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Office of Pollution Prevention and Toxics
US EPA
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RE: TSCA 8(e) ADDITIONAL INFORMATION SUBMISSION:
Docket No. 8EHQ-1200-14837

Dear Sirs:

In December of 2000, 3M submitted a draft report of a mortality study of 3M employees at a perfluorooctanesulfonyl fluoride (POSF)-based fluorochemical manufacturing facility under the above-referenced docket number. The final report of that study was submitted in September of 2001.

In April of 2005 we submitted a final report on a follow-up incidence study that was conducted to investigate the findings from the prior study.

We are now submitting a poster that was presented at the 44th annual meeting of the Society of Toxicology in March of 2005. The poster, Urinary Bladder Endpoints in Workers and Rats Exposed to Perfluoroocatanesulfonylfluoride (POSF) and Related Compounds, presents data from the incidence study mentioned above and a rat inhalation study[1] looking at urinary bladder endpoints. The data from these two studies indicate that the excess risk of bladder cancer initially reported was not associated with exposure to POSF-related substances.

Please contact John Butenhoff at 651-733-1962 should you have any questions or if we can provide additional information.

Sincerely,

TKF

Katherine E. Reed

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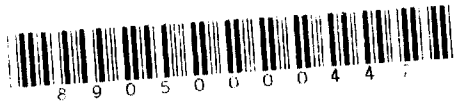
Staff Vice President
Environmental, Health and Safety Operations

Enclosure



[1] The submission regarding the rat study was submitted to EPA on July 1, 2004 as supplemental to 8EHQ-1180-373. The final report on that study has not been submitted.

Urinary Bladder Endpoints in Workers and Rats Exposed to Perfluorooctanesulfonyl Fluoride (POSF)

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Abstract

Workers with potential high exposure to POSF-related compounds, including perfluorooctanesulfonate (PFOS), had 3 bladder cancer deaths vs 0.2 expected (SMR 12.8; 95% CI 2.6 – 37.4). These compounds have not shown genotoxicity, and toxicology studies (including cancer bioassays) with PFOS and related sulfonamides have not shown bladder effects, but repeat-exposure data were not available for POSF. To further study the association of exposure to bladder cancer, we investigated potential bladder effects of POSF in rats and bladder cancer incidence in workers. Epidemiology: Because of its high survivorship, living members (N = 1895) of the study were contacted by mail to answer a questionnaire about bladder cancer, and 74% participated. Validation occurred by medical record or death certificate for those original members deceased (N = 188). Incidence of bladder cancer was compared to expected based on NCI SEER data. Results: 11 bladder cancers were identified. The Standardized Incidence Ratio (SIR) for the entire cohort was 1.3 (95% CI 0.6 – 2.3). The SIRs for ever working and working for more than one year in a high exposed job were 1.7 (95% CI 0.6 – 3.8) and 1.1 (95% CI 0.2 – 3.3), respectively. Rats were exposed by inhalation to POSF 6 hrs/day, 5 days/week for 1 and 4 weeks (0 & 300 ppm) and 13 weeks (0, 30, 100, & 300 ppm). Complete urinalysis was done on overnight urines. SEM/X-ray element analysis for crystals/calculi and immediate pH were conducted on fresh morning urines. Sagittal sections of urinary bladder were taken for SEM analysis and assessment of cell proliferation by proliferating cell nuclear antigen (3 sections, 10^3 cells/section). Results: There were no treatment-related findings with respect to urinalysis parameters, including pH and solids, nor changes of the urothelium, including no exposure-related increase in cell proliferation. These studies do not support the hypothesis that the excess risk of bladder cancer initially reported was due to exposure to POSF-related materials or PFOS.

Introduction & Objective

Introduction: The 3M facility in Decatur, Alabama was one of two major production sites of perfluorooctanesulfonyl fluoride (POSF, $C_8F_{17}SO_2F$) based specialty chemicals. These specialty chemicals have a wide range of applications, including surface treatments, paper and packaging protectants, and performance chemicals.[1] POSF based chemicals can degrade or be metabolized to perfluorooctanesulfonate (PFOS, $C_8F_{17}SO_3^-$). A cohort mortality study of current and former employees of the 3M Decatur facility was conducted to evaluate the health of workers exposed to POSF based fluorochemicals.[2] The cohort included 2083 workers who were employed for a minimum of 1 year at the Decatur facility. The standardized mortality ratio (SMR) for bladder cancer for the entire workforce was 4.8 (95% CI=1.0-14.1), and the SMR for employees who held high exposure jobs was 12.8 (95% CI=2.6-37.4). However, these results were based on only three cases. A review of the current and past use of known or suspected bladder carcinogens at this facility could not attribute exposure to a known bladder carcinogen. Due to the large difference between incidence and mortality rates for bladder cancer, a mortality study will not fully ascertain the burden of bladder cancer in a population.

Objective: The goal of the studies reported herein was to determine whether the association observed in the mortality study was representative of the entire bladder cancer experience of the employees at the 3M Decatur facility. This was accomplished through a case finding effort using direct contact with the population. In addition, bladder and urinalysis endpoints potentially related to the etiology of bladder cancer were evaluated in rats exposed to POSF by inhalation.

Methods (1)

Study Population

The 3M facility in Decatur, Alabama is divided in two major plants. The chemical plant produces specialty chemicals, including the POSF line of chemicals. The other is the film plant, where a variety of films are produced, but little or no occupational fluorochemical exposures occur. The population of interest included all current, retired, and former Decatur employees who were eligible for the original cohort mortality study. All current employees who were not part of the original cohort, those hired after January 1, 1998, were also included in the case finding exercise, but not a primary focus of the analysis as their exposure had occurred so recently that its contribution to bladder cancer is unlikely. Address information was updated through a variety of tracing resources sources available to the University of Minnesota, including TRW/Experian, Lortan Data, and National Change of Address. If a cohort member was found to have died since the mortality study was completed a copy of the death certificate was obtained from the state of record. Recruitment of all presumed living cohort members was initiated with a letter and brochure that outlined the reasons for the study, the scope of the study, what is required of participants, and assurances of confidentiality. The study questionnaire followed the recruitment letter by approximately one week. The primary purpose of the questionnaire was to identify cases of bladder cancer and the year of diagnosis. The questionnaire also recorded history of smoking; a known risk factor for bladder cancer. Non-respondents were contacted by mail and telephone. Participants reporting a bladder cancer diagnosis were contacted by letter, with telephone follow-up, to request permission to contact their physician to verify the diagnosis.

Methods (2)

Exposure Assessment

The exposure assessment followed the previously described method used in the mortality study.[3] As part of this exposure assessment, serum PFOS concentrations were measured at geometric mean serum PFOS levels (95% confidence interval) for chemical plant employees at 0.94 ppm (ug/mL) (95% CI=0.78-1.13) and for the adjacent film plant employees at 0.14 ppm (95% CI=0.11-0.16). The exposure to film plant employees is thought to be influenced by enviornmental exposure from proximity to the chemical plant. For chemical plant jobs the highest geometric mean level of serum PFOS was observed in cell operators (2.0 ppm) followed by the waste operators (1.5 ppm), chemical operators (1.5 ppm), and maintenance workers (1.3 ppm). Supervisors/managers (0.9ppm), mill operators (0.6 ppm), engineer/lab workers (0.4 ppm) and administrative assistants (0.4 ppm) had lower geometric mean serum PFOS levels.

With the knowledge of the major job-specific serum PFOS levels,[2,3] each unique job and department combination by calendar time period in the work history records were categorized into one of the following three major exposures:

1. No direct workplace exposure to POSF-based fluorochemicals (encompasses film plant jobs).
2. Low potential workplace exposure to POSF-based fluorochemicals (includes such jobs as engineers, quality control technicians, environmental, health and safety workers, administrative assistants and managers).
3. High potential workplace exposure to POSF-based fluorochemicals (includes cell operators, chemical operators, maintenance workers, mill operators, waste operators and crew supervisors).

These three categories will be referred to as the non-exposed, low exposed and high exposure. An additional classification for cumulative exposure assigned the non-exposed, low exposure, and high exposure jobs relative POSF-based job exposure value of 1, 3 and 10 respectively based on biomonitoring data. The years spent in each job were multiplied by the relative weights to develop a cumulative quantitative exposure metric.

Methods (3)

This epidemiology study was approved by the University of Minnesota Institutional Review Board.

Data Analysis

The incidence of bladder cancer in the study population was compared to the expected incidence based on rates derived from the Surveillance Epidemiology and End Results (SEER) at the National Cancer Institute.[4] The age, gender, calendar-year, and exposure-specific person-time of the cohort was tabulated using the Life Table Analysis System for the personal computer developed by the NIOSH.[5] Estimates of bladder cancer risk by relative cumulative exposure using the study population as an internal referent were made using Poisson and logistic regression. The analysis also accounted for the time-dependent nature of the PFOS exposure, as participants accrued PFOS exposure at different levels over the course of employment.

Results

Of the 2,083 original members of the cohort, 188 were determined to be deceased at the time the questionnaire was sent and 1,400 (74%) responded to the questionnaire. The remaining 495 did not respond either because they declined to participate or were not reachable.

Eleven bladder cancer cases were identified. Five of the cases were identified on the death certificates and 6 were reported on the questionnaire. The diagnosis of the self reported bladder cancers were confirmed for two cases, but four self-reported cases declined to sign the consent forms permitting validation. Two of the bladder cancer cases were women and all were over 50 years of age at the time of diagnosis or death. (Table 1)

There was no clear association between employment in PFOS exposed jobs and bladder cancer (Table 2). Similar results were observed for the time-dependent exposure analysis (Table 3).

An additional 2 cases of bladder cancer would be expected in the non-respondents based on the SEER data. The potentially missed cases in the non-respondents would only affect the results if the rate in the non-respondents was higher than the prevailing rates in the population. A sensitivity analysis showed that in order to reach nominal statistical significance at the $p < .05$ and $p < .01$ levels, the rate of bladder cancer would need to be 2.6 and 3.6 times greater than the rate of the reference population, respectively. Therefore the contribution to the risk estimate from the non-respondents would be marginal unless there were substantially higher bladder cancer rates in the non-respondent group than referent population.

Table 1

Demographic and exposure characteristics of bladder cancer cases and non-cases

		Bladder N	%	No N	%	All N
Total		11		1577		1588
Vital status	Alive	6	54.5	1394	88.4	1400
	Deceased	5	45.5	183	11.6	188
Gender	M	9	81.8	1302	82.6	1311
	F	2	18.2	275	17.5	277
Age at end of study	<30	0	0	13	0.8	13
	30-39	0	0	86	5.5	86
	40-49	0	0	334	21.2	334
	50-59	4	36.4	[illegible]	[illegible]	[illegible]
	60-69	5	45.5	[illegible]	[illegible]	[illegible]
	70+	2	18.2	[illegible]	[illegible]	[illegible]
Tobacco use*	Cigarettes	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
	Missing	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
PFOS Exposure Never		2	[illegible]	[illegible]	[illegible]	[illegible]
Low	Ever	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
	≥1 year	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
High	Ever	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
	≥1 year	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
Low or High	Ever	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
	≥1 year	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]

*[illegible]

Table 2

Standardized Incidence Ratios by Exposure Category based on expected bladder cancer Rates in US population (SEER)

	Obs	Exp	SIR	95% CI
All	11	8.4	1.30	[illegible]
Men	9	[illegible]	[illegible]	[illegible]
Women	2	[illegible]	[illegible]	[illegible]
Never exposed	2	[illegible]	[illegible]	[illegible]
Ever high	6	[illegible]	[illegible]	[illegible]
Ever low	7	[illegible]	[illegible]	[illegible]
Ever low or high	9	[illegible]	[illegible]	[illegible]
High ≥ 1 year	4	[illegible]	[illegible]	[illegible]
High or low ≥ 1 year	6	[illegible]	[illegible]	[illegible]
Weighted Exposure **				
[illegible]	2	[illegible]	[illegible]	[illegible]
[illegible]	4	[illegible]	[illegible]	[illegible]
[illegible]	3	[illegible]	[illegible]	[illegible]
[illegible]	2	[illegible]	[illegible]	[illegible]

* [illegible]

** [illegible]

Table 3

Estimated bladder cancer rates and rate ratios using the cohort as an internal referent population

	Cases	Person years	Rate	RR*	95% CI
Total	11	[illegible]	[illegible]	-	-
Weighted Exposure **					
[illegible]	2	[illegible]	[illegible]	1.0	-
[illegible]	4	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	3	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	2	[illegible]	[illegible]	[illegible]	[illegible]

* Adjusted for age and gender

** [illegible]

Inhalation Study: Experimental Conditions

Exposure

Male and female Sprague Dawley rats (Crl:CD (SD)IGS BR, Charles River (UK)) were exposed to POSF vapor by inhalation (nose-only) for 1, 4, and 13 weeks at concentrations up to 300 ppm in air by volume. Commercial grade perfluorooctane sulfonyl fluoride (POSF, FX-8-B, >99.5% purity) was supplied by 3M Company, Specialty Materials Manufacturing Division, Antwerp, Belgium. Impurities included perfluorooctanoic acid (PFOA) at 269 µg/g.

For 5 days prior to exposure, rats were acclimated to restraints and the exposure system by exposure to air only, the exposure period being increased progressively on each day (0.5, 1, 2, 4, and 6 hours). To generate exposure atmospheres, liquid POSF was metered from an infusion pump to an all glass vaporizer and the resulting atmosphere was mixed into a stream of air and passed directly into the exposure chamber (ADG snout-only exposure chambers, ADG Developments Ltd., Hitchin, Hertfordshire, England). Exposure atmospheres were monitored by fourier transformed infrared spectrometry (FTIR) and nominal exposures were calculated from airflow and delivered volume of POSF. Control groups were exposed to air only. A 5-day (6 hours/day) inhalation study was initially conducted in which rats (male and female, 5 per sex per group) were exposed to either air alone or 300 ppm POSF. Following completion of the 5-day study, rats (male and female, 5 per sex per group) were exposed 6 hrs/day, 5 days/week, to 0 & 300 ppm for 4 weeks, and 0, 30, 100, & 300 ppm for 13 weeks.

Urinalysis

Overnight urine samples were collected from all rats on study during exposure Week 13 and recovery Week 5. From approximately 16:00 to 16:30 hours, rats were placed in individual metabolism cages without food and water until approximately 07:30 to 07:45 the following morning. Individual urine samples were examined for appearance, volume, pH, specific gravity, protein, sodium, potassium, and chloride. Glucose, ketones, bile pigments, and heme pigments were qualitatively determined by Multistix (Bayer plc, Newbury, England). Heme pigments were reported as negative or positive for occurrence; whereas, glucose, ketones, and bile pigments were reported as negative, trace, or small amount in occurrence. Urine sediment was examined microscopically after centrifuging a sample of urine and spreading the deposit on a microscope slide. The deposit was examined for crystals, epithelial cells, leukocytes, erythrocytes, casts, spermatozoa and precursors, and any other abnormal components. Cell frequencies of occurrence in deposits were graded as none found, few in some fields, and few in all fields examined.

Urine SEM X-Ray Analysis

Fresh individual urine samples were collected from rats within 2 hours of lights on in the animal holding room when possible, or at necropsy from the urinary bladder. These samples were immediately assayed for pH using a microelectrode. Scanning electron microscopy (SEM) stubs were prepared and analysis for calculi and crystals by SEM X-ray element analysis.

Tissue Microscopic Analysis

Kidneys (including pelvic epithelium in the section) and urinary bladder (sections taken sagitally, one half used for SEM analysis and the other half for light microscopy and cell proliferation analysis) were prepared for microscopic analysis. Tissue samples for light microscopy were dehydrated, embedded in paraffin wax, sectioned at 4 to 5 microns thickness, and stained with hematoxylin and eosin.

Cell Proliferation Analysis

Sections of urinary bladder were taken from all rats for proliferating cell nuclear antigen (PCNA) immunostaining in order to assess the degree of cell proliferation. A total of 3,000 cells were counted from 3 separate sections (1,000 cells/section) in order to determine the cell proliferation index. Positively stained cells were counted, and examined by the pathologist to assess the sections and count S-phase positive cells, if practicle. Sections of duodenum from each rat were taken and stained to act as positive controls for the PCNA immunostaining method.

Collection of Samples for Pharmacokinetic Analysis

Blood (for serum) was obtained at sacrifice by cardiac/aorta puncture while under terminal sodium phenobarbitone anesthesia. Up to 4 mL of blood was run into tubes, allowed to clot at room temperature, and the serum was separated and frozen until analyses for POSF, PFOS, and perfluorooctanoate (PFOA).

Samples of urine remaining after urinalysis parameters assay as well as urine collected from rats at week 4 were frozen until analyses for POSF, PFOS, and PFOA.

Inhalation Study: Results

Exposure Atmosphere Generation

Overall mean chamber concentrations (± SD) achieved were 26 ± 4.3, 104 ± 6.6, and 299 ± 15.9 ppm by volume; thus, the mean concentrations achieved were close to target concentrations of 30, 100, and 300 ppm.

Urinalysis, SEM and X-Ray Analysis of Urine and Bladder Tissue, and Cell Proliferation in the Urothelium

No treatment-related differences from control in urinalysis parameters (including urine pH), microscopic effects noted for the kidney or urinary bladder were noted.

No treatment related abnormalities of the urothelial tissue were observed, and based on classification methodology[6] for subtle changes of the rat urinary bladder tissue, all bladder tissues would be classified as 1 or 2, indicating normal tissue. No abnormal urinary solids were evident in scanning electron micrographs of urinary sediment.

The percent of cells staining for PCNA (cell proliferation index) in the male and female urothelial tissue was not increased at the highest exposure concentration, 300 ppm, after 1 and 4 weeks exposure (Table 4).

Table 4. Percent Positive Cells (Mean ± SD, % Cells Staining for PCNA)

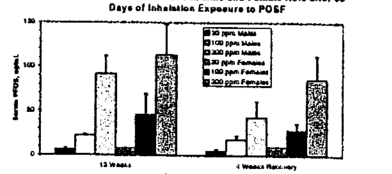
POSF Exposure (ppm by volume)	Exposure Duration: 1 Week M	1 Week F	4 Weeks M	4 Weeks F
0	2.0 ± 2.0	0.5 ± 0.3	4.0 ± 1.6	4.8 ± 1.9
300	3.9 ± 2.9	0.5 ± 0.6	4.4 ± 1.8	4.8 ± 0.7

Test Substance and Metabolite Analysis

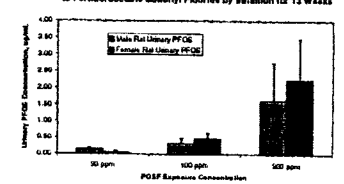
Analysis of serum and urine samples revealed the presence of PFOS and PFOA, but not POSF, suggesting hydrolytic conversion of POSF to PFOS and uptake of residual PFOA in the sample. Serum and urinary concentrations of PFOS were proportional to dose in exposed rats (Figures 1 and 2, respectively).

PFOA was present in male serum at concentration up to approximately 10 ug/mL after 13 weeks in the 300 ppm dose group. In addition, traces of PFOA and PFOS were observed in control rats, possibly from environmental and/or food sources.

Serum PFOS Concentrations in Male and Female Rats after 90 Days of Inhalation Exposure to POSF



Urinary Concentrations of PFOS in Male and Female Rats Exposed to Perfluorooctane Sulfonyl Fluoride by Inhalation for 13 Weeks



Discussion & Conclusion

The primary objective of this study was to evaluate whether the finding from the mortality study of a twelve-fold risk of risk of bladder cancer associated with ever working in a high PFOS exposed job was representative of the overall bladder cancer experience of the cohort. Overall, the results of this study do not confirm this risk level. However, the possibility remains for a smaller risk (approximately 1.5 to 2 fold) in the higher exposed workers. Bladder cancer is a relatively rare disease, and the population occupationally exposed to these compounds is limited; thus the power to detect excess risks in this study is inherently low and prohibited a convincing exposure response analysis. Future follow-up of this cohort may be limited to mortality studies, which will need to consider these results when interpreting any findings on bladder cancer.

The lack of an increase in the percent cells staining for PCNA after 1 and 4 weeks exposure to POSF, the presence of normal urine parameters, and the lack of abnormalities in SEM analysis of urothelium and urine sediments, all strongly indicate that POSF exposure does not produce effects on the urinary tract, including kidneys and bladder at exposures up to 300 ppm by volume. This is further supported by the lack of treatment-related microscopic in bladder tissue of Sprague Dawley rats exposed to PFOS[7] (the hydrolysis product of POSF), and PFOA[8,9] (a manufacturing residual of of POSF) for up to 2 years in their diet.

References

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