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TSCA Confidential Business Information Center (7407M)
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Re: Submission To TSCA 8(e)/FYI Database Re: PFOA/PFOS

To TSCA 8(e)/FYI Database:

We are hereby providing the following information for inclusion in the TSCA 8(e)/ FYI databases with respect to PFOA/PFOS:

1. Costa, G., et al., "Thirty Years of Medical Surveillance in Perfluorooctanoic Acid Production Workers," 51 J.O.E.M. 1-9 (March 2009); and

2. Joensen, U.N., et al., "Do Perfluoroalkyl Compounds Impair Human Semen Quality?," Environ. Health Persp. (doi: 10.1289/ehp.0800517) (online March 2, 2009).

Very truly yours,

Robert A. Bilott

RAB:mdm
Enclosure

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JOEM • Volume 51, Number 3, March 2009

Thirty Years of Medical Surveillance in Perfluorooctanoic Acid Production Workers

Giovanni Costa, MD
Samantha Sartori, BS
Dario Consonni, MD

Objective: *To report health outcomes of 30 years (1978–2007) of medical surveillance of workers engaged in a perfluorooctanoic acid (PFOA) production plant.* ***Methods:*** *Fifty-three males workers (20 to 63 years) were submitted every year to medical examination and blood chemical chemistry tests, and serum PFOA dosage.* ***Results:*** *In the latest survey PFOA serum levels ranged from 0.20 to 47.04 μg/mL in currently exposed workers, and from 0.53 to 18.66 μg/mL in those formerly exposed. No clinical evidence of any specific trouble or disease has been recorded over the 30 years, and all the biochemical parameters, including liver, kidney and hormonal functions, turned out to be within the reference ranges, but a significant association of total cholesterol and uric acid with and PFOA serum level was evidenced.* ***Conclusions:*** *A probable interference of PFOA on intermediate metabolism deserves further investigations.* (J Occup Environ Med. *2009;51:000–000*)

Perfluorooctanoic acid (PFOA, $C_7F_{15}COOH$, CAS No. 335-67-1) is a fully fluorinated carboxylic acid, widely used as emulsifier in fluoropolymer polymerization. It is soluble in water, where it forms a mixture with its dissociated perfluorooctanoate anion ($C_7F_{15}COO^-$); it is absorbed by oral, dermal and inhalation routes, and mainly bound to serum albumin. It is mostly found in liver, serum and kidney, and is excreted, not metabolized, mainly in urines, with large differences in elimination speeds between species and sexes. It is a mild irritant for skin and mucoses, it is not a sensitizer and has neither teratogenic nor fetotoxic effects, but it delays sexual maturation in animals.[1–5]

It is not a genotoxic, but in two chronic studies in rats it has been reported to increase the incidence of tumors (adenomas) of liver, pancreatic acinar cells, and testicular Leydig cells,[6,7] like other peroxisome proliferators, as it acts mainly as a peroxisome proliferator-activated receptor alpha (PPARα) agonist.[8,9]

In recent years, many studies have been carried out with the aim of better understanding the toxicokinetics and mode of actions, although with not clear and definite conclusions.[10–17]

Its half-life of elimination in serum markedly differs between species and sexes, being 1 to 9 days in rats, 18 days in mice, 20 to 40 days in dogs, 20 to 32 days in monkeys, and 3.8 years in humans.[18–20] Hence, its biopersistence in the human blood, its detection in the general population[21–24] and its presence in several biota and animals all over the

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world[25–27] are a concern for possible chronic effects.[28,29]

Epidemiological studies on large cohorts of occupationally exposed workers, mainly of US plants (3M and DuPont) in the period 1947–2002, could not find any association between PFOA exposure and significant adverse health effects in terms of morbidity and mortality.[30–34] For mortality in particular, neither Gilliland and Mandel[31] nor Alexander[32] and Leonard et al[33] found excess of death for cancers of any type in quite large 3M and DuPont cohorts. Also the slight excess of prostate cancer signaled by Gilliland and Mandel,[31] and of bladder cancer found in the first study by Alexander,[32] were not confirmed by subsequent more specific surveys both in 3M[34] and DuPont[33] workers.

However, some contrasting and somewhat inconsistent findings, mainly related to lipid metabolism, have been reported as concerns perturbation of biochemical variables.

The first study, made by Ubel et al[30] in 1980 in about 300 workers of 3M Cottage Grove plant, having total serum organic fluorine (90% PFOA) up to 71 μg/mL, did not report any significant deviations from the normal values as concerns hematology, liver enzymes, total cholesterol, glucose, and uric acid.

In a subsequent study on 115 workers of the same plant,[35] after adjusting for age, smoking, alcohol intake and BMI in a multivariate regression model, a total serum fluorine level above 10 ppm was associated with slight increases in hepatic enzymes through an interaction with obesity. A slight interference with HDL cholesterol was also observed, when in association with alcohol consumption.

In other two cross-sectional studies on 191 3M production workers,[36] serum PFOA was not significantly associated with any of the 11 measured hormones, except for a 10% increase in mean estradiol level among employees having the highest levels of serum PFOA (≥30 ppm), although this association was confounded by body mass index.

Extending the survey in the same production plant to 265 workers for a longer period,[37] a negative association was recorded between serum PFOA and plasma Cholecystokinin (although inside the normal range), and no effect on hepatic enzymes, bilirubin, triclycerides, HDL, and LDL cholesterol.

However, in a subsequent survey concerning 518 workers of both sexes from two US and European plants,[38] the same authors found a modest positive association between PFOA and cholesterol and triglycerides in both cross sectional and longitudinal (over a 6 year time period) analysis, after adjusting for potential confounders, such as age, BMI, smoking, alcohol, and location.

More recently, Olsen and Zobel[39] have re-examined the same parameters in 506 3M workers from three US and European 3M plants, by multiple and logistic regressions and analysis of covariance: serum PFOA concentrations were negatively associated with HDL and bilirubin, and positively with triglycerides, after adjusting for possible confounders.

Very recently two reports, one longitudinal and another cross-sectional, on DuPont production workers have been published. In the retrospective study of 454 production workers, with repeated measurements concerning liver function and lipids over a period of 25 years,[40] a positive association between serum PFOA and total cholesterol and AST was found, whereas a negative association with bilirubin, after adjustment for age, BMI, gender, and decade of hire, but without control for lipid lowering medications.

In the other cross-sectional study on 1025 active workers,[41] including hematology, liver and renal function, lipid and purine metabolism, and hormones, a modest but statistically significant positive relationship between PFOA and total cholesterol, LDL, VDLD, GGT and uric acid was recorded, after excluding people taking lipid lowering medications.

The present study is aimed at providing a further epidemiological contribution to the understanding of possible effects on humans.

The data refer to the workers of a chemical plant (Miteni, Trissino, Italy), where PFOA has been produced by electrochemical fluorination since 1968. Since 1978 all the workers engaged in PFOA production have been submitted every year to the medical surveillance program by an expert Occupational Health Physician, according to the Italian legislation concerning health and safety at work.

Materials and Methods

Subjects

As a whole, 53 workers, all males, engaged in the PFOA production department have been examined every year from 1978 to 2007, for a total of 919 persons-years. Subjects' age ranged from 20 to 63 years and length of work exposure varied from 0.5 to 32.5 years in the period.

At the latest PFOA biomonitoring, in 2007, 37 were active workers in the PFOA department, whereas 16 were no longer exposed being retired or transferred to other departments in the meantime.

All the other male workers, 12 executive clerks and 95 blue collars from the other departments of the company, who have never been exposed to PFOA but submitted to the same periodical medical surveillance, were considered as "controls."

Table 1 summarizes the main characteristics of the examined workers.

Methods

All the workers (exposed and not exposed) have been submitted every year to physical examination, including recording of blood pressure, height and weight, and to blood chemical chemistry tests, including hematology (Ht, Hb, WBC, RBC, PLTS), liver (albumin, α_1-α_2-β-γ

globulins, bilirubin, AST, ALT, ALP GGT) and renal (urea nitrogen, creatinine) functions, glucose and lipid metabolism (total and HDL Cholesterol, triglycerides), and uric acid. All the variables have been measured over the years by autoanalysers (SMA) in the same laboratory of the nearby regional public general hospital, undergoing a periodical quality control program.

In addition, in the workers of the PFOA department, also Apo-A and Apo-B lipoproteins, protein C reactive, immunoglobulins IgA-IgG-IgM, Testosterone, Estradiol, FSH, TSH, FT3, FT4, and Prostatic Serum Antigen (PSA) were tested in blood in 2002 and 2006.

The biological monitoring of serum PFOA started in April 2000 and was repeated in May 2001, December 2001, June 2002, September 2003, September 2004, May 2006, and May 2007. It included not only the workers of the PFOA department currently exposed in those years, but also those formerly exposed who had been transferred to other departments or had retired in the meantime, as well as some non-exposed workers (clerks), taken as controls.

PFOA serum levels were determined by HPLC-Electrospray-Tandem Mass Spectrometry[42] in two internationally accredited laboratories: the first one carried out the analysis in 2000, 2001, and 2002, the second one in the following years. A double blind comparison between the two laboratories carried out in 2003 gave a correlation index equal to 0.90. In 2000 and 2001, the method had an upper scale detection limit fixed at 45.5 μg/mL, thereafter no detection limit was present.

Statistical Analysis

ANOVA, *t* test and multiple linear regression models were used to evaluate the relationships between PFOA exposure and hematochemical effects while adjusting for relevant covariates. Natural log transformation of the dependent variables was performed where appropriate. Generalized Estimating Equations (GEE) models with an exchangeable correlation matrix were fitted when analyzing repeated measurements and relationships between PFOA serum level and the different (continuous or dichotomous) health outcomes, while accounting for within-subject correlations.[43] In the regression models the following covariates were included: age, years of exposure, year of PFOA sampling, BMI, smoking, and alcohol consumption.

Stata 10[44] software was used to perform all the statistical analyses.

TABLE 1
Demographic Characteristics of the Workers Examined

	PFOA Currently Workers	PFOA Previously Exposed Workers	Control Group
No	37	16	107
Sex	All males	All males	All males
Age (mean and SD)*	41.6 (8.31)	52.0 (8.7)	41.9 (9.2)
Years of work (mean and SD)*	14.3 (9.1)	16.3 (9.9)	14.0 (9.3)
Years elapsed since PFOA exposure (mean and SD)*		9.5 (8.3)	
Person-years of observation	487.2	431.8	1499.3
BMI (mean and SD)	26.2 (2.9)	25.7 (3.1)	25.1 (2.8)
Smoking (%)	38.2	47.6	36.5
Alcohol consumption (%)	73.5	71.4	62.6

*At the latest medical check.

TABLE 2
PFOA Serum Level (μg/mL), Recorded in the Year 2007, in Currently and Formerly Exposed Workers

	Currently Exposed	Formerly Exposed
No.	39	11
Min	0.20	0.53
25th percentile	2.25	2.61
Median	5.71	4.43
Mean arithmetic	12.93	6.81
Mean geometric	4.02	3.76
75th percentile	23.55	9.24
Max	47.04	18.66
Standard deviation	14.43	6.06

Results

From the clinical point of view, all the workers were in good health and no excess of morbidity for any disease has been recorded over the years.

In the latest survey, carried out in May 2007, the PFOA serum levels ranged from 0.20 to 47.04 μg/mL (ppm) in the currently exposed workers, and from 0.53 to 18.66 μg/mL in the formerly exposed workers (Table 2): in the seven non-exposed subjects (clerks/staff) examined in 2006, the PFOA serum levels ranged from 0.05 to 0.181 μg/mL.

The highest level recorded was 91.9 μg/mL in 2002. Table 3 shows the PFOA serum levels recorded over the years in the production workers. As above mentioned, in 2000 and 2001 the method had an upper scale detection limit fixed at 45.5 μg/mL; consequently, in order to compare the data over the years the maximum level in 2000 and 2001 was set by default as the highest level recorded in 2002, when the method had no upper scale detection limit.

A significant decrease of both peak and mean levels was recorded after 2002, following a renovation of the plant carried out in that year, including a partial automation of the process, and the adoption of more strict working procedures and use of suitable protective devices.

In the 27 currently production workers who had their serum PFOA measured in all the four last occasions, median value decreased from 13.6 μg/mL in 2002 to 7.1 μg/mL in 2007 (−47.8%), mean value decreased from 22.2 μg/mL to 14.0 μg/mL (−37.0%), and peak level decreased from 86.3 μg/mL to 47.0 μg/mL (−45.5%), respectively.

All the biochemical parameters, turned out to be on average within

the laboratory reference ranges, while some subjects had some parameters above the upper reference values, as shown in Table 4, which refers to the results of the latest check carried out in 2007. As compared with the control group some differences, both in terms of mean values and number of persons above the upper references limits, were noted for some parameters such as uric acid, cholesterol, triglycerides, and liver enzymes.

As the crude correlations between PFOA and the biochemical variables, recorded in the last 7 years, gave some indication on a possible association between PFOA serum levels and some lipids and uric acid (data not shown), three more in-depth analyses in this respect were performed. The aim was clarifying this possible association by controlling the most important confounding factors, and after preliminary exclusion

TABLE 3
Serum PFOA Levels (μg/mL) Recorded in Production Workers Over the Year

	2000	2001	2002	2003	2004	2006	2007
No.	25	42	46	41	34	49	50
Min	1.54	0.73	0.34	0.38	0.54	0.54	0.20
25th percentile	5.53	4.35	4.57	4.11	2.84	2.36	2.18
Median	11.92	11.07	10.15	6.25	6.82	5.27	3.89
Mean arithmetic	18.8	19.7	19.3	13.7	11.4	10.8	11.6
Mean geometric	11.7	10.2	9.3	6.9	6.5	5.8	5.4
75th percentile	32.0	19.72	20.80	14.20	18.97	16.31	18.66
Max	86.3*	91.9*	91.9	74.7	46.3	41.9	47.0
Standard deviation	20.0	23.6	23.0	16.6	12.0	11.8	13.3

*Upper scale detection limit at 45.5 μg/mL (discussed in text).

TABLE 4
Haematology: Results of the Last Check in the Currently Exposed Workers and Control Group

	Reference Values Min–Max	Exposed		Control Group	
		Mean (SD)	% Outside Reference Range	Mean (SD)	% Outside Reference Range
Glucose (mg/dL)	70–110	92.7 (8.7)	2.6	89.3 (16.5)	6.5
Urea nitrogen (mg/dL)	7–22	15.2 (3.1)	0	15.2 (3.0)	0.9
Creatinine (mg/dL)	0.80–1.30	0.92 (0.12)	0	0.93 (0.11)	0
Uric acid (mg/dL)	3.5–7.2	6.2 (1.1)	17.9	5.7 (1.2)	4.7
Cholesterol total (mg/dL)	191–240	239.7 (50.9)	35.9	213.5 (39.7)	26.2
Cholesterol HDL (mg/dL)	>39	55.9 (14.7)	0	56.7 (12.3)	6.6
Triglycerides (mg/dL)	30–180	178.9 (118.6)	7.7	146.3 (97.9)	27.1
Bilirubin total (mg/dL)	<1.0	0.58 (0.22)	0	0.67 (0.27)	10.5
Bilirubin conjugated (mg/dL)	<.30	0.15 (0.05)	2.6	0.18 (0.11)	7.6
AST (U/L)	<50	32.4 (8.8)	2.6	29.7 (10.7)	4.8
ALT (U/L)	<50	47.8 (22.3)	17.9	40.6 (21.8)	26.2
γGT (U/L)	8–61	53.8 (46.7)	28.2	44.5 (41.4)	17.9
ALP (U/L)	40–129	71.8 (22.8)	0	67.7 (19.6)	0
Proteins total (g/dL)	6.4–8.2	7.71 (0.50)	1	7.81 (0.56)	0
Albumin (%)	52–67	61.6 (3.0)	0	62.3 (3.9)	0
α1 globulins (%)	3–7	4.1 (0.9)	0	5.5 (9.4)	0.9
α2 globulins (%)	5–9	8.9 (1.1)	12.8	8.8 (1.4)	14
β globulins (%)	5–12	5.9 (0.9)	0	5.8 (0.5)	0
γ globulins (%)	10–22	14.7 (2.6)	0	14.7 (2.3)	0
WBC ($\times 10^9$/L)	4.0–10.9	7.02 (2.13)	2.6	6.96 (1.88)	5.6
RBC ($\times 10^{12}$/L)	4.5–5.9	5.06 (0.37)	0	5.11 (0.39)	1.8
Hb (g/dL)	13.5–17.5	15.6 (0.88)1	2.6	15.7 (0.86)	0.9
Ht (%)	41–53	45.9 (2.2)	0	46.0 (2.4)	0
Platelets ($\times 10^9$/L)	140–440	241.3 (55.0)	0	237.1 (54.4)	0
Protein C reactive (mg/L)	0.0–5.0	2.59 (4.21)	5.1		
Apo A (g/L)	1.10–2.05	1.43 (0.24)	5.1		
Apo B (g/L)	0.55–1.40	1.25 (0.31)	5.1		
IgG (g/L)	7.0–16.0	10.6 (2.4)	2.6		
IgA (g/L)	0.70–4.0	3.1 (1.2)	12.8		
IgM (g/L)	0.40–2.30	1.2 (0.76)	5.1		
PSA (ng/mL)	<4.0	0.80 (0.94)	2.6		
Testosterone (ng/mL)	0.1–8.0	5.88 (0.57)	5.1		
Estradiol (pg/mL)	10–50	30.8 (1.3)	2.6		
TSH (UI/mL)	0.27–4.20	1.99 (5.48)	2.6		
FT3 (pg/mL)	2.0–4.4	3.62 (0.67)	2.6		
FT4 (ng/dL)	0.80–1.70	1.31 (13.5)	2.6		

of people under treatment of primary hyperlipidemias and with a history of chronic hepatitis.

In the first analysis, 34 currently exposed workers were matched with 34 workers from the other departments with the same age (±1 year) and job seniority (±1 year) in the company, never exposed to PFOA, having the same working hours (shift work or daywork), and being resident in the same area with similar housing and living conditions. Table 5 shows that the two groups significantly differ only for total cholesterol and uric acid mean levels, being slightly higher in workers exposed to PFOA.

In the second analysis, the same 34 currently exposed workers were compared with all the other 107 male workers of the company. The multiple regression analysis, adjusted by age, job seniority, body mass index, smoking and alcohol consumption, confirmed a significant effect of PFOA exposure as concerns an increase in mean total cholesterol and uric acid (Table 6).

The third analysis considered the 56 subjects (currently, formerly and never exposed) who had serum PFOA assessed concurrently with the biochemical parameters in the last 7 years. Multivariate GEE models (including age, job seniority, body mass index, alcohol consumption, and years of observation as potential confounders) show that total cholesterol and uric acid levels were weakly but significantly correlated to serum PFOA (Table 7) as well as some liver enzymes and alpha2 globulins; however, total bilirubin appears to be inversely related to PFOA.

As expected, also body mass index was found to be significantly correlated to total (Coef. = 0.028, $P < 0.01$) and HDL (Coef. = −.029, $P < 0.001$) cholesterol, triglycerides (Coef. = 0.065, $P < 0.001$) and uric acid (Coef. = 0.022, $P < 0.001$), as well as with some liver enzymes (ALT, ALP), suggesting a possible interaction, like in previous surveys.[35]

TABLE 5
Comparison Between 34 Exposed and Non-Exposed Workers, Matched By Age, Work Seniority, Day/Shiftwork, and Living Conditions

	Non-Exposed	Exposed	*t* Test	*P*
No.	34	34		
BMI	25.94	26.16	0.34	NS
Glucose (mg/dL)	89.41	93.56	1.30	NS
Urea nitrogen (mg/dL)	14.82	19.47	1.14	NS
Creatinine (mg/dL)	0.93	0.94	0.19	NS
Uric acid (mg/dL)	5.73	6.29	2.10	0.039
Total cholesterol (mg/dL)	206.4	237.0	3.06	0.003
HDL cholesterol (mg/dL)	56.68	57.82	0.21	NS
Triglycerides (mg/dL)	150.03	155.35	0.77	NS
Total bilirubin (mg/dL)	0.58	0.55	0.18	NS
AST (U/L)	31.62	31.44	0.41	NS
ALT (U/L)	42.74	35.29	1.35	NS
GGT (U/L)	48.52	43.88	0.05	NS
ALP (U/L)	69.41	67.65	0.24	NS
Total proteins (g/dL)	7.72	7.67	0.22	NS
Albumins (%)	62.87	61.51	0.99	NS
α1 globulins (%)	3.82	3.94	0.59	NS
α2 globulins (%)	8.76	8.90	0.20	NS
β globulins (%)	5.68	5.76	0.49	NS
γ globulins (%)	14.44	14.47	0.10	NS
WBC ($\times 10^9$/L)	6.64	7.53	1.86	NS
RBC ($\times 10^{12}$/L)	5.08	5.00	0.60	NS
Haemoglobin (g/dL)	15.69	15.37	0.40	NS
Haematocrit (%)	45.86	45.36	0.74	NS
Platelets ($\times 10^9$/L)	233.03	238.97	0.84	NS

TABLE 6
Multiple Regression Analysis Comparing the 34 Exposed Workers (E) With All the Other 107 Workers (ALL) of the Plant

Parameter	Coef. (E vs ALL)	95% C.I.	*P*
Glucose (mg/dL)	3.53	−2.21/9.28	NS
Urea nitrogen (mg/dL)	3.92	−0.95/8.79	NS
Creatinine (mg/dL)	0.004	−0.04/0.05	NS
Uric acid (mg/dL)	0.50	0.06/0.94	0.027
Total cholesterol (mg/dL)	21.7	6.83/36.6	0.005
HDL cholesterol (mg/dL)	2.42	−2.30/7.13	NS
Triglycerides (mg/dL)	−.15	−34.6/34.3	NS
Total bilirubin (mg/dL)	−0.09	−0.19/0.01	NS
AST (U/L)	1.35	−2.72/5.41	NS
ALT (U/L)	−5.18	−13.7/3.32	NS
GGT (U/L)	0.32	−17.5/18.1	NS
ALP (U/L)	−0.78	−8.51/6.95	NS
Total proteins (g/dL)	−0.20	−0.57/0.17	NS
Albumin (%)	−0.73	−3.44/1.97	NS
α1 globulins (%)	−1.82	−8.18/4.54	NS
α2 globulins (%)	0.27	−0.75/1.28	NS
β globulins (%)	−0.003	−0.37/0.36	NS
γ globulins (%)	−0.53	−2.29/1.24	NS
WBC ($\times 10^9$/L)	0.58	−0.19/1.35	NS
RBC ($\times 10^{12}$/L)	−0.08	−0.23/0.07	NS
Haemoglobin (g/dL)	−0.27	−0.60/0.07	NS
Haematocrit (%)	−0.51	−1.42/0.40	NS
Platelets ($\times 10^9$/L)	1.31	−18.8/21.4	NS

TABLE 7
Results of the Multivariate Analysis (GEE Model) in the 56 Subjects (Currently, Formerly, and Never Exposed) Who Had Serum PFOA Assessed Concurrently With the Biochemical Parameters in the Last Six Yr (Controlled for Age, Job Seniority, Body Mass Index, Alcohol Consumption, and Yr of Observation)

	Coef. Pfoa	95% C.I.	P
Glucose	0.005	−0.011/0.020	NS
Urea nitrogen	0.017	−0.015/0.049	NS
Creatinine	−0.011	−0.025/0.004	NS
Uric acid	0.026	0.001/0.053	<0.05
Total cholesterol	0.028	0.002/0.055	<0.05
HDL cholesterol	−0.018	−0.047/0.012	NS
Triglycerides	0.055	−0.036/0.147	NS
Total bilirubin	−0.080	−0.137/−0.024	<0.01
Conj. bilirubin	−0.034	−0.099/0.031	NS
AST	0.038	−0.003/0.080	NS
ALT	0.116	0.054/0.177	<0.01
GGT	0.177	0.076/0.278	<0.01
ALP	0.057	0.007/0.107	<0.05
Apo A	−0.001	−0.038/0.035	NS
Apo B	0.023	−0.041/0.087	NS
IgG	−0.017	−0.115/−0.080	NS
IgM	0.048	−0.093/0.190	NS
Estradiol	−0.012	−0.080/0.057	NS
Prot C reactive	−0.020	−0.268/0.228	NS
PSA	−0.032	−0.286/0.222	NS
Testosterone	−0.005	−0.021/0.011	NS
Total proteins	−0.001	−0.010/0.008	NS
Albumins	−0.009	−0.017/0.001	NS
α1 globulins	0.026	−0.001/0.053	NS
α2 globulins	0.026	0.007/0.045	<0.01
β globulins	0.011	−0.008/0.030	NS
γ globulins	0.013	−0.005/0.031	NS
WBC	0.029	−0.011/0.071	NS
RBC	−0.002	−0.009/0.006	NS
Haemoglobin	0.008	−0.113/0.129	NS
Haematocrit	0.122	−0.132/0.376	NS
Platelets	1.987	−7.36/11.34	NS

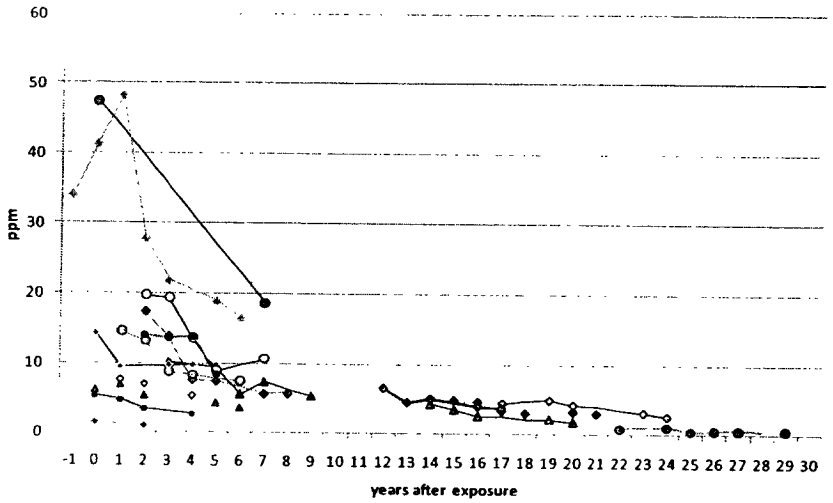

Fig. 1. PFOA serum level (μg/mL) in the 16 formerly exposed workers, who retired or were transferred to other departments.

Discussion

In our cohort neither clinical evidence of specific disturbances nor health disorders have been recorded over 30 years of medical observations of workers exposed to PFOA, having serum levels ranging from 0.20 to 91.9 μg/mL.

A significant decrease in PFOA blood levels (−37% in mean level and −45.5% in peak level) was recorded in the 4 last years after plant renovation and improvement of working conditions. However, in evaluating this trend it is necessary to take into account the long biological half-life of the substance, so that the present blood levels largely reflect the exposure conditions of the previous years.

In fact, contrary to the rather rapid rates of elimination reported in laboratory animals, PFOA appears to be slowly eliminated in humans.[3,30] Very recently Olsen et al[20] have determined the serum elimination half-life of PFOA in 26 retired fluorochemicals production workers over a 5 year span; the arithmetic mean half-life was 3.8 years (95% CI = 3.1 to 4.4) and the geometric mean half-life of 3.5 years (95% CI = 3.0 to 4.1), with a range from 1.5 to 9.1 year.

A similar trend can be found also in our data (Fig. 1). In the 16 formerly exposed workers, who retired or were transferred to other departments, the decrement of PFOA blood concentration over the last 7 years appears to be more or less steeper depending on both extent and duration of the past exposure, and years elapsed since leaving the job. Although the data are not complete and homogeneous, in terms of PFOA level, number and years of measurements, time elapsed since exposure, the observed trend in the decrement suggests an estimated half-life of 5.1 (sd 1.7) years, varying from 2.6 to 9.7 years (geometric mean 4.8). It is noteworthy that even after 20 years from exposure some people still have 1 to 3 μg/mL of PFOA in their blood.

As regards biological effects, no significant perturbation of liver and renal functions, immunology, and hormonal secretion (including estradiol, testosterone, thyroid, and prostate hormones) were recorded in our workers, except for a significant interference with lipids (cholesterol) and purines (uric acid) metabolism, beside the influence of BMI.

As mentioned in Introduction, some previous cross sectional studies in 3M workers did not find any consistent association between serum PFOA and liver, renal and lipid functions, and hormonal secretion.

As concerns liver function and lipids metabolism in particular, Gilliland and Mandel[35] did not record any significant difference between exposed and non-exposed workers in any biological parameter of liver function, and no significant correlation between total serum fluorine (above 10 μg/mL) and cholesterol, LDL or HDL, after adjusting for age, BMI, alcohol, and smoking. The same negative results were obtained in three successive cross-sectional investigations in workers with serum PFOA up to 114.1 μg/mL.[37]

More recently, in two other 3M groups, including both European and US occupationally exposed workers, Olsen et al[38] found no significant effect on liver function but a positive, although modest, association between serum PFOA (range: 0.01 to 12.7 μg/mL) and cholesterol as well as triglycerides, both in a cross-sectional and in a 6-year longitudinal analysis. Moreover, in a further study in workers with serum PFOA ranging from 0.007 to 92.03 μg/mL, Olsen and Zobel[39] recorded a weakly negative association with HDL and total bilirubin, and a positive one with triglycerides, after excluding people taking cholesterol lowering medications; however, these findings were explained by demographic differences across the three locations.

However, Sakr et al in a cross sectional study on DuPont workers,[41] having serum PFOA from 0.005 to 9.55 μg/mL, reported a modest, but significant positive correlation between serum PFOA and total cholesterol, LDL, and VLDL, whereas in another longitudinal study[40] they found a positive association of serum PFOA (range: 0 to 22.66 μg/mL) with total cholesterol and AST, and a negative association with total bilirubin, after adjusting for potential confounders (age, BMI, gender, and decade of hiring).

However, it is worth mentioning that serum cholesterol appears to move in the opposite direction than observed in experimental studies in rodents, in which PFOA has a significant hypolipidemic effect in serum, being a strong PPARα agonist.[45] No study reported any hypolipidemic effect in exposed workers, whereas three studies including the present one recorded a significant increase in cholesterol levels, besides other non-consistent increases of triglycerides and liver enzymes. Also in cynomolgus monkeys, after 6 months of oral dosing of PFOA from 3 to 30/20 mg/kg/die (with serum PFOA ranging from 20 to 467 μg/mL) no decrease of cholesterol, but a significant increase of triglycerides was observed.[18] This might be due to different mechanisms of action and/or receptor agonisms, ie, PPARα/γ and/or CAR[29] in rodents rather than in primates, which deserves further investigation. For example it is well known that there are different expressions and properties of PPAR isoforms (α, β, and γ) in animals and humans[9,13–15,46–48] as well as there might be different bindings with serum proteins (ie, albumin in animal, CETP) and in renal readsorption (Oatp1 and Oat3),[49,50] or interference with hepatic metabolism of fatty acids, ie, via CYP450.

As to the latter, it is worth mentioning that three studies (Olsen and Zabel,[39] Sakr et al,[41] and the present one), also recorded a significant negative association between serum PFOA and total bilirubin, that was not documented in rodents and primates. It is worth noting in this regard that perfluorolauric acid proved to cause a marked bilirubin destruction in vitro when added to a pure bacterial enzyme (CYP102 or BM3) specialized in fatty acid metabolism. By analogy, it is possible that a similar effect might take place in humans on interaction of PFOA with a fatty acid metabolizing CYP enzyme.[51]

Moreover, we recorded a positive significant association between serum PFOA and uric acid, that was also reported by Sakr et al in DuPont workers.[41] This may be suggestive of some interference in liver purines metabolism, which deserves specific investigations.

In our study, we tried to control as many confounding variables as possible, in particular age, BMI, smoking, alcohol consumption, and shift work. The first four factors were taken into consideration in the GEE analysis, whereas the possible interference of shift and night work on metabolic parameters, as evidenced in literature,[52,53] was controlled by comparing exposed workers and controls matched for (shift) work seniority (Table 5).

So, beside some inconsistent findings among the studies, it is probable that PFOA exerts some interference on intermediate metabolism also in humans. Owing to the small number of exposed subjects it is not possible to draw any firm conclusion, though the long time span of the careful medical surveillance may allow to exclude a severe impact of PFOA un human health. However, the possible interference on the intermediate metabolism should not be underestimated and deserves further investigations on mechanisms for action, and as a possible risk factor for metabolic disorders and/or CVD diseases.

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Do Perfluoroalkyl Compounds Impair Human Semen Quality?

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Abbreviations:

BMI:	body mass index
CI:	confidence interval
ESI:	electrospray ionization
FAI:	free androgen index
FSH:	follicle stimulating hormone
HPLC:	high performance liquid chromatography
LC-MS-MS:	liquid chromatography-tandem mass spectrometry
LH:	luteinizing hormone
LOD:	limit of detection
PFAA:	perfluoroalkyl acids
PFC:	polyfluorinated compounds
PFDA:	perfluorodecanoic acid
PFDoA	perfluorododecanoic acid
PFHpA:	perfluoroheptanoic acid
PFHxS:	perfluorohexane sulfonic acid
PFNA:	perfluorononanoic acid
PFOA:	perfluorooctanoic acid
PFOS:	perfluorooctane sulfonic acid
PFOSA:	perfluorooctane sulfonamide
PFTrA:	perfluorotridecanoic acid
PFUnA:	perfluoroundecanoic acid
SHBG:	sex hormone binding globulin
TDS:	testicular dysgenesis syndrome

Outline of section headers

Abstract

Background: Perfluoroalkyl acids (PFAAs) are found globally in wildlife and humans, and are suspected to act as endocrine disruptors. There are no reports of PFAA levels in adult men from Denmark, and no reports of a possible association between semen quality and PFAA exposure. **Objectives:** To investigate possible associations between PFAAs and testicular function. The hypothesis was that higher PFAA levels would be associated with lower semen quality and lower testosterone levels. **Methods:** We included 105 Danish men (median age 19 years) from the general population, and analyzed serum samples for levels of 10 different PFAAs and reproductive hormones, and assessed semen quality. **Results:** Considerable levels of PFOS, PFOA and PFHxS were found in all young men (median 24.5, 4.9 and 6.6 ng/mL, respectively). Men with high combined levels of PFOS and PFOA had a median of 6.2 million normal spermatozoa in their ejaculate in contrast 15.5 million among men with low PFOS-PFOA (p=0.030). In addition we found non-significant trends with regard to lower sperm concentration, lower total sperm counts and altered pituitary-gonadal hormones among men with high PFOS-PFOA levels. **Conclusion:** High PFAA levels were associated with fewer normal sperms. Thus, high levels of PFAAs may contribute to the otherwise unexplained low semen quality often seen in young men. However, our findings need to be corroborated in larger studies.

Introduction

Perfluoroalkyl acids (PFAAs) are degradation products of many man-made polyfluorinated compounds (PFCs) used in consumer and industrial products, for example for impregnation of carpets, textiles and paper (Jensen et al. 2008; Jensen and Leffers 2008; Kissa 2001). Studies of environment, wildlife and humans suggest widespread presence and exposure, as well as persistence in the environment and bioaccumulation (Giesy and Kannan 2001; Kannan et al. 2004). For perfluorooctanoic acid (PFOA), perfluorooctane sulfonic acid (PFOS) and perfluorohexane sulfonic acid (PFHxS), three of the most abundant PFAAs, half-lives for humans have been estimated as 3.8, 5.4 and 8.5 years, respectively (Olsen et al. 2007). Some studies suggest that men may have higher serum concentrations of PFAAs than women, and younger individuals may have higher levels than older (Calafat et al. 2006). Thus, young men may have particularly high levels of exposure and may therefore be a group at risk for potential adverse effects of PFAAs.

PFAAs can cross the placental barrier and therefore have the potential to affect the fetus. In humans, levels of PFOS and PFOA in umbilical cord blood have been inversely related to birth weight (Apelberg et al. 2007). In addition, PFOS, PFOA and PFHxS have been detected in human seminal plasma samples (Guruge et al. 2005). However, data on effects in humans are sparse, and most come from studies of occupationally exposed individuals. These studies have not given conclusive evidence of adverse effects. A recent study, however, measured PFAA levels in early pregnancy found that higher levels of PFOS and PFOA was associated with significantly longer waiting time to pregnancy (Fei et al. 2009).

Animal studies provide some evidence for adverse reproductive effects on animals exposed as adults or *in utero*. Exposure of adult male rats to PFOA reduced their testosterone levels and increased their estradiol levels, which may partly explain earlier

findings of induction of Leydig cell hyperplasia and/or adenomas in the testes of exposed animals (Biegel et al. 1995; Cook et al. 1992).

Our objective was to investigate the associations between PFOS, PFOA, PFHxS and other PFAAs and testicular function. Our primary hypothesis was that high concentrations of PFAAs would be associated with low testosterone levels and secondly that high PFAA levels are negatively associated to semen quality variables.

Materials and methods

Study population. Since 1996, semen quality of young men in Denmark has been surveyed in a cross-sectional study (Andersen et al. 2000; Jørgensen et al. 2002). All young Danish men must report for military draft, and annually new cohorts of approximately 300 men from the Copenhagen area in Denmark have been included. They each provided one semen sample and had a venous blood sample drawn. Of the 546 men examined in 2003, we selected 105 for the investigation of associations between PFAAs and testicular function. The 105 men included the 53 men (group 1) with the highest testosterone levels (median 31.8 nmol/l, range 30.1 - 34.8), and the 52 men (group 2) with the lowest testosterone levels (median 14.0 nmol/l, range 10.5 - 15.5). We chose the men examined in 2003, as this was the latest year from which we had completed analyses of reproductive hormones. The median age of men in group 1 was 18,9 years (range 18,2 – 24,6), median age in group 2 was 19,0 years (range 18,2 – 25,1), and median age for all 105 young men was 19.0 years (range 18.2 - 25.2) years. Information on ejaculation abstinence period and hour of blood sampling was recorded. All samples of semen and blood were collected between 8.30 a.m. and 1.15 p.m. Serum was stored at -20°C until chemical analysis.

The Danish National Committee on Biomedical Research Ethics, Copenhagen Region, approved the research, and all young men gave written informed consent.

PFAA analysis. Thawed serum samples were analyzed in January 2008 for ten different perfluorinated chemicals, with carbon chain length from C6 to C13: PFHxS (perfluorohexane sulfonic acid), PFHpA (perfluoroheptanoic acid), PFOA (perfluorooctanoic acid), PFOS (perfluorooctane sulfonic acid), PFOSA (perfluorooctane sulfonamide), PFNA (perfluorononanoic acid), PFDA (perfluorodecanoic acid), PFUnA (perfluoroundecanoic acid), PFDoA (perfluorododecanoic acid), and PFTrA (perfluorotridecanoic acid). One mL of serum was spiked with the surrogate standards $^{13}C_8$-PFOA, $^{13}C_2$-PFDA and $^{13}C_4$-PFOS and extracted according to the ion pairing method described previously (Hansen et al. 2001). Matrix-matched standards were prepared by spiking rabbit serum (Sigma Aldrich, Schnelldorf, Germany) with the analytes and the surrogate standards. Blank samples consisted of rabbit serum spiked with only surrogate standards. Standards and blanks were extracted together with each batch of samples. Instrumental analysis was performed by liquid chromatography-tandem mass spectrometry (LC-MS-MS) with electrospray ionization (ESI). The extracts (20 μL injection volume) were chromatographed on a C18 Betasil column (2.1 x 50 mm, Thermo Hypesil-Keystone, Bellafonte, PA) using an Agilent 1100 Series HPLC (Agilent Technologies, Palo Alto, CA). The high performance liquid chromatography (HPLC) was interfaced to a triple quadrupole API 2000 (Sciex, Concorde, Ontario, Canada) equipped with a TurboIon Spray source operating in negative ion mode. Chromatographic conditions and transition MS-MS ions have been described in details previously (Bossi et al. 2005). The limits of detection (LODs) ranged from 0.1 to 0.5 ng/mL.

Reproductive hormone analysis. Thawed serum samples were analyzed for the levels of testosterone, estradiol, SHBG (sex hormone binding globulin), LH (luteinizing hormone),

FSH (follicle stimulating hormone) and inhibin B as described previously (Paasch et al. 2008). Free androgen index (FAI) was calculated as [testosterone × 100 / SHBG]. Ratios between hormones were calculated by simple division.

Semen analysis. Semen volume was assessed by weight, and the sperm concentration by use of a Bürker-Türk haemocytometer. Total sperm count was calculated as semen volume x sperm concentration. The percentage of motile spermatozoa (WHO class A+B+C) was assessed on fresh samples. Sperm morphology slides were fixed, Papanicolaou stained and all assessed according to strict criteria (Menkveld et al. 1990) by one trained technician over a period of one week. Further details of the semen analysis can be seen in previously published work (Jørgensen et al. 2002).

Statistical analysis. Medians and 5-95th percentiles were used to describe the levels of PFAAs in group 1 and 2. Mann-Whitney *U*-test was used to compare the groups 1 and 2 with respect to PFAA levels, BMI (body mass index) and smoking status. Samples with values below LOD were set to 0 ng/mL. We used Pearson's correlation coefficients and related p-values to describe correlations between levels of different PFCs. Univariate regression analysis was done for comparison of hormone levels between group 1 and 2, and to describe associations between PFAAs and hormones or semen variables. Sperm concentration, semen volume and total sperm count were adjusted for the effect of ejaculation abstinence period. Sperm motility was adjusted for time between ejaculation and assessment of motility. Sex hormone concentrations were adjusted for hour of blood sampling. Semen variables and hormone levels and ratios, except sperm morphology and total testosterone, were ln transformed to obtain normality of the residuals. Smoking and

BMI were tested for confounding effects but were found to be non-significant, and therefore not included in the final analyses.

We proceeded to analyze for associations between PFAAs and testicular function (hormone levels and semen quality) for the whole group of 105 men. We singled out PFOS and PFOA, and results were calculated as estimated changes in endpoint (reproductive hormones and semen variables) with a change in serum concentration of 1 ng/mL of PFOS, PFOA concentrations, and the summed concentration of PFOS and PFOA. We divided the men into three groups from the combined concentrations of PFOS and PFOA. Each sample was given a quartile score of 1 to 4 for PFOS and PFOA levels separately. Score 1 was given to samples with levels within the lowest quartile, and score 4 within the highest quartile. We then summed the quartile scores for PFOS and PFOA, giving each sample a possible score from 2 to 8. Samples were then divided into 3 quartile groups for PFOS and PFOA combined: "Low PFAA" group (N=29) with summed quartile score from 2 to 3, "intermediate PFAA" (N=48) group with score 4 to 6, and "high PFAA" (N=28) group with score 7 to 8. Analysis for association between quartile group and hormone levels or semen variables was done using univariate regression analysis, adjusted for the above-mentioned confounders.

Statistical analysis was performed using SPSS statistical software version 16.0 (SPSS Inc., Chicago, IL, USA).

Results

Levels of PFAAs in serum. The serum levels and number of samples above LOD for all PFAAs are shown for the low- and high testosterone groups and the entire group of 105 men (Table 1). Except for PFOSA, which was detected in only 56 men, there was no significant difference in levels of any PFAA between group 1 and 2. The median PFOS,

PFOA and PFHxS concentrations for the whole group of men were 24.5, 4.9 and 6.6 ng/mL, respectively, and only these were included in the final regression analyses. The remaining PFAAs were found in much lower concentrations, and therefore these results are not discussed further. PFOS levels were positively correlated with PFOA ($r = 0.594$, $p < 0.0005$) and PFHxS ($r = 0.304$, $p = 0.002$) levels. PFOA and PFHxS levels were positively correlated, but not statistically significantly ($r = 0.136$, $p = 0.2$).

PFAAs and semen variables. In the whole group of 105 men, there was a tendency toward reduced levels of all semen variables in the "high" PFAA quartile group compared to the "low" group (groups constructed to include PFOS and PFOA levels, see statistical analysis), Table 2. The difference in percentage of morphologically normal spermatozoa as well as in the total number of normal spermatozoa (total sperm count x % morphologically normal sperms) was statistically significant ($p = 0.037$ and 0.030, respectively). In the high PFOS-PFOA group the median number of normal spermatozoa in the ejaculate was 6.2 million vs. 15.5 million in the low group (Figures 1 and 2).

When analyzing associations of semen variables to PFOS and PFOA separately, as well as the simple summed concentration of PFOS and PFOA, estimated changes in semen variables with a change in serum PFAA concentration of 1 ng/mL indicated negative but non-significant associations between the PFAAs and semen variables (Table 3).

PFAAs and reproductive hormones. There was no significant association between testosterone levels and PFAA levels, and no significant difference in PFAA levels between the high- and low-testosterone groups. For the whole group of 105 men, adjusted medians for the hormones point to a negative association to PFAA levels - however, none of these tendencies were statistically significant (Table 2).

Estimated associations between reproductive hormones and PFOS and PFOA separately and the summed concentration of PFOS and PFOA, showed no significant associations (Table 4).

Smoking and BMI. 35% of the 105 men were smokers, and there were more smokers in the high testosterone group compared to the low testosterone group (49% and 21% smokers, respectively, p = 0.003). Smoking status was significantly associated to higher testosterone, lower estradiol and higher SHBG when entered as a confounder in univariate analyses for these three variables only (p = 0.004 to 0.009). Smoking was not associated with any semen quality variables (p = 0.1 to 0.4), or levels of any PFAA (p = 0.1 to 1.0). Including smoking as a confounder did not considerably change the presented results or significance levels. Therefore, smoking was not included in the final analyses. BMI was not associated to levels of any PFAA (p = 0.1 to 1.0), nor was there any confounding effect on any semen variables (e.g. p = 0.5 for morphologically normal sperms, and p = 0.9 for total number of morphologically normal sperms in analyses for difference between high and low PFAA groups).

Discussion and conclusions

This study examined PFAA levels in young adults. High serum concentrations of PFAAs were significantly associated with reduced numbers of normal spermatozoa. In addition, sperm concentration, total sperm count and sperm motility showed some tendency towards lower levels in men with high PFAA levels, although not at a statistically significant level. A tendency toward lower inhibin B/FSH ratio with high PFAA levels was in agreement with these findings, as these hormones reflect the spermatogenetic activity.

Testosterone, FAI, testosterone/LH ratio and testosterone/estradiol ratio could suggest a poorer Leydig cell function in the "high" compared to the "low" PFAA quartile group. However, the associations between reproductive hormones and PFAAs were not completely consistent, and all were non-significant. This study therefore cannot demonstrate an adverse effect of PFAAs on Leydig cell function.

We singled out PFOS and PFOA, as there are no data that specifically support PFHxS as an endocrine disruptor, and divided the men into three groups from the combined concentrations of PFOS and PFOA to account for a potential different effect at same concentration levels. As we could find no significant association between testosterone levels and PFAA levels, and no significant difference in PFAA levels between high- and low-testosterone groups, we could analyze associations between PFOS-PFOA levels and reproductive hormones or semen variables for the whole group. Controlling for confounding effect of smoking or BMI did not change estimates or significance levels. To our knowledge, there have been no consistent reports of associations between PFAA levels and smoking or BMI.

Our material included only 105 men, and in addition, we had selected the men based on their serum testosterone values. The selection of two groups with high and low testosterone was done to test our primary hypothesis and therefore affects the homogeneity of the group when correlations are analyzed for the group as a whole. This could potentially influence the subsequent analysis of semen quality by bias or confounding, and may affect the general applicability of the results. A larger follow-up study should preferably include randomly selected men from the general population.

Our study is, to our knowledge, the first report of a correlation between semen quality and PFAAs. Very few studies of other endocrine disruptors (e.g. phthalates and pesticides) have previously demonstrated such an association (Duty et al. 2003; Hauser et

al. 2003; Hauser et al. 2007; Meeker et al. 2008; Swan et al. 2003). If the results from our preliminary study of an association between high levels of PFAAs and decreased number of normal sperms are confirmed, then high levels of PFAAs may be regarded as another endocrine disrupting factor contributing to the low semen quality seen among many young men. However, the importance of mixture effects of low-dose exposure to multiple compounds is becoming evident from animal studies but remains to be studied in humans (Hass et al. 2007; Kortenkamp et al. 2007).

The mode of action by PFAAs is not clear and only a few animal studies have explored mechanistic issues. These show decreased testosterone levels and reduced expression of steroidogenesis genes associated with Leydig cell hyperplasia in adult animals (Biegel et al. 1995; Shi et al. 2007), suggesting a direct testicular effect. Recent studies have indicated that the fetal gonad is particularly sensitive to exogenous factors (Skakkebæk et al. 2001). However, our results could indicate that exposures later in life may contribute to impairment of semen quality, in line with other recent studies (Hauser et al. 2007). We speculate that morphology is perhaps more susceptible to this than sperm concentration or total sperm count. Sperm morphology has proven an important indicator of semen quality and fertility in a clinical setting, even in men with normal sperm concentration (Guzick et al. 2001). Interestingly, a recent study showed higher levels of maternal PFOS and PFOA levels in early pregnancy was associated with significantly longer waiting time to pregnancy (Fei et al. 2009). We speculate that men and women living together may have similar exposure to PFAAs and that decreased semen quality caused by high PFAA levels may contribute to the longer waiting time to pregnancy found in that study.

The use and emission polyfluorinated compounds continue to increase, and they are not readily cleared from the environment (Jensen et al. 2008; Prevedouros et al. 2006).

Therefore, humans and wildlife worldwide will be exposed for years to come. We found positive correlations between levels of different PFAAs, as has been found previously (Apelberg et al. 2007; Calafat et al. 2007; Fei et al. 2007), suggesting common sources of exposure. The PFAAs levels we have detected are comparable to those found in other countries like Sweden and the Faroe Islands (Kärrman et al. 2007; Weihe et al. 2008), but lower than earlier results from Denmark from 1996-2002 (Fei et al. 2007). Thus, the effects we have indicated may also be true for other than the Danish population.

In conclusion, our results indicate that higher PFAA levels were associated with lower numbers of normal sperms. In addition, we found non-significant negative associations between PFAA levels and other semen variables and reproductive hormones. Thus, high levels of PFAAs may contribute to the otherwise unexplained low semen quality seen in many young men. However, results from this first and preliminary study should be corroborated in larger studies.

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Table 1

PFAA median concentrations (5-95th percentiles) for high and low testosterone groups and all study subjects (ng/mL), and p-values for difference between the two groups.

	Samples > LOD	High testosterone N=53	Low testosterone N=52	Whole group N=105	p-value
PFHxS	105	6.6 (4.0 - 13.0)	6.6 (3.5 - 12.1)	6.6 (4.0 - 12.1)	0.8
PFHpA	98	0.2 (0.01 - 0.9)	0.3 (0.00 - 1.3)	0.2 (0.00 - 1.1)	0.8
PFOA	105	4.4 (2.6 - 7.0)	5.0 (2.7 - 7.5)	4.9 (2.7 - 7. 2)	0.1
PFOS	105	25.5 (14.2 - 39.6)	23.9 (12.8 - 45.2)	24.5 (14.2 - 42.1)	0.4
PFOSA	56	0.1 (0.00 - 3.7)	0.00 (0.00 - 3.5)	0.06 (0.00 - 3.5)	0.008*
PFNA	105	0.8 (0.4 - 1.8)	0.8 (0.4 - 2.0)	0.8 (0.4 - 1.8)	0.6
PFDA	104	0.9 (0.2 - 1.1)	0.8 (0.4 - 1.2)	0.9 (0.3 - 1.1)	0.9
PFUnA	101	0.1 (0.04 - 0.3)	0.2 (0.00 - 0.4)	0.1 (0.02 - 0.4)	1.0
PFDoA	102	0.08 (0.04 - 0.8)	0.08 (0.02 - 0.8)	0.08 (0.04 - 0.8)	0.8
PFTrA	7	0.00 (0.000 - 0.4)	0.00 (0.00 - 0.06)	0.00 (0.00 - 0.2)	0.2

Table 2

Adjusted means (95% CI) for PFAA quartile groups, and p-value for difference between the low and high PFAA quartile groups.

Sex hormones[a]	Low PFAA	Intermediate PFAA	High PFAA	p-value
N	N = 29	N = 48	N = 28	
Testosterone (nmol/L)	25.2 (21.7 - 28.7)	22.3 (19.6 – 25.0)	22.3 (18.8 - 25.8)	0.2
Estradiol (pmol/L)	77.6 (70.3 - 85.8)	72.4 (67.0 – 84.7)	76.6 (69.3 - 84.7)	0.9
SHBG (nmol/L)	27.8 (24.0 - 32.2)	25.4 (22.6 – 28.5)	26.1 (22.5 - 30.3)	0.5
LH (IU/L)	3.4 (2.8 - 4.1)	2.9 (2.5 – 3.4)	3.7 (3.0 - 4.4)	0.6
FSH (IU/L)	2.7 (2.1 - 3.5)	2.7 (2.2 – 3-3)	3.0 (2.3 - 3.8)	0.6
Inhibin-B (pg/mL)	181 (142 - 232)	175 (144 - 212)	152 (119 - 195)	0.3
FAI	84.1 (74.1 - 95.5)	80.2 (72.6 – 88.7)	77.8 (68.5 - 88.3)	0.4
Testosterone/LH	6.9 (5.6 - 8.4)	7.0 (5.9 - 8.2)	5.5 (4.5 - 6.8)	0.1
FAI/LH	24.7 (19.9 - 30.7)	27.4 (23.1 – 32.5)	21.2 (17.1 - 26.4)	0.3
Estradiol/Testosterone	3.3 (3.0 - 3.7)	3.6 (3.2 – 3.9)	3.8 (3.4 - 4.2)	0.1
Inhibin/FSH	66.8 (42.1 - 106.0)	66.0 (45.9 – 94.8)	51.3 (32.3 - 81.4)	0.4
Semen quality[b]				
Volume (mL)	4.0 (3.2 - 5.0)	3.4 (2.9 – 4.1)	3.5 (2.9 - 4.4)	0.3
Concentration (mio./mL)	59 (36 - 96)	51 (35 – 74)	40 (25 - 64)	0.2
Total count (mio.)	228 (134 - 389)	172 (114 - 261)	143 (86 - 237)	0.1
Motile sperms (%)	73 (69 - 77)	70 (66 - 73)	71 (66 - 75)	0.4
Morphologically normal (%)	8.8 (7.2 - 10.4)	7.7 (6.4 – 9.0)	6.3 (4.6 - 8.0)	0.037*
Total morphologically normal (mio.)	15.5 (7.3 - 33.0)	10.0 (5.6 – 17.9)	6.23 (3.0 - 12.8)	0.030*

[a] Hormone levels are adjusted for time of blood sampling.

[b] Volume, concentration and total count are adjusted for duration of abstinence. Motility is adjusted for time between ejaculation and semen analysis. Morphology is not adjusted for confounders.

Table 3

Estimated change in semen variables[a] with a change in PFAA of 1 ng/mL (95% CI). All 105 men included.

	PFOS		PFOA		PFAA sum[b]	
ln Volume	0.000	(-0.012 - 0.011)	-0.002	(-0.070 - 0.066)	0.000	(-0.010 - 0.010)
ln Concentration	-0.020	(-0.044 - 0.005)	-0.080	(-0.230 - 0.066)	-0.018	(-0.040 - 0.004)
ln Total count	-0.018	(-0.045 - 0.010)	-0.074	(-0.230 - 0.086)	-0.016	(-0.041 - 0.008)
ln Motility	-0.006	(-0.019 - 0.007)	-0.027	(-0.110 - 0.053)	-0.006	(-0.018 - 0.007)
Morphology	-0.085	(-0.200 - 0.026)	-0.540	(-1.200 - 0.110)	-0.082	(-0.181 - 0.018)

[a] Volume, concentration and total count are adjusted for duration of abstinence. Motility is adjusted for time between ejaculation and semen analysis. Morphology is not adjusted for confounders.

[b] "PFAA sum" is PFOS and PFOA mass concentrations summed (ng/mL).

Table 4

Estimated change in reproductive hormones[a] with a change in PFAA concentration of 1 ng/mL (95% CI). All 105 men included.

	PFOS	PFOA	PFAA sum[b]
Testosterone	-0.087 (-0.32 - 0.15)	-0.98 (-2.33 - 0.37)	-0.093 (-0.303 - 0.116)
ln Estradiol	-0.001 (-0.008 - 0.005)	-0.012 (-0.051 - 0.027)	-0.001 (-0.007 - 0.005)
ln SHBG	0.002 (-0.007 - 0.012)	-0.009 (-0.067 - 0.048)	0.002 (-0.007 - 0.011)
ln LH	0.000 (-0.014 - 0.012)	-0.010 (-0.084 - 0.064)	0.000 (-0.012 - 0.010)
ln FSH	0.004 (-0.13 - 0.22)	-0.037 (-0.14 - 0.064)	0.003 (-0.013 - 0.018)
ln Inhibin-B	-0.004 (-0.21 - 0.12)	0.012 (-0.084 - 0.11)	-0.003 (-0.018 - 0.012)
ln FAI	-0.006 (-0.015 - 0.002)	-0.038 (-0.087 - 0.011)	-0.006 (-0.014 - 0.001)
ln Testosterone/LH	-0.003 (-0.017 - 0.011)	-0.037 (-0.12 - 0.045)	-0.003 (-0.016 - 0.009)
ln FAI/LH	-0.006 (-0.020 - 0.009)	-0.028 (-0.114 - 0.058)	-0.005 (-0.018 - 0.008)
ln Estradiol/ Testosterone	0.003 (-0.005 - 0.010)	0.035 (-0.010 - 0.081)	0.003 (-0.004 - 0.010)
ln Inhibin/FSH	-0.009 (-0.039 - 0.022)	0.049 (-0.13 - 0.23)	-0.006 (-0.034 - 0.022)

[a] Hormone levels are adjusted for time of blood sampling.

[b] "PFAA sum" is PFOS and PFOA mass concentrations summed (ng/mL).

Figure legends

Figure 1.

Morphologically normal spermatozoa (%) and PFAA quartile groups (adjusted means and 95% CI). All 105 men included.

* p = 0.037 for difference compared to Low PFAA quartile group.

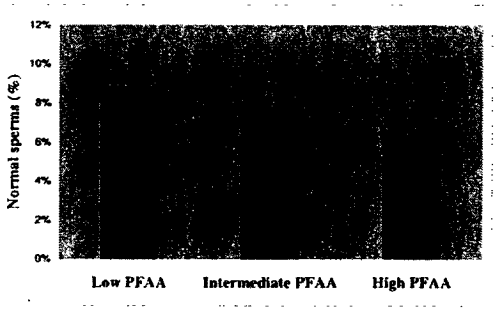
Figure 2.

Total morphologically normal spermatozoa (mio.)[a] and PFAA quartile groups (adjusted means and 95% CI). All 105 men included.

[a] Total morphologically normal spermatozoa is adjusted for duration of abstinence.

* p = 0.030 for difference compared to Low PFAA quartile group.

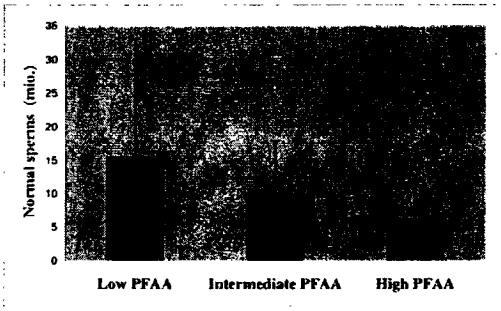
Figure 1. Morphologically normal spermatozoa (%).



Morphologically normal spermatozoa (%) and PFAA quartile groups (adjusted means and 95% CI). All 105 men included.

* p = 0.037 for difference compared to Low PFAA quartile group.
201x285mm (150 x 150 DPI)

Figure 2. Total morphologically normal spermatozoa (mio.).



Total morphologically normal spermatozoa (mio.)a and PFAA quartile groups (adjusted means and 95% CI). All 105 men included.

a Total morphologically normal spermatozoa is adjusted for duration of abstinence.
* p = 0.030 for difference compared to Low PFAA quartile group.
201x285mm (150 x 150 DPI)