data, to date, suggests the approximate average serum concentration in non-occupational adult populations may be 30 to 40 ppb with 95% of a population's serum PFOS concentrations below 100 ppb. Since serum PFOS concentrations likely reflect cumulative human exposure, this information will be useful for risk characterization.

INTRODUCTION

In May, 2000 the 3M Company (3M) announced that it would voluntarily cease manufacturing perfluorooctanesulfonyl- (POSF, $C_8F_{17}SO_2F$) related materials after the compound, perfluorooctanesulfonate (PFOS, $C_8F_{17}SO_3^-$), was found to be pervasive and persistent in human populations, wildlife, marine mammals and piscivorous birds (3M Company 2000; Hansen et al 2001; Giesy and Kannan 2001; Kannan et al 2001a; 2001b). POSF, produced by an electrochemical fluorination process, is used as the basic building block to create unique chemistries through the sulfonyl fluoride moiety using conventional hydrocarbon reactions. For example, POSF can be reacted with methyl or ethyl amines to produce either N-ethyl or N-methyl perfluorooctanesulfonamide. At this stage, these intermediates can be used to make amides, oxazolidinones, silanes, carboxylates and alkoxylates as commercial products. Also, these intermediates can be subsequently reacted with ethylene carbonate to form either N-ethyl or N-methyl perfluorooctanesulfonamidoethanol which can be used to make adipates, phosphate esters, fatty acid esters, urethane co-polymers and acrylates as commercialized products. Depending upon the specific functional derivatization or the degree of polymerization, such POSF-based products may degrade or metabolize, to an undetermined degree, to PFOS, a stable and persistent end-product that has the potential to bioaccumulate. While not a major commercial product, PFOS itself has been used in some products, including fire fighting foams.

The mechanisms and pathways leading to the presence of PFOS in human blood are not well characterized but likely involve environmental exposure to PFOS or its precursor molecules and residual levels of PFOS or PFOS precursors in industrial and commercial

products. PFOS has been detected at low parts per billion (ppb) concentrations in the general population (Hansen et al 2001; 3M Company 2000) although the scope of these investigations has been limited. Using high pressure liquid chromatography/electrospray tandem mass spectrometry, Hansen et al (2001) detected an average PFOS concentration of 28.4 ppb (SD 13.6; range 6.7-81.5) in 65 commercial individual human sera samples. An analysis of pooled blood samples (n = 3 to 6 pooled samples per location with 5 to 100 donors per pooled sample) from 18 blood banks in the United States resulted in a mean measured PFOS serum concentration of 30 ppb with a range from 9 to 56 ppb (3M Company, 2000). Serum PFOS concentrations among production employees working in POSF-related processes were approximately 2 parts per million (ppm) depending on work activity (range 0.1 to 12 ppm) (Olsen et al 1999).

The purpose of this study was to better characterize the distribution of seven fluorochemicals, including PFOS and some of its precursors in an adult population by analyzing sera samples obtained from donors at six American Red Cross blood banks. An assessment of the serum fluorochemical distribution was performed in relation to three demographic attributes (age, gender and location) of the anonymous blood donors.

METHODS

Fluorochemicals

The seven analytes detected and quantified in this study were: PFOS; N-ethyl perfluorooctanesulfonamidoacetate (PFOSAA, $C_8F_{17}SO_2N(CH_2CH_3)CH_2COO^-$); N-methyl perfluorooctanesulfonamidoacetate (M570, $C_8F_{17}SO_2N(CH_3)CH_2COO^-$); perfluorooctanesulfonamido acetate (M556, $C_8F_{17}SO_2N(CH)CH_2COO^-$);

perfluorooctanesulfonylamide (PFOSA, $C_8F_{17}SO_2NH_2$); perfluorooctanoate (PFOA, $C_7F_{13}COO^-$); and perfluorohexanesulfonate (PFHS, $C_6F_{13}SO_3^-$).

PFOSAA is an oxidation product of N-ethyl perfluorooctanesulfonamidoethanol (N-EtFOSE) and is a residual in N-EtFOSE-related chemistry which was primarily used in paper and packaging protectant applications. M570 is an oxidation product of N-methyl perfluorooctanesulfonamidoethanol (N-MeFOSE) and is a residual of N-MeFOSE-related chemistry which was used primarily in surface treatment applications (e.g., carpets, textiles). Therefore, PFOSAA and M570 can be considered markers of consumer-related exposure. Both PFOSAA and M570 can metabolize to M556 and PFOSA which, in turn can subsequently metabolize to PFOS. Unlike PFOSAA and M570, M556, PFOSA and PFOS are not specific to any one consumer application. Unlike the other analytes, PFOA and PFHS are not precursors, metabolites or residuals of PFOS. PFOA can be a residual by-product of the production of the POSF-related manufacturing electrochemical fluorination process and was produced by 3M to be an emulsifier in a variety of industrial applications (e.g., ammonium salt) (Olsen et al 2000). PFOA can also be an oxidation product or metabolite of the widely used telomer-based fluorochemicals manufactured by other companies. PFHS, the sulfonate form of perfluorohexane sulfonyl fluoride (PHSF), is a residual by-product of POSF-related production. 3M produced the PHSF as a building block compound incorporated in fire fighting foams and specific post-market carpet treatment applications.

Sample Collection

Through cooperation with six American Red Cross blood banks, 645 serum samples from adult donors (ages 20 - 69, equally represented of both sexes) were obtained for analysis. [Note: This final report supercedes the June 25, 2001 3M interim report which indicated 652 samples. Seven samples were not included in the final report because the subjects were determined to be ineligible by age (≥ 70). Additional separate 3M sponsored studies were designed to examine the distribution of serum samples among elderly adults and children.] The six American Red Cross blood banks represented donors from the following areas: Los Angeles, CA; Portland, OR; Minneapolis-St. Paul, MN; Charlotte, NC; Hagerstown, MD and Boston, MA. Samples were void of personal identifiers. The only known demographic factors were age, gender and location. Each blood bank was requested to provide approximately 10 samples per 10 year age intervals (20-29, 30-39, 40-49, 50-59 and 60-69) for each sex.

Fluorochemical Analysis

Northwest Bioanalytical (Salt Lake City, Utah) analyzed the serum for the target fluorochemicals using techniques similar to those described by Hansen et al (2001). Details of the specific analytical procedures are presented elsewhere (NWB 2002). Briefly, the analytical method consisted of a liquid:liquid extraction procedure followed by evaporation and reconstitution of the extract residue with 20 mM ammonium acetate in water:20 mM ammonium acetate in methanol (30:70, v/v). The samples were analyzed by high pressure liquid chromatography/tandem mass spectrometry. Quantitation of the target analytes in serum samples was performed by comparing the

chromatographic peak areas for each compound to those generated in a series of extracted calibration standards prepared from control Chinese plasma. The samples were injected in a systematic order. Evaluation of quality control samples injected during each analytical run indicated that the reported quantitative results may have varied, on average, up to 26 percent using human plasma calibration curves for all analytes except PFOSA which may have varied on average up to 43 percent.

Also presented in this report is a calculated total organic fluorine (TOF) index. TOF was the percent of each of the seven fluorochemicals' molecular weight that was attributed to organic fluorine [PFOS (64.7%); PFHS (61.9%); PFOA (69.0%); PFOSAA (55.3%); PFOSA (64.7%); M570 (56.6%) and M556 (58.1%)] multiplied by the ppb measured for each fluorochemical and then summed across all seven fluorochemicals.

Data Analysis

Measures of central tendency applicable to log normally distributed data (median, geometric mean) were used for descriptive analyses. In those instances where a sample was measured below the lower limit of quantitation (LLOQ), the midpoint between zero and the LLOQ was used for calculation of the geometric mean. An assessment of this midpoint assumption and how it affected the calculation of the geometric mean was performed using the 10th and 90th percentile values between zero and the LLOQ for those values <LLOQ.

In order to minimize parametric assumptions in the estimation of extreme percentiles of the population, the bootstrap method of Efron (1993) was used to generate confidence intervals around the empirical percentiles for serum concentrations. In this

method, a large number of replicated estimates of the percentile are generated from full-size samples of the original observations drawn with replacement. The distribution of the deviations of replicates from the original-sample estimate mimics the underlying sampling distribution for the estimate. Bias-corrected, accelerated percentiles were used to minimize residual bias. The bias correction factor is derived by comparing empirical percentiles to bootstrap percentiles and acceleration is accomplished by partial jackknifing.

Twenty-four samples were split and analyzed to provide an estimate of the reliability of the analyses conducted. The analytical laboratory was blind to the identity of these split samples. These analyses were performed concurrently with all other analyses of the study to minimize experimental error. Five split samples were analyzed from Charlotte, Los Angeles, Hagerstown and Portland and four split samples from Boston. Inadvertently, no reliability analyses were performed on the Minnesota samples.

RESULTS

The results for the reliability analysis for PFOS, PFOA and PFHS are displayed in Figure 1. Only six split samples for PFOSAA and seven split samples for M570 had values that were above the LLOQ. None of the PFOSA and M556 split samples were above the LLOQ. Therefore, only the reliability results for PFOS, PFOA and PFHS are displayed in Figure 1. There was a strong correlation between the split samples ($r = .9$) for each of these three fluorochemicals. It should be noted that 13 of the split sample analyses for PFHS had the identical LLOQ (2.1 ppb). This is represented in the graph as the single point near the abscissa (0,0) on the identity ($\ln y = \ln x$) line.

Provided in Table 1 is the distribution of the donor subjects by 10 year age intervals, gender and location. Altogether there were 332 male donors and 313 female donors. As could be expected with the age stratification design used for sample collection, the study subjects' mean ages were comparable by gender: 44.6 years for males and 43.9 years for females.

The measured concentrations of PFOSA and M556 were predominantly below the LLOQ. For PFOSA, there was one subject with a value above (2.1 ppb) the LLOQ, 196 subjects <LLOQ (1.0 ppb) and 448 subjects <LLOQ (1.4 ppb). The different LLOQ values were determined on different analytical runs. For M556, twelve subjects were above the LLOQ. Their M556 values ranged from 3.6 to 12.9 ppb. There were 145 subjects with M556 values <LLOQ (2.5 ppb) and 488 subjects with M556 values <LLOQ (3.2 ppb). Because of the few subjects whose serum concentrations exceeded the LLOQ, statistical analyses are not presented for PFOSA and M556. Although PFOSA and M556 are not presented in the subsequent analyses, they were included in the calculation of the TOF index. For those measured concentrations of PFOSA and M556 < LLOQ, the midpoint between zero and the LLOQ was used in the calculation of the TOF index.

The frequency distributions of the five remaining fluorochemicals, PFOS, PFOA, PFHS, PFOSAA and M570, are displayed in Figure 2. Although the graphs are suggestive of log normal distributions, only the PFOS distribution met such criteria based on the Shapiro-Wilk test. This lack of log normality is due to the greater percentage of subjects with values <LLOQ for PFOA, PFHS, PFOSAA and M570.

The range, interquartile range, number of samples < LLOQ, raw cumulative 90th percentile, median, geometric mean and 95% confidence interval of the geometric mean

for PFOS, PFOA, PFHS, PFOSAA and M570 are provided in Table 2 for all subjects, males only and females only. Overall, the geometric mean levels of PFOS was 34.9 ppb (95% CI 33.3-36.5). The range of PFOS values was < LLOQ (4.3 ppb) to 1656.0 ppb. Male subjects had significantly ($p < .05$) higher geometric means for PFOS than female subjects [male geometric mean = 37.8 ppb (95% CI 35.5-40.3) vs female geometric mean = 32.1 ppb (95% CI 30.0-34.3)]. Males also had significantly higher serum levels of PFOA and PFHS compared to females although the mean levels for both sexes were approximately one order of magnitude lower than that of PFOS. It should be noted that the overall geometric mean for the calculated TOF index was 31.7 ppb (95% CI 30.4 - 33.0) (data not shown). The calculated TOF index ranged from 5.7 ppb to 1083.2 ppb.

Provided in Figure 3 is a graphical distribution (natural log scale) of the five fluorochemicals by 10 year age intervals stratified by gender. The box covers the interquartile range of the natural log distribution. The circle within the box is the mean. The whiskers extend to the last observation within 1.5 times the interquartile range. The dots with lines through them represent observations outside the 1.5 times interquartile range. As shown in Figure 3, age was not an important predictor of adult serum fluorochemical concentrations. In those instances where there were many outliers (e.g., M570 concentrations in males aged 40-49 and 60-69), this was the result of a large percentage of values <LLOQ that were within the 1.5 x interquartile range.

As discussed previously in the Methods, the geometric mean data were calculated under the assumption that for individual serum fluorochemical values <LLOQ the midpoint between zero and the LLOQ was assigned. For PFOS, only one subject had a value <LLOQ; thus this assumption did not affect its calculation of the geometric mean.

However, considerably more subjects had LLOQs for PFOA, PFHS, PFOSAA and M570 (see Table 2). If these values were assumed to be 10% or 90% of this range between zero and the LLOQ the respective range of the geometric means (95% confidence interval in parenthesis) became: PFOA 4.0 ppb (3.7-4.1) to 4.8 ppb (4.6-5.0); PFHS 0.9 ppb (0.8-1.0) to 2.5 ppb (2.4-2.6); PFOSAA 0.8 ppb (0.7-0.9) to 2.8 ppb (2.7-3.0) and M570 0.5 ppb (0.5-0.6) to 1.9 ppb (1.8-2.0). These geometric mean values were not substantially different than those calculated using the midpoint between zero and the <LLOQ as presented in Table 2. Consequently, the midpoint between zero and the LLOQ was used for the analyses.

Presented in Table 3 are the range, interquartile range and medians for the six locations combined across age and gender for the five fluorochemicals. A graphical presentation of these data (natural log scale) is presented in Figure 4. Interpretation of the graphs is comparable to those discussed above for Figure 3. Provided in Table 4 are the results from a bootstrap analysis which calculated mean serum fluorochemical values for each of the six locations adjusted for 10 year age intervals, gender and their interaction terms. The highest mean value for PFOS was Charlotte (39.0 ppb) with the lowest being Boston (29.0 ppb). Los Angeles, Minneapolis-St. Paul and Hagerstown had comparable mean PFOS levels of approximately 35.0 ppb with Portland slightly lower (32.8 ppb). The range of means for the other fluorochemical analytes was narrow and thus difficult to distinguish any substantial differences by location. Because PFOS is the primary contributor to the calculated TOF index, the bootstrap analysis findings for TOF mirrored those of PFOS.

Scatter plots (log scale) between the five fluorochemicals are displayed in Figure 5. PFOS and PFOA were highly correlated ($r = .63$). PFOS had a lower correlation with PFOSAA ($r = .42$) and lower yet with M570 ($r = .20$). The correlation between PFOSAA and M570 was weak ($r = .12$). The remaining scatter plots display the correlation between PFOS and PFHS ($r = 0.38$) and PFOA and PFHS ($r = 0.32$). Both PFOSAA and M570, adjusted for age, gender and their interaction, were significant predictors of PFOS in a multivariable model albeit PFOSAA was the stronger of the two independent variables (Table 5). Nevertheless, approximately eighty percent of the variation of PFOS was left unexplained. Age and gender were not significant predictors in models that examined the significant association between PFOS and PFOA (Table 6) or PFHS (Table 7). None of the models in Tables 5 through 7 had lack of fit F ratios that were statistically significant ($p < .05$).

Presented in Table 8 are the results from bootstrap analyses conducted to provide mean concentrations of several tolerance limits. The tolerance limits represent the limit of each fluorochemical within which the stated proportion of the population is expected to be found. Presented are the mean values of the five serum fluorochemicals and TOF for the 90th, 95th and 99th percent tolerance limits along with the upper limit (bound) from the 95% confidence interval. For example, the mean of the 95% tolerance limit for PFOS was 88.5 ppb with an upper 95% confidence limit of 100.0 ppb. At the lowest tolerance limit analyzed (90%), the mean for PFOS was 70.7 ppb with an upper 95% confidence limit of 74.3 ppb. At the highest tolerance limit analyzed (99%), the mean was 157.3 ppb with an upper 95% confidence limit of 207.0 ppb. For other fluorochemicals analyzed, the mean of the 95% tolerance limit for PFOA was 12.1 ppb with an upper 95%

confidence limit of 13.6 ppb. For PFHS, the mean of the 95% tolerance limit was 9.5 ppb with an upper 95% confidence limit of 10.8 ppb. The mean of the 95% tolerance limit for PFOSAA was 7.6 ppb with an upper 95% confidence limit of 8.5 ppb. For M570, the mean was 5.0 ppb for a 95% tolerance limit with an upper 95% confidence limit of 5.4 ppb. Finally, for the calculated index of TOF, the mean was 75.1 ppb for the 95% tolerance limit with an upper 95% confidence limit of 80.9 ppb.

DISCUSSION

The findings from this analysis of serum PFOS concentrations in 645 adult donors are consistent with previous human data. Previous measurements of human serum samples obtained in the United States showed mean PFOS concentrations of 30 ppb in 18 pooled blood banks, 44 ppb from a pooled commercial sample of 500 donors, 33 ppb from a different pooled commercial sample of 200 donors and 28 ppb in 65 commercial individual human sera samples (3M Company 2000; Hansen et al 2001). These findings were also comparable to a limited number of European samples which found mean serum PFOS concentrations at 17 ppb in 5 pooled samples from a Belgium blood bank, 53 ppb in 6 pooled samples from the Netherlands and 37 ppb from 6 pooled blood samples from Germany and 39 individual Swedes whose serum PFOS ranged between <LLOQ 32 ppb to 85 ppb (3M Company, 2000). The mean calculated TOF index of 31.7 ppb in the present study was also consistent with the low ppb total organic fluorine measurements of general population samples that have been reported since the 1960's (Taves 1968; Taves et al 1976; Singer and Ophaug 1979; Belisle 1981).

Unique to the present study was its large individual sample size which facilitated the characterization of the serum fluorochemical distribution. This included the calculation of tolerance limits and their upper bounds. The highest serum PFOS measurement (confirmed by re-assay of the sample) was 1656.0 ppb. Because donor samples were anonymous, it is not possible to determine anything about this individual besides gender (male), age (67 years) and location (Portland). This PFOS sample approximated the average serum PFOS levels observed for POSF-related production workers (Olsen et al 1999). The next highest donor level for PFOS was considerably lower at 329 ppb (also a male, age 62 from the Portland area) and the subsequent next eight highest serum PFOS values (range 139 ppb to 226 ppb) were measured in four females and four males representing Charlotte (n = 4), Hagerstown (n = 2), Los Angeles (n = 1) and Minneapolis-St. Paul (n = 1). Also distinctive to this effort was the study's capability to examine potential associations between PFOS and age (no association) and gender (males slightly higher than females).

Our findings showed a strong correlation between PFOS and PFOA. Whereas PFOS has been routinely measured in human populations, wildlife, marine mammals and piscivorous birds (Giesy and Kannan 2001; Kannan et al 2001a; 2001b; Hansen et al 2001), serum PFOA concentrations, to date, have been consistently quantified (i.e., measured above the LLOQs) primarily in humans. This association is of significant interest because PFOA cannot convert directly from PFOS (or vice versa). Whether this association is due to the presence of PFOA as a by-product in POSF-related production or to other non-related environmental exposures or consumer products from other manufacturers (e.g., higher chain telomers) remains to be answered. Another

unanswered question is whether perfluorooctanesulfonamides can metabolize in humans to PFOA. Any of these explanations coupled with the suspected long serum half-lives in humans for PFOS (8.7 years (SD = 6.1) and PFOA (4.4 years (SD = 3.5) as reported by Burris et al (2002), could explain the strong correlation between PFOS and PFOA.

PFOS was also correlated with two fluorochemicals, PFOSAA and M570, known to be analytes from exposure to consumer products involving paper/package and carpet/textile protectants, respectively. Overall, the data, to date, indicate that PFOS bioaccumulation in animals may be primarily through environmental sources whereas both environmental and product exposures likely contribute to serum PFOS concentrations in humans (Giesy and Kannan 2001; Kannan et al 2001a; 2001b).

As with any interpretation of data obtained from a study population, questions arise regarding the representativeness and ability to generalize the data collected. Clearly, American Red Cross blood donors are a self-selected group from the United States population (Leibrecht et al 1976; Oswalt 1977; Burnett 1982; Allen and Butler 1993; Andaleeb and Basu 1995; Chiavette et al 2000). Motivations to donate have included altruism, desire for personal credit, low self-esteem, social pressure and test-seeking behavior. Motivations not to donate have included fear, medical excuses, apathy and inconvenience. Donors tend to have a greater trust in institutions, more interest in their personal health and higher risk-taking behavior. Demographically, white males and older individuals have historically constituted a larger percentage of the donor pool. Donors also tend to be more educated, married and have more children than nondonors. In the present study, donors were not asked whether a sample of their blood donation could be used for fluorochemical assay nor was there any linkage between the sample

collected and personal identifiers. No information was obtained about past exposure histories to POSF-related chemistries and materials (or the other fluorochemicals that were analyzed). Therefore, we believe the selection process of donors used in this study resulted in a reasonable representation of the overall blood donor population that was providing blood at the time when these donors were sampled. We are unaware of any database that can be considered generalizable to the diverse United States general adult population without measures of random and systematic bias incorporated in the data analysis.

Given the consistency of the data analyzed, to date, we hypothesize that the average serum PFOS concentrations in non-occupational adult populations likely ranges between 30 to 40 ppb with 95% of a population's serum PFOS concentrations below 1000 ppb. Understanding these serum PFOS levels in human populations will be useful for risk characterization since serum PFOS likely reflects cumulative human exposure (3M Company 2000). Currently available data (unpublished reports to U.S. EPA:Docket No. FYI-0500-01378) suggest that the serum concentrations observed in humans are substantially less than those required to cause adverse effects in laboratory animals (3M Company 2000).

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Table 1
Distribution of American Red Cross Blood Donor Subjects by Age, Gender and Location

Location	Males						Females						Overall Total
	20 - 29	30 - 39	40 - 49	50 - 59	60 - 69	Subtotal	20 - 29	30 - 39	40 - 49	50 - 59	60 - 69	Subtotal	
Los Angeles	14	11	15	13	10	63	16	12	12	12	10	62	125
Boston	12	11	11	11	12	57	10	11	10	11	10	52	109
Minneapolis - St. Paul	10	10	10	10	10	50	10	10	10	10	10	50	100
Charlotte	10	10	10	10	7	47	10	11	10	10	8	49	96
Portland	10	11	10	15	10	56	10	11	10	11	9	51	107
Hagerstown	10	10	10	10	19	59	10	10	10	10	9	49	108
	66	63	66	69	68	332	66	65	62	64	56	313	645

Table 2
Measures of Central Tendency of Serum Fluorochemicals for American Red Cross Blood Donors (N = 645) by Gender

	PFOS	PFOA	PFHS	PFOSAA	M570
All (N = 645)					
Range	< LOQ (4.3) – 1656.0	< LOQ (1.9) – 52.3	< LOQ (1.4) – 66.3	< LOQ (1.6) – 60.1	< LOQ (1.0) – 16.4
Q1 – Q3	24.7 – 48.5	3.4 – 6.6	< LOQ (2.1) – 3.4	< LOQ (2.8) – 3.4	< LOQ (1.8) – 2.2
< LOQ (Number)	< 4.3 (1)	< 1.9 (2)	< 1.4 (72)	< 1.6 (101)	< 1.0 (63)
		< 2.1 (48)	< 2.1 (235)	< 2.8 (271)	< 1.8 (326)
Cumulative 90%	70.7	9.4	6.3	5.2	3.8
Median	35.8	4.7	1.5	< LOQ (2.8)	< LOQ (1.8)
Geometric Mean	34.9	4.6	1.9	2.0	1.3
95% C.I. Geometric Mean	33.3 – 36.5	4.3 – 4.8	1.8 – 2.0	1.9 – 2.1	1.3 – 1.4
Males (N = 332)					
Range	< LOQ (4.3) – 1656.0	< LOQ (1.9) – 29.0	< LOQ (1.4) – 66.3	< LOQ (1.6) – 60.1	< LOQ (1.0) – 16.4
Q1 – Q3	28.3 – 49.7	3.6 – 7.0	< LOQ (2.1) – 3.8	< LOQ (2.8) – 3.3	< LOQ (1.8) – 2.2
< LOQ (Number)		< 2.1 (19)	< 1.4 (30)	< 1.6 (58)	< 1.0 (36)
			< 2.1 (104)	< 2.8 (146)	< 1.8 (163)
Cumulative 90%	72.6	10.1	7.9	4.7	3.5
Median	37.4	4.9	2.1	< LOQ (2.8)	< LOQ (1.8)
Geometric Mean	37.8	4.9	2.2	1.9	1.3
95% C.I. Geometric Mean	35.5 – 40.3	4.6 – 5.3	2.0 – 2.4	1.8 – 2.1	1.2 – 1.4

Females (N = 313)

Range	6.0 – 226.0	< LOQ (2.1) – 52.3	< LOQ (1.4) – 15.3	< LOQ (1.6) – 27.6	< LOQ (1.0) – 10.6
Q1 – Q3	22.0 – 45.8	3.1 – 6.2	< LOQ (2.1) – 2.8	< LOQ (2.8) – 3.6	< LOQ (1.8) – 2.2
< LOQ (Number)		< 1.9 (2)	< 1.4 (42)	< 1.6 (43)	< 1.0 (27)
		< 2.1 (29)	< 2.1 (131)	< 2.8 (125)	< 1.8 (163)
Cumulative 90%	69.7	8.4	5.0	6.1	4.0
Median	31.3	4.4	< LOQ (2.1)	< LOQ (2.8)	< LOQ 1.8)
Geometric Mean	32.1	4.2	1.6	2.1	1.3
95% C.I. Geometric Mean	30.0 – 34.3	3.9 – 4.5	1.5 – 1.8	2.0 – 2.3	1.2 – 1.4

Table 3
Measures of Central Tendency of Fluorochemicals by the Six American Red Cross Blood Bank Locations

	PFOS	PFOA	PFHS	PFOSAA	M570
<u>Los Angeles</u>					
Range	6.6 – 205.0	<LOQ (2.1) – 34.1	<LOQ (2.1) – 12.4	<LOQ (2.8) – 27.6	<LOQ (1.8) – 16.4
Q1 – Q3	29.5 – 53.7	2.9 – 6.6	<LOQ (2.1) – 3.0	<LOQ (2.8) – 4.2	<LOQ (1.8) – 2.2
Cumulative 90%	70.1	9.2	5.8	6.1	4.8
Median	42.2	4.6	<LOQ (2.1)	3.1	<LOQ (1.8)
Geometric Mean	40.4	4.1	1.9	2.6	1.4
95% C.I. Geometric Mean	37.0 – 44.0	3.6 – 4.7	1.7 – 2.1	2.4 – 3.0	1.2 – 1.6
<u>Boston</u>					
Range	<LOQ (4.3) – 87.2	1.5 – 13.9	<LOQ (1.4) – 12.6	<LOQ (1.6) – 6.7	<LOQ (1.8) – 13.0
Q1 – Q3	20.8 – 39.1	4.1 – 7.3	<LOQ (2.1) – 3.0	<LOQ (1.6) – 2.9	<LOQ (1.8) – 2.4
Cumulative 90%	48.7	9.7	5.4	4.1	3.5
Median	29.5	5.5	2.1	<LOQ (2.8)	1.8
Geometric Mean	28.0	5.4	1.9	1.6	1.4
95% C.I. Geometric Mean	25.4 – 31.0	5.0 – 5.8	1.6 – 2.2	1.4 – 1.8	1.2 – 1.6

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Mpls. – St. Paul

Range	7.7 – 207.0	<LOQ (1.9) – 20.0	<LOQ (1.4) – 15.2	<LOQ (1.6) – 12.9	<LOQ (1.0) – 10.6
Q1 – Q3	23.9 – 43.3	3.2 – 5.7	<LOQ (1.4) – 2.8	<LOQ (1.6) – 2.6	<LOQ (1.0) – 2.3
Cumulative 90%	71.7	9.9	5.7	4.9	4.0
Median	31.7	4.4	1.4	1.4	1.3
Geometric Mean	33.1	4.5	1.5	1.6	1.2
95% C.I. Geometric Mean	29.8 – 36.7	4.0 – 5.0	1.3 – 1.8	1.4 – 1.8	1.0 – 1.4

Charlotte

Range	19.3 – 166.0	<LOQ (2.1) – 29.0	<LOQ (1.4) – 22.4	<LOQ (1.6) – 60.1	<LOQ (1.0) – 10.8
Q1 – Q3	36.3 – 70.9	4.5 – 8.6	1.5 – 4.8	<LOQ (2.8) – 4.2	<LOQ (1.8) – 2.8
Cumulative 90%	105.3	13.3	10.9	8.6	4.7
Median	48.9	6.3	2.8	1.8	1.2
Geometric Mean	51.5	6.3	2.8	2.4	1.5
95% C.I. Geometric Mean	46.8-56.8	5.6-7.1	2.4-3.4	1.9-2.9	1.3-1.8

Portland

Range	6.0 – 1656.0	<LOQ (2.1) – 16.7	<LOQ (2.1) – 11.8	<LOQ (2.8) – 36.9	<LOQ (1.8) – 6.9
Q1 – Q3	17.2 – 37.7	2.8 – 4.7	<LOQ (2.1) – 2.5	<LOQ (2.8) – 3.8	<LOQ (1.8) – 2.9
Cumulative 90%	49.4	6.8	5.5	7.4	2.9
Median	26.0	3.8	<LOQ (2.1)	<LOQ (2.8)	<LOQ (1.8)
Geometric Mean	27.0	3.6	1.6	2.5	1.3
95% C.I. Geometric Mean	23.5 – 31.1	3.2 – 4.0	1.4 – 1.8	2.2 – 2.9	1.2 – 1.5

Hagerstown

Range	7.6 – 226.0	<LOQ (2.1) – 52.3	<LOQ (2.1) – 66.3	<LOQ (2.8) – 21.2	<LOQ (1.8) – 7.9
Q1 – Q3	24.4 – 48.1	3.2 – 5.9	<LOQ (2.1) – 3.8	<LOQ (2.8) – 1.4	<LOQ (1.8) – 1.9
Cumulative 90%	69.8	7.6	7.3	3.4	3.1
Median	35.7	4.7	<LOQ (2.1)	<LOQ (2.8)	<LOQ (1.8)
Geometric Mean	35.3	4.2	2.1	1.7	1.2
95% C.I. Geometric Mean	31.8 – 39.2	3.8 – 4.8	1.7 – 2.4	1.5 – 1.9	1.1 – 1.4

Table 4
Mean and 95% Confidence Intervals Calculated from Bootstrap Analyses
For the Six Locations, Adjusted for Age, Gender and Their Interaction Term

	PFOS		PFOA		PFHIS		PFOSAA		M570		TOF	
	Mean	95% CI	Mean	95% CI	Mean	95% CI	Mean	95% CI	Mean	95% CI	Mean	95% CI
Los Angeles	35.0	33.4 – 36.5	4.6	4.4 – 4.8	1.9	1.8 – 2.0	2.0	1.9 – 2.2	1.3	1.3 – 1.4	30.2	29.0 – 31.3
Boston	29.0	26.0 – 30.3	5.3	4.6 – 5.6	1.9	1.7 – 2.3	1.6	1.4 – 1.8	1.3	1.2 – 1.5	26.4	23.9 – 29.2
Minneapolis – St. Paul	34.8	31.9 – 36.3	4.5	4.1 – 4.7	1.8	1.6 – 2.0	1.8	1.7 – 2.1	1.3	1.1 – 1.4	29.7	27.4 – 32.1
Charlotte	39.0	36.2 – 40.7	5.0	4.6 – 5.1	2.2	2.0 – 2.4	2.1	1.9 – 2.4	1.4	1.3 – 1.5	33.4	31.3 – 35.7
Portland	32.8	30.5 – 34.2	4.3	4.0 – 4.4	1.8	1.7 – 2.0	2.1	2.0 – 2.3	1.3	1.2 – 1.4	28.5	26.7 – 30.4
Hagerstown	34.9	32.8 – 36.5	4.5	4.2 – 4.6	1.9	1.8 – 2.1	1.9	1.8 – 2.1	1.3	1.2 – 1.4	30.1	28.4 – 31.9

Table 5
Multivariable Regression Model of PFOS* by PFOSAA*, M570*,
Age, Gender and Age x Gender Interaction

	Coefficient	SE	t ratio	p value
Intercept	3.3	0.07	46.3	< .0001
PFOSAA*	0.3	0.03	11.7	< .0001
M570*	0.1	0.03	4.1	< .0001
Age	- 0.001	0.001	-0.8	.40
Gender	- 0.2	0.07	-2.4	.02
Age x Gender	0.001	0.001	0.9	.35

N = 645

*Natural log

Adjusted $r^2 = 0.22$

Gender: females =1; males = 0

t ratio = coefficient/SE (standard error)

Table 6
Multivariable Regression Model of PFOS* by PFOA*
Age, Gender and Age x Gender Interaction

	Coefficient	SE	t ratio	p value
Intercept	2.7	0.07	36.4	< .0001
PFOA*	0.6	0.03	20.3	< .0001
Age	- 0.001	0.001	-1.0	.29
Gender	0.02	0.06	0.4	.72
Age x Gender	-0.001	0.001	-1.0	.31

N = 645

*Natural log

Adjusted $r^2 = 0.40$

Gender: females = 1; males = 0

t ratio = coefficient/SE (standard error)

Table 7
Multivariable Regression Model of PFOS* by PFHS*
Age, Gender and Age x Gender Interaction

	Coefficient	SE	t ratio	p value
Intercept	3.4	0.07	45.5	< .0001
PFHS*	0.3	0.03	9.9	< .0001
Age	-0.0003	0.002	-0.2	.84
Gender	-0.06	0.07	-0.8	.43
Age x Gender	0.0002	0.002	0.2	.87

N = 645

*Natural log

Adjusted $r^2 = 0.15$

Gender: females = 1; males = 0

t ratio = coefficient/SE

Table 8. Tolerance Limits and Their Associated Mean and Upper 95th Percent Confidence Limits for Serum Fluorochemicals and Calculated Total Organic Fluorine Index

	Tolerance Limit	Mean	Upper 95 th Percent Confidence Limit
PFOS	90%	70.7	74.3
	95%	88.5	100.0
	99%	157.3	207.0
PFOA	90%	9.4	10.1
	95%	12.1	13.6
	99%	19.8	25.8
PFHS	90%	6.3	7.0
	95%	9.5	10.8
	99%	17.0	22.4
PFOSAA	90%	5.3	5.9
	95%	7.6	8.5
	99%	19.4	27.6
M570	90%	3.7	4.0
	95%	5.0	5.4
	99%	8.1	10.3
TOF	90%	59.9	63.1
	95%	75.1	80.9
	99%	137.3	187.5

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Figure 1. Analysis of Split Samples for Reliability Assessment for PFOS, PFOA and PFHS

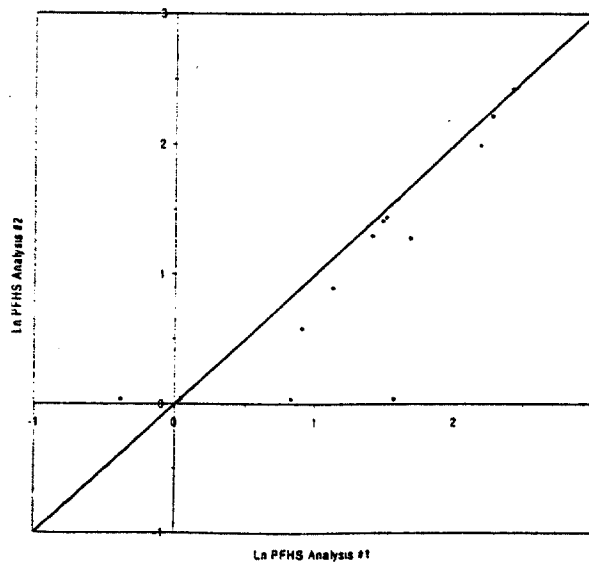
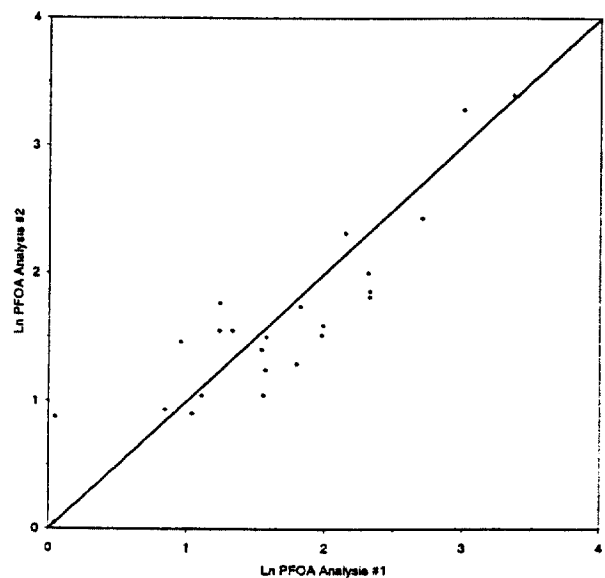
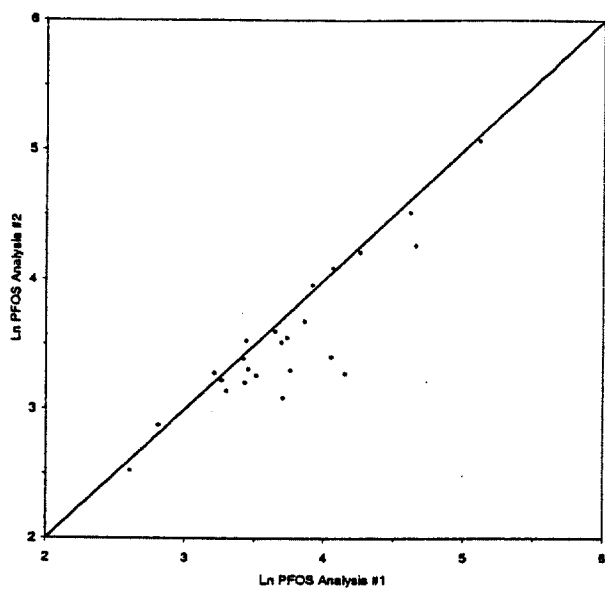
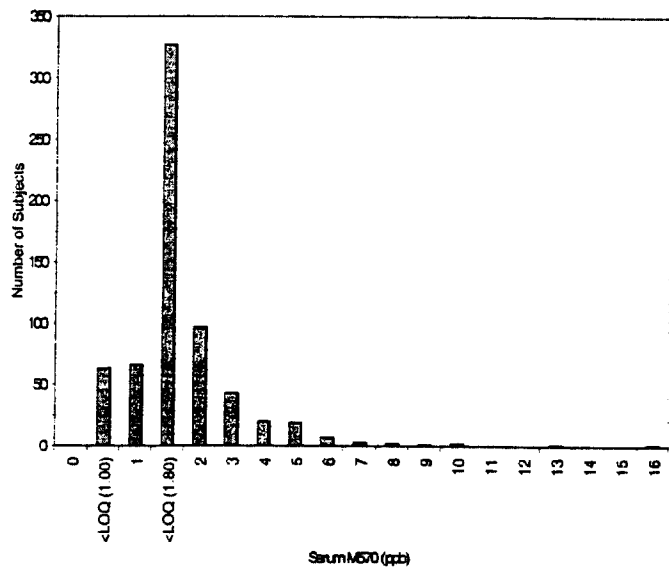
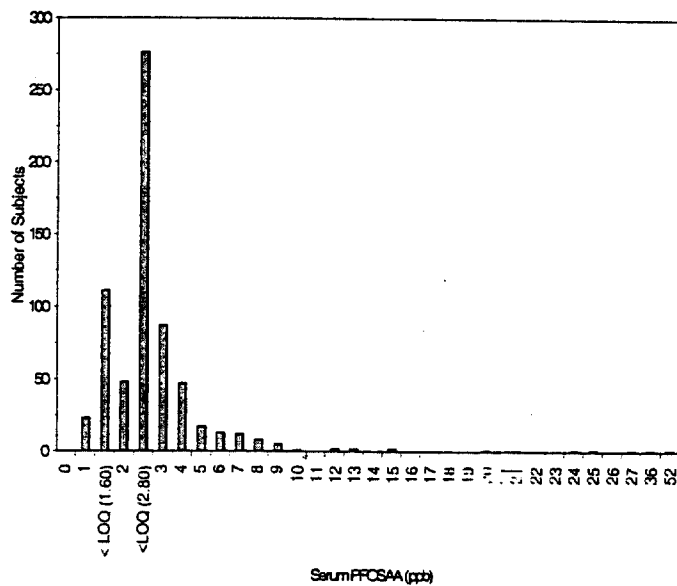
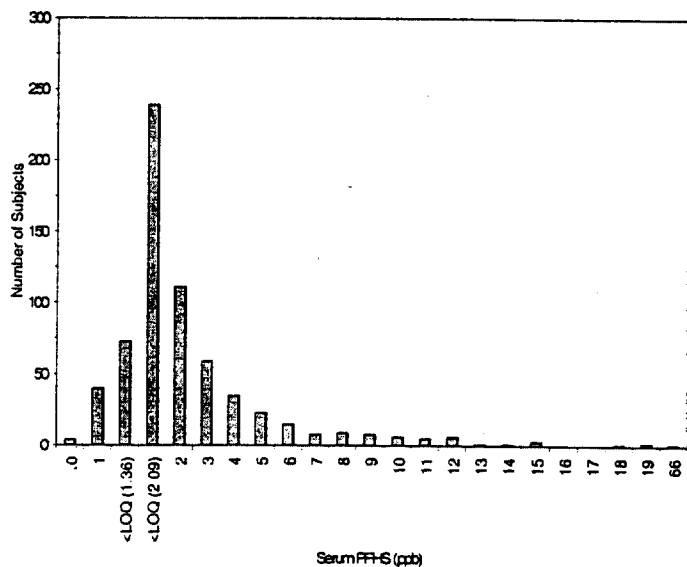
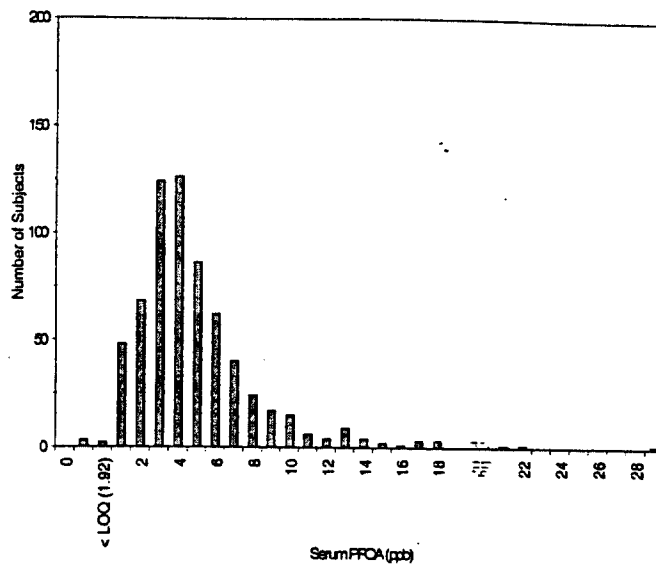
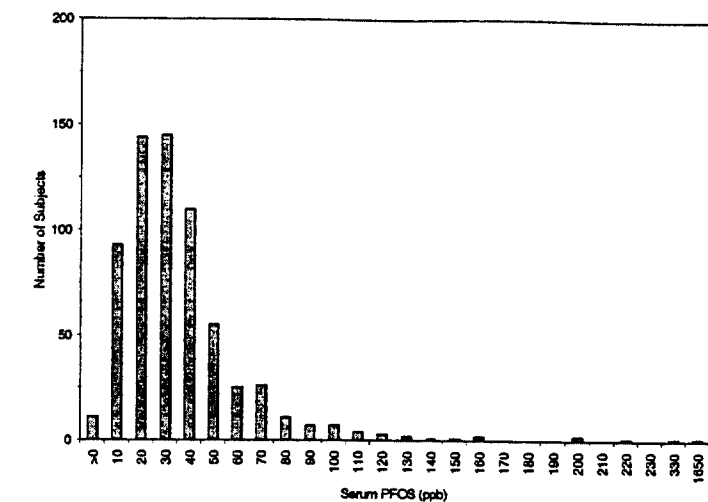


Figure 2. Adult Study Population Distribution of Measured Fluorochemical Concentrations



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Figure 3. Box and Whisker Plots of Serum Fluorochemical Concentrations by Age and Gender

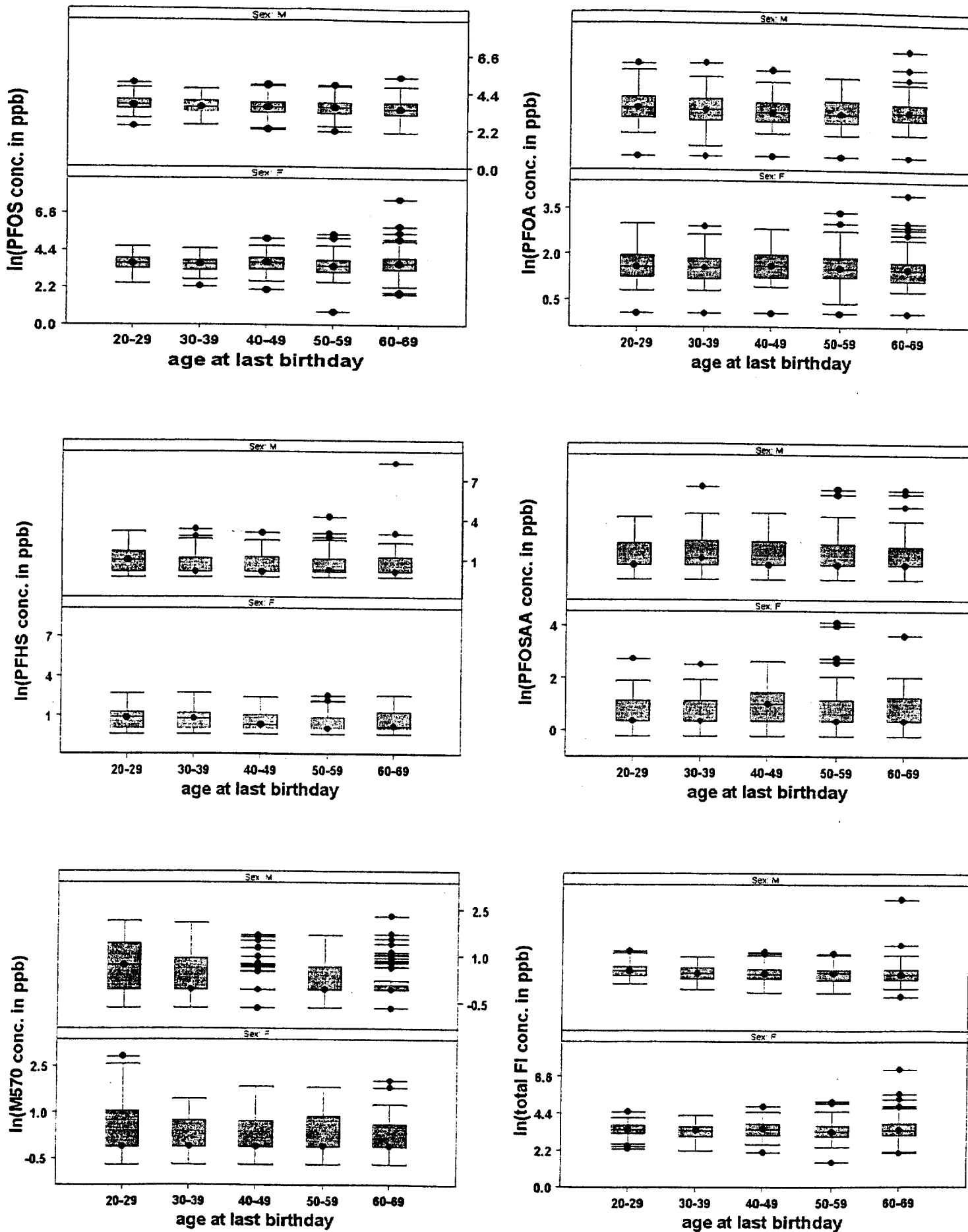


Figure 4. Box and Whisker Plots of Serum Fluorochemical Concentrations by Age and

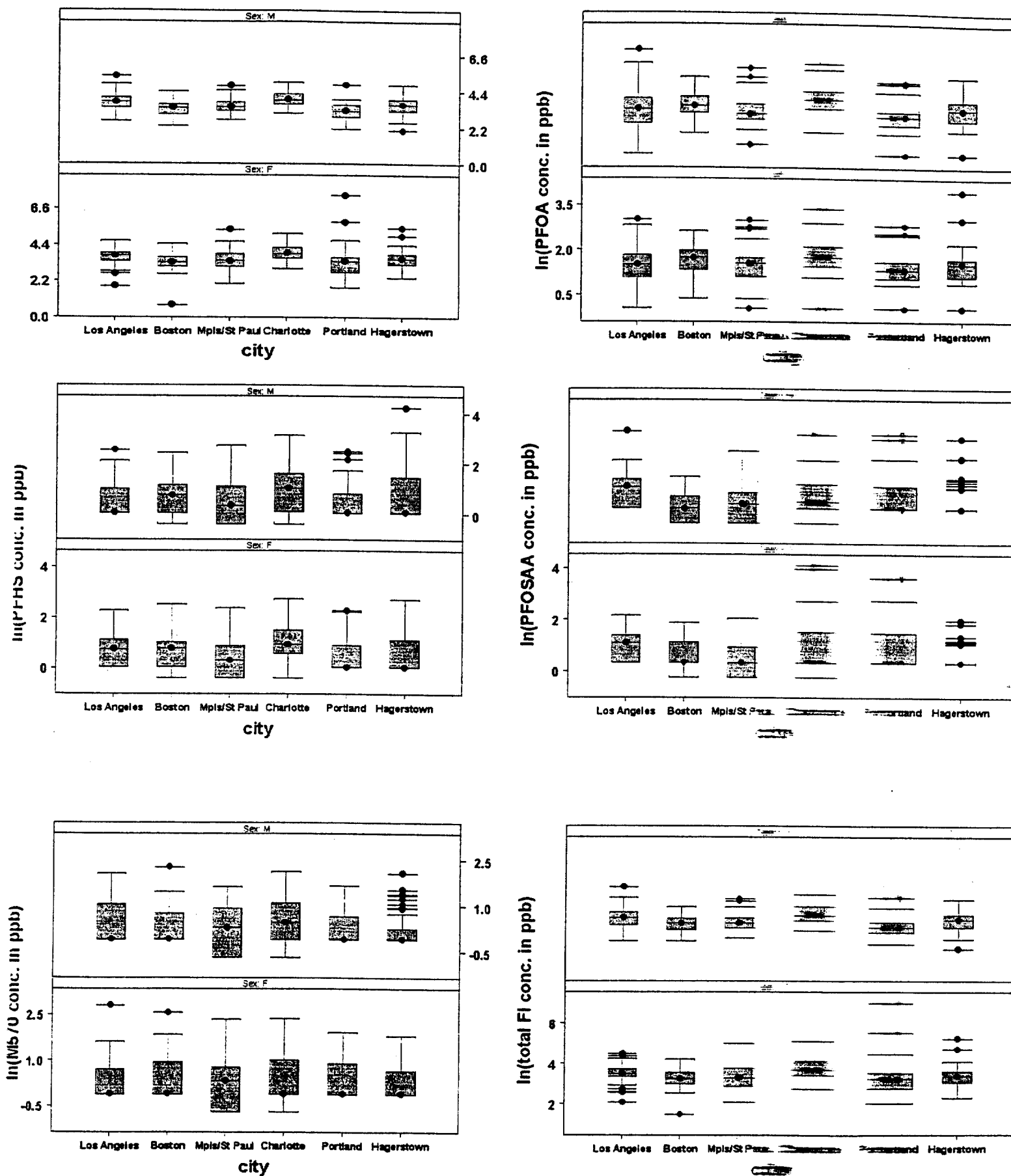


Figure 5. Scatter Plots (log scale) of Fluorochemical Associations

