

FINAL REPORT
Epidemiology
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Title: Identification of Fluorochemicals in Human Sera. II. Elderly Participants of the Adult Changes in Thought Study, Seattle, Washington

Study

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ABSTRACT

A total of 238 serum samples from elderly volunteers from a large prospective longitudinal study designed to examine cognitive function among male and female subjects, ages 65-96, in the Seattle (WA) area were obtained for fluorochemical analyses. Samples were void of personal identifiers. The only known demographic factors were: age, gender and the number of years residence in Seattle.

Sera samples were extracted and quantitatively analyzed for seven fluorochemicals using high-pressure liquid chromatography/electrospray tandem mass spectrometry. The seven fluorochemicals detected 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, $C_8F_{17}SO_2N(CH_3)CH_2COO^-$); perfluorooctanesulfonamide (PFOSA, $C_8F_{17}SO_2NH_2$); perfluorooctanoate (PFOA, $C_8F_{17}COO^-$); and perfluorohexanesulfonate (PFHS, $C_6F_{13}SO_3^-$).

Overall, the geometric mean measured concentration of PFOS was 31.0 ppb (95% CI 28.8-33.4). The measured PFOS concentration ranged from less than the lower limit of quantitation (LLOQ) of 3.4 ppb to 175.0 ppb. There was no significant difference in the PFOS geometric means by sex or years residence in Seattle. Age was negatively associated with PFOS. Bootstrap analyses were used to calculate a 95% tolerance limit for PFOS of 84.1 ppb with an upper 95% confidence limit of 104.0 ppb. Additional geometric mean and tolerance limit data are reported for PFOA, PFHS, PFOSAA and M570. The geometric means and tolerance limits for these fluorochemicals were, on average, an order of magnitude (or more) lower than PFOS. There was a strong

correlation between PFOS and PFOA ($r = .75$). PFOS had lower correlations with PFOSAA and PFHS ($r = .42$) and lower yet with M570 ($r = .29$). The number of samples with measured concentrations of PFOSA and M556 below the LLOQ prohibited meaningful statistical analysis of these compounds.

The findings from this analysis of serum PFOS concentrations are consistent with serum PFOS levels of 645 American Red Cross blood donors, ages 20-69. These and other data suggest the average serum concentration in the non-occupational adult population data approximates 30 to 40 ppb with 95% of the 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 production 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 10 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 the human population by using individual sera samples obtained from elderly subjects enrolled in the Adult Changes in Thought (ACT) study (McCurry et al 1999). An assessment of the serum fluorochemical distribution was performed in relation to three demographic attributes (age, gender and years lived in the Seattle metropolitan area) of the study subjects.

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 products. 3M produced PHSF as a building block compound incorporated in fire fighting foams and specific post-market carpet treatment applications.

Sample Collection

Through cooperation with the staff of the Adult Changes in Thought (ACT) study, 238 serum samples from elderly adult donors (ages 65-96) equally represented of both sexes were obtained for analysis. Subjects were identified during an enrollment phase of this community-based prospective cohort study of dementia and normal aging conducted collaboratively between the University of Washington and Group Health Cooperative (GHC), a major health maintenance organization in Seattle (McCurry et al 1999). Eligible individuals were those with no known history of neuropsychiatric disease or dementia. Chart reviews of these subjects' GHC medical records were conducted to confirm that the individuals did not reside in nursing homes or have a history of dementia diagnosis in their medical records. Subjects were not excluded from participation in the ACT study on the basis of common age-related chronic illnesses. Although it was desired to obtain more subjects above the age of 80, the study was truncated due to the relatively few subjects who volunteered and were eligible for this age stratum.

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 differ from the actual concentration by up to 26 percent for all analytes except PFOSA which may have differed by up to 43 percent.

Also presented in this report is a calculated index, total organic fluorine (TOF), which 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 randomly selected samples, stratified by gender, 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.

RESULTS

The results for the reliability analysis are displayed in Figure 1. None of the PFOSA and most of the M556 split samples were below the LLOQ and are therefore not displayed. There were moderately strong correlations for the split samples ($r = .7$) with either PFOS or PFOA and stronger correlations for PFHS ($r = .9$) and M570 ($r = .8$). The correlation for PFOSAA was less ($r = 0.4$). This was likely due to the fact that only four of the split samples had both values above the LLOQ. Eleven of the split samples had

one of the two values <LLOQ and nine of the split sample analyses for PFOSAA had the identical LLOQ (1.5 ppb). The midpoint between zero and the LLOQ (1.5 ppb) is represented in the graph as the single point below the abscissa (0,0) on the identity ($\ln y = \ln x$) line.

Provided in Table 1 is the distribution of the 238 elderly subjects by 10 year age intervals, gender and location. Altogether there were 118 male donors and 120 female donors. As could be expected with the age stratification design used for sample collection, the study subjects' mean ages were comparable by gender: 76.0 years for males and 76.2 years for females. Female subjects had resided, on average, slightly longer in the Seattle area.

The measured concentrations of PFOSA were below the LLOQ (1.0 ppb) for all subjects. For M556, eight subjects had measured serum concentrations above the LLOQ ranging from 2.7 to 4.8 ppb. There were 230 subjects with M556 values <LLOQ (2.5 ppb). Therefore, statistical analyses are not presented for PFOSA and M556 because of the few subjects whose serum concentrations exceeded the LLOQ. Nevertheless, PFOSA and M556 did contribute to the calculation of the TOF index by using, for those values < LLOQ, the midpoint between zero and the LLOQ.

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 normality for PFOA, PFHS, PFOSAA and M570 was likely the consequence of a greater percentage of subjects with values <LLOQ for these compounds.

The range, interquartile range, number of samples < LLOQ, 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 31.0 ppb (95% CI 28.8-33.4). The range of values was < LLOQ (3.4) to 175.0 ppb. There was no significant difference ($p < .05$) between male and female geometric means for any of the five fluorochemicals reported in Table 2. It should be noted that the geometric mean for the calculated TOF index was 28.2 ppb (95% CI 26.4 - 30.1) (data not shown). The calculated TOF index range was 3.7 ppb to 133.1 ppb.

Provided in Figure 3 is a graphical distribution (natural log scale) of the five fluorochemicals by the three age intervals (65+ thru 75, 75+ thru 85 and 85+ thru 96) 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. In simple linear regression analyses, age was significantly ($p < .05$) negatively associated with PFOS and PFOA among elderly men but only with PFOA among women. Age was not significantly associated with PFHS, PFOSAA or M570 in either sex.

There was a weak correlation between age and years residence in the Seattle area ($r = 0.2$). Analyzed independently of age, there were no significant associations between years resided in the Seattle area and PFOS, PFOA, PFHS, PFOSAA or M570.

As discussed previously, 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 (3.4 ppb)) and only five subjects were below the LLOQ (1.4 ppb) for PFOA; thus this assumption did not affect the calculation of the geometric means for these two fluorochemicals. However, considerably more subjects had values less than the LLOQs for 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: PFHS 1.5 ppb (1.2-1.8) to 2.5 ppb (2.3-2.7); PFOSAA 0.7 ppb (0.6-0.9) to 2.1 ppb (1.9-2.2) and M570 0.7 ppb (0.6-0.8) to 1.5 ppb (1.4-1.6). 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.

Scatter plots (log scale) between the five fluorochemicals are displayed in Figure 4. PFOS and PFOA were highly correlated ($r = .75$). PFOS had a lower, but similar, correlation with PFOSAA and PFHS ($r = .42$) and lower yet with M570 ($r = .29$). The correlation between PFOSAA and M570 was weak ($r = .17$). The remaining scatter plot displays the correlation between PFOA and PFHS ($r = 0.36$). Both PFOSAA and M570 were significant predictors of PFOS in a multivariable model adjusted for age, gender and their interaction (Table 3). PFOSAA was the stronger of the two independent variables. Seventy-five percent of the variation of PFOS was left unexplained. In other models, PFHS and PFOA remained significant predictors of PFOS after adjustment for age, gender and their interaction terms (Tables 4 and 5). None of the models (Tables 3 through 5) had lack of fit F ratios that were statistically significant ($p < .05$).

Presented in Table 6 are the results from bootstrap analyses conducted to provide 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 84.1 ppb with an upper 95% percent confidence limit of 104.0 ppb. At the lowest tolerance limit analyzed, (90%), the mean for PFOS was 61.1 ppb with an upper 95% confidence limit of 71.3 ppb. At the highest tolerance limit analyzed, (99%), the mean was 133.4 ppb with an upper 95% confidence limit of 169.7 ppb. For other fluorochemicals analyzed, the mean of the 95% tolerance limit for PFOA was 9.7 ppb with an upper 95% confidence limit of 11.3 ppb. For PFHS, the mean of the 95% tolerance limit was 8.3 ppb with an upper 95% confidence limit of 10.3 ppb. The mean of the 95% tolerance limit for PFOSAA was 7.8 ppb with an upper 95% confidence limit of 10.7 ppb. For M570, the mean 95% tolerance limit was 3.8 ppb with an upper 95% confidence limit of 4.3 ppb. Finally, for the calculated index of TOF, the mean was 70.2 ppb for the 95% tolerance limit with an upper 95% confidence limit of 81.2 ppb.

DISCUSSION

The findings from this analysis of serum fluorochemical concentrations in the sera of 238 elderly subjects are consistent, albeit slightly lower, than the findings reported in a companion 3M report which examined serum fluorochemical levels in 645 American Red Cross (ARC) blood donors (Olsen et al 2002). These geometric mean comparisons

(ARC vs elderly) were (95% CI in parentheses): PFOS 34.9 ppb (33.3-36.5) vs 31.0 ppb (28.8-33.4); PFOA 4.6 ppb (4.3-4.8) vs 4.2 ppb (3.9-4.5); PFHS 1.9 ppb (1.8-2.0) vs 2.2 ppb (2.0-2.4); PFOSAA 2.0 ppb (1.9-2.1) vs 1.5 ppb (1.4-1.7); and M570 1.3 ppb (1.3-1.4) vs 1.2 ppb (1.1-1.3). The 95% tolerance limits and their upper bounds were also comparable between the two study populations (ARC vs elderly): PFOS 88.5 ppb (upper 95% CI interval = 100.0 ppb) vs 84.1 ppb (104.4); PFOA 12.1 ppb (13.6) vs 9.7 ppb (11.3); PFHS 9.5 ppb (10.8) vs 8.3 ppb (10.3); PFOSAA 7.6 ppb (8.5) vs 7.8 ppb (10.7); and M570 5.0 ppb (5.4) vs 3.8 ppb (4.3). Among other limited samples obtained within the United States, mean serum PFOS concentrations in humans have been reported to be 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). The findings of this study were also comparable to a very 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, 37 ppb from 6 pooled blood samples from Germany and between <LLOQ 3.2 ppb and 85 ppb in 39 individual Swedes (3M Company, 2000).

The geometric mean calculated TOF index in the present study of elderly subjects (28.2 ppb, 95% CI 26.4 - 30.1) was also consistent with that calculated among the ARC blood donors (31.7 ppb, 95% CI 30.4 - 33.0). It was also comparable with measurements of low ppb total organic fluorine concentrations reported in a limited number of general population samples since the late 1960's using a variety of analytical methods (Taves 1968; Taves et al 1976; Singer and Ophaug 1979; Belisle 1981).

There was a strong correlation between PFOS and PFOA which was consistent with the companion research performed on ARC blood donors (Olsen et al 2002). Whereas PFOS has been routinely measured in human populations, wildlife, marine mammals and piscivorous birds (Geisy 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 to PFOS (or vice versa). Whether this association is due to the presence of PFOA as a by-product in POSF-related materials or other non-related environmental exposures or consumer products from other manufacturers (e.g., higher carbon telomers) remains to be explained. Another unanswered question is whether perfluorooctanesulfonamide residuals may metabolize in humans to PFOA as this could explain the strong association observed in this study along with the fact that both PFOS and PFOA are suspected to have long serum half-lives in humans, 8.7 years (SD = 6.1) and 4.4 years (SD = 3.5), respectively (Burris et al 2002).

PFOS was associated with two fluorochemicals, PFOSAA and M570, known to be analytes from exposure to consumer products involving paper/packaging and carpet/textile protectants, respectively. Overall, the data, to date, reveal PFOS bioaccumulation in animals may be primarily through environmental sources whereas both environmental and consumer product exposures likely contribute to serum PFOS concentrations in humans.

As with any interpretation of data obtained from a study population, questions arise regarding the representativeness and ability to generalize the data collected. Historically, the ACT study has reported a volunteer participation rate of 58 percent

(McCurry et al, 1999). Of those who have participated, 65 percent were found to be cognitively intact subjects and agreed to participate in the longitudinal portion of the ACT study. Thus, 38 percent of the GHC members, eligible by age (≥ 65), eventually participated in the ACT study. We are unaware of any database that can be considered generalizable to the diverse United States elderly population without measures of random and systematic bias incorporated in the data analysis.

We did notice a decline in measured PFOS concentrations with age among elderly men but not women. This was not observed in the ARC blood donor study which examined subjects in the age range 20-69. It is possible that this may be due to less potential for environmental or non-occupational exposures among the most elderly. Unlike the ARC blood donors, we did not observe a significant difference in PFOS levels by gender (albeit such differences were not large in the ARC study).

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 100 ppb. Understanding these serum PFOS concentrations in human populations will be useful in risk characterization since serum PFOS likely reflects cumulative human exposure. 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 Elderly Adult Subjects by Age, Years Lived in Seattle and Gender

	Male	Female	All
Number	118	120	238
Age - Number (%)			
65+ thru 75	61 (52)	60 (50)	121 (51)
75+ thru 85	46 (39)	47 (39)	93 (39)
85+ thru 96	11 (9)	13 (11)	24 (10)
Average Age (S.D.)	76.0 (7.0)	76.2 (6.4)	76.1 (6.7)
Years Lived in Seattle Area (S.D.)	50.2 (20.1)	53.3 (17.7)	51.8 (18.9)

Table 2

Measures of Central Tendency of Serum Fluorochemicals for All Elderly Subjects (N = 238) and by Gender

	PFOS	PFOA	PFHS	PFOSAA	M570
All (N = 238)					
Range	< LOQ (3.4) – 175.0	< LOQ (1.4) – 16.7	< LOQ (1.4) – 40.3	< LOQ (1.6) – 21.1	< LOQ (1.0) – 6.6
Q1 – Q3	21.6 – 44.8	3.1 – 6.0	1.4 – 3.7	< LOQ (1.6) – 2.5	< LOQ (1.0) – 2.0
< LOQ (N)	< 3.4 (1)	< 1.4 (5)	< 1.4 (58)	< 1.6 (115)	< 1.0 (83)
Cumulative 90%	61.3	7.8	6.4	5.3	3.0
Median	30.2	4.2	2.3	1.6	1.3
Geometric Mean	31.0	4.2	2.2	1.5	1.2
95% C.I. Geometric Mean	28.8 – 33.4	3.9 – 4.5	2.0 – 2.4	1.4 – 1.7	1.1 – 1.3
Males (N = 118)					
Range	< LOQ (3.4) – 161.0	< LOQ (1.4) – 14.2	< LOQ (1.4) – 40.3	< LOQ (1.5) – 19.0	< LOQ (1.0) – 6.6
Q1 – Q3	22.3 – 44.2	3.0 – 5.3	1.4 – 3.7	< LOQ (1.5) – 2.2	< LOQ (1.0) – 2.1
< LOQ (N)	< 3.4 (1)	< 1.4 (2)	< 1.4 (28)	< 1.5 (58)	< 1.0 (34)
Cumulative 90%	57.1	7.4	6.5	4.3	2.9
Median	30.3	4.0	2.5	1.5	1.4
Geometric Mean	30.2	4.0	2.3	1.4	1.3
95% C.I. Geometric Mean	27.2 – 33.5	3.7 – 4.4	1.9 – 2.6	1.3 – 1.6	1.1 – 1.5

Females (N = 120)

Range	9.6 – 175.0	< LOQ (1.4) – 16.7	< LOQ (1.4) – 17.5	< LOQ (1.6) – 21.1	< LOQ (1.0) – 5.1
Q1 – Q3	20.4 – 45.4	3.2 – 6.4	< LOQ (1.4) – 3.7	< LOQ (1.6) – 2.7	< LOQ (1.0) – 1.9
< LOQ (N)	-	< 1.4 (3)	< 1.4 (30)	< 1.6 (57)	< 1.0 (49)
Cumulative 90%	73.5	8.7	6.5	5.6	3.2
Median	30.0	4.3	2.1	1.6	1.2
Geometric Mean	31.9	4.4	2.1	1.6	1.1
95% C.I. Geometric Mean	28.6 – 35.6	4.0 – 4.9	1.8 – 2.5	1.4 – 1.9	1.0 – 1.3

Table 3
Multivariable Regression Model of PFOS* by PFOSAA*, M570*,
Age, Gender and Age x Gender Interaction

	Coefficient	SE	t ratio	p value
Intercept	4.3	0.38	11.3	< .0001
PFOSAA*	0.3	0.04	6.8	< .0001
M570*	0.2	0.05	3.7	.0003
Age	-0.01	0.005	-2.7	.008
Gender	-0.3	0.38	-0.9	.35
Age x Gender	0.005	0.005	1.0	.32

N = 238

* Natural log

Adjusted $r^2 = 0.25$

Gender: females = 1; males = 0

t ratio = coefficient/SE (standard error)

Table 4
Multivariable Regression Model of PFOS* by PFOA*
Age, Gender and Age x Gender Interaction

	Coefficient	SE	t ratio	p value
Intercept	2.2	0.3	7.0	< .0001
PFOA*	0.8	0.05	16.8	< .0001
Age	- 0.0002	0.004	-0.1	.96
Gender	- 0.2	0.3	-0.7	.47
Age x Gender	0.003	0.004	0.7	.50

N = 238

* Natural log

Adjusted $r^2 = 0.56$

Gender: females = 1; males = 0

t ratio = coefficient/SE (standard error)

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

	Coefficient	SE	t ratio	p value
Intercept	4.3	0.4	10.7	< .0001
PFHS*	0.3	0.04	6.9	< .0001
Age	- 0.01	0.005	-2.6	.01
Gender	- 0.2	0.4	-0.5	.63
Age x Gender	0.003	0.005	0.6	.57

N = 238

* Natural log

Adjusted $r^2 = 0.19$

Gender: females = 1; males = 0

t ratio = coefficient/SE (standard error)

Table 6
Tolerance Limits and Their Associated Means and Upper 95th Percent Confidence Limits
for Serum Fluorochemicals and Calculated Total Organic Fluorine Index

	Tolerance Level	Mean	Upper 95 th Percent Confidence Limit
PFOS	90%	61.1	71.3
	95%	84.1	104.0
	99%	133.4	169.7
PFOA	90%	7.9	9.0
	95%	9.7	11.3
	99%	14.3	16.2
PFHS	90%	6.3	7.2
	95%	8.3	10.3
	99%	16.3	29.6
PFOSAA	90%	5.1	6.1
	95%	7.8	10.7
	99%	16.3	20.3
M570	90%	3.0	3.4
	95%	3.8	4.3
	99%	5.7	6.5
TOF	90%	52.5	58.2
	95%	70.2	81.2
	99%	104.9	127.6

Figure 1. Analysis of Split Samples for Reliability Assessment of PFOS, PFOA, PFHS, PFOSAA and M570

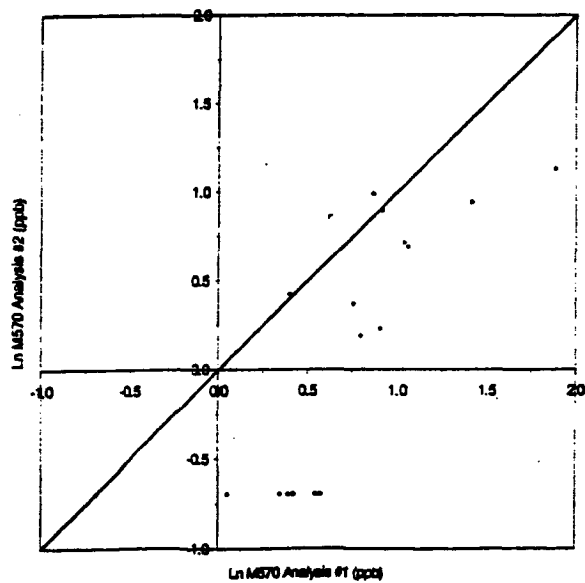
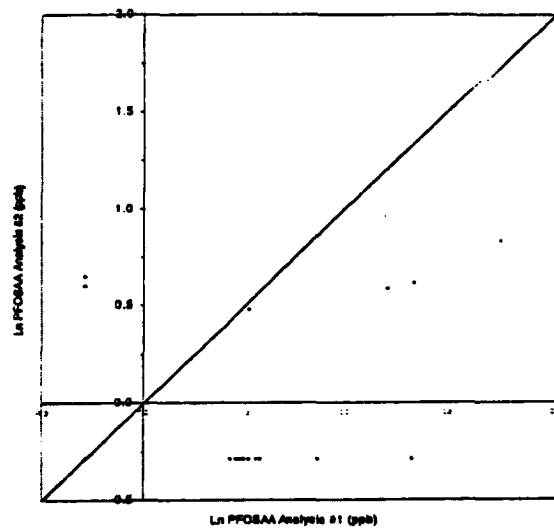
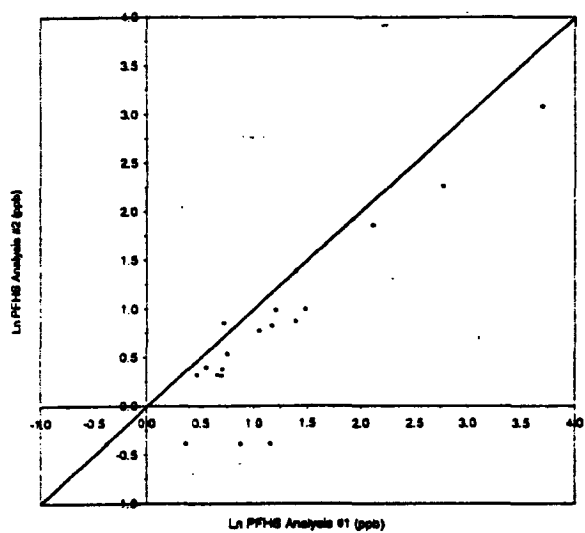
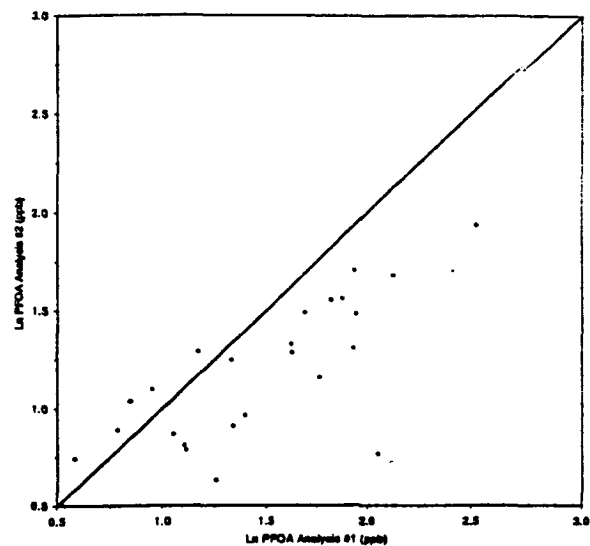
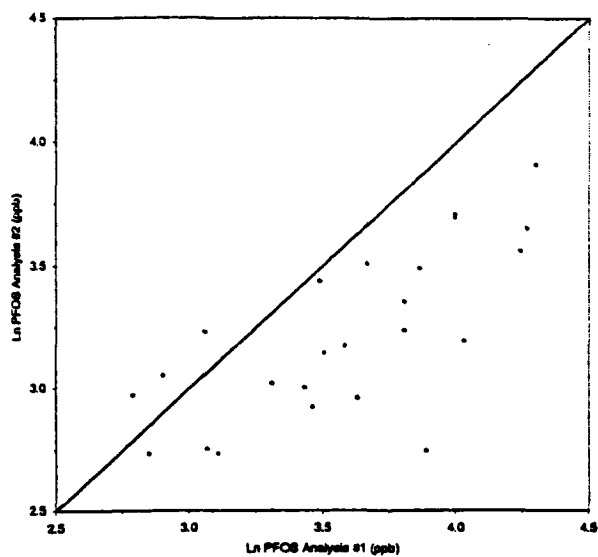


Figure 2. Elderly Study Population Distribution of Measured Fluorochemical Concentrations

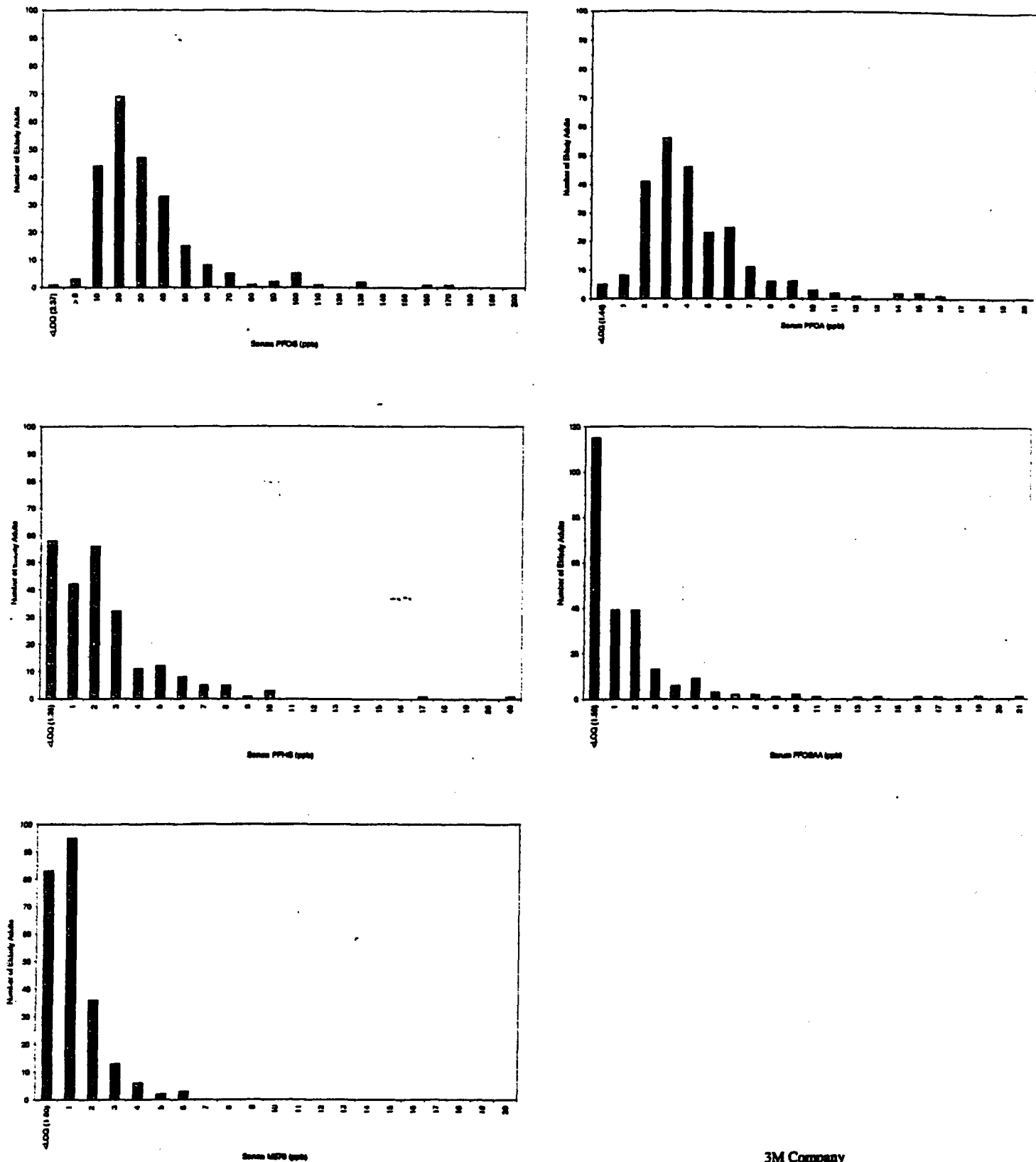


Figure 3. Box and Whisker Plots of Serum Fluorochemical Concentrations (ppb) by Age and Gender

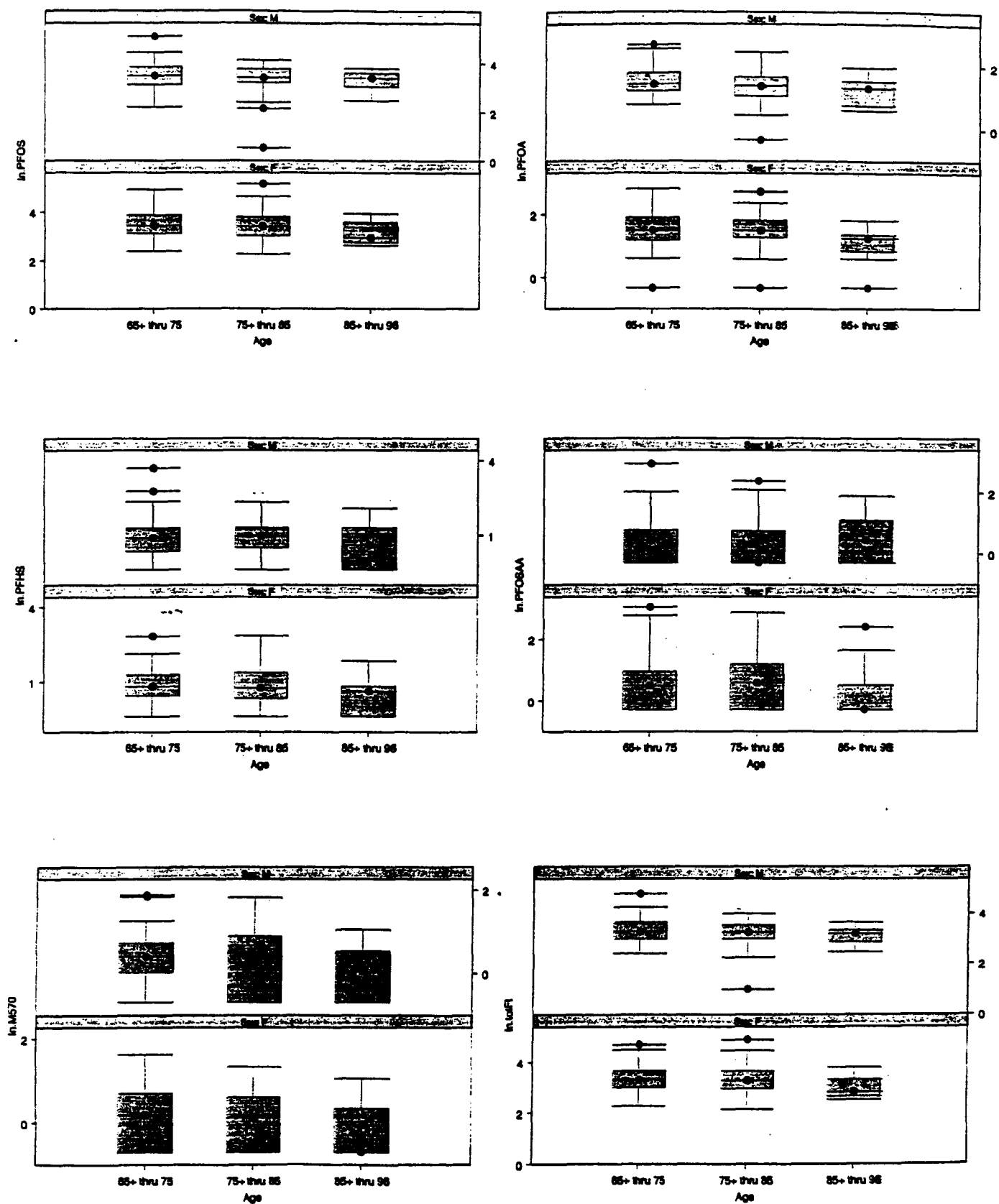


Figure 4. Scatter Plots (Log scale) of Fluorochemical Associations

