



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, $\geq$ 10 ppm) are different than those reported in the 3M final report (0 - < 1 ppm, 1 - < 10 ppm, 10 - < 30 ppm, $\geq$ 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.]

PLASMA CHOLECYSTOKININ AND HEPATIC ENZYMES, CHOLESTEROL AND LIPOPROTEINS IN AMMONIUM PERFLUOROCTANOATE 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 [$\text{CF}_3(\text{CF}_2)_6\text{CO}_2^-$], which is the anion of perfluorooctanoic acid [PFOA, $\text{CF}_3(\text{CF}_2)_6\text{COOH}$]. 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 ≥ 10 ppm.

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INTRODUCTION

Ammonium perfluorooctanoate [APFO; $\text{CF}_3(\text{CF}_2)_6\text{CO}_2\text{NH}_4^+$] is a potent synthetic surfactant used in industrial applications which rapidly dissociates in biologic media to perfluorooctanoate [$\text{CF}_3(\text{CF}_2)_6\text{CO}_2^-$], which is the anion of perfluorooctanoic acid [PFOA, $\text{CF}_3(\text{CF}_2)_6\text{COOH}$]. 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 Mandel²⁵ 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 data²⁵) 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 (≥ 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 ($BMI = 35 \text{ kg/m}^2$) but not non-obese ($BMI = 25 \text{ kg/m}^2$) 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 study¹⁷ 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 Mandel²⁵ 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 Mandel²⁵ 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.

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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				1995				1997			
	Mean	Median	S.D.	Range	Mean	Median	S.D.	Range	Mean	Median	S.D.	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				1995				1997			
	Mean	Median	S.D.	Range	Mean	Median	S.D.	Range	Mean	Median	S.D.	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				1995				1997			
	Mean	Median	S.D.	Range	Mean	Median	S.D.	Range	Mean	Median	S.D.	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

Variable	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	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
$R^2 = .17$				$R^2 = .10$			$R^2 = .30$			$R^2 = .11$		
Adj $R^2 =$				Adj $R^2 = .02$			Adj $R^2 = .19$			Adj $R^2 = .03$		
Not reported												

* 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 ($\geq$ 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
<u>Year</u>	<u>Change in HDL (mg/dl)</u>		
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		
	<i>B</i>	<i>SE(B)</i>	<i>p</i> value	<i>B</i>	<i>SE(B)</i>	<i>p</i> value	<i>B</i>	<i>SE(B)</i>	<i>p</i> value	<i>B</i>	<i>SE(B)</i>	<i>p</i> 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
<u>Year</u>	<u>Change in ALT (IU/L)</u>		
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 Natural Log CCK (pg/ml) by Serum PFOA Level (ppm)
[Linear Regression Model: $\ln \text{CCK} = 3.24 - 0.006\text{PFOA}$;
p value of PFOA coefficient = 0.19; $R^2 = .02$]

