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8. Plasma Cholecystokinin and Hepatic Enzymes, Cholesterol and Lipoproteins in Ammonium Perfluorooctanoate Production Workers.

Toxicology research suggested, to a limited extent, that the incidence of pancreas acinar cell adenomas in rats fed perfluorooctanoic acid may be the result of a mild but sustained increase in cholecystokinin (CCK) as a consequence of hepatic cholestasis. To assess this hypothesis, plasma CCK levels were measured by radioimmunoassay for those Cottage Grove employees participating in the 1997 fluorochemical surveillance examinations (n = 84). The mean serum PFOA level, as measured by high performance liquid chromatography mass spectrometry methods, was 6.8 ppm (median 1.3 ppm, range 0.1 - 81.3 ppm). CCK values approximated the assay's reference range for a 12 hour fast and were negatively, not positively, associated with employees' serum PFOA levels.

In addition to the CCK analyses, medical surveillance examinations were reviewed for the 1993, 1995 and 1997 time periods to assess the initial hypothesis generated from the 1990 fluorochemical medical surveillance data from this Cottage Grove workforce that PFOA (measured as total organic fluorine) may modulate hepatic responses to obesity and alcohol consumption (see studies # 5 and #7). In these three subsequent time periods, PFOA, assayed by mass spectrometry, did not appear to modulate hepatic responses to either obesity or alcohol consumption. Regardless of the surveillance year, there was no indication of significant clinical hepatic toxicity at the PFOA levels

observed in this workforce. The study protocol for the assessment of cholecystokinin levels, the internal 3M Final Report and a manuscript that has been accepted for publication (Drug and Chemical Toxicology) are submitted. [Note: the PFOA category levels for the univariate analyses in the publication manuscript (0 - < 1 ppm, 1 - < 10 ppm, ≥ 10 ppm) are different than those reported in the 3M final report (0 - < 1 ppm, 1 - < 10 ppm, 10 - < 30 ppm, ≥ 30 ppm). This change in the publication manuscript was done per a request made during the peer review process. This difference in categories did not affect the results or conclusions.]

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PLASMA CHOLECYSTOKININ AND HEPATIC ENZYMES, CHOLESTEROL AND LIPOPROTEINS IN AMMONIUM PERFLUOROOCTANOATE PRODUCTION WORKERS

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ABSTRACT

Ammonium perfluorooctanoate is a potent synthetic surfactant used in industrial applications. It rapidly dissociates in biologic media to perfluorooctanoate [$CF_3(CF_2)_6CO_2^-$], which is the anion of perfluorooctanoic acid [PFOA, $CF_3(CF_2)_6COOH$]. PFOA is a peroxisome proliferator known to increase the incidence of hepatic, pancreas and Leydig cell adenomas in rats. The pancreas acinar cell adenomas may be the consequence of a mild but sustained increase of cholecystokinin as a result of hepatic cholestasis. Although no significant clinical hepatic toxicity was observed, PFOA was reported to have modulated hepatic responses to obesity and alcohol consumption among production workers. To further assess these hypotheses, we examined medical surveillance data of male workers involved in ammonium perfluorooctanoate production in 1993 (n = 111), 1995 (n = 80) and 1997 (n = 74). Serum PFOA was measured by high-performance liquid chromatography mass spectrometry methods. Plasma cholecystokinin was measured (only in 1997) by the use of direct radioimmunoassay. Serum biochemical tests included hepatic enzymes, cholesterol and lipoproteins. Serum PFOA levels, by year, were: 1993 (mean 5.0 ppm, SD 12.2, median 1.1 ppm, range 0.0 - 80.0 ppm); 1995 (mean 6.8 ppm, SD 16.0, median 1.2 ppm, range 0.0 - 114.1 ppm); and 1997 (mean 6.4 ppm, SD 14.3, median 1.3 ppm, range 0.1 - 81.3 ppm). CCK values (mean 28.5 pg/ml, SD 17.1, median 22.7 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 and were negatively, not positively, associated with employees' serum PFOA levels. Our findings continue to suggest there is no significant clinical hepatic toxicity associated with PFOA levels as measured in this workforce. Unlike a previously reported observation, PFOA did not appear to modulate hepatic responses to either obesity or alcohol consumption. Limitations of these findings include: 1) the cross-sectional design as only 17 subjects were common for the three surveillance years; 2) the voluntary participation that ranged between 50 and 70 percent; and 3) the few subjects with serum levels $\geq$ 10 ppm.

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INTRODUCTION

Ammonium perfluorooctanoate [APFO; $CF_3(CF_2)_6CO_2^-NH4^+$] is a potent synthetic surfactant used in industrial applications which rapidly dissociates in biologic media to perfluorooctanoate [$CF_3(CF_2)_6CO_2^-$], which is the anion of perfluorooctanoic acid [PFOA, $CF_3(CF_2)_6COOH$]. In laboratory animals, PFOA and its salts are: 1) absorbed by ingestion, inhalation or dermal contact;[1-3] 2) distributed primarily in the liver and blood;[4] 3) not biotransformed, conjugated, incorporated into lipids or form coenzyme A conjugates;[5-8] and 4) eliminated in the female rat at a greater rate of renal excretion than the male rat although no gender differences in excretion of PFOA have been seen in other laboratory animal species.[1,4,9] In rats, administration of APFO results in peroxisome proliferation, uncoupling of mitochondrial oxidative phosphorylation and altered lipid metabolism.[10,11,9] In a 90-day gavage study of rhesus monkeys, all animals in the 100 mg/kg/day group and 3 of the 4 animals in the 30 mg/kg/day group died before the end of study.[1,12] Histopathologic examination revealed marked diffuse lipid depletion in the adrenals, slight to moderate hypocellularity of bone marrow, moderate atrophy of lymphoid follicles in the spleen and moderate atrophy of the lymphoid follicles of the lymph nodes in the two highest treatment groups. There were no histopathologic changes in the 0, 3 and 10 mg/kg/day dose groups. In lifetime feeding bioassays of rats,[13,14] APFO in the diet at 300 ppm (daily dose of 15 mg/kg/day) increased the incidence of liver, Leydig cell and pancreas acinar cell adenomas. The liver and testicular tumors most likely occur via nongenotoxic mechanisms: oxidative stress and apoptosis in the development of the liver tumors; and enhanced hepatic aromatase activity which results in a hormone-mediated mechanism (increased estradiol) for the formation of Leydig cell tumors.[15-17] The pancreas acinar cell adenomas were hypothesized to be a result of a mild but sustained increase in cholecystokinin (CCK) levels secondary to hepatic cholestasis.[18] CCK has been shown in animal models to produce pancreatic hypertrophy, hyperplasia and neoplasia.[19-23]

Hepatic toxicity and hypolipidemia have not been observed in ammonium perfluorooctanoate production workers.[24,25] Gilliland and Mandel 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 hepatic enzyme tests.[25] This workforce was not found to be at an increased mortality risk for liver cancer or liver disease.[26] However, there were four pancreatic cancer deaths compared to two expected deaths (Standardized Mortality Ratio 2.0, 95% Confidence Interval 0.5-5.0). One of these four pancreatic cancer deaths had worked in the building where ammonium perfluorooctanoate was produced.

The purpose of this analysis was to examine several additional years of medical surveillance data at this ammonium perfluorooctanoate production plant in order to determine: 1) whether CCK levels are positively associated with serum PFOA levels among production employees; 2) whether PFOA results in clinical hepatic toxicity; and 3) whether PFOA may modulate hepatic responses to obesity and alcohol.

METHODS

PFOA Production

Ammonium perfluorooctanoate production at this 3M plant began in 1947. Ammonium perfluorooctanoate, a white powder, is produced via a five-stage process: electrochemical fluorination; 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 has occurred in the drying area.

Subject Selection and Data Collection

Voluntary medical surveillance examinations were offered to the fluorochemical production workers in 1993, 1995 and 1997. The total number of male subjects, by year, who participated in these three cross-sectional investigations were: 1993 (n = 111); 1995 (n = 80); and 1997 (n = 74). (There were too few female employees to include in the data analysis.) Eligible voluntary participation rates among these production workers ranged

from approximately 50 (1997) to 70 (1993) percent. There were 68 subjects in common for 1993 and 1995; 21 subjects in common between 1995 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, serum PFOA determination and several male reproductive hormone assays. The hormone data were collected in 1993 and 1995 and the findings have been reported elsewhere.[27] Serum hepatic and lipoprotein-related biochemical tests included: alkaline phosphatase (IU/L); gamma glutamyl transferase (GGT, IU/L); aspartate aminotransferase (AST, IU/L); alanine aminotransferase (ALT, IU/L); total bilirubin (mg/dl); direct bilirubin (mg/dl); total cholesterol (mg/dl); low-density lipoproteins (LDL, mg/dl); high-density lipoproteins (HDL, mg/dl); and triglycerides (mg/dl). In 1997, employees' plasma CCK-33 (pg/ml) 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. For purposes of brevity, we will refer to CCK-33 as CCK. Employees were required to have fasted for 12 hours prior to their venipuncture. Because CCK analyses were not a standard analysis of the company's fluorochemical medical surveillance program, a study protocol was reviewed and approved by the company's human subjects committee and a signed, informed consent was obtained from each participant.

Serum chemistries and hematology were evaluated at Allina Laboratories (Minneapolis, Minnesota). Plasma CCK was measured by direct radioimmunoassay by Inter Science Institute (Inglewood, California). Serum PFOA (i.e., perfluorooctanoate) was determined by thermospray (1993 and 1995) and electrospray (1997) high-performance liquid chromatography/mass spectrometry methods.[28,29]

Data Analysis

Simple and stratified analyses, analysis of variance (ANOVA), and multivariable regression techniques were used to evaluate linear and nonlinear associations between PFOA and the biochemical parameters with adjustment for potential confounding variables.[30] Various serum category levels were used for the stratified analysis with no

significant differences observed based on cutpoints as high as ≥ 30 ppm. However, the number of employees at ≥ 30 ppm was 5 or fewer (based on year). For purposes of this report, employees were stratified into three PFOA categories (0 - <1 ppm; 1 - <10 ppm; and ≥10 ppm) in order to provide a greater number of employees in the highest (≥ 10 ppm) category. For multivariable regression analyses, 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 and non-response to this questionnaire item (almost all subjects reported between <1 - 3 drinks/day). Linear and nonlinear transformations of PFOA were used to test for associations. In particular, the multivariable models employed by Gilliland and Mandel[25] were used to determine whether PFOA has a modulating effect on obesity or alcohol consumption in regards to hepatic serum chemistries (ALT, AST and GGT) and HDL, respectively.

RESULTS

Serum PFOA levels, by year, were: 1993 (mean 5.0 ppm, SD 12.2, median 1.1 ppm, range 0.0 - 80.0 ppm); 1995 (mean 6.8 ppm, SD 16.0, median 1.2 ppm, range 0.0 - 114.1 ppm); and 1997 (mean 6.4 ppm, SD 14.3, median 1.3 ppm, range 0.1 - 81.3 ppm). Provided in Table I are the mean, standard deviation, median and range of the employees' age, BMI and hepatic, cholesterol and lipoprotein serum chemistry data stratified by PFOA level and the year of the medical surveillance examination. Depending upon the surveillance year, one to two orders of magnitude of difference were observed between the means (and medians) of the lowest and highest serum PFOA categories. There was no evidence for abnormal liver function tests, hypolipidemia or cholestasis associated with increasing employees' serum PFOA levels. Controlling for potential confounders, multivariable regression analyses did not suggest otherwise. Other measures including renal function, blood glucose and hematology were not associated with serum PFOA levels (data not shown).

The mean CCK value was 50 percent lower among employees with serum PFOA values ≥ 10 ppm than for those employees with serum PFOA levels < 1 ppm (Table I), Figure

1 is a scatter plot of the natural log of CCK and PFOA. 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 of employees with 0.6 ppm and 5.6 ppm serum PFOA levels, respectively. Adjusting for potential confounding variables, we continued to observe a negative association between the natural log of CCK and serum PFOA levels (Table II) although minimum variation was explained ($R^2 = .08$).

Provided in Table III are the multivariable regression models (as originally reported by Gilliland and Mandel with the 1990 medical surveillance data[25]) which examined the potential modulating effect of PFOA on the association between alcohol and HDL. The coefficients of determination (R^2, adjusted R^2) were not large for any model. Based on these models, Table IV shows the change in HDL levels associated with a 10 ppm increase in serum PFOA levels among light and moderate drinkers ($\geq$ 1 drink/day) compared to light drinkers ($<$ 1 drink/day). [Note: total serum organic fluorine measurements, rather than serum PFOA, were used in 1990.] Unlike the findings from the 1990 data, the effect of alcohol use on increasing HDL levels was not blunted by a 10 ppm increase in PFOA in any of the subsequent medical surveillance examination years.

Presented in Table V are multivariable regression models, including those originally reported using the 1990 surveillance data,[25] regarding the potential effect of PFOA on hepatic enzyme responses to obesity as measured by BMI. Again, the coefficients of determination were not large for any model. In the 1990 surveillance data, ALT increased among obese (BMI = 35 kg/m^2) but not non-obese (BMI = 25 kg/m^2) workers who had a 10 ppm change in serum PFOA (Table VI). However, this interaction was not observed in the 1993, 1995 or 1997 medical surveillance examinations. Likewise, we did not observe associations with AST or GGT (data not shown) as was reported in the 1990 medical surveillance examinations.[25]

DISCUSSION

We observed a negative association between serum PFOA and plasma CCK among 74 workers engaged in the production of ammonium perfluorooctanoate. This observation is opposite that proposed by Obourn et al who questioned whether chronic exposure to peroxisome proliferators, like PFOA, can cause pancreatic adenomas in the rat as the consequence of a mild but sustained increase in CCK levels secondary to hepatic cholestasis.[17] We do not believe the negative association observed in our study represents an entirely different biological relationship than what was originally postulated 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.

There are several explanations for the lack of a positive association between PFOA and CCK in our study. First and foremost, the serum PFOA measurements in these production workers may have been too low to cause an increase in CCK if such a mechanism exists in humans. Second, the mechanistic reason for the elevated CCK levels in the Obourn et al study[17] was not clearly established. Obourn et al examined the effects of Wyeth 14,643, a more potent peroxisome proliferator than PFOA; thus their findings may not be directly related to PFOA. The clinical pathology data indicative of cholestasis included alterations in bile flow and bile acid output. Absolute bile flow and flow relative-to-body weight were marginally increased and acinar cell proliferation, although numerically increased at 3 months, returned to control levels at 6 months. Obourn et al 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. Third, the primary set of biochemical and cellular events identified in rodents susceptible to the hepatic tumor effects of peroxisome proliferators has 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.[17,31] It should also be noted that unlike the pancreas of the rat, the human pancreas has no detectable CCK-A receptors and little to no mRNA for the receptor.[31-34] Finally, whether CCK initiates or promotes pancreatic cancer is a controversial issue.[35] 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.[36] CCK has promoted growth of human pancreatic cancers in cell cultures.[37] On the other hand, fasting plasma concentrations of CCK in unresected pancreatic cancer patients did not differ from healthy controls.[38] Also acinar cell malignancies in rats are rare in the human.[39] 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 acinar cell adenoma models in the rat.[40,41]

Neither Gilliland and Mandel[25] nor ourselves observed significant clinical hepatic toxicity associated with the serum PFOA levels measured in this workforce. In the three surveillance years we examined (1993, 1995 and 1997), 88 percent, 81 percent and 85 percent of those employees who volunteered had serum PFOA levels less than 10 ppm, respectively. In a laboratory study, no significant hepatic toxicity was reported for the lowest dose (3 mg/kg/day) group of rhesus monkeys administered APFO by gavage for 90 days.[1,12] One of four primates in the next highest dose (10 mg/kg/day group) developed anorexia and black stools during the course of the study. There were no other abnormalities reported for this group. Only one animal survived in the 30 mg/kg/day group and none survived in the highest (100 mg/kg/day) dose group. For the 3 and 10 mg/kg/day dose groups, their mean serum total organic fluorine levels were 54 and 67 ppm, respectively.[1,12] Sixty-nine percent of the molecular weight of PFOA is organic fluorine; therefore these total organic fluorine levels in the lower dose groups may correspond to serum PFOA levels of 80 to 100 ppm. Total organic fluorine levels were analyzed in the liver for two animals in each of the two lowest two dose groups and the means were 5 and 10 ppm, respectively.[12] Liver total organic fluorine levels were also analyzed for the 30 mg/kg/day (n = 4, mean = 98 ppm) and the 100 mg/kg/day (n = 2, mean = 213 ppm) dose groups; however, only the sole surviving animal in the 30 mg/kg/day group was analyzed for serum total organic fluorine (145 ppm which

approximates 210 ppm of PFOA). The 30 mg/kg/day group did have higher hepatic transaminase values after 30 days of compound administration than the control group. Serum chemistries were not performed for the 100 mg/kg/day group after the onset of compound administration. Relative (% body weight) liver weights were higher among the 30 mg/kg/day (3.84%) and 100 mg/kg/day (3.31%) treatment groups than the control (2.36%), 3 mg/kg/day (2.36%) and 10 mg/kg/day (2.32%) dose groups. No abnormal liver function results were observed among employees with the highest serum PFOA levels; nevertheless these workers have been restricted from potential high exposure workplace areas.

We were unable to replicate, in three subsequent medical surveillance examinations, an earlier investigation's finding that PFOA may modulate hepatic responses to obesity and alcohol.[25] Total serum organic fluorine was used as a surrogate variable for PFOA in the 1990 medical surveillance exams. The use of total serum organic fluorine constitutes additional potential exposure to perfluorocarbons; however, data suggest that PFOA would represent the greatest fraction of total serum organic fluorine levels in this employee population.[24] Another explanation for the disparate findings, in particular as related to BMI, is that there may have been measurement error regarding BMI in the original study as well as in our investigation. We have previously noted the lack of an expected positive association between BMI and estradiol in the 1990 data.[27] Yet in the present study we did not observe the anticipated strong positive correlation between BMI and ALT except in 1997. Correlation coefficients (p value in parentheses) were: 1993, r = .16, (p = .09); 1995, r = .13, (p = .27); and 1997, r = .43 (p = .0001). Self-reported alcohol data collected in the occupational setting can be questioned for its reliability as well as validity. The latter was not feasible to address; however, to partially examine the issue of reliability, we examined the analyses of the 68 employees who participated both in 1993 and 1995. The data showed good correlation between these two years for the potential confounding factors of BMI (r = .94, p = .0001), alcohol consumption (r = .67, p = .0001) and cigarette smoking (r = .84, p = .0001).

Several additional issues are worthy of consideration. The medical surveillance program is voluntary. Overall participation rates declined from approximately 70 percent in 1993 to 50 percent in 1997. Serum PFOA levels could differ between participants and nonparticipants. The high turnover of employees between 1995 and 1997 detracted from the opportunity for a longitudinal assessment. In this regard, we did examine the change in several parameters among the 68 subjects in common for 1993 and 1995. For example, the average difference in serum PFOA was + 0.1 ppm (Wilcoxin signed-rank test = -540.5, p = .0001), the mean change in ALT was +0.5 IU/L (Wilcoxin signed-rank test = 50.0, p = 0.6) and the mean change in cholesterol was -1.6 mg/dL (Wilcoxin signed-rank test = -60.0, p = 0.4). The change in serum PFOA levels did not predict, via regression analyses, the change in ALT or cholesterol. Besides the few employees in common across all three years of medical surveillance data (n = 17), another difference that occurred in 1997 was the method of analysis of PFOA changed from thermospray (1993 and 1995) to electrospray (1997) high-performance liquid chromatography mass spectrometry. Also, the laboratory reference range substantially changed for ALT in 1997 (as can be seen in Table 1 by examining the lower mean values for ALT in 1997). Finally, the issue remains that the lack of a clinical hepatotoxic effect reported by Gilliland and Mandel[25] and ourselves does not rule out the possibility that PFOA may result in a subclinical hepatic effect in this production population that we have yet to observe. Results from ongoing laboratory animal studies, including a 6 month APFO gelatin capsule feeding study of cynomolgus primates, may provide further insight into the direction of medical surveillance activities for this workforce.

REFERENCES

1. Griffith FD, Long JE. 1980. Animal toxicity studies with ammonium perfluorooctanoate. *Am Ind Hyg Assoc J* 41:576-583, 1980.
2. Kennedy G. Dermal toxicity of ammonium perfluorooctanoate. *Toxicol Appl Pharmacol* 81:348-355, 1985.
3. Kennedy G, Hall G, Brittelli J, Chen H. Inhalation toxicity of ammonium perfluorooctanoate. *Fd Chem Toxicol* 24:1325-1329, 1986.
4. Vanden Heuvel J, Kuslikis B, Van Refelghem M, Perterson R. Tissue distribution, metabolism and elimination of perfluorooctanoic acid. *J Biochem Toxicol* 6:83-92, 1991.

5. Pastoor TP, Lee KP, Perri MA, Gillies PJ. Biochemical and morphological studies of ammonium perfluorooctanoate-induced hepatomegaly and peroxisome proliferation. *Exp Mol Pathol* 47:98-109, 1987.
6. Ophaug R, Singer L. Metabolic handling of perfluorooctanoic acid in rats. *Proc Soc Exp Biol Med* 163:19-23, 1980.
7. Ylinen M, Koho A, Hanhijarvi H, Peura P. Disposition of perfluorooctanoic acid in the rat after single and subchronic administration. *Bull Environ Contam Toxicol* 44:46-53, 1990.
8. Kuslikis BI, Vanden Heuvel JP, Peterson RE. Lack of evidence for perfluoroodecanoyl- or perfluorooctanoyl-coenzyme A formation in male and female rats. *J Biochem Toxicol* 7:25-29, 1992.
9. Hanhijarvi H, Phaug R, Singer L. The sex-related difference in perfluorooctanoate excretion in the rat. *Proc Soc Exp Biol Med* 171:51-55, 1982.
10. Haughom B, Spydevold O. The mechanism underlying the hypolipemic effect of perfluorooctanoic acid (PFOA), perfluorooctane sulphonic acid (PFOSA) and clofibric acid. *Biochim Biophys Acta* 1128:65-72, 1992.
11. Keller B, Marsman D, Popp J, Thurman R. Several nongenotoxic carcinogens uncouple mitochondrial oxidative phosphorylation. *Biochim Biophys Acta* 1102:237-244, 1992.
12. Goldenthal EI, Jessup DC, Geil RG, Mehring JS. Ninety-day Subacute Rhesus Monkey Toxicity Study. Study No. 137-090. *International Research Development Corp, Mattawan (MI),* November, 1978.
13. Sibinski LJ. Two-year oral (diet) toxicity/carcinogenicity study of fluorochemical FC-143 in rats. *Riker Laboratories, St. Paul (MN),* 1987.
14. Rao MS, Reddy JK. Hepatocarcinogenesis of peroxisome proliferators. *Ann NY Acad Sci* 804:573-587, 1997.
15. Cook JC, Murray SM, Frame SR, Hurtt ME. 1992. Induction of Leydig cell adenomas by ammonium perfluorooctanoate: a possible endocrine-related mechanism. *Toxicol Appl Pharmacol* 113:209-217, 1992.
16. Cook JC, Hurtt ME, Frame SR, Biegel LB. 1994. Mechanisms of extrahepatic tumor induction by peroxisome proliferators in Crl:CD BR (CD) rats. *Toxicologist* 14:301, 1994.
17. Biegel LB, Liu RCM, Hurtt ME, Cook JC. Effects of ammonium perfluorooctanoate on Leydig cell function: in vitro, in vivo, and ex vivo studies. *Toxicol Appl Pharmacol* 134:18-25, 1995.
18. Obourn JD, Frame SR, Bell RH, Longnecker DS, Elliott GS, Cook JC. Mechanisms for the pancreatic oncogenic effects of the peroxisome proliferator Wyeth-14,643. *Toxicol Appl Pharmacol* 145:425-436, 1997.
19. Pandol SJ. Pancreatic physiology and secretory testing. (In) Sleisenger M, Fordtran JS (eds) Gastrointestinal and Liver Diseases, Volume 1, *W.B. Saunders Co., Philadelphia,* 1998, pages 771-782.
20. Longnecker DS. Experimental models of exocrine pancreatic tumors. (In) Go VLW (ed) The Exocrine Pancreas: Biology, Pathology and Diseases, *Raven, New York*, 1986, pages 443-458.
21. Longnecker DS. Experimental pancreatic cancer: role of species, sex and diet. *Bull Cancer* 77:27-37, 1990.

22. Pour PM, Runge RG, Birt D, Gingell R, Lawson T, Nagel D, Wallcave L, Salmasi S. Current knowledge of pancreatic carcinogenesis in the hamster and its relevance to the human disease. *Cancer* 47:1573-1587, 1981.
23. Pour PM, Lawson T, Helgeson S, Donnelly T, Stepan K. Effect of cholecystokinin on pancreatic carcinogenesis in the hamster model. *Carcinogenesis* 9:597-601, 1988.
24. Ubel F, Sorenson S, Roach D. Health status of plant workers exposed to fluorochemicals: A preliminary report. *Am Ind Hyg Assoc* 41:584-589, 1980.
25. Gilliland FD, Mandel JS. Serum perfluorooctanoic acid and hepatic enzymes, lipoproteins and cholesterol: a study of occupationally exposed men. *Am J Ind Med* 129:560-568, 1996.
26. Gilliland FD, Mandel JS. Mortality among employees of a perfluorooctanoic acid production plant. *J Occup Med* 35:950-954, 1993.
27. Olsen GW, Gilliland FD, Burlew MM, Burris JM, Mandel JS, Mandel JH. An epidemiologic investigation of reproductive hormones in men with occupational exposure to perfluorooctanoic acid. *J Occ Env Med* 40:614-622, 1998.
28. Johnson JD, Wolter JT, Colaizy GE, Rethwill PA, Nelson RM. Quantification of perfluorooctanoate and perfluorooctanesulfonate in human serum using ion-pair extraction and high performance liquid chromatography-thermospray mass spectrometry with automated sample preparation. *3M Company, St. Paul (MN)*, 1996.
29. Anderson DJ, Mulvana DE. Analytical report for the determination of perfluorooctanaoate and perfluorooctanesulfonate in human serum by LC/MS. *Advanced Bioanalytical Services, Inc., Ithaca (NY)*, 1997.
30. SAS Institute, Inc. SAS Users Guide: Statistics. Version 6. *SAS Institute, Inc., Cary (NC)*,1990.
31. Cattley RC, DeLuca J, Elcombe C, Fenner-Crisp P, Lake BG, Marsman DS, Pastoor TA, Popp JA, Robinson DE, Schwetz B, Tugwood J, Wahil W. Do peroxisome proliferating compounds pose a hepatocarcinogenic hazard to humans? *Reg Toxicol Pharmacol* 27:47-60, 1998.
32. Wank SA, Pisegna JR, deWeerth A . Cholecystokinin receptor family. *Ann NY Acad Sci* 713:49-66, 1994.
33. Gavin CE, Martin NP, Schlosser MJ. Absence of specific CCK-A binding sites on human pancreatic membranes. *Toxicologist* 30:334, 1996.
34. Gavin CE, Malnoske JA, White J, Schlosser MJ. Species differences in expression of pancreatic cholecystokinin-A receptors. *Toxicologist* 36:232 1997
35. Axelson J, Ihse I, Hakanson R. Pancreatic cancer: the role of cholecystokinin? Scand J Gastroenterol 1992;27:993-998. *Toxicol Appl Pharmacol* 134:18-25, 1995.
36. Herrington MK, Adrian TE. On the role of cholecystokinin in pancreatic cancer. *Int J Pancreatol* 17:121-138, 1995.
37. Palmer-Smith J, Krame ST, Solomon TE. CCK stimulates growth of six human pancreatic cancer cell lines in serum-free medium. *Reg Peptides* 22:341-349, 1991.
38. Rehfeld JF, van Solinge WW. The tumor biology of gastrin and cholecystokinin. *Adv Cancer Res* 63:295-347, 1994.

39. Anderson KE, Potter JD, Mack TM. Pancreatic cancer. (In) Schottenfeld D, Fraumeni JF (eds) Cancer Epidemiology and Prevention (2nd edition). *Oxford University Press, New York*, 1996, pages 725-771.
40. van Kranen HJ, Vermeulen E, Schoren L, Bas J, Woutersen RA, van Iersel P, van Kreijl CF, Scherer E. Activation of c-K-*ras* is frequent in pancreatic carcinomas of Syrian hamsters, but is absent in pancreatic tumors of rats. *Carcinogenesis* 12:147-1482, 1991.
41. Caldas C, Kern SE. K-ras mutation and pancreatic adenocarcinoma. *Int J Pancreatol* 18:1-6, 1995.

Table I. Mean, Median, Standard Deviation (S.D.) of Mean and Range of Demographic, Hepatic, Cholesterol and Lipoprotein Values by Serum PFOA Categories and Medical Surveillance Year (1993, 1995, 1997)

PFOA* Category (ppm)	1993 Mean	1993 Median	1993 S.D.	1993 Range	1995 Mean	1995 Median	1995 S.D.	1995 Range	1997 Mean	1997 Median	1997 S.D.	1997 Range
					PFOA (ppm)							
0 - <1	0.48	0.48	0.27	0.00 – 0.99	0.31	0.20	0.32	0.00 – 0.90	0.47	0.55	0.26	0.05 – 0.92
1 - <10	3.38	2.50	2.17	1.03 – 8.92	3.03	2.40	1.84	1.10 – 8.20	3.13	2.30	2.12	1.05 – 7.66
≥ 10	30.88	19.50	25.12	11.90 – 80.00	30.06	25.50	26.58	10.3 – 114.1	32.13	22.52	24.84	10.50 – 81.35
	F Value = 68.3, p = .0001				F Value = 39.1, p = .0001				F Value = 48.7, p = .0001			
					Age							
0 - <1	43	44	9.2	27 - 61	42	41	8.3	29 – 60	40	39	9.1	25 – 61
1 - <10	39	38	7.8	27 - 60	41	40	8.6	24 – 58	41	41	8.7	26 – 58
≥ 10	39	38	6.6	25 - 49	43	45	8.4	27 – 55	42	46	10.9	28 – 57
	F Value = 3.7, p = .02				F Value = 0.2, p = .85				F Value = 0.2, p = 0.81			
					BMI (kg/m^2)							
0 - <1	28.0	27.6	4.3	20.9 – 42.0	27.6	26.8	4.2	21.9 – 45.2	28.7	27.5	3.9	21.5 – 35.0
1 - <10	26.9	26.3	2.5	21.6 – 32.5	28.6	27.9	3.4	22.1 – 38.3	29.5	29.5	5.3	21.9 – 46.8
≥ 10	28.4	28.5	2.4	22.4 – 32.0	28.4	28.8	3.5	21.2 – 34.8	27.6	28.5	3.5	22.0 – 33.0
	F Value = 1.7, p = .14				F Value = 0.7, p = .51				F Value = 0.8, p = .47			
					CCK (pg/ml)							
0 - <1	Not done in 1993				Not done in 1995				33.4	31.3	15.7	13.4 – 80.5
1 - <10									28.0	21.1	19.2	8.8 – 86.7
≥ 10									17.4	16.4	5.6	11.4 – 29.9
									F value = 3.8, p = 0.03			

PFOA* Category (ppm)	1993 Mean	1993 Median	1993 S.D.	1993 Range	1995 Mean	1995 Median	1995 S.D.	1995 Range	1997 Mean	1997 Median	1997 S.D.	1997 Range
Alkaline Phosphatase (IU/L)												
0 - <1	88	82	26	37 – 161	78	76	18	40 – 114	79	78	19	27 – 122
1 - <10	82	78	23	47 – 151	80	76	25	48 – 165	87	84	23	47 – 164
≥ 10	83	75	24	58 – 132	89	76	31	55 - 146	80	73	23	61 - 142
	F Value = 0.7, p = .52				F Value = 1.4, p = .25				F Value = 1.3, p = .28			
GGT (IU/L)												
0 - <1	33	30	19	11 – 84	42	34	27	16 – 149	34	25	27	15 – 230
1 - <10	50	30	70	6 – 472	51	36	41	19 – 190	36	32	25	14 – 162
≥ 10	37	33	17	19 – 77	40	38	13	21 – 61	30	29	10	15 – 50
	F Value = 1.5, p = .24				F Value = 0.9, p = .41				F Value = 0.2, p = .78			
AST (IU/L)												
0 - <1	23	22	7	11 – 60	21	20	6	13 – 36	26	23	7	13 – 41
1 - <10	26	24	11	12 – 83	24	20	13	13 – 75	25	25	7	14 – 48
≥ 10	24	24	5	16 - 35	21	20	4	15 – 29	25	25	4	22 – 34
	F Value = 1.1, p = .33				F Value = 0.8. p = .45				F Value = 0.2, p = .83			
ALT (IU/L)												
0 - <1	45	42	14	22 – 88	44	40	13	27 – 80	31	30	10	13 – 59
1 - <10	48	45	29	22 – 221	53	40	34	27 – 175	33	28	15	14 – 80
≥ 10	46	47	8	35 - 62	47	48	12	28 - 71	35	31	13	18 - 57
	F Value = 0.2, p = .82				F Value = 1.2, p = .30				F Value = 0.3, p = .73			

PFOA* Category (ppm)	1993 Mean	1993 Median	1993 S.D.	1993 Range	1995 Mean	1995 Median	1995 S.D.	1995 Range	1997 Mean	1997 Median	1997 S.D.	1997 Range
					LDL (mg/dl)							
0 - <1	138	142	40	27 – 227	131	130	32	31 – 191	114	119	28	40 – 158
1 - <10	143	137	38	72 – 223	133	137	40	28 – 210	134	134	44	26 – 253
≥ 10	140	143	42	60 - 188	130	118	39	62 - 211	134	127	38	79 - 206
	F Value = 0.2, p = .84				F Value = 0.1, p = .96				F Value = 2.3, p = .11			
					Triglycerides (mg/dl)							
0 - <1	171	145	124	37 – 636	170	152	93	57 – 371	219	186	116	53 – 445
1 - <10	205	129	408	47 - 2845	175	123	144	59 – 743	176	161	85	44 – 360
≥ 10	221	223	159	41 - 564	254	183	154	77 - 563	251	188	192	63 - 718
	F Value = 0.3, p = .77				F Value = 2.7, p = .07				F Value = 2.1, p = .13			

* Study population size by PFOA category (ppm) and year

Sample Size

PFOA Category	1993	1995	1997
0 < 1	52	39	29
1 - < 10	46	26	34
≥ 10	13	15	11
Total	111	80	74

Table II. Multiple Regression Model of Factors Predicting the Natural Log of Plasma Cholecystokinin in Workers with Serum PFOA Levels

Variable	B	SE(B)	p value
Intercept	3.02	0.48	.0001
PFOA	-0.008	0.004	.07
Age	0.0001	0.007	.98
Alcohol	-0.005	0.086	.95
BMI	0.009	0.015	.53
Cigarettes	-0.009	0.007	.17

$R^2 = .08$
Adj $R^2 = .02$

Table III. Multivariable Regression Models* of Factors Predicting High Density Lipoprotein in Workers with Serum PFOA Levels

	1990			1993			1995			1997		
Variable	*B*	SE(*B*)	p value	*B*	SE(*B*)	p value	*B*	SE(*B*)	p value	*B*	SE(*B*)	p value
Intercept	65.00	10.07	.0001	55.00	16.70	.001	52.10	11.92	.0001	57.82	10.15	.0001
PFOA	-1.61	0.77	.04	-0.14	0.33	.67	-0.10	0.08	.18	-0.19	0.13	.16
Light Drink	-9.92	3.51	.006	-4.83	3.76	.20	-5.11	2.61	.05	-5.75	3.13	.07
Interaction#	1.62	0.80	.04	0.02	0.35	.96	0.02	0.13	.87	0.36	0.18	.05
	$R^2 = .17$ Adj R^2 = Not reported			$R^2 = .10$ Adj $R^2 = .02$			$R^2 = .30$ Adj $R^2 = .19$			$R^2 = .11$ Adj $R^2 = .03$		

* Adjusted for age, BMI, cigarette use, and non-respondents to alcohol question (all four years) and testosterone level (1990, 1993 and 1995). The 1990 regression model used total organic fluorine as the dependent variable (see reference #25).

Interaction = PFOA x Light Drink

Table IV. Change in HDL* from Light Alcohol Drinker (< 1 drink/day) to: 1) a Light Drinker with a 10 ppm Change in Serum PFOA; 2) a Moderate Alcohol Drinker (≥ 1 drink/day); and 3) a Moderate Alcohol with a 10 ppm Change in Serum PFOA.

	Light Drinker with a 10 pm increase in PFOA	Moderate Drinker	Moderate Drinker with 10 ppm increase in PFOA
Year		Change in HDL (mg/dl)	
1990#	0.1	9.9	-6.2
1993	-1.3	4.8	3.4
1995	-0.8	5.1	4.1
1997	1.7	5.8	3.9

*Determined from multivariable models (see table 3) adjusted for age, body mass index cigarette use, and non-respondents to alcohol question (all four years) and testosterone (1990, 1993 and 1995 only).

Data in 1990 analyzed for total serum organic fluorine (see reference #25).

Table V. Multivariable Regression Models* of Factors Predicting ALT in Workers with Serum PFOA Levels

	1990			1993			1995			1997		
	B	SE(*B*)	p value	*B*	SE(*B*)	p value	*B*	SE(*B*)	p value	*B*	SE(*B*)	p value
Intercept	58.13	24.60	.02	27.32	19.13	.16	40.93	22.90	.08	3.93	11.34	.73
PFOA	-15.80	4.58	.0008	0.89	2.88	.76	0.81	2.62	.75	2.77	1.27	.03
BMI	0.30	0.82	.72	1.07	0.67	.11	1.08	0.74	.15	1.54	0.35	.0001
Interaction#	0.62	0.17	.0004	-0.03	0.10	.79	-0.03	0.09	.76	-0.09	0.42	.04
	$R^2 = .21$ Adj R^2 = Not reported			$R^2 = .06$ Adj $R^2 = .01$			$R^2 = .11$ Adj $R^2 = .01$			$R^2 = .32$ Adj $R^2 = .26$		

*Adjusted for age, alcohol and cigarette use. The 1990 regression model used total organic fluorine as the dependent variable (see reference #25).
#Interaction = PFOA x BMI

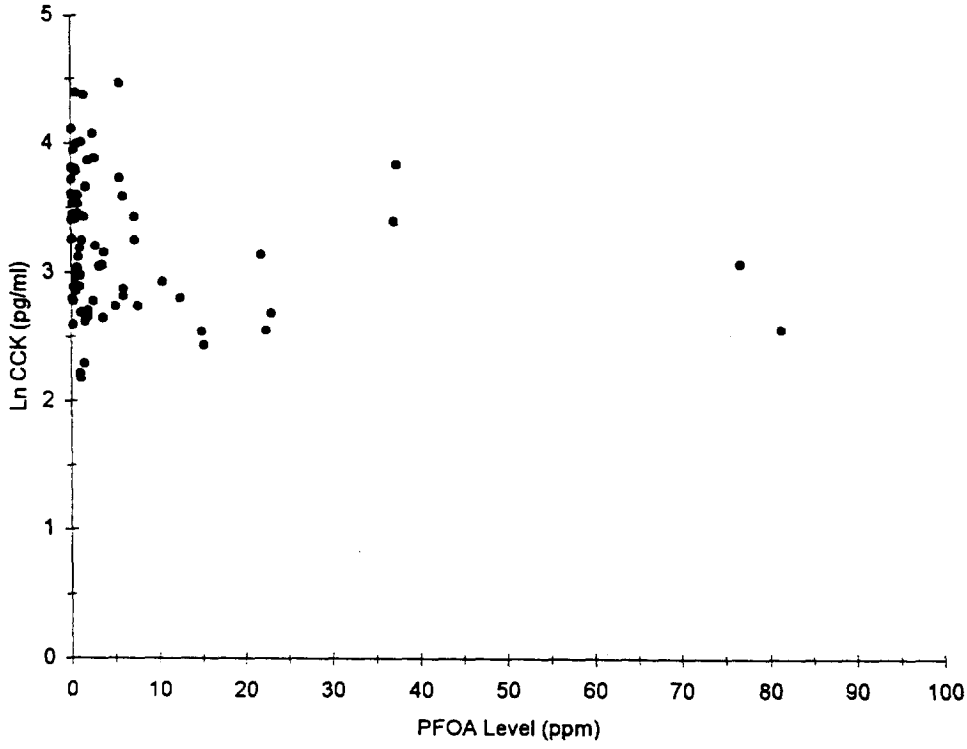
Table VI. Change in Alanine Aminotransferase (ALT)* Associated with a 10 ppm Change in Serum PFOA for Three Body Mass Indices

	BMI (kg/m^2)		
	25	30	35
Year	Change in ALT (IU/L)		
1990#	-3.0	28.0	59.0
1993	2.2	0.9	-0.5
1995	1.5	0.1	-1.2
1997	5.3	0.8	-3.7

*Determined from multivariable regression model (see table 5) adjusted for age, alcohol and cigarette use.

Data in 1990 analyzed total serum organic fluorine (see reference #26).

Figure 1.
Scatter Plot of Nautural Log CCK (pg/ml) by Serum PFOA Level (ppm)
[Linear Regression Model: Ln CCK = 3.24 - 0.006PFOA;
p value of PFOA coefficeint = 0.19; R^2 = .02]


Ln CCK (pg/ml)
5
4
3
2
1
0
0
10
20
30
40
50
60
70
80
90
100
PFOA Level (ppm)

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 Perfluoroocatanoic Acid Production Workers

Study
Start Date: September 3, 1997

Estimated Date of
Final Report: April 15, 1998

IRB Approval Date:

Protocol Number: EPI-0003
IRB Approval

Exempt	Expedited
	X

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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 cynomologus 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 perflurooctanoate (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 spectrophometric 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 choleostasis 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 choleostasis, 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, cynomologus 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 choleostasis, 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 employeees 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 sevice 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.

Compound	Cross-reactivity
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.

Specimen (pg/ml)	Amount added	Amount measured	Amount expected	Recovery (%)
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.

Mean (pg/ml)	Standard Deviation (pg/ml)	Coefficient of Variation (%)
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 cynomologus 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 choleostasis 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 ciliac 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 mEqL (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, chymotripsin 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); amyolytic enzyme (alpha amylase); lipolytic enzymes (lipase, pro-phospholipase A_2, 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 A_2 to chymotrypsin, elastase, carboxypeptidases A and B, and phospholipase A_2, 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 phospinositides 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 ^{3}[H]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 glutaramic 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

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

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

Part II Tobacco Smoking

2. Do you smoke cigarettes now? (as of one month ago)
   A. Yes
   B. No

3. Have you smoked at least 100 cigarettes during your entire life?
   A. Yes
   B. No (If 'no', please go to question 25)

4. How old were you when you first started regular cigarette smoking:_____

5. How many years have you smoked cigarettes? (Do not count the time when you periodically stopped smoking) _____

6. On average, how many cigarettes do you smoke per day now? _____

7. On **average**, the **entire time you smoked**, how many cigarettes did you smoke per day?_____

8. If you stopped smoking cigarettes completely, how old were you when you stopped?_____

Alcohol

9. 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**)

10. Age when you first began drinking alcoholic beverages:_____

11. Do you currently drink alcoholic beverages?
   A. Yes
   B. No — If 'no', how long ago did you stop (months, years)_____

12. When you **were drinking** alcoholic beverages how drinks per week did you drink?_____

13. On the average, how many alcoholic beverages per week do you **currently** drink?_____

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				
Hyperthroidism (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.

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. You 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 _____

REFERENCES

Abbruzzese JL, Gholson CF, Daugherty K, Larson E, DuBrow R, Berlin R, Levin B. A pilot clinical trial of the cholecystokinin receptor antagonist MK-329 in patients with advanced pancreatic cancer. Pancreas 7:165-171.

Adrian TE, Permet J, Wang Q, Herrington MK, Takahashi T, Larsson J, Pour PM (1994). Is CCK involved in pancreatic ductal cell cancer? Tenth International Symposium on Gastrointestinal Hormones, Santa Barbara, CA.

Anderson KE, Potter JD, Mack TM (1996). Pancreatic cancer. (In) Schottenfeld D, Fraumeni JF (eds) "Cancer Epidemiology and Prevention (2nd edition)." New York: Oxford University Press, pp 725-771.

Axelson J, Ihse I, Hakanson R (1992). Pancreatic cancer: the role of cholecystokinin? Scand J Gastroenterol 27:993-998.

Biegel LB, Liu RCM, Hurtt ME, Cook JC. Effects of ammonium perfluorooctanoate on Leydig cell function: in vitro, in vivo, and ex vivo studies. Toxicol Appl Pharmacol 1995;134:18-25.

Bryce H. Industrial and Utilitarian Aspects of Fluorine Chemistry. In: Simons J, ed. Fluorine Chemistry. New York:Academic Press, 1964:297-492.

Caldas C, Kern SE (1995). K-ras mutation and pancreatic adenocarcinoma. Int J Pancreatol 18:1-6.

Chey WY (1991). Regulation of pancreatic exocrine secretion. Int J Pancreatol 9:7-20.

Cook JC, Murray SM, Frame SR, Hurtt ME. Induction of Leydig cell adenomas by ammonium perfluorooctanoate: a possible endocrine-related mechanism. Toxicol Appl Pharmacol 1992;113:209-217.

Cook JC, Hurtt ME, Frame SR, Biegel LB. Mechanisms of extrahepatic tumor induction by peroxisome proliferators in Crl:CD BR (CD) rats. Toxicologist 1994;14:301 (abstract).

Ermak TH, Grendell JH (19??). The pancreas: anatomy, histology, embryology, and developmental anomalies. (In) Sleisenger M, Fordtran JS (eds) Gastrointestinal Diseases, Philadelphia:W.B. Saunders Co., pp 1573-1584.

Gasslander T, Axelson J, Hakanson R, Ihse I, Lilia I, Rehfeld JF. Cholecystokinin is responsible for growth of the pancreas after pancreaticobiliary diversion in rats. Scand J Gastroenterol 25:1060-1065.

Gavin CE, Martin NP, Schlosser MJ (1996). Absence of specific CCK-A binding sites on human pancreatic membranes. Toxicologist 30:334.

Gavin CE, Malnoske JA, White J, Schlosser MJ (1997). Species differences in expression of pancreatic chloecystokinin-A receptors. Toxicologist 36:1180 (abstract).

Griffith FD, Long JE. Animal toxicity studies with ammonium perfluorooctanoate. Am Ind Hyg Assoc J 1980;41:576-583.

Guy W. Fluorocompounds of human plasma: analysis, prevalence, purification, and characterization. Rochester, NY: University of Rochester, (Ph.D. disseration), 1972.

Hanhijarvi H, Phaug R, Singer L. The sex-related difference in perfluorooctanoate excretion in the rat. Proc Soc Exp Biol Med 1982;171:51-55.

Hanhijarvi H, Ylinen M, Kojo A, Kosma VM. Elimination and toxicity of perfluorooctanoic acid during subchronic administration in the Wistar rat. Pharmacol Toxicol 1987;61:66-68.

Howatson AG, Carter DC. Pancreatic carcinogenesis - enhancement of cholecystokinin in the hamster-nitrosamine model. Br J Cancer 51:1-7-114.

Herrington MK, Permert J, Kazakoff KR, Zucker KA, Bilchik AJ, Pour PM, Adrian TE. Effects of raw soya diet and cholecystokinin receptor blockade on pancreatic growth and tumor initiation in the hamster. Cacner Lett 82:7-16.

Herrington MK, Adrian TE (1995). On the role of cholecystokinin in pancreatic cancer. Int J Pancreatol 17:121-138.

Ikeda T, Aiba K, Fukuda K, Tanaka M . The induction of peroxisome proliferation in rat liver by perfluorinated fatty acids, metabolically inert derivatives of fatty acids. J Biochem 1985;98:475-482.

Johnson FE, LaRegina MC, Martin SA, Bshiti HM (1983). Cholecystokinin inhibits pancreatic and hepatic carcinogenesis. Cancer Detect Prev 6:389-402.

Kennedy G. Dermal toxicity of ammonium perfluorooctanoate. Toxicol Appl Pharmacol 1985;81:348-355.

Kennedy G, Hall G, Brittelli J, Chen H.. Inhalation toxicity of ammonium perfluorooctanoate. Fd Chem Toxicol 1986;24:1325-1329.

Kuslikis BI, Vanden Heuvel JP, Peterson RE. Lack of evidence for perfluorodecanoyl- or perfluorooctanoyl-coenzyme A formation in male and female rats. J Biochem Toxicol 1992;7:25-29.

Lemberger T, Desvergne B, Wahli W. Peroxisome proliferator-activated receptors: a nuclear receptor signaling pathway in lipid physiology. Ann Rev Cell Dev Biol 1996;12:335-363.

Liu RCM, Hurtt ME, Cook JC, Biegel LB. Effect of the peroxisome proliferator, ammonium perfluorooctanoate (C8) on hepatic aromatase activity in adult male CRl:CD BR (CD) rats. Fund Appl Toxicol 1996;30:220-228.

Longnecker DS (1986). Experimental models of exocrine pancreatic tumors. (In) Go VLW (ed) The Exocrine Pancreas: Biology, Pathology and Diseases, New York: Raven, pp 443-458.

Longnecker DS (1990). Experimental pancreatic cancer: role of species, sex and diet. Bull Cancer 77:27-37.

Longnecker DS (1991). Hormones and pancreatic cancer. Int J Pancreatol 9:81-86.

Lu L, Louie D, Owyang C (1989). A cholecystokinin releasing peptide mediates feedback regulation of pancreatic secretion. Am J Physiol 256:G430-G435.

Mainz DL, Black O, Webster PD (1973). Hormonal control of pancreatic growth. J Clin Invest 52:2300-2306.

McGuinness EE, Morgan GH, Levison DA, Frape DL, Hopwood D, Wormsley KG (1980). The effects of long-term feeding of soya flour on the rat pancreas. Scand J Gastroenterol 15:407-502.

Nickles M. Presence du fluor dans la sang. Compt Rend 1856;43:885.

Obourn JD, Frame SR, Elliot GS, Cook JC (1997a). Pancreatic oncogenic effects of Wyeth 14,643. Toxicologist 36:1181 (abstract).

Obourn JD, Frame SR, Elliot GS, Cook JC (1997b). Pancreatic oncogenic effects of Wyeth 14,643. Haskell Laboratory for Toxicology and Industrial Medicine, E.I. du Pont de Nemours & Company, (unpublished manuscript).

Olsen GW, Gilliland FD, Burlew MM, Burris JM, Mandel JS, Mandel JH. An epidemiologic investigation of reproductive hormones in men with occupational exposure to perfluorooctanoic acid. (Submitted for publication.)

Ophaug R, Singer L. Metabolic handling of perfluorooctanoic acid in rats. Proc Soc Exp Biol Med 1980;163:19-23.

Palmer-Smith J, Kramer ST, Solomon TE (1991). CCK stimulates growth of six human pancreatic cancer cell lines in serum-free medium. Regulatory Peptides 32:341-349.

Pandol SJ. Pancreatic physiology. (In) Sleisenger M, Fordtran JS (eds) Gastrointestinal Diseases, Philadelphia:W.B. Saunders Co., pp 1585-1600.

Pastoor TP, Lee KP, Perri MA, Gillies PJ. Biochemical and morphological studies of ammonium perfluorooctanoate-induced hepatomegaly and peroxisome proliferation. Exp Mol Pathol 1987;47:98-109.

Petersen H, Solomon TE, Grossman MI. Effect of chronic petnagastrin, cholecystokinin and secretin on pancreas of rats. Am J Physiol 234:E283-286.

Poston GJ, Gillspie J, Guillou PJ (1991). Biology of pancreatic cancer. Gut 32:800-812.

Pour P, Kruger FW, Althoff J, Cardesa A, Mohr U (1975). A new approach for induction of pancreatic neoplasms. Cancer Res 35:2259-2268.

Pour PM, Runge RG, Birt D, Gingell R, Lawson T, Nagel D, Wallcave L, Salmasi S (1981). Current knowledge of pancreatic carcinogenesis in the hamster and its relevance to the human disease Cancer 47:1573-1587.

Pour PM, Lawson T, Helgeson S, Donnelly T, Stepan K (1988). Effect of cholecystokinin on pancreatic carcinogenesis in the hamster model. Carcinogenesis 9:597-601.

Rao MS, Reddy JK. Hepatocarcinogenesis of peroxisome proliferators. Ann NY Acad Sci 1996;804:573-587.

Rehfeld JF, van Solinge WW (1994). The tumor biology of gastrin and cholecystokinin. Adv Cancer Res 63:295-347.

SAS Institute, Inc. SAS Users Guide: Statistics. Version 6. Cary, NC: SAS Institute, Inc., 1990.

Satake K, Mukai R, Kato Y, Umeyama K. Effects of cerulein on the normal pancreas and on experimental pancreatic carcinoma in the Syrian golden hamster. Pancreas 1:246-253.

Sibinski LJ. Two-year oral (diet) toxicity/carcinogenicity study of fluorochemical FC-143 in rats. St. Paul, MN:Riker Laboratories, 1987.

Singh P, Townsend CM, Upp J, Laridjani A, Thompson JC. Characterization of cholecystokinin receptors (CCK-r) in normal and cancerous human pancreas. Fed Proc 45:291.

Smith JP, Liu G, Soundararajan V, McLaughlin PJ, Zagon IS. Identificaiton and characterization of CCK-B/gastrin receptors in human pancreatic cancer cell lines. Am J Physiol 266:R277-R283.

Stewart ID, Flaks B, Watanapa P, Davies PW, Williamson RC. Pancreatobiliary diversion enhances experimental pancreatic carcinogenesis. Br J Cancer 63:63-66.

Takjad VD, Fortune KP, Pollo DA, Shah GN, Wank WA, Gardner JD (1994). Direct demonstration of three different states of the pancreatic cholecystokinin receptor. Proc Natl Acad Sci 91:1868-1872.

Taves D. Evidence that there are two forms of fluoride in human serum. Nature 1968:217:1050-1051.

Taves D. Electrophoretic mobility of serum fluoride. Nature 1968;220:582-583.

Vanden Heuvel J, Kuslikis B, Van Refelghem M, Peterson R. Tissue distribution, metabolism and elimination of perfluorooctanoic acid. J Biochem Toxicol 1991;6:83-92.

van Kranen HJ, Vermeulen E, Schoren L, Bas J, Woutersen RA, van Iersel P, van Kreijl CF, Scherer E (1991). Activation of c-K-*ras* is frequent in pancreatic carcinomas of Syrian hamsters, but is absent in pancreatic tumors of rats. Carcinogenesis 12:147-1482.

Wank SA, Pisegna JR, deWeerth A (1994). Cholecystokinin receptor family. Ann NY Acad Sci 713:49-66.

Ylinen M, Koho A, Hanhijarvi H, Peura P. Disposition of perfluorooctanoic acid in the rat after single and subchronic administration. Bull Environ Contam Toxicol 1990;44:46-53.

Zucker KA, Adrian TE, Bilchik AJ, Modlin IM. Effects of the CCK receptor antagonist L364,718 on pancreatic growth in adult and developing animals. Am J Physiol 257:G511-G516.

FINAL REPORT

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

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Study
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Protocol Number: EPI-0003
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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; Haughom 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 serlf-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, ≥ 1drink 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 CCK = 3.3 - 0.008 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 Obourn 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 peroxsisome 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 Obourn 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 $\geq$ 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.

REFERENCES

Anderson KE, Potter JD, Mack TM (1996). Pancreatic cancer. (In) Schottenfeld D, Fraumeni JF (eds) "Cancer Epidemiology and Prevention (2nd edition)." New York: Oxford University Press, pp 725-771.

Axelson J, Ihse I, Hakanson R (1992). Pancreatic cancer: the role of cholecystokinin? Scand J Gastroenterol 27:993-998.

Biegel LB, Liu RCM, Hurtt ME, Cook JC (1995). Effects of ammonium perfluorooctanoate on Leydig cell function: in vitro, in vivo, and ex vivo studies. Toxicol Appl Pharmacol 1995;134:18-25.

Bryce H (1964). Industrial and Utilitarian Aspects of Fluorine Chemistry. In: Simons J, ed. Fluorine Chemistry. New York:Academic Press, pages 297-492.

Burns CJ, Boswell JM, Olsen GW (1996). Liver enzyme activity and body mass index. J Occ Env Med 38:1248-1252.

Caldas C, Kern SE (1995). K-ras mutation and pancreatic adenocarcinoma. Int J Pancreatol 18:1-6.

Cattley RC, DeLuca J, Elcombe C, Fenner-Crisp P, Lake BG, Marsman DS, Pastoor TA, Popp JA, Robinson DE, Schwetz B, Tugwood J, Wahil W (1998). Do peroxisome proliferating compounds pose a hepatocarcinogenic hazard to humans? Reg Toxicol Pharmacol 27:47-60.

Cook JC, Murray SM, Frame SR, Hurtt ME (1992). Induction of Leydig cell adenomas by ammonium perfluorooctanoate: a possible endocrine-related mechanism. Toxicol Appl Pharmacol 1992;113:209-217.

Cook JC, Hurtt ME, Frame SR, Biegel LB (1994). Mechanisms of extrahepatic tumor induction by peroxisome proliferators in Crl:CD BR (CD) rats. Toxicologist14:301 (abstract).

Gavin CE, Martin NP, Schlosser MJ (1996). Absence of specific CCK-A binding sites on human pancreatic membranes. Toxicologist 30:334.

Gavin CE, Malnoske JA, White J, Schlosser MJ (1997). Species differences in expression of pancreatic chloecystokinin-A receptors. Toxicologist 36:1180 (abstract).

Gilliland FD, Mandel JS (1993). Mortality among employees of a perfluorooctanoic acid production plant. JOM 35:950-954.

Gilliland FD, Mandel JS (1996). Serum perfluorooctanoic acid and hepatic enzymes, lipoproteins and cholesterol: a study of occupationally exposed men. Am J Ind Med 129:560-568.

Griffith FD, Long JE (1980). Animal toxicity studies with ammonium perfluorooctanoate. Am Ind Hyg Assoc J 41:576-583.

Hanhijarvi H, Phaug R, Singer L (1982). The sex-related difference in perfluorooctanoate excretion in the rat. Proc Soc Exp Biol Med;171:51-55.

Hanhijarvi H, Ylinen M, Kojo A, Kosma VM (1987). Elimination and toxicity of perfluorooctanoic acid during subchronic administration in the Wistar rat. Pharmacol Toxicol 61:66-68.

Herrington MK, Adrian TE (1995). On the role of cholecystokinin in pancreatic cancer. Int J Pancreatol 17:121-138.

Haughom B, Spydevold O (1992). The mechanism underlying the hypolipemic effect of perfluoocatnoic acid (PFOA), perfluorooctane sulphonic acid (PFOSA) and clofibric acid. Biochim Biophys Acta 1128:65-72.

Johnson JD, Wolter JT, Colaizy GE, Rethwill PA, Nelson RM (1996). Quantification of perfluorooctanoate and perfluorooctanesulfonate in human serum using ion-pair extraction and high performance liquid chromatography-thermospray mass spectrometry with authomated sample preparation. 3M Environmental Laboartory Report. St. Paul:3M Company.

Just WW, Gorgas K, Hartl FU, Heinemann P, Salzer M, Schimassek H (1989). Biochemical effects and zonal heterogeneity of peroxisome proliferation induced by perfluorocarboxylic acids in rat liver. Hepatology 9:570-581.

Keller B, Marsman D, Popp J, Thurman R (1992). Several nongenotoxic carcinogens uncouple mitochnodrial oxidative phosphorylation. Biochim Biophys Acta 1102:237-244.

Kennedy G. Dermal toxicity of ammonium perfluorooctanoate. Toxicol Appl Pharmacol 1985;81:348-355.

Kennedy G, Hall G, Brittelli J, Chen H. (1986). Inhalation toxicity of ammonium perfluorooctanoate. Fd Chem Toxicol 24:1325-1329.

Longnecker DS (1986). Experimental models of exocrine pancreatic tumors. (In) Go VLW (ed) The Exocrine Pancreas: Biology, Pathology and Diseases, New York: Raven, pp 443-458.

Longnecker DS (1990). Experimental pancreatic cancer: role of species, sex and diet. Bull Cancer 77:27-37.

Longnecker DS (1991). Hormones and pancreatic cancer. Int J Pancreatol 9:81-86.

Obourn JD, Frame SR, Bell RH, Longnecker DS, Elliott GS, Cook JC (1997). Mechanisms for the pancreatic oncogenic effects of the peroxisome proliferator Wyeth-14,643. Toxicol Appl Pharmacol 145:425-436.

Olsen GW, Gilliland FD, Burlew MM, Burris JM, Mandel JS, Mandel JH (1998). An epidemiologic investigation of reproductive hormones in men with occupational exposure to perfluorooctanoic acid. J Occ Env Med 1998;40:614-622

Ophaug R, Singer L (1980). Metabolic handling of perfluorooctanoic acid in rats. Proc Soc Exp Biol Med 163:19-23.

Palmer-Smith J, Krame ST, Solomon TE (1991). CCK stimulates growth of six human pancreatic cancer cell lines in serum-free medium. Reg Peptides 22:341-349.

Pandol SJ (1998). Pancreatic physiology and secretory testing. (In) Sleisenger M, Fordtran JS (eds) Gastrointestinal and Liver Diseases, Volume 1, Philadelphia:W.B. Saunders Co., pp 771-782.

Pastoor TP, Lee KP, Perri MA, Gillies PJ (1987). Biochemical and morphological studies of ammonium perfluorooctanoate-induced hepatomegaly and peroxisome proliferation. Exp Mol Pathol 47:98-109.

Pour PM, Runge RG, Birt D, Gingell R, Lawson T, Nagel D, Wallcave L, Salmasi S (1981). Current knowledge of pancreatic carcinogenesis in the hamster and its relevance to the human disease Cancer 47:1573-1587.

Pour PM, Lawson T, Helgeson S, Donnelly T, Stepan K (1988). Effect of cholecystokinin on pancreatic carcinogenesis in the hamster model. Carcinogenesis 9:597-601.

Rao MS, Reddy JK (1996). Hepatocarcinogenesis of peroxisome proliferators. Ann NY Acad Sci 804:573-587.

Rehfeld JF, van Solinge WW (1994). The tumor biology of gastrin and cholecystokinin. Adv Cancer Res 63:295-347.

SAS Institute, Inc. (1990). SAS Users Guide: Statistics. Version 6. Cary, NC: SAS Institute, Inc.

Sibinski LJ (1987). Two-year oral (diet) toxicity/carcinogenicity study of fluorochemical FC-143 in rats. St. Paul, MN:Riker Laboratories.

Ubel F, Sorenson S, Roach D (1980). Health status of plant workers exposed to fluorochemicals: A preliminary report. Am Ind Hyg Assoc 41:584-589.

Vanden Heuvel J, Kuslikis B, Van Refelghem M, Peterson R (1991). Tissue distribution, metabolism and elimination of perfluorooctanoic acid. J Biochem Toxicol 6:83-92.

van Kranen HJ, Vermeulen E, Schoren L, Bas J, Woutersen RA, van Iersel P, van Kreijl CF, Scherer E (1991). Activation of c-K-*ras* is frequent in pancreatic carcinomas of Syrian hamsters, but is absent in pancreatic tumors of rats. Carcinogenesis 12:147-1482.

Wank SA, Pisegna JR, deWeerth A (1994). Cholecystokinin receptor family. Ann NY Acad Sci 713:49-66.

Ylinen M, Koho A, Hanhijarvi H, Peura P (1990). Disposition of perfluorooctanoic acid in the rat after single and subchronic administration. Bull Environ Contam Toxicol 44:46-53.

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

PFOA ppm	1993 Data Mean	SD	Range	1995 Data Mean	SD	Range	1997 Data Mean	SD	Range
				PFOA					
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
	F value = 248.5, p = .0001			*F value = 77.6, p = .0001*			*F value = 145.5, p = .0001*		
				Age					
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
	F value = 3.3, p = .02			*F value = 0.9, p = .46*			*F value = 0.3, p = .84*		
				BMI					
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
	F value = 1.1, p = .33			*F value = 0.8, p = .52*			*F value = 1.4, p = .24*		
				Alcohol					
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
	F value = 2.9, p = .04			*F value = 0.9, p = .43*			*F value = 0.7, p = .58*		
				Cigarettes					
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	-	-
	F value = 1.3, p = .26			*F value = 1.3, p = .29*			*F value = 1.7, p = .18*		
				CCK					
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
							F value = 2.5, p = .06		

Table L
continued

PFOA ppm	1993 Data Mean	SD	Range	1995 Data Mean	SD	Range	1997 Data Mean	SD	Range
				Alkaline Phosphatase					
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
	F value = 1.6, p = .19			*F value = 1.0, p = .39*			*F value = 0.9, p = .45*		
				GGT					
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
	F value = 1.0, p = .41			*F value = 0.6, p = .61*			*F value = 0.3, p = .91*		
				SGOT					
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
	F value = 1.1, p = .36			*F value = 0.5, p = .66*			*F value = 0.3, p = .84*		
				SGPT					
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
	F value = 0.4, p = .75			*F value = 0.9, p = .46*			*F value = 1.0, p = .42*		
				Total Bilirubin					
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
	F value = 0.7, p = .55			*F value = 1.5, p = .22*			*F value = 0.4, p = .76*		
				Direct Bilirubin					
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
	F value = 0.1, p = .94			*F value = 2.2, p = .10*			*F value = 0.2, p = .89*		

Table L
continued

PFOA ppm	1993 Data Mean	SD	Range	1995 Data Mean	SD	Range	1997 Data Mean	SD	Range
				Cholesterol					
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
	F value = 0.6, p = .65			*F value = 0.6, p = .63*			*F value = 1.8, p = .16*		
				HDL					
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
	F value = 2.0, p = .11			*F value = 0.9, p = .46*			*F value = 0.6, p = .62*		
				LDL					
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
	F value = 0.2, p = .91			*F value = 0.2, p = .92*			*F value = 1.5, p = .22*		
				Triglycerides					
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
	F value = 0.5, p = .67			*F value = 2.0, p = .13*			*F value = 1.4, p = .25*		
				BUN					
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
	F value = 0.9, p = .44			*F value = 0.4, p = .75*			*F value = 0.9, p = .44*		
				Creatinine					
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
	F value = 0.5, p = .68			*F value = 1.4, p = .25*			*F value = 1.1, p = .37*		

Table L
continued

PFOA ppm	1993 Data Mean	SD	Range	1995 Data Mean	SD	Range	1997 Data Mean	SD	Range
				Glucose					
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
	F value = 0.2, p = .87			*F value = 0.7, p = .59*			*F value = 0.9, p = .47*		
				Hematocrit					
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
	F value = 1.6, p = .20			*F value = 0.6, p = .63*			*F value = 0.5, p = .66*		
				Hemoglobin					
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
	F value = 1.9, p = .14			*F value = 1.0, p = 0.40*			*F value = 0.4, p = .79*		
				RBC					
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
	F value = 2.3, p = .08			*F value = 0.4, p = .78*			*F value = 1.4, p = .25*		
				Platelets					
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
	F value = 0.5, p = .72			*F value = 0.5, p = .72*			*F value = 1.0, p = .42*		

Table L
continued

PFOA	1993 Data			1995 Data			1997 Data		
ppm	Mean	SD	Range	Mean	SD	Range	Mean	SD	Range
				WBC					
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
	F value = 0.1, p = .99			*F value = 0.3, p = .82*			*F value = 1.0, p = .39*		

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

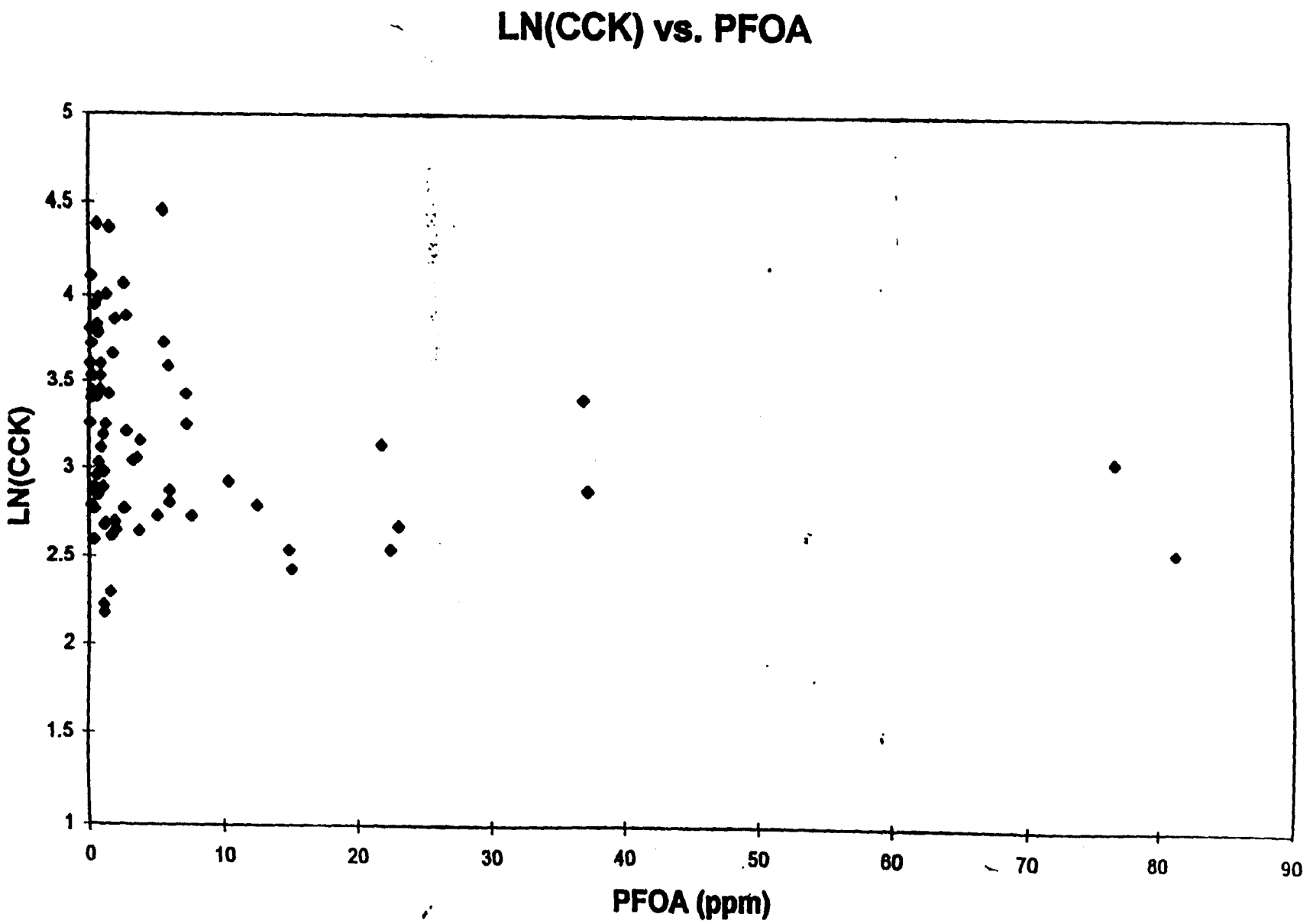
LN(CCK) vs. PFOA
LN(CCK)
5
4.5
4
3.5
3
2.5
2
1.5
1
0
10
20
30
40
50
60
70
80
90
PFOA (ppm)

Table II. Change in HDL* from Light Alcohol Drinker (< 1 drink/day) to Moderate Alcohol Drinker (≥ 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 toal 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/m^2)	25	30	35
SGOT			
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
SGPT			
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).