

Internal Correspondence

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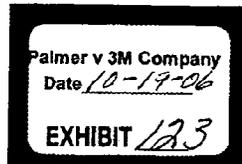
TO: Fluorochemical Steering Committee Members
FROM: Jeff Mandel, M.D., 333-8670 (220-3W-05)
SUBJECT: Papers/Dr. Gilliland
DATE: December 7, 1993

These are two additional papers that are being prepared by Dr. Gilliland. They need our input/approval before being submitted for publication.

The papers are negative for the most part. We're working with him regarding some of the wording. We're operating under the premise that these sorts of topics need 3M support.

Please send your comments back to me by the end of the month so I can communicate with Dr. Gilliland.

JHM/vk



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CONFIDENTIAL - SUBJECT TO A PROTECTIVE ORDER ENTERED IN HENNEPIN COUNTY DISTRICT COURT, NO. 27-CV-10-28862

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**Peripheral Blood Lymphocyte Count in Men Occupationally Exposed to
Perfluorooctanoic Acid**

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Medical Department**

Running title: Lymphocyte count and PFOA

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ABSTRACT

Studies in Rhesus monkeys suggest that perfluorooctanoic acid (PFOA) has immunotoxic effects on the cell-mediated immune system in primates. The predominant histopathological lesion in PFOA-treated monkeys was diffuse atrophy of lymph nodes and splenic germinal centers. Although PFOA accounts for the majority of fluorine present in the serum of the general population and in occupationally exposed workers, little information is available concerning human responses to PFOA exposure. To assess whether PFOA exposure is associated with effects on the human cell-mediated immune system, we examined the cross-sectional associations between peripheral blood lymphocyte counts and PFOA in 115 workers employed at a PFOA production plant. Total serum fluorine was used as a surrogate measure for serum PFOA levels. Peripheral blood lymphocyte count was significantly associated with total serum fluoride level; however, the magnitude and direction of the relationship was dependent on smoking, alcohol use, and obesity status. For example, for non-smokers and moderate drinkers, an increase of 10 ppm in total serum fluoride was associated with a decrease in lymphocyte count of 1640 cells in non-obese ($BMI=25 \text{ kg/m}^2$) workers and by 925 cell in obese workers ($BMI=35 \text{ kg/m}^2$). PFOA is associated with alterations in peripheral blood lymphocyte numbers in PFOA production workers, suggesting that cell-mediated immunity may be affected by PFOA.

KEY WORDS perfluorooctanoic acid, epidemiology, immune toxicity, lymphocyte count

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INTRODUCTION

Perfluorooctanoic acid (PFOA) is widely used in industrial processes and consumer products as a result of its unique chemical properties and potent surface activity (Griffith, 1980). Because PFOA has a long biological half-life, small frequent doses can accumulate to appreciable levels (Ubel, 1980). As a result, PFOA has been found in the serum of all human population studied, and accounts for the majority of fluorine present in the serum of populations in industrialized countries¹⁻⁷.

Little is known about the toxic potential of PFOA in humans; however, studies have suggested that the cell-mediated immune system may be a site of toxicity in primates. Rhesus monkeys treated with oral PFOA developed histologic changes in spleen and lymph nodes. The primary histopathologic lesion was atrophy in lymph node and splenic germinal centers¹. The immune system of rodent species has not been reported to undergo similar histopathologic changes in subacute and chronic feeding studies¹. No data are available concerning immunotoxicity in humans. Because PFOA is present in the serum of exposed workers and in the general population³⁻⁷, it is a matter of concern whether the findings in monkeys indicate the potential for human immunotoxicity. To assess whether PFOA could affect the cell-mediated immune system in humans, we studied the association of PFOA, as measured by total serum fluorine, with peripheral blood lymphocyte count (PBL) in 115 occupationally exposed employees at a plant that produces PFOA.

MATERIALS AND METHODS

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All current workers employed in PFOA production over the 5 previous years and a sample of workers in jobs with no apparent PFOA exposure for the previous 5 years were invited to participate. Participants had vital parameters measured in the plant medical department by an occupational health nurse, completed a medical history questionnaire, and underwent venipuncture. Blood was drawn for assays of peripheral lymphocyte count. A fluorine-free 15 ml vacutainer was used to collect blood for total serum fluorine determination. Total serum fluorine was used as the measure of PFOA in this occupational group. Total serum fluorine was determined using the sodium biphenyl extraction and atomic absorption spectrometry⁸.

Pearson correlation coefficients were calculated to assess the univariate associations between PBL and age, body mass index (BMI), cigarette use, and alcohol use. Linear multivariate regression models were fit to estimate the associations of PBL count with PFOA, adjusted for age, BMI, cigarettes use, and alcohol use. Two way interactions between total serum fluorine and the four covariates were evaluated using residual analysis, model fit, and tests of significance. Interaction terms were included in the final model if biologically plausible and if the parameter estimate for the interaction term was significant at the $\alpha = .10$ level.

RESULTS

Participant characteristics are displayed in Table 1. Total serum fluorine values ranged from 0 and 26 ppm with a mean of 3.3 ppm. Twenty-three

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(20.0%) participants had serum values less than 1 ppm, 6 (5.2%) had values between 10 and 15 ppm, and 5 (4.4%) had values greater than 15 ppm.

Table 2 presents the correlation coefficients between PBL and total serum fluorine, age, BMI, alcohol use, and cigarette consumption. Peripheral blood lymphocyte count was significantly correlated with total serum fluorine ($r=.19$, $p=.04$). The final regression model for PBL, which adjusted for age, BMI, cigarette use, and alcohol use, included data for 111 workers; 4 of the 115 workers had missing assay values and were excluded from the analysis (Table 3). Total serum fluorine was inversely associated with PBL; however, the relationship was complex, with significant interactions between total serum fluorine and BMI, cigarette use, and alcohol use. Table 4 illustrates the relationship for a 10 ppm change in total serum fluorine for combinations of smoking, alcohol consumption, and BMI status. In moderate drinkers (1-3oz alcohol per day), PBL decreased in the categories of smoking and of obesity; in light drinkers (<1oz per day), PBL decreased in non-obese smokers only.

Discussion

Serum PFOA level is associated with changes in the cell-mediated immune system, as evidenced by changes in PBL; however, the relationship depends upon smoking, alcohol use, and obesity. PFOA could modulate cell counts by altering the known effects of smoking, alcohol consumption, and adiposity on peripheral leukocyte counts (refs). No human studies of the effect of PFOA on the immune system are available for comparison; however, a study of this group of workers suggests that PFOA may modify the hepatic response to

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alcohol and obesity. Modulation of endobiotic and xenobiotic metabolism could represent a common mechanism for the observed association of PFOA with alterations in hepatic and immune response.

The relationship between the changes in PBL in humans and the lymph node and splenic germinal center atrophy observed in monkeys is not clear. Changes in PBL may be unrelated to germinal center atrophy. Alternatively, small changes in PBL could reflect larger changes in T cell subsets. In addition, the modification of the relationship between smoking and PBL by PFOA could be the result of changes in the number of particular T cell subsets. Furthermore, PFOA may be associated with changes in immune function beyond simple changes in cell number. Cytokine signaling is important in immune function and could be altered by PFOA exposure ²⁷. The response to antigen binding depends upon rearrangement of membrane proteins. Changes in the membrane physical characteristics produced by the potent surfactant action of PFOA could alter immune responses.

Interpretation of the findings requires careful consideration of the study limitations. Given the occupational study setting, the voluntary participation, and the requirements for blood sample collection, the overall participation was unexpectedly high, and non-response bias is likely to be small. Workers not included may have had a different response pattern than those who were included. Migration out of the high exposure jobs is unlikely to be the result of subclinical changes in PBL counts. The vast majority of workers who had significant exposure over the previous 5 years would be included in the study sample as the turn-over rate in plant employees was low (3% per year) and the study included all current employees with appropriate

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job histories. Selection bias is not a likely explanation for the findings in this study.

Total serum fluorine was used as a surrogate variable for PFOA exposure. The use of total serum fluorine has been validated in past biological monitoring in the plant and other plants using PFOA². Approximately 90% of total serum fluorine in workers was reported to be in the form of PFOA^{2,8}. Total serum fluorine is likely to be highly correlated with serum PFOA in this cohort. The coefficient of variation for total serum fluorine was 66%. At the low end of the spectrum (< 1 ppm), where the assay is limited by sensitivity, the total serum fluorine values may overestimate the true value. The measurement errors are likely to lead to an underestimate of the effect of PFOA on the physiologic endpoints. The duration of exposure may be an important determinant of PFOA level and effect; however, information on the duration of employment in exposed jobs was not available, because plant records did not contain sufficient information to reconstruct exposures.

Inflammatory and infectious processes, which are major determinants of PBL, were not assessed in this study. Because there is no evidence that these processes are related to total serum fluorine or serum PFOA, they are unlikely to confound the estimated relationships.

The changes in PBL counts associated with PFOA exposure present a complex picture. Alcohol use, cigarette use, and BMI modified the association of cell count with PFOA. The magnitude of these associations is not clinically significant from an infectious disease perspective; however, judgment as to the clinical relevance of such changes must await further study. More

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research is needed in the area of PFOA immunotoxicity. The findings of the present study need to be confirmed. Changes in T cell subsets could be confirmed by immunophenotyping lymphocytes using well established flow cytometry methods ^{28, 29}. In addition, the standard immunotoxicologic assessment defined by the National Toxicology Program ³⁰ needs to be conducted for PFOA.

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Table I Participants characteristics

	<i>mean</i>	<i>range</i>
total fluorine (ppm)	3.3	0-26
age (years)	39.2	24-59
BMI (kg/m ²)	26.9	18.8-40.5
	<i>number</i>	<i>percent</i>
Alcohol use		
<1 ounce per day	87	75.6
1-3 ounces per day	20	17.4
>3 ounces per day	0	0.0
non-response	8	7.0
Cigarettes use		
non-smoker	85	73.9
current smoker	28	24.4
non-response	2	1.7

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TABLE2 Pearson correlation coefficients between total serum fluoride, age, body mass index (BMI), daily alcohol use, daily tobacco consumption, and peripheral blood lymphocyte count

	Total fluorine (ppm)	Age (years)	BMI (kg/m ²)	Alcohol (oz/day)	Tobacco (cigs/day)
LYMPHOCYTES	.19 p=.04	-.05	.04	.15	.28 p=.002

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TABLE 3 Linear multivariate regression model of factors predicting the lymphocyte count among ill workers.

Variable	β	SE(β)	p-value
Intercept	2205.6	611.1	.0005
Total Fluorine (ppm)	-342.7	125.3	.007
Alcohol *			
low (<1oz/day)	-326.6	222.7	.02
nonresponse (NR)	-977.1	355.7	.007
low X Fluorine	189.0	52.3	.0005
NR X Fluorine	247.9	103.9	.02
Cigarettes/day	34.0	6.9	.0001
Cigs/day X Fluorine**	-3.3	1.45	.02
BMI (kg/m ²)	1.58	19.6	.94
BMI X Fluorine**	7.15	4.1	.08

R² = .35

#Reference category is drinkers who consumed 1-3 oz ethanol/day.

**interaction terms alcohol category by total fluoride; cigarettes/day by total fluoride, BMI by total fluoride.

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Table 4 Change in lymphocyte count for 10 ppm increase in total serum fluorine

< 1 oz. alcohol/day		
	Non-smoker	Smoker (20 cigs/day)
BMI 25 mg/Kg²	+750	-406
BMI 35 mg/Kg²	+965	+306
1-3 oz. of alcohol/day		
	Non-smoker	Smoker (20 cigs/day)
BMI 25 mg/Kg²	-1640	-2300
BMI 35 mg/Kg²	-924	-1585

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