

FINAL REPORT

**Epidemiology
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3M Company
220-3W-05
St. Paul, MN 55144**

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Title: An Epidemiologic Investigation of Plasma Cholecystokinin and Hepatic Function in Perfluorooctanoic Acid Production Workers

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Principal Investigator: Geary W. Olsen, DVM, PhD¹

Co-investigators: Jean Burris, RN, MPH¹
 Michele M. Burlew, MS¹
 Jeffrey H. Mandel, MD, MPH¹

Study Director: Jeffrey H. Mandel, MD, MPH¹

1. Occupational Medicine, 3M Company, 220-3W-05, St. Paul, MN 55115

ABSTRACT

Perfluorooctanoic acid (PFOA) is a peroxisome proliferator which increased the incidence of pancreas acinar cell adenomas in rats. Recent research suggested that these tumors may be the consequence of a mild but sustained increase in cholecystokinin (CCK) as a consequence of hepatic cholestasis. In addition, an epidemiologic investigation had suggested that PFOA may modulate hepatic responses to obesity and alcohol consumption in these production workers.

To further assess these hypotheses, we conducted three cross-sectional analyses of the employees' serum PFOA levels and medical surveillance data collected in 1993 (n = 111), 1995 (n = 80) and 1997 (n = 74). Plasma CCK was only measured in 1997. Serum PFOA was measured by mass spectrophotometry methods and plasma CCK was assayed by radioimmunoassay. Mean serum PFOA levels, by year, were: 1993, mean = 5.0 ppm (range 0.0 - 80.0 ppm); 1995, mean 6.8 ppm (range 0.0 - 114.1 ppm); and 1997, mean = 6.4 ppm (range 0.1 - 81.3 ppm). CCK values (mean = 28.5 pg/ml, range 8.8-86.7 pg/ml) approximated the assay's reference range (up to 80 pg/ml) for a 12 hour fast. Employees' serum PFOA levels were not positively associated with either clinical hepatic toxicity as measured by various serum liver enzyme tests, cholestasis or elevated plasma CCK levels. Nor did serum PFOA levels modulate hepatic responses (e.g., liver enzymes and high density lipoprotein) to obesity and alcohol, respectively.

INTRODUCTION

Perfluorocarbons are structurally analogous to hydrocarbons, except the hydrogens are replaced by fluorine [Bryce, 1964] and may contain other elements such as oxygen, nitrogen and sulfur. Ammonium perfluorooctanoate is a potent synthetic surfactant used in industrial applications which rapidly dissociates in aqueous solution to perfluorooctanoic acid (PFOA, $C_7F_{15}CO_2H$).

In laboratory animals, PFOA and its salts are: 1) absorbed by ingestion, inhalation or dermal; 2) not metabolized; 3) distributed primarily in the plasma and liver of male rats and the liver, plasma and kidney in female rats ; and 4) eliminated in the male rat via feces and urine whereas in the female rat there is a greater rate in renal excretion [Griffith and Long, 1980; Ophaug and Singer, 1980; Hanhijarvi et al., 1982; 1987; Just et al., 1989; Kennedy 1985; Kennedy et al., 1986; Ylinen et al., 1990; Vanden Heuvel et al., 1991]. In rats, PFOA results in peroxisome proliferation, uncoupling of mitochondrial oxidative phosphorylation, altered lipid metabolism, hypolipidemia and an increased incidence of liver, Leydig cell and pancreas acinar cell adenomas [Griffith and Long 1980; Kennedy, 1985; Kennedy et al., 1986; Sibinski 1987; Haugom and Spydevold, 1992; Keller et al., 1992; Cook et al., 1992; 1994]. The induction of these tumors most likely occurs via nongenotoxic mechanisms because PFOA is not mutagenic [Griffith and Long, 1980; Biegel et al., 1995]. Causal mechanisms may include the role of oxidative stress in the liver tumors and increased estradiol levels, via induction of hepatic aromatase activity, in the development of Leydig cell tumors [Cook et al, 1992; 1994; Rao and Reddy, 1996]. The pancreas acinar adenomas were hypothesized to be a result of a mild but sustained

increase in cholecystokinin (CCK) levels secondary to hepatic cholestasis [Obourn et al., 1997]. CCK is released from the "M" cells in the duodenal mucosa in response to the presence of food, binds to receptors on the pancreas acinar cells and subsequently stimulates the release of pancreatic enzymes into the duodenum [Pandol, 1998]. It is controlled by a negative feedback cycle involving monitor peptide and trypsin. CCK has been shown, in some animal models, to produce pancreatic hypertrophy, hyperplasia and neoplasia [Longnecker, 1986; 1990; 1991 Pour et al, 1981; 1988].

Hepatic toxicity, hypolipidemia and abnormal hormone levels have not been observed in PFOA production workers [Ubel et al, 1980; Gilliland and Mandel, 1996; Olsen et al., 1998]. Gilliland and Mandel [1996] did report that PFOA may negatively modulate the effect alcohol has on high density lipoprotein (HDL) levels and exacerbate the effect that obesity has on liver enzyme tests. However, this workforce was not found to be at an increased mortality risk for liver cancer or liver disease [Gilliland and Mandel, 1993]. There were 4 pancreatic cancer deaths compared to 2 expected (Standardized Mortality Ratio 1.96, 95% Confidence Interval 0.53-5.01). One of these four pancreatic cancer deaths had worked in the building where PFOA is produced at this chemical plant.

The purpose of this epidemiologic investigation was to re-examine the workforce in this PFOA production plant in order to determine: 1) whether CCK levels are positively associated with serum PFOA levels among production employees; and 2) whether PFOA may modulate hepatic responses to obesity and alcohol.

METHODS

PFOA Production

PFOA production at this 3M plant began in 1947. PFOA, a white powder, is produced by an electrochemical process [Bryce, 1954]. Production involves a four-stage process: isolating and converting the chemical to a salt slurry, converting the slurry to a salt cake, drying the cake, and packaging. The greatest likelihood for exposure to PFOA occurred in the drying area although job history was not predictive of total serum fluorine levels (a surrogate for serum PFOA) [Gilliland and Mandel, 1996].

Subject Selection and Data Collection

Voluntary medical surveillance examinations were offered biennially (1993, 1995 and 1997) to the fluorochemical production workers. The total number of subjects, by year, who participated in these three cross-sectional investigations were: 1993 (n = 111); 1995 (n = 80); and 1997 (n = 74). Eligible voluntary participation rates among these production workers approximated 70 percent. There were 68 subjects in common for 1993 and 1995; 20 subjects in common between 1993 and 1997 (lower number due to employee turnover and re-assignments); and 17 subjects in common for all three years. Surveillance activities included a self-administered questionnaire, measurement of height, weight and pulmonary function, standard biochemical and urinalysis tests, PFOA determination and several male reproductive hormone assays. The hormone data were collected only in 1993 and 1995 and results have been reported elsewhere [Olsen et al., 1998]. Serum biochemical tests included: alkaline phosphatase, gamma glutamyl

transferase (GGT), serum glutamyl oxaloacetic transaminase (SGOT), serum glutamyl pyruvic transaminase (SGPT), total bilirubin, direct bilirubin, cholesterol, low-density lipoproteins (LDL), high-density lipoproteins (HDL), triglycerides, blood urea nitrogen (BUN), creatinine and glucose. Hematology tests included: hematocrit, hemoglobin, red blood cells (RBC), platelets and white blood cells (WBC). In 1997, employees' plasma CCK-33 levels were determined. CCK exists in various forms and lengths although sulfated CCK-33 (i.e., a 33 amino acid arrangement) appears to be the predominant form. Employees were required to have fasted for 12 hours prior to their venipuncture. One employee self-reported that he did not fast and thus he was excluded from the study. His CCK level was 123 pg/dl. This exclusion left 74 employees available for analysis in 1997.

Serum chemistries and hematology were evaluated at United Hospitals (St. Paul, Minnesota). Plasma CCK-33 was measured by direct radioimmunoassay by Inter Science Institute (Inglewood, California). Serum PFOA was determined by thermospray (1993 and 1995) and electrospray (1997) high performance liquid chromatography mass spectrometry methods [Johnson et al., 1996; Advanced Bioanalytical Services Inc., 1997].

Data Analysis

Simple and stratified analysis, Pearson correlation coefficients, analysis of variance (ANOVA), and ordinary multivariate regression were used to evaluate linear and nonlinear associations between PFOA and the biochemical parameters with adjustment for potential confounding variables [SAS, 1990]. For stratified analyses, employees were divided into four PFOA categories: 0 - <1 ppm, 1 - <10 ppm, 10 - <30 ppm, and ≥ 30 ppm in order to determine if an effect existed at the highest serum levels. These categories had been

previously used to examine associations between male reproductive hormones and PFOA among these workers in 1993 and 1995 [Olsen et al., 1998]. For multivariable regression evaluation, PFOA, age, body mass index (BMI), alcohol use, and cigarette use were examined as both categorical and continuous variables. Alcohol use was analyzed as less than 1 drink per day, ≥ 1 drink per day (with almost all subjects between 1-3 drinks/day), and non-response to the questionnaire item. Linear and nonlinear transformations of PFOA were used to test for associations. In particular, the multivariable models employed by Gilliland and Mandel [1996] were re-examined to determine whether PFOA has a modulating effect on obesity and alcohol consumption in regards to hepatic serum chemistries (SGOT and SGPT) and HDL, respectively.

RESULTS

Mean serum PFOA levels, by year, were: 1993, mean = 5.0 ppm (SD = 12.3, range 0.0 - 80.0 ppm); 1995, mean 6.8 ppm (SD = 16.0, range 0.0 - 114.1 ppm); and 1997, mean = 6.4 ppm (SD = 14.3, range 0.1 - 81.3 ppm). In 1997, the mean CCK value was 28.5 pg/ml (SD = 17.1 pg/ml, range 8.8-86.7 pg/ml). All but two CCK values were within the assay's reference range (up to 80 pg/ml). These two CCK values (80.5 pg/ml and 86.7 pg/ml) were from employees with 0.6 ppm and 5.6 ppm serum PFOA levels, respectively.

Serum PFOA levels were not consistently correlated with any of the potential confounding variables, serum chemistries or hematological parameters. The Pearson correlation coefficients (in parentheses) between PFOA and the variables for 1993, 1995 and 1997 respectively, were: age (-.22, -.14, .02); alcohol (.10, .18, .01), BMI (.10, .10,

-.01), cigarettes (.07, .11, -.02), alkaline phosphatase (.11, .14, -.07), SGOT (.12, -.01, .02), SGPT (.10, .04, .14), GGT (.07, -.01, -.05), total bilirubin (-.02, -.14, -.08), direct bilirubin (.01, -.32, -.04), cholesterol (.15, .14, .18), LDL (-.01, -.07, .11), HDL (-.11, -.19, .03) triglycerides (.17, .37, .11), glucose (-.08, .04, -.04), BUN (-.12, -.11, .05), creatinine (.07, .17, -.01), hematocrit (.22, .08, -.10), hemoglobin (.22, .11, -.11), RBC (.09, -.01, -.19), platelets (-.10, .04, .11) and WBC (-.01, .06, -.01). In 1997, the Pearson correlation coefficient for PFOA and CCK was -.20 ($p = .09$).

Table I provides the mean, standard deviation and range of the potential confounders, serum chemistries and hematologies by four levels of PFOA categorization (0-<1, 1-<10, 10-<30, and ≥ 30 ppm) for the three years (1993, 1995 and 1997) of medical surveillance examinations. The mean of the PFOA ppm categories differed significantly with each other and there were two orders of magnitude difference between the lowest and highest PFOA categories in each year. There were no statistically significant ($p < .05$) F values for any clinical chemistry test or hematological parameter examined for any of the three surveillance years. It should be noted that the mean CCK values were 50 percent lower among employees with serum PFOA values ≥ 10 ppm.

Figure 1 is a scatterplot of the relation between CCK (transformed via natural log) and PFOA. The linear regression equation was: $\ln \text{CCK} = 3.3 - 0.008 \text{ PFOA}$ (p value of PFOA coefficient = .07; r^2 of model = .04). We did not observe any significant differences in mean serum chemistry values for those employees with high CCK values (e.g., ≥ 40) compared to those with lower CCK values or for those subjects with high PFOA values (e.g., ≥ 10 ppm) compared to those with lower values. Use of multivariable regression

models (data not shown) continued to indicate a weak negative association between CCK and PFOA adjusting for potential confounding variables (e.g., age, body mass index, alcohol, cigarettes and clinical chemistry measures of hepatic function).

Based on the multivariable model used by Gilliland and Mandel [1996], Table II provides the change in HDL levels associated with a 10 ppm increase in serum PFOA levels among moderate drinkers (≥ 1 drink/day) compared to light drinkers (< 1 drink/day). Included in Table II is the change originally reported by Gilliland and Mandel [1996] in this workforce with their 1990 surveillance data. It should be noted, however, that their 1990 model was based on total serum organic fluorine measurements rather than serum PFOA levels. Unlike 1990, there was not a substantial modulation in HDL levels with increased PFOA serum levels among moderate drinkers.

Likewise, Table III presents the results of multivariable analyses, including those originally reported using the 1990 surveillance data [Gilliland and Mandel, 1996], regarding the potential modulating effect of PFOA on hepatic responses to obesity in the three subsequent surveillance years. Whereas SGPT levels increased considerably with a 10 ppm change in total serum organic fluorine when the BMI was ≥ 30 , this association was not observed in 1993, 1995 or 1997.

DISCUSSION

We observed a weak negative association between serum PFOA and plasma CCK among 74 workers engaged in the production of ammonium perfluorooctanoate. This finding was opposite that hypothesized based on the toxicological findings of Obour et al [1997] who fed diets to rats containing either 0 or 100 ppm of Wyeth-14,643, a potent

peroxisome proliferator, which causes the same triad of tumors, including pancreas acinar cell adenomas, as PFOA. After six months, the mean pancreatic weights of the treated rats were 17 percent above control animals ($p < .05$), mean plasma CCK levels were 44 percent higher ($p < .05$) and markers of cholestasis (total bile acids, alkaline phosphatase and bilirubin) were also significantly elevated. The clinical pathology data indicative of cholestasis were associated with alterations in bile flow and bile acid output. Obourn et al [1997] had also conducted *in vitro* experiments of both Wyeth-14,643 and PFOA which argued against other biological pathways known to elevate plasma CCK levels including CCK_A receptor agonism, trypsin inhibition and increased dietary fat content. Obourn et al [1997] concluded that chronic exposure to Wyeth-14,643 may induce pancreatic adenomas via a mild but sustained increase in CCK levels secondary to hepatic cholestasis.

We offer several explanations for the lack of a positive association between PFOA and CCK in our study. First, the primary set of biochemical and cellular events identified in rodents susceptible to the hepatocarcinogenic effects of peroxisome proliferators have not been identified in either liver biopsies from humans exposed to peroxisome proliferators or in *in vitro* studies with human hepatocytes; however, the peroxisome proliferator-activated receptor (PPAR- α) is expressed at very low levels in the human liver [Obourn et al., 1997; Cattley et al., 1998]. Consequently, an expert panel has recently opined that it is unlikely that peroxisome proliferators are carcinogenic to humans under anticipated conditions and levels of exposure, however, their carcinogenic potential cannot be ruled out under extreme conditions of exposure [Cattley et al., 1998]. Second, even if the mechanism existed in humans, the serum measurements in these production workers may have been too low to cause an effect. Third, CCK receptors appear to be

different between the rat and human. Recent studies indicate, that unlike the pancreas of the rat and dog, the human pancreas has no detectable CCK_A receptors and little to no mRNA for the receptor [Wank et al., 1994]. Human, cynomologus and rhesus monkeys lack specific binding sites for the selective CCK_A ligand ³[H]L-364,718 [Gavin et al., 1996, 1997]. Because the CCK receptor activity of the rat may be quite dissimilar to the human, a cynomologus monkey may be a more appropriate animal model to study the pancreatic pathophysiology consequence of exposure to PFOA in the human. Fourth, whether CCK initiates or promotes pancreatic cancer is not a new question and the research published, to date, remains controversial [Axelson et al., 1992]. Data from more than seventy laboratory animal studies have variably suggested that CCK has positive trophic effects, inhibitory effects, or no involvement in pancreatic tumor growth [Herrington and Adrian, 1995]. CCK has promoted growth of human pancreatic cancers in cell cultures [Palmer-Smith et al., 1991]. On the other hand, fasting plasma concentrations of CCK in unresected pancreatic cancer patients did not differ from healthy controls [Rehfeld et al., 1994]. Fifth, the rat may be an inappropriate model in the study of human pancreas carcinogenesis. Carcinogens in rats induce acinar cell malignancies which are rare in the human [Anderson et al., 1996]. Hamster pancreas cancer models are of ductal cell origin which resemble human pancreatic cancer (adenocarcinomas of the ductules) [Pour et al., 1981]. Activation of the c-K-ras gene is frequent in both human and hamster pancreatic cancer but is not found in azaserine-induced pancreatic cancer models in the rat [van Kranen et al., 1991; Caldas and Kern, 1995]. Finally, we must ask whether the weak negative association observed in our study represents an entirely different biological relationship than what was originally postulated based on the findings

by Obourm et al [1997]. We do not believe so because: 1) all CCK values observed in this study were within the assay's reference except for two values (which were not associated with high serum PFOA values); and 2) there was no suggestion of cholestasis which was considered the underlying reason for the elevated CCK levels in the rat.

We were unable to replicate in three separate years the original suggestion that PFOA may modulate hepatic responses to obesity and alcohol. Several explanations for the disparate findings exist. First, there may be an association that was not observed by us. In the original report [Gilliland and Mandel, 1990], total serum organic fluorine was used as a surrogate variable for PFOA exposure because the assay was less expensive and technically easier to perform at the time. The use of a total serum organic fluorine may represent other perfluorocarbons, which could be peroxisome proliferators; however, data suggest that PFOA would represent the greatest fraction of total serum organic fluorine levels in this employee population [Ubel, 1980]. Another explanation for the disparate BMI findings is that there may have been measurement error regarding body mass index in the original study or in our study. We have previously noted the lack of an expected positive association between BMI and estradiol in the 1990 data [Olsen et al., 1998]. In only one of the years was there the expected [Burns et al., 1997] strong positive correlation between BMI and SGPT values (1990, $r = .20$, $p = .02$; 1993, $r = .16$, $p = .09$; 1995, $r = .13$, $p = .27$; 1997, $r = .43$, $p = .0001$). Self-reported alcohol data collected in the occupational setting should also be questioned for its reliability as well as validity. To partially address the issue of reliability, we examined the analyses of the 68 employees who participated both in 1993 and 1995. The data showed good correlation for the confounding factors of BMI ($r = .94$, $p = .0001$), alcohol consumption ($r = .67$, $p = .0001$)

and cigarette smoking ($r = .84, .0001$). The few employees in common for all three years ($n = 17$) prevent any conclusions regarding the reliability of self-reported data across all three years. The trend in the correlations was comparable for the 1993 and 1995 analyses (e.g., 1995/1997 correlations were BMI: $r = .91, p < .0001$; alcohol $.37, r = .37, p < .15$; cigarettes, $r = .99, p < .0001$).

A few additional issues need to be considered in evaluating the results from this study. The cross-sectional design does not allow for a direct analysis of the temporality of an association. Given that the half-life of PFOA is estimated to be 18 to 24 months [Ubel et al., 1980], it is conceivable that there may be some biological accommodation to the effects of PFOA as suggested by Biegel et al [1995]. Also, there were fewer employees analyzed in 1995 and 1997 reducing the statistical power of the study although the number of subjects with serum PFOA measurements ≥ 10 ppm remained comparable. Finally, the issue remains that the lack of a clinical hepatotoxic effect observed by Gilliland and Mandel [1996] and ourselves does not negate the possibility that PFOA may have a subclinical effect in this production population that has yet to be observed. Results from additional laboratory animal studies may provide further insight.

In conclusion, our data do not suggest that, at the serum levels measured, PFOA is associated with a mild increase in plasma CCK levels. Neither the original epidemiological findings [Gilliland and Mandel, 1996] or our findings suggest clinical hepatic toxicity at the PFOA levels observed. The fact that we were unable to demonstrate, on three separate occasions, PFOA modulation of hepatic responses to obesity and alcohol, leads us to believe that this, too, is unlikely at the serum PFOA levels measured in this study.

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Table I. Mean Standard Deviation of Mean (SD) and Range of Perfluorooctanoic Acid (PFOA), Demographic, Clinical Chemistries & Hematology, by Serum PFOA Levels, and Year of Data Collection

PFOA ppm	1993 Data			1995 Data			1997 Data		
	Mean	SD	Range	Mean	SD	Range	Mean	SD	Range
<u>PFOA</u>									
0 - <1 ppm*	0.48 ¹	0.27	0.00-0.99	0.31 ²	0.32	0.00-0.90	0.47 ²	0.26	0.05-0.92
1 - <10 ppm	3.38 ¹	2.17	1.03-8.92	3.03 ²	1.84	1.10-8.20	3.13 ²	2.12	1.05-7.66
10 - <30 ppm	16.26 ¹	3.39	11.90-21.00	17.11 ¹	6.90	10.30-28.20	17.27 ¹	5.19	10.50-23.13
≥ 30 ppm	60.13 ¹	24.01	31.60-80.0	55.96 ¹	33.29	34.20-114.10	58.14 ¹	24.21	37.11-81.35
	<i>F value = 248.5, p = .0001</i>			<i>F value = 77.6, p = .0001</i>			<i>F value = 145.5, p = .0001</i>		
<u>Age</u>									
0 - <1 ppm	43	9.2	27-61	42	8.3	29-60	40	9.1	25-61
1 - <10 ppm	39	7.8	27-60	41	8.6	24-58	41	8.7	26-58
10 - <30 ppm	41	5.0	34-49	45	7.4	30-55	44	11.3	28-57
≥ 30 ppm	33	7.4	25-43	38	9.2	27-50	40	11.2	29-52
	<i>F value = 3.3, p = .02</i>			<i>F value = 0.9, p = .46</i>			<i>F value = 0.3, p = .84</i>		
<u>BMI</u>									
0 - <1 ppm	28.0	4.3	20.9-42.0	27.6	4.2	21.9-45.2	28.7	3.9	21.5-35.0
1 - <10 ppm	26.9	2.5	21.6-32.5	28.6	3.4	22.1-38.3	29.5	5.3	21.9-46.8
10 - <30 ppm	28.3	2.8	22.4-32.0	27.8	4.0	21.2-34.8	25.9	3.0	22.0-29.2
≥ 30 ppm	28.5	1.6	26.9-30.2	29.8	1.8	28.2-32.6	30.6	2.0	28.2-33.0
	<i>F value = 1.1, p = .33</i>			<i>F value = 0.8, p = .52</i>			<i>F value = 1.4, p = .24</i>		
<u>Alcohol</u>									
0 - <1 ppm	0.4	0.5	0.0-1.9	0.5	0.7	0.0-2.9	0.7	1.0	0.0-3.4
1 - <10 ppm	0.7	0.7	0.0-3.4	0.5	0.5	0.0-1.9	0.5	0.6	0.0-2.6
10 - <30 ppm	0.8	0.6	0.4-2.1	0.8	0.7	0.0-2.1	0.4	0.6	0.0-1.4
≥ 30 ppm	0.9	0.8	0.0-2.0	0.5	0.6	0.0-1.4	0.7	0.6	0.1-1.6
	<i>F value = 2.9, p = .04</i>			<i>F value = 0.9, p = .43</i>			<i>F value = 0.7, p = .58</i>		
<u>Cigarettes</u>									
0 - <1 ppm	2	7.2	0-30	4	9.4	0-40	3	7.7	0-30
1 - <10 ppm	6	10.1	0-40	3	6.0	0-20	7	10.4	0-30
10 - <30 ppm	6	11.3	0-30	9	15.2	0-40	9	16.4	0-40
≥ 30 ppm	5	10.0	0-20	6	8.9	0-20	0	-	-
	<i>F value = 1.3, p = .26</i>			<i>F value = 1.3, p = .29</i>			<i>F value = 1.7, p = .18</i>		
<u>CCK</u>									
0 - <1 ppm	Not done			Not done			33.4	15.7	13.4-80.5
1 - <10 ppm							28.0	19.2	8.8-86.7
10 - <30 ppm							15.7	4.1	11.4-23.0
≥ 30 ppm							20.6	7.2	12.9-29.9
							<i>F value = 2.5, p = .06</i>		

Table L
continued

PFOA ppm	1993 Data			1995 Data			1997 Data		
	Mean	SD	Range	Mean	SD	Range	Mean	SD	Range
<u>Alkaline Phosphatase</u>									
0 - <1 ppm	88	26	37-161	78	18	40-114	79	19	27-122
1 - <10 ppm	82	23	47-151	80	25	48-165	87	23	47-164
10 - <30 ppm	75	14	58-107	88	29	59-146	81	28	61-142
≥ 30 ppm	103	33	74-132	93	38	55-136	78	16	64-100
	<i>F value = 1.6, p = .19</i>			<i>F value = 1.0, p = .39</i>			<i>F value = 0.9, p = .45</i>		
<u>GGT</u>									
0 - <1 ppm	33	19	11-84	42	27	16-149	34	27	15-130
1 - <10 ppm	50	70	6-472	51	41	19-190	36	25	14-162
10 - <30 ppm	36	14	19-59	39	13	21-61	28	11	15-50
≥ 30 ppm	40	26	19-77	42	15	23-58	32	11	16-40
	<i>F value = 1.0, p = .41</i>			<i>F value = 0.6, p = .61</i>			<i>F value = 0.3, p = .91</i>		
<u>SGOT</u>									
0 - <1 ppm	23	7	11-60	21	6	13-36	26	7	13-41
1 - <10 ppm	26	11	12-83	24	13	13-75	25	7	14-48
10 - <30 ppm	23	4	16-28	21	4	15-29	24	2	22-28
≥ 30 ppm	28	6	23-35	20	4	15-25	27	5	22-34
	<i>F value = 1.1, p = .36</i>			<i>F value = 0.5, p = .66</i>			<i>F value = 0.3, p = .84</i>		
<u>SGPT</u>									
0 - <1 ppm	45	14	22-88	44	13	27-80	31	10	13-59
1 - <10 ppm	48	29	22-221	53	34	27-175	33	15	14-80
10 - <30 ppm	43	6	35-52	45	12	28-70	30	11	18-51
≥ 30 ppm	54	5	51-62	51	13	39-71	43	13	27-57
	<i>F value = 0.4, p = .75</i>			<i>F value = 0.9, p = .46</i>			<i>F value = 1.0, p = .42</i>		
<u>Total Bilirubin</u>									
0 - <1 ppm	0.63	0.28	0.20-1.30	0.84	0.32	0.40-2.20	0.84	0.48	0.40-2.30
1 - <10 ppm	0.58	0.22	0.20-1.30	0.75	0.26	0.40-1.30	0.79	0.42	0.30-2.40
10 - <30 ppm	0.53	0.17	0.20-0.80	0.67	0.21	0.40-1.00	0.66	0.33	0.30-1.20
≥ 30 ppm	0.65	0.13	0.50-0.80	0.64	0.17	0.50-0.90	0.73	0.22	0.40-0.90
	<i>F value = 0.7, p = .55</i>			<i>F value = 1.5, p = .22</i>			<i>F value = 0.4, p = .76</i>		
<u>Direct Bilirubin</u>									
0 - <1 ppm	0.18	0.07	0.10-0.40	0.22	0.04	0.20-0.30	0.10	0.10	0.00-0.60
1 - <10 ppm	0.17	0.07	0.10-0.30	0.22	0.05	0.10-0.30	0.09	0.05	0.00-0.20
10 - <30 ppm	0.18	0.07	0.10-0.30	0.19	0.06	0.10-0.30	0.09	0.04	0.00-0.10
≥ 30 ppm	0.18	0.10	0.10-0.30	0.18	0.04	0.10-0.20	0.08	0.05	0.00-0.10
	<i>F value = 0.1, p = .94</i>			<i>F value = 2.2, p = .10</i>			<i>F value = 0.2, p = .89</i>		

Table L
continued

PFOA ppm	1993 Data			1995 Data			1997 Data		
	Mean	SD	Range	Mean	SD	Range	Mean	SD	Range
<u>Cholesterol</u>									
0 - <1 ppm	215	44	136-297	207	37	115-284	199	30	129-257
1 - <10 ppm	219	39	155-309	212	36	143-284	213	41	121-315
10 - <30 ppm	230	38	171-305	225	45	132-288	228	57	164-334
≥ 30 ppm	236	42	175-268	214	36	181-257	230	21	212-258
<i>F value = 0.6, p = .65</i>			<i>F value = 0.6, p = .63</i>			<i>F value = 1.8, p = .16</i>			
<u>HDL</u>									
0 - <1 ppm	43	11	22-83	42	8	22-59	41	9	26-61
1 - <10 ppm	47	13	28-90	43	12	22-67	44	12	23-94
10 - <30 ppm	52	23	34-97	43	8	28-54	45	11	24-60
≥ 30 ppm	37	10	27-47	36	9	26-46	45	10	30-52
<i>F value = 2.0, p = .11</i>			<i>F value = 0.9, p = .46</i>			<i>F value = 0.6, p = .62</i>			
<u>LDL</u>									
0 - <1 ppm	138	40	27-227	131	32	31-191	114	28	40-158
1 - <10 ppm	143	38	72-223	133	40	28-210	134	44	26-253
10 - <30 ppm	144	37	65-185	134	46	62-211	135	46	79-206
≥ 30 ppm	131	57	60-188	121	20	107-157	132	24	110-166
<i>F value = 0.2, p = .91</i>			<i>F value = 0.2, p = .92</i>			<i>F value = 1.5, p = .22</i>			
<u>Triglycerides</u>									
0 - <1 ppm	171	124	37-636	170	93	57-371	219	116	53-445
1 - <10 ppm	205	408	47-2845	175	144	59-743	176	85	44-360
10 - <30 ppm	168	110	41-362	239	147	77-539	241	241	63-718
≥ 30 ppm	341	204	70-564	286	180	145-563	269	79	188-360
<i>F value = 0.5, p = .67</i>			<i>F value = 2.0, p = .13</i>			<i>F value = 1.4, p = .25</i>			
<u>BUN</u>									
0 - <1 ppm	15	4	9-25	15	4	8-24	16	3	9-22
1 - <10 ppm	14	4	5-22	15	4	6-26	15	4	7-24
10 - <30 ppm	14	4	7-17	16	4	6-20	17	4	11-24
≥ 30 ppm	13	1	11-14	13	2	12-16	16	6	9-23
<i>F value = 0.9, p = .44</i>			<i>F value = 0.4, p = .75</i>			<i>F value = 0.9, p = .44</i>			
<u>Creatinine</u>									
0 - <1 ppm	0.9	0.2	0.6-1.4	1.0	0.1	0.8-1.3	1.0	0.1	0.8-1.2
1 - <10 ppm	1.0	0.1	0.7-1.3	0.9	0.1	0.6-1.2	1.0	0.1	0.7-1.3
10 - <30 ppm	0.9	0.1	0.8-1.1	1.0	0.1	0.9-1.2	1.0	0.2	0.8-1.4
≥ 30 ppm	1.0	0.1	0.8-1.1	1.0	0.1	0.9-1.1	1.0	0.1	0.9-1.1
<i>F value = 0.5, p = .68</i>			<i>F value = 1.4, p = .25</i>			<i>F value = 1.1, p = .37</i>			

Table L
continued

PFOA ppm	1993 Data			1995 Data			1997 Data		
	Mean	SD	Range	Mean	SD	Range	Mean	SD	Range
<u>Glucose</u>									
0 - <1 ppm	89	8	75-115	91	14	75-148	92	16	63-153
1 - <10 ppm	89	32	66-288	91	11	74-128	100	38	71-255
10 - <30 ppm	84	10	67-97	88	12	77-116	84	5	76-90
≥ 30 ppm	83	9	71-90	97	10	86-111	90	12	72-97
	<i>F value = 0.2, p = .87</i>			<i>F value = 0.7, p = .59</i>			<i>F value = 0.9, p = .47</i>		
<u>Hematocrit</u>									
0 - <1 ppm	45	2	40-51	44	2	41-51	45	3	38-52
1 - <10 ppm	45	2	42-50	44	2	41-49	45	2	42-49
10 - <30 ppm	45	2	41-48	44	2	40-48	45	2	40-47
≥ 30 ppm	48	5	44-56	45	5	41-54	44	3	41-46
	<i>F value = 1.6, p = .20</i>			<i>F value = 0.6, p = .63</i>			<i>F value = 0.5, p = .66</i>		
<u>Hemoglobin</u>									
0 - <1 ppm	15.6	0.8	13.6-17.2	15.1	0.8	13.7-17.1	15.3	0.9	13.2-18.0
1 - <10 ppm	15.6	0.8	14.2-17.4	14.9	0.8	13.2-16.7	15.4	0.8	13.7-17.0
10 - <30 ppm	15.4	0.8	14.2-16.9	15.2	0.8	14.0-16.7	15.2	0.6	13.9-15.8
≥ 30 ppm	16.6	1.9	15.0-19.3	15.7	2.0	14.2-19.1	15.0	0.9	14.2-16.0
	<i>F value = 1.9, p = .14</i>			<i>F value = 1.0, p = 0.40</i>			<i>F value = 0.4, p = .79</i>		
<u>RBC</u>									
0 - <1 ppm	5.1	0.3	4.6-6.3	4.9	0.3	4.5-5.4	5.1	0.5	4.1-7.0
1 - <10 ppm	5.0	0.3	4.5-5.9	4.9	0.3	4.5-6.0	5.1	0.4	4.3-6.1
10 - <30 ppm	4.9	0.3	4.4-5.4	4.8	0.3	4.4-5.2	4.8	0.3	4.3-5.2
≥ 30 ppm	5.4	0.5	4.8-6.0	5.0	0.5	4.5-5.6	4.8	0.3	4.5-5.1
	<i>F value = 2.3, p = .08</i>			<i>F value = 0.4, p = .78</i>			<i>F value = 1.4, p = .25</i>		
<u>Platelets</u>									
0 - <1 ppm	240	51	141-374	223	49	162-327	234	32	162-288
1 - <10 ppm	241	48	156-370	236	46	163-337	250	55	129-378
10 - <30 ppm	249	85	185-466	230	48	180-339	237	44	172-308
≥ 30 ppm	213	30	189-255	217	40	179-267	260	36	215-300
	<i>F value = 0.5, p = .72</i>			<i>F value = 0.5, p = .72</i>			<i>F value = 1.0, p = .42</i>		

Table L
continued

PFOA ppm	1993 Data			1995 Data			1997 Data		
	Mean	SD	Range	Mean	SD	Range	Mean	SD	Range
<u>WBC</u>									
0 - <1 ppm	6.5	2.1	3.3-15.0	6.0	1.8	3.4-11.8	6.3	1.1	4.3-8.9
1 - <10 ppm	6.5	1.9	3.8-12.7	6.3	1.8	4.1-10.8	7.1	2.3	4.2-16.5
10 - <30 ppm	6.6	2.0	4.6-11.4	6.3	1.2	4.9-8.6	6.8	2.2	5.0-10.9
≥ 30 ppm	6.3	1.9	4.4-8.7	6.5	1.2	5.1-8.3	6.1	0.9	5.0-7.2
	<i>F value = 0.1, p = .99</i>			<i>F value = 0.3, p = .82</i>			<i>F value = 1.0, p = .39</i>		

1. Mean significantly different (Bonferroni t-test, $p < .05$) than each of the other PFOA ppm categories.
2. Mean significantly different (Bonferroni t-test, $p < .05$) than the 10-30 ppm and ≥ 30 ppm PFOA categories.

*Study Population by PFOA (ppm) Category and Year

PFOA Category	1993	1995	1997
0 - <1 ppm	52	39	29
1 - <10 ppm	46	26	34
10 - <30 ppm	9	10	7
≥ 30 ppm	4	5	4

Figure 1

LN(CCK) vs. PFOA

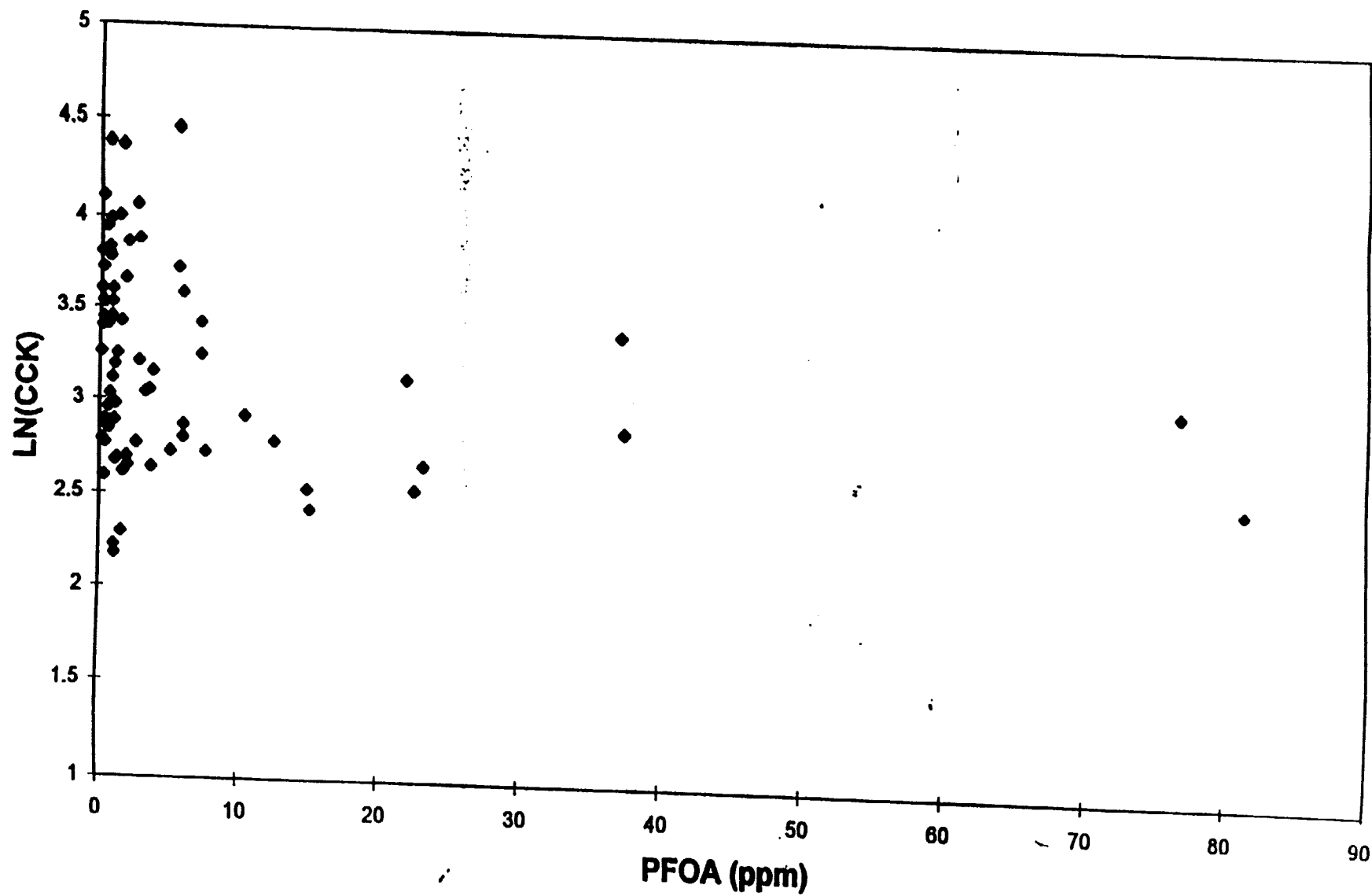


Table II. Change in HDL* from Light Alcohol Drinker (< 1 drink/day) to Moderate Alcohol Drinker ($\geq$ 1 drink/day) Associated with a 10 ppm Change in Serum PFOA Level

Year	Moderate Drinker	Moderate Drinker with 10 ppm increase in PFOA
1990 [#]	+9.9	-6.2
1993	+4.8	+3.4
1995	+5.1	+4.1
1997	+5.7	+3.8

*Determined from multivariable model adjusted for age, body mass index and smoking (all four years) and testosterone (1990, 1993 and 1995 only).

[#] Data in 1990 analyzed total serum organic fluorine (see Gilliland and Mandel, 1996).

Table III. Change in Serum Glutamic Oxaloacetic Transaminase and Serum Glutamic Pyruvic Transaminase Associated with a 10 ppm Change in Serum PFOA

BMI (kg/m²)	25	30	35
<u>SGOT</u>			
1990 [*]	-2.4	3.7	9.7
1993	1.3	0.5	-2.5
1995	-0.3	0.1	0.5
1997	2.1	0.1	-1.9
<u>SGPT</u>			
1990 [*]	-3.0	28.0	59.0
1993	2.5	1.5	0.5
1995	0.8	-1.0	-2.1
1997	5.3	0.7	-3.8

^{*}Determined from multivariable regression model adjusted for age, body mass index and smoking.

^{*} Data in 1990 analyzed total serum organic fluorine level (see Gilliland and Mandel, 1996).

PROTOCOL

Epidemiology
Medical Department
3M Company
220-3W-05
St. Paul, MN 55144

Date: September 3, 1997

Title: An Epidemiologic Investigation of Plasma Cholecystokinin and Hepatic Function in ,
Perfluorooctanoic Acid Production Workers

Study
Start Date: September 3, 1997

Protocol Number: EPI-0003

IRB Approval

Exempt Expedited
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Estimated Date of
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Principal Investigator:

Geary W. Olsen, DVM, PhD¹

Co-investigators:

Jean Burris, RN, MPH¹

Michele M. Burlew, MS¹

Jeffrey H. Mandel, MD, MPH¹

/

Study Director:

Jeffrey H. Mandel, MD, MPH¹

1. Occupational Medicine, 3M Company, 220-3W-05, St. Paul, MN 55115

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ABSTRACT

Two-year feeding studies in Crl:CD BR (CD) rats at a maximum amount of 300 ppm perfluorooctanoic acid (PFOA) showed, in addition to liver adenomas and Leydig cell adenomas, an increased incidence of pancreas acinar cell adenomas. However, PFOA was not found to be mutagenic; thus the induction of these tumors likely occurs via nongenotoxic mechanisms. Recent research has suggested that the pancreas adenomas are a secondary effect from elevated cholecystokinin (CCK) levels due to hepatic cholestasis. Increased plasma CCK levels has produced pancreatic hypertrophy, hyperplasia and neoplasia in some, but not all, animal models. A cynomolgus monkey is currently being used in a PFOA feeding study to further assess the relation with CCK because the rat pancreas CCK receptor may be quite dissimilar to that of the human and monkey.

Because the role of CCK in the promotion of pancreatic cancer remains quite controversial in laboratory animals, additional insight is warranted into any possible association between occupational exposure to PFOA and plasma CCK levels. Therefore, the purpose of this cross sectional epidemiological study design is to determine whether there is an association between plasma CCK-33 levels, as measured by radioimmunoassay, and serum PFOA levels among 3M Cottage Grove fluorochemical production workers. We will also examine hepatic enzymes, bilirubin and lipoproteins in relation to the employees' serum PFOA levels due to the toxicological issue regarding hepatic cholestasis.

INTRODUCTION

Fluorocarbons are compounds of fluorine, carbon and other elements such as oxygen, nitrogen and sulfur. Perfluorocarbons are structurally analogous to hydrocarbons, except the hydrogens are replaced by fluorine [Bryce, 1964]. In general, perfluorocarbons are inert and heat stable; thus they are often used in high temperature applications and make excellent insulators and surfactants. Synthesis has been accomplished by electrochemical fluorination, direct fluorination, teleomerization, and catalytic methods using high valence metals.

Although fluoride (inorganic ionic fluoride) was identified in human blood 140 years ago [Nickles, 1856], the presence of fluorine in a free ionic state as well as a covalently bound organic state was first reported in 1968 [Taves, 1968a; 1968b]. Guy [1972] subsequently identified perfluorooctanoic acid (PFOA, $C_7F_{15}CO_2H$) as a major component of the serum organic fluorine fraction. Ammonium perfluorooctanoate, a potent synthetic surfactant used in industrial applications, rapidly dissociates in aqueous solution to PFOA. Since Tave's and Guy's observations [Taves, 1968a; 1968b; Guy 1972], PFOA has been the subject of several toxicologic studies.

In laboratory animals, PFOA acid or its salts is absorbed by ingestion, inhalation or dermal exposure [Griffith and Long, 1980; Kennedy 1985; Kennedy et al., 1986]. PFOA is not metabolized [Ophaug and Singer, 1980; Ylinen et al., 1990; Vanden Heuvel et al., 1991; Kuslikis et al., 1992]. PFOA is distributed primarily in the plasma and liver of male rats and the liver, plasma and kidney in female [Vanden Heuvel et al., 1991]. The major route of elimination in the male rat is via urine and feces whereas in the female rat there is

a 10-fold greater rate in renal excretion [Vanden Heuvel et al., 1991; Hanhijarvi et al., 1982; 1987]. Castrated male rats treated with estradiol have PFOA urinary excretion rates similar to female rats [Ylinen et al., 1990; Vanden Heuvel et al., 1991].

Peroxisome proliferators, like PFOA, are a diverse class of chemicals that cause hepatic peroxisome proliferation and enzyme induction, liver hyperplasia and, in some instances, hepatocarcinogenesis in rats and mice [Ikeda et al., 1985; Pastoor et al., 1987; Sibinski 1987; Cook et al., 1992; Biegel et al., 1995; Liu et al., 1995; Lemberger et al., 1996]. Peroxisome proliferators bind to and activate peroxisome proliferator-activated receptors (PPAR) belonging to the superfamily of nuclear hormone receptors. Upon binding of a peroxisomal proliferator or other ligands, such as fatty acids, PPAR interacts with RXR, another nuclear hormone receptor activated by 9-CIS retinoic acid. This heterodimer binds to specific hormone recognition elements called peroxisome proliferator response elements (PPRE) in the promoter of target genes, resulting in the coordinated transactivation of a set of genes in peroxisomal, mitochondrial, microsomal and cytosolic cell compartments involved in lipid homeostasis.

Two-year feeding studies in Crl:CD BR (CD) rats at a maximum amount of 300 ppm PFOA showed, in addition to liver adenomas and Leydig cell adenomas [Sibinski 1987; Cook et al., 1994], an increased incidence of pancreas acinar cell adenomas [Cook et al., 1994]. PFOA was not found to be mutagenic [Griffith and Long, 1980]; thus the induction of these tumors most likely occurs via nongenotoxic mechanisms [Biegel et al., 1995]. Evidence strongly implicates the role of oxidative stress in liver tumor development for peroxisome proliferators [Rao and Reddy, 1996]. Cook et al [1994]

showed that the Leydig cell tumors are likely the result of increased estradiol levels due to induction of hepatic aromatase activity.

The pancreas tumors have been hypothesized to be a secondary consequence of PFOA's effect on the rat liver via cholestasis-induced increased plasma cholecystokinin (CCK) concentrations [Obourn et al., 1997a; 1997b]. Specifically, Obourn et al [1997a;1997b] examined the possible mechanisms for the pancreatic oncogenetic effects of two potent peroxisome proliferators: Wyeth 14,643 and ammonium perfluorooctanoate (C8). Both compounds *in vitro* failed to: 1) bind to the CCK-A receptor in a competition binding assay; and 2) inhibit trypsin in a continuous spectrophotometric assay. Rats fed 100 ppm Wyeth 14,643 for 60 days were found to have no pancreatic weight effects, increases in plasma CCK, acinar cell proliferation or increased fecal fat. However, rats fed 100 ppm Wyeth 14,643 for 3 and 6 months had increased pancreatic weight (6% and 17% above control, respectively), increased CCK plasma levels and acinar cell proliferation. Increased serum concentrations of serum bile acids, alkaline phosphatase and bilirubin suggested cholestasis and this was confirmed by measuring bile flow. Relative bile flow (relative to liver weight) was decreased at 6 months to 72% of the control group. Obourn et al concluded that Wyeth 14,643 (and also inferred for PFOA) causes liver effects, including cholestasis, that result in increased plasma CCK levels. Increased CCK levels have been shown in other animal models to produce pancreatic hypertrophy, hyperplasia and neoplasia [Longnecker, 1986; 1990; Pour et al, 1981].

Appendix A provides a literature review which offers a more detailed appreciation of the role CCK plays in pancreatic physiology, hypertrophy and neoplasia. Included in Appendix A are reviews of the following topics: 1) an overview of the anatomy and

histology of the pancreas; 2) pancreatic exocrine physiology as it relates primarily to CCK; 3) the epidemiology of pancreatic cancer; 4) animal models of pancreatic cancer; 5) CCK receptors and the pancreas; and 6) the role of CCK and pancreatic cancer. A very brief review of the information provided in Appendix A is summarized below.

The principal inorganic components of exocrine pancreatic secretions are water, sodium, potassium, chloride and bicarbonate [Pandol, 1993]. The principal organic constituents are proteins, primarily digestive enzymes, produced from the acinar cells. The enzymes are secreted into the pancreatic ductules in an inactive form with activation occurring in the intestinal lumen primarily by cleavage with trypsin. Regulation of enzyme secretions is controlled by both neuro- and humoral stimulation of the acinar cells. Receptors located on the basolateral surface of the acinar cells have been reported for CCK, acetylcholine, bombesin, substance P and vasoactive intestinal peptide (VIP) in the guinea pig, rat and mouse. However, CCK-A receptors were not found in human, cynomolgus and rhesus monkeys [Gavin et al., 1997a; 1997b]. The intestinal phase represents the most important aspect of pancreatic enzyme secretion which is mediated by both enteropancreatic vagovagal reflexes and hormones. Secretin is the major humoral mediator of ductal bicarbonate and water secretion whereas CCK is the major humoral mediator of meal-stimulated enzyme secretion. CCK is a potent regulator peptide that also stimulates gall bladder contraction, potentiates secretin-induced pancreatic bicarbonate secretion and slows gastric emptying time. CCK is also a major neurotransmitter in the brain. CCK exists in various forms and lengths although sulfated CCK-33 appear to be the predominant form. The octa peptide retains full activity of the 33 peptide molecule. CCK in the duodenum is inhibited by a negative feedback mechanism

involving primarily trypsin. Whether CCK initiates or promotes pancreatic cancer remains highly controversial. It is clear that administration of exogenous CCK or its analog cerulein does induce pancreatic hypertrophy and hyperplasia in several species as measured by increased DDN synthesis, DNA content, RNA content and glandular weight. Various methodologic approaches have examined the issue of whether CCK causes pancreatic neoplasia. These endeavors have included the administration of exogenous CCK, manipulation of endogenous CCK, administration of CCK receptor antagonists, measurement of CCK receptor binding activity and detection of CCK receptor mRNA. Data from more than seventy laboratory animal studies have provided mixed results [Herrington and Adrian, 1995]. CCK-8, CCK-9 and CCK-39 have promoted growth of human pancreatic cancers in cell culture in serum-free media [Palmer-Smith et al., 1991]. However, fasting plasma concentrations of CCK in unresected pancreatic cancer patients did not differ from healthy controls [Rehfeld et al., 1994; Adrian et al., 1994].

Because the CCK receptor activity of the rat may be quite dissimilar to the human [Gavin et al., 1997a;1997b], a cynomologus monkey may be a more appropriate animal model to use for a pancreatic pathophysiology consequence of exposure to PFOA in the human. In this regard, 3M, in conjunction with the APME, has initiated a minimum 3 month feeding study of PFOA to cynomologus monkeys. A primary study hypothesis is whether increased CCK levels and pancreatic hypertrophy are associated with serum PFOA levels in these cynomologus monkeys. Because the pancreatic effects (e.g., increased CCK levels, increased pancreas weights) in rats were not observed by Obourn et al [1997a;1997b] until the sixth study month, it is anticipated that at least the same

comparable time period, if not much more, will be needed to determine whether such an effect exists, if at all, in cynomologus monkeys.

Because the role of CCK with pancreatic cancer remains quite controversial in laboratory animals, further insight into any role exposure to PFOA may have in relation to CCK in humans should be considered. In this regard, we propose to examine, via a cross sectional epidemiological study design, the plasma CCK levels of 3M Cottage Grove fluorochemical production workers in relation to their serum PFOA levels. Due to the hypothesis that elevated levels of CCK in the rat are a result of cholestasis, we will also examine hepatic enzyme function, bilirubin and lipoproteins.

METHODS

Study Design and Population

The study is a cross-sectional design. Cottage Grove production have biennial medical surveillance examinations as specified in the 3M Medical Surveillance Protocol for fluorochemicals. Eligible employees are all building 15 employees (department numbers 3020 and 3060), building 6 employees (department number 3036) and building 3 employees (they will automatically be included as they are staffed from buildings 6 and 15). In addition, all plant engineering employees and craftworkers who service buildings 6 and 15 are eligible for the study (departments 0688 and 0690). It is anticipated that there will be a maximum of 100 employees eligible for the medical surveillance examination.

Data Collection

As specified by the 3M Medical Surveillance Protocol for fluorochemicals, the medical history, tests and physical examination requirements include: a medical questionnaire along with a self-administered special questionnaire designed for the fluorochemical production area (Appendix B), spirometry, serum chemistries, hematology, urinalysis, serum PFOA measurements, and the measurement of the employee's height, weight, blood pressure and pulse. These medical surveillance examinations are scheduled to take place between September 15, 1997 and October 31, 1997. Clinical laboratory evaluations will occur at United Hospitals (St. Paul, Minnesota). Serum PFOA levels will be evaluated by Dr. Jack Henion at the Advanced Bioanalytical Services Inc. (Ithaca, New York).

In addition to the above medical surveillance parameters, we will also draw 10 ml of blood for plasma CCK-33 measurements. CCK-33 will be measured by direct radioimmunoassay by Inter Science Institute (Inglewood, California). The Inter Science Institute's technical coordinator for this project will be Alan Kacena. The requirements for plasma CCK determination are a 10-12 hour fast prior to collection of the specimen. Antacid medication or medications affecting intestinal motility should also be discontinued, if possible, for at least 48 hours prior to collection. The Inter Science Institute will supply 3M a 10 ml EDTA collection tube which contains the special G.I. preservative. Trasylol. Plasma should be separated from the cells immediately after collection and then frozen in a plastic vial. Specimens should be shipped on dry ice to the laboratory. Specimens can be shipped in a batch sample.

The expected upper reference level of CCK at this laboratory is 80 pg/ml. The laboratory has provided the following data regarding quality assurance aspects of its CCK-33 radioimmunoassay.

Specificity - Cross reactivity was determined at the 50% inhibition of binding level.

<u>Compound</u>	<u>Cross-reactivity</u>
CCK	1.00
Gastrin	0.04
Secretin	0.02
Glucagon	<0.01
Insulin	<0.01
Vasoactive Intestinal Polypeptide	< 0.01
Gastric Inhibitory Polypeptide	< 0.01
Pancreatic Polypeptide	< 0.01
Motilin	< 0.01
Other compounds tested	< 0.01

Recovery - Specimens were spiked with a known quantity of CCK and measured to determine the amount of recovery.

<u>Specimen (pg/ml)</u>	<u>Amount added</u>	<u>Amount measured</u>	<u>Amount expected</u>	<u>Recovery (%)</u>
17	20	34	37	91.9
18	50	66	68	97.0
92	50	131	142	92.2

Intra-assay Variability - The mean, standard deviation and coefficient of variation for three controls assayed in one run are listed below.

<u>Mean (pg/ml)</u>	<u>Standard Deviation (pg/ml)</u>	<u>Coefficient of Variation (%)</u>
16	2.3	14.4
94	7.4	7.9
159	13.0	8.2

Inter-assay Variation - The mean, standard deviation, and coefficient of variation for three controls assayed in different runs are listed below.

Mean (pg/ml)	Standard Deviation (pg/ml)	Coefficient of Variation (%)
18	2.7	15.0
91	8.1	8.9
163	15.2	9.3

A consent form will be signed by all fluorochemical production employees who participate in the determination of their plasma CCK levels (Appendix C). In essence, the only additional item that will be collected beyond that already specified in the Medical Surveillance Protocol Manual for fluorochemical workers is the 10 ml of blood necessary for plasma CCK radioimmunoassay analysis.

Data Analysis

After consideration of normality of the data, stratified analyses, ANOVA, Pearson correlation coefficients, and linear multivariate regressions will be used to evaluate for positive or negative associations between PFOA and CCK-33, hepatic enzymes and lipoproteins. It is anticipated that the following PFOA levels will be used for the categorical analyses: 0 - < 1 ppm, 1 - < 10 ppm, 10 - < 30 ppm and $\geq$ 30 ppm. These levels were used in a previous study of reproductive hormone levels and PFOA among the same Cottage Grove fluorochemical production workforce [Olsen et al., 1997]. Four potential confounders will be considered in the analyses: alcohol consumption, cigarette smoking, body mass index and the employee's age. Study results will be tabulated and analyzed by packaged procedures in the SAS System [SAS, 1990].

DISCUSSION

The purpose of this epidemiologic investigation is to determine whether plasma CCK-33 levels are associated with serum PFOA levels among fluorochemical production employees at the 3M Cottage Grove plant. There are several strengths to the proposed study. First, although it is hypothesized that PFOA may cause CCK-induced pancreatic acinar adenomas in the rat, this model may be irrelevant as the histology and CCK-A receptor activity of the rat appears to be quite different than that observed for humans. Second, this study will measure plasma CCK and serum PFOA levels. This level of specificity of exposure is often missing in occupational epidemiology investigations. Qualitative/subjective data will only be used in the analysis of alcohol consumption and cigarette smoking. Third, the results of this study may have considerable importance in the interpretation of CCK-related data obtained from the cynomolgus monkey study. Although an infrequent occurrence, results from this epidemiologic investigation may direct further toxicological studies. Fourth, in addition to plasma CCK-33 levels, we will also examine hepatic enzymes, bilirubin and lipoprotein levels in relation to serum PFOA levels because cholestasis is the proposed mechanism for enhanced CCK levels in the rat.

Several methodological issues will need to be considered in evaluating the results from this study. First, the cross-sectional design does not allow for a direct analysis of the temporality of an association. Given the long-half life of PFOA, it is conceivable that there may be some biological accommodation to the effects of PFOA, as suggested by Biegel et al [1995]. Second, although there may be upwards of 80 employees eligible for CCK-33 determination, not all subjects may participate. Any refusals will decrease the

statistical power of the study and result in nonresponse bias. Third, there exists potential measurement error for the confounders of interest. Currently, alcohol consumption and cigarette usage are politically sensitive issues and thus the accuracy of these self-reported data must be questioned. Fourth, it is recommended that CCK measurements be done after a 10-12 hour fast. Employees will be reminded of this fact; however, whether they adhere to this remains-to-be-seen.

The protocol, any addenda to the protocol, data analyses, and a copy of the final report will undergo a Quality Assurance audit. Permanent records of all other data generated during the course of this study are subject to privacy and confidentiality considerations. All data gathered or generated including protocol addendum and the final report will be archived by the Medical Department, 3M Company, St. Paul, Minnesota.

Costs for routine medical surveillance and the epidemiologic analyses will be borne by the Medical Department. Costs for CCK-33 analysis will be covered by the Specialty Chemicals Division. Inter Science Laboratory has quoted \$120 per sample for CCK-33 radioimmunoassay based on an 100 person batch sample. Thus, costs should not exceed \$12,000 for the CCK-33 analyses.

It is estimated that data collection will be completed by November 15, 1997. Sample analyses should be completed by January 15, 1998. Data analysis and report writing will take approximately 3 months. Thus, a draft manuscript ready for review and approval should be available by April 1, 1998.

Upon completion of this study, results will be communicated to management, fluorochemical production employees and the technical community. A manuscript for

publication consideration in a scientific journal will be prepared and submitted for peer-review.

APPENDIX A

Pancreas Anatomy and Histology

The pancreas first appears embryologically at the fourth week of gestation [Ermak and Grendell, 1993]. The pancreas arises from a dorsal and ventral outpouching. The tail, body and part of the head of the pancreas are formed by the dorsal component. The remainder of the head of the pancreas develops from the ventral outpouching. In the adult, the pancreas measures 12 to 15 cm in length and weighs between 70 and 100 gm. The circulation of the pancreas is derived from branches of the celiac and superior mesenteric arteries. The venous drainage flows into the portal system. The visceral efferent innervation of the pancreas is through the vagi and splanchnic nerves via the hepatic and celiac plexuses, respectively.

The pancreas functions as both an exocrine and endocrine organ. The endocrine pancreas is located in the islets of Langerhans. Glucagon, insulin, somatostatin and pancreatic polypeptide are produced in the A, B, D and PP cells of the islets of Langerhans, respectively. B cells are the most abundant representing 50 to 80 percent of islet volume. The exocrine pancreas consists of countless acini with their accompanying ductules. The islets are intermixed with the acini. Acinar cells surrounding the islets of Langerhans appear to be morphologically and biochemically different from acini further removed from the islets. An acinus is a network of adjoining acinar cells which can take on a variety of shapes (e.g., spherical, tubular or irregular). The intercellular connection between acinar cells is the gap junction which functions as a pore to allow small molecules (500 to 1000 daltons) to pass between the cells. The acinar cells synthesize, store and secrete digestive enzymes into the lumen of the acinus. On an acinar cell's basolateral membrane are receptors for hormones and neurotransmitters that stimulate enzyme

secretion. The basal region (furthest away from the lumen of the acinus) of the acinar cell contains rough endoplasmic reticulum for protein synthesis and comprises about 20 percent of the cell volume. The apical region (nearest the lumen of the acinus) of the acinar cell contains zymogen granules which hold the digestive enzymes. The Golgi complex is located between the nucleus and zymogen granules. The lumen of the acinus is the origin of the secretory duct and contains centroacinar cells. The centroacinar cells function similarly to the ductule epithelial cells by secreting ions and water. The lumen of the acinus leads to intralobular ducts which eventually anastomose to create interlobular ducts. The interlobular ducts anastomose to form the main pancreatic duct. The primary function of the ductal system is to secrete an alkaline solution which aids in the transport of digestive enzymes to the lumen of the intestine. This alkalinity also raises the intralumen pH of the intestine necessary for digestive enzyme action.

Pancreas Physiology

The principal inorganic components of exocrine pancreatic secretions are water, sodium, potassium, chloride and bicarbonate [Pandol, 1993]. Smaller quantities of calcium, magnesium, zinc, phosphate and sulfate are also secreted. The purpose of these secretions is to aid in the delivery of digestive enzymes to the intestinal lumen and neutralize gastric acid emptied into the duodenum. These inorganic pancreatic secretions, produced primarily from the ductal system as the result of secretin stimulation in the upper intestinal mucosa, vary from 0.2 ml/min in the resting state to 4.0 ml/min during stimulation with the total daily volume equal to 2.5 liters. The bicarbonate concentrate varies between 25 mEq/L (low flow) to 120 mEq/L (high flow). Chloride concentrations

vary inversely. Secretin stimulates secretion by activating adenylate cyclase and increasing cyclic adenosine monophosphate (cAMP) which ultimately results in an exchange of chloride for bicarbonate ions in the lumen of the pancreatic ductule.

The principal organic constituents of the pancreas are proteins, primarily digestive enzymes, produced from the acinar cells [Pandol, 1993]. The major enzymes function in the following manner: digest starch and glycogen (amylase); hydrolyze triglycerides (lipase and phospholipase A₂) as well as cholesterol esters, lipid soluble vitamin esters, diglyceride and monoglyceride (carboxylesterase); and cleave peptide bonds (trypsin, chymotrypsin and elastase).

The concentration of protein in the pancreatic secretions depends on the output rate from the acinar cells. The enzymes are secreted into the pancreatic duct in an inactive precursor form because they could potentially digest the pancreas. Digestive enzymes in the acinar cell include: proteolytic enzymes (trypsinogen, chymotrypsinogen, proelastase, procarboxypeptidase A, procarboxypeptidase B); amylolytic enzyme (alpha amylase); lipolytic enzymes (lipase, pro-phospholipase A₂, carboxylesterase lipase), nucleases (deoxyribonuclease, ribonuclease) and other enzymes (pro-colipase and trypsin inhibitor).

Activation of the precursor form to the final enzyme occurs in the intestinal lumen.

Trypsinogen is activated to trypsin via the action of enterokinase. The trypsin then, in turn, activates other types of proenzymes to their final enzyme form (e.g.,

chymotrypsinogen, proelastase, procarboxypeptidases A and B, prophospholipase A₂ to chymotrypsin, elastase, carboxypeptidases A and B, and phospholipase A₂, respectively).

In addition to the proenzyme activation of these other enzymes, trypsin also activates the conversion of trypsinogen to trypsin.

The synthesis of these digestive enzymes occurs in the rough endoplasmic reticulum of the acinar cells. Once synthesized, these proteins are transported to the acinar cell's Golgi complex where further glycosylation and concentration occur. Finally the enzymes are transported to the zymogen granules via vesicles that cycle back and forth between the Golgi complex and the granules. It appears that each zymogen granule contains the entire complement of secretory enzymes although their concentrations may differ between granules. The percent cell volume that the zymogen granules represents can vary from 15 to 20 percent (fasted adult) to less than one percent after stimulation with a cholinergic drug or the gastrointestinal hormone, cholecystokinin. The enzymes are believed to be released from the zymogen granules by exocytosis (the fusion of the granule membrane with the apical cell membrane and subsequent release of the granule content into the ductule lumen). It is thought that different stimuli result in the selective secretion of specific enzymes from the acinar cells. Furthermore, the regulation of protein (enzyme) synthesis can be altered by dietary intake. Increased concentrations of amylase and decreased levels of chymotrypsinogen have been reported with carbohydrate-rich diets.

Studies on the regulation of enzyme secretion in humans are limited [Lu et al., 1989; Chey, 1991; Pandol, 1993]. The majority of information known, to date, is from animal models. Regulation of enzyme secretion is controlled by neuro- and humoral stimulation of the acinar cells. Receptors, located on the basolateral surface of the acinar cell, have been reported for cholecystokinin (CCK), acetylcholine, bombesin, substance P, vasoactive intestinal peptide (VIP) in the guinea pig, rat, and mouse. Depending on their mode of stimulus-secretion coupling, the receptors have been divided into two categories.

VIP and secretin increase cellular cAMP via activation of adenylate cyclase. An increase in cAMP mediates the secretory response. The acinar cell also contains receptors for CCK, acetylcholine, bombesin and substance P. Rather than stimulating cAMP, these compounds increase cellular metabolism of membrane phosphoinositides and calcium. A major effect of CCK, acetylcholine, bombesin and substance P is the mobilization and release of intracellular stores of calcium. These agonists also increase cellular cyclic guanosine monophosphate, arachidonate release from membrane phospholipids, and electrical membrane potential changes. The exact mechanisms by which these steps lead to enzyme secretion remain to be fully elucidated. There does appear to be a greater than additive response to the joint effects of agonists which alter cAMP (e.g., VIP and secretin) and calcium (e.g., CCK and acetylcholine).

Exocrine pancreatic secretion occurs during fasting (interdigestive) as well as after ingestion of a meal (digestive). The digestive state is divided into three phases: cephalic, gastric and intestinal. The cephalic and gastric phases are neuro-regulated whereas the intestinal phase is regulated by neurological and hormonal factors.

In the cephalic phase, increased pancreatic secretions of bicarbonate and digestive enzymes will occur after smelling, tasting, chewing and swallowing food with or without duodenal chyme or acidification. This is due to increased vagal tone which increases intrapancreatic postganglionic cholinergic input which subsequently stimulates the release of the bicarbonate and enzymes. These neurons in the pancreas are activated by central input during the cephalic phase and by vagovagal reflexes initiated by the stimulation during gastric and intestinal phases. Besides acetylcholine, there are other neurotransmitters in the pancreas that contain peptides, VIP, gastrin-releasing peptide,

CCK, neuropeptide Y, neuortensin, substance P, enkephalins, calcitonin gene related peptide and galanin. These peptides may coexist with nonpeptide transmitters in autonomic nerves and thus appear to play significant roles in the regulation of the exocrine pancreas.

The gastric phase of pancreatic secretion results from food stimuli in the stomach. The gastric stimuli cause primarily enzymatic release with minimum secretion of water and bicarbonate.

The intestinal phase represents the most important aspect of pancreatic enzyme secretion. The intestinal phase begins upon entry of chyme into the duodenum. The intestinal phase is mediated by both enteropancreatic vagovagal reflexes and hormones. There are two major mediators of these pancreatic enzymatic secretions: secretin and CCK. The type of pancreatic secretion depends on whether it is released by secretin or CCK. Secretin is the major mediator of ductal bicarbonate and water secretion. CCK is the major mediator of pancreatic enzyme secretion.

The release of secretin from the duodenal mucosa depends upon the acid load (minimum pH of 4.5) that is delivered to the duodenum. Fatty acids greater than eight carbons in length and bile acids are likely secondary stimulants for secretin release. Secretion of bicarbonates also occurs via cholinergic input. Although CCK by itself does not invoke bicarbonate release, it can augment secretin-induced bicarbonate secretion.

CCK is the major humoral mediator of meal-stimulated enzyme secretion. CCK is released from the 'I' (or sometimes called 'M') cells of the upper small intestinal mucosa by products from fat and protein digestion. Phenylalanine, valine, methionine and tryptophan are the most potent amino acids for CCK release (and subsequent pancreas

enzyme secretion). A low level of intestinal contents mediate pancreatic enzyme secretion by an enteropancreatic neural reflex whereas high loads of intestinal contents mediate enzyme secretion predominantly by the hormonal effects of CCK. The release of secretin and CCK is mediated by releasing peptides secreted from the intestinal mucosa. These releasing peptides are, in turn, inactivated in the upper small intestinal lumen by pancreatic proteases.

Specifically, the regulation of CCK occurs in the following manner: 1) chyme " enters the duodenum which results in the releasing peptides secreted from the intestinal mucosa which subsequently release CCK from the 'I' cells; 2) intestinal CCK is absorbed and travels, via the circulatory system, to the pancreas where it binds to receptors; 3) the activated receptors causes the release of pancreatic precursor enzymes from the acinar cells' zymogen granules into the pancreatic ductules which subsequently drain into pancreatic duct and then empties into the intestinal lumen; 4) the precursor enzymes are activated in the intestinal lumen where one of these enzymes, trypsin, assists in the digestion of protein; 5) upon cessation of eating, the concentration of free trypsin increases in the intestinal lumen with the decreased lumen contents; 6) this free trypsin inhibits two substances that act as stimuli for CCK release (monitor peptide and CCK-releasing factor); 7) this inhibition of monitor peptide and CCK-releasing factor subsequently results in the cessation of CCK release from the duodenal 'I' cells; and finally 8) a decreased CCK circulatory concentration results in less CCK binding activity at the acinar membrane which ultimately decreases pancreatic pro-enzyme secretions from the zymogen granules. Of course, this negative feedback cycle begins again with the next stimuli entering the duodenum (i.e., the next meal). It appears, albeit to a much lesser

extent, that circulating insulin, starch, glucose and calcium may also produce an pancreatic enzyme secretory response.

The evaluation of exocrine pancreatic function is accomplished by both direct and indirect tests. Because of its large functional reserve, malabsorption does not occur until CCK-stimulated digestive enzyme secretion is reduced to 10 percent of normal; thus most tests have low sensitivity to detect mild to moderate degrees of pancreatic insufficiency. The direct function tests are based on the measurement of enzyme and bicarbonate secretion upon duodenal intubation and stimulation of secretin and/or CCK. A variety of indirect tests are available which examine surrogates of either stimuli and/or the actual digestive enzyme secretions [Pandol, 1993].

Epidemiology of Pancreatic Cancer

Briefly, cancer of the pancreas is the ninth leading cause of cancer and the fourth leading cause of cancer death for men and women in the United States [Anderson et al., 1996]. Five-year survivorship is less than 5 percent. Pancreatic cancer occurs fifty percent more frequently in males than females and blacks than whites. Adenocarcinoma of the pancreatic ductules is the primary histologic type. The proportion of pancreatic cancer from acinar origin is estimated at 1 to 15 percent. Numerous pancreatic cancer case-control studies published over the last 15 years have failed to show any strong associations. The most consistent association is, on average, a two-fold increased risk with cigarette smoking. Several lines of epidemiologic evidence suggest diets high in animal fat and low in vegetable and fruits are weakly associated with pancreatic cancer.

No consistent associations have been reported for industry, occupation, or specific chemical exposures.

The most important discovery during the past 15 years has been the accumulation of data which shows that mutations in cellular proto-oncogenes and tumor suppressor genes are important events in pancreatic carcinogenesis [Caldas and Kern, 1995]. *K-ras* mutations are a frequent finding in adenocarcinomas of the pancreas in humans with the great majority of these mutations found in codon 12 of c-Kirsten *ras*. In fact, pancreatic cancer is the human tumor with the highest incidence of *ras* mutations. The *ras* gene family encodes proteins involved in cell growth and differentiation. Thus, point mutations in *ras* genes may play an important role in early pancreatic carcinogenesis. Other evidence suggest roles for mutations in other oncogenes (*myc*, *erbB-2*) and tumor suppressor genes (*apc*, *p53*). These findings support a genetic model of pancreatic tumorigenesis: ductal cells driven by almost universal mutation of a dominant oncogene, *K-ras* and deregulation of cell-cycle control. Whether multiple carcinogens may cause pancreatic cancer remains a plausible hypothesis. However, the degree of consistency in the mutations, transversions and transitions of nucleotides from G to T or G to A in codon 12 of *K-ras* may eventually point to only a few specific causes.

Animal Models of Pancreatic Cancer

There are two animal models of pancreatic cancer [Longnecker, 1990]. A number of nitroso compounds have been used to produce ductal pancreatic cancer in hamsters including N-nitrosobis(2-oxopropyl)amine (BOP), N-nitrosobis(2-hydroxypropyl)amine (BHP), and N-nitroso(2-hydroxypropyl)(2-oxopropyl)amine (HPOP) [Pour et al.,

1975;1981]. In the rat, azaserine (o-diazoacetyl-L-serine) is the most commonly used carcinogen [Longnecker, 1986]. However the hamster and rat models are of different cellular origin. Carcinogens in rats induce acinar cell malignancies which are rare in the human. Hamster models are of ductal cell origin and thus resemble human pancreatic cancer. Furthermore, the pattern of genetic mutations found in tumors from BOP-treated hamsters parallels that of human cancers. Activation of the c-K-ras gene is frequent in both human and hamster pancreatic cancer but is not found in azaserine-induced pancreatic cancer in the rat [van Kranen et al., 1991].

CCK Receptors, and the Pancreas

As discussed previously, CCK is a potent regulatory peptide that is the major stimulus for pancreatic enzyme secretion. CCK-33 refers to its 33 amino acid arrangement:

Lys-Ala-Pro-Ser-Gly-Arg-Met-Ser-Ile-Val-
Lys-Asn-Leu-Gln-Asn-Leu-Asp-Pro-Ser-His-
Arg-Ile-Ser-Asp-Arg-Asp-Tyr-Met-Gly-Trp-
Met-Asp-Phe

where Ala = alanine
Arg = arginine
Asn = asparagine
Asp = aspartic acid
Gln = glutamine
Gly = glycine
His = histidine
Ile = isoleucine
Leu = leucine
Lys = lysine
Met = methionine
Phe = phenylalanine
Pro = proline
Ser = serine
Trp = tryptophan
Tyr = tyrosine
Val = valine

CCK stimulates gallbladder contraction, potentiates secretin-induced pancreatic bicarbonate secretion, and slows gastric emptying. CCK also exists in various forms and lengths from metabolites CCK-4 to a pro-CCK peptide, CCK-58. The octapeptide retains full activity of the 33 peptide molecule. Sulphated CCK-8 and CCK-33 appear to be the predominant forms. CCK acts through more than one type of receptor. In animals, CCK-A receptors are found on pancreatic acinar cells, smooth muscles, vagal afferent fibers and some central neurons. CCK-A receptors have a high affinity for sulphated

CCK-8 and recognize gastrin poorly. On the other hand, CCK-B receptors are abundantly found in the central nervous system and on gastric glands and recognizes both sulfated and nonsulfated CCK-8 and gastrin. Because circulating levels of gastrin are appreciably higher than those of CCK, the CCK-B receptors are usually activated by gastrin leaving the CCK-A receptors as the predominant form for CCK binding.

CCK-A and CCK-B receptors are not consistently found in all species. Recent studies indicate, that unlike rat and dog pancreas, the human pancreas has no detectable // CCK-A receptors and little to no mRNA for the receptor [Wank et al., 1994]. In particular, human, cynomolgus and rhesus monkeys lack specific binding sites for the selective CCK-A ligand ^3H L-364,718 [Gavin et al., 1997]. In contrast, the baboon pancreas exhibited a significant number of specific CCK-A bindings sites although not as much as the rat or guinea pig pancreas.

As also stated previously, CCK is inhibited by a negative feedback mechanism involving primarily trypsin: lack of trypsin in the duodenum and jejunum results in CCK release, whereas the presence of free trypsin inhibits CCK release. Thus, any interference with this feedback mechanism by orally administered trypsin inhibitors will result in continued pancreatic secretion via the release of CCK. These trophic effects of CCK have been demonstrated in both rats and hamsters [Pour et al., 1981; Longnecker, 1990].

The Role of CCK and Pancreatic Cancer

Whether CCK initiates or promotes pancreatic cancer is not a new question and the research published, to date, remains highly controversial with elusive answers [Axelson et al., 1992; Herrington and Adrian, 1995]. What is clear is that administration of exogenous CCK or its analog cerulein does induce pancreatic hypertrophy and hyperplasia in several species as measured by increased DNA synthesis, DNA content, RNA content, total protein content and glandular weight [Mainz et al., 1973; Petersen et al., 1978; Zucker et al., 1989]. Increased endogenous plasma CCK levels, via pancreaticobiliary diversion (thought to inhibit the negative feedback regulation of CCK release), have also resulted in pancreatic hypertrophy and hyperplasia [Gasslander et al., 1990]. Furthermore, administration of a CCK-A receptor antagonist will abolish or markedly reduce the pancreatic trophic response of CCK. Feeding rats or hamsters a high-fat or high-protein diet induces pancreatic hypertrophy and hyperplasia [Longnecker et al., 1990].

Various methodologic approaches have examined the issue of whether CCK causes pancreatic neoplasia, not just hypertrophy and hyperplasia. These research endeavors have included the administration of exogenous CCK, manipulation of endogenous CCK, administration of CCK receptor antagonists, measurement of CCK receptor binding activity, and detection of CCK receptor mRNA. Herrington and Adrian [1995] have summarized this body of research by stating, "in spite of extensive research, the role of CCK in pancreatic cancer is still very unclear, and the subject is highly controversial. Data from more than seventy studies have variably suggested that CCK has no positive trophic effects, inhibitory effects, or no involvement in pancreatic tumor growth."

Provided below for illustrative purposes are five examples offered by Herrington

and Adrian [1995] regarding the controversial issues surrounding CCK and pancreatic cancer.

- 1.) Several *in vivo* experiments with exogenous CCK or cerulein (a CCK analog derived from frog skin) enhanced the development of pancreatic cancer in hamsters treated with carcinogens [Howatson and Carter, 1985; Satake et al., 1986; Pour et al., 1988] yet other hamster studies showed CCK reduced the incidence of pancreatic carcinomas in hamsters or produced no effect [Pour et al., 1988, Johnson et al., 1988].
- 2.) Endogenous CCK levels have been associated with pancreatic tumors. For example, pancreaticobiliary diversion enhanced azaserine-treated pancreatic cancer development in rats [Stewart et al., 1991]. Long-term feeding of raw soya flour to rats, which contains protease inhibitors, produced pancreas acinar adenomas and adenocarcinomas [McGuinness et al., 1980]. However, feeding raw soya flour or synthetic protease inhibitors to hamsters did not result in increased pancreatic tumors even though plasma CCK levels were increased [Herrington et al., 1994].
- 3.) CCK receptor antagonists have been used to investigate the role of CCK, exogenously or endogenously, in pancreatic cancer. These antagonists include glutamic acid derivatives (proglumide, lorglumide, loxiglumide) and nonpeptide compounds (asperlicin, devazepide, L365,260). The results of these studies varied considerably depending upon animal model, the timing of the administration of the antagonist in relation to administration of the carcinogen, dosage administered and cultured cells. Studies of the CCK receptor antagonist devazepide in patients with pancreatic cancer have not shown significant effects on survival [Abbruzzese et al., 1992].
- 4.) CCK binding was consistently demonstrated in whole cells and membranes from acinar cell tumors in the rat azaserine model. However, specific CCK receptors have not been demonstrated on intact, cultured human tumor cells of ductal cell origin [Adrian et al., 1994]. Nevertheless, CCK binding has been demonstrated in membrane fractions from several different pancreatic cancer cell lines [Singh et al 1986; Smith et al., 1994] but this significance is uncertain when receptors cannot be demonstrated on the cell surface [Herrington and Adrian, 1995].
- 5.) CCK (CCK-8, CCK-9 and CCK-39) promoted growth of pancreatic cancer in cell culture in serum-free media [Palmer-Smith et al., 1991]. On the other hand, fasting plasma concentrations of CCK in unresected pancreatic cancer patients did not differ from levels in healthy controls or in patients with colon or lung cancer [Rehfeld et al.,

1994;Adrian et al., 1994]. CCK levels did decline in pancreatic cancer patients after pancreatoduodenectomy due to the loss of CCK-producing I cells located in the duodenum [Adrian et al., 1994].

In conclusion, CCK likely promotes pancreatic tumors in some animals but there is no evidence that it is an inducer of pancreatic tumors. Although CCK receptors have not been extensively investigated in the normal human pancreas, there may well be a different pattern of receptor expression in humans than other species. If true, then results from laboratory animals, in particular the azaserine-rat and nitrosamine-hamster models, may be of minimum applicability to understanding CCK and its relation to abnormal pancreatic function in humans.

APPENDIX B

3M Health Questionnaire

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Last Name	First Name	Middle Name	Employee Number	Today's Date	Date of Hire

Directions: There are three parts to this questionnaire. Read the directions to each part of the questionnaire. Answer the questions in sequence starting with Part I. Circle your response on the questionnaire. For questions that require a fill in the blank response, please provide your answer on the space provided on the questionnaire. Erase cleanly any answer you wish to change. We greatly appreciate your assistance in completing this questionnaire.

Part I General Work History

- Have you ever worked in the Chemical Division?
 A. Yes If 'yes', how many years did you work there? _____
 B. No What year did you start working in the Chemical Division? _____

Part II Tobacco Smoking

- Do you smoke cigarettes now? (as of one month ago)
 A. Yes
 B. No
- Have you smoked at least 100 cigarettes during your entire life?
 A. Yes
 B. No (If 'no', please go to question 25)
- How old were you when you first started regular cigarette smoking: _____
- How many years have you smoked cigarettes? (Do not count the time when you periodically stopped smoking) _____
- On average, how many cigarettes do you smoke per day now? _____
- On average, the entire time you smoked, how many cigarettes did you smoke per day? _____
- If you stopped smoking cigarettes completely, how old were you when you stopped? _____

Alcohol

- Do you now, or did you ever drink at least 12 alcoholic beverages in a one year period of time? (1 alcohol beverage = 1 beer, 1 glass of wine; or 1 shot of hard liquor)
 A. Yes
 B. No (If no, please go to Part VI)
- Age when you first began drinking alcoholic beverages: _____
- Do you currently drink alcoholic beverages?
 A. Yes
 B. No If 'no', how long ago did you stop (months, years) _____
- When you were drinking alcoholic beverages how drinks per week did you drink? _____
- On the average, how many alcoholic beverages per week do you currently drink? _____

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Part III

Directions: The next set of questions are about your health. The information you provide will be kept strictly confidential. We would like to know if a doctor or other health care professional has ever told you that you have any of the medical problems listed below. We need to know the diagnosis, the year you were first diagnosed and the name of the doctor who saw you for this medical problem. Please mark each question.

A. Cancer

Type of Cancer	Yes	No	If Yes, year you were first diagnosed	Name and address of Doctor
Bone				
Thyroid				
Lymphoma (Non-Hodgkins)				
Hodgkins				
Leukemia				
Multiple Myeloma				
Lung				
Colon				
Pancreas				
Liver				
Kidney				
Bladder				
Testicular				
Prostate				
Breast				
Ovarian				
Uterine				
Other Cancers				
Identify the cancer types				

B. Liver Disease

Diagnosis	Yes	No	If, Yes, year you were first diagnosed	Name and address of Doctor who diagnosed you with this condition
Hepatitis A (Infectious hepatitis)				
Hepatitis B (Serum hepatitis)				
Hepatitis caused by medications				
Cirrhosis of the liver				
Abnormal liver tests				
Other liver conditions				
Identify other liver conditions				

C. Problems with Immune System or Endocrine (Hormone) System

Diagnosis	Yes	No	If Yes, year you were first diagnosed	Name and address of doctor who diagnosed you with this condition
Low gamma globulin				
Autoimmune disorders (Rheumatoid arthritis, lupus, scleroderma)				
Hyperthyroidism (overactive thyroid)				
Hypothyroidism (underactive thyroid)				
Benign prostatic hypertrophy (prostatic enlargement)				
Osteoporosis				
Diabetes				
Other conditions				
Identify other conditions				

For Nursing use only:

Height in inches,
without shoes

Weight in pounds

Blood Pressure

Urinalysis
Blood
Sugar
Albumin

Please staple pulmonary function test results to the back of this page.

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APPENDIX C

CONSENT FORM

Title of Study Protocol

An Epidemiologic Investigation of Cholecystokinin and Hepatic Function
among Perfluorooctanoic Acid Production Workers

Introduction

You and your colleagues involved in fluorochemical production at Cottage Grove are being invited to participate in a research study. Please review this consent form carefully and be sure your questions are answered before you make a decision to participate. The nurse and/or physician can provide you additional information at the time of your medical surveillance examination.

Purpose of Study

The purpose of this study is to conduct the routine medical surveillance exam that is offered to you every two years. In addition to this exam we will also check your blood cholecystokinin (CCK) and FC-143 levels. CCK is a hormone that is necessary in pancreatic enzyme production. The information gained from this study will further help us understand any human exposure to FC-143.

Study Procedures

If you choose to participate we will be asking that you fill out the Health Questionnaire and provide blood samples for laboratory testing. The exam will consist of a medical surveillance form, blood pressure, height, weight and lung function testing. Laboratory testing will include hematology: complete blood count and differential, platelet count; and blood chemistries: total, HDL and LDL, cholesterol, triglycerides, glucose (blood sugar), liver and kidney function tests and a pancreas function test. In addition we will measure for FC-143 in your blood. The blood can be drawn with one needle stick and will require approximately 4 tubes. *Please remember to fast for at least 12 hours prior to the test. Also, please do not use, if at all possible, any antacid medications for 48 hours prior to the test.* Scheduling will be coordinated by the Medical Department through the coordinator for each division.

In the questionnaire you will be asked if you have specific medical diseases. For certain problems, we need to be absolutely sure that your listed condition actually exists. For that reason we may be getting in touch with you to ask for your permission to contact your physician. This would be ONLY about the medical condition of interest to us.

Potential Risks/Discomforts

The only discomfort you may feel is from the needle stick. You may also have some temporary redness and/or slight swelling in this area after the blood collection.

Benefits

There will be no direct benefit from your participation in this study. However, the information gained from this study will further help us understand any human exposure to FC-143. Your individual results as well as the overall results of this study will be communicated to you.

Compensation/Medical Treatment From Related Illness or Injury

If you suffer injury or a medical condition that appears to be the result of participating in this study, you will be directed to the 3M Medical Department for observation and diagnosis. At your request or at the recommendation of the 3M Medical Department, you will be referred to another health care professional at no cost to you. In the event of a research related injury, compensation will be determined on a case by case basis by 3M. The contact for medical compensation is Jeff Mandel, M.D., 733-8670 of the 3M Medical Department.

Confidentiality

The information you provide on the questionnaire, or any information provided to us, will be kept strictly confidential and will be used for group analysis only. The data collected in this study may be used in publications or public presentations. Your name will not be revealed in any publication or other documents intended for publication examination.

You will be given feedback about your individual results as has been done in the past. The periodic health examination is not a substitute for an examination by your doctor. We encourage you to share the results of your tests with your private doctor.

Subject Rights/Availability of Information

If you have any questions about the study now, or later, or in the event of a research related injury or emergency, contact Dr. Jeff Mandel (733-8670) or Jean Burris, R.N. (737-7867). For answers to questions about your rights in regard to this research, you may contact Dr. Larry R. Zobel, Chair, 3M Institutional Review Board at 733-5181.

Voluntary Participation and Withdrawal

Participation in this study is voluntary. Refusal to participate will involve no penalty or loss of benefits to which you are otherwise entitled. You are free to withdraw at any time for any reason. Your decision to participate or withdraw from this study will not affect your work status or performance appraisals. The investigator may stop your participation in this study at any time if it is determined that your continued participation would be detrimental to your health or if the study objectives are changed.

Subject Consent

By signing this consent form, I certify that I am at least 18 years old. I confirm that I have read this consent form, and that I have been given adequate opportunity to ask any questions I may have about this consent form or about the Study. I also confirm that I understand the scope of my participation in this Study, and that all of my questions have been answered to my satisfaction. I am signing this consent form voluntarily, and I desire to participate in this Study. I understand that I am not waiving or releasing any of my legal rights by signing this consent form, or by participation in this Study. I understand that I will receive a copy of this signed consent form.

Signature and Date

3M Employee Number

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