Serum Perfluorooctane Sulfonate and Hepatic and Lipid Clinical Chemistry Tests in Fluorochemical Production Employees

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The 3M Company manufactures fluorochemicals, which have as a precursor perfluorooctane sulfonyl fluoride ($C_8F_{17}SO_2F$). These compounds may be expected to transform metabolically, to an undetermined degree, to perfluorooctane sulfonate (PFOS, $C_8F_{17}SO_3^-$) as an end-stage metabolite. Subchronic studies in rats and primates indicate a potential for cumulative toxicity with PFOS with the primary effect related to metabolic wasting with hypolipidemia as a consistent finding. Biennial medical surveillance has been offered to the company's fluorochemical production workers located in Decatur, Alabama, and Antwerp, Belgium. In 1995, the mean serum PFOS level, as measured by high-performance liquid chromatography mass spectrometry, for 178 male employees was 2.19 parts per million (ppm; range, 0.00 to 12.83 ppm), and in 1997, for 149 male employees, it was 1.75 ppm (0.10 to 9.93 ppm). Our analyses suggest that among these production employees, there were no substantial changes in serum hepatic enzymes, cholesterol, or lipoproteins associated with PFOS levels less than 6 ppm. It was not possible to derive inferences from the few employees who had serum PFOS levels ≥ 6 ppm. These results may be due to the lower levels of serum PFOS measured among these production employees, compared to those suspected to cause effects in laboratory animals.

The 3M Company manufactures products that contain fluorochemicals, either as intentional components or residual impurities, that have as a precursor molecule perfluorooctane sulfonyl fluoride ($C_8F_{17}SO_2F$). Workplace exposure may occur by inhalation, ingestion, and dermal routes. These fluorochemicals may transform metabolically, to an undetermined degree, to perfluorooctane sulfonate (PFOS; $C_8F_{17}SO_3^-$) as an end-stage metabolite.[1] Potassium perfluorooctane sulfonate ($C_8F_{17}SO_3^-K^+$) is itself a surfactant used as a wetting and foaming agent in industrial and commercial processes.

PFOS is known to concentrate primarily in the liver and, to a 10-fold lesser extent, in the plasma of rats.[2] There appears to be significant enterohepatic circulation of PFOS with both urinary and fecal excretion.[2,3] Cholestyramine decreased the retention of radiolabeled PFOS in the liver and plasma and increased its elimination via feces.[3] Subchronic studies in rats and primates suggest there is cumulative toxicity with PFOS.[4–7] Lowered serum total cholesterol levels appear to be an early consistent finding, with cumulative toxicity resulting in metabolic wasting and ultimately death. Although the mechanism of toxicity in laboratory animals is not fully understood, it may be due to an effect on fatty acid transport and metabolism, membrane function, peroxisome proliferation, and/or mitochondrial bioenergetics.[8–11]

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Voluntary biennial medical surveillance of fluorochemical production employees has been routinely performed since the late 1970s at 3M's Decatur, Alabama, and Antwerp, Belgium, locations. Total serum organic fluorine levels were analyzed until the mid-1990s when serum PFOS determination, quantifiable by high-performance liquid chromatography mass spectrometry, became available. The purpose of this study was to provide a cross-sectional analysis of the medical surveillance data in relation to the employees' serum PFOS levels.

Methods

Fluorochemical Production

Fluorochemical production began in Decatur in 1961 and Antwerp in 1976. In general, perfluorinated chemicals are produced via an electrochemical process: a solution of organic substrate is electrolyzed in anhydrous hydrogen fluoride at a low voltage.[12] The products of this electrolysis cell reaction are highly fluorinated compounds, with the end-product defined by the starting material. Potential for workplace exposure to those fluorochemicals that metabolize to PFOS can occur in the electrochemical cell, the reactor, mixing, drumming, and packaging areas, as well as in the plant's quality assurance and research and development laboratories.

Subject Selection

Voluntary fluorochemical medical surveillance examinations are offered biennially to approximately 200 Antwerp and 300 Decatur production employees. In 1995, a total of 178 male employees (Antwerp: $n = 88$; Decatur: $n = 90$) participated in the medical surveillance examinations. In 1997, 149 male employees (Antwerp: $n = 65$; Decatur: $n = 84$) participated. (There were too few female employees to include in the data analysis.) Sixty-one employees participated in both years. This reduction was due to a large turnover of employees at both plant locations during 1996 and 1997. The surveillance consisted of a medical questionnaire; measurement of height, weight, and blood pressure; standard clinical chemistry and hematology tests; and determination of serum PFOS levels.

PFOS Analysis

In 1995, the analysis for serum PFOS was conducted by 3M's Environmental Laboratory in St. Paul, Minnesota. The method used tetrabutylammonium to ion-pair with PFOS in serum.[13] The ion-pairs were then extracted with ethyl acetate. The abstraction product was then analyzed using high-performance liquid chromatography–thermospray mass spectrometry. In 1997, the serum samples were analyzed by liquid chromatography/mass spectrometry, using selected ion monitoring in the negative-ion mode.[14,15]

Laboratory Analyses

For both time periods and plant locations, United Laboratory Services (St. Paul, MN) performed the standard hematological and serum chemistry tests. These included the following: alkaline phosphatase (IU/L), gamma glutamyl transferase (IU/L), aspartate aminotransferase (IU/L), alanine aminotransferase (IU/L), total and direct bilirubin (mg/dL), cholesterol (mg/dL), low-density protein (mg/dL), high-density cholesterol (HDL; mg/dL) and triglycerides (mg/dL). Clinical chemistry, hematology, and serum PFOS determinations were performed on overnight fasted blood samples.

Data Analysis

Descriptive, simple, and stratified analyses, analyses of variance, and ordinary multivariable regression were used to evaluate associations between PFOS and each hematological and clinical chemistry test. Age, body mass index (BMI; kg/m^2), current alcohol consumption (drinks per day), and cigarette use (cigarettes smoked per day) were potential confounding factors that were considered in the analyses. For stratified analyses, employees were divided into four PFOS categories (0 to <1 ppm, 1 to <3 ppm, 3 to <6 ppm, and ≥6 ppm) to determine whether an effect could be detected at the highest serum PFOS levels. Other categorical levels were used that provided similar results. For multivariable regression analyses, serum PFOS and the potential confounders of age, BMI, alcohol use, and cigarette use were examined as continuous and categorical explanatory variables in the models. Multivariable regression models were fitted, with PFOS analyzed as a continuous variable, using linear as well as non-linear transformations in order to maximize the possibility of finding associations between PFOS and the dependent variable of interest. Natural log transformations of the dependent variables were performed, when necessary, to normalize variables and to enhance model fit. Traditional stepwise selection procedures were also utilized (selection in and out of model was set at $P = 0.1$), as well as taking into account other covariates that may be on the biologic pathway of effect.[16] We did not examine changes in measured PFOS between the two time periods because the estimated half-life of PFOS in the serum is believed to range between 1000 and 1500 days (J. H. Mandel, MD, unpublished data based on 4 years of serum measurements of three retirees, 1998). Study results were analyzed using the SAS System.[17]

Results

The distribution of employees, by serum PFOS exposure categorization, is presented in Table 1. Whereas 20% of the Decatur employees had exposures at ≥3 ppm for both years, this proportion in Antwerp declined from 25% in 1995 to 13% in 1997. For both years, 95% of the employees' serum PFOS levels were below 6 ppm. There were no PFOS measurements ≥6 ppm in Antwerp in 1997.

TABLE 1
Distribution of Employees, by Year, Location, and Perfluorooctane Sulfonate (PFOS) Exposure Level (in parts per million [ppm])

	1995 Data						1997 Data					
	All Employees		Antwerp		Decatur		All Employees		Antwerp		Decatur	
PFOS	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%
0 to <1 ppm	45	25	34	39	11	12	60	40	31	48	29	35
1 to <3 ppm	91	51	32	36	59	66	63	43	25	38	38	45
3 to <6 ppm	35	20	19	22	16	18	21	14	9	14	12	14
≥6 ppm	7	4	3	3	4	4	5	3	0	0	5	6
Total	178	100	88	100	90	100	149	100	65	100	84	100

TABLE 2
Mean Values of PFOS, Demographic, Serum Chemistry, and Hematologic Parameters for Antwerp and Decatur, 1995 and 1997 Examinations[a]

	1995 Data		1997 Data	
Variable	Antwerp	Decatur	Antwerp	Decatur
PFOS, ppm	1.93	2.44	1.48	1.96
Age, years	37***	45	33***	44
BMI, kg/m^2	23.9***	29.2	23.5***	30.0
Cigarettes, no. per day	4.7*	7.9	5.5	6.6
Alcohol, drinks per day	1.3***	0.2	1.1***	0.1
Alk Phos, IU/L	75***	97	70***	87
GGT, IU/L	41	48	26*	36
AST, IU/L	26*	29	27	26
ALT, IU/L	44	47	31	34
T. bilirubin, mg/dL	0.86***	0.58	0.80***	0.58
D. bilirubin, mg/dL	0.22	0.21	0.15***	0.12
Cholesterol, mg/dL	214	218	206	215
LDL, mg/dL	138	136	134	137
HDL, mg/dL	54***	43	50***	42
Triglycerides, mg/dL	115***	187	111***	192

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

[a] BMI, body mass index; Alk Phos, alkaline phosphatase; GGT, gamma glutamyl transferase; AST, aspartate aminotransferase; ALT, alanine aminotransferase; T., total; D., direct; LDL, low-density lipoprotein; HDL, high-density lipoprotein.

The mean values for PFOS, demographic, and liver and lipid test results, by location, are presented in Table 2. The Antwerp male employee population was significantly younger than that at Decatur, had lower BMIs, and had higher self-reported daily consumption of alcohol. In addition, their clinical profiles were different for several tests. For both time periods, the Antwerp employees had lower mean alkaline phosphatase and triglyceride values and higher total bilirubin and HDL values.

Table 3 lists the mean, median, standard deviation, and range of the covariates and hepatic enzymes, cholesterol, and lipoproteins by four levels of PFOS categorization (0 to <1, 1 to <3, 3 to <6, and ≥6 ppm) for the combined populations for each surveillance year. (Hematology and other clinical chemistry data were unremarkable and are not shown.) Several observations are noteworthy. First, the mean for the ≥6 ppm PFOS category was one order of magnitude higher than the lowest PFOS category (0 to <1 ppm) for both years. Also, the means of the four PFOS categories were significantly different from each other. Second, there was only one variable, total bilirubin, that had significant ($P < 0.05$) F tests for differences in means in both years of analysis. Stratification by plant location and surveillance year did not result in significant findings for total bilirubin or direct bilirubin (Table 4). Third, mean serum cholesterol levels remained constant (1995) or increased (1997) with higher PFOS category levels, although the HDL mean values trended lower among those employees with the highest PFOS categorizations. Stratification by plant location and surveillance year did not result in significantly different mean cholesterol or HDL values by PFOS categories (Table 5). Finally, it should be noted that employees in the highest PFOS category were older and had higher BMIs than did employees in the lowest PFOS category. Furthermore, in 1997, the employees with ≥6 ppm serum PFOS levels were only from Decatur (Table 1); thus the analyses may be confounded because Decatur employees were generally older and heavier than Antwerp employees. For example, in 1997 the mean HDL level for the ≥6 ppm group was 40 mg/dL (Table 3). However, this mean value was solely from Decatur employees.

Linear and nonlinear relationships between PFOS and the dependent variables of interest, taking into account the potential confounding affects of age, BMI, alcohol, and cigarettes, resulted in many analyses. Total bilirubin showed a significant

TABLE 3
Mean, Median, Standard Deviation (SD) of Mean and Range of PFOS, by Demographic and Serum Chemistries for Antwerp and Decatur Employees Combined, for 1995 (n = 178) and 1997 (n = 147)

	1995 Data				1997 Data			
PFOS* (ppm)	Mean	Median	SD	Range	Mean	Median	SD	Range
PFOS, ppm								
0 to <1	0.49[1]	0.50	0.27	0.00 to 0.90	0.52[1]	0.52	0.27	0.10 to 0.97
1 to <3	1.82[1]	1.77	0.58	1.00 to 2.91	1.78[1]	1.64	0.56	1.02 to 2.89
3 to <6	4.12[1]	3.97	0.81	3.00 to 5.80	3.87[1]	3.59	0.70	3.09 to 5.30
≥6	8.17[1]	7.73	2.52	6.06 to 12.83	7.20[1]	6.68	1.59	6.05 to 9.3
	F value = 321.9, P < 0.0001				F value = 367.6, P < 0.0001			
Age, years								
0 to <1	37	36	8	21 to 58	36	34	11	21 to 62
1 to <3	42[2]	41	9	25 to 60	42[2]	41	9	24 to 63
3 to <6	40	40	7	26 to 55	41	42	5	32 to 54
≥6	45	43	7	37 to 56	42	45	9	29 to 52
	F value = 3.7, P = 0.02				F value = 5.1, P = 0.002			
Alcohol, drinks per day								
0 to <1	0.8	0.6	0.9	0 to 3.6	0.5	0.1	0.8	0 to 4.3
1 to <3	0.5	0.1	0.7	0 to 3.6	0.5	0.1	0.8	0 to 5.0
3 to <6	1.2	0.3	1.9	0 to 6.0	1.0	0.1	1.8	0 to 7.1
≥6	0.7	0.0	1.1	0 to 2.9	0.2	0.1	0.2	0.1 to 0.8
	F value = 4.0, P = 0.009				F value = 1.8, P = 0.15			
BMI, kg/m^2								
0 to <1	25.5	24.8	4.2	17.9 to 38.7	26.0	24.9	4.9	20.1 to 41.7
1 to <3	27.7	26.3	5.8	19.6 to 60.7	27.7	26.4	5.7	18.1 to 48.5
3 to <6	24.9[3]	25.0	3.8	17.9 to 32.5	27.3	27.9	4.4	19.1 to 36.0
≥6	27.7	29.4	4.2	20.6 to 33.0	30.8	29.7	4.0	26.1 to 36.2
	F value = 3.7, P = 0.02				F value = 2.1, P = 0.10			
Cigarettes, no. per day								
0 to <1	2.6	0.0	6.4	0.0 to 25.0	4.7	0.0	9.4	0 to 40
1 to <3	6.8	0.0	11.3	0.0 to 40.0	8.2	0.0	11.3	0 to 40
3 to <6	10.6	8.0	12.4	0.0 to 40.0	4.1	0.0	8.3	0 to 30
≥6	0.4	0.0	1.1	0.0 to 3.0	6.0	0.0	13.4	0 to 30
	F value = 4.8, P = 0.003				F value = 1.5, P = 0.23			
Alkaline phosphatase, IU/L								
0 to <1	80	78	22	31 to 158	77	73	17	49 to 132
1 to <3	89	89	27	49 to 191	83	79	23	41 to 163
3 to <6	86	85	21	32 to 124	76	74	22	29 to 120
≥6	88	85	24	63 to 136	88	84	18	65 to 114
	F value = 1.3, P = 0.28				F value = 1.2, P = 0.32			
GGT, U/L								
0 to <1	43	31	28	16 to 155	28	22	20	10 to 142
1 to <3	47	36	39	2 to 293	36	25	33	10 to 179
3 to <6	40	39	15	21 to 80	28	27	14	13 to 71
≥6	43	33	18	28 to 79	33	37	12	17 to 48
	F value = 0.5, P = 0.71				F value = 1.1, P = 0.34			
AST, U/L								
0 to <1	27	25	13	15 to 96	27	25	7	13 to 53
1 to <3	29	26	12	14 to 90	26	25	7	15 to 56
3 to <6	25	24	5	13 to 37	25	23	7	14 to 43
≥6	33	33	6	26 to 43	29	28	3	26 to 34
	F value = 1.8, P = 0.14				F value = 0.5, P = 0.67			
ALT, IU/L								
0 to <1	48	43	20	27 to 118	31	30	11	13 to 60
1 to <3	46	42	21	18 to 183	33	29	16	10 to 89
3 to <6	42	41	7	30 to 59	34	31	18	14 to 82
≥6	51	49	17	29 to 82	41	45	10	25 to 49
	F value = 1.0, P = 0.38				F value = 0.9, P = 0.46			
T. bilirubin, mg/dL								
0 to <1	0.88	0.70	0.50	0.40 to 2.90	0.77	0.60	0.40	0.30 to 2.30
1 to <3	0.66[2]	0.60	0.30	0.20 to 1.50	0.61[2]	0.60	0.21	0.30 to 1.30
3 to <6	0.64[2]	0.60	0.28	0.20 to 1.40	0.63	0.50	0.31	0.40 to 1.30
≥6	0.76	0.70	0.23	0.50 to 1.20	0.58	0.50	0.24	0.40 to 1.00
	F value = 4.4, P = 0.005				F value = 2.9, P = 0.04			

TABLE 3
Continued.

PFOS* (ppm)	1995 Data Mean	Median	SD	Range	1997 Data Mean	Median	SD	Range
D. bilirubin, mg/dL								
0 to <1	0.22	0.20	0.05	0.02 to 0.40	0.15	0.10	0.07	0.10 to 0.40
1 to <3	0.21	0.20	0.06	0.10 to 0.40	0.12[2]	0.10	0.04	0.10 to 0.20
3 to <6	0.21	0.20	0.04	0.20 to 0.30	0.12	0.10	0.04	0.10 to 0.20
≥6	0.20	0.20	0.02	0.10 to 0.30	0.10	0.10	0.00	0.10 to 0.10
	F value = 0.6, *P* = 0.58				*F* value = 3.5, *P* = 0.02			
Cholesterol, mg/dL								
0 to <1	219	215	47	100 to 340	198	197	40	110 to 280
1 to <3	216	213	43	118 to 315	216	219	42	116 to 365
3 to <6	214	214	35	128 to 278	229[2]	224	29	192 to 321
≥6	213	221	36	160 to 251	229	238	26	186 to 250
	F value = 0.1, *P* = 0.96				*F* value = 4.3, *P* = 0.006			
LDL, mg/dL								
0 to <1	140	137	43	29 to 261	124	128	34	50 to 205
1 to <3	134	134	40	44 to 234	141	134	38	61 to 290
3 to <6	137	135	34	65 to 190	148[2]	142	24	111 to 196
≥6	142	136	32	95 to 178	145	156	26	103 to 164
	F value = 0.2, *P* = 0.87				*F* value = 3.7, *P* = 0.01			
HDL, mg/dL								
0 to <1	53	53	13	31 to 94	46	48	11	19 to 74
1 to <3	48	47	13	26 to 94	44	45	10	28 to 69
3 to <6	45	46	11	23 to 74	48	47	10	32 to 69
≥6	45	46	9	34 to 61	40	38	4	37 to 45
	F value = 2.9, *P* = 0.04				*F* value = 1.1, *P* = 0.34			
Triglycerides, mg/dL								
0 to <1	129	96	98	41 to 622	148	107	162	38 to 1209
1 to <3	161	133	107	41 to 651	156	122	108	44 to 534
3 to <6	158	142	88	34 to 413	166	158	92	45 to 394
≥6	132	151	45	64 to 187	220	191	83	149 to 352
	F value = 1.1, *P* = 0.35				*F* value = 0.5, *P* = 0.67			

* See Table 1 for sample size by year.
[1] Mean is significantly different ($P < 0.05$, Bonferroni (Dunn) test) from the mean of the other PFOS categories.
[2] Mean is significantly different ($P < 0.05$, Bonferroni (Dunn) test) from the mean of 0 to <1 ppm PFOS category.
[3] Mean is significantly different ($P < 0.05$, Bonferroni (Dunn) test) from the mean of 1 to <3 ppm PFOS category.

quadratic association with PFOS for Decatur employees. That is, total bilirubin levels initially declined but subsequently increased with increasing PFOS levels. This association was not observed in the Antwerp employee population, which had higher total bilirubin levels. In 1997, serum cholesterol levels were positively associated (linearly) with serum PFOS, after adjustment for potential confounding factors among Decatur employees (data not shown). This association was not observed with serum PFOS and cholesterol in 1995 among Decatur employees or in either time period with the Antwerp employees. Stratification of the data by plant (Table 5) and multivariable analyses (data not shown) showed no consistent associations between PFOS and HDL. For both plants combined, HDL levels were significantly negatively associated (linearly) with PFOS in 1995, after adjustment for possible confounding factors, but this was not observed in 1997.

Discussion

We conducted two cross-sectional analyses of surveillance data to examine the associations between serum PFOS levels and several hematological and clinical chemistry tests in male fluorochemical production employees. For both years, 95% of the measured serum PFOS levels were below 6 ppm. Because the Antwerp and Decatur employees were dissimilar by age, BMIs, and self-reported alcohol use, we conducted combined as well as separate analyses by plant location. These three demographic factors explain, at least partially, why the Antwerp employees had lower mean serum triglycerides and higher HDL levels than the Decatur employees.[18–22]

Clinical hepatic enzyme tests were not associated with the employees' serum PFOS levels. This was an important question to address because PFOS has been reported (1) to be a peroxisome proliferator in rats[8,10]; (2) to result in increased plasma liver transaminase tests in a

TABLE 4
Mean, Median (Med), Standard Deviation (SD) of Mean and Range of PFOS, and Total and Direct Bilirubin Levels, by Year and Plant Location

	Antwerp				Decatur			
PFOS* (ppm)	Mean	Med	SD	Range	Mean	Med	SD	Range
1995 Data								
T. bilirubin, mg/dL								
0 to <1	0.96	0.80	0.55	0.40 to 2.90	0.65	0.60	0.16	0.40 to 0.90
1 to <3	0.83	0.80	0.26	0.40 to 1.30	0.57	0.50	0.28	0.20 to 1.50
3 to <6	0.75	0.70	0.30	0.30 to 1.40	0.51	0.50	0.18	0.20 to 1.00
≥6	0.93	0.90	0.25	0.70 to 1.20	0.63	0.65	0.10	0.50 to 0.70
	F value = 1.2, P = 0.31				F value = 0.7, P = 0.54			
D. bilirubin, mg/dL								
0 to <1	0.23	0.20	0.06	0.20 to 0.40	0.20	0.20	0.00	0.20 to 0.20
1 to <3	0.22	0.20	0.04	0.20 to 0.30	0.20	0.20	0.06	0.10 to 0.40
3 to <6	0.21	0.20	0.03	0.20 to 0.30	0.20	0.20	0.04	0.20 to 0.30
≥6	0.20	0.20	0.00	0.20 to 0.20	0.20	0.20	0.08	0.10 to 0.30
	F value = 0.7, P = 0.55				F value = 0.4, P = 0.74			
1997 Data								
T. bilirubin, mg/dL								
0 to <1	0.90	0.80	0.46	0.40 to 2.30	0.63	0.60	0.30	0.30 to 1.40
1 to <3	0.68	0.70	0.23	0.30 to 1.30	0.56	0.50	0.18	0.30 to 1.00
3 to <6	0.79	0.70	0.40	0.30 to 1.30	0.51	0.50	0.16	0.30 to 0.90
≥6	—	—	—	—	0.58	0.50	0.24	0.40 to 1.00
	F value = 2.3, P = 0.11				F value = 1.0, P = 0.41			
D. bilirubin, mg/dL								
0 to <1	0.16	0.20	0.08	0.10 to 0.40	0.13	0.10	0.06	0.10 to 0.30
1 to <3	0.13	0.10	0.05	0.10 to 0.20	0.11	0.10	0.03	0.10 to 0.20
3 to <6	0.14	0.10	0.05	0.10 to 0.20	0.11	0.10	0.03	0.10 to 0.20
≥6	—	—	—	—	0.10	0.10	0.00	0.10 to 0.10
	F value = 2.2, P = 0.12				F value = 1.3, P = 0.28			

* See Table 1 for sample sizes by year.

subchronic rat study[4]; and (3) to increase serum aspartate aminotransferase and lower alkaline phosphatase levels in a subchronic rhesus monkey study.[5]

There appears to be significant enterohepatic circulation of PFOS with both urinary and fecal excretion in the rat.[2,3] Although we observed a quadratic relation between PFOS and total bilirubin in our multivariable analyses with the Decatur employee population, interpretation of these data is difficult, given the narrow range of PFOS serum levels associated with the total bilirubin values. Interestingly, the Antwerp employees' total bilirubin levels were significantly higher than the Decatur employees' levels. We suspect there may be a greater prevalence of Gilbert's syndrome[20,23] among the Antwerp employees. In 1995, 15 (17%) Antwerp employees had total bilirubin values ≥1.2 mg/dL, compared with three (3%) Decatur employees. Comparable percentages also existed in 1997. We are uncertain whether post-collection factors, including hemolysis, light, and heat, could have contributed to the differences in total bilirubin levels between the Antwerp and Decatur employees.[18] Unfortunately, total bilirubin was not analyzed in two subchronic rhesus monkey studies, which resulted in death of all animals in the 4.5 mg/kg/day PFOS dose group or higher.[5,6] Ongoing toxicology studies in rats and primates should provide additional perspectives regarding any biological effect between PFOS and bilirubin levels.

Our data do not suggest a reduction in total serum cholesterol with PFOS at the serum levels measured among these production employees. PFOS is a peroxisome proliferator in the rat, and hypolipidemia has been consistently observed in subchronic rat and primate toxicology studies.[5–8,10] Rhesus monkeys that had been administered PFOS at 4.5 mg/kg/day had mean serum cholesterol values that decreased from 183 mg/dL to 99 mg/dL within 30 days.[5] Rhesus monkeys administered 1.5 mg/kg/day had mean cholesterol levels that decreased from 195 mg/dL to 111 mg/dL within 90 days. A no-observable-effect level was seen for the 0.5 mg/kg dose group at 90 days. Serum PFOS measurements were not determined in these rhesus monkeys. However, data from a recent cynomolgus monkey dose-range-finding study suggested that hypolipidemia may be initially associated with serum PFOS levels in the range of 100 to 200 ppm.[7] Haughom and Spydevold suggested that the hypolipidemic effect of PFOS may be due to reduced liver activity of hydroxymethyl glutaric acid-CoA reductase and acyl-CoA cholesterol acyltransferase, with enhanced fatty acid oxidation in the liver.[9] Nabbefeld et al observed that PFOS has a high affinity for the fatty acid carrier proteins albumin and L-fatty acid–binding

TABLE 5
Mean, Median (Med), Standard Deviation (SD) of Mean and Range of PFOS, and Total and HDL Cholesterol, by Year and Plant Location

	Antwerp				Decatur			
PFOS* (ppm)	Mean	Med	SD	Range	Mean	Med	SD	Range
1995 Data								
Cholesterol, mg/dL								
0 to <1	220	219	50	100 to 340	215	208	39	154 to 276
1 to <3	206	211	49	118 to 315	221	218	39	132 to 300
3 to <6	217	215	30	178 to 266	209	213	42	128 to 278
≥6	223	221	16	208 to 240	206	206	47	160 to 251
	F value = 0.6, P = 0.61				F value = 0.5, P = 0.69			
HDL, mg/dL								
0 to <1	56	57	13	31 to 94	43	41	9	31 to 59
1 to <3	53	51	13	33 to 79	45	44	12	26 to 94
3 to <6	50	49	11	31 to 74	39	39	9	23 to 51
≥6	53	49	7	48 to 61	39	39	5	34 to 46
	F value = 1.1, P = 0.35				F value = 1.4, P = 0.25			
1997 Data								
Cholesterol, mg/dL								
0 to <1	193	190	41	110 to 277	204	208	38	145 to 277
1 to <3	213	205	48	116 to 365	218	226	39	152 to 290
3 to <6	228	223	38	192 to 321	230	230	23	197 to 280
≥6	—	—	—	—	229	238	26	186 to 250
	F value = 2.9, P = 0.07				F value = 2.0, P = 0.13			
HDL, mg/dL								
0 to <1	51	50	12	19 to 74	42	41	9	26 to 59
1 to <3	48	46	10	34 to 68	42	41	10	28 to 69
3 to <6	51	50	10	39 to 69	45	45	10	32 to 62
≥6	—	—	—	—	40	38	4	37 to 45
	F value = 0.8, P = 0.47				F value = 0.5, P = 0.67			

* See Table 1 for sample sizes by year.

protein in vivo. This could potentially alter fatty acid transport, biochemistry, and metabolism, which conceivably could then lead to a decrease in cholesterol esterification and metabolic wasting.[11]

Several methodological issues should be considered in evaluating the results from our study. First, the cross-sectional design does not allow for a direct analysis of the temporality of an association. Second, the voluntary participation rates in the fluorochemical medical surveillance were not ideal among the eligible fluorochemical production employees. Third, the serum levels of PFOS that were measured may be below the no-effect level in laboratory animals. Fourth, PFOS concentrates primarily in the liver of laboratory animals. Serum measurements of PFOS may not adequately reflect body burden. Fifth, the two cross-sectional analyses cannot be viewed as independent populations. Sixty-one employees were studied in both years. This was due, in part, to a large turnover of employees at both plants between examinations. Sixth, there could be measurement error in important confounding variables. Analysis of the data of the 61 subjects who participated in both years showed that there was excellent correlation for the confounding factors of BMI (r = .92, P = 0.0001), self-reported aspects of alcohol consumption (r = .88, P = 0.0001), and cigarette smoking (r = .79, P = 0.0001). As expected, these 61 employees' serum PFOS levels for the 2 years were highly correlated (r = .92, P = 0.0001). Their results did not differ from those of the entire study population. Finally, the quality of medical surveillance data, prior to its use for studying an a priori hypothesis, can often be evaluated by whether known positive associations are observed. In this regard, we did observe various expected associations, such as cigarette smoking and elevated white blood cell counts and large BMIs, associated with elevated liver transaminase levels.[24,25]

In summary, our findings suggest that among these Antwerp and Decatur male fluorochemical production employees, there were no substantial changes in serum hepatic enzymes, cholesterol, or lipoproteins associated with PFOS levels less than 6 ppm. It is not possible to derive inferences from the few employees with serum levels ≥6 ppm. Limitations of this study include its cross-sectional design, the low voluntary participation rates among the employees, and the lower levels of serum PFOS measured among these employees, compared with those suspected to cause effects in laboratory animals.

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