Kara L. Andres/US-Corporate/3M/US 01/10/2008 10:44 PM	To Geary Olsen/US-Corporate/3M/US@3M-Corporate cc bcc Subject CG Draft Report

Geary:

Here is a copy of the updated DRAFT Cottage Grove Study Report (Methods and Results). I have put the electronic file out on the p:drive in the following location:

P:\Projects\COM_Epi\Kara Andres\CG Report Draft

The text highlighted in yellow is my original questions to you from the draft copy you already reviewed. The text highlighted in blue contains new questions for you, is just newly added text, or serves as a reminder to add new text for the new tables for predicted lipid values and scatterplots that I did.

I printed a new set of updated tables as well as this methods/results document for you. I am also going to return your original draft copy with your initial comments.

Any questions, let me know.

CG Methods.doc

Kara

Methods

Selection of Study Participants

In October 2005, a letter was sent to all current and retiree or former fluorochemical production employees of the 3M plant in Cottage Grove inviting them to participate in a medical monitoring program. Did current employees have to have a minimum time to qualify also? For retirees or former Cottage Grove employees, anyone who worked in the chemical operation for at least one year during their tenure at the site qualified for participation. The purpose of this program was to monitor the presence of two specific fluorochemicals known as PFOA and PFOS. The letter explained the specifics of the monitoring program, the voluntary nature of participation, and instructions for study participation. A second letter was sent in January 2006 to employees who had not responded to the initial letter inquiring again whether they would like to participate in the monitoring study.

Data Collected

The following data was collected from study participants:

- Clinical laboratory tests, including uric acid and blood chemistry (lipid and liver measurements). These measurements were performed at Allina Laboratories (St. Paul, MN). LDL was an indirect calculation using the Friedwald formula [LDL = total cholesterol - HDL − (triglycerides/5)] when triglycerides were ≤ 400 mg/dL.
- Vital sign measurements, including height, weight, blood pressure, and pulse.
- Medical Surveillance questionnaire, which included medical history (history of 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.

Location of blood draw for labs?...

The questionnaire was mailed to participants and vitals were completed and signed by a health care provider.

Exam date for this study was based on the date that the employee filled out the questionnaire and had vital measurements performed by a health care provider? Or had blood drawn for labs?

PFOA/PFOS Determinations
Information on how/when the serum PFOA/PFOS measurements were done?

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.

Mention anything about how results (both lab and PFOA/PFOS) being communicated to employees?

Statistical Analysis
The analyses included 3 employee groups: "current" employees, "former" employees (qualifying retiree or former fluorochemical production employees), and "all" employees ("current" and "former" employees combined). Due to PFOA and PFOS exposure differences between the current and former employees, as well as age differences, most of the analyses were done for all 3 employee groups.

Descriptive statistics, including the means, standard deviations, medians, and ranges, were calculated for PFOA, PFOS and clinical lab 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 unrounded 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 sex and 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 lab 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 had at least 3 of the following: (1) BMI $\geq$ 30; (2) triglycerides $\geq$ 150 mg/dL; (3) HDL $<$ 40 mg/dL for males or $<$ 50 mg/dL for females; (4) blood pressure systolic $\geq$ 130 mm Hg or diastolic $\geq$ 85 mm Hg; (5) glucose $\geq$ 100 mg/dL (from "Socioeconomic Position and the Metabolic Syndrom in Early, Middle and Late Life: Evidence from NHANES 1999-2002" in Annepid. Based on AHA/Nat'l HLB Institute guidelines). A "current smoker" was defined as anyone who answered "yes" to survey questions 11 or 12. A "former smoker" was defined as anyone who answered "yes" to survey questions 13 or 14. (Put copy of survey in the report appendix and refer to appendix?)

Descriptive statistics (mean, standard deviation, median, and range) were calculated for PFOA and PFOS by employee group for both males and females.

Means and 95% confidence intervals were computed for PFOA and PFOS by binary result of survey question or metabolic syndrome 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 PFOA and PFOS were calculated by PFOA and PFOS quintile. Differences in quintile means were tested using t-tests. The number and percent of employees by PFOA and PFOS quintiles and reference points for clinical lab 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 PFOA quintiles and the proportion of employees above the reference point for clinical lab parameters.

Univariate and mulitvariate regression analyses were used to estimate the relationships between both PFOA and PFOS 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, uric acid, glucose, BUN, creatinine, cholesterol, LDL, HDL, triglycerides, alkaline phosphatase, AST, ALT, GGT, total bilirubin, and direct bilirubin. Presented in this report are LDL values where triglycerides were ≤ 400 mg/dL. In Olsen paper pg. 12 states bias for LDL with higher trigs. Consistent with a previous study report (add reference for Olsen and Zobel report, May 2006) 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. Age is known to be positively associated with cholesterol, BMI with triglycerides and (to some degree) cholesterol, and alcohol consumption with increased HDL. Triglycerides have been shown to be important predictors of liver enzyme values and should be controlled in analyses that examine the relationship between PFOA and PFOS and liver enzymes. (Olsen, 2006) Log transformations of both response and explanatory variables improved normality assumptions and were used in all models. Necessary to say muliplicative models here?

The following table illustrates how alcohol as a covariate was defined using the response to the survey question regarding drinks per week. For the log transformation of alcohol, 0.1 was added to prevent the log of 0. (note to Geary: I noticed I goofed and used 1.5 instead of 2 for 2nd interval, but not going to redo all analyses)

Alcohol	Survey Question Response
0	None or less than 1 drink per week
1.5	1-3 drinks per week

5.5	4-7 drinks per week
11	8-14 drinks per week
15	Over 14 drinks per week

Linear regression analyses for lipid variables (cholesterol, LDL, HDL, and triglycerides) were repeated after stratifying by self-reported high cholesterol medication status (currently taking high cholesterol medications = yes or no). Regression analyses were also done for cholesterol after stratifying by PFOA $\leq$ 1 ppm and PFOA > 1 ppm for all males and males who self-reported they were not taking high cholesterol medication.

Add paragraph regarding new scatterplots and tables showing predicted lipid values.

There were 7 male employees (5 former, 2 current) who did not answer the question regarding alcohol consumption and thus were excluded from the multivariate regressions and analyses involving alcohol consumption. Three male former employees were missing partial or complete clinical lab parameter data and the analyses involving these parameters automatically adjusted the sample sizes. There were 12 total employees, 10 males and 2 females (each with an equal number of current and former employees), who had triglyceride levels > 400 mg/dL and thus were excluded from the analyses involving LDL.

There were 5 employees with partial data (they either only had PFOA/PFOS measurements or questionairre and clinical chemistry data), thus were not included in the analyses for this study.

Results

In total there were 437 Cottage Grove employee participants in this study, 123 current employees and 314 former employees. Overall, there were 382 (87%) males and 55 (13%) females. The gender distribution was similar among the current and former employee groups. (Do we know how many employees received letters? Could add a

The mean PFOA and PFOS levels were significantly higher ($p \leq 0.05$) among current employees compared to former employees (Table 1). The range of PFOA levels for current employees is fairly large and includes the largest PFOA value of 17.5 ppm. However, the median PFOA value is also higher for current employees, indicating that it isn't just the highest 1 or 2 PFOA values causing the higher mean PFOA level for current employees. The distributions for PFOA and PFOS were skewed to the right for both employee groups. Former employees were significantly older, as expected, and BMI was similar between the two groups. Mean systolic and diastolic blood pressure were both significantly higher among former employees, as well as mean glucose, BUN, and creatinine. However, all of these mean values were within the reference range. For the lipid parameters, mean cholesterol, LDL, and triglyceride levels were significantly higher for current employees, while mean HDL levels were significantly higher among former employees. With the exception of triglycerides, these mean lipid values were within the reference range.

The number and percent of employees by participant characteristic and employee group are presented in Table 2. As expected based on an older former employee group, there were significantly more former employees with a medical history of high blood pressure (42% vs. 14%), hepatitis or yellow jaundice (5% vs. 1%), and diabetes (11% vs. 3%). As far as current medications, there were significantly more former employees taking medications for high blood pressure (42% vs. 11%), high cholesterol (39% vs. 15%), and sugar diabetes (9% vs. 3%). The proportion of employees falling into the various categories of alcohol drinks per week was different between the 2 groups. There was a higher proportion of current employees who reported drinking 4-7 drinks per week (24% vs. 13%).

Table 3 displays the number and percent of employees by reference point of lab parameter or health factor. There were significantly more former employees above the

reference point for glucose, BUN, creatinine, and blood pressure. Current employees had significantly more employees above the reference point for cholesterol (≥ 200 mg/dL: 51% vs. 38%; ≥ 240 mg/dL: 12% vs. 6%), LDL (38% vs. 24%), and triglycerides (46% vs. 33%). There were significantly more current employees who reported drinking > 3 drinks per week and who were current smokers.

New Table: 4AAA (renumber subsequent tables if decide to keep)

Descriptive statistics for PFOA and PFOS by employee group and gender are provided in Table 4AAA. For PFOA, there was some variation in PFOA levels for both females and males. For females, mean PFOA was slightly higher for the former employee group, which contained the maximum PFOA value of 7.15 ppm. For males, mean PFOA was higher in the current employee group, which had the maximum PFOA value of 17.5 ppm. For PFOS, there was little variation in PFOS levels and the mean PFOS was only slightly higher in the current employee group for both females and males.

Presented in Tables 4-7 are the mean PFOA and PFOS levels by the binary result of specific survey questions or metabolic syndrome and employee group. This analysis was done separately for males and females. Due to the limited number of employees in some response groups, the analyses may lack sensitivity and should be interpreted cautiously. Among males, mean PFOA was significantly higher for current employees who answered "yes" to medical history of gall bladder disease compared to those who answered "no", however, the confidence interval for the "yes" group is very imprecise due to the small number of employees, one of which had the highest PFOA level of 17.5 ppm (Table 4). For the question regarding taking high blood pressure medications, there was a significant difference between the "yes" and the "no" group for all employees, however, the mean PFOA level of the "no" group was higher than the "yes" group. There were no statistically significant differences in the mean PFOS levels for males (Table 5). Due to the small overall number of females (n=55), some comparisons between the "yes" and "no" groups were not possible (when the responses were all "yes" or all "no"). Mean PFOA was significantly higher for all and former employees who answered "yes" to medical history of gall bladder disease compared to those who answered "no", however,

as with the males, these tests were likely influenced by the high PFOA value of 7.15 ppm in the former group (Table 6). Is this minimizing the fact that history of gall bladder had significance for both males and females? For employees who met the study definition of metabolic syndrome, mean PFOS was significantly higher for all and current employees compared to those employees who didn't meet the definition (Table 7). Due to n of 1 in yes group, didn't mention significant p for current employees taking high cholesterol meds. remove p-vals from table where n=1? Geary, Per your comment, I removed p-vals from tables 4-7 if only had n=1 in Yes or NO group.

Table 8 shows descriptive statistics for PFOA by PFOA quintile for all employees. There were no significant differences in mean PFOA values for quintiles 1 through 4, where mean PFOA ranged from quintile 1 of 0.005 ppm to quintile 4 of 0.133 ppm. The mean PFOA level for quintile 5 was 1.433 ppm, which was significantly different from quintiles 1 through 4. The corresponding median value for quintile 5 was 0.496 ppm, which, as mentioned earlier, suggests the distribution of PFOA was skewed to the right.

The number and percent of all employees by PFOA quintile and health factor or lab parameter are presented in Table 9. There was a significant difference in proportional distribution across quintiles by status. In the lower quintiles, there was a higher percent of former employees, but in the fifth quintile there was a shift to a higher percent of current employees. Due to this uneven distribution and from what was seen in Table 3 (significantly more current employees drank > 3 drinks per week), alcohol consumption is higher in the upper quintiles. According to the Cochran-Armitage Test for Trend, as the PFOA quintile increased, there was a significant increase in the proportion of employees above the reference point for cholesterol $\geq$ 200 mg/dL, LDL, triglycerides, and ALT. There was a significant decrease in the proportion of employees above the reference point for BUN and direct bilirubin (and almost significant for glucose) as PFOA quintile level increased.

Table 10 shows descriptive statistics for PFOS by PFOS quintile for all employees. Mean PFOS ranged from quintile 1 of 0.016 ppm (range 0.001-0.025 ppm) to quintile 5

of 0.302 ppm (range 0.132-1.500 ppm). Corresponding median values were 0.017 ppm to 0.195 ppm, respectively.

The number and percent of all employees by PFOS quintile and health factor or lab parameter are displayed in Table 11. There was a significant difference in proportional distribution across quintiles by status. In the lower quintiles, there was a higher percent of former employees, but in the fourth quintile there was an increase in the proportion of current employees and in the fifth quintile there was a balance (equal proportion of current and former employees). As with PFOA, alcohol consumption is higher in the upper quintiles, which correlated with the fact that there was a higher proportion of current employees in the fourth and fifth quintiles. 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 cholesterol $\geq$ 200 mg/dL, LDL, and triglycerides. There was a significant decrease in the proportion of employees above the reference point for glucose as PFOS quintile level increased.

Presented in Tables 12-15 are the non-adjusted and adjusted PFOA and PFOS coefficients for lab parameter result by employee group separately for males and females. The multivariate regression models were adjusted for age, BMI and alcohol. The unadjusted PFOA and PFOS coefficients in the univariate regressions were significant for one or more employee groups for several lab parameters, however, when age, BMI, and alcohol were included as covariates the adjusted PFOA and PFOS coefficients were not significant. The results presented here include the lab parameters where the adjusted PFOA or PFOS coefficient from the multivariate (say multiplicative model here instead to clarify looking at the adjusted model involving natural logarithms??) regression models were significant.

Geary, for the regression results below I focused on the last column of results for the adjusted coefficient in the multiplicative model (natural logs of PFOA and all covariates) so perhaps the above sentence needs to clarify that.

Table 12 presents non-adjusted and adjusted PFOA coefficients for lab parameter result by employee group for males. The adjusted PFOA coefficient in the multivariate model was significant for all and current employee groups (p=0.04 and p=0.006, respectively) for cholesterol, and explained < 1% of the cholesterol variance in the full model for all employees (R^2=.11) and 7% of the cholesterol variance in the full model for current employees (R^2=.09).

Ask Geary about the following and if it is something we may want to include here or in discussion: Also, for the median PFOA level of 0.20 ppm for current male employees (and using age=48, BMI=28.8, and alcohol=2 (medians for current employees)) the predicted value of cholesterol from the multivariate model was 196 mg/dL, which is within the reference range. [For PFOA=0.01, 8.01,17.5, predicted cholesterol was 180, 219, 224] Using the ln vs. ln models for these predicted values.

The adjusted PFOA coefficient was also significant (p=0.05) for current employees for LDL, and explained approximately 4% of the LDL variance in the full model (R^2=.05).

Non-adjusted and adjusted PFOS coefficients for lab parameter result for males are presented in Table 13. The adjusted PFOS coefficient in the multivariate model was significant for current employees for alkaline phosphatase (p=0.04), and explained approximately 4% of the variance of the response variable in the full model (R^2=.09).

Table 14 presents non-adjusted and adjusted PFOA coefficients for lab parameter result by employee group for females. The adjusted PFOA coefficient in the multivariate model was significant for current employees (p=0.03) for cholesterol, and explained 20% of the cholesterol variance in the full model (R^2=.73). The adjusted PFOA coefficient was also significant for current employees for triglycerides (p=0.01) and AST (p=0.03). The amount of triglyceride variance in the full model (R^2=.69) explained by PFOA was 32% and the amount of AST variance in the full model (R^2=.46) explained by PFOA was 40%. Mention the n here is only 14? Analyses also showed statistically negative associations for BUN, total bilirubin, and direct bilirubin.

Non-adjusted and adjusted PFOS coefficients for lab parameter result for females are presented in Table 15. The adjusted PFOS coefficient in the multivariate model was significant for current employees (p=0.02) for triglycerides, and explained 29% of the triglyceride variance in the full model (R^2=.65). Analyses also showed statistically negative associations for BUN, total bilirubin, and direct bilirubin.

Table 16 displays the number and percent of employees by employee group and self-reported high cholesterol medication status separately for males and females. Overall, there were 34% of males and 25% of females who reported they were taking high cholesterol medication.

New Table 16AAA?? Discuss with Geary and see if this table is more beneficial for the report than Table 16.

Presented in Tables 17-20 are the non-adjusted and adjusted PFOA and PFOS coefficients for the lipid response variables (cholesterol, LDL, HDL and triglycerides) stratified by self-reported high cholesterol medication status (currently taking high cholesterol medications = yes or no). Separate tables were done for males and females. Analyses for males and females who were taking high cholesterol medication did not result in any significant associations, therefore, the results presented here focus on the multivariate regression models for males and females who self-reported they were not taking high cholesterol medication.

Looking at the regression results for males (Table 17), the adjusted PFOA coefficients were significant for all and current employee groups (p=0.01 and p=0.002, respectively) for cholesterol, and for current employees for LDL (p=0.03). These results were similar to those from the analyses in Table 12 where all males were included, regardless of their cholesterol medication status. In addition, the adjusted PFOA coefficient was significant for all employees for triglycerides (p=0.04). The amount of variance of the lipid response variables explained by PFOA in these models ranged from < 2% to 11%.

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Table 18 shows the non-adjusted and adjusted PFOS coefficients for lipid parameters for males. The models including only those males who were not taking cholesterol lowering medications indicated a significant PFOS coefficient for all and current employee groups for cholesterol (p=0.01 and p=0.009, respectively) and LDL (p=0.02 and p=0.01, respectively). The amount of variance of the lipid response variables explained by PFOS in these models ranged from 2% to 8%.

Presented in Table 19 are the non-adjusted and adjusted PFOA coefficients for lipid parameters for females. Similar to Table 14 where all females were included in the analysis, regardless of their cholesterol medication status, the adjusted PFOA coefficients were significant for current employees for cholesterol (p=0.04) and triglycerides (p=0.006). The amount of cholesterol variance explained by PFOA was 21% and the amount of triglyceride variance explained by PFOA was 45%.

The adjusted PFOS coefficient was significant for current female employees (p=0.05) for triglycerides (Table 20). Again, this was similar to the multivariate regression result in Table 15 where all females were included in the analysis, regardless of cholesterol medication status. The amount of triglyceride variance explained by PFOS was 28%.

ADD text regarding the new tables for male predicted lipid parameter resuls based on the ln vs. ln regression models (Tables X1-X8) as well as the scatterplots. Is this the appropriate place to add this text?

Table 21 shows the number and percent of all males and males who self-reported they were not taking high cholesterol medication that had a PFOA level $\leq$ 1 ppm and > 1 ppm by employee group. There were only 25 total males and 21 males who were not taking cholesterol medication that had a PFOA level >1 ppm.

Presented in Tables 22 and 23 are the multivariate regression results for cholesterol for all males and males who were not taking cholesterol lowering medication and stratified by

PFOA level ($\leq$ 1 ppm and > 1 ppm. Due to the limited number of female employee participants, these analyses were only done for males.

Table 22 shows for PFOA level $\leq$ 1 ppm, the adjusted PFOA coefficient for current male employees not taking cholesterol lowering medications was significant (p=0.01) for cholesterol, and explained 9% of the cholesterol variance in the full model (R^2=.10). Is it right to say then that the association between cholesterol and PFOA isn't driven by those employees with high PFOA levels, the association exists even when PFOA is low? Note, predicted chol level is 206 when PFOA = .99.
For PFOA level > 1 ppm, analyses did not result in any significant associations (Table 23).

Presented in Appendix tables 1-4 are the non-adjusted and adjusted PFOA and PFOS coefficients for liver parameters (alkaline phosphatase, AST, ALT, and GGT) from the multivariate regression models adjusted for age, triglycerides (in place of BMI) and alcohol. Analyses were similar whether BMI was included as a covariate (Tables 12-15) or triglycerides were included as a covariate (Appendix 1-4).

µg/mL rather than ppm?