

A 01

CODING FORMS FOR SRC INDEXING

Microfiche No.			OTS0559843								
New Doc ID		89000000084		Old Doc ID		8EHQ-0200-14596					
Date Produced		09/04/98		Date Received		02/08/00		TSCA Section		8E	
Submitting Organization			3M CO								
Contractor											
Document Title			SUPPORT: AN EPIDEMIOLOGIC INVESTIGATION OF PLASMA CHOLE- CYSTOKININ AND HEPATIC FUNCTION IN PERFLUOROCTANOIC ACID PRODUCTION WORKERS, WITH COVER LETTER DATED 012800								
Chemical Category			PERFLUOROCTANOATE								

B 01

A 02

A

S

SUPPORT

A 03

Larry B. Zobel, M.D., MPH
Staff Vice President
and Medical Director

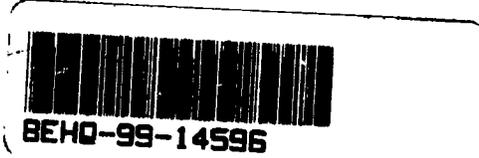
3M Medical Department

9EHQ-0200-14596

3M Center, Building 220-2E-02
PO Box 33220
St. Paul, MN 55133-3220
651 733 5181 Office
651 733 5152 Facsimile

3M

January 28, 2000



RECEIVED
OPPT/CBIC
2000 FEB -8 AM 11:46

Document Control Office (7407)
Room G99 East Tower Attn: 8(e)
Office of Pollution Prevention and Toxics
US Environmental Protection Agency
401 M Street, SW
Washington, DC 20460-0001

MR 32051

RE: 8EHQ-1199-14596 *dcw*



Dear Mr. O'Bryan:

Four papers are attached in response to your request (8EHQ-1199-14596) for a description and results of the 3M medical surveillance program for employees exposed to perfluorooctanoate. When these papers refer to total organic fluorine, it is believed to be primarily perfluorooctanoate both on the basis of known exposure and later direct measurement of perfluorooctanoate. These papers present the most recent results of a program that began in the mid seventies.

Following reports of the finding of "organic fluorine" in sera samples, and the tentative identification of perfluorooctanoic acid as a component of this organic fluorine by Taves in 1976, sera measurements and medical surveillance began at 3M's perfluorooctanoate production facility in Cottage Grove, Minnesota. The surveillance program has generally consisted of annual or biannual tests of clinical chemistries, pulmonary function, chest xray, blood counts and a biomonitor of fluorochemical exposure. A "total organic fluorine" measurement was done until 1993. This measures the amount of fluorine that was covalently bound to carbon in the serum sample. In 1993 and later, perfluorooctanoate was measured directly by a method developed by 3M scientists and transferred to an outside laboratory.

When test data were available, a company physician reviewed each employee's results. These physicians did not, and have not, found abnormalities in individuals that they felt were related to fluorochemical exposure. That is, medical conditions, medications and lifestyle factors adequately explained the laboratory abnormalities (which one expects to find in this type of program). Aggregate analyses of clinical data have also been conducted. Detailed technical reports are available beginning with the 1990 medical surveillance examinations, and are summarized in the attached papers. The findings specifically indicate no significant clinical hepatic toxicity associated with the perfluorooctanoate levels observed in this workforce.

RECEIVED
OPPT/HCIC
00 MAR -1 AM 11:19

Contain NO CBI

B 03

B

Mortality Among Employees of a Perfluorooctanoic Acid Production Plant

Frank D. Gilliland, MD, PhD
Jack S. Mandel, PhD, MPH

Perfluorooctanoic acid (PFOA) has been found at low levels (10 to 100 parts per billion) in sera of the general population and at higher levels in occupationally exposed workers. Although PFOA has been reported to be a promoter of rodent hepatocarcinogenesis and to alter reproductive hormones in humans and rodents, there is little information on human health effects associated with PFOA exposure. The present study examined the relationship between PFOA and mortality using a retrospective cohort mortality design. The cohort consisted of 2788 male and 749 female workers employed between 1947 and 1983 at a plant that produced PFOA. The all-causes standardized mortality ratio was .75 (95% confidence interval [CI], .56 to .99) for women and .77 (95% CI, .69 to .86) for men. Among men the cardiovascular standardized mortality rate was .68 (95% CI, .58 to .80) and the all-gastrointestinal diseases was .57 (95% CI, .29 to .99). There was no significantly increased cause-specific standardized mortality ratio for either men or women. Ten years of employment in exposed jobs was associated with a 3.3-fold increase (95% CI, 1.02 to 10.6) in prostate cancer mortality compared to no employment in PFOA production. There were only six prostate cancer deaths overall and four among the exposed workers; thus, the results must be interpreted cautiously. If prostate cancer mortality is related to PFOA, PFOA may increase prostate cancer mortality by altering reproductive hormones in male workers.

Perfluorooctanoic acid (PFOA) and its salt, ammonium perfluorooctanoate, are perfluorinated surfactants. Because of their unique surface active properties they are used in a large number of industrial applications and consumer products including plasticizers, lubricants, wetting agents, and emulsifiers.¹⁻³ Despite their widespread use, little is known about potential adverse health effects.

PFOA induced marked hepatomegaly and peroxisome proliferation in rodent livers.³⁻⁶ The chemically diverse group of xenobiotics that induce peroxisomes is of concern because of its association with nongenotoxic hepatocarcinogenesis.⁷⁻¹⁰ PFOA did not produce an increased number of hepatocellular carcinomas in a 2-year rat feeding study.⁸ However, biphasic (initiation and promotion) and triphasic (initiation, selection, and promotion) hepatic carcinogenesis studies in rodents have shown significantly increased numbers of carcinomas in the PFOA-treated rats.^{11,12} It has been suggested that the marked rodent hepatomegaly produced by PFOA is a marker for carcinogenic potential.¹³ The observations of increased Leydig cell tumors in a 2-year rat PFOA feeding study and of disruption of the hypothalamic-pituitary-gonad axis in PFOA-treated rats¹⁴ are consistent with the hypothesis that PFOA-associated tumors are mediated by a hormonal nongenotoxic mechanism.

PFOA has a long half-life in humans. A study of occupationally exposed workers showed that the half-life in men is greater than 1.5 years.¹⁵ Hence, accumulation of PFOA may occur from small, frequent PFOA doses. PFOA in the serum of the gen-

From the Division of Environmental and Occupational Health, School of Public Health, University of Minnesota, Minneapolis, Minnesota (Dr Gilliland, Dr Mandel); and Department of Internal Medicine, Occupational and Environmental Medicine Section, St Paul Ramsey Medical Center, St Paul, Minnesota (Dr Gilliland).

Address correspondence to: Frank D. Gilliland, MD, University of New Mexico School of Medicine, New Mexico Tumor Registry, 990 Camino de Salud NE, Albuquerque, NM 87131, 0096-1736/93/3509-0950\$03.00/0.

Copyright © by American College of Occupational and Environmental Medicine

Contain NO CR

and populations of industrialized countries" is likely to be the result of an accumulation of small PFOA doses.

No health problems related to PFOA exposure were observed in a cross-sectional study among workers employed at the PFOA production plant.²¹ Cross-sectional studies of PFOA-exposed workers at this plant have shown that PFOA was associated with decreased free testosterone and increased estradiol.²⁰

To determine whether mortality from any cause was associated with occupational exposure to PFOA, a retrospective cohort mortality study was conducted at a plant that has produced PFOA since 1947.

Methods

The plant consists of several divisions, with PFOA production restricted to the Chemical Division. A number of other specialty chemicals have been produced in this division. The study cohort consisted of workers who were employed at the plant for at least 6 months between Jan 1, 1947, and Dec 31, 1983. Data were abstracted from plant personnel records, which were maintained on all workers ever employed at the plant. Vital status was ascertained from the Social Security Administration for the period 1947 to 1982 and from the National Death Index for the period 1979 to 1989. All workers with unknown vital status were traced using a variety of tracing strategies such as directory assistance, Metronet and TRW searches, reverse directories, motor vehicle registration lists, contacting neighbors and relatives, and the post offices. Death certificates were obtained from the appropriate state health departments for those identified as, or presumed to be, deceased. Information concerning the data and cause of two deaths which occurred outside the United States was obtained from family members. A nosologist coded the death certificates for underlying cause of death according to the International Classification of Diseases, 8th revision. The reliability of the coding was evaluated by resubmitting a random sample of

death certificates for coding by the same nosologist. In the 25 death certificates from 1970 to 1989 resubmitted to the nosologist for ICD coding, there were no changes in the major categories of cause of death.

Workers were categorized as exposed or unexposed to PFOA based on their job histories. Exposed workers were defined as all workers employed for 1 month or more in the Chemical Division. Unexposed workers were employees who either never worked in the Chemical Division or worked in the Chemical Division for less than 1 month. Cumulative exposure to PFOA was estimated using the surrogate measure of months of Chemical Division employment.

The observed numbers of cause-specific deaths were compared to the expected numbers of deaths obtained by applying sex- and race-specific quinquennial age, calendar period, and cause-specific mortality rates for the United States and Minnesota populations to the distribution of observed person-time.^{21,22} Because less than 1% of plant employees were non-white, white male and white female rates were used for comparison. For women, only United States rates were used because cause- and calendar period-specific Minnesota rates for women were not available. The effects of latency, duration of employment, and work in the Chemical Division were examined using stratified standardized mortality ratio (SMR) analyses. Cause-specific mortality rates were compared between exposed and unexposed workers using stratified SMRs.²³ SMRs were calculated for

men based on US and Minnesota white male mortality rates for three latency intervals (10, 15, and 20 years) and three categories of duration of employment (5, 10, and 20 years). The SMRs were calculated using the program developed by Monson.²³

The relative risk (RR) and 95% confidence interval (CI) for deaths from all causes, cancer, cardiovascular diseases, and other selected causes were estimated using proportional hazard models.^{24,25} The time to event or censoring was defined as time from first employment to event or to December 31, 1989. In models for specific causes of death, deaths from other causes were censored at the time of death. Age at first employment, year of first employment, and duration of employment were included as covariates in the model. The analyses were stratified by gender. The appropriateness of the proportional hazard assumptions was tested using stratified models with graphical analysis of $\log(-\log(\text{survival}))$ versus follow-up time relationships and models that tested the significance of a product term between exposure and log follow-up time.^{24,25} Proportional hazard calculations were conducted using SAS.²⁶

Results

A total of 3537 workers employed at the plant between Jan 1, 1947 and Dec 31, 1983 were identified from company records. Six workers who had incomplete employment records were excluded from the study. The cohort consisted of 2788 (79%) men and 749 (21%) women (Table 1). Men

TABLE 1
Characteristics of Female and Male Employees, 1947-1989

	Chemical Division		Non-Chemical Division		Total	
	Female	Male	Female	Male	Female	Male
Number of workers	245	1339	504	1449	749	2788
Person-years of observation	6629.0	33385.3	13280.4	37732.4	19309.4	71117.7
Mean follow-up (y)	24.6	24.8	26.4	26.0	25.8	25.5
Mean age at employment (y)	28.8	25.6	26.9	28.9	27.6	27.3
Mean year of death	1965.0	1963.8	1962.8	1962.3	1963.5	1963.0
Mean year of death	1981.3	1978.3	1979.2	1978.1	1979.6	1978.2
Mean age at death (y)	58.7	54.2	54.4	58.1	55.4	56.4

contributed 71,117 person-years of observation, which were equally divided between the Chemical Division and non-Chemical Division. Women contributed 19,309.4 person-years, two-thirds of which were in the non-Chemical Division.

Vital status was obtained for 100% of the cohort (Table 2). There were 50 deaths among the women (11 in the Chemical Division cohort and 39 in the non-Chemical Division cohort) and 348 deaths among the men (148 deaths in the Chemical Division group and 200 in the non-Chemical Division group). Death certificates were obtained for 99.5% of deaths.

For women, the SMR for all causes of death (SMR = .75; 95% CI, .56 to .99) was significantly lower than expected (Table 3). There was no association with duration of employment or latency for deaths from all-causes, cancer, and cardiovascular diseases (data not shown). Mortality among Chemical Division women was less than expected. In Chemical Division women, the all-causes SMR was .46 (95% CI, .23 to .86) and the cancer

SMR was .36 (95% CI, .07 to 1.05). The all-causes SMR for the non-Chemical Division women was .91 (95% CI, .64 to 1.24) and the cancer SMR was .91 (95% CI, .49 to 1.52) (data not shown).

Using Minnesota rates for comparison, the SMR for men for all causes, for cardiovascular diseases, and for all gastrointestinal diseases was significantly less than 1 (Table 4). None of the cause-specific SMRs was large nor was any significantly different from 1. The results were similar when the expected numbers of male deaths were based on US mortality rates. For the three latency intervals, the SMRs for deaths from all causes ranged from .75 to .77. For all cancers, the SMRs ranged from 1.06 to 1.12 and were nonsignificant.

Among men, there was no association between any cause of death and duration of plant employment. The all-causes SMRs were .86 (95% CI, .72 to 1.01) for the Chemical Division group and .69 (95% CI, .59 to .79) for the non-Chemical Division group (data not shown). The SMRs for pros-

tate cancer were 2.03 (95% CI, .55 to 4.50) in the Chemical Division group and .58 (95% CI, .07 to 2.09) in the non-Chemical Division cohort. In the Chemical Division group, there were 4 observed and 2 expected deaths from prostate cancer. There was no significant association between any cause of death and latency in either exposure group. For the Chemical Division cohort, the prostate cancer SMR was 1.61 (95% CI, .32 to 4.70) in the greater than 15-year latency group.

Table 5 presents the final proportional hazard model for all-causes, all-cancer, and prostate-cancer mortality among the 2788 male workers employed for more than 6 months. The estimated relative risk for all-cause mortality for a 1-year increase in age at first employment was 1.08 (95% CI, 1.07 to 1.09). Year of first employment and duration of employment were negatively associated with deaths from all causes. The risk associated with months employed in the Chemical Division was small and nonsignificant.

In the final prostate cancer mortality model, length of employment in the Chemical Division was positively and significantly associated with prostate cancer risk. The relative risk for a 1-year increase in Chemical Division employment time was 1.13 (95% CI, 1.01 to 1.27). For 10 years' employment in the Chemical Division, the relative risk was estimated to be 3.3 (95% CI, 1.02 to 10.6) compared with workers never employed in the Chemical Division. Age at first employment was positively associated with prostate cancer mortality. Length of time employed in the Chemical Division was not significantly related to mortality from lung cancer, gastrointestinal cancer, pancreatic cancer, or diabetes mellitus.

Discussion

This was the first retrospective cohort mortality study of workers employed in a PFOA production plant. Mortality from all causes in both men and women was significantly less than expected. Because of the healthy worker effect, internal comparisons

TABLE 2
Vital Status and Cause of Death Ascertainment among Female and Male Employees, 1947-1989

Vital Status	Chemical Division				Non-Chemical Division				Total			
	Female		Male		Female		Male		Female		Male	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Alive	234	95.3	1191	88.9	465	91.6	1249	86.2	699	93.3	2440	87.5
Dead	11	4.7	148	11.1	39	8.4	200	13.8	50	6.7	348	12.5
Total	245	100.0	1339	100.0	504	100.0	1449	100.0	749	100.0	2788	100.0

TABLE 3
Observed (Obs) and Expected (Exp) Deaths, Standardized Mortality Ratios (SMR) and 95% Confidence Intervals (CI) for 749 Female Employees

Cause of Death	Obs	Exp	SMR	95% CI
All causes	50	66.74	0.75	0.56-0.99
Cancer	17	23.04	0.71	0.42-1.14
Gastrointestinal	2	4.54	0.44	0.05-1.59
Respiratory	4	4.72	0.95	0.26-2.43
Breast	3	5.87	0.51	0.10-1.49
Genital	2	3.37	0.59	0.07-2.14
Lymphopietic	3	2.04	1.47	0.30-4.29
Cardiovascular	10	12.39	0.81	0.49-1.29
Cerebrovascular	3	3.51	0.86	0.01-4.80
Gastrointestinal	3	3.41	0.88	0.18-2.57
Injuries	4	6.23	0.64	0.17-1.64
Suicide	1	1.78	0.56	0.01-3.13

and Minnesota...
ity rates for three...
. 15, and 20 years...
s of duration of...
0, and 20 years...
culated using the...
by Monson...
(RR) and 95%...
(CI) for deaths...
of cardiovascular...
selected causes...
ing proportional...
e time to event...
ed as time from...
event or to De...
models for spe...
n, deaths from...
ored at the time...
t employment...
ent, and dura...
included as...
l. The analyses...
er. The appro...
rtional hazard...
d using stratifi...
rtial analysis of...
rsus follow-up...
models that...
of a product...
and log follo...
tional hazard...
ducted using

ers employed...
1, 1947 and...
ntified from...
orkers who...
ent records...
study. The...
(79%) men...
ble 1). Men

Male...
2788...
71117.7...
25.5...
27.3...
1963.0...
1978.2...
56.4

TABLE 4
Deaths and Standardized Mortality Ratios (SMR) Based on Minnesota White Male Rates, Among 2788 Male Employees 1947-1989 and 1339 Men Ever Employed in the Chemical Division, 1947-1989

Causes of Death	All Male Employees				Men Employed in Chemical Division			
	Obs	Exp	SMR	95% CI	Obs	Exp	SMR	95% CI
All causes	347	450.79	0.77	0.69-0.86	148	172.96	0.86	0.72-1.01
Cancer	103	97.29	1.05	0.86-1.27	40	36.31	1.10	0.79-1.50
Gastrointestinal	24	26.78	0.90	0.57-1.33	9	9.77	0.92	0.42-1.75
Colon	9	9.42	0.96	0.44-1.81	4	3.46	1.15	0.31-4.01
Pancreas	8	5.58	1.43	0.62-2.83	4	2.04	1.96	0.53-5.01
Respiratory	31	30.42	1.02	0.69-1.45	12	11.26	1.07	0.55-1.86
Lung	29	28.94	1.00	0.67-1.44	11	10.70	1.03	0.51-1.84
Prostate	6	6.07	0.99	0.36-2.15	4	1.97	2.03	0.55-4.59
Testis	1	0.92	1.09	0.01-6.05	1	0.44	2.28	0.03-12.66
Bladder	3	2.18	1.37	0.28-4.01	1	0.75	1.33	0.02-7.40
Lymphopoietic	13	12.07	1.09	0.57-1.84	5	4.76	1.05	0.34-2.45
Cardiovascular	145	212.19	0.68	0.58-0.80	54	76.65	0.70	0.53-0.92
CHD*	110	159.09	0.69	0.57-0.83	43	57.74	0.74	0.54-1.00
Cerebrovascular	10	24.66	0.60	0.32-1.02	4	8.53	0.47	0.13-1.20
All gastrointestinal	12	21.13	0.57	0.29-0.99	8	8.27	0.97	0.42-1.91
All respiratory	13	21.75	0.60	0.32-1.06	7	7.77	0.91	0.36-1.87
Diabetes	8	6.52	1.23	0.53-2.42	3	2.55	1.18	0.24-3.44
Injuries	38	47.74	0.80	0.56-1.08	31	31.72	0.98	0.66-1.39
Suicide	12	15.09	0.79	0.41-1.39	10	6.99	1.43	0.68-2.63

*CHD, coronary and atherosclerotic heart disease.

were made between Chemical Division and non-Chemical Division employees. There were no significantly elevated SMRs in Chemical Division or non-Chemical Division employees. However, prostate cancer mortality was associated with length of employment in the Chemical Division in proportional hazard analysis. Ten years of employment in the Chemical Division was associated with an estimated 3.3-fold increase (95% CI, 1.02 to 10.60) in prostate cancer mortality. The use of prostate cancer mortality

to assess the association between PFOA and prostate cancer occurrence is problematic. Age-adjusted prostate cancer mortality rates from 1983 to 1989 (949 per 100,000) were only 25% of the incidence rates (99.4).²⁷ This low proportion of deaths among cases attributed to prostate cancer reflects the high risk of death for competing causes for this disease of elderly men. Given the small number of observed deaths from prostate cancer in the study, and the observed difference in incidence and mortality rates, the

suggested association between PFOA exposure and prostate cancer must be viewed as hypothesis generating and should not be overinterpreted. The association may be real, may have been a chance finding, or may be the result of an unrecognized environmental factor. However, the biologic plausibility for any association between PFOA employment and prostate cancer is provided by animal toxicologic and human epidemiologic data that show an association between PFOA and reproductive hormone changes.²⁰

The all-causes, all-cancer, and all-cardiovascular mortality among women was less than expected in the overall cohort. The low SMRs are most likely to be a result of the healthy worker effect. Latency and duration of plant employment did not have a strong relationship with the healthy worker effect.

The interpretation of this study requires consideration of methodological issues. SMRs for the subgroups of workers are not strictly comparable. We attempted to calculate standardized rate ratios; however, the rates were based on small numbers and produced unstable ratios. Estimates of PFOA exposure were based on job history, and categorization of workers into ever versus never employed in the Chemical Division may not reflect the biologic effective dose of PFOA. PFOA exposure was apparently widespread among employees not directly exposed to PFOA,¹⁴ and the exposure categorization may misclassify workers as unexposed when they were ex-

TABLE 5
Proportional Hazard Regression Model of Factors Predicting Mortality among All Male Employees*

Variable	All Causes of Death				Cancer Deaths				Prostate Cancer Deaths			
	β	SE(β)	P	RR†	β	SE(β)	P	RR†	β	SE(β)	P	RR†
Year of first employment	-0.55	0.009	0.0001	0.946	-0.031	0.019	0.11	0.969	0.010	0.081	0.9	1.011
Age at first employment (y)	0.079	0.006	0.0001	1.08	0.078	0.011	0.0001	1.081	0.082	0.045	0.06	1.085
Duration of employment (y)	-0.34	0.001	0.0001	0.967	-0.028	0.009	0.002	0.972	-0.07	0.052	0.18	0.932
Months in chemical division	0.001	0.001	0.24	1.001	0.002	0.001	0.2	1.002	0.01	0.005	0.03	1.01

* Abbreviations used are: β , regression parameter; SE(β), standard error of the slope parameter; RR, relative risk
† Relative risk for one unit change in independent variable

posed. Such misclassification would be expected to bias the effect estimates toward the null if increased exposure increases death rates. Months employed in the Chemical Division may better reflect the biologic effective dose because cumulative exposure reflects the bioaccumulation of PFOA. Workers were exposed to many other xenobiotics, such as benzene and asbestos, during their employment at the plant. However, none of these materials has been associated with prostate cancer.

Although the mean age at first employment and mean year of first employment are similar in the Chemical Division and non-Chemical Division cohorts of men and women, the comparisons of the rates of disease are confounded by differences in the distribution of age at risk. The use of an internal comparison group may reduce, but not eliminate, confounding if the internal comparison groups have different distributions of these time factors. Because the disease occurrence relationship is defined in terms of cumulative exposure, the true effect of PFOA exposure may have been biased toward or away from the null by uncontrolled confounding by time factors.^{28,29}

Further research is needed to evaluate and confirm the association between PFOA and prostate cancer. The findings in this study are based on a small number of cases and could have resulted from chance or unrecognized confounding from exposure to other factors. Studies of prostate cancer incidence in this and other PFOA-exposed work forces may clarify the suggested increase in prostate cancer risk.

Acknowledgments

This work was supported in part by National Institute for Occupational Safety and Health Grant T150H07098-10, and the 3M Corporation.

References

1. Guenther R, Vietor M. Surface active materials from perfluorocarboxylic and

Gilliland & Mandel - Mortality in a PFOA Production Plant

2. perfluorosulfonic acid. *IAAC. Technical Research and Development* 1967; 165-169.
3. Goffith I, Long J. Animal toxicity studies with ammonium perfluorooctanoate. *Arch Ind Hyg Toxicol* 1980;41:576-583.
4. Olson C, Andersen M. The acute toxicity of perfluorooctanoic and perfluorodecanoic acids in male rats and their effects on fatty tissue. *Toxicol Appl Pharmacol* 1983;70:362-372.
5. Pastoor T, Lee K, Perri M, Gillies P. Biochemical and morphological studies of ammonium perfluorooctanoate-induced hepatomegaly and peroxisome proliferation. *Exp Mol Pathol* 1987;47:98-109.
6. Just W, Gorgas K, Hartl F, Heinemann P, Salazar M, Schimasek H. Biochemical effects and zonal heterogeneity of peroxisome proliferation induced by perfluorocarboxylic acids in rat liver. *Hepatology* 1989;9:570-581.
7. Kennedy G. Dermal toxicity of ammonium perfluorooctanoate. *Toxicol Appl Pharmacol* 1985;81:348-355.
8. Kennedy G, Hall G, Brittelli J, Chen H. Inhalation toxicity of ammonium perfluorooctanoate. *Food Chem Toxicol* 1986;24:1325-1329.
9. Cook J, Murray S, Frame S, Hunt M. Induction of Leydig cell adenomas by ammonium perfluorooctanoate: A possible endocrine related mechanism. *Toxicol Appl Pharmacol* 1991;113:209-213.
10. Reddy J, Azarnoff D, Hignite C. Hypolipidemic hepatic peroxisome proliferators form a novel class of chemical carcinogens. *Nature* 1980;283:397-398.
11. Reddy J, Lalwani N. Carcinogenesis by hepatic peroxisome proliferators: evaluation of the risk of hypolipidemic drugs and industrial plasticizers to humans. *Crit Rev Toxicol* 1983;12:1-85.
12. Nilsson R, Beije B, Preat V, Ersson K, Ramel C. On the mechanism of the hepatocarcinogenicity of peroxisome proliferators. *Chem Biol Interact* 1991; 78:235-250.
13. Abdellatif A, Preat V, Vamecq J, Nilsson R, Roberfroid M. Peroxisome proliferation and modulation of rat liver carcinogenesis by 2,3-dichlorophenoxyacetic acid, 2,4,5-trichlorophenoxyacetic acid, perfluorooctanoic acid and nafenopin. *Carcinogenesis* 1990;11:1899-1902.
14. Takagi A, Sai K, Umemura T, Hasegawa R, Kurokawa Y. Short-term exposure to the peroxisome proliferators, perfluorooctanoic acid and perfluorodecanoic acid, causes significant increases of 8-hydroxydeoxyguanosine in liver DNA of rats. *Cancer Lett* 1991;57:55-60.
15. Uhlir J, Sorenson S, Reuch D. Health status of plant workers exposed to fluorocarbon chemicals: a preliminary report. *Arch Ind Hyg Toxicol* 1980;41:584-589.
16. Guy W. Fluorocompounds of human plasma: analysis, prevalence, purification, and characterization. PhD thesis. Rochester, NY: University of Rochester; 1972.
17. Guy W, Taves D, Brey W. Organic fluorocompounds in human plasma: prevalence and characterization. In: Filler R, ed. *Biochemistry involving carbon-fluorine bonds ACS Symposium Series*. New York: American Chemical Society; 1976:117-134.
18. Guy W. Ionic and organic fluorine in blood. In: Johansen E, Taves D, Olsen T, ed. *Continuing evaluation of the use of fluoride*. Boulder, CO: Westview Press; 1979.
19. Taves D. Evidence that there are two forms of fluoride in human serum. *Nature* 1968;217:1050-1051.
20. Taves D. Comparison of "organic" fluoride in human and nonhuman serums. *J Dent Res* 1971;50:783.
21. Gilliland F. Fluorocarbons and human health: studies in an occupational cohort. PhD thesis. Minneapolis, MN: University of Minnesota; 1992.
22. Berry G. The analysis of mortality by the subject-years method. *Biometrics* 1983; 39:173-184.
23. Monson R. Analysis of relative survival and proportional mortality. *Comput Biomed Res* 1974;7:325-332.
24. Miettinen O. Standardization of risk ratios. *Am J Epidemiol* 1972;96:383-388.
25. Cox D. Regression models and life tables. *J R Stat Soc (B)* 1972;34:187-220.
26. SAS Institute. SAS User's Guide: Statistics. 6th ed. Cary, NC: SAS Institute Inc; 1990.
27. Kalbfleisch J, Prentice R. *The statistical analysis of failure time data*. New York: John Wiley & Sons; 1980.
28. Miller R. Cancer statistics review 1973-1989. Bethesda, Maryland: US Department of Health and Human Services, National Institutes of Health, National Cancer Institute. NIH Pub. No. 92-2789; 1992.
29. Checkoway H, Pierce N, Crawford-Brown D. Research methods in occupational epidemiology. New York: Oxford University Press; 1989.
30. Robins J. A new approach to causal inference in mortality studies with a sustained exposure period. *Math Model* 1986;7:1393-1512.

Serum Perfluorooctanoic Acid and Hepatic Enzymes, Lipoproteins, and Cholesterol: A Study of Occupationally Exposed Men

Frank D. Gilliland, MD, PhD and Jack S. Mandel, PhD, MPH

Perfluorooctanoic acid (PFOA) produces marked hepatic effects, including hepatomegaly, focal hepatocyte necrosis, hypolipidemia, and alteration of hepatic lipid metabolism in a number of animal species. In rodents, PFOA is a peroxisome proliferator, an inducer of members of the cytochrome P450 superfamily and other enzymes involved in xenobiotic metabolism, an uncoupler of oxidative phosphorylation, and may be a cancer promoter. Although PFOA is the major organofluorine compound found in humans, little information is available concerning human responses to PFOA exposure. This study of 115 occupationally exposed workers examined the cross-sectional associations between PFOA and hepatic enzymes, lipoproteins, and cholesterol. The findings indicate that there is no significant clinical hepatic toxicity at the PFOA levels observed in this study. PFOA may modulate the previously described hepatic responses to obesity and xenobiotics. © 1996 Wiley-Liss, Inc.

KEY WORDS: perfluorooctanoic acid, human, hepatic enzymes, cholesterol, HDL

INTRODUCTION

Perfluorooctanoic acid (PFOA) is a potent synthetic surfactant that is used in a wide variety of industrial processes and products. Organic fluorine has been found in the serum of all human populations studied (Ubel et al., 1980; Taves, 1971; Taves et al., 1976; Guy, 1979; Belisle, 1981). Guy and Taves reported that PFOA was the principal organic fluorine compound in human serum (Taves, 1971; Taves et al., 1976; Guy, 1979). PFOA is found in serum because PFOA has a long biological half-life, allowing accumulation of small doses over time (Ubel et al., 1980)

Little is known about the toxic potential of PFOA in humans; however, studies have shown that the liver is an important site of toxicity in animals (Griffith and Long, 1980; Kennedy, 1985; Kennedy et al., 1986; Pastoor et al., 1987; Van Raefelghem et al., 1987; Just et al., 1989).

Animals treated with PFOA rapidly develop hepatomegaly with focal necrosis and show marked hepatic physiologic responses that include hypolipidemia, peroxisome proliferation, induction of xenobiotic metabolic enzymes, increased hepatic tumor incidence, uncoupling of mitochondrial oxidative phosphorylation, and alterations in lipid metabolism (Griffith and Long, 1980; Kennedy, 1985; Kennedy et al., 1986; Pastoor et al., 1987; Van Raefelghem et al., 1987; Just et al., 1989; Takagi et al., 1991; Permadi et al., 1992; Haughom et al., 1992; Sohlenius et al., 1992; Keller et al., 1992; Handler, 1992). Rats treated with PFOA and other peroxisome proliferators (PPs), such as clofibrate, show a 50% reduction of serum cholesterol and changes in the hepatic production and processing of lipoproteins. Haughom et al. (1992) showed that the hypolipidemic response results from downregulation of HMG-CoA reduc-

Division of Environmental and Occupational Health, School of Public Health, University of Minnesota, Minneapolis (F.D.G., J.S.M.)

Department of Internal Medicine, Occupational and Environmental Medicine Section, St. Paul Ramsey Medical Center, St. Paul, Minnesota (F.D.G.)

Address reprint requests to Frank D. Gilliland, University of New Mexico School of Medicine, Epidemiology and Cancer Control Program, 900 Camino de Salud NE, Albuquerque, NM 87131

Accepted for publication April 25, 1995.

© 1996 Wiley-Liss, Inc.

TABLE I. Distribution of Exposed Workers by Total Serum Fluorine Category in 3M Chemolite Plant, Cottage Grove, MN

	Total serum fluorine (ppm)					Total
	<1	1-3	>3-10	>10-15	>15-26	
Age ^a	39.9 (10.2)	39.6 (8.5)	36.0 (7.5)	39.3 (11.1)	41.6 (10.5)	39.2
BMI (kg/m ²) ^a	27.6 (5.3)	26.6 (2.6)	26.3 (3.3)	29.4 (3.7)	26.0 (1.4)	26.9
Alcohol use ^b						
<1 oz/day	17 (73.9)	51 (78.5)	9 (56.3)	5 (83.3)	5 (100)	87 (75.5)
1-3 oz/day	2 (8.7)	13 (20.0)	4 (25.0)	1 (16.7)	0 (0)	20 (17.4)
Nonresponse	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Tobacco use ^b	4 (17.4)	1 (1.5)	3 (18.7)	0 (0)	0 (0)	8 (7.0)
Smoker	3 (13.0)	16 (24.6)	6 (37.5)	2 (33.3)	1 (20.0)	85 (73.9)
Nonsmoker	19 (82.7)	49 (75.4)	9 (56.2)	4 (66.7)	4 (80.0)	28 (24.4)
Nonresponse	1 (4.3)	0 (0)	1 (6.3)	0 (0)	0 (0)	2 (1.7)
Total	23 (100)	65 (100)	16 (100)	6 (100)	5 (100)	115

BMI, body mass index
^aValues are mean (SD).
^bValues are n (percent).

tase. In addition, PFOA has been associated with hepatocyte necrosis and increased hepatic enzymes, suggesting that irreversible cell damage occurs (Kennedy, 1985; Just et al., 1989). Hepatomegaly and alterations in lipid metabolism appear to be rapidly reversible; however, other hepatic changes are not rapidly reversed (Perkins, 1992; Sohlenius et al., 1992).

Based on findings from the studies of rodents and in vitro experiments, some investigators have suggested that PFOA is likely to present a health risk to humans (Just et al., 1989; Takagi et al., 1991). If the observations in rodent species are relevant to humans exposed to PFOA, it is reasonable to hypothesize that changes in human hepatic enzymes and lipid metabolism are similar to those observed in rodents. Limited data are available to assess the hepatic responses to PFOA in humans. Ubel and coworkers (1980) and Griffith and Long (1980) reported that PFOA-exposed workers showed no clinical evidence of adverse hepatic effects. Furthermore, a retrospective cohort mortality study of exposed workers found no excess mortality from liver cancer or liver disease (Gilliland and Mandel, 1993). To assess whether the changes in cholesterol, lipoproteins, and hepatic enzymes observed in rodents treated with PFOA occur in humans, we studied 115 occupationally exposed employees at a plant that produces PFOA. Production workers with the highest PFOA exposures had serum PFOA levels similar to those in rodents that developed hepatomegaly when treated orally with low doses of PFOA (Ubel et al., 1980). We examined the cross-sectional association between serum PFOA, a validated surrogate measure of total serum fluorine, and cholesterol, lipoproteins, and hepatic enzymes in this group of occupationally exposed men.

TABLE II. Distribution of Age, Alcohol, and Tobacco Use in Participants by Body Mass Index in Study of Workers Exposed to PFOA

	BMI mg/kg ²		
	<25	25-30	>30
Total	41 (100%)	57 (100%)	17 (100%)
Tobacco use			
Smoker	11 (26.8%)	15 (26.3%)	2 (11.8%)
Nonsmoker	29 (70.7%)	41 (71.9%)	15 (88.2%)
Nonresponse	1 (2.5%)	1 (1.8%)	0 (0%)
Alcohol use			
<1 oz/day	31 (75.6%)	43 (75.4%)	13 (76.4%)
1-3 oz/day	6 (14.6%)	11 (19.3%)	3 (17.7%)
Nonresponse	4 (9.8%)	3 (5.3%)	1 (5.9%)
Age			
<40 years	31 (75.6%)	8 (49.1%)	6 (35.3%) ^a
≥40 years	10 (24.4%)	29 (50.9%)	11 (64.7%)
Total serum fluorine			
Mean ppm (SD)	2.8 (3.7)	4.0 (5.5)	2.1 (3.5)

^ap = 005
 BMI, body mass index

MATERIALS AND METHODS

Subject Selection

Participants were recruited from current employees at a PFOA production plant that has operated since 1947. The

0 568 (1 990)

ly

OA in hu-
 liver is an
 and Long,
 toor et al.,
 1989).
 hepatome-
 ic physio-
 eroxisome
 enzymes,
 mitochon-
 lipid me-
 y, 1985;
 afelghem
 ermadi et
 al., 1992;
 th PFOA
 lofibrate,
 anges in
 proteins.
 emic re-
 A reduc-

TABLE III. Serum Cholesterol, Low Density Lipoprotein, and High Density Lipoprotein by Total Serum Fluoride in Study of Workers Exposed to PFOA

Total fluoride	N	Mean	SD	Median	Range	Test*
Cholesterol (mg/dl)						
<1 ppm	23	201	34.7	203	132-268	F = .065 p = .62
≥1-3	65	211	40.0	212	130-349	
>3-10	16	206	37.7	198	150-277	
>10-15	6	226	40.0	216	183-298	
>15-26	5	214	27.0	204	184-244	
Total	115	210	38.1	210	130-349	
LDL (mg/dl)						
<1 ppm	23	132	32.4	137	70-196	F = 0.31 p = .87
≥1-3	65	136	34.5	131	70-264	
>3-10	16	134	34.5	133.5	83-217	
>10-15	6	124	44.0	139	36-156	
>15-26	5	143	20.8	144	117-171	
Total	115	135	33.8	134	36-264	
HDL (mg/dl)						
<1 ppm	23	45.9	11.7	47	19-67	F = 0.66 p = .66
≥1-3	65	46.1	10.0	44	30-79	
>3-10	16	41.8	10.2	40	29-68	
>10-15	6	46.5	6.8	44	40-59	
>15-26	5	45.6	10.2	49	29-54	
Total	115	45.4	10.2	43	19-79	

*Anova.
LDL, low density lipoprotein; HDL, high density lipoprotein.

TABLE IV. Pearson Correlation Coefficients Between Total Serum Fluoride, Age, Body Mass Index, Daily Alcohol Use, Daily Tobacco Consumption, and Lipoproteins

	Total fluoride (ppm)	Age (years)	BMI (kg/m ²)	Alcohol (oz/day)	Tobacco (cigs/day)
CHOLESTEROL	.07	.25	.19	.09	.35
		p = .008	p = .05		p = .0001
LDL	.02	.13	.06	-.008	.28
					p = .00
HDL	-.01	.03	-.13	.18	-.09
					p = .06

LDL, low-density lipoprotein; HDL, high-density lipoprotein; BMI, body mass index.

plant produces a number of specialty chemicals in addition to PFOA. Details about the plant have been described previously (Gilliland and Mandel, 1993). All workers employed in PFOA production during the period 1985-1989 were invited to participate in the study. Workers with jobs involving direct contact with PFOA during the 1985-1989

TABLE V. Serum Cholesterol by Body Mass Index, Age, Smoking, and Drinking Status: 3M Chemoite Plant, Cottage Grove, Minnesota

	Cholesterol (mg/dl)					Test*
	N (%)	Mean	SD	Median	Range	
BMI						
<25	41 (35.7)	195	40.1	175	130-277	F = 5.10 p = .008
25-30	57 (49.6)	219	36.2	220	146-349	
>30	17 (14.8)	214	29.3	216	163-268	
Age						
≤30	21 (18.3)	196	37.8	201	130-254	F = 1.60 p = .19
>30-40	48 (41.7)	219	43.8	204	132-349	
>40-50	27 (23.5)	216	30.2	216	163-263	
>50-60	19 (16.5)	219	29.7	224	164-268	
Alcohol						
<1 oz/d	87 (81.3)	209	38.6	204	135-349	F = .63 p = .43
1-3 oz/d	20 (18.7)	216	33.5	218	130-277	
Missing	8	207	45.5	213	132-261	
Tobacco						
Smoker	28 (24.8)	233	41.6	238	167-349	F = 15.63 p = .0001
Nonsmoker	85 (75.2)	203	32.9	203	130-268	
Missing	2	198	83.1	198	135-261	
Total	115					

*Anova.
BMI, body mass index.

period were considered highly exposed. This group included maintenance and engineering supervisors, as well as production workers. Forty-eight (96%) of 50 exposed workers agreed to participate in the study. In addition, a sample of workers employed in jobs with no apparent PFOA exposure was asked to participate. Those without direct contact with PFOA for at least 5 years were considered to have low exposure. A randomly selected low-exposure group of workers was frequency matched in 5-year age groups to the high-exposure workers. Sixty-five employees from jobs thought to involve no PFOA exposure volunteered for the study. The total number of the presumed unexposed employees invited to participate was not recorded; however, few individuals in this group declined to participate. We estimate that more than 80% of those invited agreed to participate in the study.

Total serum fluoride was used as a surrogate variable for PFOA exposure. We assayed total serum fluoride rather than measuring PFOA directly because the assay was less expensive and technically easier to perform on the large number of samples collected in this study. Furthermore, the use of total serum fluoride has been validated as a surrogate marker for PFOA in past biological monitoring in the plant and other plants using PFOA (Ubel et al., 1980). Approximately 90% of total serum fluoride in workers was reported

TABLE VI. Serum Low Density Lipoprotein by Body Mass Index, Age, Smoking, and Drinking Status: 3M Chemolite Plant, Cottage Grove, Minnesota

	N (%)	LDL (mg/dl)				Test*
		Mean	SD	Median	Range	
BMI						
<25	41 (35.7)	130	22.8	133	70-217	F = .65 p = .52
25-30	57 (49.6)	138	34.2	135	36-264	
>30	17 (14.8)	136	33.0	137	71-196	
Age						
≤30	21 (18.3)	130	29.6	131	75-177	F = .37 p = .77
>30-40	48 (41.7)	136	36.2	137	70-264	
>40-50	27 (23.5)	133	34.5	137	36-193	
>50-60	19 (16.5)	140	32.3	137	20-196	
Alcohol						
<1 oz/d	87 (81.3)	135	34.5	133	36-264	F = .01 p = .93
1-33 oz/d	20 (18.7)	135	31.4	137	76-217	
Missing	8	134	35.6	141	70-174	
Tobacco						
Smoker	28 (24.8)	152	35.6	146	99-264	F = 9.42 p = .003
Nonsmoker	85 (75.2)	130	31.3	133	78-217	
Missing	2	115	55.9	115	70-174	
Total	115					

*Anova
BMI, body mass index; LDL, low density lipoprotein.

TABLE VII. Serum High Density Lipoprotein by Body Mass Index, Age, Smoking, and Drinking Status

	N (%)	HDL (mg/dl)				Test*
		Mean	SD	Median	Range	
BMI						
<25	41 (35.7)	46.0	10.7	43	19-68	F = .38 p = .69
25-30	57 (49.6)	45.6	10.5	44	22-79	
>30	17 (14.8)	43.6	7.7	43	32-55	
Age						
≤30	21 (18.3)	43.5	14.3	40	19-79	F = .72 p = .55
>30-40	48 (41.7)	46.7	9.9	46	22-65	
>40-50	27 (23.5)	46.0	8.3	45	29-61	
>50-60	19 (16.5)	46.6	7.9	43	32-67	
Alcohol						
<1 oz/d	87 (81.3)	44.3	9.2	43	19-65	F = 3.98 p = .05
1-33 oz/d	20 (18.7)	49.3	13.5	45	29-79	
Missing	8	46.3	9.3	53	32-55	
Tobacco						
Smoker	28 (24.8)	44.3	8.9	43	29-68	F = .35 p = .56
Nonsmoker	85 (75.2)	45.6	10.6	43	19-79	
Missing	2	54.5	21.2	55	53-56	
Total	115					

*Anova
BMI, body mass index; HDL, high density lipoprotein.

to be in the form of PFOA (Venkateswarlu, 1982). Because the vast majority of total serum fluorine in plant employees is in the form of PFOA, total serum fluorine closely reflects serum PFOA in production workers, and its use is unlikely to introduce substantial error into the study.

We expected the group of workers who were selected for the unexposed group based on job history to have total serum fluorine levels similar to the general population. However, we found that this group of workers was not unexposed, having levels 20-50 times higher than levels reported for the general population. We concluded that job history was not an accurate metric for exposure. Because job history performed poorly for exposure assessment, we used measured total serum fluorine to classify individuals in the analyses.

Data Collection

Participants completed a medical history questionnaire, were measured for height and weight, and donated a blood sample by venipuncture for assays of total serum fluorine, serum glutamyl oxaloacetic transaminase (SGOT), serum glutamyl pyruvic transaminase (SGPT), gamma glutamyl transferase (GGT), cholesterol, low-density lipoproteins

(LDL), and high-density lipoproteins (HDL). The blood sample for total serum fluorine (TSF) was collected in a fluorine-free 15-ml Vacutainer. Divided aliquots of serum collected for total fluorine assay were frozen at -70°C. After all total fluorine samples had been received, batches of 15 samples were assayed on successive work days. Total serum fluorine, reported as a mean value, was determined using sodium biphenyl extraction and atomic absorption spectroscopy (Venkateswarlu, 1982). Each sample was assayed twice. Each batch included high- and low-quality control samples.

Analysis

Stratified analysis. Anova, Pearson correlation coefficients, and linear multivariate regression were used to evaluate associations between PFOA and the biochemical endpoints. For stratified analyses, Anova procedures were used to assess differences in mean values. Total serum fluorine was divided a priori into five categories—<1 ppm, 1-3 ppm, >3-10 ppm, >10-15 ppm, and >15 ppm—based on the distribution of previous monitoring data. Age, body mass index (BMI), alcohol use, and tobacco use were included in regression models as potential confounders. Number of cigarettes smoked per day was used as a continuous

TABLE VIII. Linear Multivariate Regression Model of Factors Predicting the High Density Lipoprotein in Study of Workers Exposed to PFOA

Variable	B	SE(B)	p value
Intercept	65.00	10.07	0.001
Total fluorine	-1.61	.77	.04
Alcohol ^a			
Low (<1 oz/day)	-9.92	3.51	.006
Nonresponsive (NR)	-6.77	5.73	.24
Low x total fluorine ^b	1.62	.80	.04
NR x total fluorine ^b	2.05	1.63	.21

R² = .17

^aReference category is drinkers who consumed 1-3 oz ethanol/day

^bInteraction terms between total fluorine and alcohol category adjusted for age, body mass index, smoking, and testosterone

TABLE IX. Serum Glutamic Oxaloacetic Transaminase, Serum Glutamic Pyruvic Transaminase, and Gamma Glutamyl Transferase by Total Serum Fluorine in Study of Workers Exposed to PFOA

Total fluorine	N	Mean	SD	Median	Range	Test ^a
SGOT (IU/dl)						
<1 ppm	23	22.5	4.1	22	13-29	F = 0.41 p = .80
≥1-3	65	24.1	8.6	23	10-74	
>3-10	16	25.8	14.5	22.5	17-77	
>10-15	6	25.7	11.3	22.5	17-47	
>15-26	5	22.2	5.1	22	14-27	
SGPT (IU/dl)						
<1	23	47.7	10.7	46	30-69	F = 1.19 p = .32
≥1-3	65	51.3	30.2	45	4-263	
>3-10	16	53.0	14.0	50.5	29-40	
>10-15	6	70.2	53.2	52.5	38-177	
>15-26	5	44.6	8.6	42	34-54	
GGT (IU/dl)						
<1 ppm	23	37.2	29.4	27	6-117	F = 0.39 p = .81
≥1-3	65	32.4	26.7	25	5-174	
>3-10	16	35.4	35.4	26	10-158	
>10-15	6	38.3	16.7	36.5	19-60	
>15-26	5	22.2	11.5	20	11-37	
Total	115	33.7	27.6	26	5-174	

^aAnova.

SGOT, serum glutamic oxaloacetic transaminase; SGPT, serum glutamic pyruvic transaminase; GGT, gamma glutamyl transferase.

TABLE X. Pearson Correlation Coefficients Between Total Serum Fluorine, Age, Body Mass Index, Daily Alcohol Use, Daily Tobacco Consumption, and Hepatic Parameters in Study of Workers Exposed to PFOA

	Total fluorine (ppm)	Age (years)	BMI (kg/m ²)	Alcohol (oz/day)	Tobacco (cigs/day)
SGOT	.01	-.10	.09	.12	-.11
SGPT	.01	.01	.20	.03	-.11
			p = .02		
GGT	.04	.12	.27	.15	.03
			p = .004		

SGOT, serum glutamic oxaloacetic transaminase; SGPT, serum glutamic pyruvic transaminase; GGT, gamma glutamyl transferase; BMI, body mass index

TABLE XI. Serum Glutamic Oxaloacetic Transaminase by Body Mass Index, Age, Smoking, and Drinking Status in Study of Workers Exposed to PFOA

	N (%)	SGOT (IU/dl)					Test ^a
		Mean	SD	Median	Range		
BMI							
<25	41 (35.7)	24	12.4	22	13-77	F = .92	
25-30	57 (49.6)	23	5.8	23	10-42	p = .40	
>30	17 (14.8)	27	8.1	26	17-47		
Age							
≤30	21 (18.3)	25	12.7	23	17-77	F = .78	
>30-40	48 (41.7)	24	9.1	23	10-74	p = .51	
>40-50	27 (23.5)	22	5.4	23	13-40		
>50-60	19 (16.5)	26	7.8	23	14-47		
Alcohol							
<1 oz/d	87 (81.3)	26	13.5	22	16-77	F = .61	
1-3 oz/d	20 (18.7)	24	8.0	23	10-74	p = .44	
Missing	8	23	4.3	21	19-31		
Tobacco							
Smoker	28 (24.8)	24	8.4	23	13-77	F = .02	
Nonsmoker	85 (75.2)	24	11.0	22	10-42	p = .89	
Missing	2	20	3.5	20	17-47		
Total	115						

^aAnova.

SGOT, serum glutamic oxaloacetic transaminase; BMI, body mass index

variable if model fit was improved compared with the model using categorical variables. BMI was categorized into three categories: <25 kg/m², 25-30 kg/m², and >30 kg/m². Alcohol use was divided into three categories: <1 drink per day, 1-3 drinks per day, and no response to the questionnaire item, and was entered into the models as a set of indicator variables. Significant nonlinear dose-response

relationships were evaluated by comparing model fit using residual analysis and by comparing parameter estimates using indicator variables and continuous variables. Interactions between total serum fluorine and the covariates were evaluated based on biologic plausibility. Interaction terms were included in the final model if the parameter estimate had a p value ≤ 0.05. The two nonrespondents to the smok-

TABLE XII. Serum Glutamic Pyruvic Transaminase by Body Mass Index, Age, Smoking, and Drinking Status. 3M Chemolite Plant, Cottage Grove, Minnesota

	N (%)	SGPT (IU/dl)				Test
		Mean	SD	Median	Range	
BMI						
<25	41 (35.7)	49	35.4	41	29-263	F = 2.1 p = 12
25-30	57 (49.6)	50	14.2	49	4-95	
>30	17 (14.8)	64	32.8	55	38-177	
Age						
≤30	21 (18.3)	49	11.5	45	31-80	F = .61 p = .61
>30-40	48 (41.7)	53	33.6	47	29-263	
>40-50	27 (23.5)	47	15.2	46	4-99	
>50-60	19 (16.5)	57	32.0	50	34-177	
Alcohol						
<1 oz/d	87 (81.3)	53	29.35	47	29-263	F = .68 p = .41
1-3 oz/d	20 (18.7)	47	16.9	46	4-99	
Missing	8	51	10.9	52	35-67	
Tobacco						
Smoker	28 (24.8)	48	15.2	47	4-90	F = .76 p = .39
Nonsmoker	85 (75.2)	53	29.6	48	30-263	
Missing	2	49	25.5	49	31-67	
Total	115					

*Anova.
BMI, body mass index; SGPT, serum glutamic pyruvate transaminase.

TABLE XIII. Gamma Glutamyl Transferase by Body Mass Index, Age, Smoking, and Drinking Status in Study of Workers Exposed to PFOA

	N (%)	GGT (IU/dl)				Test*
		Mean	SD	Median	Range	
BMI						
<25	41 (35.7)	28	31.1	17	5-174	F = 3.54 p = .03
25-30	57 (49.6)	34	23.1	19	8-158	
>30	17 (14.8)	48	28.6	44	19-117	
Age						
≤30	21 (18.3)	32	23.4	25	11-111	F = 1.56 p = .36
>30-40	48 (41.7)	31	32.7	22	5-174	
>40-50	27 (23.5)	33	17.2	29	8-72	
>50-60	19 (16.5)	44	29.3	35	11-117	
Alcohol						
<1 oz/d	87 (81.3)	40	25.5	35	8-89	F = 1.64 p = .36
1-3 oz/d	20 (18.7)	32	25.3	26	6-174	
Missing	8	41	50.4	23	12-158	
Tobacco						
Smoker	28 (24.8)	36	21.3	33	5-89	F = .55 p = .46
Nonsmoker	85 (75.2)	32	26.3	25	6-174	
Missing	2	85	103.2	85	12-158	
Total	115					

*Anova.
BMI, body mass index; GGT, gamma glutamyl transferase.

ing questions were not included in the analysis. All analyses were conducted using the statistical computing package SAS (Statistical Analysis Systems, 1992).

RESULTS

Participant characteristics are shown in Tables I and II. Total serum fluorine values for the 115 participants varied between 0 and 26 ppm, with a mean of 3.3 ppm. Twenty-three (20.0%) participants had serum values <1 ppm, and 11 (9.6%) had values >10 ppm (Table I). The distributions for age, BMI, alcohol use, and tobacco use did not differ significantly among total serum fluorine categories. Mean total serum fluorine, tobacco use, and alcohol use did not differ significantly between obese and non-obese workers. Obese (BMI > 30) participants were significantly older than non-obese participants (Table II).

In univariate analyses, the marked hypolipidemic effect of PFOA observed in rodents was not apparent in exposed workers (Table III). As shown in Table IV, total serum fluorine was not significantly correlated with cholesterol, LDL, or HDL; however, several expected correlations were present. Alcohol consumption was associated with higher HDL levels. Age and body mass index (BMI) were signif-

icantly correlated with cholesterol. The number of cigarettes smoked per day was significantly correlated with cholesterol and LDL. A similar pattern of associations was seen in the stratified analyses for these variables (Tables V-VII).

We found that PFOA was associated with HDL levels in moderate drinkers. After adjusting for alcohol use, age, BMI, cigarette use, and testosterone levels, moderate alcohol use was associated with an increase in HDL (9.9 mg/dl) compared with light drinkers or abstainers (Table VIII). As total serum fluorine increased, the effect of moderate alcohol use on HDL was blunted; a 10-ppm rise in total serum fluorine reversed the effect of moderate alcohol on HDL. In light drinkers, little change in HDL was observed as total fluorine increased. After adjusting for alcohol use, age, BMI, and cigarette use, total serum fluorine was not significantly associated with cholesterol, or LDL (not shown).

SGOT, SGPT, and GGT did not significantly differ among the five categories of total serum fluorine (Table IX). As expected, SGPT and GGT were significantly correlated with BMI, but were not significantly correlated with total serum fluorine, age, alcohol consumption, or cigarette consumption (Table XI). Stratified analyses indicated the same pattern, except GGT was also associated with BMI (Tables IX-XIII).

After adjusting for age, cigarette use, alcohol use, and

TABLE XIV. Linear Multivariate Regression Model of Factors Predicting Serum Glutamic Oxaloacetic Transaminase in Study of Workers Exposed to PFOA

Variable	β	SE(β)	p value
Intercept	26.71	7.1	.0003
Total fluorine (ppm)	-3.23	1.31	.02
BMI (kg/m ²)	-.0004	.23	.99
BMI \times total fluorine ^a	.12	.05	.015

R² = .17
^aInteraction term between total serum fluorine and BMI
 Adjusted for age, alcohol use, and smoking
 BMI, body mass index.

TABLE XV. Linear Multivariate Regression Model of Factors Predicting Serum Glutamic Pyruvic Transaminase in Study of Workers Exposed to PFOA

Variable	β	SE(β)	p value
Intercept	53.13	24.6	.02
Total fluorine (ppm)	-15.80	4.58	.0008
BMI (kg/m ²)	.30	.82	.72
BMI \times total fluorine ^a	.62	.17	.0004

R² = .21
^aInteraction term between total serum fluorine and BMI.
 Adjusted for age, alcohol use, and smoking.
 BMI, body mass index.

TABLE XVI. Change in Serum Glutamic Oxaloacetic Transaminase and Serum Glutamic Pyruvic Transaminase Associated With a 10 ppm Change in Total Serum Fluorine in Study of Workers Exposed to PFOA

BMI (kg/m ²)	25	30	35
SGOT	-2.4	3.7	9.7
SGPT	-3.0	28.0	59.0

BMI, body mass index; SGOT, serum glutamic oxaloacetic transaminase; SGPT, serum glutamic pyruvic transaminase.

BMI, total serum fluorine was associated with changes in SGOT, SGPT, and GGT through interactions with known determinants of hepatic enzymes. SGOT and SGPT showed a different association in obese and non-obese workers (Tables XIV and XV). A 10 ppm difference in total serum fluorine resulted in a slight decrease in SGOT and SGPT for non-obese (BMI = 25 kg/m²) workers (Table XVI). In obese (BMI = 35 kg/m²) workers, an increase in SGOT and SGPT was associated with a 10 ppm difference. In the regression model for GGT (Table XVII), moderate alcohol consump-

TABLE XVII. Linear Multivariate Regression Model of Factors Predicting Gamma Glutamyl Transferase in Study of Workers Exposed to PFOA

Variable	β	SE(β)	p value
Intercept	-12.59	22.62	.58
Total fluorine (ppm)	-1.93	2.11	.36
Alcohol ^a			
Low (<1 oz/day)	-12.37	9.50	.20
Nonresponse (NR)	-28.13	15.46	.07
Low \times fluorine ^b	1.59	2.18	.47
NR \times fluorine ^b	13.90	4.48	.003

R² = .18.
^aReference category is drinkers who consumed 1-3 oz ethanol/day.
^bInteraction terms between total fluorine and alcohol category.
 Adjusted for age, body mass index, and smoking.

tion was positively associated with GGT. In moderate drinkers, GGT decreased as total fluorine increased; the decrease was less in light drinkers.

All hepatic enzyme assays were in a clinically acceptable range, and no workers reported hepatic disease diagnoses or signs, or symptoms consistent with hepatic disorders. No clinical cases of liver dysfunction associated with PFOA exposure have been found by the medical surveillance program at the plant.

DISCUSSION

PFOA is an alleged cancer promoter in rats (Reddy et al., 1980). In biphasic liver carcinogenesis protocols (initiation and promotion) and triphasic protocols (initiation, selection, and promotion), PFOA produced increased numbers of malignant hepatocellular carcinomas (Abdellatif et al., 1990; Nilsson et al., 1991). Takagi et al. (1991) have suggested that because the intensity of hepatic response may be an early marker for liver carcinogenic potential, PFOA has a high potential for liver carcinogenesis. The hypolipidemia observed in PFOA-treated rodents was not observed in PFOA-exposed workers. At the levels of exposure in this study, PFOA is not associated with a marked hepatic response and is not likely to have a significant carcinogenic potential in humans. Obese workers may be a susceptible population for subclinical hepatic changes.

In rodents, PFOA alters endobiotic and xenobiotic hepatic metabolic enzyme profiles (Pastoor et al., 1987). Few studies of the human response to PFOA exposure have been published. In a study at the same plant, Ubel et al. (1980) reported no association between PFOA and hepatic enzymes. However, their analysis did not consider the joint effects of obesity or alcohol with PFOA exposure. In the present study, changes in SGOT and SGPT were associated with PFOA through an interaction with adiposity. In obese

Predicting
PFOA

p value
.58
.36
.20
.07
.47
.003

Moderate
ed: theaccept-
e diag-
disor-
d with
surveil-ddy et
(initi-
on, se-
mbers
et al.,
e sug-
may be
A has
demia
ed in
n this
ic re-
genic
ptiblec he-
Few
been
(980)
e en-
joint
n the
ated
base

participants only. SGOT and SGPT increased with increasing PFOA. The hypothesis that PFOA may modulate the hepatic effects of obesity is consistent with these changes in enzyme profile. This hypothesis has biologic plausibility because obesity has been associated with elevation of transaminases through fatty infiltration (Ludwig et al., 1980; Hodgson et al., 1989). PFOA may directly or indirectly potentiate this effect in susceptible individuals. PFOA alters hepatic lipid metabolism and may block the metabolism of accumulated fatty acids, resulting in an exacerbation of the pathologic process (Haughom et al., 1992).

PFOA may also modulate the effect of alcohol on hepatic metabolism. PFOA is associated with changes in the effect of alcohol consumption on HDL levels, essentially blocking the rise in HDL associated with alcohol consumption. GGT was inversely associated with PFOA in drinkers. Perfluorooctanoic acid may decrease serum GGT by altering cell membrane permeability, by reducing the alcohol-mediated induction of GGT, or by changing alcohol oxidation pathways and reducing the production of such toxic intermediates as acetaldehyde (Bates, 1981; Schuckit and Griffiths, 1982; Orrego et al., 1985; Schuckit and Irwin, 1988). These findings support the hypothesis that PFOA modulates the effects of endogenous and exogenous determinants of hepatic metabolism.

Interpretation of these findings is limited by a number of factors. Only active workers in PFOA production were included in this study. It is unlikely that workers who had significant exposure during the previous 5 years would have been lost to this study because of transfer out of the PFOA production division. Transfer as a result of subclinical changes in such biochemical parameters as SGOT is unlikely. Because of the low turnover rate in plant employees (3% per year) and the inclusion of most current employees with appropriate job histories, selection bias is not a likely explanation for the findings in this study. Given the high participation (>80%), nonresponse bias is likely to be small. Information on smoking and alcohol consumption was collected and used in the analyses; however, measurement error for these variables could allow residual confounding. Because smoking and alcohol consumption are not strong determinants for the endpoints in this study, the magnitude of any residual confounding is likely to be small. The duration of exposure may be an important determinant of PFOA level and effect; however, information on the duration of employment in exposed jobs was not available because plant records did not contain sufficient information to reconstruct exposures. Furthermore, the use of job history resulted in marked misclassification of exposure status, indicating that the use of job duration would be of limited value in determining duration of exposure.

Many of the participants were employed in the production of compounds other than PFOA; however, none of these processes involve substantial exposure to known he-

patotoxins. Because PFOA has a long biological half-life in humans, is absorbed easily, and is hepatotoxic in rodents, PFOA production workers have been under medical surveillance for more than 20 years. No adverse clinical outcomes related to PFOA exposure have been observed in these employees.

In summary, PFOA was not associated with the marked hepatic changes in humans that have been observed in rodents. This finding is consistent with the results of a retrospective mortality study that found no increased mortality from liver disease (Gilliland and Mandel, 1993) and with the results from an earlier morbidity study that found no adverse hepatic effects (Ubel et al., 1980). PFOA may modulate the effect of alcohol use and obesity on hepatic lipid and xenobiotic metabolism. Continued epidemiologic surveillance is appropriate in workers exposed to PFOA.

ACKNOWLEDGMENTS

This study was supported in part by NIOSH grant T150H07098-16 and the 3M Medical Department.

REFERENCES

- Abdellatif A, Preat V, Vamecq J, Nilsson R, Roberfroid M (1990): Peroxisome proliferation and modulation of rat liver carcinogenesis by 2,3-dichlorophenoxyacetic acid, 2,4,5 trichlorophenoxyacetic acid, perfluorooctanoic acid and nafenopin. *Carcinogenesis* 11:1899-1902.
- Bates H (1981): GGTP and alcoholism: A sober look. *Lab Management* 19:1-3.
- Belisle J (1981): Organic fluoride in human serum: Natural versus industrial sources. *Science* 212:1509-1510.
- Gilliland F, Mandel J (1993): Mortality among employees in a PFOA production plant. *J Occup Med* 35:950-954.
- Griffith F, Long J (1980): Animal toxicity studies with ammonium perfluorooctanoate. *Am Ind Hyg Assoc* 41:576-583.
- Guy W (1979): Ionic and organic fluorine in blood. In Johansen E, Taves D, Olsen T (eds): *Continuing Evaluation of the Use of Fluoride*. Boulder, CO: Westview Press.
- Haughom B, Spydevold O (1992): The mechanism underlying the hypolipemic effect of perfluorooctanoic acid (PFOA), perfluorooctane sulfonic acid (PFOSA) and clotibric acid. *Biochim Biophys Acta* 1128:65-72.
- Hodgson M, Van Thiel D, Lauschus K, Karpf M (1989): Liver injury tests in hazardous waste workers: The role of obesity. *J Occup Med* 31:238-242.
- Just W, Gorgas K, Hartl F, Heinemann P, Salazar M, Schimmassek H (1989): Biochemical effects and zonal heterogeneity of peroxisome proliferation induced by perfluorocarboxylic acids in rat liver. *Hepatology* 9:570-581.
- Keiler B, Marsman D, Popp J, Thurman R (1992): Several nongenotoxic carcinogens uncouple mitochondrial oxidative phosphorylation. *Biochim Biophys Acta* 1102:237-244.
- Kennedy G (1985): Dermal toxicity of ammonium perfluorooctanoate. *Toxicol Appl Pharmacol* 81:348-355.
- Kennedy G, Hall G, Britelli J, Chen H (1986): Inhalation toxicity of ammonium perfluorooctanoate. *Food Chem Toxicol* 24:1325-1329.

- Ludwig J, Viggiano T, McGill D, Ott B (1980): Nonalcoholic steatohepatitis. *Mayo Clin Proc* 55:434-438.
- Nilsson R, Beije B, Prent V, Ervén K, Ramel C (1991): On the mechanism of the hepatic carcinogenicity of peroxisome proliferators. *Chem Biol Interact* 78:235-50.
- Orrego H, Blake J, Israel Y (1985): Relationship between gamma glutamyl transpeptidase and mean urinary alcohol levels in alcoholics while drinking and in withdrawal. *Alcohol Clin Exp Res* 9:10-13.
- Pastoor T, Lee K, Perri M, Gillies P (1987): Biochemical and morphological studies of ammonium perfluorooctanoate-induced hepatomegaly and peroxisome proliferation. *Exp Mol Pathol* 47:98-109.
- Perkins R (1992): Investigation of ammonium perfluorooctanoate effect on hormone levels and peroxisome proliferation in the rat. *Toxicologist* 12:38.
- Permad H, Lundgren B, Andersson K, DePierre, J. (1992): Effects of perfluoro fatty acids on xenobiotic-metabolizing enzymes, enzymes which detoxify reactive forms of oxygen and lipid peroxidation in mouse liver. *Biochem Pharmacol* 44:1183-1191.
- Reddy J, Azarnoff D, Hignite C (1980): Hypolipidemic hepatic peroxisome proliferators form a novel class of chemical carcinogens. *Nature* 283:397-398.
- Schuckit M, Griffiths J (1982): Gamma glutamyltransferase values in non-alcoholic drinking men. *Am J Psychiatry* 139:227-228.
- Schuckit M, Irwin M (1988): Diagnosis of alcoholism. *Med Clin North Am* 72:1133-1153.
- Sohlemus K, Andersson K, DePierre J (1992): The effects of perfluorooctanoic acid on hepatic peroxisome proliferation and related parameters show no sex-related differences in mice. *Biochem Toxicol* 285:779-783.
- Statistical Analysis System (1992). "SAS User's Guide, Version 6." Cary, NC: SAS Institute, Inc.
- Takagi A, Sai K, Umemura T, Hasegawa R, Kurakawa Y (1991): Short-term exposure to the peroxisome proliferators, perfluorooctanoic acid and perfluorodecanoic acid, causes significant increases of 8-hydroxydeoxyguanosine in liver DNA of rats. *Cancer Lett* 57:55-60.
- Taves D (1971): Comparison of "organic" fluoride in human and nonhuman serums. *J Dent Res* 50:783.
- Taves D, Guy W, Brey W (1976): Organic fluorocarbons in human plasma: Prevalence and characterization. In Filler R (ed): "Biochemistry Involving Carbon-Fluorine Bonds." Washington DC: American Chemical Society, pp 117-134.
- 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.
- Van Rafelghem M, Matte D, Bruner R, Andersen M (1987): Pathological and hepatic ultrastructural effects of a single dose of perfluoro-n-decanoic acid in the rat, hamster, mouse and guinea pig. *Fundam Appl Toxicol* 9:522-540.
- Venkateswarlu P (1982): Sodium biphenyl method for determination of covalently bound fluorine in organic compounds and biological materials. *Anal Chem* 54:1132-1137.

in
do
ad
est
19
pre
PF
suc
wit
me
lev
bod
othe
tha
cha
tion
subj
mea
labo

From
J.S. Ma
Mexico
School
Addr
Paul, M
1076
Copy

An Epidemiologic Investigation of Reproductive Hormones in Men with Occupational Exposure to Perfluorooctanoic Acid

Geary W. Olsen, DVM, PhD
Frank D. Gilliland, MD, PhD
Michele M. Burlew, MS
Jean M. Burris, MPH
Jack S. Mandel, PhD
Jeffrey H. Mandel, MD

Perfluorooctanoic acid (PFOA), a potent synthetic surfactant used in industrial applications, is a peroxisome proliferator that has resulted in dose-related increases in hepatic, pancreatic acinar, and Leydig cell adenomas in laboratory animals. In addition, PFOA increased serum estradiol levels through the induction of hepatic aromatase activity. In 1993 and 1995, we conducted two cross-sectional studies of 111 and 80 production workers, respectively, and specifically measured their serum PFOA in relation to several reproductive hormones to determine whether such an effect occurs in humans. PFOA was not significantly associated with estradiol or testosterone in either year's study. A 10% increase in mean estradiol levels was observed among employees who had the highest levels of serum PFOA, although this association was confounded by body mass index. Neither was PFOA consistently associated with the other measured hormones. Our results provide reasonable assurance that, in this production setting, there were no significant hormonal changes associated with PFOA at the serum levels measured. Limitations of this investigation include its cross-sectional design, the few subjects exposed at the highest levels, and the lower levels of serum PFOA measured, compared with those levels reported to cause effects in laboratory animal studies.

Although fluoride (inorganic ionic fluoride) was identified in human blood 140 years ago,^{1,2} the presence of fluoride in a covalently bound organic state was first reported in 1968.³⁻⁴ Guy subsequently identified perfluorooctanoic acid (PFOA, C₇F₁₅CO₂H) as a major component of the serum organic fluorine fraction.⁵ Ammonium perfluorooctanoate, a potent synthetic surfactant used in industrial applications, rapidly dissociates in aqueous solution to PFOA.

In laboratory animals, PFOA acid, or its salts, is absorbed by ingestion, inhalation, or dermal exposure⁶⁻⁸ and is not metabolized.⁹⁻¹² PFOA is distributed primarily in the plasma and liver of male rats and the liver, plasma, and kidney in female rats.¹¹ The major route of elimination is via urine and feces. In the female rat, there is a tenfold-greater renal excretion rate.^{11,13,14} Castrated male rats treated with estradiol have PFOA urinary excretion rates similar to those of female rats.^{10,11}

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.¹⁵⁻²¹ Two-year feeding studies in CrI:CD BR (CD) rats at a maximum amount of 300 parts per million (ppm) PFOA showed liver adenomas and an increased incidence of pancreas acinar cell adeno-

From the Medical Department, 3M Company, St. Paul, Minn. (Dr Olsen, Ms Burlew, Ms Burris, Dr J.S. Mandel); the Epidemiology and Cancer Control Program, School of Medicine, University of New Mexico, Albuquerque, NM (Dr Gilliland); and the Division of Environmental and Occupational Health, School of Public Health, University of Minnesota, Minneapolis, Minn. (Dr J.H. Mandel)

Address correspondence to: Geary W. Olsen, DVM, PhD, Medical Department, 3M, 220-3W-05, St. Paul, MN 55144.

1076-2752/98/4007-0614\$3.00/0

Copyright © by American College of Occupational and Environmental Medicine

mas^{22,23} and Leydig cell adenomas.^{17,22} PFOA is not mutagenic⁶ and thus the induction of these tumors most likely occurs through nongenotoxic mechanisms such as oxidative stress.^{19,24}

To determine whether the Leydig cell adenomas were the result of an endocrine-related mechanism, Cook et al¹⁸ gavaged CD rats for 14 days with up to 50 mg/kg of ammonium perfluorooctanoate. A significant increase in serum estradiol and decrease in testosterone levels were observed. The estradiol increase may be due to an induction of hepatic aromatase activity.¹⁸ The decrease in serum testosterone levels might be the result of reduced conversion of 17-alpha hydroxyprogesterone (17-HP) to androstenedione (via the inhibition of the C-17,20 lyase enzyme). However, Biegel et al¹⁹ were unable to replicate the negative testosterone association.

CD rats fed 100 ppm PFOA for a maximum of 13 weeks showed increased estradiol but not testosterone levels.^{25,26} Elevated estradiol levels were found among CD rats fed 300 ppm during a 2-year bioassay, with no dose-related differences for testosterone, luteinizing hormone (LH), and follicle-stimulating hormone (FSH).²²

The 3M Company has conducted medical surveillance of employees involved in PFOA production. Levels of serum organic fluorine (1.00–71.00 ppm) in production employees were 10- to 50-fold greater than values (0.01–0.13 ppm) reported from human sera.²⁷ Since PFOA could be an endocrine modulator,¹⁸ a cross-sectional study among workers with potential exposure to PFOA was conducted in 1990.²⁸ Among 115 men engaged in PFOA production, total serum fluorine levels (sodium biphenyl extraction method²⁹) ranged from 0 to 26 ppm (mean = 3.27 ppm; standard deviation [SD] = 4.68 ppm). It had been estimated that 80%–90% of human total serum fluorine levels consisted of PFOA.²⁷ Adjusting for potential covariates

and hormones of a priori interest resulted in a positive nonlinear (quadratic) association with estradiol, positive linear associations with prolactin and TSH, and negative nonlinear (square root) associations with free or bound testosterone.²⁸

A mortality study of employees in the chemical division, which included the PFOA production buildings, found no significantly increased cause-specific standardized mortality ratio for either male or female employees.³⁰ There were four deaths from prostate cancer, compared with 1.97 expected (95% confidence interval [CI], 0.55–4.59). Only one employee had worked directly in the PFOA production buildings. An association between PFOA exposure and prostate cancer was considered biologically plausible based on the animal and human data, which showed associations between PFOA and reproductive hormones.

The purpose of this report is to describe the results from two cross-sectional studies from the same plant that were done in 1993 and 1995.

Methods

PFOA Production

PFOA production at this plant began in 1947. PFOA, a white powder, is produced by an electrochemical process.³¹ The products of this electrolysis cell reaction are highly fluorinated compounds, with the end-product defined by the starting material. 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.

Subject Selection

General medical surveillance is performed biennially for employees at this plant. There were 111 male employees in 1993 and 80 male employees in 1995 who participated in medical surveillance, hormone testing, and serum PFOA determination.

Sixty-eight employees participated in both years. The surveillance consisted of a medical questionnaire; measurement of height, weight, and pulmonary function; standard biochemical and urinalysis tests; PFOA determination; and several hormone assays.

PFOA Analysis

A thermospray mass spectrophotometry assay was used to determine serum PFOA levels in 1993 and 1995.³² The range of serum PFOA was 0 to 80 ppm in 1993 and 0 to 115 ppm in 1995. The upper limit of detection in 1993 was 80 ppm, whereas there was no upper limit of detection in 1995. Levels were highly correlated among the 68 employees studied in 1993 and 1995 ($r = .91$, $P = 0.0001$). There was also high correlation between total serum fluorine level measured with the 1990 study²⁸ and the PFOA measured in 1993 ($r = .72$, $P = 0.0001$, $n = 94$ subjects) and in 1995 ($r = .84$, $P = 0.0001$, $n = 63$ subjects). These findings were not unexpected, because of the estimated 18- to 24-month half-life of PFOA in humans.²⁷

Hormone Assays

Serum samples were analyzed by the University of Minnesota's Endocrinology Laboratory (Minneapolis, MN) or the Endocrine Science Reference Laboratory (Tarzana, CA). Eleven hormones were assayed: cortisol, dehydroepiandrosterone sulfate (DHEAS), estradiol, FSH, 17 α -hydroxyprogesterone (17-HP), free testosterone, total testosterone, LH, prolactin, thyroid-stimulating hormone (TSH) and sex hormone-binding globulin (SHBG). All but SHBG were analyzed at the University of Minnesota's Endocrinology Laboratory.

Cortisol was assayed using a fluorescence polarization immunoassay (Abbott TDx, North Chicago, IL). Radioimmunoassays (RIA) were used for DHEAS (Pantex, Santa Monica, CA), estradiol (modified

Par
tota
agn
CA
min
LH,
usin
noas
term
nigh
Juan
asses
ter ch
tion
Labor
calcul
free t
were
analys

Data A

Sim
ysis of
correlat
multiva
evaluate
and each
potential
stratified
vided in
ppm, 1-
≥30 ppm
effect ex
levels. P
PFOA, a
alcohol u
examined
tinuous v
alyzed as
drinks per
questionn
recorded a
nonsmoker
fitted with
uous variat
square root
assure tha
missed. Th
ation of e
and bound
Nonlinear c
were exami

participated
efficacy questionnaire;
weight, and
standard bio-
tests; PFOA
total hormone

spectropho-
to determine
1993 and
serum PFOA
and 0 to 115
per limit of
80 ppm.
per limit of
levels were
the 68 em-
and 1995
There was
between total
measured with
PFOA mea-
= 0.0001.
1995 ($r =$
subjects).
expected,
18- to 24-
A in hu-

alyzed by
ta's Endo-
neapolis,
ence Ref-
na, CA).
ayed: cor-
ne sulfate
H, 17 α -
HP), free
one, LH,
ing hor-
ormone-
All but
e Univer-
crinology
g a fluo-
unoassay
go, IL).
A) were
x, Santa
modified

Paritex). 17-HP (modified CIS) and total testosterone (Coat-A Count; Diagnostic Product Corp., Los Angeles, CA). Free testosterone was determined using equilibrium dialysis. LH, FSH and prolactin were assayed using a microparticle enzyme immunoassay (Abbott Imx). TSH was determined using a chemiluminescence immunometric assay (Nichols, San Juan Capistrano, CA). SHBG was assessed via a radioimmunoassay after chromatographic sample purification (Endocrine Science Reference Laboratory). Bound testosterone was calculated as total testosterone less free testosterone. The same assays were used for both 1993 and 1995 analyses.

Data Analysis

Simple and stratified analyses, analysis of variance (ANOVA), Pearson correlation coefficients, and ordinary multivariable regression were used to evaluate associations between PFOA and each hormone, with adjustment for potential confounding variables. 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. For multivariable evaluation, PFOA, age, body mass index (BMI), alcohol use, and cigarette use were examined as both categorical and continuous variables. Alcohol use was analyzed as less than 1 drink per day, 1-3 drinks per day, and non-response to the questionnaire item. Cigarette use was recorded as either current smoker or nonsmoker. Regression models were fitted with PFOA entered as a continuous variable, using linear, square, and square root transformations in order to assure that associations were not missed. The possible nonlinear association of estradiol, free testosterone, and bound testosterone was evaluated. Nonlinear dose-response relationships were examined by model fit and by

comparing parameter estimates, using indicator and continuous variables. Stepwise selection procedures were also used. Study results were analyzed by the SAS System.¹¹

We did not examine hormone changes between the two examinations because of the estimated half-life of PFOA (approximately two years) and intraindividual variability in hormones. Since the results for the 68 employees who participated in both years were similar to those obtained for the entire study, only the results for all employees are presented below.

Results

Serum PFOA levels were not highly correlated with either the covariates or the hormones. These correlation coefficients (in parentheses) for 1993 and 1995 data, respectively, were the following: age (-.22, .14); alcohol (.10, .18); BMI (.11, .10); cigarettes (.05, .11); cortisol (.07, -.05); DHEAS (.13, .12); estradiol (.12, .15); FSH (-.12, -.13); 17-HP (.11, .30); LH (-.06, .13); prolactin (.04, -.04); SHBG (-.07, .03); bound testosterone (.01, .02); free

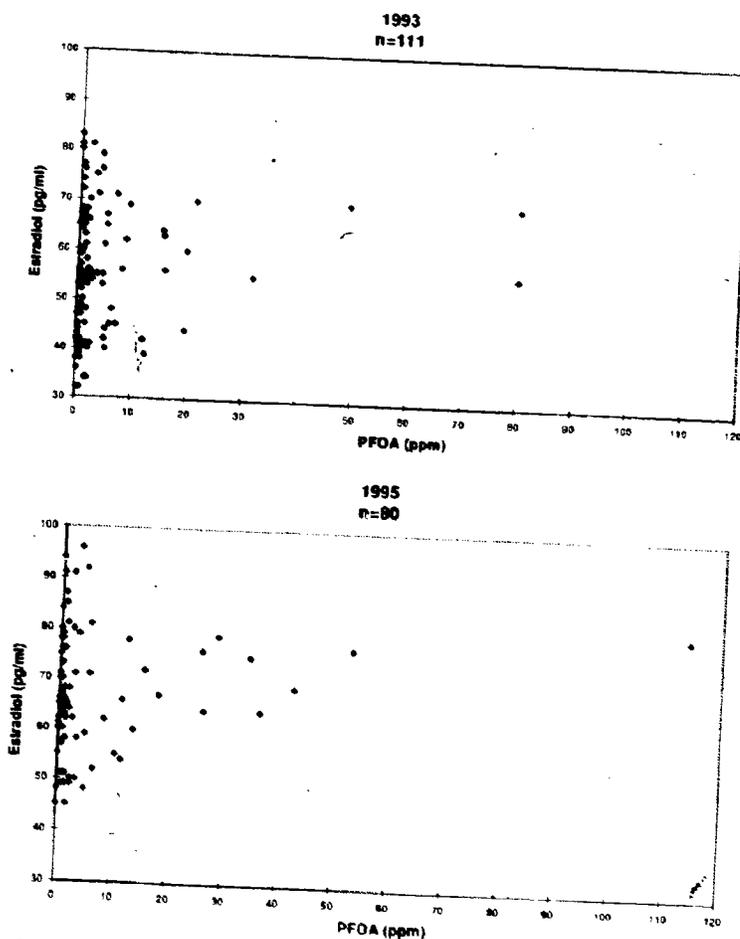


Fig. 1. Scatterplot of serum estradiol (pg/ml) by perfluorooctanoic acid (PFOA, in parts per million [ppm]) for employees in 1993 and 1995.

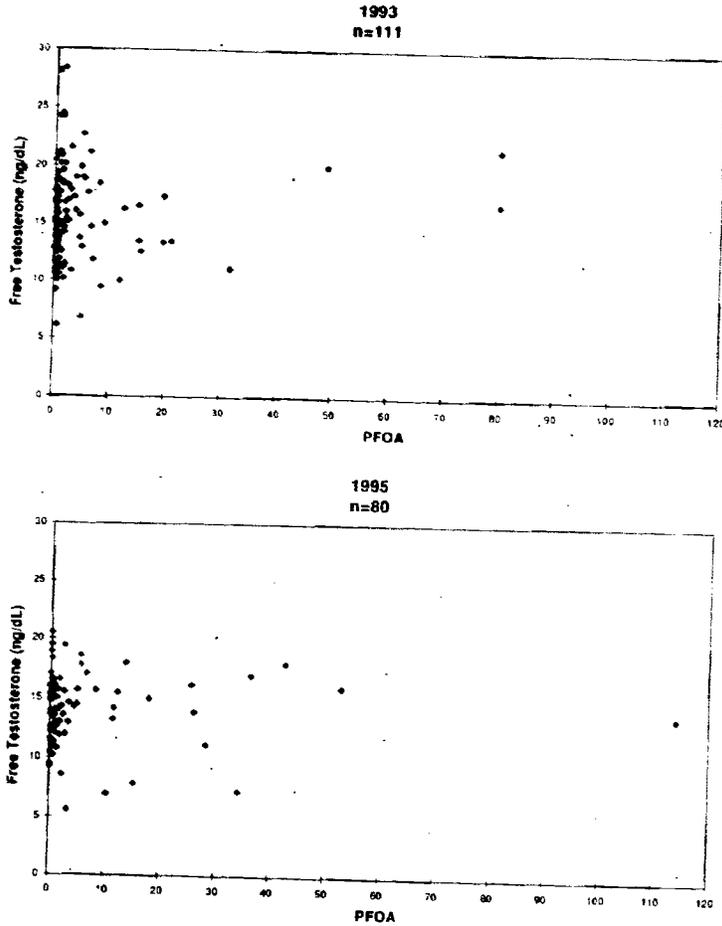


Fig. 2. Scatterplot of serum free testosterone (ng/dl) by PFOA (ppm) for employees in 1993 and 1995.

testosterone (.09, .01); and TSH (.03, .15).

Figures 1 and 2 are scatterplots for estradiol and free testosterone for each year. Simple linear regression of the natural log of each hormone with PFOA, treated as a continuous variable, resulted in no statistically significant coefficients in 1993 for any hormone and only one in 1995: 17-HP (beta coefficient = 0.006, $P = 0.03$, $R^2 = .06$). This result was dependent upon one person. In 1995, this person had a level of 198 ng/dL of 17-HP and 114 ppm of serum PFOA. In 1993, this person's values

were 206 ng/dL of 17-HP and 80 ppm (upper limit of detection in 1993) for PFOA.

Table 1 provides the mean, median, standard deviation, and range of the covariates and several hormones, by four levels of PFOA categorization (0-<1, 1-<10, 10-<30, and ≥ 30 ppm). Seventy-five percent of the employees with serum PFOA levels at 10 ppm or greater participated in both years. From Table 1, several observations are noteworthy. First, the mean of the PFOA ppm categories differed by two orders of magnitude between the lowest and

highest categories for both years. Second, the ≥ 30 -ppm PFOA category had the youngest mean employee age in both years. Third, BMI was the greatest among employees in the ≥ 30 -ppm PFOA category in 1995. Fourth, mean estradiol levels were not significantly different between PFOA levels in either year, although the ≥ 30 -ppm PFOA categories had mean estradiol levels that were 10% higher than the other PFOA levels. Fifth, there were no discernible trends between PFOA and either bound or free testosterone. Sixth, 17-HP levels were highest in the ≥ 30 -ppm PFOA group in both years. No significant associations were observed for cortisol, DHEAS, FSH, LH, and SHBG (data not shown).

As expected,¹⁴ estradiol was highly correlated with BMI (1993: $r = .41$, $P < 0.001$; 1995: $r = .30$, $P < 0.01$) and free testosterone with age (1993: $r = -.48$, $P < 0.001$; 1995: $r = -.40$, $P < 0.001$); thus Table 2 provides mean estradiol and free testosterone values stratified by BMI and age, respectively. It should be noted that all five employees in 1995 with serum PFOA levels ≥ 30 ppm had BMIs ≥ 28 .

Linear and nonlinear relationships, taking into account potential confounders (especially age and BMI) as well as other covariates that may be on the biologic pathway of effect, resulted in no significant associations with PFOA except for 17-HP in the 1995 analyses (data not shown). Again, this association was dependent on the one employee discussed earlier.

Because a primary hypothesis of the present study was whether PFOA increased estradiol and decreased testosterone serum levels in a nonlinear fashion, we replicated these prior models²⁸ with our 1993 and 1995 data. PFOA was not significantly associated with serum estradiol, free testosterone, or bound testosterone (data not shown). There was no significant association (data not shown) between PFOA and prolactin among

TA
Ma
Va
PF
Ag
0
1
1
1
≥
Alco
0-
1-
10-
≥30
BMI
0-
1-
10-
≥30
Cigar
0-
1-
10-
≥30
Estrad
0-
1-
10-
≥30
17-HP
0-
1-
10-
≥30
Prolact
0-
1-
10-
≥30
Bound t
0-
1-
10-
≥30

TABLE 1
Mean, Median, Standard Deviation (SD) of Mean and Range of Perfluorooctanoic Acid (PFOA), Demographic and Hormonal Values by Serum PFOA Levels, and Year of Data Collection*

PFOA (ppm)	1993 Data				1995 Data			
	Mean	Median	SD	Range	Mean	Median	SD	Range
PFOA (ppm)								
0-<1 ^a	0.48 ^b	0.47	0.27	0.00-0.99	0.31	0.2	0.32	0.00-0.90
1-<10	3.34 ^b	2.49	2.17	1.03-8.92	3.03	2.4	1.84	1.10-8.20
10-<30	16.26 ^b	15.40	3.39	11.90-21.00	17.11 ^b	14.3	6.90	10.30-28.20
≥30	60.13 ^b	64.45	24.01	31.60-80.00	55.96 ^b	42.4	33.29	34.20-114.10
	$F = 253.25, P = 0.0001$				$F = 77.57, P = 0.0001$			
Age (yr)								
0-<1	43.6	45.0	9.2	27.0-61.0	42.0	41.0	8.3	29.0-60.0
1-<10	39.2	38.0	7.7	27.0-60.0	41.3	40.0	8.6	24.0-58.0
10-<30	39.9	39.5	4.2	34.0-45.0	45.1	46.0	7.4	30.0-55.0
≥30	33.3	32.5	7.4	25.0-43.0	38.2	35.0	9.2	27.0-50.0
	$F = 3.67, P = 0.01$				$F = 0.88, P = 0.46$			
Alcohol (drinks/day)								
0-<1	0.4	0.3	0.5	0.0-1.9	0.5	0.3	0.7	0.0-2.9
1-<10	0.7	0.5	0.7	0.0-3.4	0.5	0.4	0.5	0.0-1.9
10-<30	0.9	0.7	0.6	0.4-2.1	0.8	0.7	0.7	0.0-2.1
≥30	0.9	0.7	0.8	0.0-2.0	0.5	0.4	0.6	0.0-1.4
	$F = 3.05, P = 0.03$				$F = 0.94, P = 0.43$			
BMI (kg/m ²)								
0-<1	28.0	27.5	4.2	20.9-42.0	27.6	26.8	4.2	21.9-45.2
1-<10	26.8	26.3	2.5	21.6-32.5	28.6	27.9	3.4	22.1-38.3
10-<30	29.1	28.8	1.8	27.1-32.0	27.8	27.7	4.0	21.2-34.8
≥30	28.5	28.4	1.6	26.9-30.2	29.8	28.9	1.8	28.2-32.6
	$F = 1.60, P = 0.19$				$F = 0.77, P = 0.52$			
Cigarettes (cigarettes/day)								
0-<1	2.6	0.0	7.5	0.0-30.0	3.8	0.0	9.4	0.0-40.0
1-<10	6.0	0.0	10.5	0.0-40.0	2.6	0.0	6.0	0.0-20.0
10-<30	2.5	0.0	7.1	0.0-20.0	9.1	0.0	15.2	0.0-40.0
≥30	5.0	0.0	10.0	0.0-20.0	6.0	0.0	8.9	0.0-20.0
	$F = 1.26, P = 0.29$				$F = 1.26, P = 0.30$			
Estradiol (pg/mL)								
0-<1	54.7	53.0	13.5	32.0-83.0	68.1	66.0	11.7	45.0-94.0
1-<10	56.0	55.0	12.0	34.0-81.0	65.2	62.0	14.9	45.0-96.0
10-<30	54.8	58.0	11.6	39.0-70.0	67.1	66.5	9.1	54.0-79.0
≥30	62.8	63.0	8.4	55.0-70.0	73.2	75.0	6.7	64.0-81.0
	$F = 0.54, P = 0.66$				$F = 0.69, P = 0.56$			
17-HP (ng/dL)								
0-<1	106.8	106.0	34.9	44.0-203.0	91.6	94.0	32.2	39.0-190.0
1-<10	120.2	115.5	41.5	45.0-249.0	110.6	105.5	35.6	54.0-179.0
10-<30	97.9	105.5	28.4	54.0-134.0	110.3	85.5	77.5	46.0-297.0
≥30	126.5	123.0	66.8	54.0-206.0	123.0	102.0	54.7	72.0-198.0
	$F = 1.55, P = 0.21$				$F = 1.67, P = 0.18$			
Prolactin (μg/L)								
0-<1	8.2	8.0	3.5	2.0-18.0	10.9	10.0	5.1	4.0-23.0
1-<10	8.8	8.0	4.6	2.0-22.0	11.8	10.0	6.0	5.0-28.0
10-<30	15.0 ^b	9.0	15.2	6.0-51.0	12.9	14.0	5.3	3.0-21.0
≥30	7.5	7.5	0.6	7.0-8.0	9.4	9.0	2.7	7.0-14.0
	$F = 3.67, P = 0.01$				$F = 0.66, P = 0.58$			
Bound testosterone (ng/dL)								
0-<1	528.7	513.7	178.0	220.9-1059.5	534.8	518.5	150.4	278.7-1059.5
1-<10	609.7	609.2	168.2	212.2-1021.6	567.7	564.9	152.3	216.4-898.4
10-<30	485.2	477.5	113.9	301.0-651.6	554.4	549.7	185.5	238.1-823.8
≥30	569.6	596.5	81.6	450.9-634.4	567.8	623.0	155.0	341.7-703.0
	$F = 2.48, P = 0.07$				$F = 0.26, P = 0.85$			

TABLE 1
Continued

PFOA (ppm)	1993 Data				1995 Data			
	Mean	Median	SD	Range	Mean	Median	SD	Range
Free testosterone (ng/dL)								
0-<1	15.0	14.8	4.0	6.1-28.1	14.2	14.0	2.7	9.3-20.5
1-<10	16.6	16.5	4.4	6.8-28.4	14.2	14.4	3.1	5.6-19.4
10-<30	14.2	13.5	2.5	10.0-17.4	13.2	14.1	3.5	7.0-18.0
≥30	17.4	18.5	4.7	11.1-21.6	14.4	16.0	4.3	7.3-18.0
	F = 1.81, P = 0.15				F = 0.31, P = 0.82			
TSH (mU/L)								
0-<1	1.4	1.3	0.8	0.2-4.3	1.7	1.5	0.8	0.6-4.0
1-<10	1.4	1.2	0.7	0.5-3.1	1.7	1.5	0.9	0.5-3.7
10-<30	2.1	2.2	0.8	1.2-2.9	2.9 [§]	2.5	1.1	1.9-5.8
≥30	1.2	1.1	0.4	0.6-1.8	1.7	1.3	0.6	1.1-2.5
	F = 2.21, P = 0.09				F = 5.47, P = 0.002			

* BMI, body mass index; 17-HP, 17-alpha hydroxyprogesterone; TSH, thyroid-stimulating hormone.
 † Samples sizes: 0-<1 ppm: 1993, n = 53; 1995, n = 39.
 1-<10 ppm: 1993, n = 46; 1995, n = 26.
 10-<30 ppm: 1993, n = 8; 1995, n = 10.
 ≥30 ppm: 1993, n = 4; 1995, n = 5.
 ‡ Mean significantly different (Bonferroni t-test, p < .05) than the three other PFOA ppm levels
 § Mean level significantly different (Bonferroni t-test, p < .05) than the 0-<1 ppm and 1-<10 ppm PFOA categories

TABLE 2
Mean, Median, Standard Error (SE) of Mean and Range of Estradiol by Body Mass Index and Free Testosterone by Age, Stratified by Serum PFOA Level and Year of Data Collection

BMI (kg/m ²) by PFOA (ppm)	1993 Data					1995 Data				
	n	Mean	Median	SE	Range	n	Mean	Median	SE	Range
Estradiol (pg/mL)										
BMI <28										
0-<1 ppm	30	48.4	47.0	1.7	32.0-68.0	23	66.0	66.0	2.2	48.0-87.0
1-<10	30	55.0	55.0	2.2	34.0-81.0	13	62.0	62.0	3.7	48.0-91.0
10-<30	3	54.3	56.0	5.5	44.0-63.0	5	64.6	64.0	4.1	54.0-79.0
≥30	2	62.5	62.5	7.5	55.0-70.0	0	—	—	—	—
BMI ≥28										
0-1 ppm	23	63.0	66.0	2.9	32.0-83.0	16	71.1	72.0	3.2	45.0-94.0
1-<10	16	57.8	55.5	3.1	34.0-79.0	13	68.3	65.0	4.5	45.0-96.0
10-<30	5	55.0	60.0	6.1	39.0-70.0	5	69.6	72.0	4.1	55.0-78.0
≥30	2	63.0	63.0	7.0	56.0-70.0	5	73.2	75.0	3.0	64.0-81.0
Free testosterone (ng/dL)										
Age <40										
0-<1 ppm	20	17.3	16.8	0.9	10.5-28.1	18	15.3	15.2	0.6	11.3-20.5
1-<10	28	16.8	17.4	0.7	10.1-24.4	13	14.7	14.6	0.6	10.8-17.8
10-<30	4	15.2	14.9	1.0	13.4-17.4	2	15.7	15.7	2.4	13.3-18.0
≥30	3	19.5	20.1	1.4	16.9-21.6	3	15.9	16.0	1.2	13.8-18.0
Age ≥40										
0-<1 ppm	33	13.6	13.4	0.6	6.1-21.2	21	13.2	13.4	0.6	9.3-18.3
1-<10	18	16.2	15.8	1.3	6.8-28.4	13	13.7	14.3	1.0	5.6-19.4
10-<30	4	13.2	13.1	1.4	10.0-16.6	8	12.6	14.1	1.2	7.0-16.2
≥30	1	11.1	11.1	—	—	2	12.2	12.2	4.9	7.3-17.0

moderate drinkers, as was previously reported.²⁸

Discussion

We conducted two cross-sectional studies of PFOA production workers

to investigate the relation between serum PFOA levels and several reproductive hormones: in particular, estradiol and testosterone. Although we did not observe a significantly positive association between PFOA

exposure and estradiol, mean estradiol levels were 10% greater among employees with the highest serum PFOA levels (≥30 ppm); however, this was confounded by BMI, and any interpretation is limited by the

C
n
k
h
g
p
pr
ab
po
di
rin
fer
(1)
exp
rin
199
that
199
mos
sion
sific
(eg.
twec
obse
ficien
jects
ipate
the 15
empla
and 19
estradi
Dose
sensiti
parent
mal an
observ
tween
obse
serum l
in these
the obse
studies;
the CD
mean
PFOA.
suremen
study w
The 10%
levels of
with the
PFOA (≥
threshold
the conve
liferators
their nuclei

Range
9.3-20.5
5.6-19.4
7.0-18.0
7.3-18.0
0.6-4.0
0.5-3.7
1.9-5.8
1.1-2.5

by Age.

Range
48.0-87.0
48.0-91.0
54.0-79.0

45.0-94.0
45.0-96.0
55.0-78.0
64.0-81.0

11.3-20.5
10.8-17.8
13.3-18.0
13.8-18.0

9.3-18.3
5.6-19.4
7.0-18.2
7.3-17.0

mean estradiol among the highest serum; however, BMI, and titated by the

few subjects at this PFOA level. Gilliland also observed an approximate 10% increase in mean estradiol levels from his lowest (0-1 ppm) to highest (15-26 ppm) total serum organic fluorine levels among these production employees.²⁸ Unlike the present study, this previous report also observed a significant nonlinear positive association between estradiol and total serum organic fluorine.²⁸ Possible reasons for the different results include the following: (1) use of different measurements of exposure (total serum organic fluorine in 1990 and serum PFOA in 1993 and 1995); (2) the possibility that the multivariate model used in 1990 may have transgressed the homoscedasticity assumption of regression analysis³⁵; (3) possible misclassification of confounding variables (eg, the expected relationship between BMI and estradiol was not observed in 1990; correlation coefficient = -.01); (4) different subjects analyzed (94 employees participated in both the original 1990 and the 1993 surveys, compared with 61 employees who participated in 1990 and 1995); and (5) differences in the estradiol assays.

Dose, threshold effect, and species sensitivity may account for the apparent differences between the animal and human studies. We did not observe a significant association between estradiol and PFOA but did observe a 10% increase at the highest serum levels of PFOA. Serum PFOA in these workers was likely below the observable effect levels in animal studies: the observable effect level in the CD rat is somewhere above a mean serum level of 55 ppm PFOA.^{18,25} All but three PFOA measurements from employees in our study were below 55 ppm PFOA. The 10% increase in mean estradiol levels observed among employees with the highest levels of serum PFOA (≥ 30 ppm) could suggest a threshold response. The discovery of the convergence of peroxisomal proliferators and estradiol at the level of their nuclear hormone receptors pro-

vides a plausible mechanism for a possible threshold relationship between PFOA and estradiol.^{36,37} While responses to peroxisome proliferators, like PFOA, are readily observed in rats and mice, other species—including humans—have shown no such responses to many types of peroxisome proliferators at equivalent dose levels.³⁸⁻⁴¹

We did not observe any significant associations between PFOA and free or bound testosterone. However, we did observe a significant positive association between 17-HP and PFOA in the 1995 but not 1993 analyses. We examined 17-HP, a precursor of testosterone, because Cook et al¹⁸ suggested that PFOA may affect the conversion of 17-HP to testosterone via inhibition of 17,20-lyase. If this enzyme was inhibited, the expected result would be an increase in 17-HP levels, which was observed in both years' studies, although significantly in only the 1995 study. Recent laboratory work suggests that there may be an accommodation by the CD rat to the initial testosterone-lowering effect of PFOA.¹⁹ A previous report²⁸ observed a significant negative nonlinear association between total serum fluorine and free or bound testosterone. This observation was dependent upon one influential data point, that of an employee who had no detectable total serum organic fluorine level but had the highest free testosterone level measured.

Several methodological issues should 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.¹⁹ Second, the two cross-sectional analyses cannot be viewed as independent populations because 68 employees were studied in both years. Fewer employees participated in serum measurements in the 1995 assessment, although the majority of

those with the highest serum PFOA exposure levels in 1993 also participated in 1995. This reduced sample size resulted in lower statistical power. Third, we specifically measured serum PFOA levels. Use of total serum organic fluorine may represent other perfluorocarbons, which could be peroxisome proliferators, although data suggest that PFOA would represent the greatest fraction of total serum organic fluorine levels in this employee population.^{27,29} Fourth, there could be measurement error in important confounding variables. Analysis of the 68 subjects who participated in both years showed good correlation for the confounding factors of BMI ($r = .93$, $P = 0.0001$) and the self-reported aspects of alcohol consumption ($r = .67$, $P = 0.0001$) and cigarette smoking ($r = .84$, $P = 0.0001$). Fifth, the quality of medical surveillance data can be evaluated by whether known associations are observed.⁴² In this regard, we observed various expected associations (eg, estradiol and BMI, free testosterone and age). Finally, the pulsatile nature of some of the hormones studied (eg, FSH, LH, testosterone) has resulted in prior recommendations that mean hormone measurements should be the result of pooled blood from multiple samples taken at short intervals.⁴³ In our study, multiple samples were not feasible because of the low probability of employees voluntarily giving three samples over a 45- to 60-minute time period.

In summary, we conducted two cross-sectional studies in 1993 and 1995 and did not observe a significantly positive association between PFOA exposure and estradiol or a significantly negative association with testosterone. Our study may not have been sensitive enough to detect whether an association between PFOA and estradiol could exist in humans because measured serum PFOA levels were likely below the observable effect levels suggested in the animal studies. Our results provide reasonable assurance that sig-

nificant hormonal changes among these male production employees were not apparent in relation to their measured serum PFOA levels.

Acknowledgments

The authors gratefully acknowledge the contributions of the following individuals to this study: Frances Curtis, Mary Fowler, Mary Hansen, and Drs James Johnson, Roger Perkins, James Wolter, and Larry Zobel

References

- Bryce H. Industrial and utilitarian aspects of fluorine chemistry. In: Simons J, ed. *Fluorine Chemistry*. New York: Academic Press, 1964:297-492.
- Nickles M. Presence du fluor dans la sang. *Compt Rend* 1856:43-885
- 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
- Guy W. *Fluorocompounds of Human Plasma Analysis Prevalence, Purification, and Characterization*. [Doctoral dissertation.] Rochester, NY: University of Rochester, 1972.
- Griffith FD, Long JE. Animal toxicity studies with ammonium perfluorooctanoate. *Am Ind Hyg Assoc J*. 1980;41:576-583.
- Kennedy G. Dermal toxicity of ammonium perfluorooctanoate. *Toxicol Appl Pharmacol*. 1985;81:348-355.
- Kennedy G, Hall G, Britelli J, Chen H. Inhalation toxicity of ammonium perfluorooctanoate. *Food Chem Toxicol*. 1986; 24:1325-1329.
- Ophaug R, Singer L. Metabolic handling of perfluorooctanoic acid in rats. *Proc Soc Exp Biol Med*. 1980;163:19-23.
- Ylino M, Kojo 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.
- 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.
- Kuslikis BI, Vanden Heuvel JP, Peterson R. Lack of evidence for perfluorodecanoyl or perfluorooctanoyl-coenzyme A formation in male and female rats. *J Biochem Toxicol*. 1992;7:25-29
- 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, Ylino M, Kojo A, Kosma VM. Elimination and toxicity of perfluorooctanoic acid during subchronic administration in the Wistar rat. *Pharmacol Toxicol* 1987;61:66-68.
- 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.
- 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.
- Sabinski LJ. *Two-Year Oral (Diet) Toxicity/Carcinogenicity Study of Fluorochemical FC-143 in Rats*. St. Paul, MN: Riker Laboratories; 1987.
- 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.
- 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.
- 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. *Fundam Appl Toxicol*. 1996;30:220-228
- 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
- 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).
- Oghouran JD, Frame SR, Elliot GS, Cook JC. Pancreatic oncogenic effects of Wyeth 14,643. *Toxicologist* 1997;36:232 (abstract)
- Rao MS, Reddy JK. Hepatocarcinogenesis of peroxisome proliferators. *Ann NY Acad Sci*. 1996;804:573-587
- Palazzolo MJ. *13-Week Dietary Toxicity Study with T-5180 Ammonium Perfluorooctanoate (CAS No. 3825-26-1)*, in Male Rats. Madison, WI: Hazelton Laboratories; 1991.
- Perkins RG. Investigation of ammonium perfluorooctanoate effect on hormone levels and peroxisomal proliferation in the rat. *Toxicologist* 1992;12:38 (abstract).
- Ubel FA, Sorenson SD, Roach DE. Health status of plant workers exposed to fluorochemicals, a preliminary report. *Am Ind Hyg Assoc J*. 1980;41:584-589.
- Gilliland FD. *Fluorocarbons and Human Health: Studies in an Occupational Cohort*. [Doctoral dissertation.] Minneapolis, MN: University of Minnesota; 1992.
- Pothapragada V. Sodium biphenyl method for determination of covalently bound fluorine in organic compounds and biological materials. *Anal Chem*. 1982; 54:1132-1137.
- Gilliland FD, Mandel JS. Mortality among employees of a perfluorooctanoic acid production plant. *J Occup Med*. 1993;35:950-954.
- Simons J, Bryce T. Electrochemical fluorination. In: Simons J, ed. *Fluorine Chemistry*. New York: Academic Press; 1954:340-377.
- 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 Environmental Laboratory Report.] St. Paul, MN: 3M Company; 1996
- SAS Institute, Inc. *SAS Users Guide: Statistics*. Version 6. Cary, NC: SAS Institute, Inc.; 1990.
- Griffin JE, Wilson JD, Disorders of the testes. In: Isselbacher KJ, Braunwald E, Wilson JD, Martin JB, Fauci AS, Kasper DL, eds. *Harrison's Principles of Internal Medicine*. New York: McGraw-Hill, 1994:2006-2017.
- Montgomery DC, Peck EA. *Introduction to Linear Regression Analysis*. New York: John Wiley and Sons, Inc.; 1992: 67-117.
- Keller H, Dreyer C, Medin J, Mahfoudi A, Ozato K, Wahli W. Fatty acids and retinoids control lipid metabolism through activation of peroxisome proliferator-activated receptor-retinoid X receptor heterodimers. *Proc Natl Acad Sci* 1993;90:2160-2164.
- Hunter J, Kassam A, Winrow CJ, Rachubinski RA, Capone JP. Crosstalk between the thyroid hormone and peroxisome proliferator-activated receptors in regulating peroxisome proliferator-responsive genes. *Mol Cell Endocrinol*. 1996;116: 213-221
- Stringer DA. *Hepatic Peroxisome Proliferation* [Monograph No. 17] Brussels: European Centre for Ecotoxicology and Toxicology of Chemicals (ECETOC), 1992

Epidemiologic Study of Perfluorooctanoic Acid • Olsen et al

39. De la Iglesia FA, McGuire ED, Haskins JR, Lalwani ND. Structural diversity of peroxisome proliferators and their effects on mammalian liver cells in vivo. *Ann NY Acad Sci.* 1996;804:310-327.
40. Tugwood JD, Aldridge TC, Lambe KG, MacDonald N, Woodyatt NJ. Peroxisome proliferator-activated receptors: structures and function. *Ann NY Acad Sci.* 1997;804:252-265.
41. Tugwood JD, Lambe KG, Woodyatt NJ. Inter-individual differences in human peroxisome proliferator-activated receptor alpha structure and function. *Toxicologist.* 1997;36:236 (abstract).
42. Olsen GW, Kusch GD, Stafford BA, Gudmundsen SL, Carrier MF. The positive known association design: a quality assurance method for occupational health surveillance data. *J Occup Med.* 1991;33:998-1000.
43. Goldzieher JW, Dozier TS, Smith KD, Steinberger E. Improving the diagnostic reliability of rapidly fluctuating plasma hormone levels by optimized multiple-sampling techniques. *J Clin Endocrinol Metab.* 1976;43:824-830.

821
D. Rouch DE.
Workers exposed to
Primary report.
10:41:584-589.
Occupational Co-
operation.] Minneapo-
nesota: 1992.
um biphenyl
of covalent
Compounds and
Chem. 1982;

5. Mortality
fluorooctanoic
Occup Med.

Chemical fluo-
ed. Fluorine
ademic Press;

Colaizy GE.
Quantification
of perfluoro-
serum using
performance
thermospray
Automated sam-
mental Lab-
N: JM Com-

Users Guide:
NC: SAS

Orders of the
unwald E.
AS, Karper
s of Inter-
Graw-Hill;

roduction
ysis. New
nc.: 1992;

Mahfoudi
acids and
metabolism
the prolifer-
id X re-
Acad Sci.

J. Rachu-
between
ome pro-
regulating
pensive
996;116:

e Prolif-
Brussels:
ogy and
ETOC);

FINAL REPORT

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

Date: September 4, 1998

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

Study
Start Date: September 3, 1997

Protocol Number: EPI-0003
IRB Approval
Exempt Expedited
 X

IRB Approval Date: September 3, 1997

Principal Investigator: Geary W. Olsen, DVM, PhD¹

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

Study Director: Jeffrey H. Mandel, MD, MPH¹

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

ABSTRACT

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

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

INTRODUCTION

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

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

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

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

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

METHODS

PFOA Production

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

Subject Selection and Data Collection

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

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

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

Data Analysis

Simple and stratified analysis, Pearson correlation coefficients, analysis of variance (ANOVA), and ordinary multivariate regression were used to evaluate linear and nonlinear associations between PFOA and the biochemical parameters with adjustment for potential confounding variables [SAS, 1990]. For stratified analyses, employees were divided into four PFOA categories: 0 - <1 ppm, 1 - <10 ppm, 10 - <30 ppm, and ≥ 30 ppm

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

RESULTS

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

Serum PFOA levels were not consistently correlated with any of the potential confounding variables, serum chemistries or hematological parameters. The Pearson correlation coefficients (in parentheses) between PFOA and the variables for 1993, 1995

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

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

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

PFOA values (e.g., ≥ 10 ppm) compared to those with lower values. Use of multivariable regression models (data not shown) continued to indicate a weak negative association between CCK and PFOA adjusting for potential confounding variables (e.g., age, body mass index, alcohol, cigarettes and clinical chemistry measures of hepatic function).

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

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

DISCUSSION

We observed a weak negative association between serum PFOA and plasma CCK among 74 workers engaged in the production of ammonium perfluorooctanoate. This finding was opposite that hypothesized based on the toxicological findings of Obourm et

al [1997] who fed diets to rats containing either 0 or 100 ppm of Wyeth-14,643, a potent peroxisome proliferator, which causes the same triad of tumors, including pancreas acinar cell adenomas, as PFOA. After six months, the mean pancreatic weights of the treated rats were 17 percent above control animals ($p < .05$), mean plasma CCK levels were 44 percent higher ($p < .05$) and markers of cholestasis (total bile acids, alkaline phosphatase and bilirubin) were also significantly elevated. The clinical pathology data indicative of cholestasis were associated with alterations in bile flow and bile acid output. Obourn et al [1997] had also conducted *in vitro* experiments of both Wyeth-14,643 and PFOA which argued against other biological pathways known to elevate plasma CCK levels including CCK_A receptor agonism, trypsin inhibition and increased dietary fat content. Obourn et al [1997] concluded that chronic exposure to Wyeth-14,643 may induce pancreatic adenomas via a mild but sustained increase in CCK levels secondary to hepatic cholestasis.

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

et al., 1998]. Second, even if the mechanism existed in humans, the serum measurements in these production workers may have been too low to cause an effect.

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

and Kern, 1995]. Finally, we must ask whether the weak negative association observed in our study represents an entirely different biological relationship than what was originally postulated based on the findings by 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 ≥ 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.
- Axelsson 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, DeLucia 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. *Toxicologist* 14:301 (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 cholecystokinin-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, Spydevo JO (1992). The mechanism underlying the hypolipemic effect of perfluorooctanoic acid (PFOA), perfluorooctane sulphonic acid (PFOSA) and clofibrate 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 automated sample preparation. *3M Environmental Laboratory 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 mitochondrial 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.
- Obourm 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 Refeighem 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 L. Mean Standard Deviation of Mean (SD) and Range of Perfluorooctanoic Acid (PFOA), Demographic, Clinical Chemistry and Hematology, by Serum PFOA Levels, and Year of Data Collection

PFOA ppm	1993 Data			1995 Data			1997 Data		
	Mean	SD	Range	Mean	SD	Range	Mean	SD	Range
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
	<i>F value = 248.5, p = .0001</i>			<i>F value = 77.6, p = .0001</i>			<i>F value = 145.5, p = .0001</i>		
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
	<i>F value = 3.3, p = .02</i>			<i>F value = 0.9, p = .46</i>			<i>F value = 0.3, p = .84</i>		
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
	<i>F value = 1.1, p = .33</i>			<i>F value = 0.8, p = .52</i>			<i>F value = 1.4, p = .24</i>		
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	0.5	0.1-1.6
	<i>F value = 2.9, p = .04</i>			<i>F value = 0.9, p = .43</i>			<i>F value = 0.7, p = .58</i>		
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	-	-
	<i>F value = 1.3, p = .26</i>			<i>F value = 1.3, p = .29</i>			<i>F value = 1.7, p = .18</i>		
CCK									
0 - <1 ppm	Not done			Not done			33.4	15.7	13.4-80.5
1 - <10 ppm	Not done			Not done			28.0	19.2	8.8-86.7
10 - <30 ppm	Not done			Not done			15.7	4.1	11.4-23.0
≥ 30 ppm	Not done			Not done			20.6	7.2	12.9-29.9
	Not done			Not done			<i>F value = 2.5, p = .06</i>		

Table L
continued

PFOA
ppm

1993 Data
Mean SD Range

1995 Data
Mean SD Range

1997 Data
Mean SD Range

Alkaline Phosphatase

	1993 Data	1995 Data	1997 Data
	Mean SD Range	Mean SD Range	Mean SD Range
0 - <1 ppm	88 26 37-161	78 18 40-114	79 19 27-122
1 - <10 ppm	82 23 47-151	80 25 48-165	87 23 47-164
10 - <30 ppm	75 14 58-107	88 29 59-146	81 28 61-142
≥ 30 ppm	103 33 74-132	93 38 55-136	78 16 64-100
	<i>F value = 1.6, p = .19</i>	<i>F value = 1.0, p = .39</i>	<i>F value = 0.9, p = .45</i>

GGT

	1993 Data	1995 Data	1997 Data
	Mean SD Range	Mean SD Range	Mean SD Range
0 - <1 ppm	33 19 11-84	42 27 16-149	34 27 15-130
1 - <10 ppm	50 70 6-472	51 41 19-190	36 25 14-162
10 - <30 ppm	36 14 19-59	39 13 21-61	28 11 15-50
≥ 30 ppm	40 26 19-77	42 15 23-58	32 11 16-40
	<i>F value = 1.0, p = .41</i>	<i>F value = 0.6, p = .61</i>	<i>F value = 0.3, p = .91</i>

SGOT

	1993 Data	1995 Data	1997 Data
	Mean SD Range	Mean SD Range	Mean SD Range
0 - <1 ppm	23 7 11-60	21 6 13-36	26 7 13-41
1 - <10 ppm	26 11 12-83	24 13 13-75	25 7 14-48
10 - <30 ppm	23 4 16-28	21 4 15-29	24 2 22-28
≥ 30 ppm	28 6 23-35	20 4 15-25	27 5 22-34
	<i>F value = 1.1, p = .36</i>	<i>F value = 0.5, p = .66</i>	<i>F value = 0.3, p = .84</i>

SGPT

	1993 Data	1995 Data	1997 Data
	Mean SD Range	Mean SD Range	Mean SD Range
0 - <1 ppm	45 14 22-88	44 13 27-80	31 10 13-59
1 - <10 ppm	48 29 22-221	53 34 27-175	33 15 14-80
10 - <30 ppm	43 6 35-52	45 12 28-70	30 11 18-51
≥ 30 ppm	54 5 51-62	51 13 39-71	43 13 27-57
	<i>F value = 0.4, p = .75</i>	<i>F value = 0.9, p = .46</i>	<i>F value = 1.0, p = .42</i>

Total Bilirubin

	1993 Data	1995 Data	1997 Data
	Mean SD Range	Mean SD Range	Mean SD Range
0 - <1 ppm	0.63 0.28 0.20-1.30	0.84 0.32 0.40-2.20	0.84 0.48 0.40-2.30
1 - <10 ppm	0.58 0.22 0.20-1.30	0.75 0.26 0.40-1.30	0.79 0.42 0.30-2.40
10 - <30 ppm	0.53 0.17 0.20-0.80	0.67 0.21 0.40-1.00	0.66 0.33 0.30-1.20
≥ 30 ppm	0.65 0.13 0.50-0.80	0.64 0.17 0.50-0.90	0.73 0.22 0.40-0.90
	<i>F value = 0.7, p = .55</i>	<i>F value = 1.5, p = .22</i>	<i>F value = 0.4, p = .76</i>

Direct Bilirubin

	1993 Data	1995 Data	1997 Data
	Mean SD Range	Mean SD Range	Mean SD Range
0 - <1 ppm	0.18 0.07 0.10-0.40	0.22 0.04 0.20-0.30	0.10 0.10 0.00-0.60
1 - <10 ppm	0.17 0.07 0.10-0.30	0.22 0.05 0.10-0.30	0.09 0.05 0.00-0.20
10 - <30 ppm	0.18 0.07 0.10-0.30	0.19 0.06 0.10-0.30	0.09 0.04 0.00-0.10
≥ 30 ppm	0.18 0.10 0.10-0.30	0.18 0.04 0.10-0.20	0.08 0.05 0.00-0.10
	<i>F value = 0.1, p = .94</i>	<i>F value = 2.2, p = .10</i>	<i>F value = 0.2, p = .89</i>

Table I
continued

PFOA ppm	1993 Data			1995 Data			1997 Data		
	Mean	SD	Range	Mean	SD	Range	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
<i>F value = 0.6, p = .65</i>									
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
<i>F value = 2.0, p = .11</i>									
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
<i>F value = 0.2, p = .91</i>									
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
<i>F value = 0.5, p = .67</i>									
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
<i>F value = 0.9, p = .44</i>									
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
<i>F value = 0.5, p = .68</i>									

Table I.
continued

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

Table L
continued

PFOA ppm	1993 Data			1995 Data			1997 Data		
	Mean	SD	Range	Mean	SD	Range	Mean	SD	Range
	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
	<i>F value = 0.1, p = .99</i>			<i>F value = 0.3, p = .87</i>			<i>F value = 1.0, p = .39</i>		

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

*Study Population by PFOA (ppm) Category and Year

PFOA Category	1993	1995	1997
0 - <1 ppm	52	39	29
1 - <10 ppm	46	25	34
10 - <30 ppm	9	10	7
≥ 30 ppm	4	5	4

Figure 1

LN(CCK) vs. PFOA

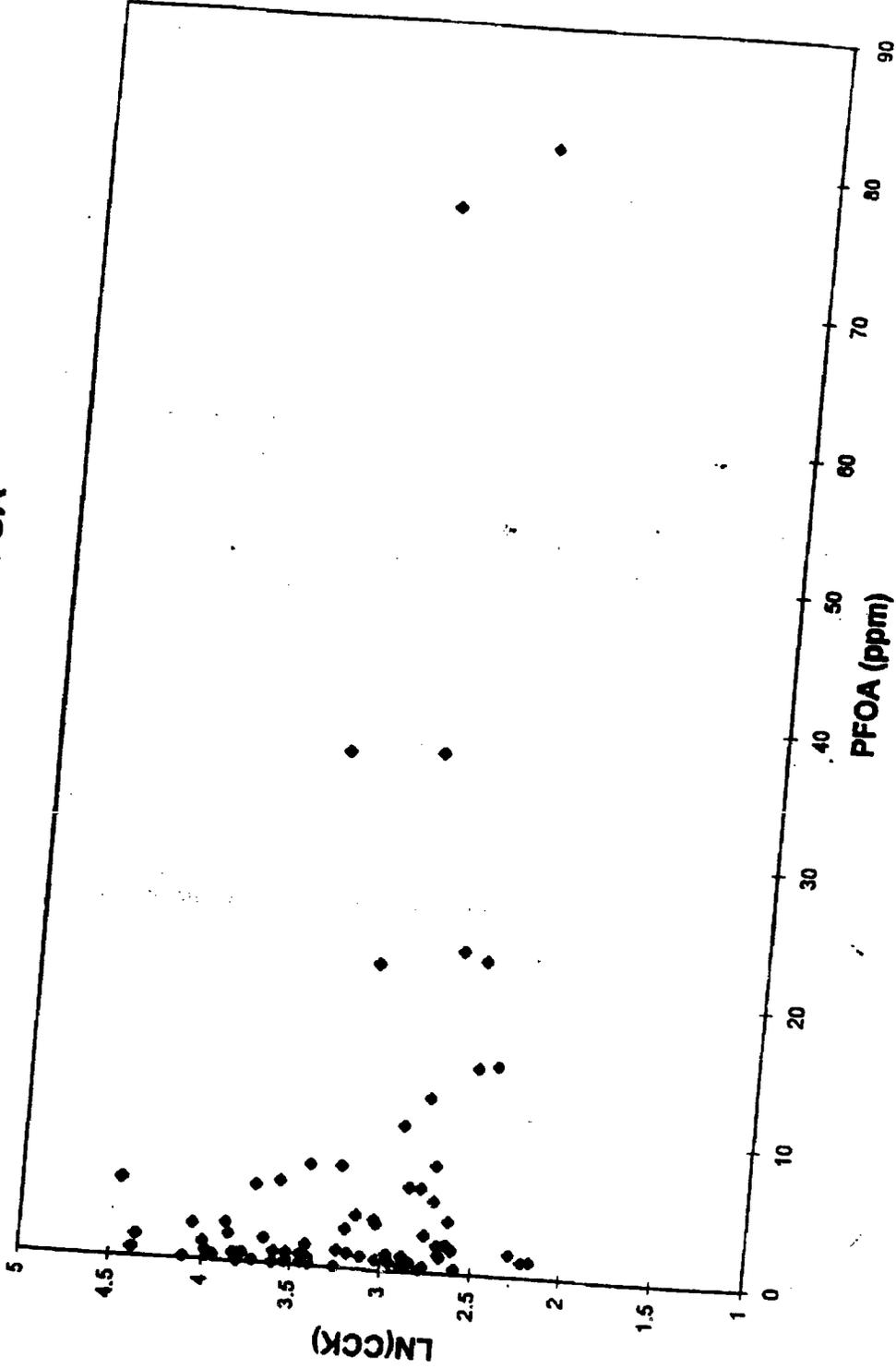


Table II. Change in HDL* from Light Alcohol Drinker (< 1 drink/day) to Moderate Alcohol Drinker (\geq 1 drink/day) Associated with a 10 ppm Change in Serum PFOA Level

Year	Moderate Drinker	Moderate Drinker with 10 ppm increase in PFOA
1990 ^a	+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).

^a Data in 1990 analyzed total serum organic fluorine (see Gilliland and Mandel, 1996).

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

BMI (kg/m ²)	25	30	35
<u>SGOT</u>			
1990 [#]	-2.4	3.7	9.7
1993	1.3	0.5	-2.5
1995	-0.3	0.1	0.5
1997	2.1	0.1	-1.9
<u>SGPT</u>			
1990 [#]	-3.0	28.0	59.0
1993	2.5	1.5	0.5
1995	0.8	-1.0	-2.1
1997	5.3	0.7	-3.8

[#]Determined from multivariable regression model adjusted for age, body mass index and smoking.

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

CERTIFICATE OF AUTHENTICITY

THIS IS TO CERTIFY that the microimages appearing on this microfiche are accurate and complete reproductions of the records of U.S. Environmental Protection Agency documents as delivered in the regular course of business for microfilming.

Date produced 10 18 00
(Month) (Day) (Year)

Mary J. Julek
Camera Operator

Place Syracuse New York
(City) (State)



END