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FINAL REPORT

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Title: Identification of Fluorochemicals in Human Sera. III. Pediatric Participants in a Group A Streptococci Clinical Trial Investigation

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ABSTRACT

The purpose of this study was to better characterize the distribution of seven fluorochemicals, including perfluorooctanesulfonate (PFOS, $C_8F_{17}SO_3^-$) in 599 pediatric samples obtained from a multi-center clinical trial of group A streptococcal infections. Serum samples were collected in 1994-1995 and frozen at -20 degrees Celsius. The samples were void of personal identifiers. The only known demographic factors were: age (2-12), gender and the state residence (n = 23 states and the District of Columbia).

Sera samples were extracted and quantitatively analyzed for seven fluorochemicals using high-pressure liquid chromatography/electrospray tandem mass spectrometry. 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 37.5 ppb (95% CI 33.3-36.5). The measured PFOS concentrations ranged from 6.7 ppb to 515.0 ppb. Male children had a significantly ($p < .01$) higher geometric mean serum PFOS level compared to female children [male children geometric mean = 40.1 ppb (95% CI 37.7-42.6) vs female geometric mean = 35.2 ppb (95% CI 33.3-37.2)]. Bootstrap analysis was used to calculate a mean 95% tolerance limit of 88.5 ppb with an upper 95% confidence limit of 97.0 ppb. Additional geometric mean and tolerance limit data are reported for PFOA, PFHS, PFOSAA and M570. A unique finding observed in these pediatric data

that was not observed in the adult or elderly data reported elsewhere, were the higher 95% tolerance limit mean concentrations for PFHS (64.5 ppb) and M570 (11.9 ppb) with upper 95% confidence limits of 80.6 ppb and 14.8 ppb, respectively. It is unlikely that these findings are a consequence of analytical systematic error between these three studies. There was a strong correlation between PFOS and both PFOA (r = .70) and PFHS (r = .66) with lower correlations with PFOSAA (r = .43) and M570 (r = .42). The number of samples with measured concentrations of PFOSA and M556 less than the LLOQ prohibited meaningful statistical analyses for these compounds.

The findings from this analysis of serum PFOS concentrations are consistent with serum PFOS levels of 645 American Red Cross blood donors and 238 elderly subjects from a longitudinal study of cognitive function. Along with other human data, the average serum PFOS concentration in non-occupational human populations may approximate 30 to 40 ppb with 95% of the population's serum PFOS concentrations below 100 ppb. Since serum PFOS concentration likely reflects cumulative human exposure, this information will be useful for risk characterization. The higher mean 95% tolerance limits for PFHS and M570 suggest that some children may have had a greater exposure experience than adults and the elderly to products containing perfluorohexanesulfonyl fluoride (PHSF) and N-methyl perfluorooctanesulfonamidoethanol (N-MeFOSE) surface protectants.

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 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, using individual pediatric samples obtained from a multi-center clinical trial of group A streptococcal infections. The present study is the third formal assessment undertaken by the 3M Company to examine the distribution of PFOS in human sera. The previous two assessments examined serum fluorochemical levels among American Red Cross adult blood donors (Olsen et al 2000a) and elderly participants of a longitudinal cognitive function study in the Seattle (WA) area (Olsen et al 2000b).

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) may be 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

The sera analyzed in this study were collected as part of a large multi-center clinical trial of 1,131 children, ages 2 to 12 years, who presented with signs and symptoms of acute-onset pharyngitis (Kaplan et al 1998). All 1,131 children had positive throat cultures for group A streptococci at an initial visit. The objective of the original research was to determine age-specific geometric mean titers and upper limits of normal for antistreptolysin O and anti-deoxyribonuclease B. Sera for the clinical trial were obtained between January 1994 and March 1995. Sera were kept frozen at -20 degrees Celsius by the University of Minnesota Department of Pediatrics prior to the 3M request of an alloquot of 0.1 ml per sample for the present study (additional amounts were obtained for the reliability analysis - see below). Because of the uncertainty regarding the population distribution of PFOS, sample size was estimated by the use of tolerance limits (Natrella 1966). Provided below is the sampling distribution that was used. Percent sampled was the highest for the younger ages and included all samples four years of age and less.

Age Group	Total N	Sampled (%)
2	27	27 (100)
3	51	51 (100)
4	81	81 (100)
5	122	100 (82)
6	146	80 (55)
7	161	60 (37)
8	131	40 (31)
9	135	40 (30)
10	109	40 (37)
11	87	40 (46)
12	81	40 (49)
Total	1131	599 (53)

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 the 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 10^{th} and 90^{th} 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.

An analysis of the reliability of the assay was conducted after the original samples were analyzed. The laboratory was blind to the identity of these samples as they related to the original values reported. Triplicate samples were analyzed for the highest one percent of the measured concentrations of PFOS, PFOA and PFHS. If there was insufficient serum sample left for analysis, the next highest sample was included for analysis. A 20 percent random sample of the next highest nine percent samples was also conducted but with only a single measurement. Finally, a five percent sample was randomly chosen of the remaining 90 percent of all samples. This five percent sample was also analyzed only once. Altogether, there were 62 samples reanalyzed representing sera from 44 unique children.

RESULTS

The results for the reliability analysis for PFOS, PFOA, PFHS, PFOSAA and M570 using the reanalyzed samples is displayed in Figure 1. There were no measured concentrations of PFOSA that were above the LLOQs. Only 12 of the 62 M556 concentration comparisons were above the LLOQ; thus, these graphs are not displayed. There were strong correlations for PFOS ($r = .98$), PFOA ($r = .96$) and PFHS ($r = .93$). Correlations were slightly less for PFOSAA ($r = .69$) and for M570 ($r = .80$). Both PFOSAA and M570 had many comparisons below the LLOQ as represented in the graphs near the abscissa (0,0) on the identity ($\ln y = \ln x$) line.

Provided in Table 1 is the distribution of the 599 children by age and gender. Altogether there were 299 males and 300 females. Presented in Table 2 is the distribution by states (n = 23) and the District of Columbia. One subject (female) was not analyzed due to an insufficient quantity of serum sample.

The measured concentrations of PFOSA and M556 were predominantly below the LLOQ. For PFOSA, there were no subjects with a concentration above the LLOQ, 457 subjects had concentrations <LLOQ (1.0 ppb), 82 subjects had concentrations <LLOQ (2.0 ppb) and 50 subjects had analyses below the LLOQ but failed to meet the performance standards of the analytical method. As there was only 0.1 ml, on average, per sample, no subsequent analyses were conducted on these 50 samples for PFOSA. For M556, 258 subjects had concentrations that ranged between 2.5 ppb and 9.9 ppb, 263 subjects had concentrations <LLOQ (2.5 ppb) and 77 subjects had concentrations < LLOQ (5.0 ppb). Assuming the midpoint between zero and the LLOQ, the geometric mean for M556 was 2.4 ppb (95% CI 2.2 - 2.5). Because PFOSA and M556 had many analyses <LLOQ, statistical analyses are not presented for these compounds. They were included in the calculation of the TOF index using, for those PFOSA or M556 values <LLOQ, the midpoint between zero and the LLOQ.

The distributions of the five remaining fluorochemicals, PFOS, PFOA, PFHS, PFOSAA and M570, are displayed in Figure 2 for the 598 children samples analyzed. 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 may be due to the greater proportion of subjects with values <LLOQ for PFOA, PFHS, PFOSAA and M570.

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 3 for all children (N = 598), males only (N = 300) and females only (N = 298). Overall, the geometric mean concentration of PFOS was 37.5 ppb (95% CI 36.0-39.1). The PFOS values ranged from 6.7 ppb to 515.0 ppb. Male children had a significantly ($p < .01$) higher geometric mean serum PFOS level compared to female children although the absolute difference was not substantial [male children geometric mean = 40.1 ppb (95% CI 37.7-42.6) vs female geometric mean = 35.2 ppb (95% CI 33.3-37.2)]. Male children also had significantly higher geometric mean serum levels of PFOA and PFHS compared to female children. There were not gender-related geometric mean differences for PFOSAA and M570. The geometric mean for the calculated TOF index was 38.9 ppb (95% CI 37.2-40.7). The calculated TOF index range was 9.6 ppb to 803.7 ppb. Geometric means of male children (41.6 ppb, 95% CI 38.8-44.5) were significantly ($p < .01$) higher than female children (36.4 ppb, 95% CI 34.3-38.7).

Measures of central tendency for each of the ages, 2 to 12, are presented in Table 4. Provided in Figure 3 is a graphical distribution (natural log scale) of the five fluorochemicals by each age 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. Analyzed as a continuous variable in simple regression models, age was significantly ($p < .05$)

negatively associated with PFOA and M570 in both males and females but not with PFOS, PFHS or PFOSAA.

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, no subject had a value <LLOQ; thus this assumption did not affect its calculation of the geometric mean. However, many subjects had values less than the 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 4.6 ppb (4.3-4.9) to 5.0 ppb (4.8-5.2); PFHS 3.2 ppb (2.8-3.8) to 5.2 ppb (4.7-5.7); PFOSAA 2.5 ppb (2.2-2.7) to 3.7 ppb (3.6-3.9) and M570 1.1 ppb (1.0-1.3) to 2.3 ppb (2.2-2.5). 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.

Provided in Figure 4 is a graphical presentation of the fluorochemical data (natural log scale) by the 23 states and the District of Columbia stratified by gender. Interpretation of the graphs is comparable to those discussed above for Figure 3. For PFOS, mean values were comparable for the various locations. Statistical analyses by state were problematic because of the limited sample size for any given age and gender combination.

Scatter plots (log scale) between the five fluorochemicals are displayed in Figure 5. PFOS and PFOA were highly correlated (r = .70). PFOS had a lower correlation with

PFOSAA (r = .43) and M570 (r = .42). The correlation between PFOSAA and M570 was less (r = .27). The remaining scatter plots display the correlations between PFOS and PFHS (r = 0.66) and PFOA and PFHS (r = 0.48). Both PFOSAA and M570, adjusted for age, gender and their interaction, were significant predictors of PFOS in a multivariable model (Table 5). Almost seventy percent of the variation of PFOS, however, was left unexplained. Adjusted for age, gender and their interaction, PFOA remained a significant predictor of PFOS (Table 6). A quadratic term was significant in the model which examined the association between PFOS and PFHS adjusted for age, gender and their interactions (Table 7).

Presented in Table 8 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 90^{th}, 95^{th} and 99^{th} 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% percent confidence limit of 97.0 ppb. At the lowest tolerance limit analyzed, (90%), the mean for PFOS was 70.6 ppb with an upper 95% confidence limit of 75.2 ppb. At the highest tolerance limit analyzed, the (99%), the mean was 140.6 ppb with an upper 95 percent confidence limit of 217.0 ppb. For other fluorochemicals analyzed, the mean of the 95% tolerance limit for PFOA was 10.1 ppb with an upper 95% confidence limit of 11.0 ppb. For PFHS, the mean of the 95% tolerance limit was 64.5 ppb with an upper 95% confidence limit of 80.6 ppb. The mean of the 95% tolerance limit for PFOSAA was 10.4 ppb with an upper 95% confidence limit of 11.2 ppb. For M570, the mean was

11.9 ppb for a 95% tolerance limit with an upper 95% confidence limit of 14.8 ppb. Finally, for the calculated index of TOF, the mean was 112.1 ppb for the 95% tolerance limit with an upper 95% confidence limit of 125.0 ppb.

DISCUSSION

As seen in Figure 6, the geometric mean measured concentrations for these pediatric samples is consistent with those reported for adult blood donors (Olsen et al 2000a) and elderly participants of a longitudinal study of cognitive function (Olsen et al 2002b). No substantial differences were observed for PFOS or PFOA between the three study populations. Interpretation of the PFHS, PFOSAA and M570 is more problematic because the LLOQs varied slightly between studies and thus the assumption of a midpoint value may unduly influence a geometric mean calculation when comparing mean measured concentrations for the three studies.

Displayed in Figure 7 is another perspective regarding the differences in measured fluorochemical concentration distributions between the pediatric, adult and elderly population data. It is clearly evident that the 95% tolerance limits for PFHS and, to a lesser extent M570, were substantially different in children than compared to the adult and the elderly populations whereas the mean concentrations of the 95% tolerance limits were similar for PFOS, PFOA and PFOSAA. These findings suggest a different exposure pattern for some children compared to the adult and the elderly populations. While residual PFHS related chemistry may have existed in POSF related materials, it was an intentional major ingredient only in fire fighting foam and an after market carpet protector, which was discontinued in 1999. One potential hypothesis to explain the

difference between adult and children sera PFHS levels could be the differential exposure to carpet known to exist between these two population groups. The mean 95 percent tolerance limit for M570 was also greater in children than in the adults and the elderly. M570 can be a residual analyte of N-methyl perfluorooctanesulfonamidoacetate surface protectants which would include carpet and textile applications. An alternative hypothesis, which we suspect is much less likely, is that a segment of the pediatric population clears PFHS and M570 differently than adults or the elderly. There appeared to be similar comparisons between the three populations for the mean 95% tolerance limit for PFOSAA which may be a residual analyte associated with the N-EtFOSE paper and packaging protectant products.

Previous measurements of human serum samples obtained in the United States have been comparable to what has been reported in the children, adult and elderly studies. The mean PFOS serum level was 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, 37 ppb from 6 pooled blood samples from Germany and ranged between <LLOQ (3.2 ppb) to 85 ppb in 39 individual Swedes (3M Company, 2000). The mean calculated TOF index used 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).

As was also observed in the adult and elderly studies (Olsen et al 2002a; 2002b), we found a strong correlation between PFOS and PFOA in the children sera. 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; 3M Company 2000), 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 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 perfluoroctanesulfonamides 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. It should also be noted that the serum PFHS half-life reported by Burris et al (2002) was uninterpretable (-2.27 years, SD = 23.1) but possibly indicative of a long (years) serum half-life.

PFOS was also correlated 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, indicate that 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 its representativeness and the ability to generalize from the data collected. We are confident that our sampling procedures allowed for an adequate representation of the original study database. We believe this population of children is not unique due to the high prevalence of group A streptococcal infections in children. The only other information available for analysis were the age, gender and residence (state) of the children. We are unaware of any database that can be considered generalizable to the diverse United States pediatric general 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 below 100 ppb. Understanding these serum PFOS levels in human populations will be useful in 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, to date, that the serum concentrations observed in humans are substantially less than those required to cause adverse effects in laboratory animals (3M Company 2000). The data in the present study regarding the higher mean 95% tolerance limits for PFHS and M570, compared to those found in the adults and the elderly, suggest that some children may have had a greater exposure experience to products containing PHSF and N-MeFOSE surface protectants.

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Table 1
Distribution of All Children (N = 599) by Age and Gender

Age	Male	Female	Total
2	18	9	27
3	25	26	51
4	36	45	81
5	40	40	80
6	40	40	80
7	30	30	60
8	30	30	60
9	20	20	40
10	20	20	40
11	20	20	40
12	20	20	40
TOTAL	299	300	599

Table 2
Distribution of Children (N = 599) by Location, Gender and Mean Age

State	Males	Females	Total	Mean Age
Alabama	10	12	22	8.3
Arizona	7	11	18	6.8
California	25	22	47	6.0
Colorado	22	17	39	6.6
District of Columbia	17	11	28	6.1
Florida	16	26	42	6.4
Idaho	10	6	16	8.1
Illinois	1	1	2	5.0
Kansas	10	3	13	6.4
Kentucky	11	7	18	6.9
Massachusetts	24	21	45	6.3
Michigan	5	4	9	8.3
Missouri	4	6	10	7.8
North Carolina	15	18	33	6.7
Nebraska	2	3	5	6.8
New Jersey	23	15	38	6.9
New Mexico	14	14	28	7.6
New York	17	13	30	6.3
Ohio	16	17	33	6.3
Oklahoma	10	18	28	6.3
Pennsylvania	6	3	9	5.9
Texas	20	27	47	6.0
Utah	10	16	26	6.8
Virginia	4	19	23	8.3
TOTAL	299	300	599	6.7

Table 3
Measures of Central Tendency of Serum Fluorochemicals for All Children (N = 598) and by Gender

	PFOS	PFOA	PFHS	PFOSAA	M570
All Children (N = 598)					
Range	6.7 – 515.0	< LOQ (1.9) – 56.1	< LOQ (1.4) – 711.7	< LOQ (1.6) – 23.8	< LOQ (1.0) – 48.0
Q1 – Q3	27.6 – 51.0	3.8 – 6.7	1.6 – 10.8	2.1 – 5.6	< LOQ (1.0) – 3.8
< LOQ (N)	-	< 1.9 (5)	< 1.4 (92)	< 1.6 (67)	< 1.0 (140)
		< 2.9 (20)	< 2.4 (37)	< 2.6 (47)	< 2.0 (60)
Cumulative 90%	70.8	8.5	35.3	8.8	7.3
Median	36.7	5.1	3.8	3.7	1.8
Geometric Mean	37.5	4.9	4.5	3.3	1.9
95% C.I. Geometric Mean	36.0 – 39.1	4.7 – 5.1	4.1 – 5.1	3.1 – 3.6	1.7 – 2.1
Male Children (N = 300)					
Range	11.4 – 515.0	<LOQ (2.9) – 56.1	< LOQ (1.4) – 711.7	< LOQ (1.6) – 20.7	< LOQ (1.0) – 48.0
Q1 – Q3	28.7 – 53.9	3.9 – 6.9	1.9 – 12.2	2.0 – 5.8	< LOQ (1.0) – 4.2
< LOQ (N)	-	< 2.9 (11)	< 1.4 (34)	< 1.6 (40)	< 1.0 (66)
			< 2.4 (20)	< 2.6 (22)	< 2.0 (31)
Cumulative 90%	75.6	9.0	38.5	8.8	7.5
Median	39.4	5.2	4.4	3.7	2.0
Geometric Mean	40.1	5.2	5.3	3.3	2.0
95% C.I. Geometric Mean	37.7 – 42.6	4.9 – 5.2	4.5 – 6.3	3.0 – 3.6	1.8 – 2.3

Female Children
(N = 298)

Range	6.7 – 165.0	< LOQ (1.9) – 18.6	< LOQ (1.4) – 170.0	< LOQ (1.6) – 23.8	< LOQ (1.0) – 38.1
Q1 – Q3	27.0 – 46.3	3.5 – 6.3	< LOQ (2.4) – 10.2	2.2 – 5.6	< LOQ (1.0)– 3.7
< LOQ (N)	-	< 1.9 (5)	< 1.4 (58)	< 1.6 (27)	< 1.0 (74)
		< 2.9 (9)	< 2.4 (17)	< 2.6 (25)	< 2.0 (29)
Cumulative 90%	64.8	8.0	22.5	8.7	6.9
Median	34.7	4.9	3.3	3.8	1.7
Geometric Mean	35.2	4.7	3.9	3.4	1.8
95% C.I. Geometric Mean	33.3 – 37.2	4.4 – 4.9	3.3 – 4.5	3.1 – 3.7	1.6 – 2.0

Table 4. Measures of Central Tendency of Serum Fluorochemicals for All Children (N = 598) by Age

Age	PFOS	PFOA	PFHS	PFOSAA	M570
Age 2					
Range	8.8 – 217.0	<LOQ (1.9) – 34.2	<LOQ (1.4) – 497.0	<LOQ (1.6) – 10.5	<LOQ (1.0) – 16.3
Q1 – Q3	16.8 – 41.4	2.5 – 6.2	<LOQ (1.4) – 17.1	1.7 – 5.7	<LOQ (2.0) – 5.1
Cumulative 90%	88.2	17.7	74.5	9.5	10.0
Median	27.1	4.1	3.6	3.4	2.5
Geometric Mean	28.6	4.5	4.1	3.3	2.5
95% C.I. Geometric Mean	21.4 – 38.1	3.3 – 6.3	2.0 – 8.5	2.4 – 4.5	1.6 – 3.5
Age 3					
Range	6.7 – 184.0	<LOQ (1.9) – 16.1	<LOQ (1.4) – 170.0	<LOQ (1.6) – 15.8	<LOQ (1.0) – 34.4
Q1 – Q3	24.3 – 50.6	4.2 – 6.7	<LOQ (2.4) – 12.2	2.1 – 5.4	<LOQ (2.0) – 3.9
Cumulative 90%	104.1	9.8	89.0	7.0	9.0
Median	30.3	5.4	3.9	3.7	2.0
Geometric Mean	34.9	5.1	4.8	3.1	2.1
95% C.I. Geometric Mean	29.0 – 41.8	4.4 – 5.9	3.0 – 7.5	2.5 – 3.8	1.6 – 2.8

Age 4

Range	11.6 – 325.0	2.0 – 56.1	<LOQ 1.4) – 416.0	<LOQ (1.6) – 23.8	<LOQ (1.0) – 48.0
Q1 – Q3	27.6 – 43.1	4.2 – 7.2	1.9 – 9.2	2.0 – 6.0	<LOQ (2.0) – 4.6
Cumulative 90%	60.5	10.1	28.3	7.8	8.0
Median	34.0	5.5	3.5	3.9	2.2
Geometric Mean	35.3	5.7	4.5	3.4	2.2
95% C.I. Geometric Mean	31.8 – 39.2	5.1 – 6.4	3.4 – 6.0	2.9 – 4.0	1.7 – 2.8

Age 5

Range	15.8 – 96.5	<LOQ (2.9)– 11.5	<LOQ (1.4) – 80.6	<LOQ (1.6) – 19.6	<LOQ (1.0) – 38.1
Q1 – Q3	30.3 – 56.4	4.3 – 7.1	1.8 – 11.0	2.3 – 6.6	<LOQ (2.0) – 4.7
Cumulative 90%	73.1	9.0	27.8	10.3	10.7
Median	39.7	5.5	4.1	4.7	2.3
Geometric Mean	40.5	5.4	4.4	4.0	2.3
95% C.I. Geometric Mean	36.8 – 44.5	4.9 – 5.9	3.3 – 5.8	3.3 – 4.7	1.8 – 3.0

Age 6

Range	12.2 – 515.0	<LOQ (2.9) – 20.2	<LOQ (1.4) – 711.7	<LOQ (1.6) – 12.9	<LOQ (1.0) – 23.0
Q1 – Q3	30.0 – 56.3	4.2 – 6.9	2.3 – 10.5	2.2 – 6.2	<LOQ (1.0) – 4.9
Cumulative 90%	76.4	8.6	27.3	9.4	13.5
Median	40.0	5.3	4.3	4.0	2.0
Geometric Mean	41.0	5.3	5.1	3.5	2.1
95% C.I. Geometric Mean	36.2 – 46.5	4.8 – 5.9	3.8 – 6.8	3.0 – 4.2	1.6 – 2.8

Age 7

Range	16.7 – 134.0	<LOQ (2.9) – 11.0	<LOQ (1.4) – 94.2	<LOQ (1.6) – 9.6	<LOQ (1.0) – 17.1
Q1 – Q3	27.3 – 49.7	3.5 – 6.1	2.6 – 19.5	1.8 – 4.4	0.6 – 3.3
Cumulative 90%	74.2	7.7	51.0	6.0	6.6
Median	36.7	4.6	6.6	2.8	1.8
Geometric Mean	38.4	4.6	7.3	2.7	1.7
95% C.I. Geometric Mean	34.3 – 43.0	4.1 – 5.1	5.2 – 10.3	2.3 – 3.2	1.3 – 2.2

Age 8

Range	17.2 – 116.0	2.1 – 16.4	<LOQ (1.4) – 180.0	<LOQ (1.6) – 21.7	<LOQ (1.0) – 17.8
Q1 – Q3	32.5 – 55.3	4.1 – 7.2	1.6 – 9.4	2.6 – 6.7	<LOQ (2.0) – 4.0
Cumulative 90%	78.1	8.9	50.5	11.5	7.0
Median	38.1	5.2	4.3	3.8	1.7
Geometric Mean	41.8	5.4	4.7	3.9	1.9
95% C.I. Geometric Mean	37.3 – 46.8	4.9 – 6.0	3.3 – 6.9	3.1 – 4.7	1.5 – 2.5

Age 9

Range	17.5 – 122.0	<LOQ (2.9) – 11.6	<LOQ (1.4) – 145.0	<LOQ (1.6) – 11.6	<LOQ (1.0) – 6.4
Q1 – Q3	34.1 – 54.7	3.8 – 6.3	2.2 – 13.6	<LOQ (2.6) – 5.2	<LOQ (2.0) – 3.0
Cumulative 90%	67.2	7.0	36.8	8.9	4.3
Median	44.1	5.3	4.7	3.1	1.5
Geometric Mean	42.8	4.9	5.6	2.7	1.5
95% C.I. Geometric Mean	37.6 – 48.7	4.3 – 5.6	3.6 – 8.6	2.1 – 3.5	1.2 – 2.0

Age 10

Range	10.2 – 98.9	<LOQ (2.9) – 8.9	<LOQ (1.4) – 88.7	<LOQ (1.6) – 20.7	<LOQ (1.0) – 7.0
Q1 – Q3	29.1 – 50.2	3.6 – 6.2	1.2 – 7.9	2.1 – 5.1	<LOQ (2.0) – 3.8
Cumulative 90%	70.1	7.2	35.3	8.7	5.7
Median	33.9	4.7	2.5	3.6	1.9
Geometric Mean	37.7	4.6	3.2	3.2	1.8
95% C.I. Geometric Mean	32.5 – 43.7	4.1 – 5.2	2.0 – 4.9	2.5 – 4.1	1.4 – 2.4

Age 11

Range	10.4 – 106.0	<LOQ (1.9) – 9.0	<LOQ (1.4) – 75.4	<LOQ (1.6) – 18.7	<LOQ (1.0) – 11.3
Q1 – Q3	25.4 – 48.9	2.6 – 5.4	<LOQ (1.4) – 6.6	<LOQ (2.6) – 6.8	<LOQ (1.0) – 2.5
Cumulative 90%	68.7	7.0	37.0	14.7	5.0
Median	35.8	3.8	1.8	4.2	<LOQ (2.0)
Geometric Mean	33.5	3.6	2.7	3.7	1.2
95% C.I. Geometric Mean	28.2 – 39.7	3.0 – 4.3	1.7 – 4.3	2.7 – 5.0	0.9 – 1.6

Age 12

Range	11.4 – 124.0	<LOQ (1.9) – 14.6	<LOQ (1.4) – 35.7	<LOQ (1.6) – 11.3	<LOQ (1.0) – 31.0
Q1 – Q3	22.7 – 43.3	2.6 – 4.9	0.8 – 10.1	1.5 – 5.3	<LOQ (1.0) – 2.6
Cumulative 90%	65.9	5.8	21.2	7.7	4.7
Median	34.0	3.8	3.9	3.7	1.3
Geometric Mean	32.8	3.5	3.5	3.0	1.4
95% C.I. Geometric Mean	27.9 – 38.5	3.0 – 4.2	2.3 – 5.4	2.4 – 3.9	1.0 – 1.8

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

	Coefficient	SE	t ratio	p value
Intercept	3.1	0.05	58.1	< .0001
PFOSAA*	0.2	0.02	9.7	< .0001
M570*	0.2	0.02	9.3	< .0001
Age	0.02	0.006	2.5	.01
Gender	- 0.04	0.05	-0.8	.40
Age x Gender	- 0.003	0.006	-0.6	.58

N = 598

*Natural log

Adjusted r^2 = .31

Gender: females = 1; males = 0

t ratio = coefficient/SE (standard error)

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

	Coefficient	SE	t ratio	p value
Intercept	2.2	0.07	33.5	< .0001
PFOA*	0.8	0.03	25.4	< .0001
Age	0.03	0.005	6.1	< .0001
Gender	0.0008	0.04	0.02	.98
Age x Gender	- 0.004	0.005	-0.8	.41

N = 598

*Natural log

Adjusted r^2 = .53

Gender: females = 1; males = 0

t ratio = coefficient/SE (standard error)

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

	Coefficient	SE	t ratio	p value
Intercept	3.2	0.04	72.4	< .0001
PFHS*	0.1	0.03	4.6	< .0001
$[PFHS]^{2}$ *	0.03	0.006	5.1	< .0001
Age	0.01	0.005	2.2	.03
Gender	0.03	0.04	0.7	.47
Age x Gender	- 0.008	0.005	1.6	.12

N = 598

*Natural log

Adjusted r^2 = .47

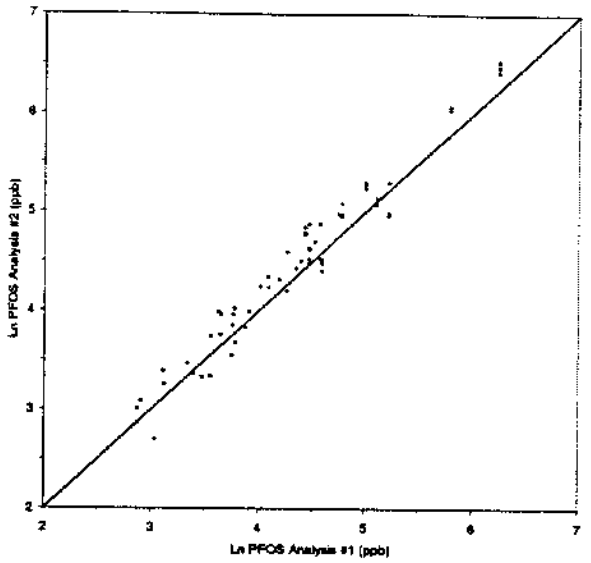
Gender: females = 1; males = 0

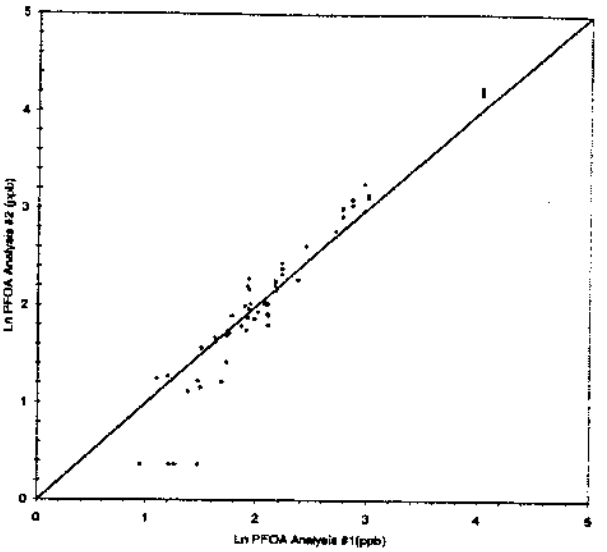
t ratio = coefficient/SE (standard error)

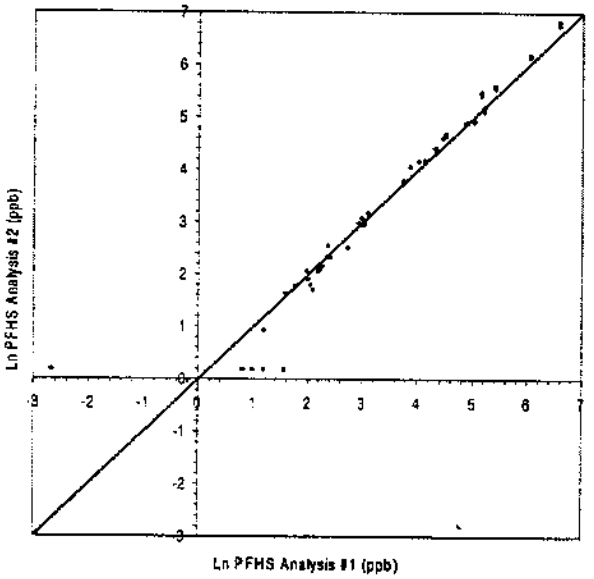
Table 8
Tolerance Limits and Their Associated Means and Upper 95th Percent Confidence Limits for Serum Fluorochemicals and Calculated Total Organic Fluorine Index

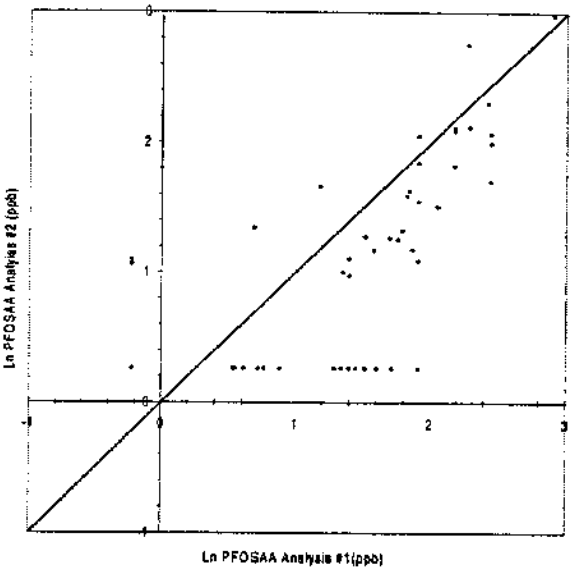
	Tolerance Limit	Mean	Upper 95th Percent Confidence Limit
PFOS	90%	70.6	75.2
	95%	88.5	97.0
	99%	140.6	217.0
PFOA	90%	8.4	9.0
	95%	10.1	11.0
	99%	16.6	20.2
PFHS	90%	33.9	38.7
	95%	64.5	80.6
	99%	156.3	416.0
PFOSAA	90%	8.6	9.1
	95%	10.4	11.2
	99%	17.8	20.7
M570	90%	7.2	8.2
	95%	11.9	14.8
	99%	25.7	38.1
TOF	90%	77.8	91.5
	95%	112.2	125.0
	99%	203.0	482.1

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









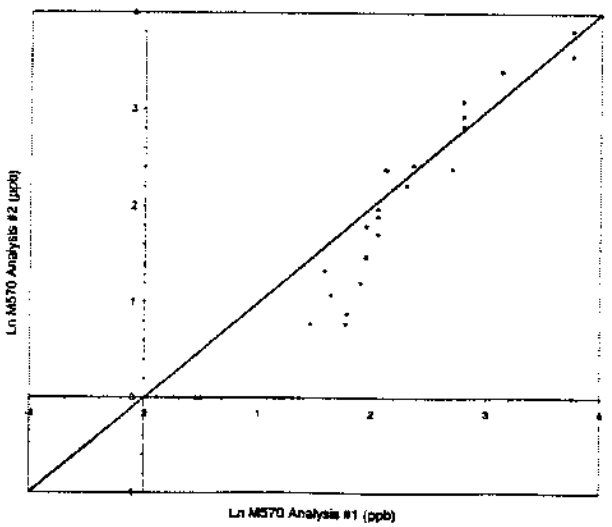
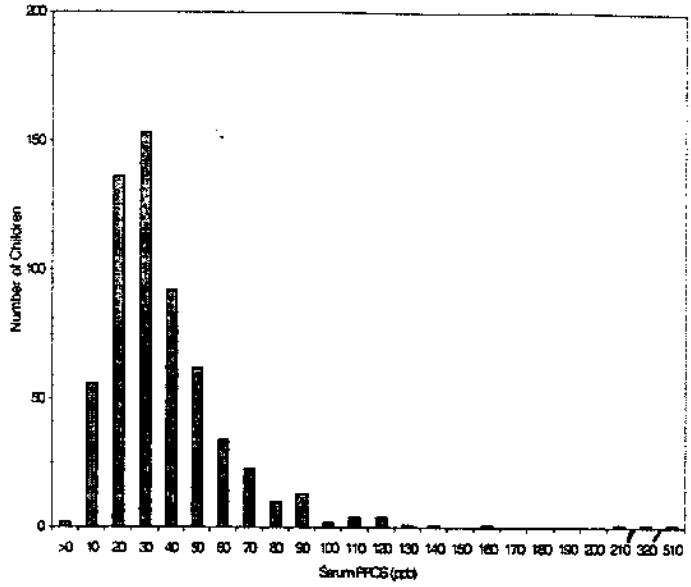
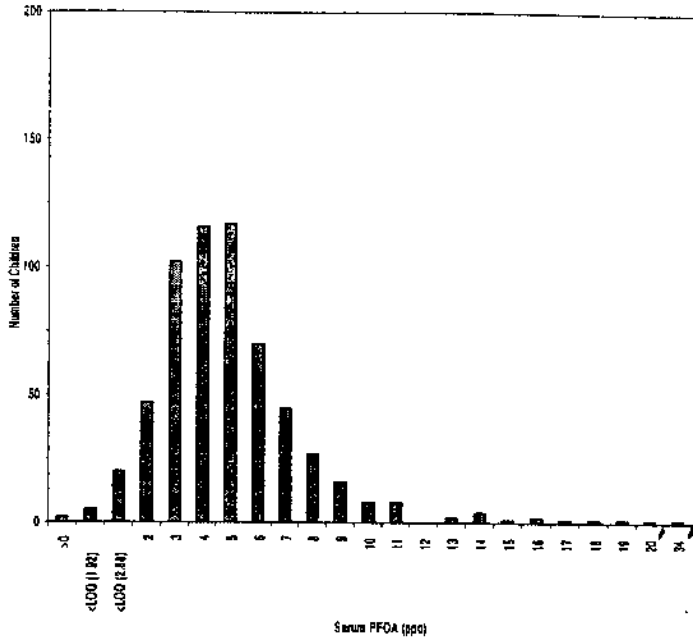
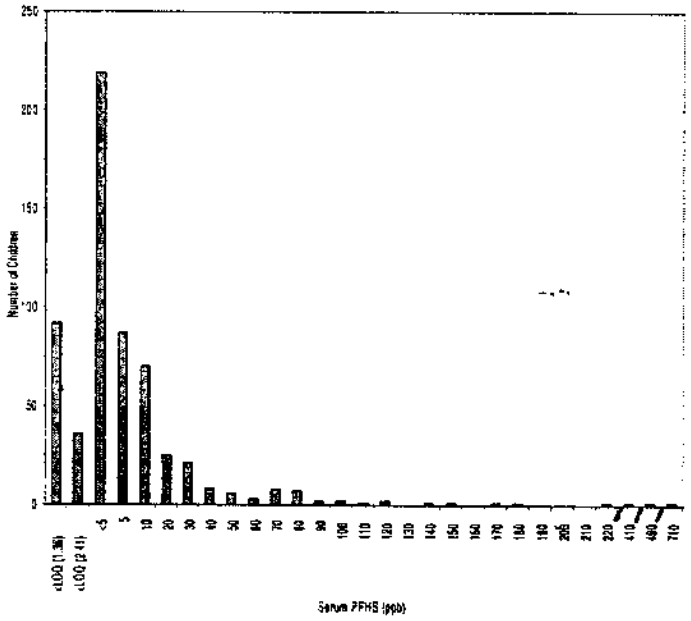
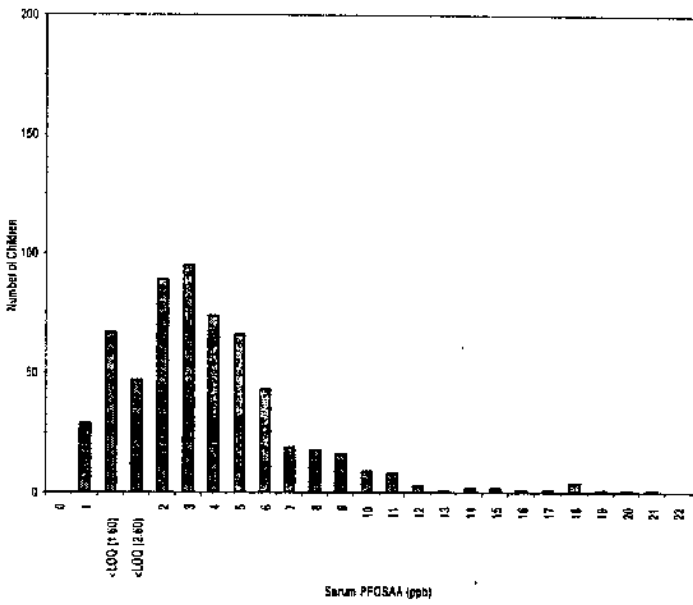


Figure 2. Pediatric Study Population Distribution of Measured Fluorochemical Concentrations









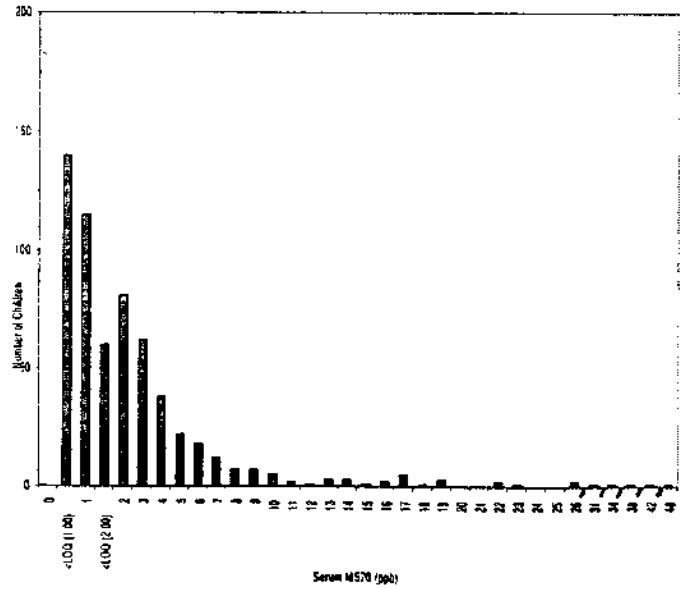
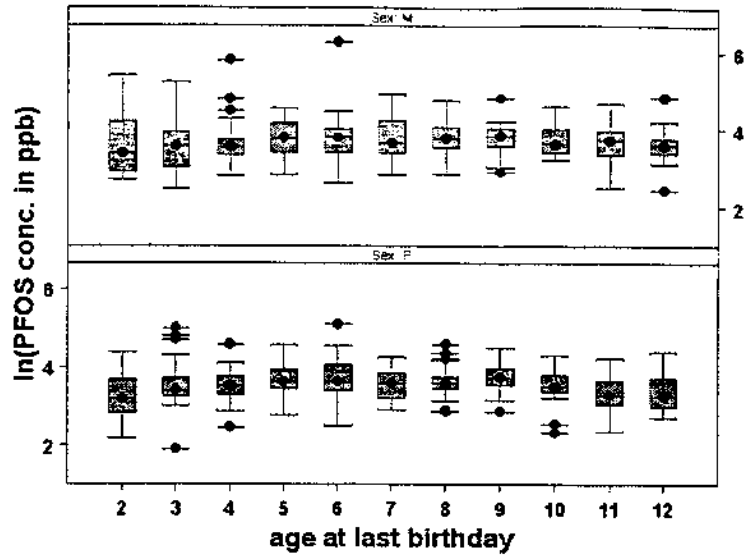
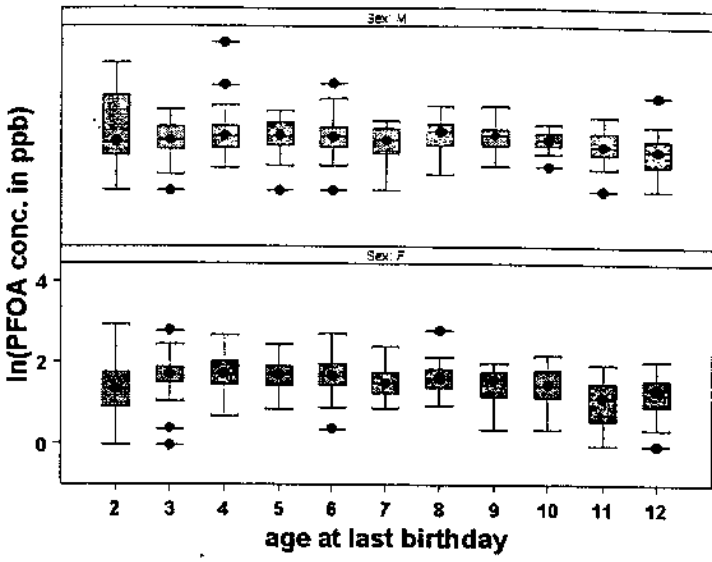
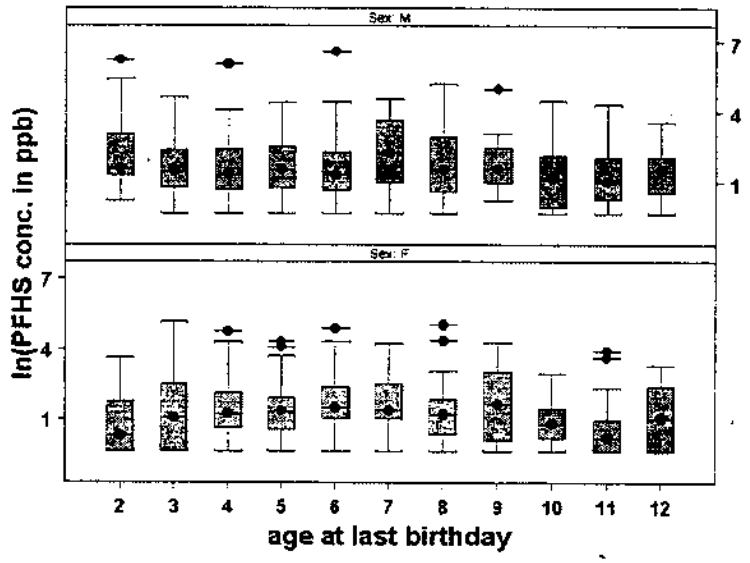
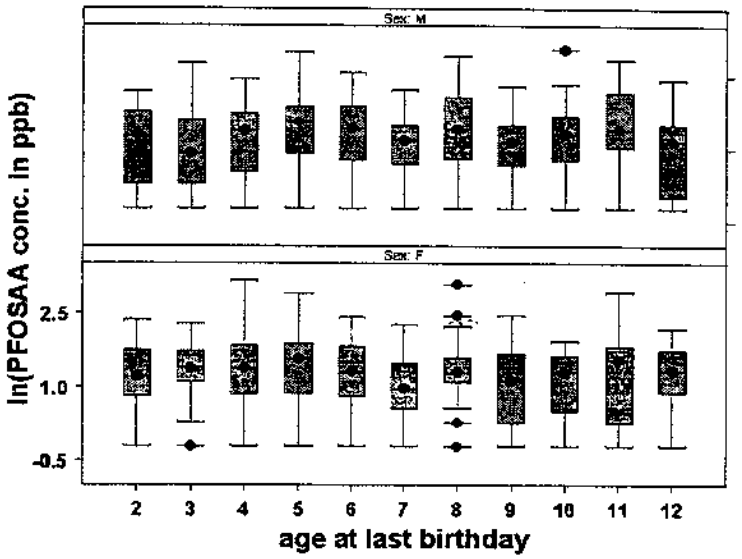


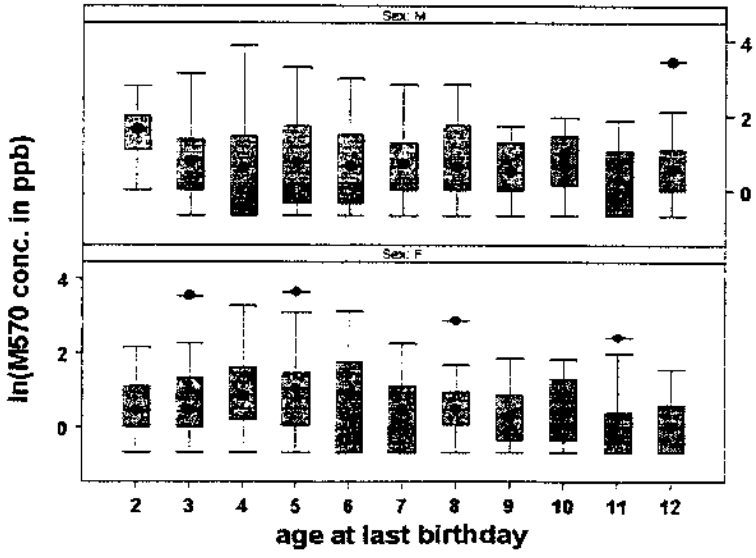
Figure 3. Box and Whisker Plots of Serum Fluorochemical Concentrations by Age and Gender











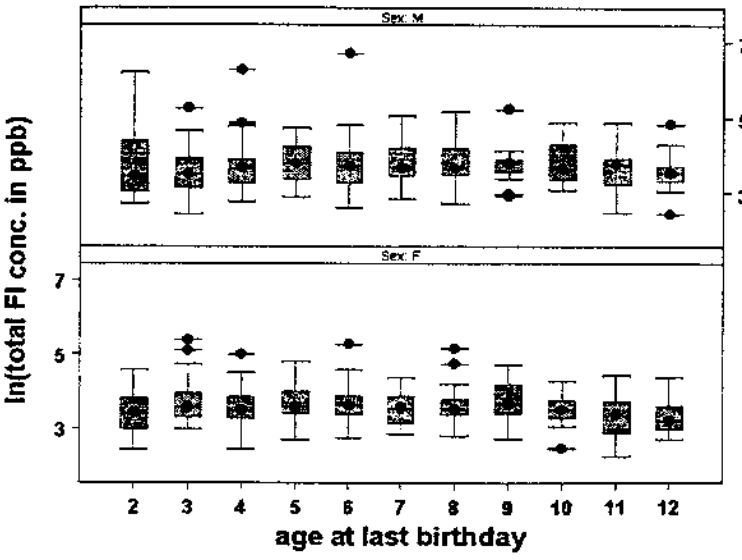


Figure 4. Box and Whisker Plots of Serum Fluorochemical Concentrations by Gender and State

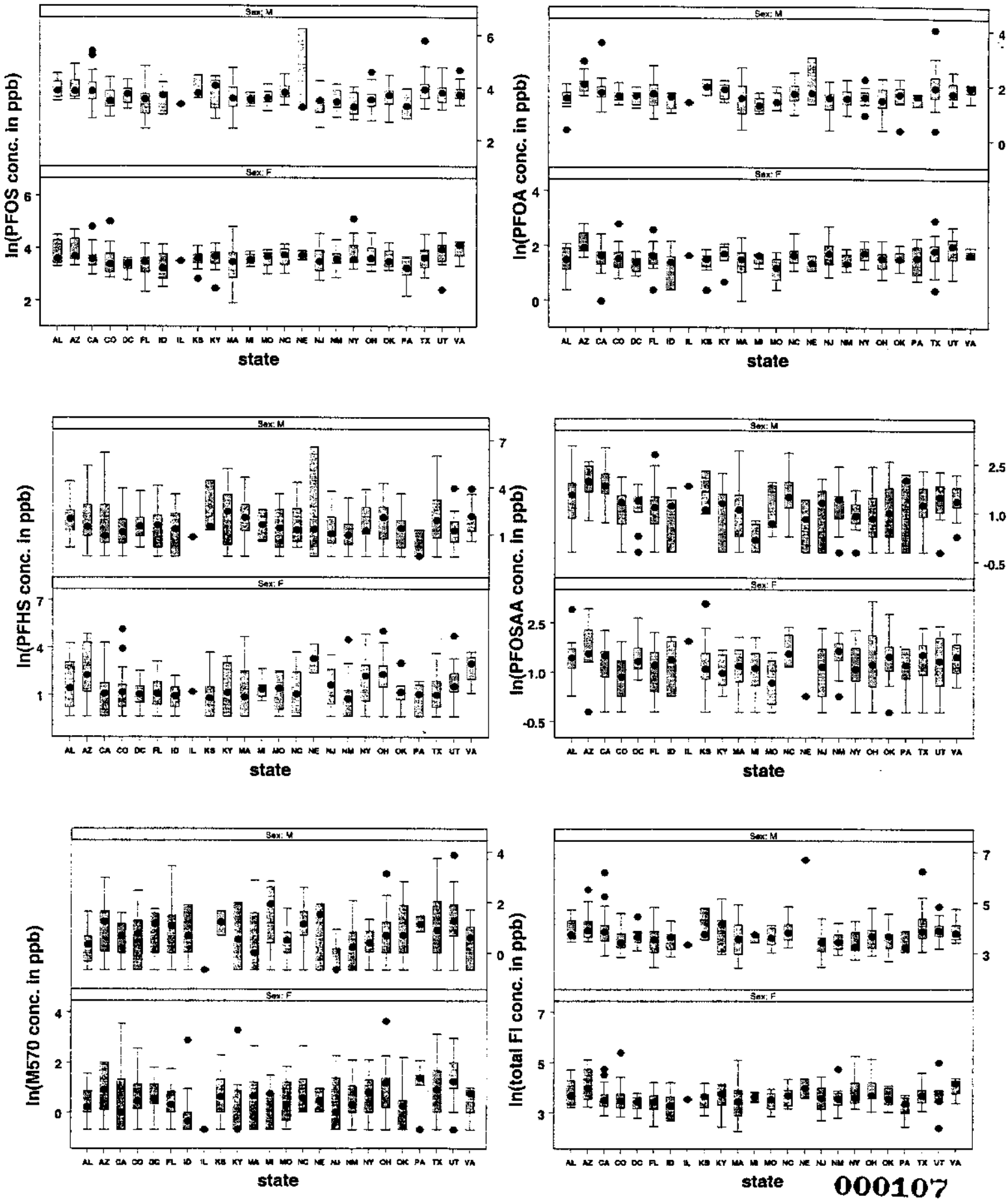
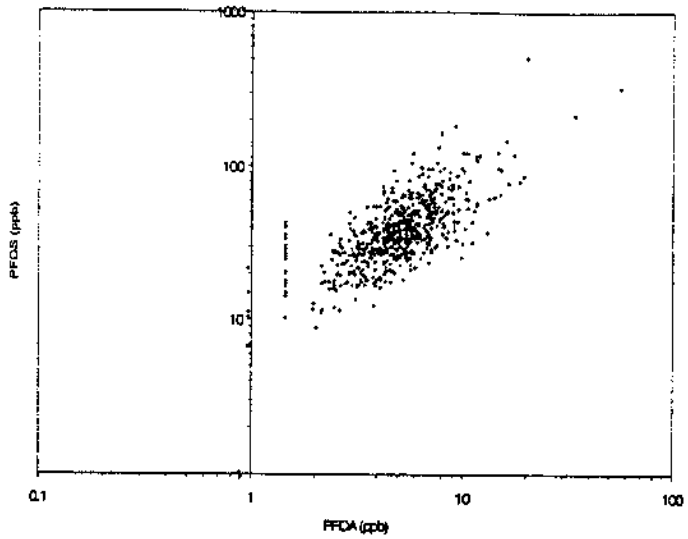
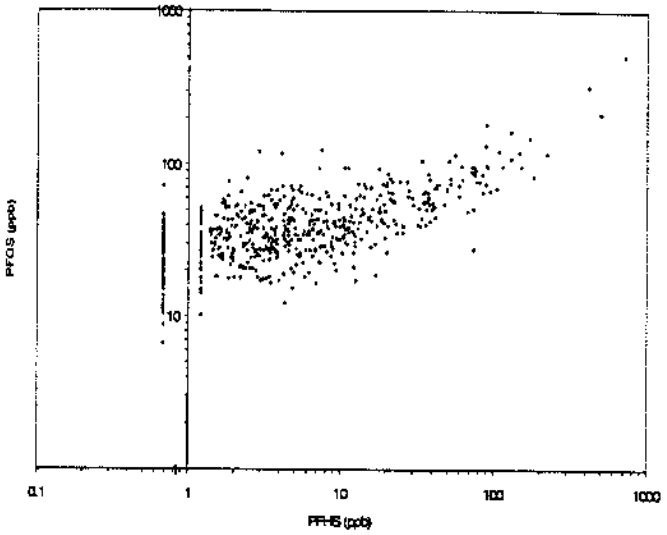
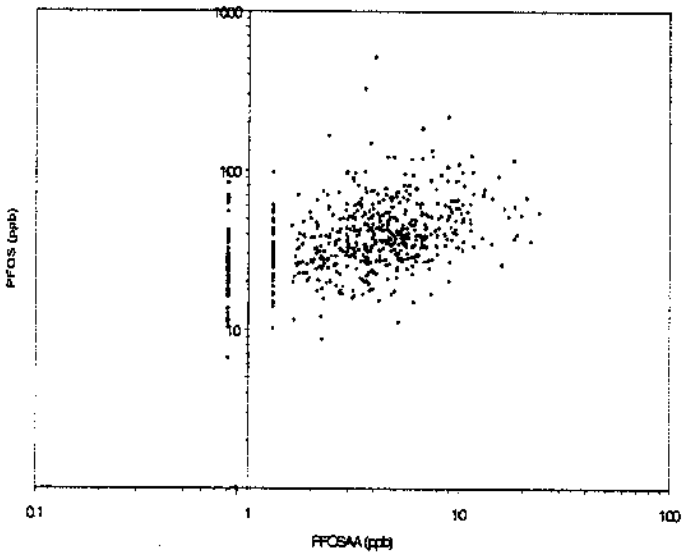
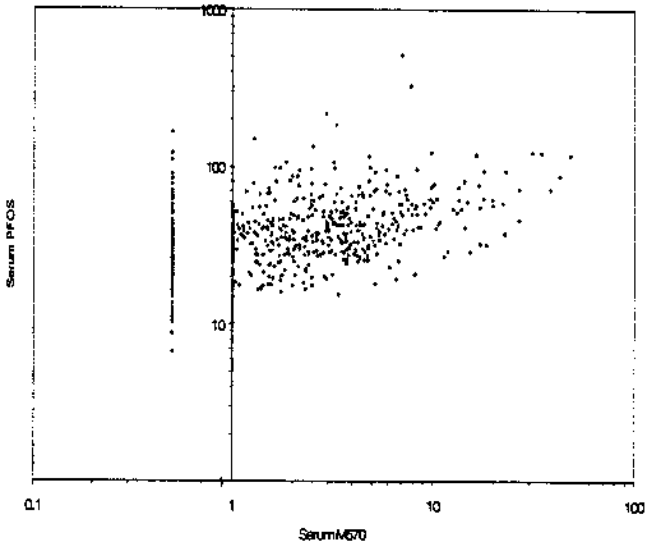


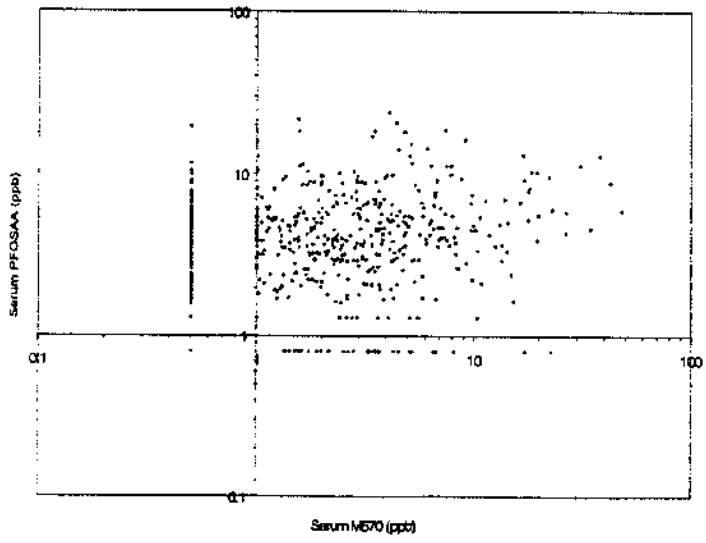
Figure 5. Scatter Plots (log scale) of Fluorochemical Associations











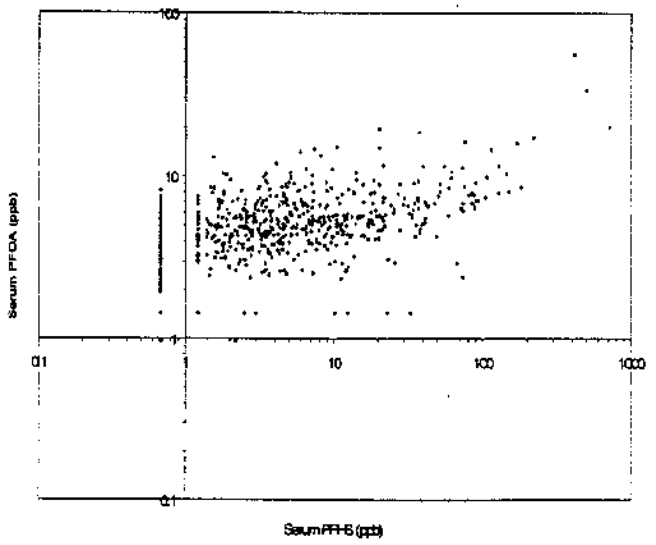


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Figure 6. Comparison of Geometric Means and 95% Confidence Intervals for PFOS, PFOA, PFHS, PFOSAA and M570 for the Pediatric (N = 598), Adult (N = 645) and Elderly (N = 238) Studies

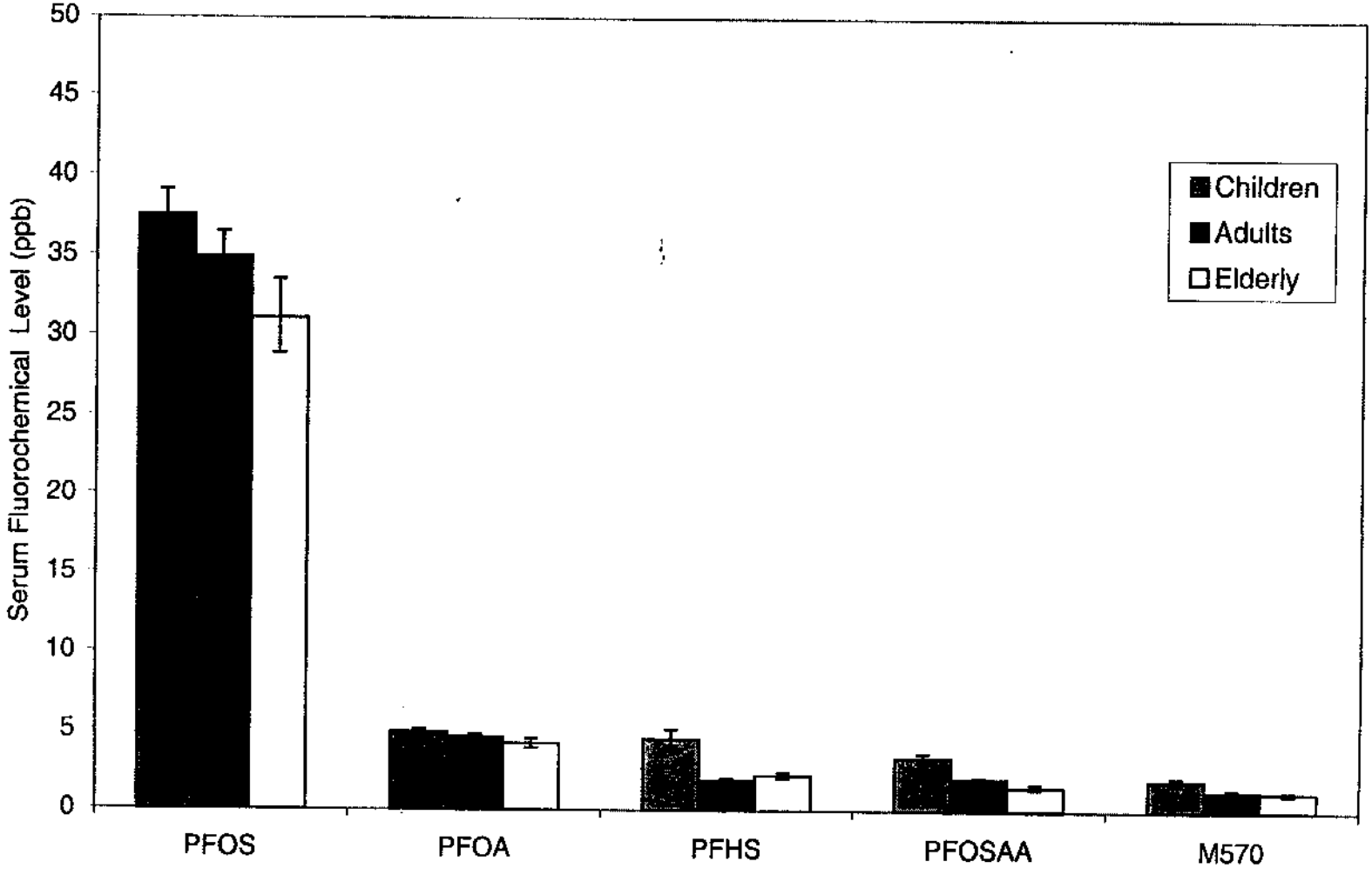

Figure 7. Comparison of the Mean 95% Tolerance Limits and the Upper 95% Confidence Limits for PFOS, PFOA, PFHS, PFOSAA and M570 for the Pediatric (N = 598), Adult (N = 645) and Elderly (N = 238) Studies