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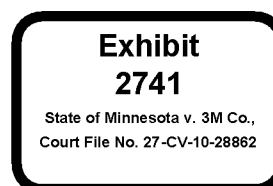
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Abstract

Periodic medical surveillance pertaining to the potential occupational exposure to perfluorochemicals has been conducted at the 3M Decatur facility since the 1980s. The 3M Decatur fluorochemical medical surveillance program has primarily focused on the analysis of serum PFOS and PFOA, in relation to the evaluation of standard clinical chemistry tests including serum lipids, hepatic enzymes, and renal function. In 2004-2005, current and former employees, with a minimum of one year employment at the 3M Decatur fluorochemical manufacturing site, were offered an opportunity to voluntarily participate in a medical monitoring program. Current employees (approximately 500 eligible) were notified by plant communications. Former employees (approximately 800 eligible) received a mailed letter of invitation to participate.

A total of 91 current employees chose to participate. Among former employees, 236 (30%) responded to the letter of invitation with 181 requesting more information and 55 desiring not to participate. Of those requesting more information, 123 participated in the medical monitoring program. Total participants therefore numbered 214 (177 males, 37 females).

The medical monitoring program consisted of the participant responding to a medical questionnaire, having their height, weight, and blood pressure measured, and agreeing to have two tubes of blood (serum) collected via a venipuncture which allowed for measurement of PFOS and PFOA concentrations as well as standard set of clinical chemistries.

Among the 91 current employees, median serum PFOS and PFOA concentrations were 0.42 µg/mL (range 0.04 – 5.68) and 0.45 µg/mL (range 0.02 – 2.83), respectively.

Among all former employees, median serum PFOS and PFOA concentrations were 0.11 $\mu\text{g/mL}$ (0.01 – 4.70) and 0.04 $\mu\text{g/mL}$ (0.00 – 1.99), respectively.

Statistical analyses considered all 214 participants as well as stratified by employee group (current or former) and sex (male or female). Inferences based on these stratified analyses for females are not possible due to relatively few females who participated ($n = 37$).

Arithmetic mean serum PFOS and PFOA concentrations measured in the current and former male employees were not significantly different between individuals who self-reported medical histories of high blood pressure, hepatitis, liver diseases, gall bladder disease or diabetes. The cross-sectional design of this medical monitoring program, however, prevents any assessment of temporality between exposure and disease onset.

Adjusting for potential confounders, there were no statistical associations ($p < 0.05$) between PFOS or PFOA and the lipid parameters, including total cholesterol, LDL, HDL, and triglycerides among all male participants or by employee group. Stratifying the analyses by the respondents' lipid-lowering medication status did not alter these observations.

Adjusting for potential confounders, there were no associations between PFOS or PFOA with the hepatic clinical parameters measured in this medical monitoring program when analyzed among all male participants. Among current male employees, PFOA, but not PFOS, was statistically significantly ($p < 0.05$) positively associated with GGT but not alkaline phosphatase, AST or ALT. This association was not observed for with PFOS nor was it observed between former employees and either PFOA or PFOS.

No consistent associations were observed for renal function, blood glucose or measured systolic or diastolic blood pressure for current and/or former employees with either PFOS or PFOA.

In summary, the analysis of the 3M Decatur medical monitoring program of 214 current and former employees does not demonstrate associations between PFOS or PFOA serum concentrations at the concentrations measured in this program and either hypo- or hyper- cholesterolemia. The results also contribute to the weight of the evidence that serum PFOS and PFOA concentrations, at the concentrations measured in this program, are not associated with hepatic injury as assessed by self-reports and/or a set of hepatic clinical chemistries.

Introduction

Periodic medical surveillance pertaining to the potential occupational exposure to perfluorochemicals has been conducted at the 3M Decatur facility since the 1980s. Employees were initially measured for serum total organic fluorine which subsequently changed in the 1990s to specific analytical measurements of serum perfluorooctanesulfonate (PFOS, $C_7F_{15}COO^-$) and perfluorooctanoate (PFOA, $C_7F_{15}COO^-$) using high performance liquid chromatography mass spectrometry (Olsen et al. 1999). The 3M Decatur fluorochemical medical surveillance program has primarily focused on the analysis of serum PFOS and PFOA, in relation to the evaluation of standard clinical chemistry tests including hepatic enzymes, renal function, and lipids (Olsen et al. 1999; 2003; Olsen and Zobel 2007). In 1994 and 1997, no substantial changes in serum hepatic enzymes, cholesterol, or lipoproteins were associated with PFOS at concentrations less than 6 $\mu\text{g/mL}$ (Olsen et al. 1999). There were too few employees above 6 $\mu\text{g/mL}$ to derive inferences. A total of 253 Decatur male and female employees participated in the 2000 fluorochemical medical surveillance program and results have focused on PFOS (Olsen et al. 2003) and PFOA (Olsen and Zobel 2007). When analyzed collectively with data from 3M's Cottage Grove and Antwerp fluorochemical manufacturing sites for those male participants who did not take lipid-lowering medications, Olsen and Zobel (2007) reported PFOA was not significantly ($P > 0.05$) associated with total cholesterol or low-density lipoproteins (LDL) but was negatively associated with high-density lipoproteins (HDL) but not for any of the individual sites. Olsen and Zobel suggested this was likely due to the consequence of residual confounding due to different demographic profiles at these sites. Similar

inconsistencies were observed for serum triglycerides. There were no significant associations observed between PFOA and hepatic enzymes for the three facilities combined although modest positive associations were observed at the Decatur facility. Analyses of all locations showed no associations with TSH or thyroxine (T4) and those associations observed for free T4 or triiodothyronine (T3) were well within these assays' normal reference ranges.

A cohort mortality study of the 3M Decatur employee workforce was conducted of 2,083 past and present employees with one or more years of employment duration (Alexander et al. 2003). Among those who worked one or more years in a high exposure job, there were 14 observed deaths from all malignant neoplasms compared to 16.7 expected [Standardized Mortality Ratio (SMR) = 0.84, 95% CI 0.46-1.41]. Three of these deaths were attributed to bladder cancer compared to 0.2 expected (SMR = 16.1, 95% CI 3.3 – 47.1). This association, however, was not confirmed when the incidence of bladder cancer was evaluated (Alexander and Olsen 2007). A health evaluation was mailed to 1,895 eligible past and current Decatur employees who worked for at least one year at the 3M facility. A total of 1,400 (74%) participated by responding to the health questionnaire that inquired about several cancer and non-cancer conditions. No associations were reported between working in a PFOS-exposed job and the risk of any of the surveyed conditions including cystitis, bladder calculi, liver disease, benign prostatic hyperplasia, cholecystitis, and cancers of the colon, liver, prostate, breast, and thyroid (Grice et al. 2007).

Lau et al. (2007) reviewed the toxicological findings of perfluoroalkyl acids focusing primarily on PFOS and PFOA. Both PFOS and PFOA are associated with liver

enlargement in rodents and nonhuman primates and also result in hepatocellular adenomas in rats. Dose-dependent PFOS results in lowered serum cholesterol in rats and monkeys. The dose-dependent toxicological studies have also indicated PFOA can result in hypolipidemia in rats and is likely a consequence of a PPAR α -mediated response (Kennedy et al. 2004) however the data for PFOS are not as definitive because liver toxicity and carcinogenicity of PFOS are evident at doses lower than those that induce peroxisome proliferation in short-term studies in rats (Lau et al. 2007). The relevance of this PPAR α -mediated response in humans is considered much less because humans have considerably less expression of the receptor.

3M announced its phase-out of the production of PFOS-based materials in 2000 with complete cessation of production by 2002. In addition, production and use of PFOA was phased-out. In 2004-2005, 3M Decatur employees in the chemical plant, and former employees (including retirees) who were identified as having previously worked in chemical operations for one year or more, were offered an opportunity to voluntarily participate in a medical monitoring program designed to measure serum PFOS and PFOA concentrations in relation to similar standard clinical chemistry tests that have been previously analyzed. A brief medical history questionnaire was also part of this medical monitoring which inquired about selected diseases, medication usage including lipid-lowering medications, and lifestyle habits.

Methods

Selection of Decatur Current Employees

Beginning in December 2004, current employees of the 3M Decatur facility, who may have had current or past workplace exposure experience to fluorochemicals in the

chemical plant, were offered an opportunity to participate in a fluorochemical medical monitoring program. Although a specific census of the number of eligible individuals was not quantified, it was estimated that approximately 500 employees would have considered themselves with having such potential exposure experience (approximately 50% of the total Decatur site employee population at the time). These employees would have included those individuals previously involved as cell and chemical operators, foremen, engineers, maintenance, and administrative workers including environmental health and safety specialists, supervisors, and clerical. Current employees who participated went to the 3M Decatur occupational health clinic to have their blood samples collected and responded to the 3M standard medical surveillance questionnaire that had been used in prior medical surveillance programs at this, and other 3M manufacturing facilities. All current employees signed an informed consent form that was approved by the 3M Institutional Review Board.

Selection of Decatur Retired and Former Employee Participants

Communication of the program to Decatur retirees and former employees was via a mailed letter of invitation from the 3M Medical Director (Dr. Larry Zobel) that accompanied the notification of the University of Minnesota Decatur Health Facility Survey results regarding bladder cancer incidence. This notification was authored by the principal investigator Dr. Bruce Alexander. The reason for inclusion of the letter of invitation from Dr. Zobel in the University of Minnesota mailing was the fact that the 3M Medical Department did not have a list of addresses for most former employees. This address list, however, had been compiled by the University of Minnesota for the Decatur

Health Facility Survey. This address list was not provided to the 3M Medical Department by the University of Minnesota to prevent any confidentiality concerns that might arise. The University of Minnesota did agree to include a letter of invitation from Dr. Zobel when they notified participants and nonparticipants of their study results from the Decatur Health Facility Survey. The letter from Dr. Zobel explained the specifics of the medical monitoring program, the voluntary nature of participation, and instructions for study participation. Retirees and former employees were asked to respond to the letter of invitation by providing a written response (yes or no on a form letter) and their current address to the 3M Medical Department. Eligibility for the medical monitoring program for retired and former employees of the 3M Decatur chemical plant was defined as having worked at the plant for one year or more, same as it was in the Decatur Health Facility Survey. Altogether, approximately 800 retired and former employees of the 3M Decatur chemical plant received an invitation to participate in the voluntary medical monitoring program.

There were a total of 236 responses returned (30% of the 800 letters that were mailed). A total of 181 individuals requested to participate while 55 did not. For the 181 affirmative responses, the 3M Medical Department subsequently contacted these individuals via a second letter to the provided address that then explained much more specific logistics of participation.

Approximately 95% of the individuals who desired to participate lived within one hour drive of Decatur, Alabama and were therefore requested to make an appointment at a medical clinic in the Decatur area. Participation included the collection of two tubes of blood (serum) collected and responding to a brief medical history questionnaire

(Appendix A). All participants signed an informed consent form that was approved by the 3M Institutional Review Board. Instructions and materials were shipped to those participants who chose to use their own medical provider and to those who did not live in the Decatur area. For these individuals, supplies were provided to the participant to take to this medical care provider. Upon collection of the blood samples and questionnaire data, the medical care provider then shipped the samples and information to the appropriate laboratory for analysis of the clinical chemistries and for PFOS and PFOA. Of the 181 initial respondents who indicated they desired to participate, 123 (68 percent) provided all requested data (blood samples and questionnaire information). Combined with the 91 current employees, these 214 individuals were defined as the final database.

Data Collected

The following data were collected from study participants whether current employees or former employees:

- Serum PFOA and PFOS concentrations (reported as $\mu\text{g/mL}$);
- Clinical laboratory tests including the following: alkaline phosphatase (IU/L), gamma glutamyl transferase (GGT, IU/L), aspartate aminotransferase (AST, IU/L), alanine aminotransferase (ALT, IU/L), total and direct bilirubin (mg/dL), cholesterol (mg/dL), high density lipoprotein (HDL, mg/dL), triglycerides (mg/dL), blood glucose (mg/dL), blood urea nitrogen (BUN, mg/dL), and serum creatinine (mg/dL). These measurements were performed at Allina Laboratories (St. Paul, MN). LDL was an indirect calculation using

the Friedwald formula [$\text{LDL} = \text{total cholesterol} - \text{HDL} - (\text{triglycerides}/5)$]

when triglycerides were ≤ 400 mg/dL;

- Vital sign measurements, including height, weight, blood pressure, and pulse;
- Information pertaining to certain medical conditions as well as current medication information. Health habit information, including alcohol consumption and whether or not the employee was a current or former smoker and packs per day smoked, was also collected.

Analytical Methods for PFOA and PFOS

All samples were analyzed for PFOA and PFOS by the 3M Environmental Laboratory. The samples were collected over time and analyzed in three batches, thus three interim reports were generated which detail methods and quality control results (Appendix B). Briefly, PFOA and PFOS were extracted from serum by protein precipitation in acetonitrile via a MultiPROBE II robotic liquid handling system utilizing 96 well-plates. Calibration standards, quality control spikes, and blanks were extracted in control rabbit serum. Additionally, $^{13}\text{CPFOA}$ was spiked into all samples, control human serum and the calibration curve prior to extraction and was used as a surrogate to monitor extraction efficiency. Quantitation was accomplished by high performance liquid chromatography tandem mass spectrometry.

Data Management

Questionnaires and clinical lab reports were mailed to the 3M Medical Department and the data were entered into Microsoft Excel. The data were then

electronically transferred into a JMP dataset (SAS Institute, Inc, Cary, NC) for analyses.

The JMP dataset underwent a 100% quality assurance against the original Allina lab reports and medical surveillance questionnaires to ensure accuracy of the final analysis dataset.

Statistical Analyses

The analyses included 2 employee groups: “current” employees and “former” employees (retired and former (non-retired) fluorochemical production employees). Also analyses involving “all” participants (“current” and “former” employees combined) were conducted. Due to PFOS and PFOA exposure differences between the current and former employees, as well as age differences, most of the analyses were done for all 3 categories.

Descriptive statistics, including means, standard deviations, medians, and ranges, were calculated for PFOS, PFOA and clinical chemistry parameter values, as well as for age and BMI. Differences in means between current and former employees were tested using t-tests. Age was calculated as the non-rounded age in years on the exam date for this study. BMI was computed using the formula $[(\text{weight in pounds}/(\text{height in inches})^2) \times 703]$.

Categorical analyses were done for each sex and by questionnaire responses concerning history of certain medical conditions, current medications, alcohol consumption, and smoking. The number and percent of employees were calculated by reference points for clinical parameters and health factors (BMI, blood pressure,

metabolic syndrome, alcohol use, and smoking status). Chi-square tests were performed to test for differences in proportions between current and former employees.

Employees were considered as having metabolic syndrome in this study if they met at least 3 of the following 5 conditions: (1) BMI ≥ 30 ; (2) triglycerides ≥ 150 mg/dL; (3) HDL < 40 mg/dL for males or < 50 mg/dL for females; (4) blood pressure systolic ≥ 130 mm Hg or diastolic ≥ 85 mm Hg; and (5) glucose ≥ 100 mg/dL.

A “current smoker” was defined as anyone who answered survey question 4 or 5 on the medical surveillance questionnaire (current employees) or question 11 or 12 on the former employee questionnaire (Appendix A). A “former smoker” was defined as anyone who answered question 6 or 7 on the medical surveillance questionnaire (current employees) or question 13 or 14 on the former employee questionnaire.

Descriptive statistics (including mean, standard deviation, median, and range) were calculated for PFOA, PFOS, clinical parameter values, as well as for age and BMI by employee group for both males and females.

Means and 95% confidence intervals were computed for PFOS and PFOA by binary response (yes/no) of survey questions regarding selected medical conditions and also for the definition of metabolic syndrome used in these analyses. Analyses were done separately for males and females. Differences in means (“yes” group vs. “no” group) were tested using t-tests.

Means, 95% confidence intervals, medians, and ranges of PFOS and PFOA were calculated by PFOA and PFOS quintile categories. Differences in quintile means were tested using t-tests. The number and percent of employees by PFOS and PFOA quintiles and reference points for clinical parameters and health factors (BMI, metabolic

syndrome, and alcohol use) as well as employee status (current or former) were calculated. Chi-square tests were performed to test for differences in proportions for health factor variables. Cochran-Armitage Tests for Trend (two-sided) were used to test for increasing (or decreasing) trends between PFOS/PFOA quintiles and the proportion of employees above the reference point for clinical parameters.

Simple and multivariable regression analyses were used to estimate the relationships between both PFOS and PFOA and the outcome variables of interest. Regression analyses were done separately for males and females in order to control for the confounding effect of gender. The following were considered dependent variables for these analyses: systolic and diastolic blood pressure, alkaline phosphatase, AST, ALT, GGT, total bilirubin, direct bilirubin, glucose, BUN, creatinine, cholesterol, LDL, HDL, and triglycerides. Presented in this report are LDL values where triglycerides were ≤ 400 mg/dL. Consistent with a previous study report (Olsen and Zobel 2007) age, BMI, and alcohol (average drinks per week) were considered as covariates in all multivariate analyses. In addition, for analyses of hepatic variables (alkaline phosphatase, AST, ALT, and GGT), triglycerides were also considered as a covariate in place of BMI. Log transformations of both response and explanatory variables improved normality assumptions and were used in all models.

Alcohol as a covariate was defined using the response to the survey question regarding drinks per week. The five responses for the survey question regarding alcohol consumption were averaged as follows: None or less than 1 drink per week (0 drinks/week); 1-3 drinks per week (2 drinks per week); 4-7 drinks per week (5.5 drinks/week); 8-14 drinks per week (11 drinks per week); and over 14 drinks per week

(15 drinks per week). For the log transformation of alcohol, 0.1 was added to prevent the log of 0.

The number and percent of employees by employee group and self-reported lipid-lowering medication status were calculated for males and females. Descriptive statistics for the four lipid parameters (cholesterol, LDL, HDL, and triglycerides) were provided by sex and employee group for both levels of participant lipid-lowering medication status. Linear regression analyses for lipid variables were repeated after stratifying by self-reported lipid-lowering medication status.

There was 1 male current employee with partial data (had PFOS/PFOA measurements, but no questionnaire or clinical chemistry data), thus he was not included in the analyses for this study. There were 14 employees (12 males, 2 females) who did not answer the question regarding alcohol consumption and thus were excluded from the regression analyses. There were 7 male employees, 5 former and 2 current, who had triglyceride levels > 400 mg/dL and thus were excluded from the analyses involving the calculated measurement of LDL.

Results

Descriptive Results

There were 214 Decatur employee participants in this study who had both PFOS and PFOA measurements and clinical parameters: 91 current employees and 123 former employees. Overall, there were 177 (83%) males and 37 (17%) females. The gender distribution was similar among the current and former employee groups.

The arithmetic mean PFOS and PFOA concentrations were statistically significantly higher ($p \leq 0.05$) among current employees compared to former employees (Table 1) and the distributions were skewed to the right as the median values were at least 1.5 times lower and 2.5 times lower than the means for current and former employees, respectively. Geometric means (95% CI in parenthesis) by employee group for PFOS were: current employees 0.47 $\mu\text{g/mL}$ (95% CI 0.38 – 0.59); former employees 0.13 $\mu\text{g/mL}$ (95% CI 0.10 – 0.16); and all participants 0.22 $\mu\text{g/mL}$ (95% CI 0.18 – 0.26). For PFOA, the geometric means were: current employees 0.43 $\mu\text{g/mL}$ (95% CI 0.34 – 0.54); former employees 0.04 $\mu\text{g/mL}$ (95% CI 0.3 – 0.06); and all participants 0.11 $\mu\text{g/mL}$ (95% CI 0.09 – 0.15).

Former employees were significantly older (60 years) than current employees (47 years) (Table 1). BMI was similar between these two groups (Table 1). Mean systolic and diastolic blood pressure were both significantly higher among former employees, as well as mean glucose, BUN, and direct bilirubin (Table 1). With the exception of blood glucose for former employees (mean value of 104 mg/dL), these mean values were within the reference range. For the lipid parameters, mean cholesterol and LDL levels were significantly higher for current than former employees. However, these data in Table 1 do not account for prevalence of lipid-lowering medications between the two groups. There was not a statistically significant difference in mean triglyceride levels between current and former employees.

The number and percentage of employees by participant characteristic and employee group are presented in Table 2. As expected because of their older age, there were significantly more former than current employees with a medical history of high

blood pressure (55% vs. 32%) and diabetes (18% vs. 6%). There were significantly more former employees taking medications for high blood pressure (53% vs. 32%), high cholesterol (45% vs. 27%), and diabetes (16% vs. 4%).

Table 3 displays the number and percent of employees by the upper (or lower) part of the reference range of clinical parameters and health-related factors. There were significantly more former employees above the reference for glucose (45% vs. 20%), BUN (6% vs. 0%), HDL (40% vs. 23%), triglycerides (35% vs. 22%) as well as the metabolic syndrome (42% vs. 22%). Current employees had significantly more employees above the reference for ALT (26% vs. 10%), cholesterol (≥ 200 mg/dL: 53% vs. 33%; ≥ 240 mg/dL: 22% vs. 10%), and LDL (40% vs. 27%). There were significantly more former employees who reported drinking > 3 drinks per week and who were former smokers.

Descriptive statistics for PFOS, PFOA, age, BMI, and clinical parameter results by employee group are provided in Table 4A for males and in Table 4B for females. Geometric means (95% CI in parenthesis) for PFOS by employee group were: current male employees 0.49 $\mu\text{g/mL}$ (95% CI 0.39 – 0.63); former male employees 0.15 $\mu\text{g/mL}$ (95% CI 0.12 – 0.18); and all male participants 0.25 $\mu\text{g/mL}$ (95% CI 0.20 – 0.30). For PFOA, the geometric means were: current male employees 0.47 $\mu\text{g/mL}$ (95% CI 0.36 – 0.61); former male employees 0.05 $\mu\text{g/mL}$ (95% CI 0.04 – 0.07); and all male participants 0.13 $\mu\text{g/mL}$ (95% CI 0.10 – 0.17).

For female employee groups, geometric means (95% CI in parenthesis) were for PFOS: current female employees 0.38 $\mu\text{g/mL}$ (95% CI 0.24 – 0.61); former female employees 0.06 $\mu\text{g/mL}$ (95% CI 0.03 – 0.10); and all female participants 0.13 $\mu\text{g/mL}$

(95% CI 0.08 – 0.21). For PFOA, the geometric means were: current female employees 0.28 µg/mL (95% CI 0.15 – 0.50); former female employees 0.02 µg/mL (95% CI 0.01 – 0.05); and all female participants 0.07 µg/mL (95% CI 0.03 – 0.13).

For males in Table 4A, the results were similar, as seen in Table 1. Current employee mean values were significantly higher for PFOS, PFOA, cholesterol, and LDL and former employee mean values were significantly higher for age, blood pressure, glucose, BUN, and direct bilirubin. The mean glucose (106 mg/dL) and mean triglyceride (165 mg/dL) values for former male employees were influenced by a former male employee who had both the maximum glucose (357 mg/dL) and triglyceride (732 mg/dL) values. The results were similar for females, but there were not significant differences between current and former female employees for BUN, direct bilirubin, cholesterol, or LDL (Table 4B). In addition, the former female employee mean value was significantly higher for BMI and the current female employee mean value was significantly higher for total bilirubin.

Presented in Tables 5-8 are the mean PFOS and PFOA concentrations by the sex-specific binary categorizations to specific survey questions for each employee group. Due to the limited number of employees in some response groups, the analyses should be interpreted cautiously. No comparisons were performed when response sample size was less than 2. There were few statistically significant differences in serum PFOS or PFOA concentrations by these disease or medication use factors. Mean PFOS was significantly higher for former employees who met the study definition of metabolic syndrome compared to those who didn't meet the definition (Table 5). Among males, there were no statistically significant differences in the mean PFOA levels (Table 6). Due to the small

total number of females (n=37), some comparisons between the “yes” and “no” groups were not possible (when the responses were all “yes” or all “no”). There were no statistically significant differences in mean PFOS levels for females (Table 7). Mean PFOA was significantly higher for former female employees who answered “yes” to a medical history of diabetes as well as taking diabetes medications compared to those who answered “no”, however, the confidence intervals for the “yes” groups were fairly imprecise due to the small number of employees (Table 8). For female employees who met the study definition of metabolic syndrome, mean PFOA was significantly higher for former female employees compared to those female employees who didn’t meet the definition.

Table 9 shows descriptive statistics by PFOS quintile for all employees. There were no significant differences in mean PFOS concentrations for quintiles 1 through 3. Mean PFOS concentrations ranged from quintile 1 of 0.040 µg/mL to quintile 5 of 1.597 µg/mL. Corresponding median values were 0.040 µg/mL to 1.380 µg/mL, respectively, for the five quintiles.

Displayed in Table 10 are the number and percent of all employees, by PFOS quintile, for each lifestyle factor, health factor, and clinical parameter reference point. There was a significant difference in proportional distribution across quintiles by the employee status (current or former). In the lower quintiles, there were a higher percentage of former employees, but in the fourth quintile there was a shift to a higher percentage of current employees. According to the Cochran-Armitage Test for Trend, as the PFOS quintile increased, there was a significant increase in the proportion of employees above the reference point for alkaline phosphatase, ALT, and nearly

significant for cholesterol ≥ 200 mg/dL. There was a significant decrease in the percentage of current employees above the reference point for glucose as PFOS quintile level increased. None of these statistical comparisons, however, are adjusted for potential confounding variables and therefore should be interpreted cautiously.

Similar to Table 9, Table 11 displays descriptive statistics by PFOA quintile for all employees. Mean PFOA concentrations ranged from quintile 1 of 0.009 $\mu\text{g/mL}$ to quintile 5 of 1.383 $\mu\text{g/mL}$, with corresponding median values of 0.009 $\mu\text{g/mL}$ to 1.240 $\mu\text{g/mL}$, respectively.

Similar to Table 10 for PFOS, displayed in Table 12 are the number and percent of all employees by PFOA quintile for each lifestyle factor, health factor, and clinical parameter reference point. Again, there was a significant difference in proportional distribution across quintiles by status. In the lower quintiles, there were a higher percentage of former employees, but in the third quintile there was a shift to a higher percentage of current employees. According to the Cochran-Armitage Test for Trend, as the PFOA quintile increased, there was a significant increase in the percentage of employees above the upper reference range points for cholesterol ≥ 200 mg/dL, cholesterol ≥ 240 mg/dL, LDL, alkaline phosphatase, and ALT. There was a significant decrease in the percentage of employees above the reference points for glucose, BUN, and direct bilirubin as PFOA quintile level increased. None of these statistical comparisons, however, are adjusted for potential confounding variables.

Multivariable Results

Presented in Tables 13-16 stratified by sex are the non-adjusted and adjusted natural log PFOS and natural log PFOA coefficients for clinical chemistry parameter results by employee group. These multivariate regression models were adjusted for age, BMI, and alcohol or their natural logs (multiplicative model). Models using both transformations are displayed in order to compare with each other on the affect that the confounders had (and their transformation) on the coefficient of the explanatory variable. The lipid analyses include all subjects whether taking or not taking lipid lowering medications. The PFOA and PFOS coefficients in the unadjusted regression models were statistically significant for one or more employee groups for several clinical parameters, however, when age, BMI, and alcohol were included as covariates (regardless of transformation) the adjusted PFOA and PFOS coefficients generally became statistically nonsignificant ($p > 0.05$). Exceptions will be noted. The PFOA or PFOS coefficients did not differ substantially between the two models based on different transformations of the covariates.

Non-adjusted and adjusted PFOS coefficients for clinical parameter results for males are presented in Table 13. None of the adjusted coefficients for PFOS were statistically significant for any of the clinical parameters in the all and former employee analyses and only for creatinine in the current employee model. The adjusted PFOS coefficient for current employees for creatinine ($p=0.02$) explained approximately X% of the variance of the response variable in the full model ($R^2=.$).

Table 14 presents non-adjusted and adjusted PFOA coefficients for clinical parameter results by employee group for males. Adjusted PFOA coefficients were not

statistically significant for glucose, BUN, creatinine, cholesterol, LDL, HDL, triglycerides, alkaline phosphatase, AST, ALT, total bilirubin, or direct bilirubin. The adjusted PFOA coefficient in the multiplicative model was significant for the current employee group only ($p=0.04$) for GGT and explained X% of the GGT variance in the full model ($R^2=.$). [Note: the adjusted PFOA coefficient was also significant for diastolic blood pressure ($p=0.02$) for current employees but not for systolic blood pressure ($p = 21$).

Non-adjusted and adjusted PFOS coefficients for clinical parameter results for females are presented in Table 15 and for PFOA in Table 16. These models should be cautiously interpreted as only 37 individuals contributed to the analyses, 21 females to the former analyses and 16 females to the current analyses. As a result, the measures of variation (i.e., SE) are larger than seen in the corresponding tables for male subjects.

In Table 15, the adjusted PFOS coefficient in the multiplicative model was significant for current female employees for alkaline phosphatase ($p=0.02$), and explained X% of the alkaline phosphatase variance in the full model ($R^2=.$). The adjusted PFOS coefficient was significant for all and former employees for total bilirubin ($p=0.001$ and $p=0.003$, respectively). For former female employees, the adjusted PFOS coefficient was also significant for glucose ($p=0.03$) and BUN ($p=0.01$). A significantly negative association was between PFOS and ALT for former female employees ($p = 0.04$). No statistically significant coefficients for PFOS were found for creatinine, cholesterol, LDL, HDL, triglycerides, AST, or GGT.

In Table 16, the adjusted PFOA coefficient in the multiplicative model was significant for former female employees for glucose ($p=0.02$), and explained X% of the

glucose variance in the full model ($R^2=.$). The adjusted PFOA coefficient was also significant for all and former female employees for total bilirubin ($p=0.002$ and $p=0.004$, respectively). For current employees, the adjusted PFOA coefficient was significant for alkaline phosphatase ($p=0.0004$). Analyses showed no statistically significant associations for the lipid clinical parameters (cholesterol, LDL, HDL, and triglycerides), BUN, creatinine, AST, ALT, or GGT.

Because triglycerides may also be an important covariate in the analysis of clinical hepatic chemistries, BMI was replaced with triglycerides as a covariate in Tables 17 – 20 and alkaline phosphatase, AST, ALT, and GGT were reanalyzed. None of the adjusted coefficients for PFOS or PFOA were statistically significant for the current male employee group (Tables 17 and 18). Among former male employees, negative associations between PFOS with alkaline phosphatase or AST (Table 17), and between PFOA and AST (Table 18), were statistically significant. Among female employees, the associations observed between PFOS and alkaline phosphatase (Table 19) or PFOA and alkaline phosphatase (Table 20) were significant ($p=0.0005$ and $p=0.02$, respectively), otherwise all other coefficients of PFOS or PFOA were not significant.

Table 21 displays the number and percent of employees by employee group and sex, and their self-reported lipid-lowering medication status. 42% of males and 14% of females reported taking lipid-lowering medications with the largest percentages occurring among former employees, as would be expected given their significantly older age.

Table 22A provides the descriptive statistics for the four lipid clinical parameters by those participants who self-reported they were not taking lipid-lowering medications for each employee group by sex. Likewise, Table 22B provides the same data for those

participants who self-reported that they were taking lipid-lowering medications. Among males, stratified by employee group, those taking lipid-lowering medications had lower total cholesterol and LDL values than those who did not these medications. There were too few females who self-reported taking lipid lowering medications to evaluate the data.

Presented in Tables 23 - 26 are the non-adjusted and adjusted PFOS and PFOA coefficients for the lipid clinical parameters (cholesterol, LDL, HDL and triglycerides) stratified by self-reported lipid-lowering medication status. Regardless of the lipid lowering medication, for males, no significant adjusted coefficients for PFOS (Table 23) or PFOA (Table 24) occurred with cholesterol, LDL, HDL, or triglycerides. Among females not taking lipid-lowering medications, no significant coefficients for PFOS (Tables 25) or for PFOA (Table 26) were observed. Insufficient sample size prevented analysis of those females taking lipid lowering medications.

Discussion

This was a cross-sectional analysis of a voluntary medical monitoring program that was offered to current and former employees of the 3M Decatur fluorochemical manufacturing plant in 2004-2005. A total of 214 current and former employees participated representing approximately 20 % of eligible current employees and 15% of eligible former employees who chose to participate. The majority (n = 177) were males (83%) thus making it difficult to derive inferences from the female data.

The median serum PFOS (0.42 µg/mL) and PFOA (0.45 µg/L) concentrations measured among the participating current male employees are approximately 10 and 100 times higher, respectively, than those reported in general population studies in the United

States (Olsen et al. 2003; Calafat et al. 2007). Among the former employees, the median PFOS (0.11 µg/mL) and PFOA (0.04 µg/mL) concentrations are approximately 2 and 10 times higher than the general population. These differences in magnitude of PFOS and PFOA concentrations between these current and former employees and the general population may vary slightly depending upon the date of the collection of the general population blood samples. PFOS and PFOA serum/plasma concentrations appear to be declining since the announced phase-out of perfluorooctanyl compounds by 3M in 2000 (Olsen et al. 2007; Calafat et al. 2007).

Adjusting for potential confounders, there were no associations between PFOS or PFOA and the lipid parameters reported, including total cholesterol, LDL, HDL, and triglycerides among male participants. Stratifying the analyses by the respondents' lipid-lowering medication status did not alter these observations. Therefore, these data do not support laboratory animal studies that have shown hypolipidemia with sufficiently high enough serum concentrations of PFOS or PFOA. Neither do the present data support the inference of a positive association between PFOA and serum total cholesterol reported in some epidemiologic studies (Olsen et al. 2003; Sakr et al. 2007a; 2007b) but not others (Ubel et al. 1980; Gilliland and Mandel 1996; Olsen et al. 2000; Emmett et al. 2006). If a positive association does exist between PFOA and serum cholesterol, the longitudinal analyses conducted by Sakr et al. 2007a suggests such an association would not be clinically relevant in the general population. In their longitudinal analysis, Sakr et al. reported a 1000 ng/mL increase in PFOA was associated with a 1 mg/dL increase in PFOA. This association, if biologically plausible, would be unlikely to be observed in either the general population which has a median serum PFOA concentration of 5 ng/mL

or in affected communities with groundwater contamination of PFOA concentrations that have been reported to have median PFOA concentrations approximating 350 ng/mL (Emmett et al. 2006). In fact, total cholesterol was not associated with the latter serum PFOA concentrations (Emmett et al. 2006).

Adjusting for potential confounders, there were no associations between PFOS or PFOA with any of the hepatic clinical parameters measured in this medical monitoring program when analyzed among all male participants of this medical monitoring program. Among current male employees only, PFOA, but not PFOS, was statistically significantly positively associated with GGT but not alkaline phosphatase, AST, or ALT. In the regression models, the PFOA coefficient became statistically nonsignificant with GGT when adjusted for age, BMI, and triglycerides although the magnitude of change in the statistical significance of the model was minimal. Whereas a previous cross-sectional analysis of fluorochemical medical surveillance data collected in 2000 at Decatur suggested modest positive associations between PFOA and alkaline phosphatase, ALT, and GGT (Olsen and Zobel 2007), no associations were observed with the current employees for alkaline phosphatase in the present analyses and those observed for ALT were statistically nonsignificant whether adjusted for BMI or triglycerides. Among former employees there were statistically significant negative associations observed with PFOA and alkaline phosphatase and AST in the regression models.

The overall results from the present medical monitoring program lends further support to the conclusion that there is no consistent evidence that average serum PFOS or PFOA concentrations reported in occupationally exposed workers, are associated with clinically relevant perturbations in hepatic enzymes indicative of liver injury (Gilliland

and Mandel 1993; Olsen et al. 1999; 2000; 2003; Olsen and Zobel 2007; Sakr et al. 2007a; 2007b). Our findings are supported by the lack of associations regarding hepatic diseases reported by Grice et al. in their interview assessment of 1400 (74%) of 3M Decatur past and present employees who had worked at least one year at this facility since its beginning in the 1960s. In addition, Emmett et al. (2006) did not observed associations with hepatic enzymes among residents of a community affected by groundwater contamination of PFOA. These residents had serum PFOA concentrations that averaged approximately 350 ng/mL.

In the present medical monitoring program, serum PFOS and PFOA concentrations measured in these current and former male employees were not significantly different between individuals who self-reported medical histories involving high blood pressure, hepatitis, liver diseases, gall bladder disease and diabetes. The cross-sectional design of this medical monitoring program, however, prevents any assessment of temporality between exposure and disease onset. Other limitations include the 15 to 20 percent participation rate of eligible past and present employees who were offered an opportunity to participate. This participation rate was markedly less than that reported by Grice et al. in their self-reported assessment of health conditions of past and present employees at the 3M Decatur facility. A major reason for this discrepancy is the fact that Grice et al. only involved a mailed questionnaire in their study whereas the present medical monitoring program required former employees to make an appointment at a medical clinic and to have a venipuncture with two tubes of blood collected. No financial incentive to participate was offered to current or former employees. The great majority of former employees who participated lived within a one hour drive of Decatur,

Alabama and was therefore able to utilize the medical clinic that was contracted with to conduct the examination. Because of their age, a greater percentage of retired employees, might have health conditions that prevented them from participating. Former employees who were still active in the workforce, and therefore relatively healthier, may not have desired to participate because of the need to make a medical appointment during work hours. This medical monitoring program should be viewed as a service offered to former employees rather than a focused research study. Follow-up of nonrespondents was more problematic because 3M investigators did not know their address. However, the fact that only a few mailings were considered undeliverable and returned back to the University of Minnesota (personal communication Dr. Bruce Alexander), the program investigators considered it quite likely that the great majority of eligible respondents had received their letter of invitation.

In summary, the analysis of the 3M Decatur medical monitoring program of 214 current and former employees does not offer support that PFOS and PFOA concentrations, as measured, were associated with hypo- or hyper- cholesterolemia. The results also contribute to the weight of the evidence that serum PFOS and PFOA concentrations, as measured, are not associated with hepatic injury. Limitations of this analysis include the low response rate to the letter of invitation to participate and the cross-sectional design which prevents any assessment of temporality.

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