Epidemiologic Assessment of Worker Serum Perfluorooctanesulfonate (PFOS) and Perfluorooctanoate (PFOA) Concentrations and Medical Surveillance Examinations

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Perfluorooctanesulfonyl fluoride (POSF, $C_8F_{17}SO_2F$) is used to create applications for surfactants and paper, packaging, and surface (eg, carpets, textiles) protectants. Such POSF-based products or their residuals may degrade or metabolize to PFOS ($C_8F_{17}SO_3^-$). PFOS concentrates in liver and serum and results in hypolipidemia as an early effect of cumulative dosages. Male and female employees of two perfluorooctanyl-manufacturing locations (Antwerp, Belgium and Decatur, Alabama) participated in a periodic medical surveillance program that included hematology, clinical chemistry, thyroid hormone, and urinalysis testing. Serum concentrations of PFOS and perfluorooctanoate (PFOA, $C_7F_{15}CO_2^-$, used as a fluoropolymer emulsifier) were measured via mass spectrometry methods. The mean serum PFOS and PFOA concentrations for 263 Decatur employees were 1.32 parts per million (ppm; geometric mean 0.91, range 0.06–10.06 ppm) and 1.78 ppm (geometric mean 1.13, range 0.04–12.70 ppm), respectively. Mean concentrations were approximately 50% lower among 255 Antwerp workers. Adjusting for potential confounding factors, there were no substantial changes in hematological, lipid, hepatic, thyroid, or urinary parameters consistent with the known toxicological effects of PFOS or PFOA in cross-sectional or longitudinal analyses of the workers' measured serum fluorochemical concentrations. (J Occup Environ Med. *2003;45:260–270*)

Perfluorooctanesulfonyl fluoride (POSF, $C_8F_{17}SO_2F$), which is produced by an electrochemical fluorination process, is used as the basic building block to create unique chemistries through the sulfonyl fluoride moiety using conventional hydrocarbon reactions. Applications include surfactants and paper, packaging, and surface (eg, carpet, upholstery, textile) protectants. Depending upon the specific functional derivatization or the degree of polymerization, such POSF-based products or their residuals may degrade or metabolize to an undetermined degree to PFOS ($C_8F_{17}SO_3^-$). PFOS is a stable and persistent end-product that has the potential to bioaccumulate. Although not a major commercial product, PFOS has been used in some products, including firefighting foams. Another fluorochemical, the ammonium salt of perfluorooctanoate (PFOA, $C_7F_{15}CO_2^-$) is produced to be an emulsifier in the polymerization of fluoropolymers. In May 2000, the 3M Company announced that it would voluntarily cease manufacturing perfluorooctanyl-related materials after PFOS was found to be pervasive and persistent in human populations and wildlife.[1–5] Nonoccupational exposures to PFOS or precursors are not well understood at this time but could include environmental sources, consumer products, or as indirect food additives.

PFOS concentrates primarily in the liver and, to a lesser extent, in the

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plasma, of rats.[6] There appears to be significant enterohepatic circulation of PFOS with both urinary and fecal excretion.[7] Subchronic studies in rats and primates suggest that the toxicity of PFOS is dependent upon cumulative dose.[8–12] Decreased serum cholesterol was the earliest clinical chemistry response observed in primates and occurred at serum PFOS (potassium salt) concentrations above 100 parts per million (ppm).[12] Rats fed PFOS (potassium salt) at 20 ppm in the diet over a 2-year time period experienced a marginal increase in liver tumors, primarily adenomas.[13] Although the mechanism of toxicity in laboratory animals remains to be fully elucidated, PFOS toxicity may involve: 1) the inhibition of HMG CoA reductase and acyl CoA cholesterol acyl transferase activity;[14] 2) activation of the PPARα receptor;[15,16] 3) disturbances in fatty acid transport and metabolism;[17] and/or 4) altered membrane function changes and mitochondrial bioenergetics.[18,19]

There are substantial sex and species differences in elimination of PFOA with the longest serum elimination half-life reported for humans.[20–23] Besides urinary excretion, biliary excretion and reabsorption of PFOA occurs.[7] The liver is the primary target organ for PFOA-induced toxicity. PFOA produced hypolipidemia in rodents[14,24] but not in a 6-month capsule feeding study of cynomolgus monkeys.[22] Increased liver weight as a result of mitochondrial proliferation occurred in all three monkey dose groups (3, 10, and 20 mg/kg/day), although histopathologic evidence of liver injury occurred only in the highest dose group. Two-year lifetime bioassays of rats fed 300 ppm PFOA in the diet resulted in the increased incidence of tumors (adenomas) of the liver, pancreas (acinar cell), and testes (Leydig cell).[25] The hepatocellular tumors may result from a combination of oxygen stress and cell proliferation that accompanies an increase in peroxisomes. The tumors observed in the testis may be the consequence of sustained increases in estradiol as a result of aromatase induction[25–27] whereas those in the pancreas may involve the release of cholecystokinin as a result of cholestasis.[28] It remains to be determined whether these possible mechanisms are relevant to humans.

Serum measurements of PFOS and PFOA have been obtained as part of the 3M Company's effort to assure worker safety as related, in particular, to lipid and hepatic parameters because of the toxicological evidence that indicates the liver is the target organ of effect in rats and primates. The company's fluorochemical medical surveillance program is offered employees on a routine periodic basis at three 3M manufacturing facilities: Antwerp (Belgium), Cottage Grove (Minnesota), and Decatur (Alabama). Electrochemical fluorination at the Cottage Grove site involved the production of PFOA and its associated salts but not POSF.[29–31] The present study focused on the company's Antwerp and Decatur facilities, where POSF and PFOA have been produced. Although a cross-sectional assessment of the 1995 and 1997 Antwerp and Decatur medical surveillance program data was previously reported, the data were limited for several reasons: low voluntary employee participation, no inclusion of female employees, inherent limitations of the cross-sectional design, and analysis of only serum PFOS concentrations.[32] Increased employee participation in the year 2000 medical surveillance program allowed for a much larger cross-sectional analysis of both male and female employees and also enabled a 6-year longitudinal assessment of clinical chemistries in relation to PFOS, PFOA, and a calculated total organic fluorine value.

Materials and Methods

Manufacturing Sites

The manufacturing operations of the two sites are similar. Fluorochemical production occurs in several buildings. In one building, where the base product is manufactured, POSF, occurs via electrochemical fluorination. At another building, the POSF starting material is reacted to form fluorochemical amines and then further to other fluorochemicals, including alcohols and acrylates with subsequent polymerization. Synthetic fluoroelastomers are also produced at both facilities. PFOA has been routinely produced at Antwerp but only since 1999 in Decatur. However, PFOA can be a byproduct of POSF-related manufacturing at both facilities.

The fluorochemical medical surveillance program is available on a periodic voluntary basis to all Antwerp and Decatur chemical plant employees and those employees with site-wide responsibilities (eg, environmental, health and safety workers). In 2000, approximately 340 Antwerp and 500 Decatur chemical plant and site employees were eligible to participate. The surveillance program measured several serum fluorochemicals, including PFOS and PFOA as well as hematology, clinical chemistries, and thyroid hormones. Urinalyses were conducted only in Decatur.

Hematology, Clinical Chemistry, Thyroid Function, and Urinalysis

Upon collection and shipment of specimens, Allina Laboratory Services (St. Paul, MN) performed standard hematological and clinical chemistry tests for both manufacturing sites. These included the following hematological tests: hematocrit (percent), hemoglobin (gm/dL), red blood cells (RBC, 1000/mm^3), white blood cells (WBC, 1000/mm^3) and platelet count (1000/mm^3); and the following clinical chemistry tests: alkaline phosphatase (IU/L), gamma glutamyl transferase (GGT, IU/L),

aspartate aminotransferase (AST, IU/L), alanine aminotransferase (ALT, IU/L), total and direct bilirubin (mg/dL), cholesterol (mg/dL), high-density lipoprotein (HDL, mg/dL), triglycerides (mg/dL), blood glucose (mg/dL), blood urea nitrogen (BUN, mg/dL), and serum creatinine (mg/dL). Reference ranges have been relatively constant since 1994/95, although for ALT the range declined from 20–65 IU/L in 1994/95 to 1–40 IU/L in 1997 and 2000. Six thyroid tests were conducted by LabCorp (Kansas City, MO): thyroid-stimulating hormone (TSH; μIU/mL); serum thyroxine (T4; μg/dL); free thyroxine (free T4; ng/dL); serum triiodothyronine (T3; ng/dL); thyroid hormone binding ratio (THBR, previously referred to as T3 Uptake); and free thyroxine index (FTI). TSH, free T4, and T3 were determined by an immunochemiluminometric assay. T4 and THBR were determined by a cloned enzyme donor immunoassay. FTI was calculated by multiplying T4 and THBR. Urinalyses were only performed on Decatur employees via the standard urine microstick analysis, which tested for urine glucose, albumin and red blood cells.

Fluorochemical Analyses

In the 2000 fluorochemical medical surveillance program, the employees' sera samples were extracted and quantitatively analyzed for PFOS and PFOA using high-pressure liquid chromatography electrospray tandem mass spectrometry and evaluated versus an extracted curve from a human plasma matrix. All serum fluorochemical analyses were determined by Northwest Bioanalytical Laboratory Inc. (Salt Lake City, UT). Sera samples were extracted using an ion-pairing extraction procedure.[2] Evaluation of quality control samples injected during each analytical run indicated that the reported quantitative results may have varied, on average, up to 20% although most analyses were within ±10%. For all employees, serum values for PFOS and PFOA values were above the lower limit of quantitation. Results are reported in ppm.

Five other fluorochemical analytes were also analyzed in the 2000 medical surveillance program: perfluorohexanesulfonate ($C_6F_{13}SO_3^-$); N-ethyl perfluorooctanesulfonamidoacetate ($C_8F_{17}SO_2N(CH_2CH_3)CH_2COO^-$); *N*-methyl perfluorooctanesulfonamidoacetate ($C_8F_{17}SO_2N(CH_3)CH_2COO^-$); perfluorooctanesulfonamidoacetate ($C_8F_{17}SO_2NHCH_2COO^-$); and perfluorooctanesulfonamide ($C_8F_{17}SO_2NH_2$). These fluorochemicals were measured at concentrations 1 to 3 orders of magnitude lower than PFOS and PFOA and are therefore not presented. A total organic fluorine (TOF) value was determined by calculating the percent of each of the seven specific fluorochemical's molecular weight that was attributed to organic fluorine (eg, PFOS, 64.7%; PFOA, 69.0%) multiplied by the ppm measured for each fluorochemical and then summed across all seven fluorochemicals.

Serum fluorochemical analyses were conducted at different laboratories in the previous surveillance years (1994 [Decatur], 1995 [Antwerp], and 1997 [both facilities]) using slight variations of the Hansen et al. method.[2] In those years, only PFOS and PFOA were quantified. Therefore, TOF for the longitudinal assessment was based only on PFOS and PFOA.

Data Analyses

For the cross-sectional analyses, associations between PFOS, PFOA, or TOF and each hematological and clinical chemistry test and thyroid hormone assay were evaluated with descriptive statistics, analysis of variance, and multivariable regression. For stratified analyses, employees were divided into quartiles of their serum PFOS distribution. Age, body mass index, current alcohol consumption (drinks per day) and cigarette use (cigarettes smoked per day), years worked at Antwerp or Decatur, and type of job (production versus nonproduction) were potential confounding factors that were considered in the analyses. Production jobs included cell operators, chemical operators, mill operators, and crew supervisors. Nonproduction jobs included engineers, quality-assurance laboratory and research workers, and administration (eg, managers, clerical staff).

Logistic regression was used to calculate adjusted odds ratios for the quartile distribution of employees who had liver function tests above the laboratory's reference ranges. In addition, the individual continuous dependent variables (lipids, hepatic enzymes or thyroid hormones) were fitted in multivariable regression analyses with PFOS, PFOA, or TOF analyzed as an independent continuous variable(s). The statistical significance of the fluorochemical coefficient was considered at $P < 0.05$. Natural log transformations of the dependent variables were performed when necessary to normalize variables and to enhance model fit. Study results were analyzed using the SAS System (SAS Institute, Cary, NC).[33,34]

For the longitudinal assessment, lipid and hepatic clinical chemistry tests were evaluated as repeated measures incorporating the random subject effect fitted to a mixed model by the MIXED procedure in the SAS statistical package.[35] Restricted maximum likelihood estimates of variance parameters were computed. Adjusted regression models were built by introducing all covariates (see below) and testing the covariance structure. Equal spacing was assumed given there were approximately 3 years between each medical surveillance examinations. Based on goodness-of-fit tests, the autoregressive was routinely considered the best covariance structure for the mixed models. Variables included PFOS (or PFOA or TOF), years of observation (ie, follow-up), the interaction term of PFOS and years of observation, age, body mass index

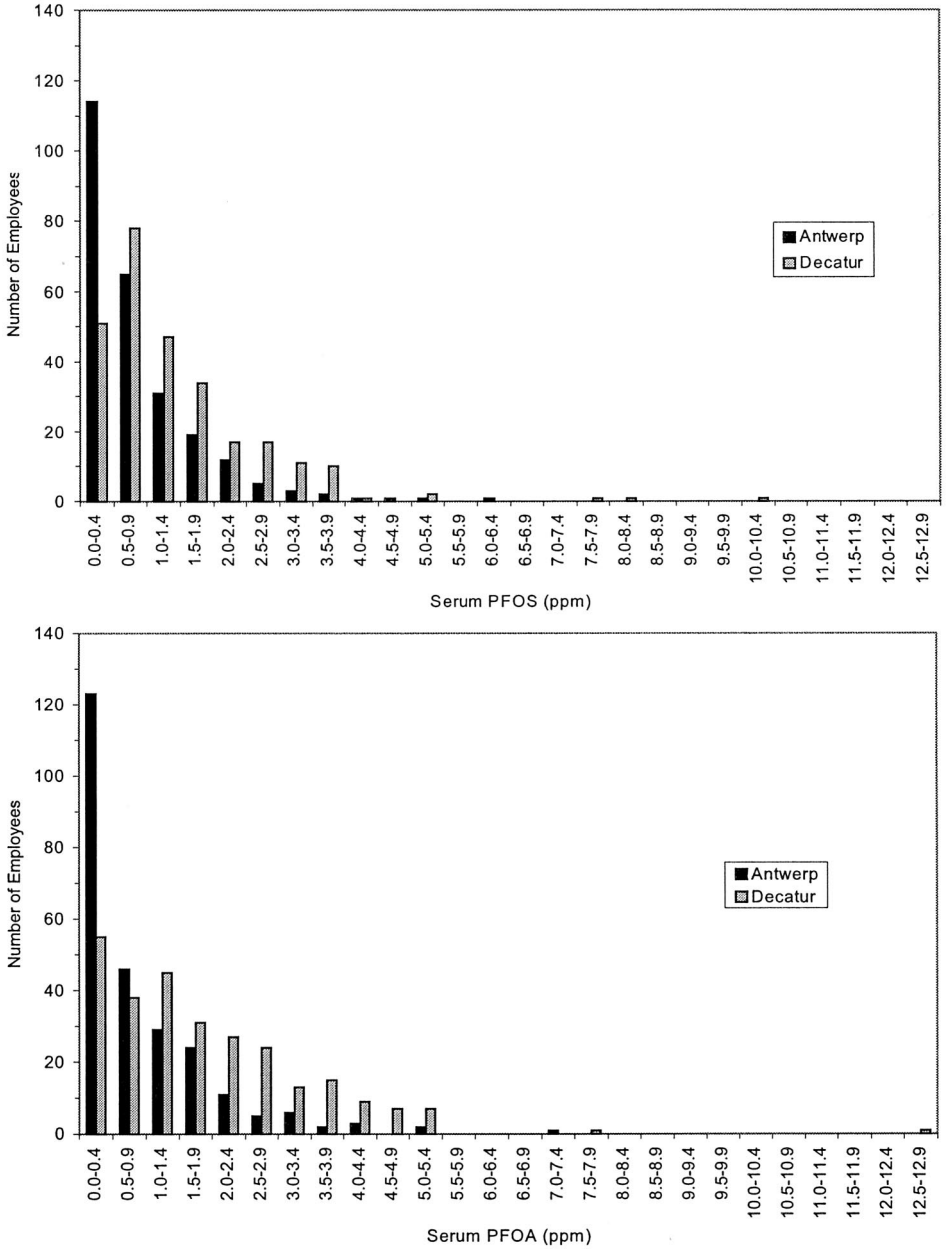

Fig. 1. Distribution of Antwerp and Decatur employees by their serum PFOS and PFOA concentrations.

(BMI), cigarettes smoked per day, alcohol drinks per day, location, year at first entry, and baseline years worked. Serum triglycerides was also considered a potential confounding factor for all hepatic clinical chemistry analyses (cross sectional and longitudinal).

Results

Cross-sectional Analysis

Altogether, there were 255 (75%) Antwerp employees (206 male and 49 female) and 263 (52%) Decatur employees (215 male, 48 female) who voluntarily participated in the 2000 fluorochemical medical surveillance program. Seventy-three percent of the Antwerp male employee participants and 75% of the Decatur employee participants worked in production activities. Only 12% of the Antwerp female employees worked in production activities compared with 63% of the Decatur female employees.

Presented in Fig. 1 are the distributions of serum PFOS and PFOA concentrations among the Antwerp and Decatur employee participants. The arithmetic mean serum PFOS concentration among all Antwerp subjects was 0.80 ppm (range 0.04–6.24 ppm) with a geometric mean of 0.44 ppm (95% CI = 0.38–0.51). For PFOA, the arithmetic mean was 0.84 ppm (range 0.01–7.04 ppm) with a geometric mean of 0.33 ppm (95% CI = 0.27–0.40). The arithmetic mean serum PFOS concentration among all Decatur participants

TABLE 1
Mean and Standard Deviation Comparisons Between Antwerp and Decatur Employees' Serum PFOS, PFOA, TOF, Demographic and Clinical Chemistry Results, by Gender

	Males				Females			
	Antwerp (N = 206)		Decatur (N = 215)		Antwerp (N = 49)		Decatur (N = 48)	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
PFOS	0.96[d]	0.97	1.40	1.15	0.13[d]	0.10	0.93	0.81
PFOA	1.03[d]	1.09	1.90	1.59	0.07[d]	0.17	1.23	1.18
TOF	1.60[d]	1.34	2.65	2.00	0.17[d]	0.20	1.76	1.50
Age	37[d]	9	43	9	36	7	42	9
BMI	24.8[d]	3.0	28.8	4.4	22.8[d]	3.2	27.7	5.9
Years worked	13[c]	8	16	13	12[a]	7	13	10
Alcoholic drinks/day	1.1[d]	1.1	0.1	0.3	0.5[d]	0.4	0.1	0.1
Cholesterol	218	41	215	42	208	36	200	39
HDL	55[d]	15	44	10	68[a]	16	59	12
Triglycerides	124[d]	87	191	124	94[d]	45	133	152
Alk Phos	60[d]	15	74	20	46[a]	13	65	18
GGT	23[d]	17	31	18	12[d]	7	18	15
AST	23[c]	6	26	8	18	6	20	7
ALT	23[d]	10	35	16	13[d]	6	19	10
Total Bilirubin	1.0[d]	0.3	0.7	0.2	0.8[b]	0.3	0.6	0.2

[a] $p < .05$ compared to Decatur (student t test).
[b] $p < .01$ compared to Decatur (student t test).
[c] $p < .001$ compared to Decatur (student t test).
[d] $p < .0001$ compared to Decatur (student t test).

was 1.32 ppm (range 0.06 to 10.06 ppm) with a geometric mean of 0.91 ppm (95% CI = 0.82–1.02). For PFOA, the arithmetic mean was 1.78 ppm (range 0.04–12.70 ppm) with a geometric mean of 1.13 ppm (95% CI = 0.99–1.30).

Antwerp male employees compared with their Decatur counterparts had lower mean serum PFOS and PFOA concentrations; were younger; had lower BMIs; drank more alcoholic beverages per day; and had higher mean HDL and total bilirubin and lower triglyceride and hepatic clinical chemistry values (Table 1). Similar findings were observed between Antwerp and Decatur female employees (Table 1).

Presented in Table 2 are the mean, standard deviation, and range of demographic, clinical chemistry, and thyroid hormone results stratified by the serum PFOS quartile distribution for the combined 421 Antwerp and Decatur production and nonproduction male employees. (Note: The median values [data not shown in Table 2] by PFOS quartile, for PFOS, PFOA, and TOF were, respectively: quartile 1: [0.29, 0.25, 0.43 ppm]; quartile 2 [0.59, 0.86, 1.14 ppm]; quartile 3 [1.17, 1.20,1.88 ppm]; and quartile 4 [2.46, 2.43, 4.06 ppm].) As noted in the footnote to Table 2, the demographic and clinical values reflect the higher percentage of Antwerp employees in the lowest PFOS quartile and the higher percentage of Decatur employees in the upper PFOS quartiles. The fourth quartile had statistically significant higher mean values than the first quartile for triglycerides, alkaline phosphatase, ALT and T3. The fourth quartile had significantly lower mean values for drinks per day and total bilirubin compared to the first quartile. There were no significant mean differences between quartiles for cigarettes smoked, hematology, blood glucose, BUN, serum creatinine or urinalyses (data not shown).

Similar to Table 2, presented in Table 3 are the serum PFOS quartile distributions for Antwerp and Decatur production and nonproduction female employees. Again, the Antwerp employees predominated in the lowest serum PFOS quartile and Decatur female employees predominated in the highest quartile. [Note: The median values [data not shown], by quartile, for PFOS, PFOA and TOF were, respectively: quartile 1: [0.08, 0.02, 0.09 ppm]; quartile 2 [0.13, 0.05, 0.14 ppm]; quartile 3 [0.37, 0.36, 0.59 ppm]; and quartile 4 [1.34, 1.39, 2.66 ppm].) The fourth quartile had significantly higher mean values than the first quartile for age, BMI, alkaline phosphatase, and GGT. The fourth quartile had significantly lower mean values for alcoholic drinks per day and total bilirubin. There were no significant mean differences for cigarettes smoked, hematology, blood glucose, BUN, serum creatinine, or urinalyses (data not shown).

Summarized in Table 4 are the number of Antwerp and Decatur employees (and percentages) who had hepatic enzyme tests above reference range values, stratified by the quartiles of the serum PFOS distribution. Among male employees, 12% of the employees in the fourth quartile had above reference range values for ALT and GGT compared with 4 to 8% in the first through third quartiles. For the total liver panel, 23% of

TABLE 2
Antwerp and Decatur Production and Non-Production* (N = 421) Male Employees' PFOS, PFOA, TOF, Demographic, Clinical Chemistry and Thyroid Hormone Results by Quartile of Serum PFOS Distribution*

	Quartile 1 (N = 105)			Quartile 2 (N = 105)			Quartile 3 (N = 106)			Quartile 4 (N = 105)		
	Mean	SD	Range	Mean	SD	Range	Mean	SD	Range	Mean	SD	Range
PFOS	0.27[2,3,4]	0.11	0.04–0.42	0.60[1,3,4]	0.12	0.43–0.81	1.19[1,2,4]	0.24	0.82–1.68	2.69[1,2,3]	1.09	1.69–10.06
PFOA	0.54[2,3,4]	0.77	0.01–4.03	1.21[1,4]	1.19	0.06–7.04	1.45[1,4]	1.10	0.12–7.48	2.70[1,2,3]	1.63	0.25–12.70
TOF	0.62[2,3,4]	0.58	0.05–3.03	1.40[1,3,4]	0.89	0.38–5.69	2.12[1,2,4]	0.87	0.98–6.61	4.41[1,2,3]	1.72	1.92–12.23
Age	38[3]	10	23–60	41	10	21–63	42[1]	9	22–61	40	9	27–60
BMI	25.8	4.0	19.2–40.8	26.9	4.0	19.0–37.3	27.3	4.5	17.2–50.1	27.2	4.5	17.8–45.5
Years worked	12[3]	10	1–38	15	12	2–38	16[1]	11	1–38	15	10	2–38
Drinks/day	0.9[3,4]	1.0	0–5	0.6	0.9	0–4	0.5[1]	0.9	0–6	0.5[1]	0.9	0–5
Cholesterol	214	41	140–331	214	43	121–308	215	39	105–303	222	44	122–384
HDL	54	15	31–121	47	11	29–80	48	13	24–100	48	15	26–119
Triglycerides	131[4]	95	32–527	155	102	35–633	169	123	32–731	177[1]	123	39–796
Alk Phos	61[3,4]	16	26–98	67	18	30–142	69[1]	21	30–160	70[1]	19	21–126
GGT	24	16	7–111	29	22	7–144	26	15	6–89	30	17	7–85
AST	25	8	13–58	25	6	16–49	24	7	7–51	25	9	13–69
ALT	26[4]	13	10–91	28	11	10–63	28	14	6–103	33[1]	19	8–99
Total Bilirubin	1.0[3,4]	0.3	0.5–2.0	0.9	0.3	0.3–2.0	0.8[1]	0.3	0.4–2.0	0.8[1]	0.3	0.4–2.2
TSH	2.0	1.2	0.03–5.7	3.1	6.6	0.5–65.3	2.1	2.0	0.2–18.8	2.5	2.8	0.5–21.5
T4	8.3	1.4	0.5–11.5	8.2	1.4	4.2–12.0	8.3	1.5	3.3–12.9	8.4	1.4	4.7–11.4
Free T4	1.1	0.2	0.9–1.5	1.1	0.1	0.6–1.4	1.1	0.2	0.4–1.6	1.1	0.2	0.8–1.6
T3	124[4]	17	94–164	128	20	86–186	127	21	91–196	132[1]	22	87–190

* Number of male employees by location, production (P) and non-production (NP) category and quartile (percent in parenthesis).

	Q1		Q2		Q3		Q4	
	P	NP	P	NP	P	NP	P	NP
Antwerp	38	38	38	12	38	4	36	2
Decatur	7	22	40	15	51	13	63	4
Total	45 (43)	60 (57)	78 (74)	27 (26)	89 (84)	27 (16)	99 (94)	6 (6)

[1] Mean is significantly different ($P < .05$, Bonferroni (Dunn) t test) from the mean of the 1st quartile; [2] 2nd quartile; [3] 3rd quartile; [4] 4th quartile.

the male employees had one or more liver clinical chemistry tests above the reference range value compared with 14 to 16% of the male employees in the lower three quartiles. Odds ratios were calculated for each quartile for those employees above or below reference ranges as listed in Table 4 (quartile one reference odds ratio = 1.0). The odds ratios were adjusted for the potential confounding effects of age, BMI, alcohol, cigarettes, and location. For ALT, the odds ratios for the second, third, and fourth quartiles were (95% CI in parentheses) 0.6 (95% CI = 0.1–2.8), 1.2 (0.3–4.8), and 2.1 (0.6–7.3), respectively. For GGT, the odds ratios were 1.3 (0.4–4.1), 0.9 (0.3–3.1), and 2.0 (0.7–5.8), respectively. For the total liver panel, the odds ratios were: 1.1 (0.5 to 2.3), 1.1 (0.4–2.3), and 1.6 (0.7–3.3), respectively. None of the odds ratios were statistically significant ($P < 0.05$). Logistic models did not meet satisfactory convergence criteria because of the few male subjects with values above the reference ranges for alkaline phosphatase or AST or for female employees for any liver function analysis in Table 4.

Using multivariable regression analysis and adjusting for potential confounders, serum PFOS was positively associated with the natural log of serum cholesterol (PFOS coefficient = 0.020, P value = 0.04) and triglycerides (PFOS coefficient = 0.066, P value = 0.01), although these associations contributed minimally (partial $R^2 < 0.01$ and 0.03, respectively) to the variation explained in the models (cholesterol model adjusted $R^2 = 0.06$; triglyceride model adjusted $R^2 = 0.27$). PFOA and TOF were also positively associated with cholesterol and triglycerides with similar variations explained. These modest positive associations for PFOS or PFOA, however, were opposite the expected direction based on the known toxicity of these compounds. HDL was not significantly associated with PFOS or PFOA. Adjusting for their potential confounders, the hepatic enzyme and bilirubin analyses were not significantly associated with PFOS or PFOA. Multivariable regression analyses of the thyroid hormones resulted in no significant associations with PFOS or PFOA except for a positive association with the natural log of T3 (PFOS coefficient = 0.015, P value = 0.04; PFOA coefficient = 0.016, P value = 0.01), which, again, contributed minimally to the variation explained in the model (partial $R^2 = 0.01$).

Longitudinal Analysis

A total of 174 Antwerp and Decatur male employees participated in

TABLE 3
Antwerp and Decatur Production and Non-Production* (n = 97) Female Employees' PFOS, PFOA, TOF, Demographic, Clinical Chemistry and Thyroid Hormone Results by Quartile of Serum PFOS Distribution*

	Quartile 1 (n = 24)			Quartile 2 (n = 24)			Quartile 3 (n = 25)			Quartile 4 (n = 24)		
	Mean	SD	Range	Mean	SD	Range	Mean	SD	Range	Mean	SD	Range
PFOS	0.07[3,4]	0.02	0.04–0.10	0.13[4]	0.03	0.10–0.19	0.39[1]	0.15	0.20–0.70	1.51[1,2,3]	0.76	0.77–3.62
PFOA	0.04[3,4]	0.04	0.01–0.23	0.07[4]	0.07	0.02–0.34	0.61[1]	0.74	0.04–3.50	1.88[1,2,3]	1.20	0.25–5.41
TOF	0.09[3,4]	0.04	0.05–0.26	0.17[3,4]	0.07	0.09–0.35	0.80[1,2,4]	0.61	0.21–3.02	2.77[1,2,3]	1.44	0.86–7.81
Age	34[4]	9	24–52	37[4]	7	25–52	39	9	25–58	44[1,2]	6	30–52
BMI	22.8[4]	2.7	18.4–28.3	23.9[4]	4.3	17.3–32.3	25.5	6.1	18.3–45.3	28.7[1,2]	5.7	20.3–41.5
Years Worked	11	8	1–29	15	7	3–29	12	9	2–27	14	10	3–32
Drinks/day	0.4[4]	0.4	0–1	0.4[4]	0.4	0–1	0.3	0.4	0–2	0[1,2]	0.1	0–1
Cholesterol	207	39	132–274	203	39	138–302	200	32	139–271	208	42	129–313
HDL	66	16	46–121	65	16	33–104	63	15	38–104	60	13	36–91
Triglycerides	93	48	26–248	91	41	24–172	107	53	32–233	164	206	42–1049
Alk Phos	50[4]	16	22–81	44[3,4]	11	20–65	59[2]	16	32–91	69[1,2]	18	41–100
GGT	11[4]	7	2–32	13	8	5–41	14	6	7–30	22[1]	21	6–97
AST	19	5	11–31	18	7	9–43	19	5	11–33	19	7	7–39
ALT	13	5	8–35	16	11	6–58	16	6	7–36	19	10	6–47
Total Bilirubin	0.8[3,4]	0.2	0.5–1.2	0.8[3,4]	0.3	0.2–1.7	0.6[1,2]	0.2	0.3–1.0	0.5[1,2]	0.1	0.3–0.8
TSH	2.2	1.2	0.03–4.9	2.2	1.5	0.03–6.7	2.5	1.4	0.7–6.5	2.3	1.0	1.0–5.2
T4	10.2	2.0	6.6–13.8	9.8	3.1	4.6–18.3	9.9	2.3	5.8–15.1	9.1	2.1	5.8–14.2
Free T4	1.1	0.1	0.8–1.3	1.2	0.7	0.7–4.6	1.1	0.1	0.9–1.3	1.0	0.1	0.7–1.2
T3	145	28	98–191	147	53	81–345	133	31	86–228	127	28	86–196

* Number of female employees by location, production (P) and non-production (NP) category and quartile (percent in parenthesis).

	Q1		Q2		Q3		Q4	
	P	NP	P	NP	P	NP	P	NP
Antwerp	3	20	2	17	1	6	0	0
Decatur	0	1	1	4	7	11	22	2
Total	3 (12)	21 (88)	3 (12)	21 (88)	8 (32)	17 (68)	22 (92)	2 (8)

[1] Mean is significantly different ($P < .05$, Bonferroni (Dunn) t test) from the mean of the 1st quartile; [2]2nd quartile; [3]3rd quartile; [4]4th quartile.

the year 2000 medical surveillance program and at least one of the two previous program years: 64 (37%) participated in both 1994/95 and 2000 (Antwerp = 45, Decatur = 19); 69 (39%) of the longitudinal group participated in both 1997 and 2000 (Antwerp = 34, Decatur = 35); and 41 (24%) participated in all 3 years (Antwerp = 21, Decatur = 20). For purposes of brevity, these three subpopulations are referred to as subgroups A, B, and C, respectively.

Presented in Table 5 are the mean PFOS, PFOA, and TOF concentrations for these three subgroups by manufacturing site and year of the medical surveillance program. Serum PFOS declined over the 6-year time period whereas mean serum PFOA concentrations increased for subgroups B and C.

Serum PFOS was not a significant predictor of cholesterol or triglycerides in the longitudinal analyses (Table 6). PFOA and TOF, however, were positively associated with cholesterol as well as triglycerides. This association was primarily attributed to the 21 Antwerp employees represented in subgroup C whose mean serum PFOA levels increased over the 6-year period from 1.32 ppm to 2.06 ppm whereas their serum PFOS levels declined from 2.10 ppm to 1.53 ppm. During the same time period, their mean cholesterol values increased from 208 mg/dL to 229 mg/dL and their triglyceride levels increased from 85 mg/dL to 123 mg/dL. Their BMIs increased from 23.4 to 24.3. There were no significant PFOS, PFOA, or TOF coefficients associated with changes in HDL or the various liver function tests (data not shown) adjusting for the potential confounders.

Discussion

Medical surveillance programs in the workplace, including the present fluorochemical program, are usually voluntary. High participation rates in these voluntary programs are important to identify employees with abnormal test results, minimize participation bias, and increase the statistical power to detect subtle abnormalities.[36] Also, nonparticipation may not allow for an adequate characterization of the distribution of chemical-specific serum concentrations in employee biomonitoring analyses. We previously addressed this latter question by conducting a random sample biomonitoring study of Decatur employees and 80% of those eligible participated.[37] The serum distributions of PFOS and PFOA concentrations were comparable to previous data collected

TABLE 4
Number of Participants (Percent in Parenthesis) by Employee Population Who Had Above Reference Range Values for Hepatic Clinical Chemistry Tests by Quartile of Serum PFOS Distribution

	Alkaline Phosphatase				AST				ALT				GGT				Total Liver Panel*			
	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4
Antwerp & Decatur																				
Male Employees Production and[1] Non-Production	0 (0)	1 (1)	3 (3)	2 (2)	3 (3)	1 (1)	1 (1)	4 (4)	4 (4)	4 (4)	7 (7)	13 (12)	6 (6)	8 (8)	6 (6)	12 (12)	15 (14)	17 (16)	17 (16)	24 (23)
Female Employees Production and[2] Non-Production	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (4)	0 (0)	0 (0)	0 (0)	1 (4)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	2 (8)	0 (0)	2 (8)	0 (0)	2 (8)

* Includes Alkaline Phosphatase, AST, ALT, GGT, Total and Direct Bilirubin (at least one of these tests were above upper reference range).
[1] See Table 2 PFOS quartile distribution.
[2] See Table 3 PFOS quartile distribution.

TABLE 5
Mean and Standard Deviation of PFOS, PFOA, TOF for Three Employee Subgroups Who Participated in Fluorochemical Medical Surveillance Program Between 1994 and 2000

	Subgroup A (N = 64)				Subgroup B (N = 69)				Subgroup C (N = 41)					
	1994/1995		2000		1997		2000		1994/1995		1997		2000	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
PFOS	2.13	2.16	1.36	1.24	1.36	1.37	1.20	0.91	2.13	1.46	2.09	1.54	1.65	1.62
PFOA	1.36	1.63	1.59	1.72	1.22	0.97	1.49[a]	1.05	1.41	1.09	1.90[a]	1.87	1.77[a]	1.22
TOF	2.32	2.09	1.98	1.69	1.73	1.47	1.80	1.16	2.35	1.36	2.66[a]	1.72	2.29	1.60

[a]comparison between 1994/1995 and 2000 mean data, $P < .05$

through past, as well as the present fluorochemical medical surveillance programs; thus, we have concluded that this voluntary fluorochemical medical surveillance program has likely presented a nonbiased assessment of the employees' serum fluorochemical distribution.

The company's 2000 fluorochemical medical surveillance program had three times the number of participants compared to previous surveillance years. The voluntary participation rates for the present assessment were 53% (Decatur) and 75% (Antwerp) and were higher than those in previous surveillance years. This increase in employee participation was likely the consequence of at least two factors: 1) heightened employee awareness about their serum fluorochemical values; and 2) the company's announcement that it would voluntarily cease production of perfluorooctanyl chemistry in certain repellents and surfactants because of the pervasive and persistent nature of PFOS.

We observed several demographic and lifestyle differences between the Antwerp and Decatur employees. In particular, Antwerp male employees were younger, had lower BMIs, and drank more alcoholic beverages than their Decatur counterparts. These differences can be important confounding variables when analyzing lipid and hepatic clinical chemistries.

In the cross-sectional analysis, the positive association in the multivariable models between PFOS and serum cholesterol is contrary to the substantial body of toxicological literature that suggests a negative association in laboratory animals.[12,13,18,38] In a 6-month primate PFOS capsule study, triglycerides were unaffected but decreased serum cholesterol was an early toxicological response that occurred at serum PFOS levels above 100 ppm.[12] This serum PFOS concentration is approximately ten times greater than the highest employee value (10.06 ppm) measured in the present study. Accordingly, the positive association we observed between measured serum PFOS concentrations and total cholesterol appears spurious.

The serum PFOA concentrations measured in these 518 employees were lower than those measured among the company's manufacturing employees at its Cottage Grove facility (the primary site of PFOA manufacture) whose serum PFOA levels have been assayed as high as 114 ppm (mean = 5 ppm; median = 1 ppm).[31] Mean serum trigylceride levels were greater among the Cottage Grove APFO production workers with the highest (≥10 ppm) PFOA serum concentrations although adjustment for potential confounders have provided inconclusive

TABLE 6
Longitudinal Analyses of Serum Cholesterol or Triglycerides Levels by PFOS, PFOA or TOF and Other Covariates for 174 Male Employees

	Cholesterol[#]		Triglycerides[#]	
	Coefficient	95% C.I.	Coefficient	95% C.I.
PFOS Model*				
PFOS	0.010	(−0.005)–0.025	0.025	(−0.015)–0.065
Years Observed	0.0009	(−0.008)–0.010	−0.004	(−0.029)–0.021
PFOS × Years Obs	−0.0004	(−0.004)–0.003	0.006	(−0.004)–0.015
PFOA Model*				
PFOA	0.032	0.013–0.051	0.094	0.045–0.144
Years Observed	0.005	(−0.004)–0.014	0.007	(−0.017)–0.031
PFOA × Years Obs	−0.005	(−0.001)–(−0.002)	−0.008	(−0.018)–0.002
TOF Model*				
TOF	0.021	0.006–0.035	0.053	0.014–0.093
Years Observed	0.004	(−0.005)–0.014	−0.0005	(−0.027)–0.026
TOF × Years Obs	−0.003	(−0.005)–0.0003	−0.0005	(−0.008)–0.007

[#] Natural log
* Adjusted for age, BMI, drinks/day, cigarettes/day, location, entry period and baseline years worked.

results.[31] The positive associations observed between PFOA and triglycerides in the present study are inconsistent with the known hypolipidemic effect of this compound in rats that is thought to be associated with activation of the nuclear receptor, peroxisome proliferator-activated receptor (PPARα).[14,16,39] Haughom and Spydevold have suggested that this hypolipidemic effect results from an overall decrease in lipoprotein formation due to decreased activities of hydroxymethylglutaryl CoA reductase and acyl-CoA:cholesterol acyltransferase as well as a reduction in fatty acid synthesis.[14] The relevance of the PPARα-mediated response in humans has been debated because of the greatly decreased expression of PPARα in humans and other nonresponsive species.[40,41] On the other hand, PPARγ, a nuclear receptor expressed mainly in adipose tissue, has been shown to be activated by the antidiabetic thiazolidinendiones, which upregulates glycerol kinase activity stimulating increased hepatic triglycerides.[42] Whether this mode of action is plausible for PFOA in humans is not known. Mouse and human PPARγ$_1$ were unresponsive to PFOA when tested at a range of 0.5 to 40 μM in a cell transfection assay.[43] In a 6-month oral toxicity study of PFOA (ammonium salt) in male cynomolgus monkeys, PFOA was significantly associated with triglycerides in the high-dose group.[22] This association was observed in measurements taken after one month of dosing at which time group mean triglyceride was significantly higher than control values as well as within group pretreatment values. At the end of the study mean triglyceride was elevated compared to time related controls but not to the animals' pretreatment values. However, only two primates were evaluated in the high-dose group at the end of study. Inspection of individual values for PFOA serum concentration and serum triglyceride values did not reveal a meaningful association between these two parameters (John Butenhoff, personal communication). Therefore, although the possibility cannot be discounted that PFOA may activate the PPARγ nuclear receptor with a resulting increase in triglycerides, the evidence for this mechanism remains equivocal.

Adjusting for potential confounding factors, there were no substantial associations between hepatic enzymes and the employees' serum PFOS concentrations. This observation is consistent with results from the 6-month PFOS capsule feeding study where no overt hepatic toxicity was observed in the two lower dose groups (0.03 or 0.15 mg/kg/day) whose mean serum concentrations measured 16 and 83 ppm (males) and 13 and 67 ppm (females), respectively.[12] The 0.75 mg/kg/day treatment group did show hepatocellular hypertrophy and lipid vacuolation along with other signs of toxicity and two of six male monkeys died (no female deaths). No significantly increased differences from control or pretreatment values were observed at end-of-study for serum levels of alkaline phosphatase or ALT in the high dose group. Mean serum concentrations for male and female monkeys at this dose was 170 ppm. The liver:serum concentration ratio in the primate was approximately 1:1 to 2:1 for all treatment groups.

We observed a positive association between PFOS and T3 in the longitudinal assessment. There is unlikely any clinical relevance for this association. No other thyroid hormone was associated with PFOS and the observation is contrary to the primate study, which showed lowered T3 values without an indication of a hypothyroid compensatory increase in TSH, hypolipidemia or thyroid gland histological changes.

Retrospective cohort mortality studies have not reported statistically

significant standardized mortality ratios for all cancer or liver cancer deaths at either the Decatur or Cottage Grove manufacturing sites.[44–46] The Decatur mortality study did observe three deaths from bladder cancer compared to 0.2 expected (standarized mortality ratio = 12.8; 95% CI = 2.6–37.4) in the subgroup of workers with the highest potential exposure to perfluorooctanesulfonyl fluoride (POSF)-based materials.[44] Alexander et al. did not determine whether this association was fluorochemical-related or possibly due to other nonfluorochemical occupational exposures, nonoccupational exposures or chance. PFOS did not result in any bladder tumors in the high dose group (20 ppm) of a 2-year bioassay of rats.[13] The present study found no differences among Decatur employees' serum PFOS concentrations and their urinalyses. Additional epidemiologic research is ongoing to further understand the bladder cancer mortality association among the Decatur workforce.

Although we were able to perform a longitudinal assessment of the medical surveillance data, several limitations remained in our analyses. Only 41 of the 175 employees in the longitudinal analysis participated in all three surveillance years. Insufficient numbers prevented longitudinal analyses of the female employees. Because 3M announced a phase-out of the production of perfluorooctanyl chemistry-related materials, it is doubtful whether there will be more employees who can be included in future longitudinal assessments. Given the variability inherent in the analytical method and the different laboratories used, serum measurements of PFOS and PFOA may have varied ±20% although most were ±10%. This experimental error may have masked associations with lipid or hepatic clinical chemistries although the range of PFOS and PFOA measured was relatively consistent throughout the study time period. Our findings suggest that employees' measured serum PFOS concentrations have either remained constant or declined slightly during this 6-year time period. However, serum PFOA levels trended slightly upwards during the study period, which may have been the result of increased production.

In summary, a cross-sectional analysis of 421 male and 97 female POSF and PFOA production and nonproduction employees, as well as a longitudinal analysis over a 6-year time period of 174 male employees, have not shown substantial changes in lipid or hepatic clinical chemistry test results that are consistent with the known toxicological effects of these compounds. This finding was not unexpected because these employees' average serum concentrations were considerably lower than those known to cause the earliest clinical effects in laboratory animals.

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