



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

This published paper by Olsen et al (J Occup Env Med 1998;40:614-622) further examined the observation initially reported by Gilliland in his doctoral thesis that serum PFOA levels (as measured by total organic fluorine) may be associated with reproductive hormone changes. In 1993 and 1995, the fluorochemical medical surveillance program had 111 and 80 male production workers (Cottage Grove, MN) voluntarily participate. Unlike the 1990 program which analyzed for serum total organic fluorine, serum PFOA was measured in 1993 and 1995 by mass spectrometry methods. Serum PFOA levels were then compared to several reproductive hormones. Serum PFOA was not significantly associated with estradiol or testosterone. 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 any of the other measured hormones.

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 fluorine in a covalently bound organic state was first reported in 1968.³⁻⁴ Guy subsequently identified perfluorooctanoic acid (PFOA, $C_7F_{15}CO_2H$) 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 Crl: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 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- α 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

Pantex). 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.¹³

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, for PFOA and the variable of interest 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

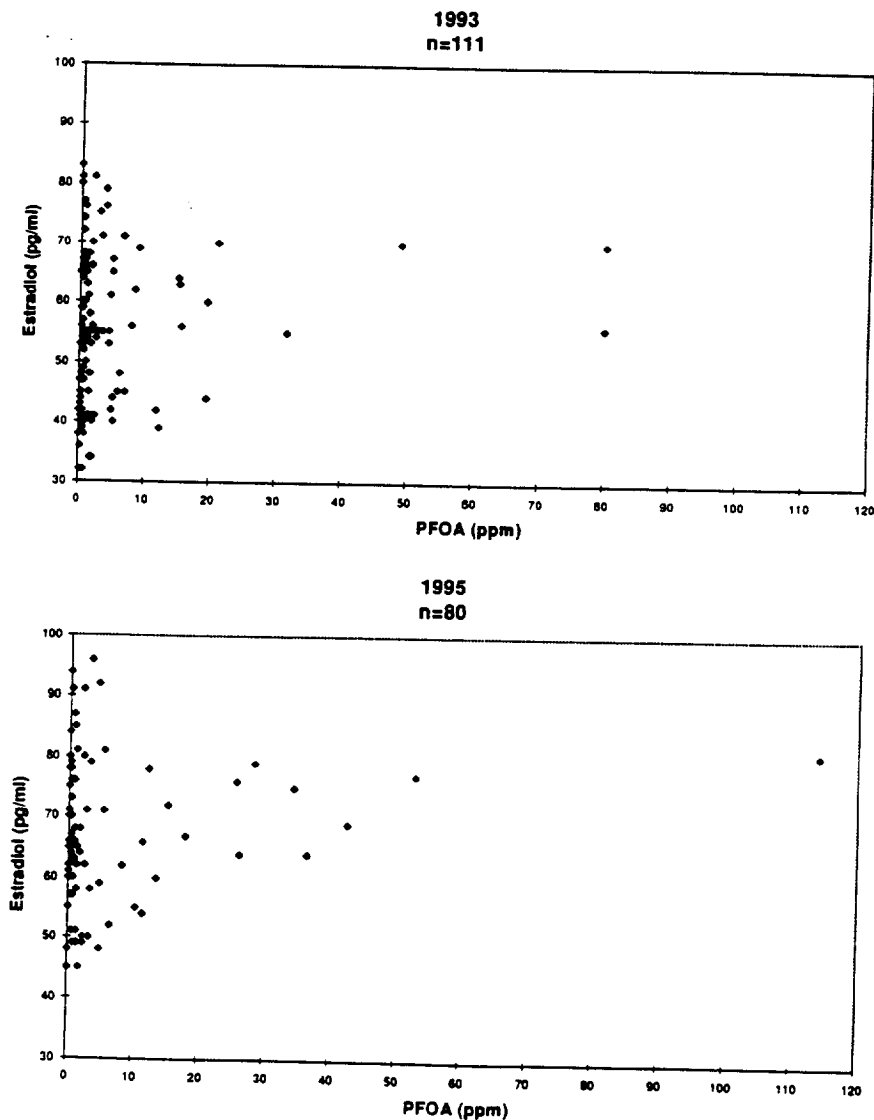


Fig. 1. Scatterplot of serum estradiol (pg/ml) by perfluorooctanoic acid (PFOA, in parts per million [ppm]) for employees in 1993 and 1995.

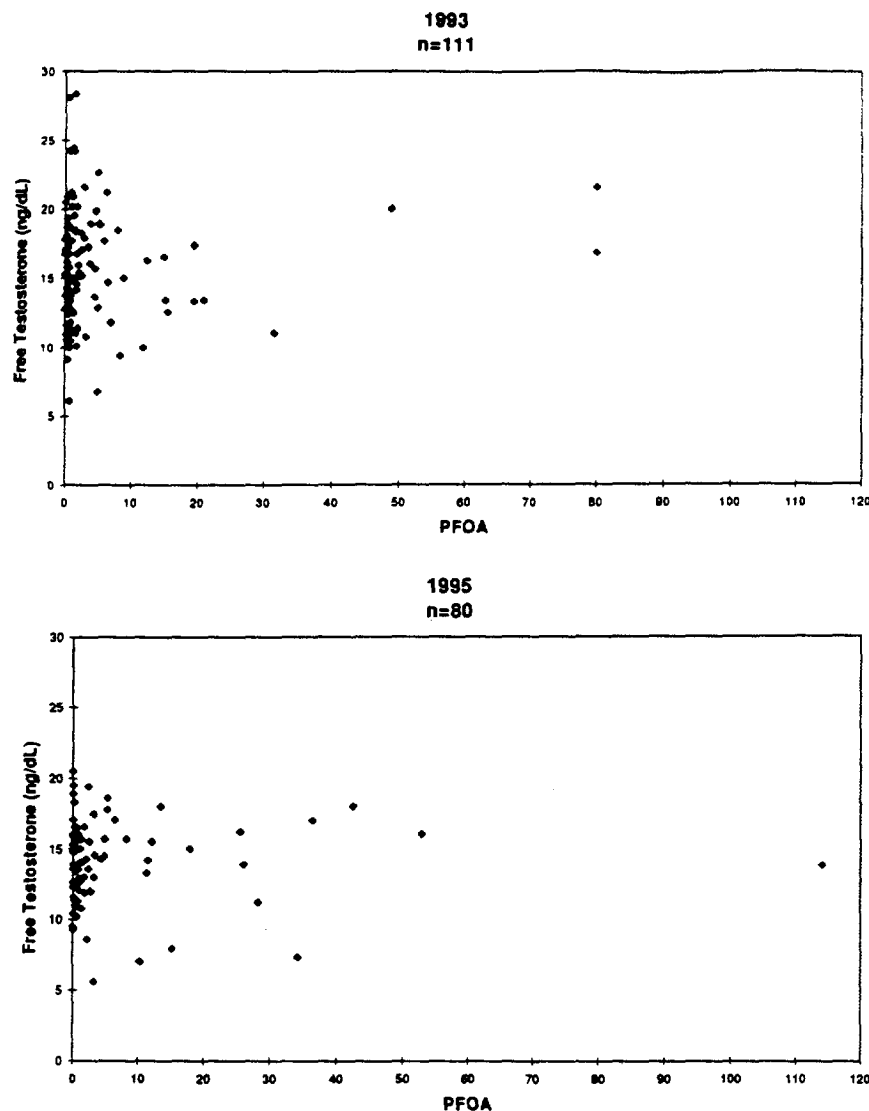


Fig. 2. Scatterplot of serum free testosterone (ng/dl) by PFOA (ppm) for employees in 1993 and 1995.

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,³⁴ 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

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†	0.48 [‡]	0.47	0.27	0.00-0.99	0.31	0.2	0.32	0.00-0.90
1-<10	3.34 [‡]	2.49	2.17	1.03-8.92	3.03	2.4	1.84	1.10-8.20
10-<30	16.26 [‡]	15.40	3.39	11.90-21.00	17.11 [‡]	14.3	6.90	10.30-28.20
≥30	60.13 [‡]	64.45	24.01	31.60-80.00	55.96 [‡]	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 [§]	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	0.8-1.8	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				
	<i>n</i>	Mean	Median	SE	Range	<i>n</i>	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

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 = -0.01); (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, Brittelli 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.
- Ylinen M, Koho A, Hanhijarvi H, Peura P. Disposition of perfluorooctanoic acid in the rat after single and subchronic administration. *Bull Environ Contam Toxicol*. 1990;44:46-53.
- 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 RE. 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, Ylinen M, Kojo A, Kosma VM. Elimination and toxicity of perfluorooctanoic acid during subchronic administration in the Wistar rat. *Pharmacol Toxicol*. 1987;61:66-68.
- 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.
- Sibinski 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).
- Ogboorn 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, Martini 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.

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, Currier 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.