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February 11, 2005

E-MAIL AND FEDERAL EXPRESS

Dr. Sue Shallal
U.S. Environmental Protection Agency
EPA Science Advisory Board Staff (1400F)
1200 Pennsylvania Avenue, N.W.
Washington, DC 20460

Re: Science Advisory Board Perfluorooctanoic Acid Risk Assessment (PFOA)
Review Panel: Comments And Additional Information For Review Prior To
February 22-23, 2005 Public Meeting

Dear Dr. Shallal:

We serve as class counsel for a class of tens of thousands of individuals who have consumed or are consuming PFOA-contaminated drinking water in the West Virginia and Ohio communities near a manufacturing facility owned by E.I. duPont de Nemours and Company in Wood County, West Virginia, where DuPont has used PFOA since the 1950s (the "DuPont Plant"). On behalf of those individuals, we are hereby submitting information for consideration by the Science Advisory Board Perfluorooctanoic Acid Risk Assessment (PFOA) Review Panel (the "Panel") in connection with its upcoming review of USEPA's January 4, 2005, "Draft Risk Assessment Of The Potential Human Healths Effects Associated With Exposure To Perfluorooctanoic Acid And Its Salts" (the "Draft Risk Assessment").

According to the Federal Register Notice published January 12, 2005, we understand that written comments on the Draft Risk Assessment will be accepted until the public meeting on February 22-23, 2005. However, during the January 25, 2005, public teleconference to discuss the agenda for the upcoming meeting, including "additional information needs" of the Panel, several Panel members asked about the ability of the Panel to consider information not

referenced in the current Draft Risk Assessment, including new information that may have been generated or otherwise became available after the date USEPA completed its current draft. (In that regard, we note that the current Draft Risk Assessment indicates that it is "based on information available to the agency as of June 2004." (OPPT-2003-0012-839)) We understand that USEPA agreed that the Panel could consider such additional information. Thus, although we reserve the right to submit additional, more detailed written comments before the February 22-23, 2005, meeting, we thought it might be helpful to identify for the Panel some of the relevant information that is available but not referenced within the current Draft Risk Assessment. We have provided a brief summary of some of the data below, and have attached copies of some of the available documents to assist the Panel's review of the information before the public meeting later this month. Citations also are provided, where available, to indicate where the documents (and related information) can be located within either of two public dockets created by USEPA relating to PFOA: AR-226 and OPPT-2003-0012.

I. ADDITIONAL HUMAN HEALTH EFFECTS/EPIDEMIOLOGY DATA IS AVAILABLE.

- In addition to the findings of elevated cholesterol and triglyceride levels among 3M PFOA workers referenced in the Draft Risk Assessment, (Draft Risk Assessment, at 13-21), there have now been similar findings reported among both Italian PFOA workers, (*see* Exhibit 1; AR-226-1865-69, 1886; and OPPT-2003-0012-813-817), and among the PFOA workers at the DuPont Plant in West Virginia (*see* Exhibit 2; AR-226-1899). This data became available after the June 2004 date referenced in EPA's Draft Risk Assessment and should be considered for purposes of the Draft Risk Assessment. Because of these cholesterol findings, the Panel also should consider the results of the much earlier research DuPont conducted on myocardial infarction ("MI") and coronary heart disease ("CHD") rates among workers at the DuPont Plant in West Virginia, (*see, e.g.*, Exhibit 3; AR-226-1448, 1462, 1465, 1495-96), along with the results of a recent survey of health conditions among residents of the communities surrounding the DuPont Plant, in which significantly elevated incidences of angina, MI, and stroke (among other conditions) were reported among the community residents exposed to PFOA in their drinking water (*see* Exhibit 7^{1/}). The earlier MI and CHD data for the DuPont Plant in West Virginia should also be reevaluated in light of the data

^{1/} Although this new community study data was recently submitted to USEPA for inclusion in the USEPA's public dockets for PFOA, it had not yet been posted to the dockets at the time of this submission.

recently released by DuPont (referenced below) indicating that all of the workers at the DuPont Plant, including the workers that DuPont considered "unexposed" or "controls" for purposes of its earlier, internal MI and CHD studies, most likely were also significantly exposed to PFOA. (*See, e.g., Exhibit 13*)

- With respect to liver effects, the Draft Risk Assessment references the 3M PFOA worker studies in which the authors suggest negative findings with respect to liver effects. (Draft Risk Assessment, at 13-21). There is, however, a paper available in the EPA public docket (but not referenced in the current Draft Risk Assessment) that raises questions about the accuracy of those findings and concludes that liver effects are, in fact, indicated by the data. (*See Exhibit 4; OPPT-2003-0012-249/250*) The Draft Risk Assessment also does not reference the much earlier research by DuPont that investigated liver enzyme levels among its workers at the DuPont Plant in West Virginia. (*See, e.g., Exhibit 5; AR-226-1457/1462*) This data also should be reevaluated in light of the data recently released by DuPont (referenced below) indicating that all of the workers at the DuPont Plant, including those that DuPont considered "unexposed" or "controls" for purposes of its earlier, internal liver studies, most likely were also significantly exposed to PFOA. (*See, e.g., Exhibit 13*)
- There is no reference in EPA's Draft Risk Assessment to the reproductive issues and elevated incidence of cancer reported in a recent study of hundreds of people living in the communities near the DuPont Plant in West Virginia where they are exposed to PFOA-contaminated drinking water. (*See, e.g., Exhibit 6; AR-226-1714-1716; and OPPT-2003-0012-607/677/836*) More detailed data from this study has become available since June of 2004, including an analysis of certain types of cancer claims submitted by workers at the DuPont Plant, including prostate cancer claims, which should be considered by the Panel in connection with the Draft Risk Assessment and its current discussion of prostate cancer findings among 3M PFOA workers. (*See, e.g., Exhibit 7; OPPT-2003-0012-836; and AR-226-1893/4*) In addition, the reproductive health issues raised by male employees at the DuPont Plant in West Virginia in 1984 may be of interest, although not referenced in the current Draft Risk Assessment. (*See Exhibit 8*)
- There is no reference in the current Draft Risk Assessment to the study designed by DuPont in 1981 to determine whether exposure to PFOA among workers at the DuPont Plant in West Virginia was "causally related" to adverse pregnancy outcomes among children born to those workers, nor to the results DuPont obtained that same year indicating a statistically significant increase in birth defects among the children born to those employees. (*See Exhibit 9, at*

EID106200 (concluding that a finding of 2 malformations in 10 live births would be a "statistically significant excess") and EID090083 (referencing 2 malformations among 5 live births to PFOA-exposed workers at the DuPont Plant in West Virginia)).

- In connection with the cancer discussion provided in the current Draft Risk Assessment and EPA's position on the relevance to humans of three tumor types observed in PFOA rodent studies, the Panel should consider the findings of the FIFRA Scientific Advisory Panel from December of 2003 when considering key aspects of EPA's position in this regard. (See Exhibit 10) The minutes from that SAP meeting are publicly available at (<http://www.epa.gov/oscpmont/sap/2003/december9/meetingminutes.pdf>) and may be useful in evaluating these issues, particularly with respect to the applicability of USEPA's arguments to children.

II. ADDITIONAL HUMAN BIOMONITORING DATA IS AVAILABLE.

- Since the June 2004 date referenced in the Draft Risk Assessment, new information has become available confirming significantly elevated levels of PFOA in the blood of individuals living in one of the communities exposed to PFOA-contaminated drinking water near the DuPont Plant in West Virginia. This data confirms levels of PFOA as high as 128 ppb in non-occupationally-exposed residents exposed to PFOA through contaminated drinking water, where the average level of PFOA in the drinking water over the last several years has been approximately 0.5 ppb. (See Exhibit 11; AR-226-1441-71/1863-64/1870-71; and OPPT-2003-0012-725/26) Residents of this particular community were included in the recent community study referenced above in which elevated incidence of cancer, angina, MI, stroke, and reproductive issues (among others) were reported, as were residents of communities exposed to even higher levels of PFOA through their drinking water (some over 4 ppb in the water), for which blood data is not yet currently available. Additional information is also available indicating similar levels of PFOA in the blood of residents near the same DuPont Plant who consumed water from private wells with levels of PFOA between 0.12 ppb to 0.17 ppb. (See Exhibit 12^{2/}) Given this data indicating levels of PFOA in the blood of residents of a community exposed to PFOA in their drinking water that are

^{2/} Although provided to USEPA earlier this month, USEPA had not yet posted the information to public docket AR-226 or OPPT-2003-0012 at the time of this submission.

significantly higher than the general population PFOA blood levels referenced in the Draft Risk Assessment, the Draft Risk Assessment should be revised to incorporate consideration of the risks to such exposed subpopulations.

- Since the June 2004 date referenced in the Draft Risk Assessment, additional PFOA blood data also has become available for the workers at the DuPont Plant in West Virginia that should be considered in connection with the Draft Risk Assessment. This data seems to confirm that the average level of PFOA in the blood of workers at the DuPont Plant who were directly exposed to PFOA through their job duties is 494 ppb and the average level of PFOA in the blood of the workers at the same plant who should not have had any such direct occupational exposure to PFOA is 114 ppb. (*See Exhibit 13; AR-226-1884/85*) This data also seems to confirm that PFOA was found as high as 963 ppb in this same group of workers who should not have had any direct occupational exposure to PFOA at the DuPont Plant. (*See id.*) This new data should be considered in conjunction with the recent blood data from one of the neighboring residential communities where over 120 ppb PFOA was found in the blood of residents exposed to drinking water containing, on average, less than 1 ppb of PFOA, (*see discussion above*), and in conjunction with a blood model developed by DuPont that estimates that the level of PFOA in the blood of the residents who are drinking water from one of the other nearby communities with over 4 ppb PFOA in the water would be approximately 1000 ppb. (*See, e.g., Exhibit 14; AR-226-1478*) This new blood data should be considered in connection with the Panel's review of the Draft Risk Assessment.

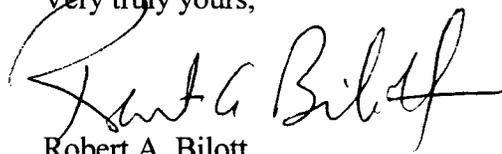
III. INFORMATION ON CUMULATIVE TOXICITY OF PERFLUORINATED CHEMICALS IS AVAILABLE.

- The Current Draft Risk Assessment does not consider risks from PFOA in context with the cumulative risks from exposure to similar perfluorinated chemicals, which are often also present where PFOA is found. Information is available concerning such cumulative risks and should be considered by the Panel in connection with its evaluation of the current Draft Risk Assessment. (*See, e.g., Exhibit 15; AR-226-1532*) In fact, USEPA itself nominated the entire class of perfluorinated compounds to the National Toxicology Program for a class-study of the "hazard/risk across [the] entire structural class." (Exhibit 16) In response, the NTP Interagency Committee for Chemical Evaluation and Coordination ("ICCEC") recently recommended various toxicological studies of the class. (*Id.*)

Dr. Sue Shallal
February 11, 2005
Page 6

As requested in the Federal Register Notice, we have enclosed 35 copies of this submission for distribution to all Panel members for review in advance of the February 22-23, 2005, public meeting. As mentioned above, we hereby reserve our right to submit additional written comments to the Panel before that meeting. In the meantime, we request the opportunity to provide oral comments during the February 22-23, 2005, public meeting. Thank you.

Very truly yours,

A handwritten signature in black ink, appearing to read "Robert A. Bilott". The signature is fluid and cursive, with a large initial "R" and "B".

Robert A. Bilott

RAB/mdm
Enclosures

cc: Dr. Charles M. Auer (w/ encls.)
Dr. Jennifer Seed (w/ encls., by e-mail)
Mark J. Garvey, Esq. (w/ encls.)
R. Edison Hill, Esq. (w/ encls.)
Larry A. Winter, Esq. (w/ encls.)
Gerald J. Rapien, Esq. (w/ encls.)

AR226-1867



8EHQ-0904-00373

DuPont Haskell Laboratory
for Health and Environmental Sciences
Elkton Road, P.O. Box 50
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September 17, 2004

32pp.

Via Federal Express

8EHQ-80-373

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Room 6428

Attention: 8(e) Coordinator

Office of Pollution Prevention and Toxics

U.S. Environmental Protection Agency, ICC Building

1201 Constitution Ave., NW

Washington, DC 20460

CONTAINING NO CBI

Dear 8(e) Coordinator:

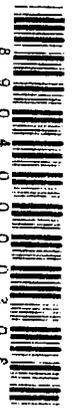
Ammonium Perfluorooctanoate
8EHQ-0381-0394

This letter is submitted "For Your Information" to supplement the letter of September 7, 2004 submitted by Mr. Edward E. Shea, representing MIC Specialty Chemicals, Inc. (Attachment I). Overall, the letter and accompanying trip report are an accurate reflection of the proceedings of the meeting held at the Inn at Montchanin Village on August 20-21, 2004. Our conclusions from the meeting are summarized as follows:

1. No effects were observed on the general health of the workers at the Miteni plant.
2. No changes in clinical chemistry parameters were observed with the exception of an apparent slight alteration of serum lipid levels that correlated with exposure. The cause and biological significance of this observation is unclear and requires further analysis.
3. Although the observed changes appear to be correlated with exposure, it does not demonstrate a causal association with exposure to these substances.
4. Average serum levels of PFOA were higher in the Miteni workers than have been reported for occupational exposure, and significantly higher than levels reported in the general population.

For the sake of completeness, we are submitting with this letter a copy of the data slides that summarize DuPont's preliminary analysis of the serum lipid data (Attachment II). We note that 3M actually led the initial analysis of the Miteni data including the 37 blood parameters mentioned in Dr. Costa's trip report. After seeing the preliminary analysis of the serum lipid data, DuPont subsequently undertook an independent statistical analysis of the serum lipid data only, and presented our preliminary analysis at the August 20-21 meeting. With respect to the data referenced in Mr. Shea's letter we note the following:

1. In Table 1 it should be noted that analyses of the sera of the "PFOA-exposed" group also revealed the presence of PFOS (perfluorooctanoic sulfonate). Dr. Costa confirmed that the plant worked with both perfluorinated compounds. The average serum PFOA concentration was ~ 16 ppm with a range of 0.04-92 ppm and for serum PFOS the average was ~ 0.5 ppm with a range of 0.06-3.3 ppm. It should be noted that no analyses for these same perfluorinated compounds was undertaken for the sera of workers in the non-PFOA/non-PFOS area ("Non-PFOA" group).



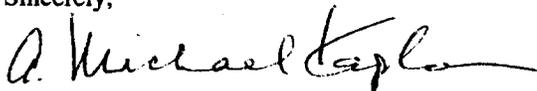
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2. There are a number of caveats that are germane to the interpretation of the data in addition to the fact that workers were exposed to both PFOA and PFOS; notably:
 - a. The dataset consists of a small and arbitrary collection of subjects (~35 employees).
 - b. There are no pre-employment/baseline lipid levels for historical reference in the study population.
 - c. Concomitant exposure to other chemicals in the plant is an unknown variable.
 - d. Serum lipids are well known to be affected by many different factors including family history, diet, and lifestyle; data on these factors were not available for inclusion for analysis.
3. There was a strong correlation observed between PFOA and PFOS levels. Therefore, we analyzed the relationship between the various serum lipids with respect to three dependent variables: total perfluorinated compound (the sum of PFOA + PFOS), PFOA alone and PFOS alone.
4. The data have been analyzed in terms of a linear model; however, other models may fit the data better. In general, there is no *a priori* reason to expect a linear dose-response relationship in a biological system.

As indicated in Mr. Shea's letter, DuPont has additional ongoing studies that may enable a broader interpretation of this small study in the Miteni workers. Notably, we are conducting a study, "Ammonium Perfluorooctanoate: Cross-Sectional Surveillance of Clinical Measures of General Health Status Related to a Serum Biomarker of Exposure and Retrospective Cohort Mortality Analyses in a Polymer Production Plant" of over 1,000 employees at our Washington Works plant. Currently we are analyzing the data and expect to issue a final report by year's end. In addition, we are evaluating the effects of perfluorinated compounds on the activation of various nuclear receptors, e.g., PPAR α , in an effort to better understand the biological activity of this class of chemicals. Finally, we are exploring the hypothesis that hyperlipidemia prolongs retention of PFOA in the serum, thus accounting for the observed correlations.

A copy of the final report(s)/manuscript(s) for the DuPont studies will be submitted to the Agency when available.

Sincerely,



A. Michael Kaplan, Ph.D.
Director – Regulatory Affairs and Occupational Health

AMK/RWR/PJG:clp
(302) 366-5260

Attachments: (I) "Mr. Edward E. Shea's TSCA Letter, September 7, 2004"
(II) "DuPont Statistical Analysis of Serum Lipid Data of Miteni Workers"

BEHQ - 0904 - 15663

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9pp.

AR226-1865

September 7, 2004

TSCA Confidential Business Information Center (7407M)
EPA East - Room 1428
U.S. Environmental Protection Agency
1201 Constitution Avenue, N.W.
Washington, DC 20004-3302

CONTAINS NO CBI

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RECEIVED

Ladies and Gentlemen:

We represent MIC Specialty Chemicals, Inc. Pursuant to Section 8(e) of the Toxic Substances Control Act (TSCA), we submit on behalf of our client a copy of a report dated August 23, 2004 to Miteni S.p.A by Dr. Giovanni Costa of the University of Verona describing a meeting with 3M and du Pont to discuss biological monitoring of Miteni workers exposed to perfluorooctanoic acid (PFOA). A Department Manager of our client received a copy of the memorandum on August 23, 2004 during an overseas trip.

Miteni is an Italian company which manufactures PFOA in Italy. Our client imports PFOA purchased from Miteni into the United States.

In general, the information described in the enclosed report appears to be favorable and our client does not know that any information in the report indicates a substantial risk. However, our client is not in a position to make that determination and, therefore, decided to make the submission on a precautionary basis. Our client advises that the enclosed information is the only information which it has about the biological monitoring described in the report.

If you have any questions, please call me at 212-237-1140.

Very truly yours,

Edward E. Shea

Edward E. Shea

2004 SEP 22 PM 12:44

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EES:mg

cc: Hitoshi Inada, Esq.
Takehiro Fujimura, Esq.
Ms. Marian Roach

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MR 279024

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AR226-1866

Verona, 23.08.2004

Spett.
Direzione
MITENI S.p.A.
Trissino (VI)

Report on the meeting held on Friday 20th and Saturday 21st 2004 at the Inn at Montchanin Village (Wilmington, USA) with 3M and DuPont delegations.

1. Participants:

- John Butenhoff	3M, toxicologist
- Geary Olsen	3M, clinical epidemiologist
- Larry Zobel	3M, occupational health physician
- Peter Gillies	DuPont, expert in lipid metabolism
- John Green	DuPont, statistician
- Gerald Kennedy	DuPont, toxicologist
- Robin Leonard	DuPont, epidemiologist
- Robert Rickard	DuPont, toxicologist
- Giovanni Costa	Miteni, occupational health physician

2. Background

On August 9th I have been invited by G. Olsen (3M) to participate in this meeting, organised jointly with P. Gillies (DuPont), aimed at discussing the results of the analysis of the data collected by me at Miteni plant in Trissino, concerning the workers of the PF department.

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As it was been agreed last year in the ambit of the *APME-APFO ad hoc toxicological working group*, I have started a scientific collaboration with them aimed at evaluating the data related to the periodical, biological monitoring of Miteni workers exposed to PFOA.

Despite the small number of workers involved (compared to 3M and DuPont workers), such data were considered very helpful in understanding any possible interaction of PFOA with human physiology, as such cohort of workers has been checked regularly since 1979 by annual medical examinations, integrated by several blood and urine analysis. In the case of 3M workers, some workers (on voluntary basis) have been checked occasionally (1993, 1996, 2000), whereas DuPont did not carry out regular checks of such kind in the past, but it is now carrying on a general examination of more than 400 workers, the results of which are due by the end of this year.

Therefore, the statistical analysis of Miteni data was considered very useful for checking whether or not any pre-clinical adverse effect could be detected, in order to better address the checks of larger groups at 3M and DuPont plants, and for further more detailed investigations on some specific biological parameters.

So, in December 2003 (after discussion and agreement with Miteni general management in the meeting held in Frankfurt on November 21st) I sent G. Olsen the first database of the biological monitoring of Miteni workers (in anonymous format) and, in February 2004, on occasion of the SOT Conference held in Baltimore, I had a first meeting with them and other 3M and DuPont experts (see my report dated 29.03.04 and abstract below) for a preliminary analysis of the data.

Thereafter, we decided to integrate the dataset with some more specific analysis concerning the lipids metabolism, that I collected during the periodical, annual blood check carried out in Spring this year.

Consequently, two months ago I sent G. Olsen and P. Gillies two updated datasets (still in anonymous format), one concerning the biological data available since 1987 of all exposed workers to PFOA, and the second one concerning the last blood analysis carried out in Spring 2004.

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3. Preliminary results of the statistical analysis

The analysis of the first database was aimed at evaluating any possible abnormality of biological parameters, occurred in the last 17 years, in relation to the PFOA blood levels measured in the last 4 years (2000-2003); the second one was aimed at comparing exposed and non-exposed workers to find out any difference that could be statistically associated to PFOA exposure.

The statistical analysis, carried out by means of appropriate statistical programs by DuPont expert statisticians, confirmed the negative results for almost all the 37 blood parameters considered: in particular no significant effects were detected as concerns haematology, proteins metabolism, immunology, liver, kidney and prostate function.

Only some slight effects on lipids metabolism were detected, which deserve further analysis and proper interpretations.

In fact, a slight increase of total cholesterol in workers exposed to PFOA was observed, which also appeared to show an increasing trend associated with the highest blood PFOA levels.

Table 1 shows the comparison of exposed and non-exposed workers and the slight significant increase of total cholesterol in exposed workers. There is no increase of other lipids, such as tryglicerides in particular, but the fraction of "Non-HDL Cholesterol" seems that concerned.

Figure 1 shows the positive correlation between total cholesterol and PFOA blood levels in the last 4 years (when PFOA was measured): a slight association between PFOA blood level and total cholesterol concentration seems to be consistent over the years. Figure 2 shows the same trend as concerns "Non-HDL Cholesterol".

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Table 1: Comparison of the main lipid components in exposed and non-exposed workers to PFOA

Endpoints	Manufacturing Area		P-value
	Non -PFOA	PFOA	
Serum Lipids			
Total Cholesterol	214 ± 4	233 ± 9	0.03
HDL Cholesterol	53 ± 1	51 ± 2	0.37
LDL Cholesterol	133 ± 3	146 ± 8	0.09
Non-HDL Cholesterol	160 ± 4	182 ± 10	0.03
Non-HDL/HDL	3.3 ± 0.2	3.9 ± 0.3	0.09
Total Triglycerides	141 ± 12	169 ± 19	0.22
Demographic Characteristics			
Age	39.5 ± 1.0	40.7 ± 1.5	0.53
BMI	25.3 ± 0.3	25.7 ± 0.5	0.52
Alcohol Consumption	0.27 ± 0.02	0.36 ± 0.04	0.07

Values are expressed as the mean ± SEM for approximately n = 94 non -PFOA workers and n = 35 PFOA workers.

Data are from a 2004 sample collection

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Figure 1: Correlations between Total Cholesterol (log) and PFOA (log) levels in the 4 years

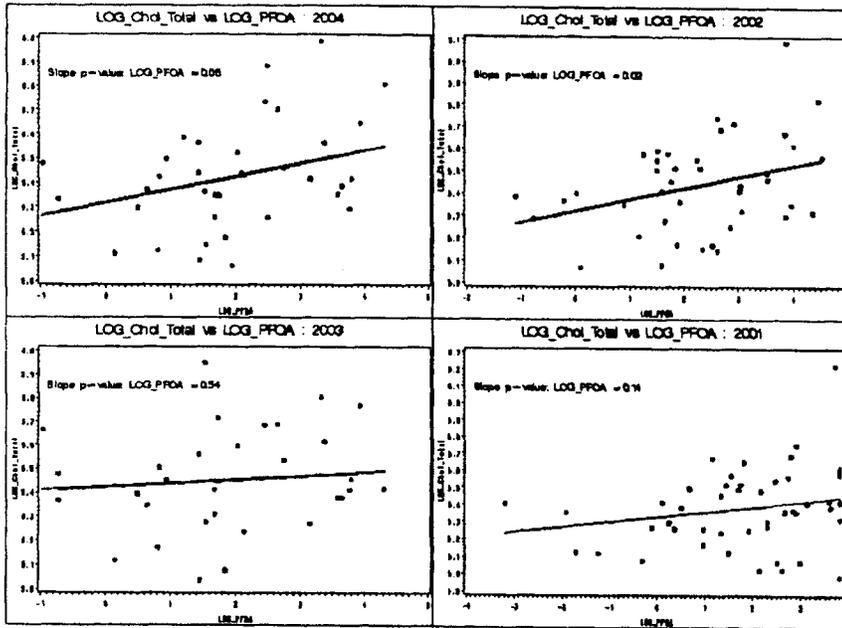
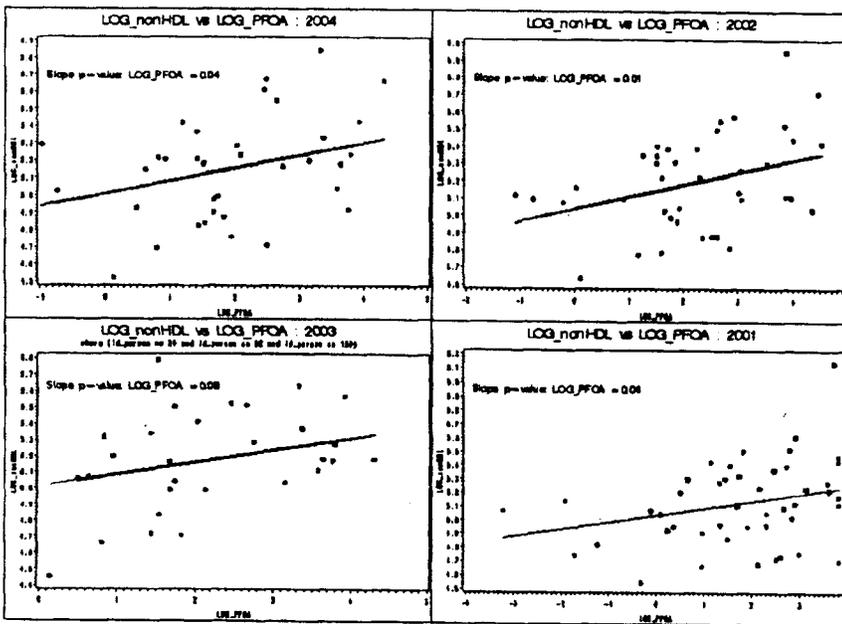


Figure 2: Correlations between non-HDL Cholesterol (log) and PFOA (log) levels in the 4 years



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In the light of these findings, G. Olsen (3M epidemiologist) reviewed the 3M datasets related to their surveys carried out in 1993, 1996 and 2000, and a preliminary analysis concerning cholesterol levels appears to be in agreement with the Miteni data.

In other words, it seems that PFOA (probably only at high blood concentrations) can interfere with metabolism of Cholesterol, in particular by increasing the fraction of "Non-HDL Cholesterol".

Such findings need a precise interpretation, also because they are in contrast with the animal experimental data (rats), where PFOA causes a decrease of cholesterol levels. That can be related to interspecies differences in drug metabolism, which are also raised for the different findings in carcinogenicity (it is carcinogen in rats, but not in primates and humans).

In order to elucidate better the possible mechanisms underlying such effect P. Gillies, DuPont expert on lipid metabolism, made an updated review of the current knowledge on lipids metabolism, trying to make some hypothesis about possible mechanisms. According to his analysis, such effect cannot be mediated by a PPAR α mechanism (as suggested for rats), but it is probably due to an interference with a protein (CEPT) able to transfer of cholesterol in blood and liver. He is going to have a deeper insight on such matter both by further discussion with the best academy experts on lipids and by a bio-molecular study concerning the nuclear receptors for such protein.

4. Communication and regulatory aspects.

Robert Rickard (DuPont) said that is going to have a FYI ("For your information") meeting with EPA next Wednesday, August 25th.

According to the TSCA 8e rules he has to report to EPA any new data concerning possible toxicological characteristics of PFOA DuPont may know, with particular reference to human health.

He exposed his agenda, which includes an updating of the recent toxicological studies carried out at the Haskell Lab concerning the exposure of rats and mice to linear, branched

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P.IVA 0237309 023 8

and mixed (linear/branched) PFOA, as well as the presentation of some ongoing studies, dealing with the health examination of more the 400 workers at DuPont "Washington" plant in West Virginia, and the review of epidemiological data concerning mortality in general population of West Virginia. G. Olsen and L. Zobel (3M) will also communicate their epidemiological data concerning 3M workers at Decatur and Antwerp plants.

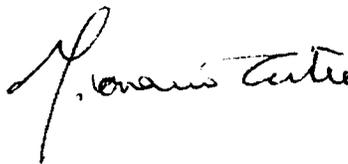
In such agenda he would like to mention also some findings related to the data on lipid metabolism mentioned above, related to Miteni workers, keeping anonymous the source of data. In particular, he would like to report the positive findings of a long lasting medical surveillance of workers exposed to PFOA, showing no effects on general health and also on biological parameters, concerning the main target organs and functions, except for a mild possible interference with lipid metabolism, which deserves further analysis.

So, he asked me whether he can report such data, in particular he would like to show one or two charts related to the lipid parameters (such as figures 1 and 2) in his presentation, without mentioning the source and the name of Miteni, and without giving EPA any written document.

As I replied that I could not deal with such request, but he must ask and get the formal permission from Miteni Management, due to the short time available he asked me to pass you such request in order to get your response (whatever it is) as soon as possible.

With kind regards

Prof. Giovanni Costa



Prof. Giovanni Costa
* Medico Chirurgo
Specialista in Medicina del Lavoro
Cattedra di Medicina del Lavoro
Università degli Studi di Verona
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Abstract of my report dated 29.03.04 concerning the meeting of the APME-APFO ad hoc toxicology group, held in Baltimore on March 24.03.04.

5.2. Workers.

The Chairman noted that most of the companies represented in the WG had commenced or were about to embark on a blood level monitoring programme in their current employees (Asahi Glass has just started it and asked me some advice concerning the parameters to be collected from the workers). He encouraged all companies to share their data in the way that Miteni and 3M had done in the past, and he also asked me to co-ordinate the outcomes for the APME group; I gave him my willingness for that.

As also Asahi Glass is sending its sample to the German lab already used by Solvay, I suggested that all European should join this lab, provided that it is quite reliable, in order to limit the factors that can confound the results.

As concerns our data, I informed the group that we analysing them with reference to the interaction with lipid metabolism in collaboration with Geary Olsen (3M) and Peter Gillies (DuPont); see the enclosed report of the meeting held at Marriott hotel on Wednesday morning with G. Olsen and J. Buthenoff (3M) and P. Gillies and G. Kennedy (DuPont).

G. Kennedy confirmed that DuPont is starting its biomonitoring according to the protocol he circulated to the group in the last week. On Wednesday evening I have been invited for a dinner by Larry Jansen (Lawyer) and Robert Rickard (Science Director) of DuPont for exchanging information about the workers' biomonitoring (see attached report).

Attachment 1.

Meeting with G. Olsen and J. Butenhoff (3M toxicologists), Peter Gillies and J. Kennedy (DuPont toxicologists)

On Wednesday 24th morning, I had a 3-hour meeting with with 3M and DuPont toxicologist to discuss the preliminary findings of the data collected in MITENI workers and concerning the possible interference of PFOA with lipid metabolism.

G. Olsen have carried out a preliminary statistical analysis of the biochemical data related to year 2002 and 2003, which showed some possible slight effects on HDL and LDL cholesterol.

P. Gillies (DuPont expert on lipids) described the meaning of the different blood lipid components and their possible interaction with PFOA.

After a long discussion and a careful analysis of the present data, it has been convened to add further data to the dataset, in particular those related to the ongoing biomonitoring which is due to end by April. In this survey further analysis of lipids and proteins (LDL.Cholesterol, APO-A2, APO-B, reactive C-protein) have been added in order to clarify better such interaction.

So, I agreed in sending them such new data by the end of April and then start a deeper statistical analysis.

The results are expected to be sent to a toxicology journal for publication by the end of the year.

Attachment II

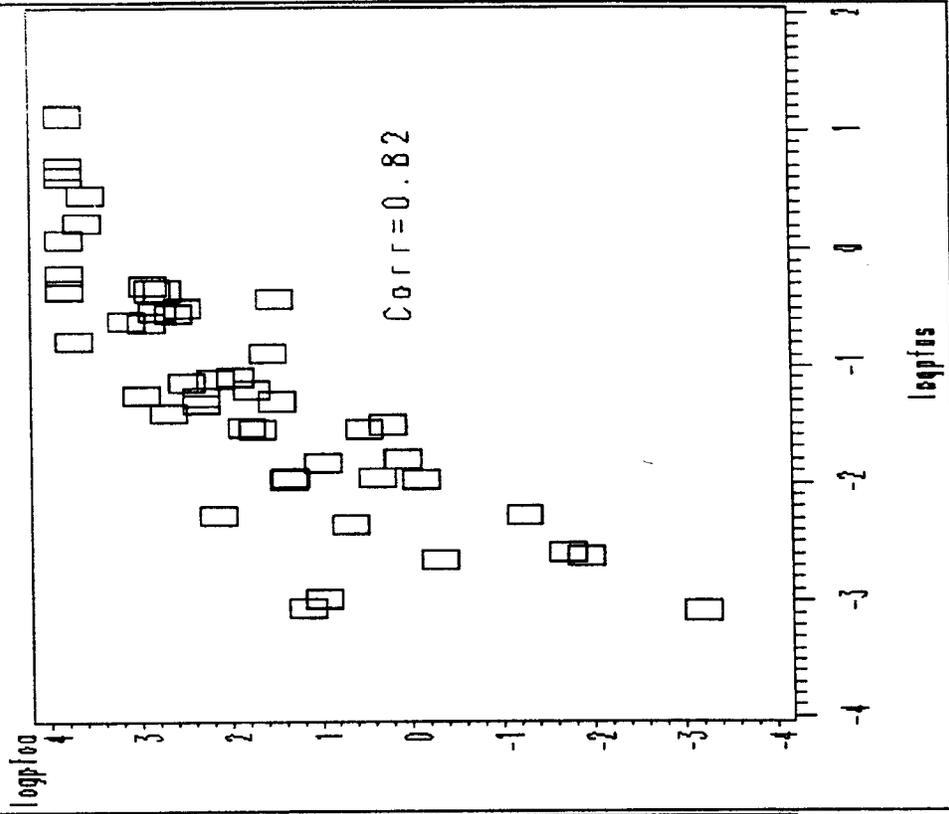
DuPont Statistical Analysis

of Serum Lipid Data

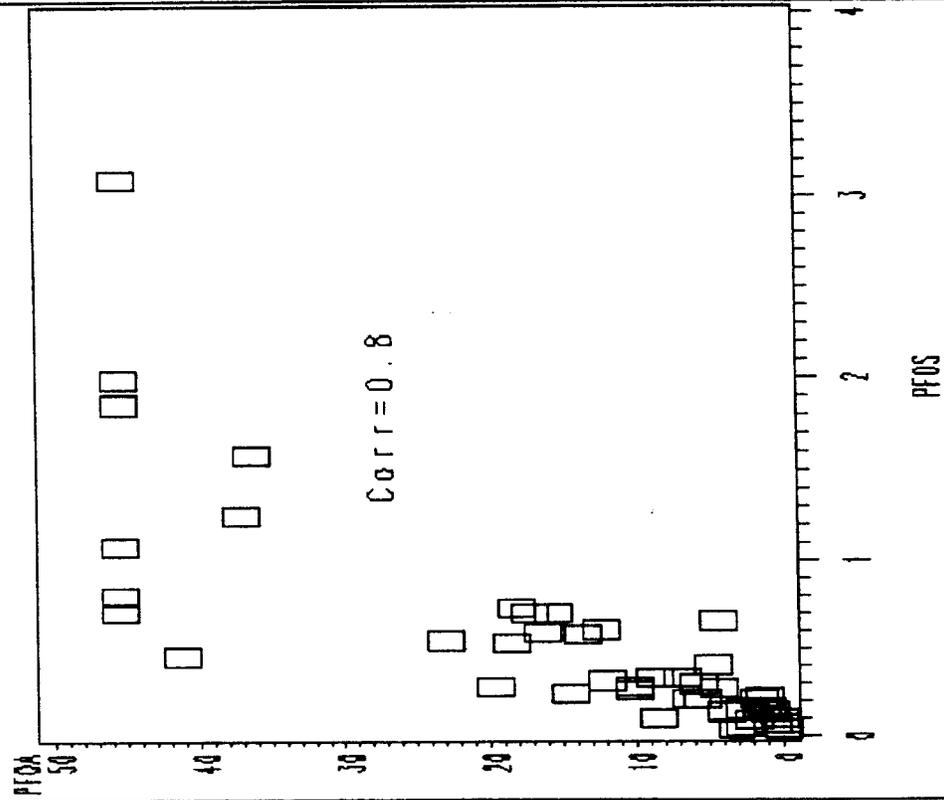
of Miteni Workers

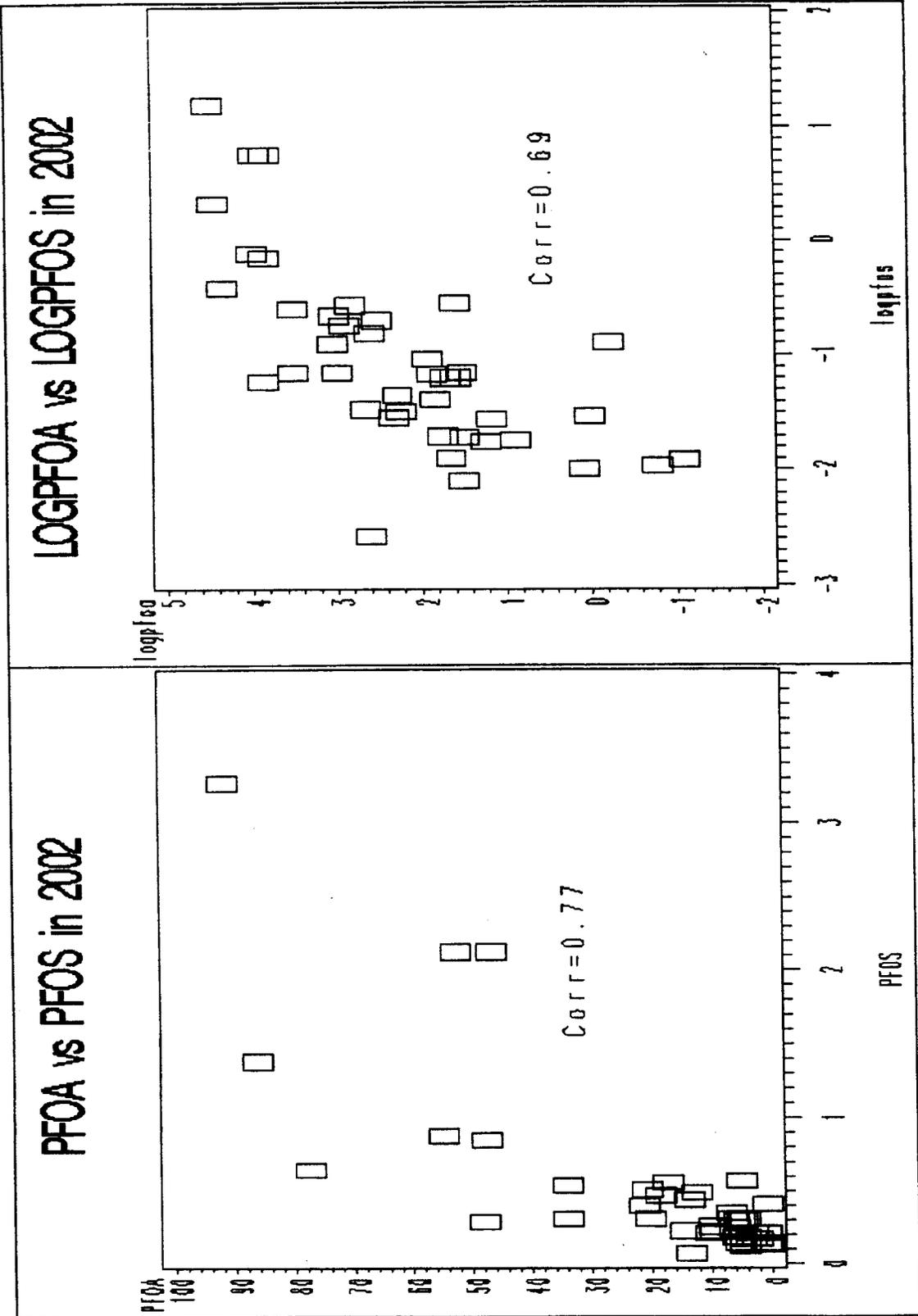
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LOGPFOA vs LOGPFOS in 2001



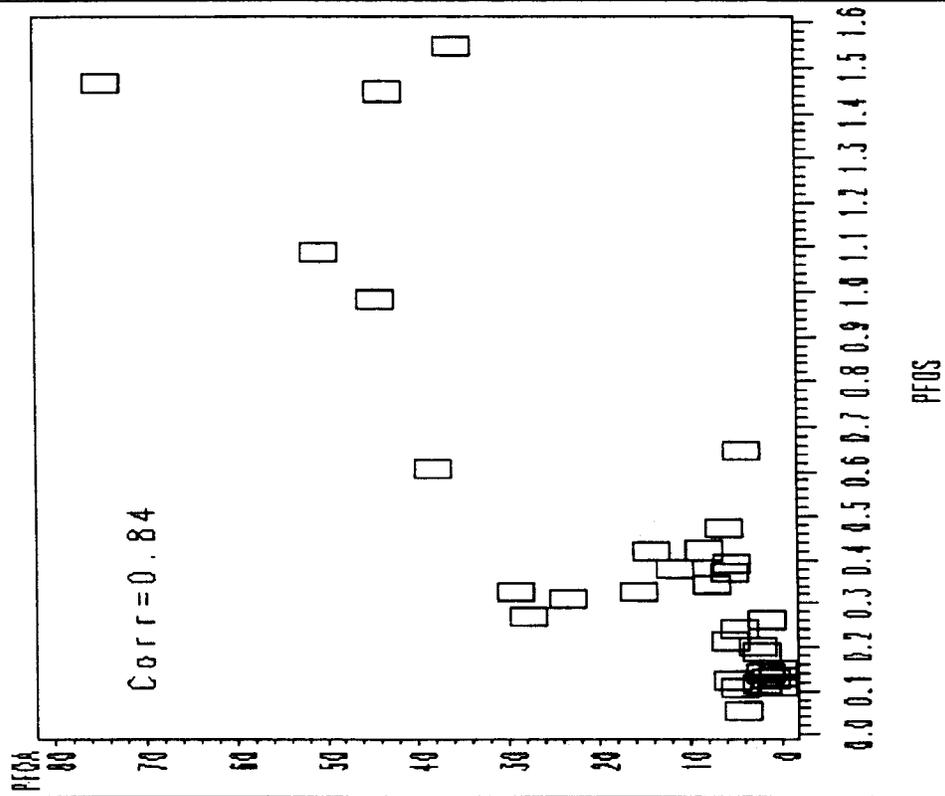
PFOA vs PFOS in 2001



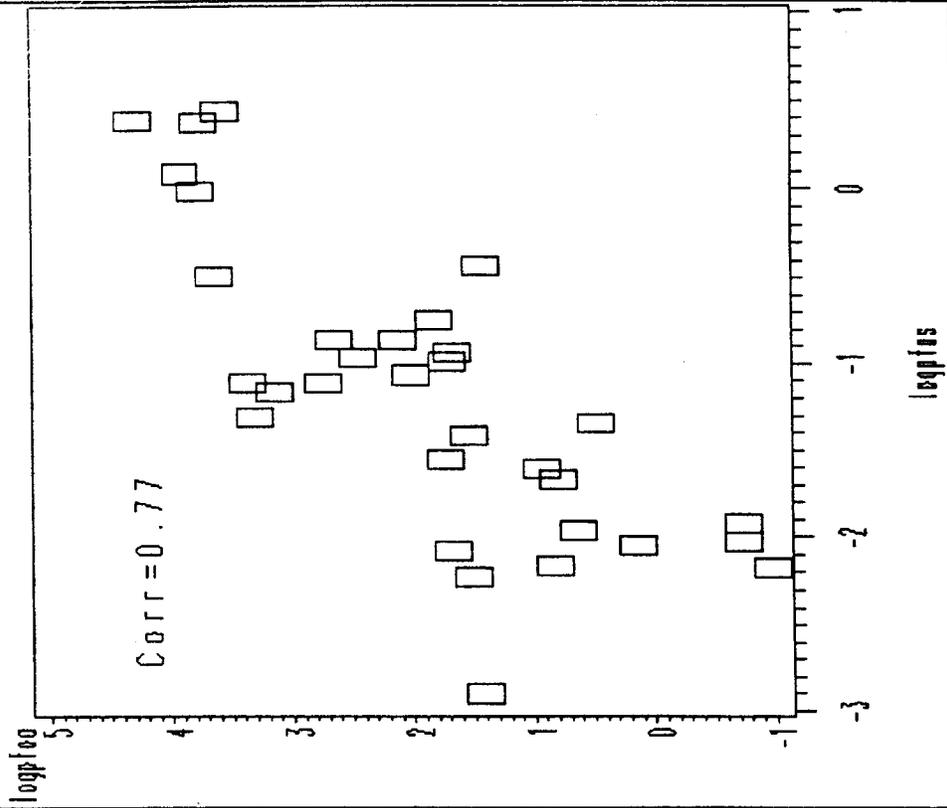


Confidential DuPont Draft Work Product

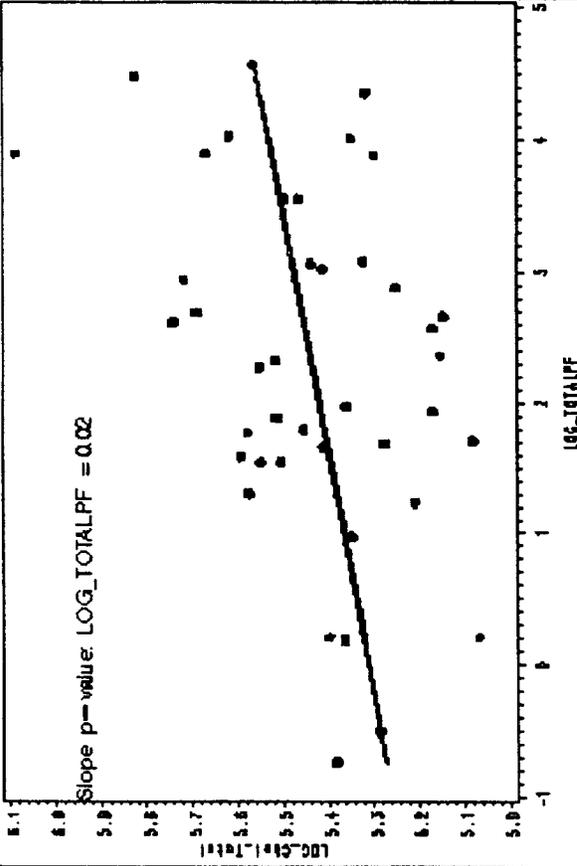
PFOA vs PFOS in 2003



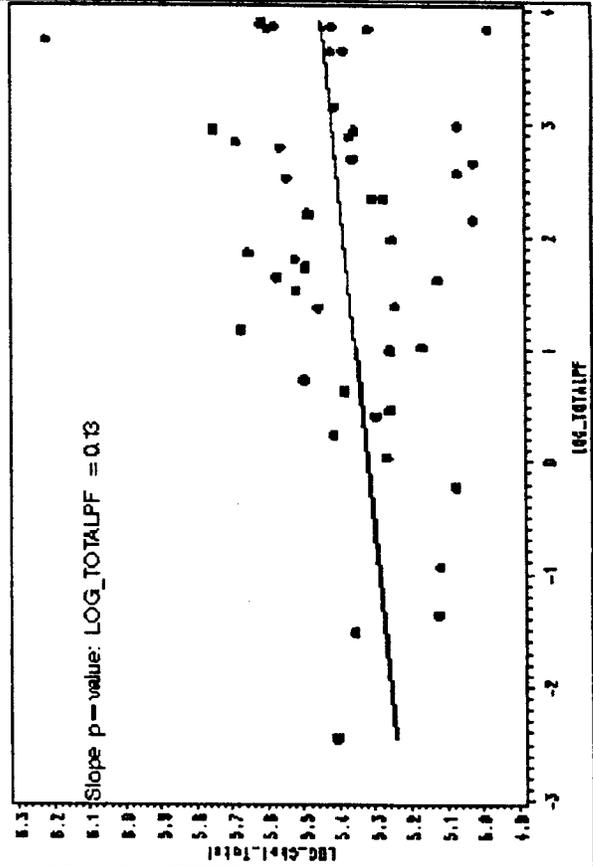
LOGPFOA vs LOGPFOS in 2003



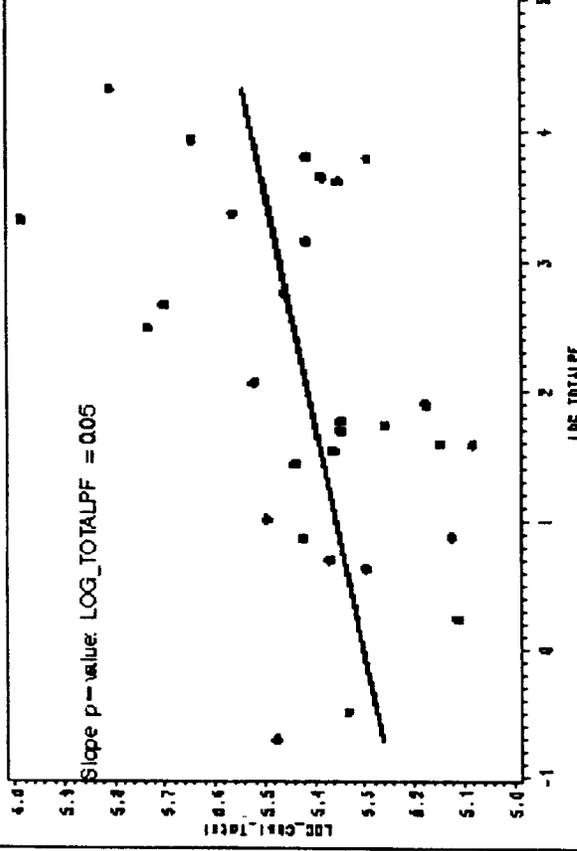
LOG_Chol_Total vs LOG_TOTALPF : 2002



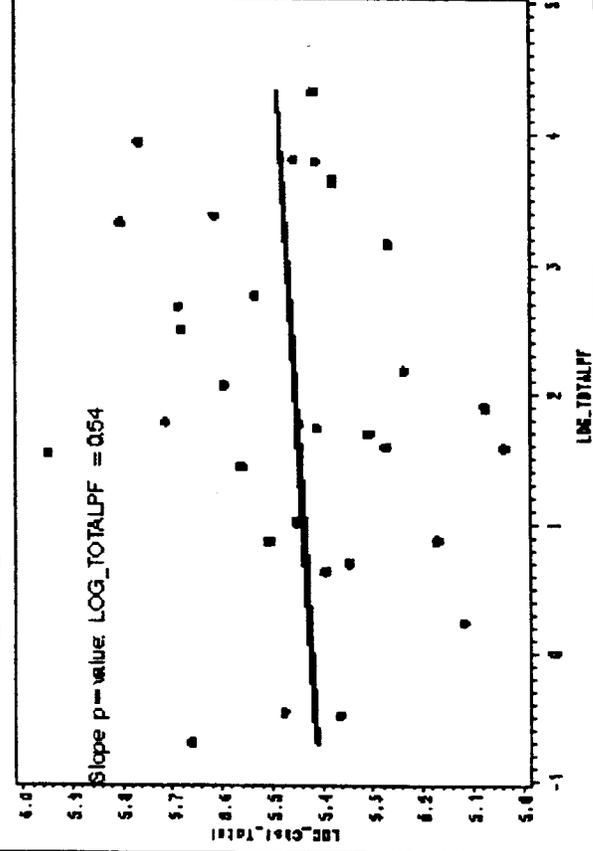
LOG_Chol_Total vs LOG_TOTALPF : 2001



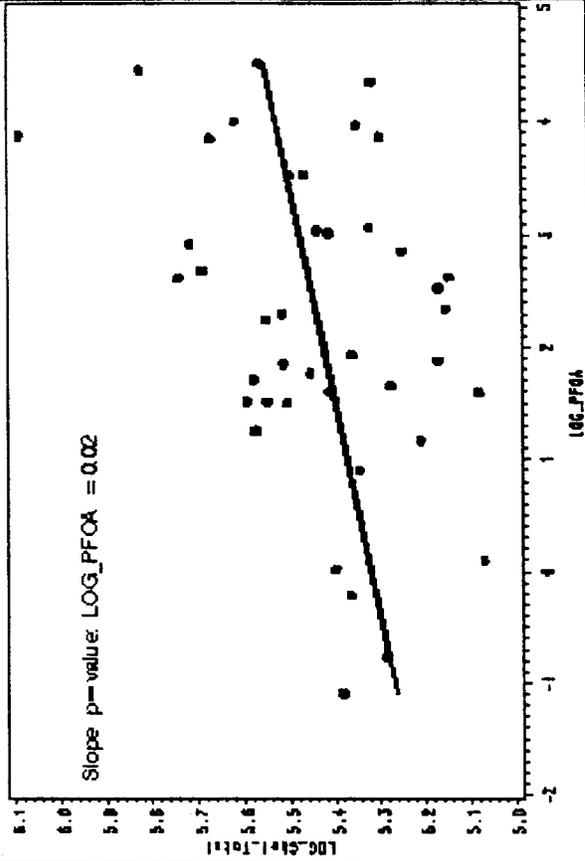
LOG_Chol_Total vs LOG_TOTALPF : 2004



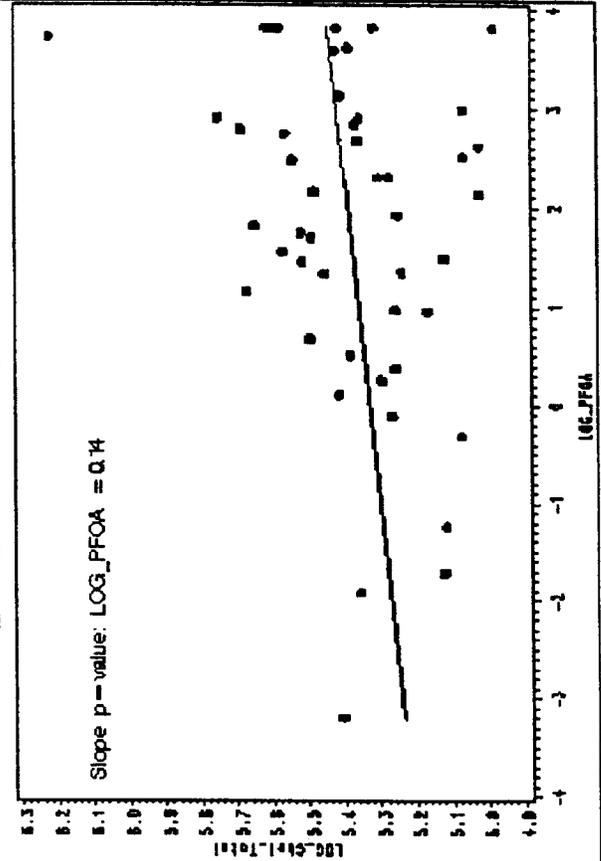
LOG_Chol_Total vs LOG_TOTALPF : 2003



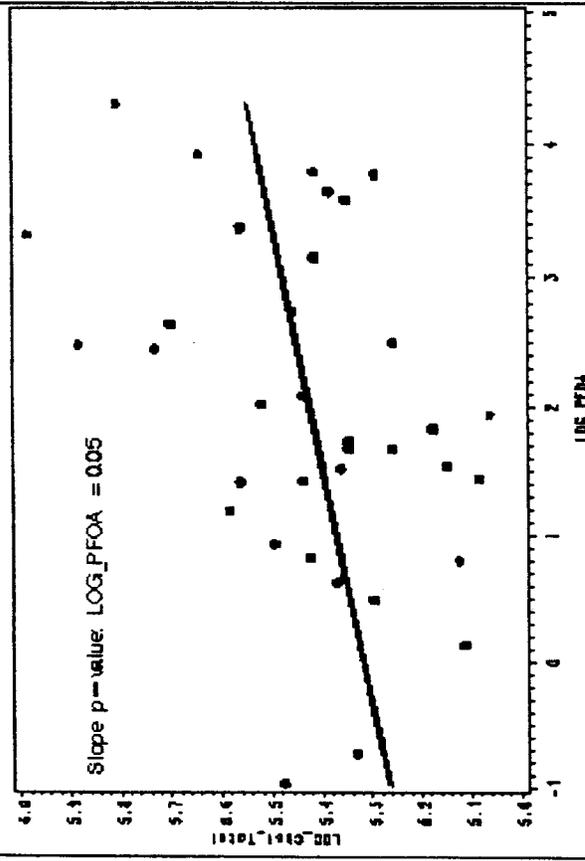
LOG_Chol_Total vs LOG_PFOA : 2002



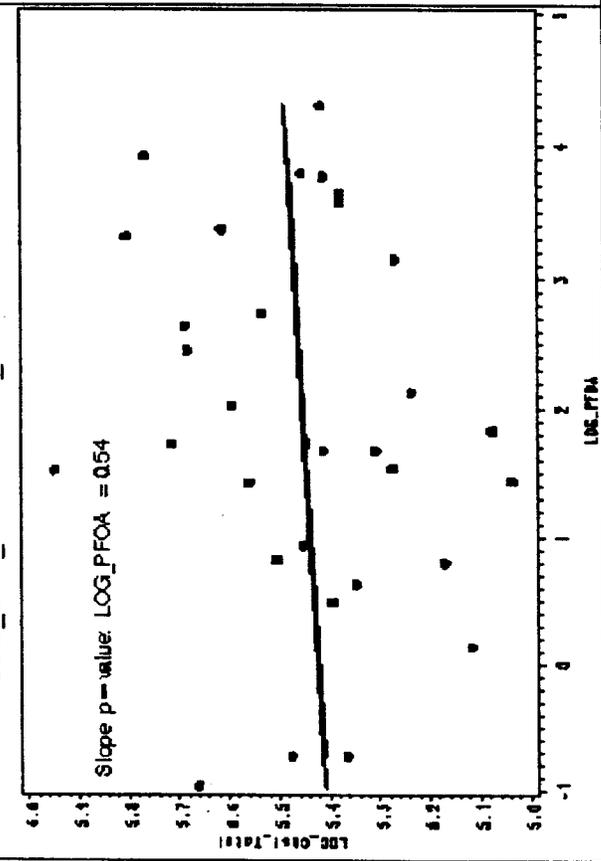
LOG_Chol_Total vs LOG_PFOA : 2001



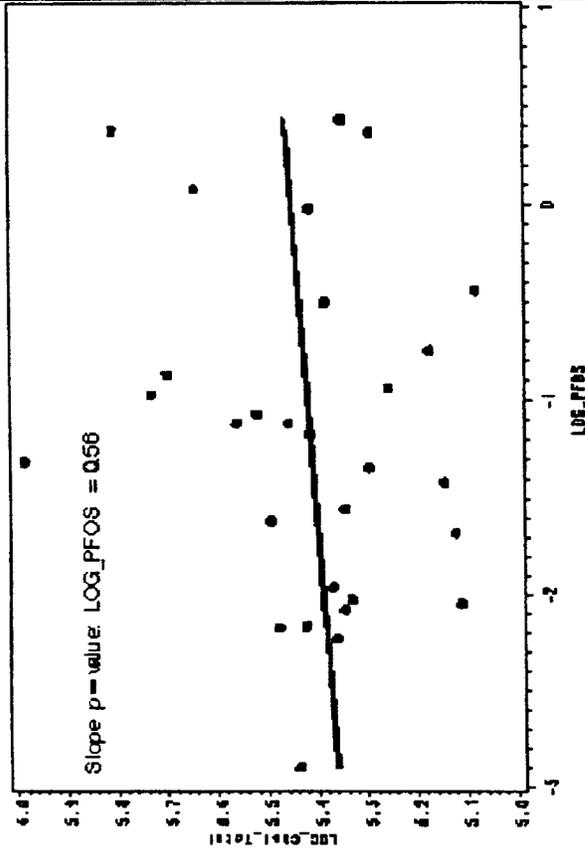
LOG_Chol_Total vs LOG_PFOA : 2004



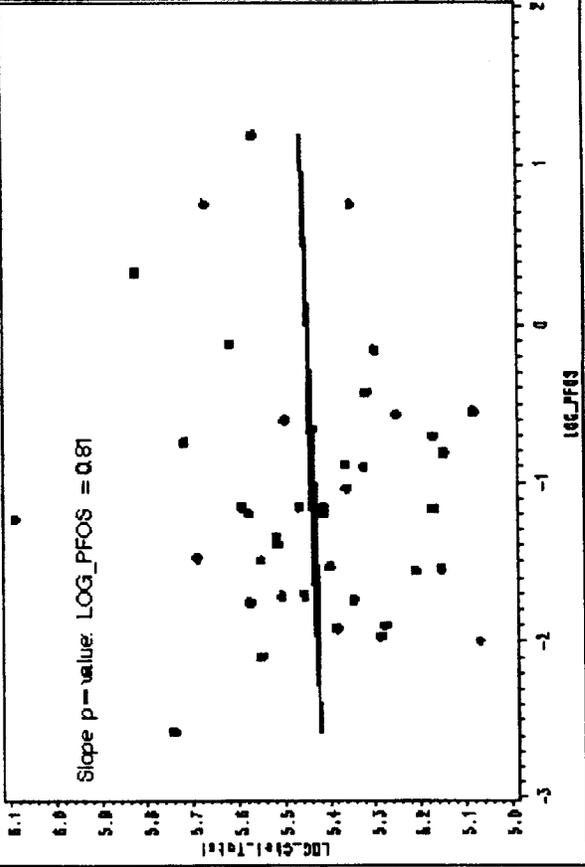
LOG_Chol_Total vs LOG_PFOA : 2003



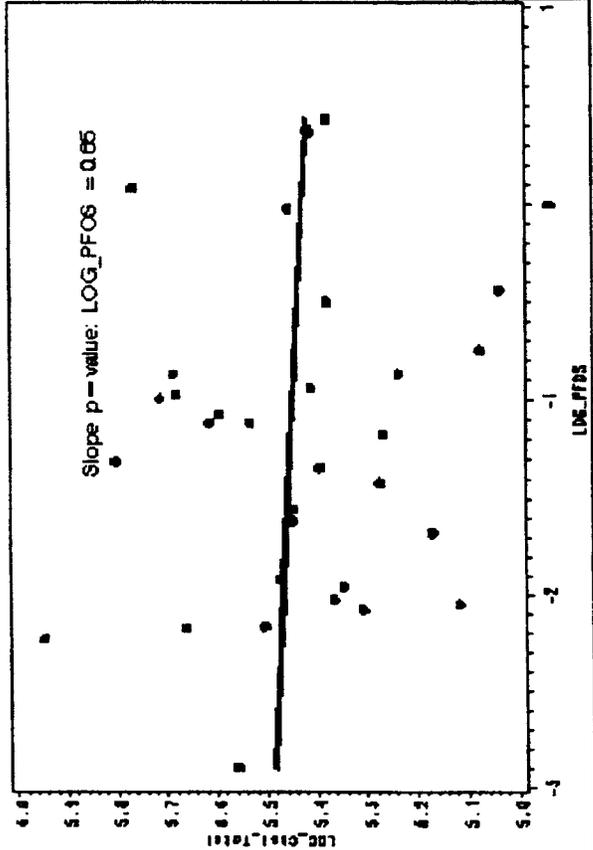
LOG_Chol_Total vs LOG_PFO8 : 2004



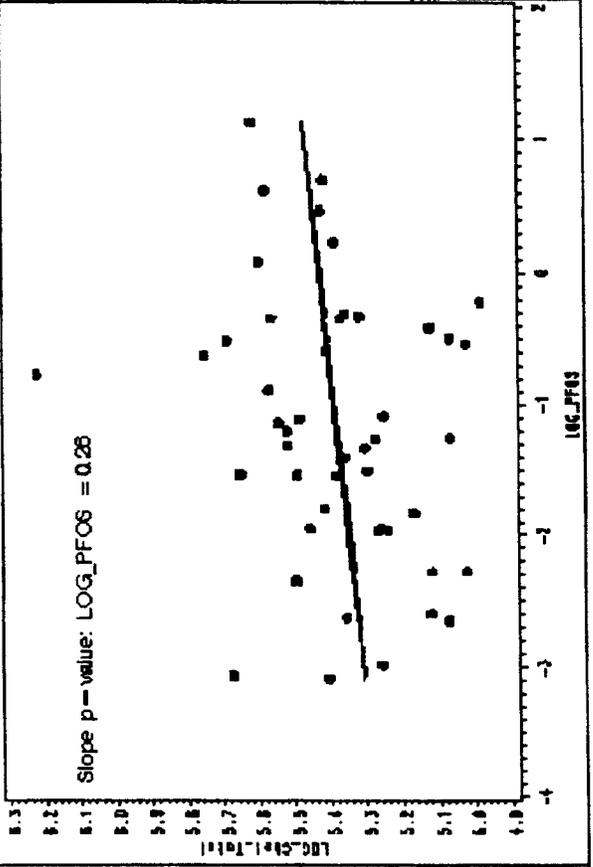
LOG_Chol_Total vs LOG_PFO8 : 2002



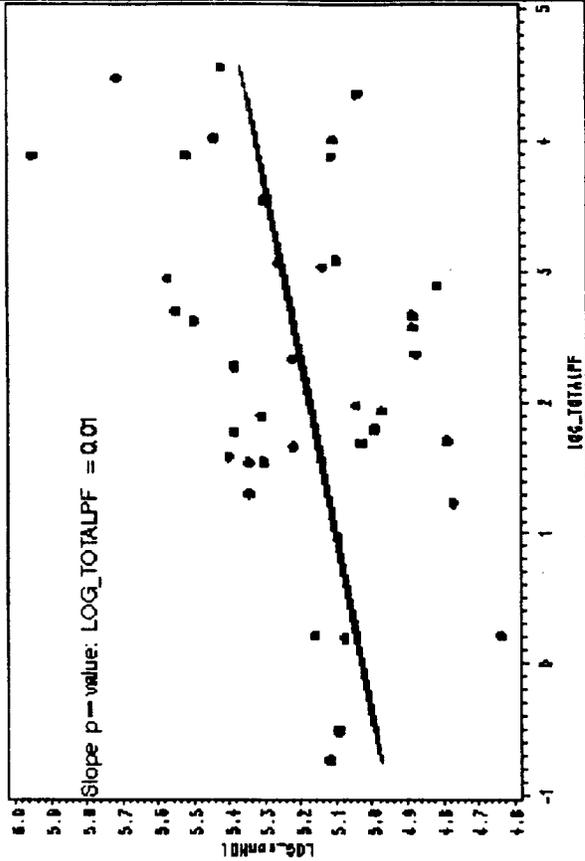
LOG_Chol_Total vs LOG_PFO8 : 2003



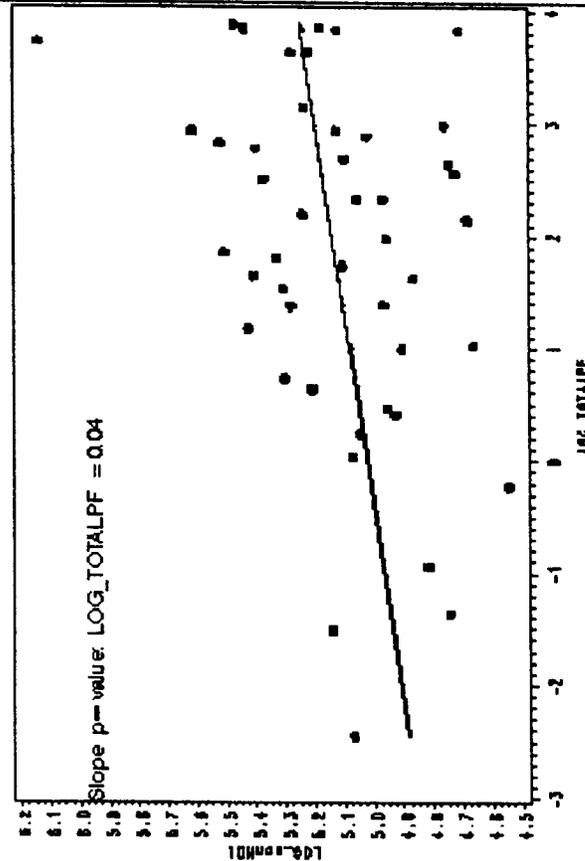
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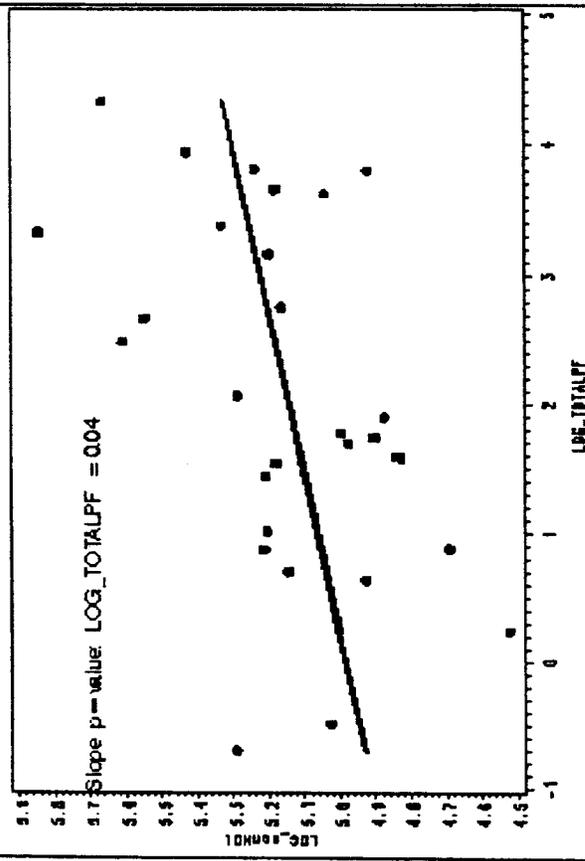
LOG_nonHDL vs LOG_TOTALPF : 2002



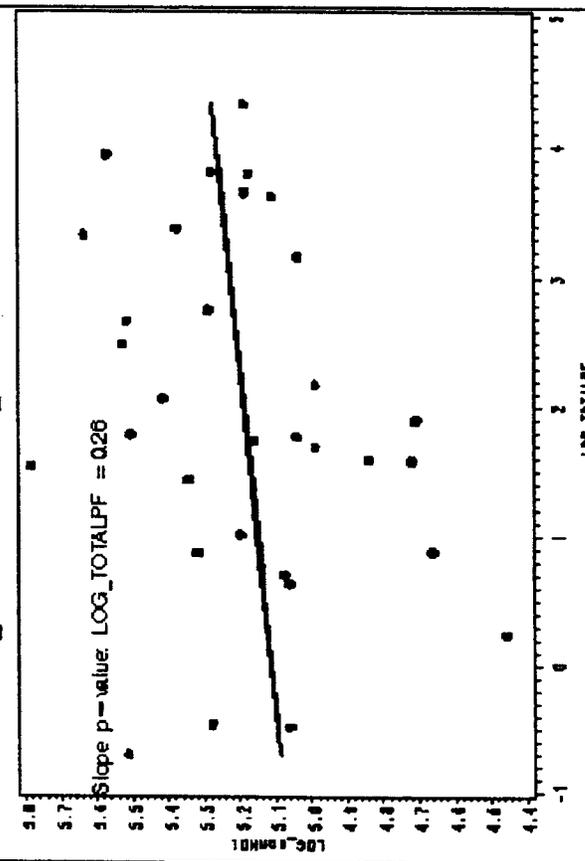
LOG_nonHDL vs LOG_TOTALPF : 2001

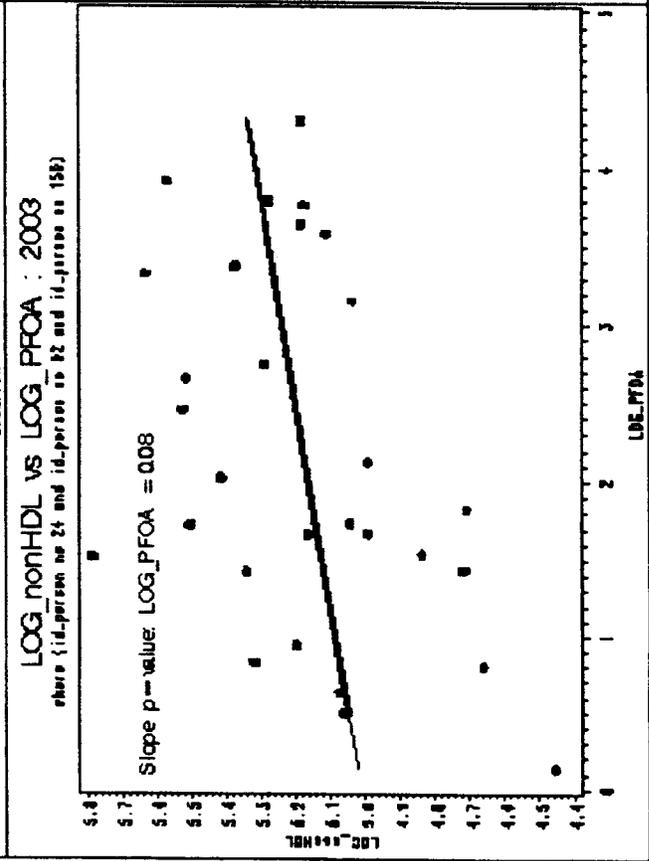
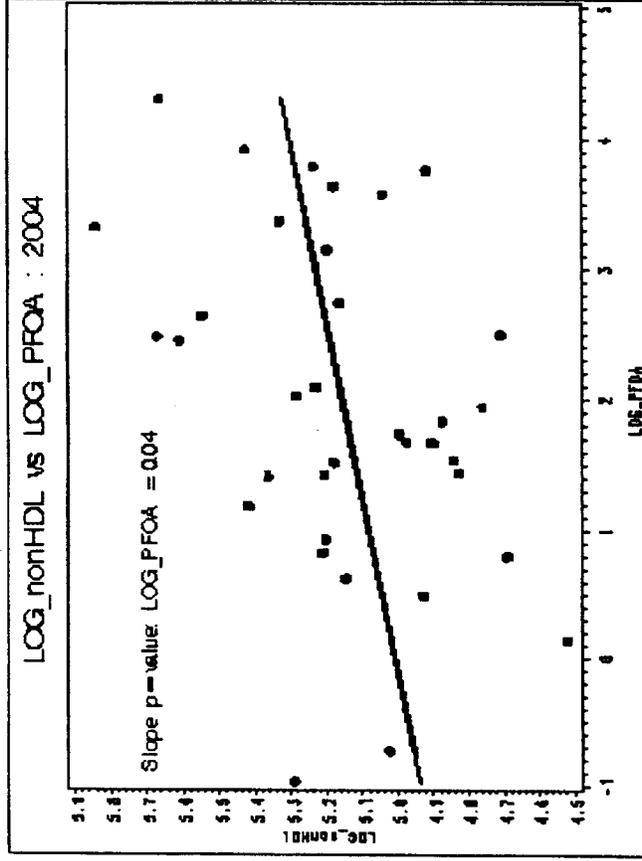
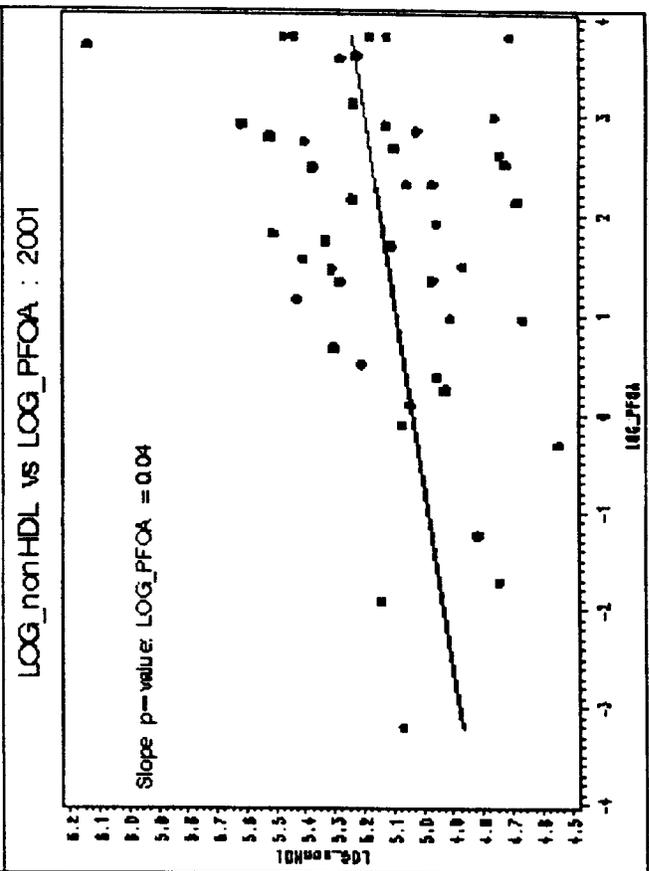
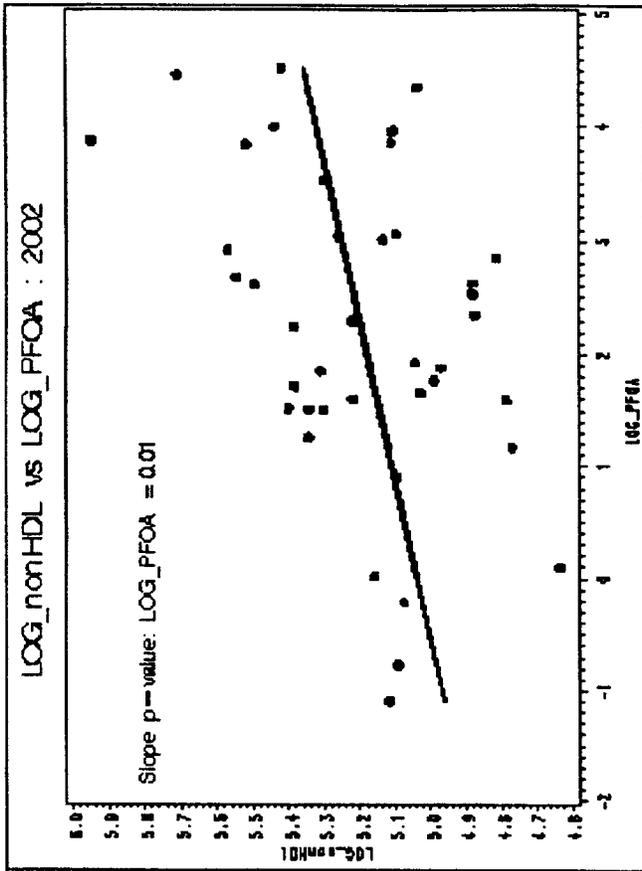


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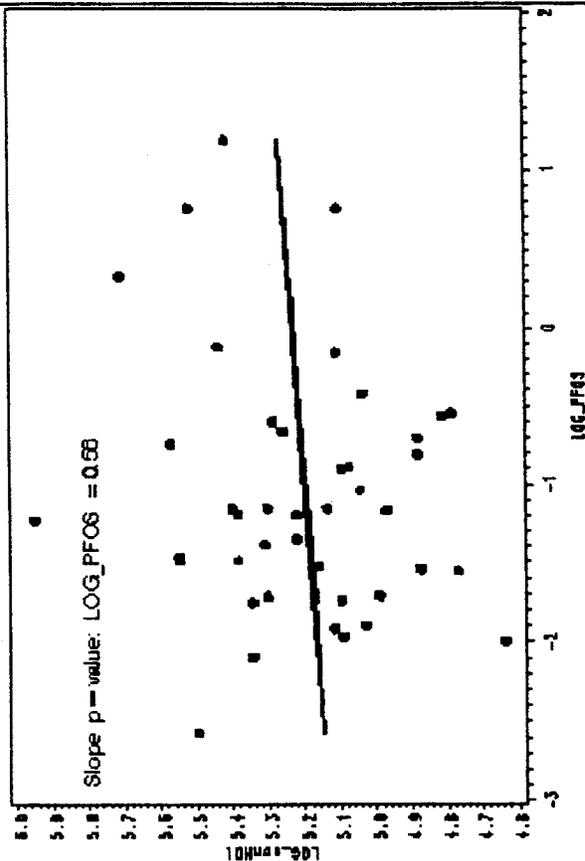


LOG_nonHDL vs LOG_TOTALPF : 2003

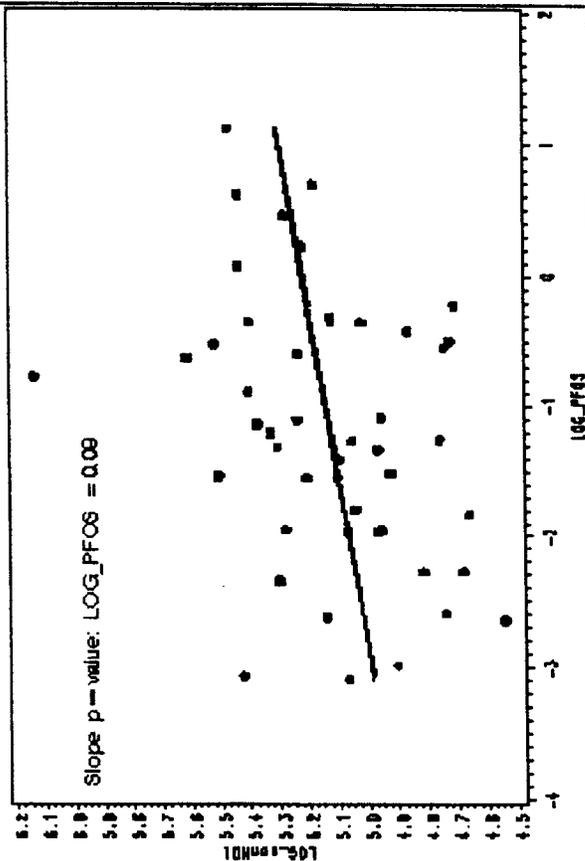




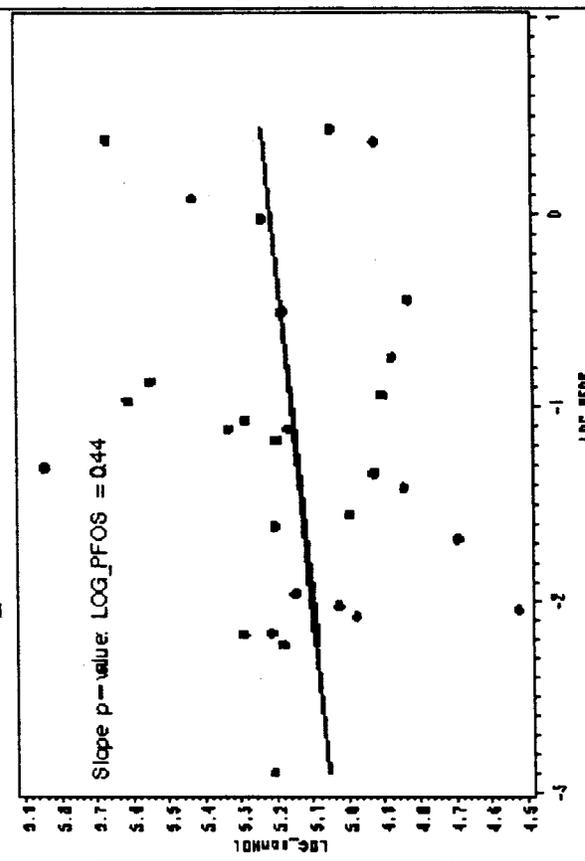
LOG_nonHDL vs LOG_PFO5 : 2002



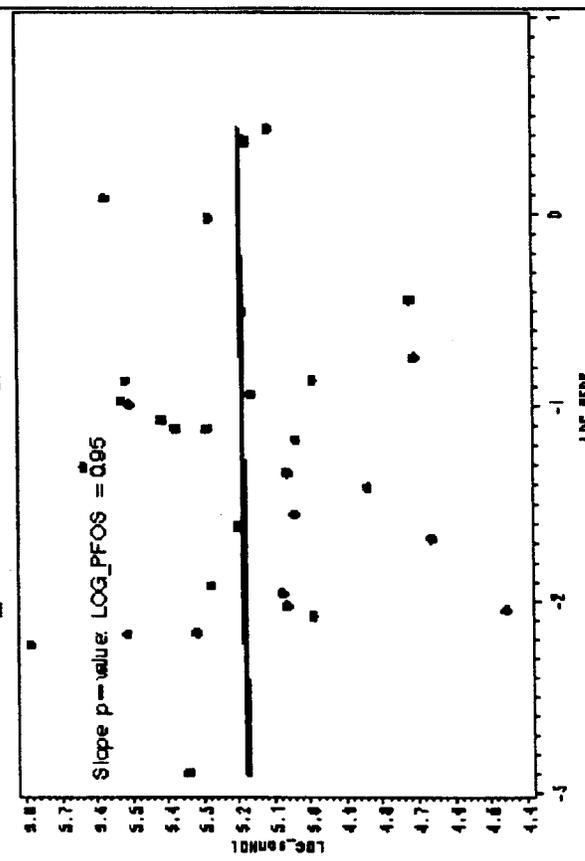
LOG_nonHDL vs LOG_PFO5 : 2001

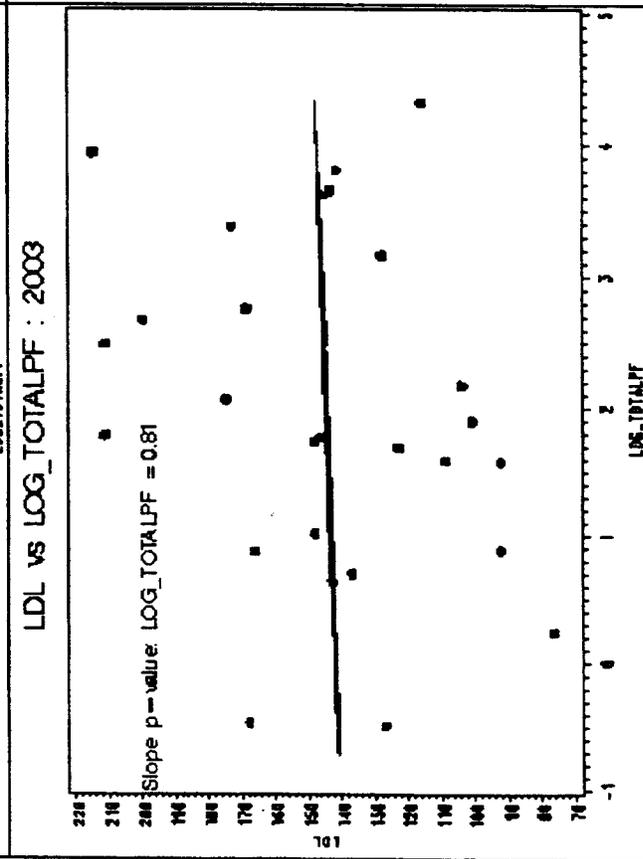
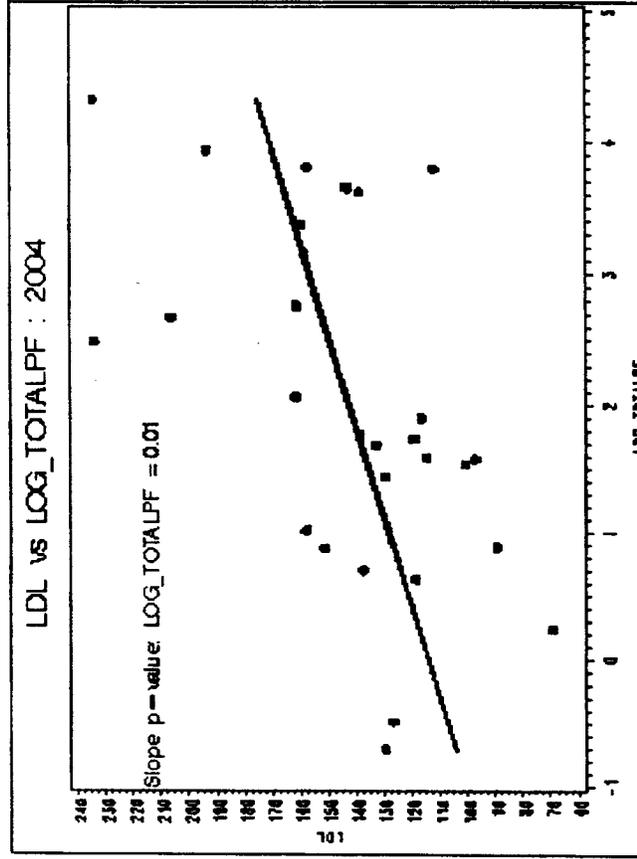
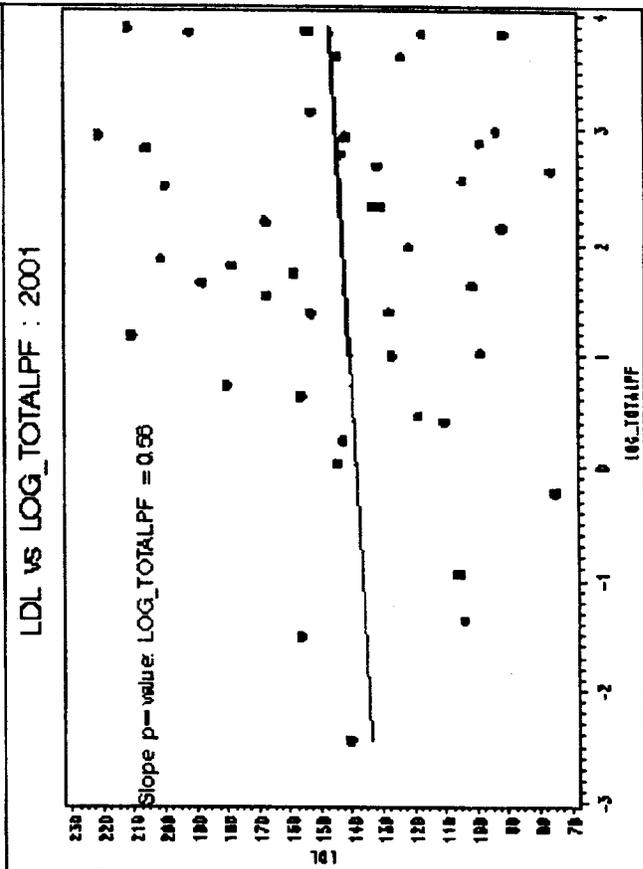
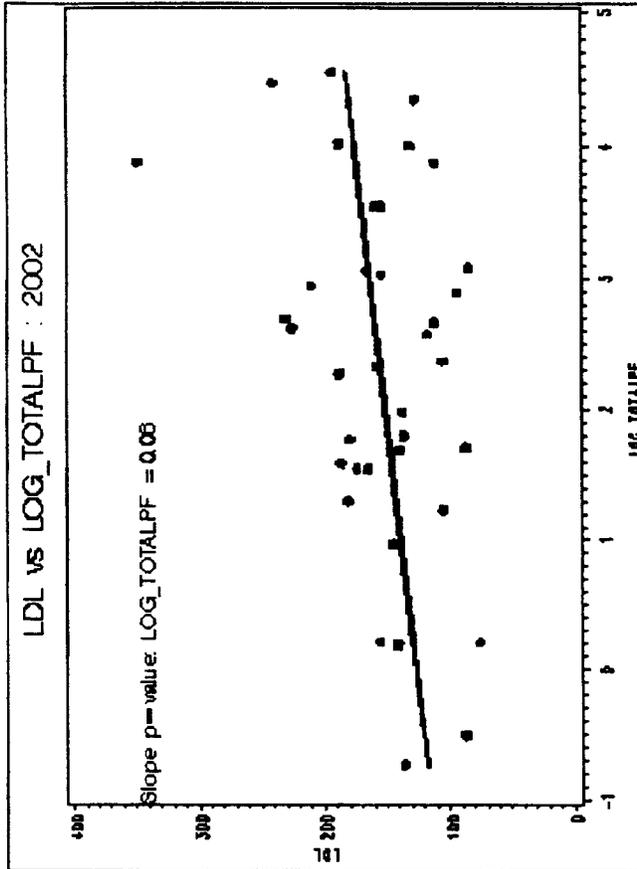


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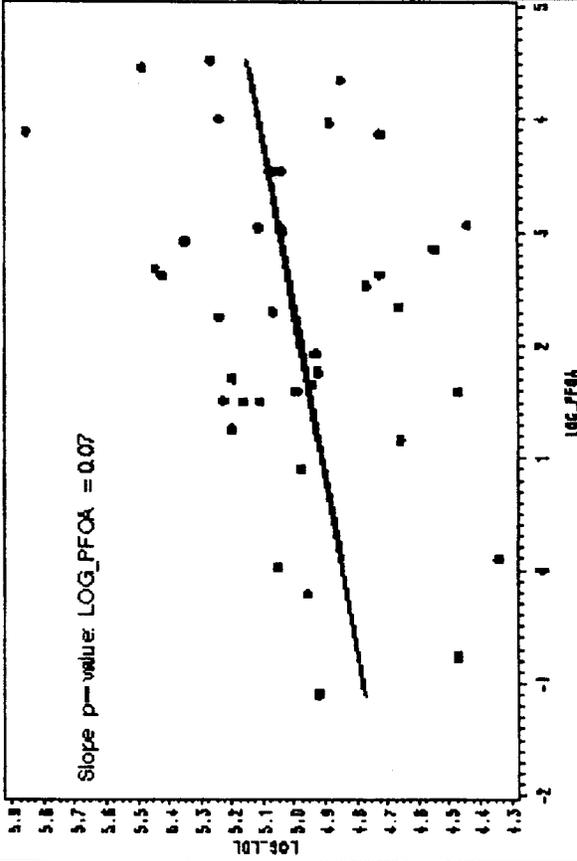


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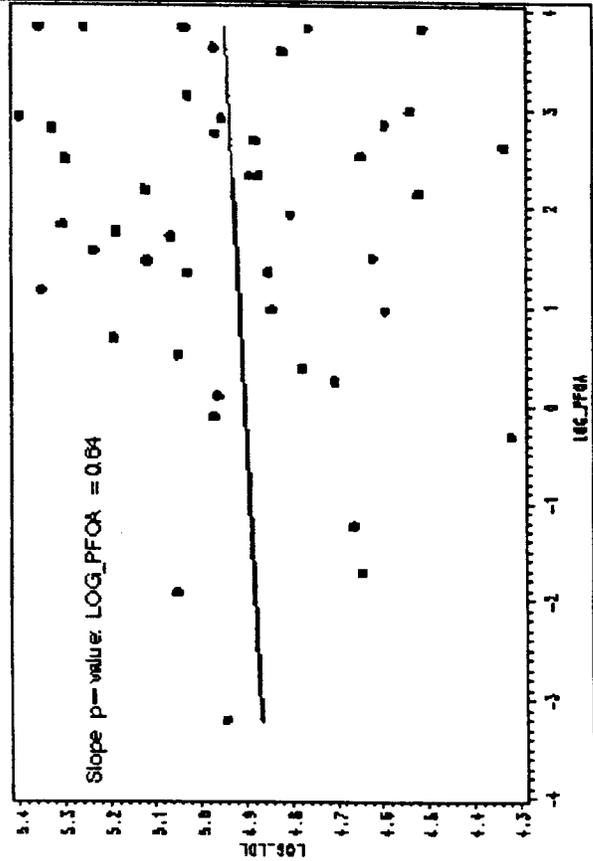




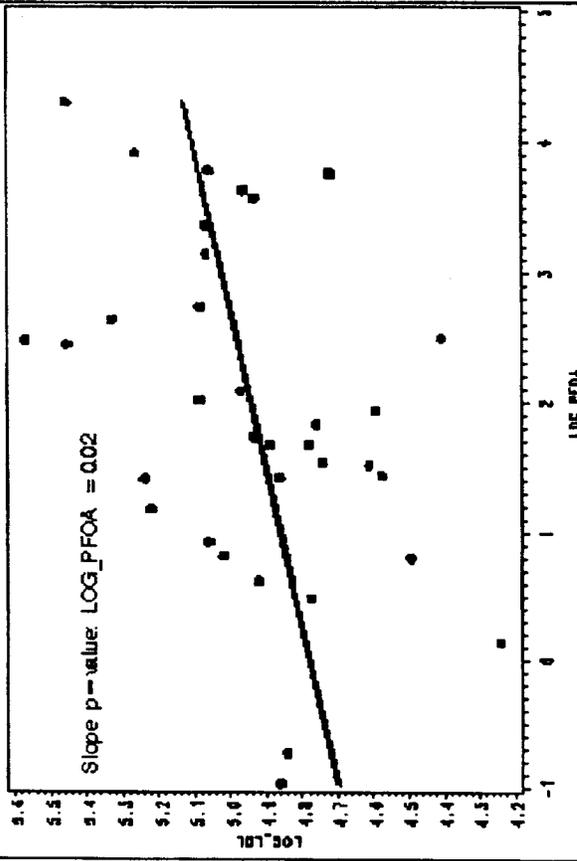
LOG_LDL vs LOG_PFOA : 2002



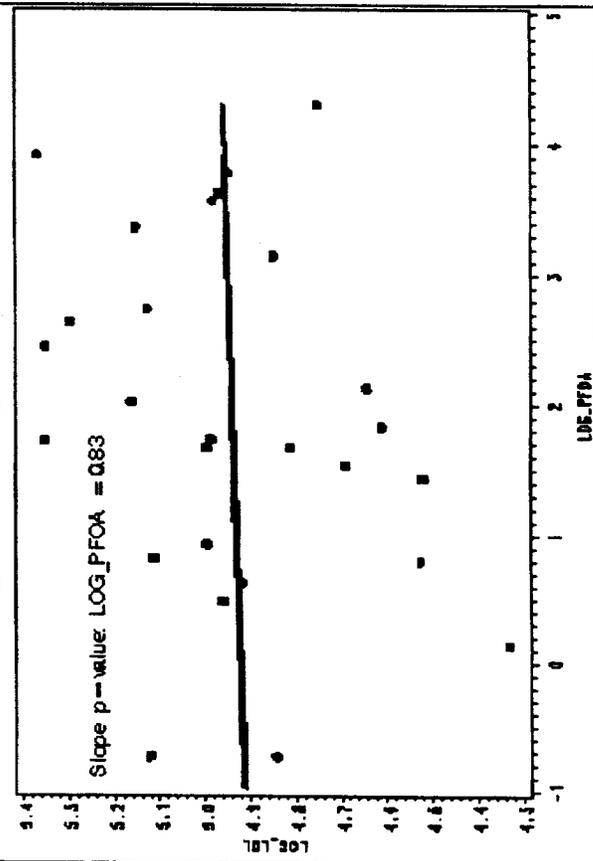
LOG_LDL vs LOG_PFOA : 2001



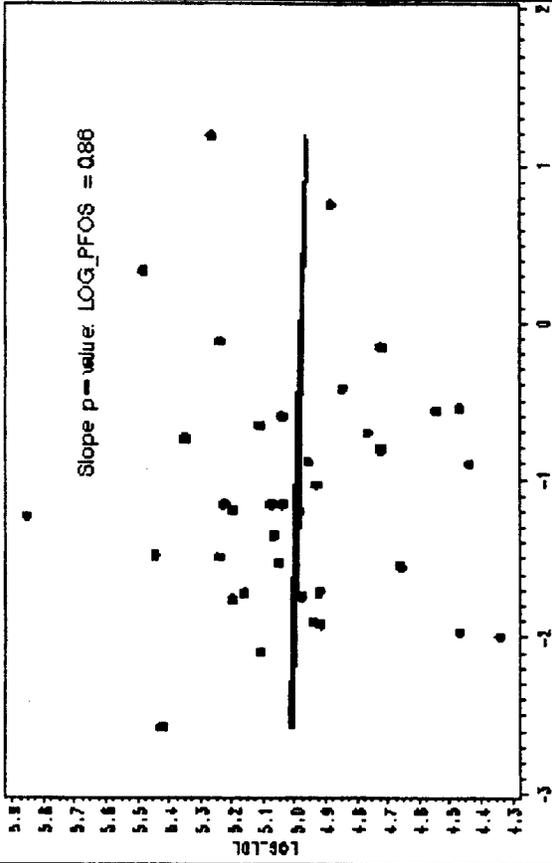
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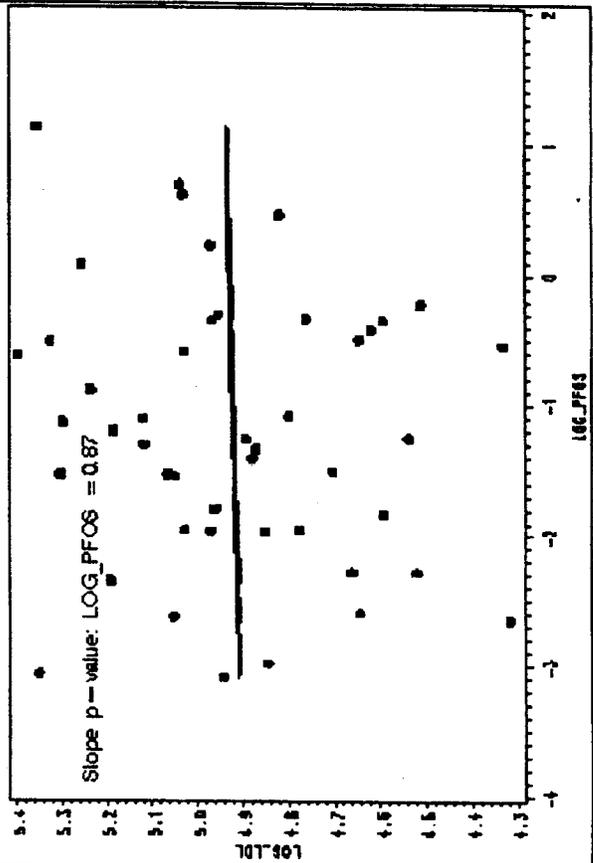
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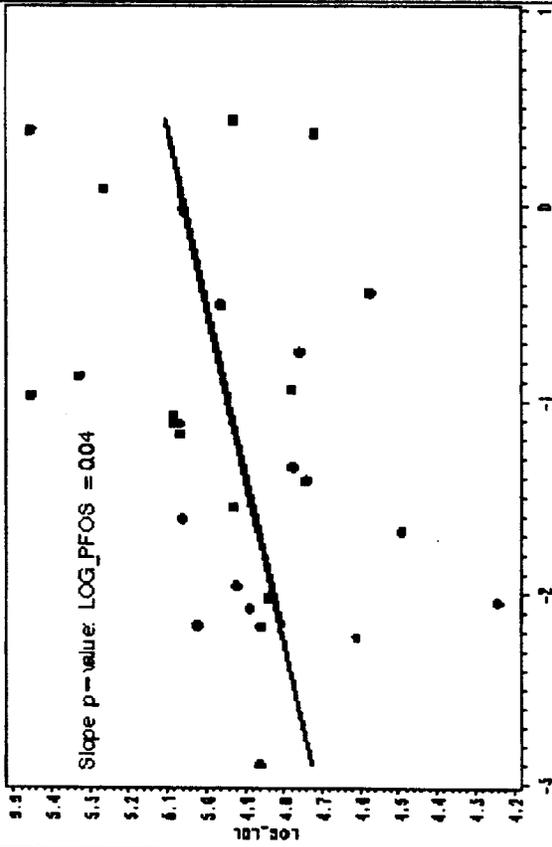
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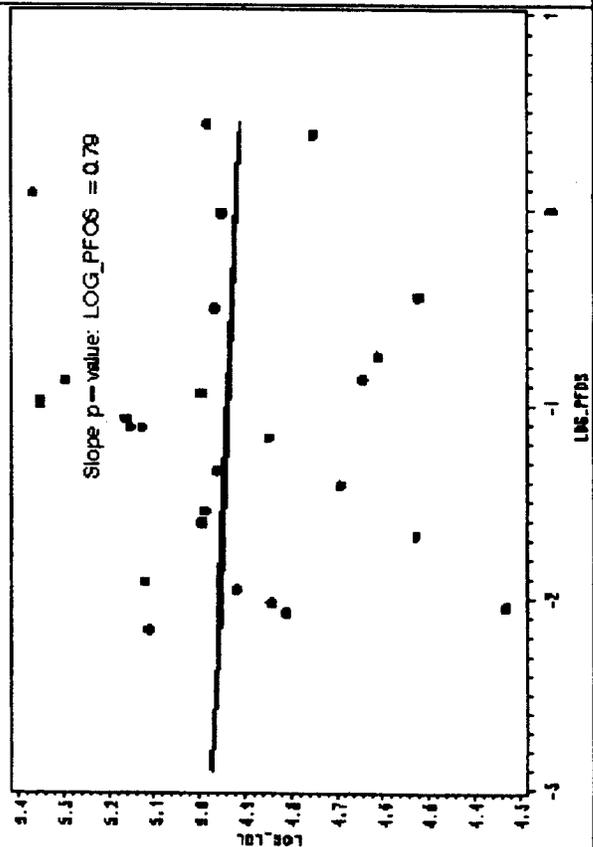
LOG_LDL vs LOG_PFO5 : 2001



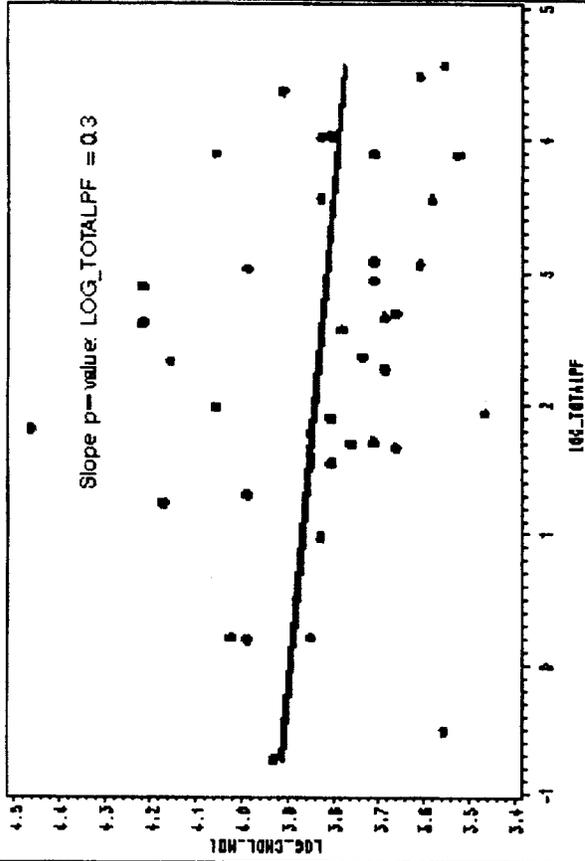
LOG_LDL vs LOG_PFO5 : 2004



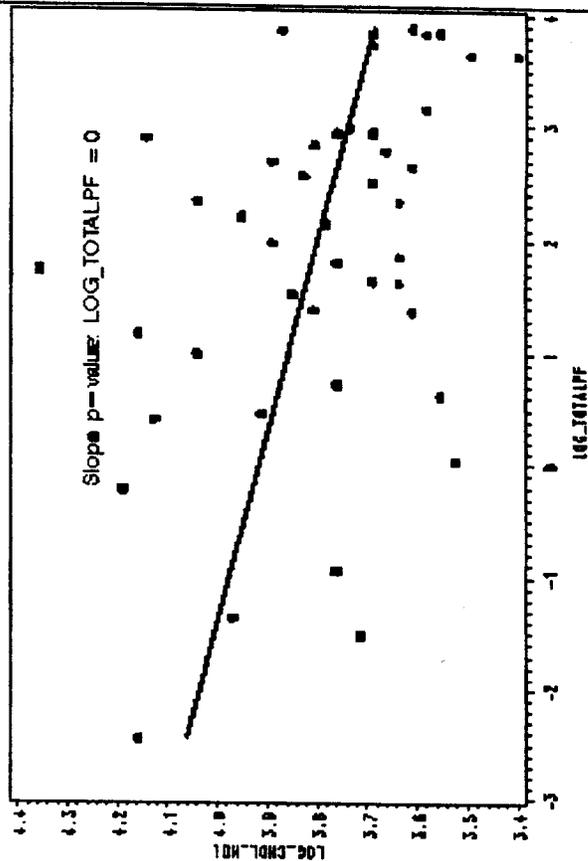
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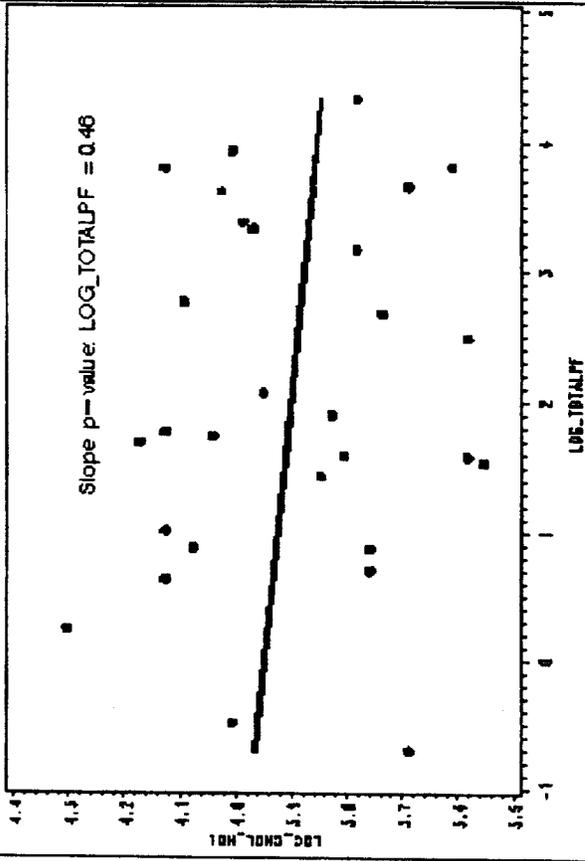
LOG_CHOL_HDL vs LOG_TOTALPF : 2002



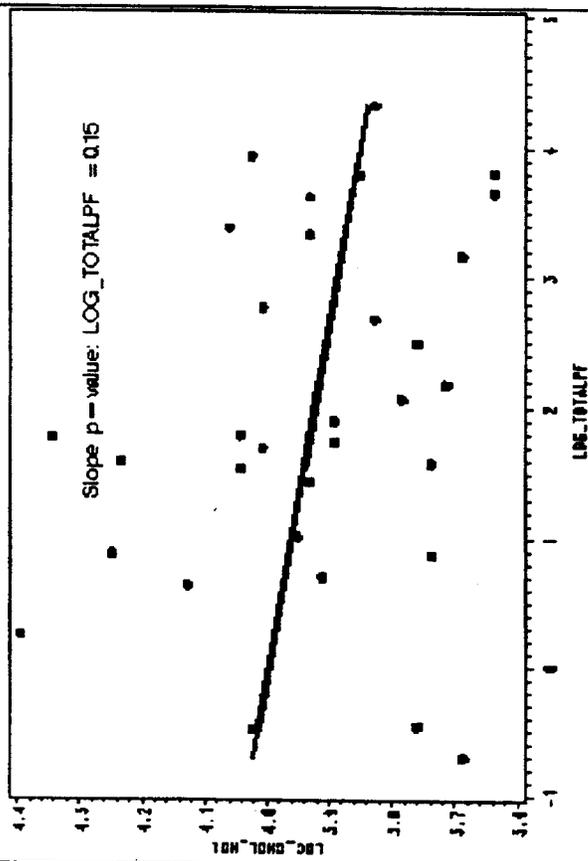
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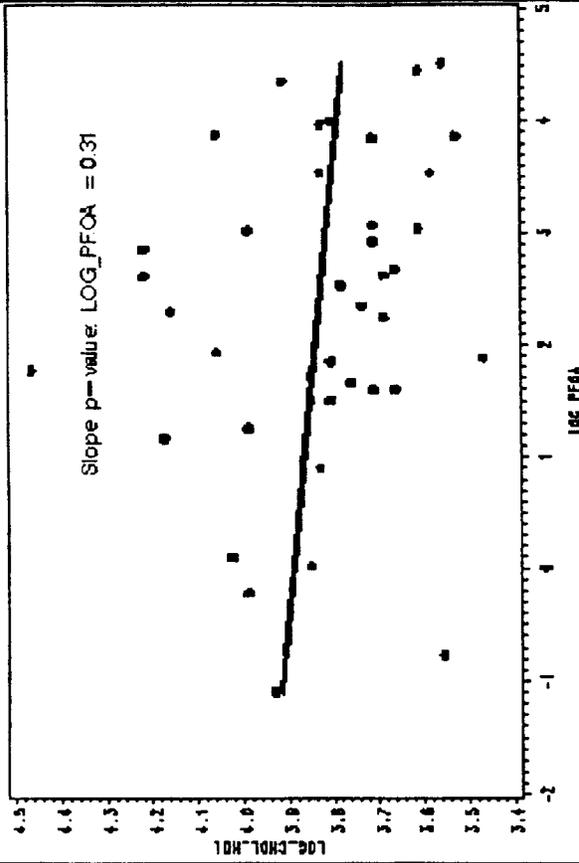
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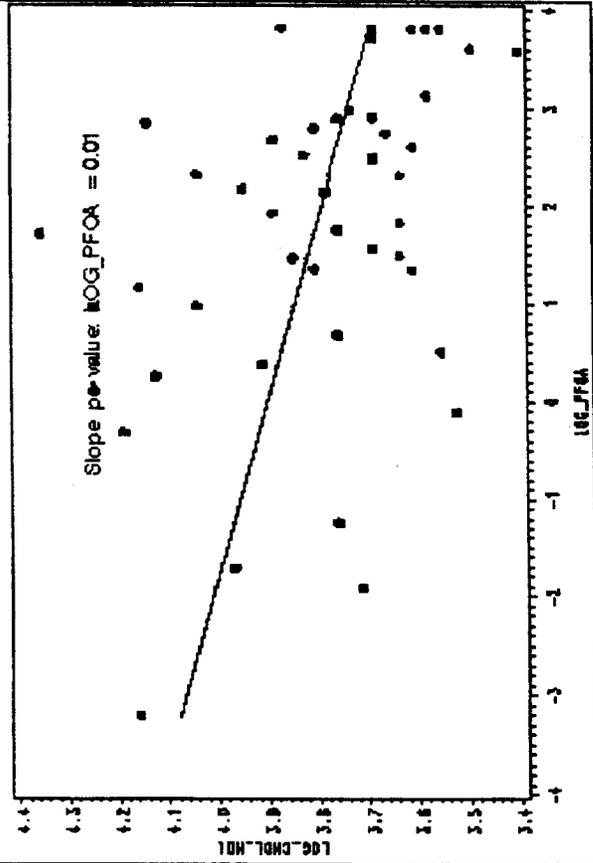
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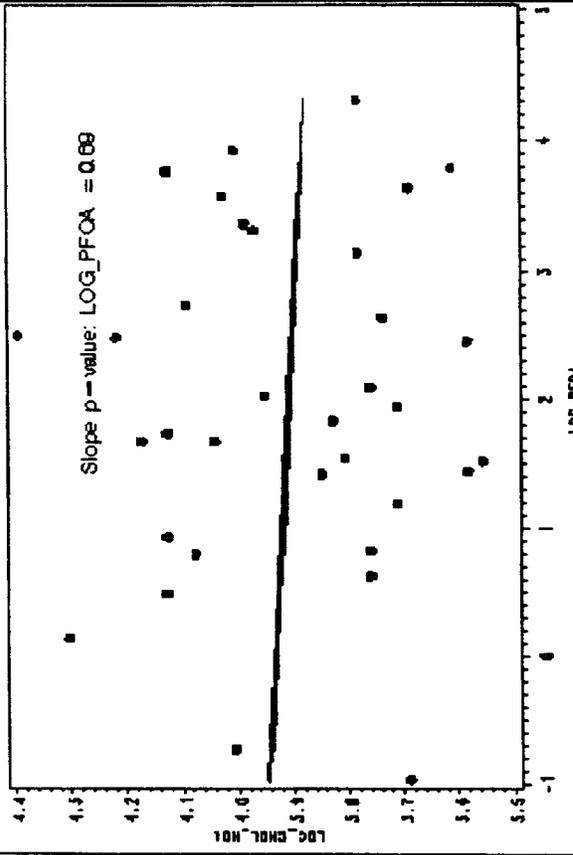
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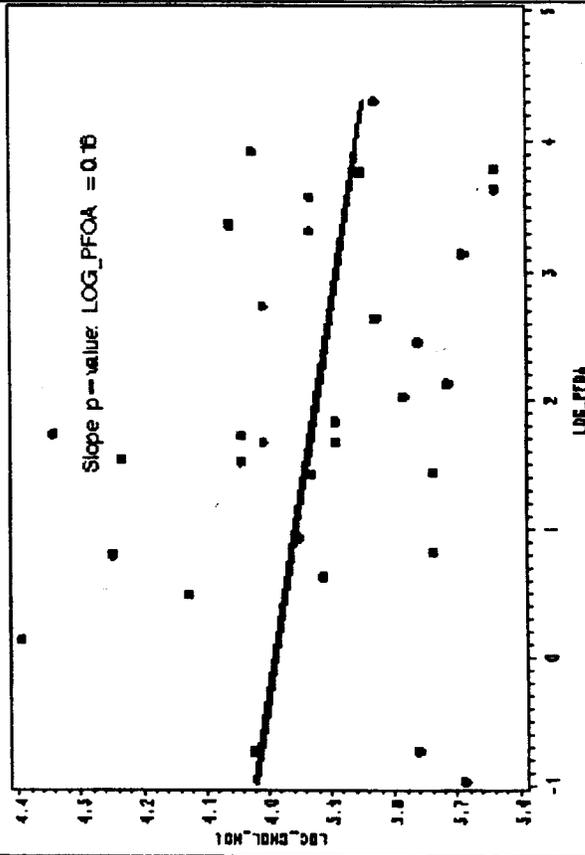
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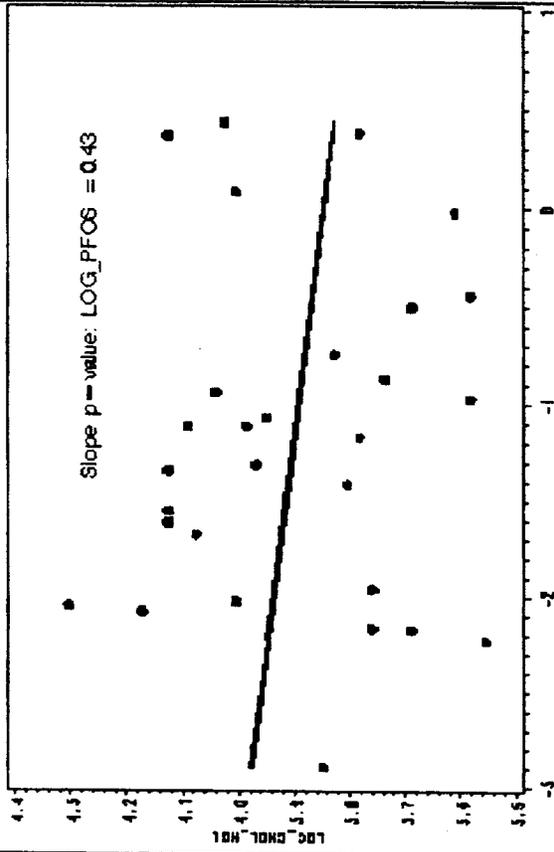
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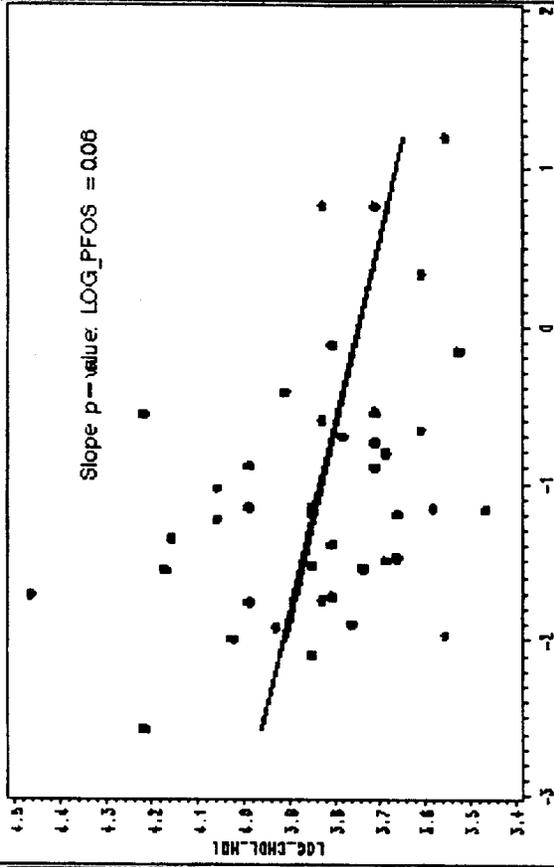
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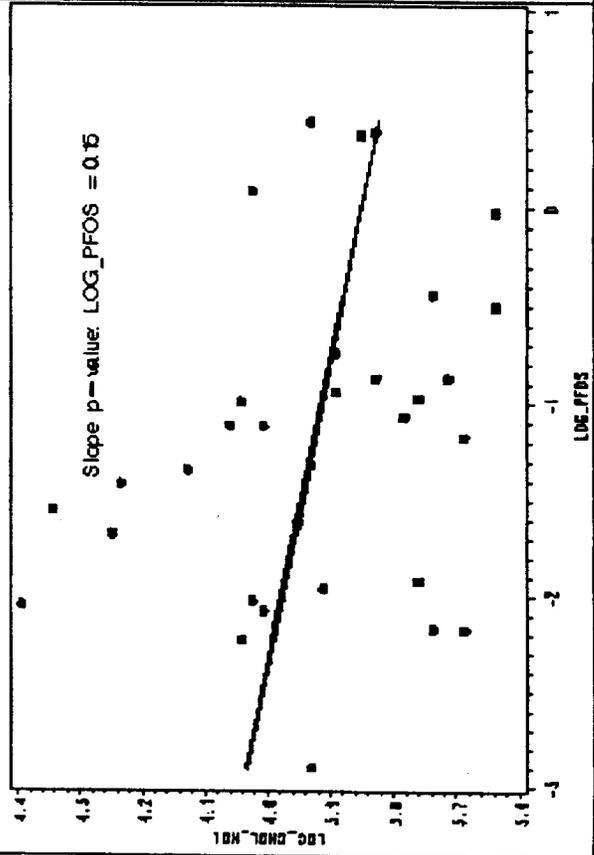
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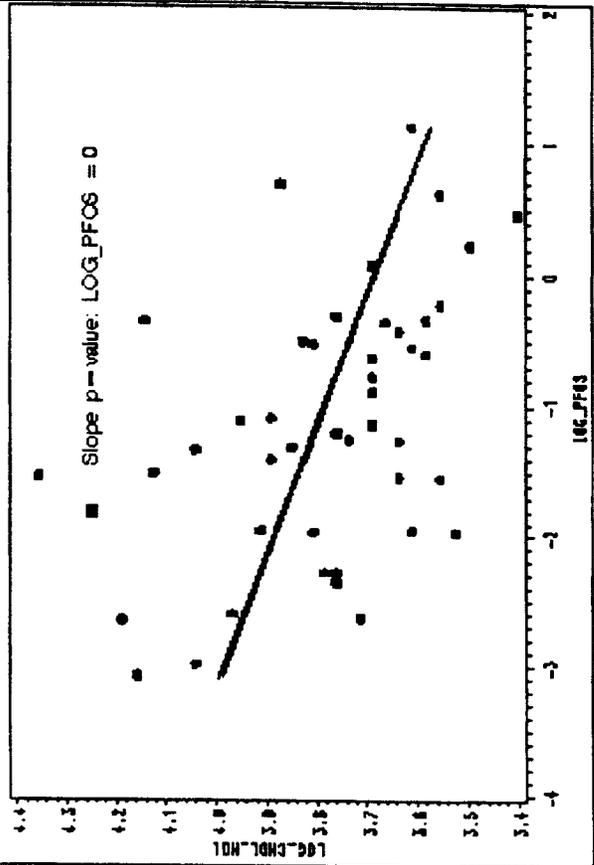
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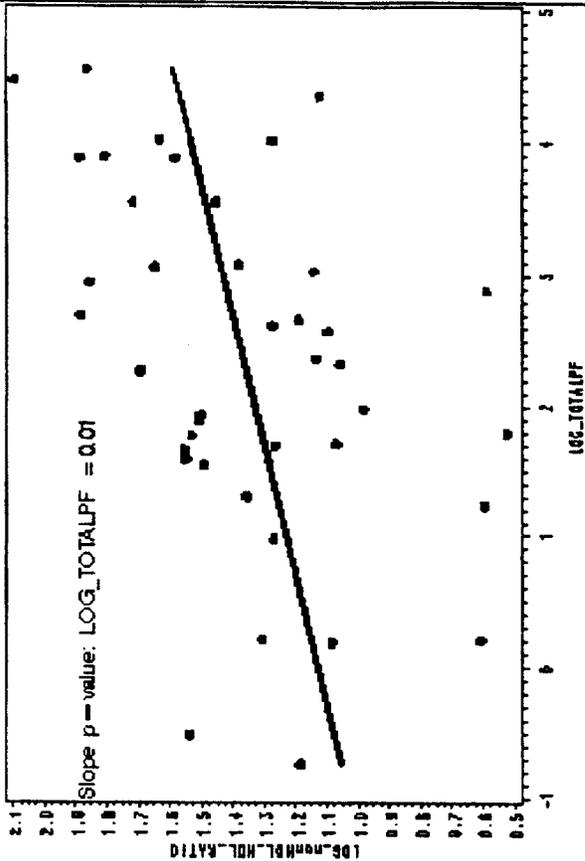
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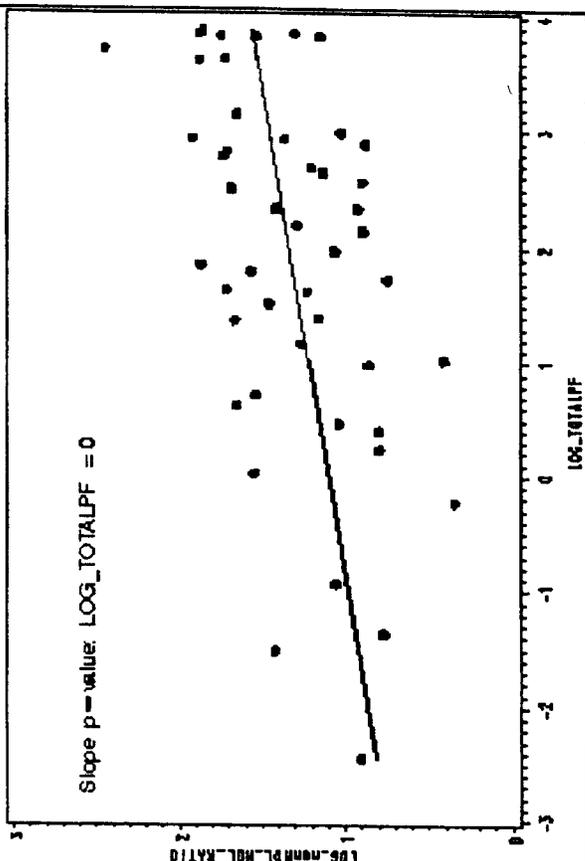
LOG_CHOL_HDL vs LOG_PFO5 : 2001



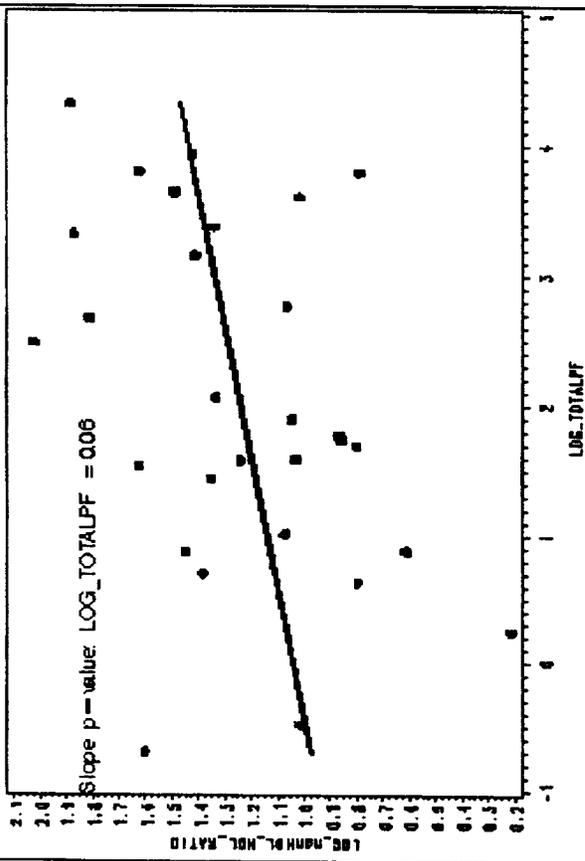
LOG_nonHDL_HDL_RATIO vs LOG_TOTALPF : 2002



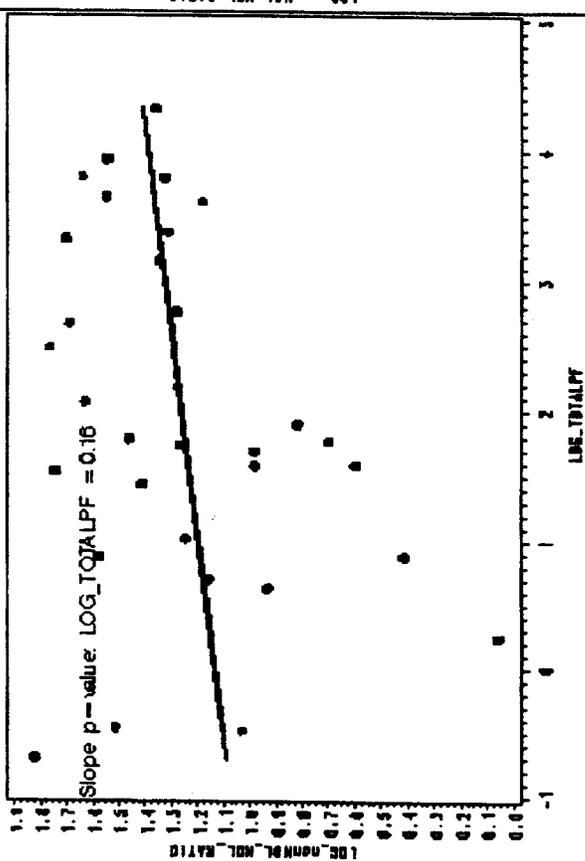
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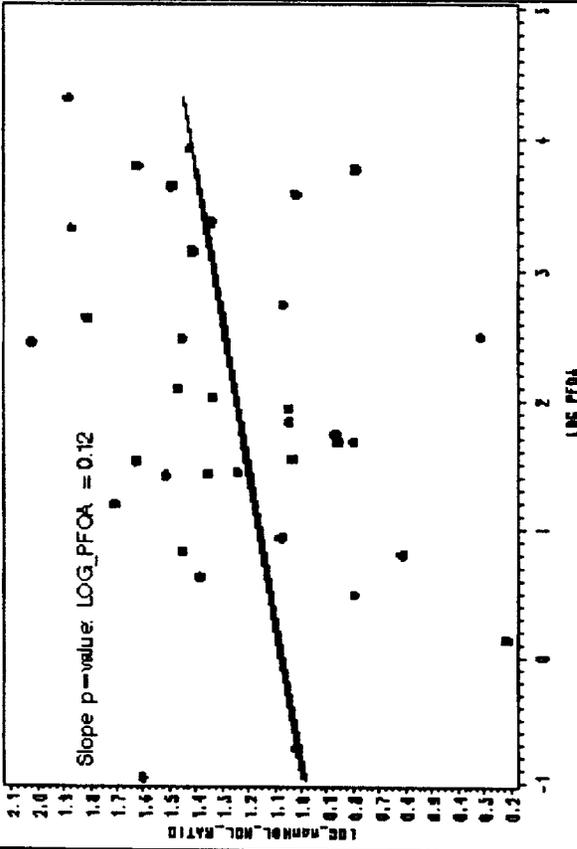
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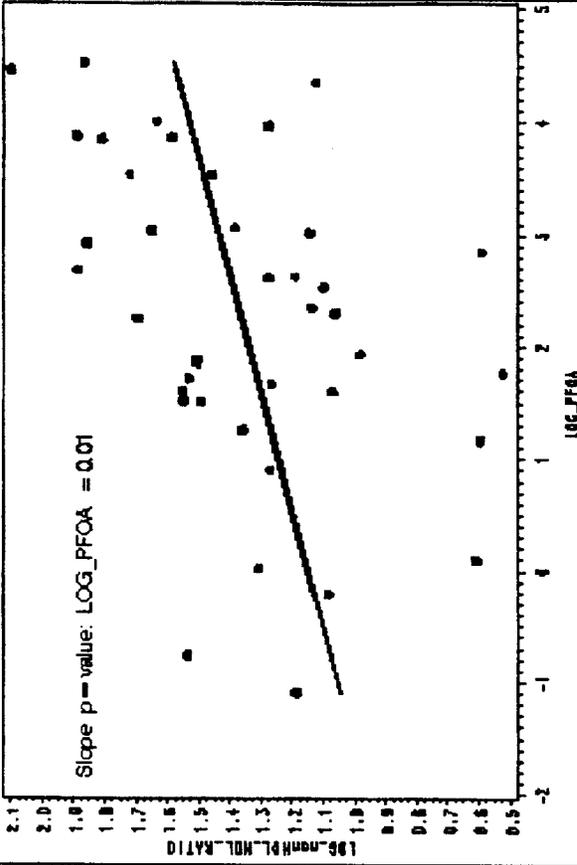
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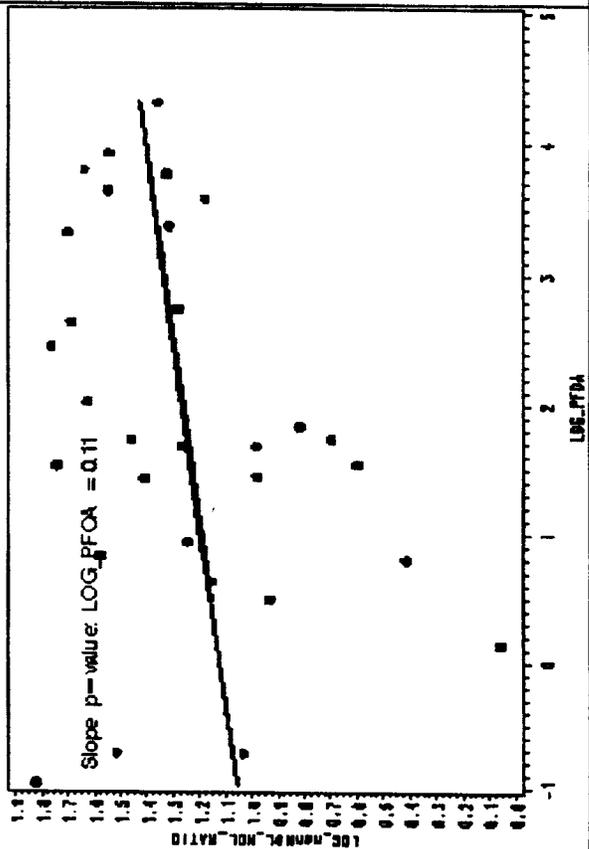
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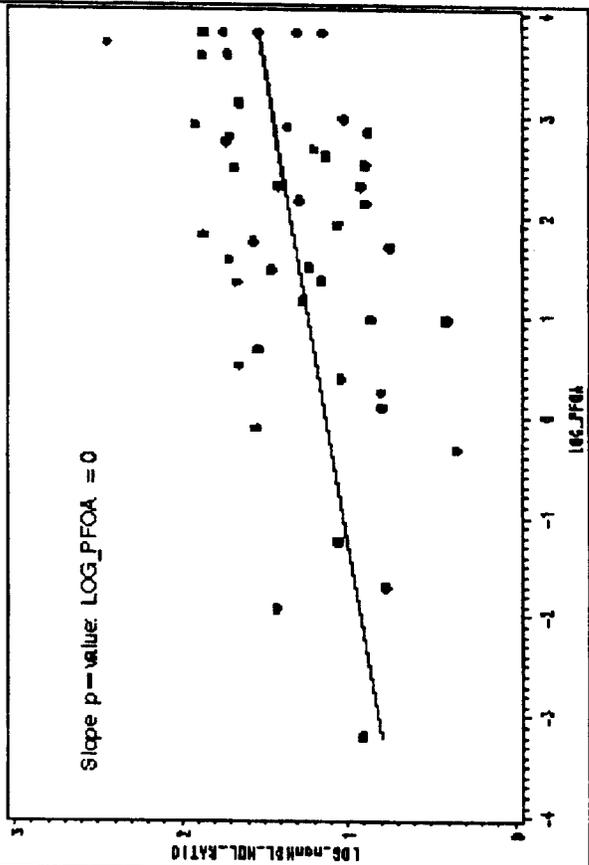
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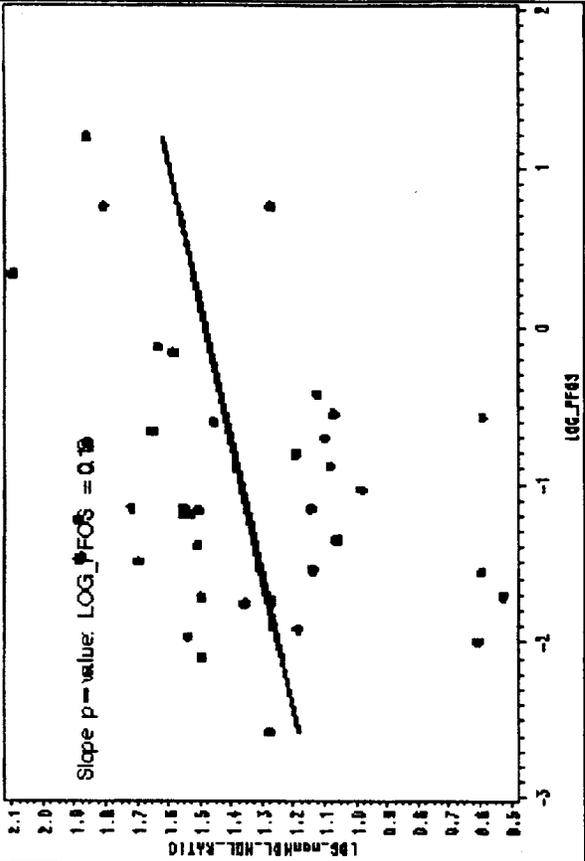
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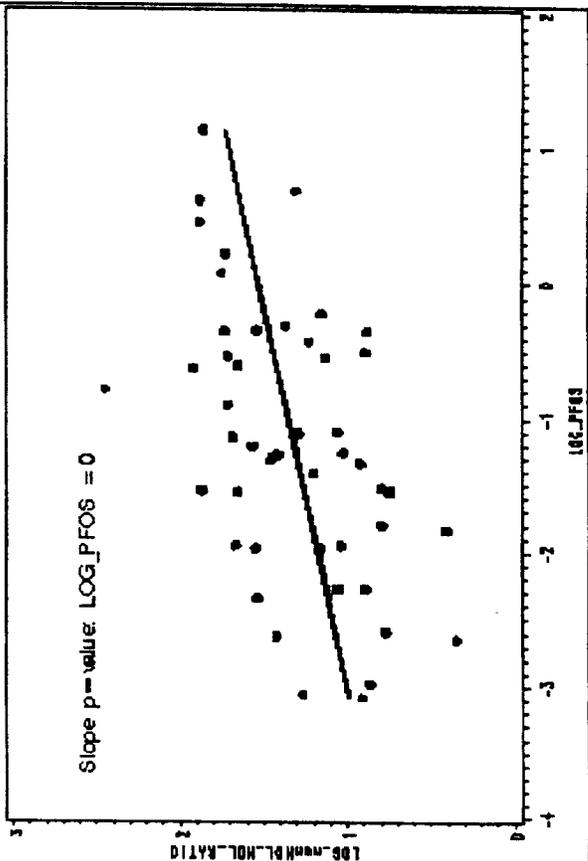
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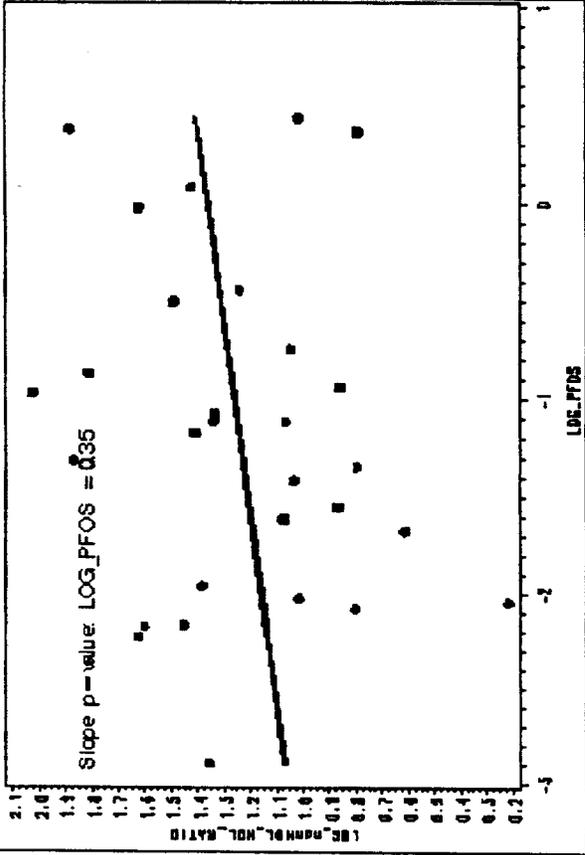
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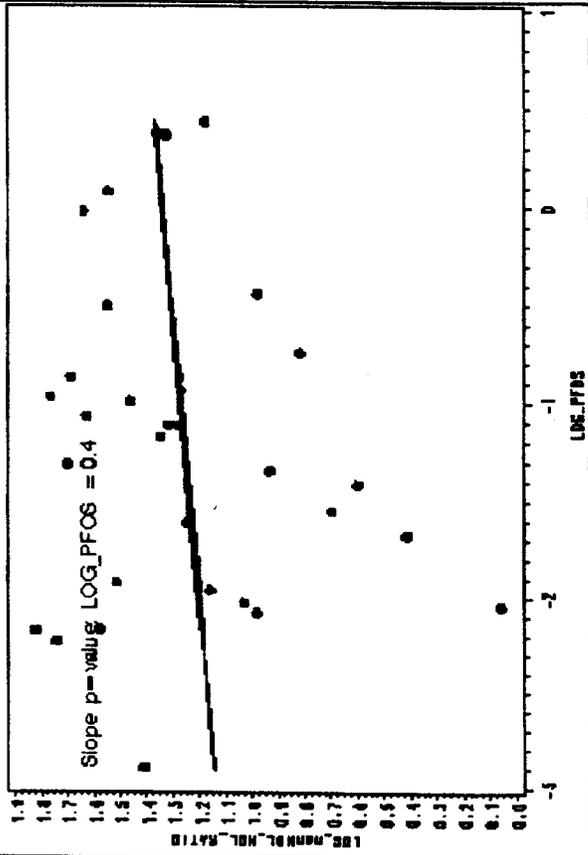
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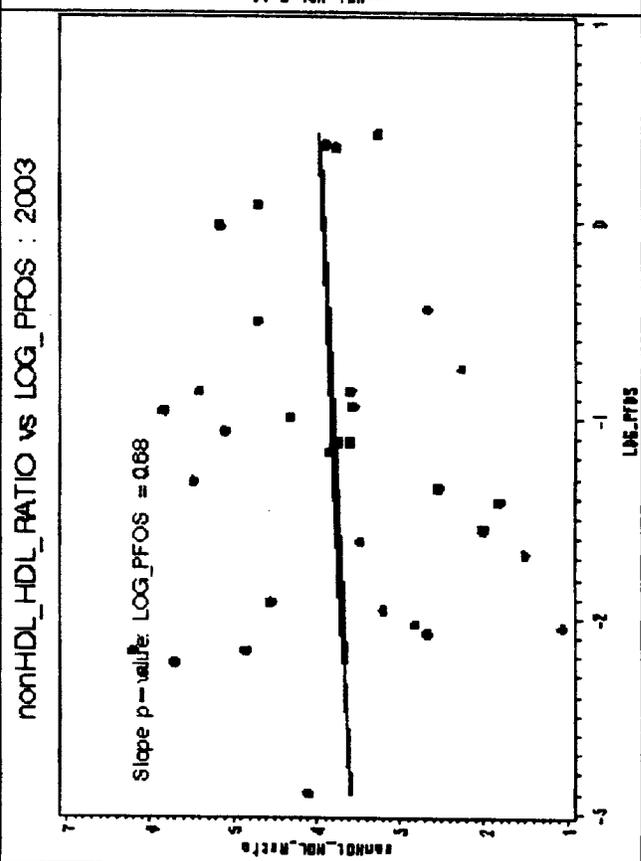
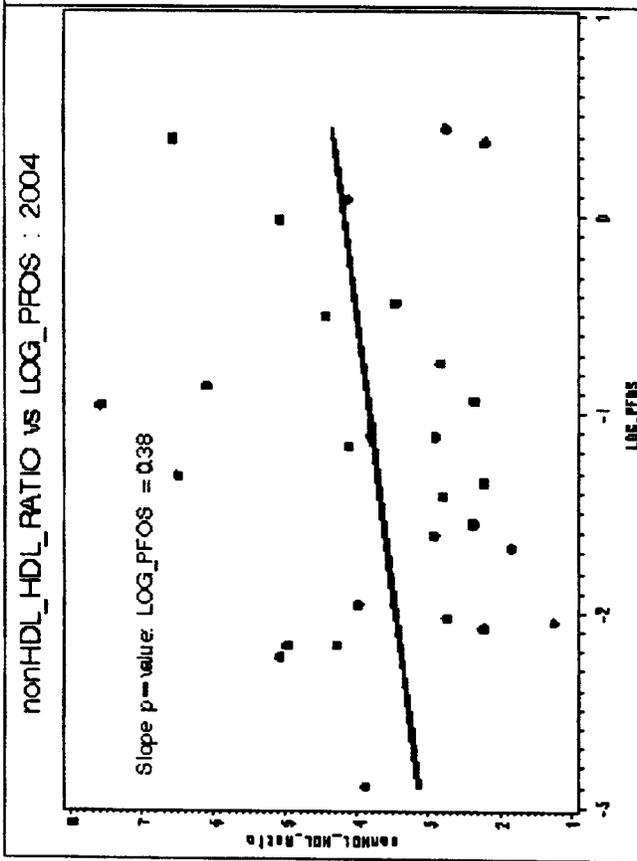
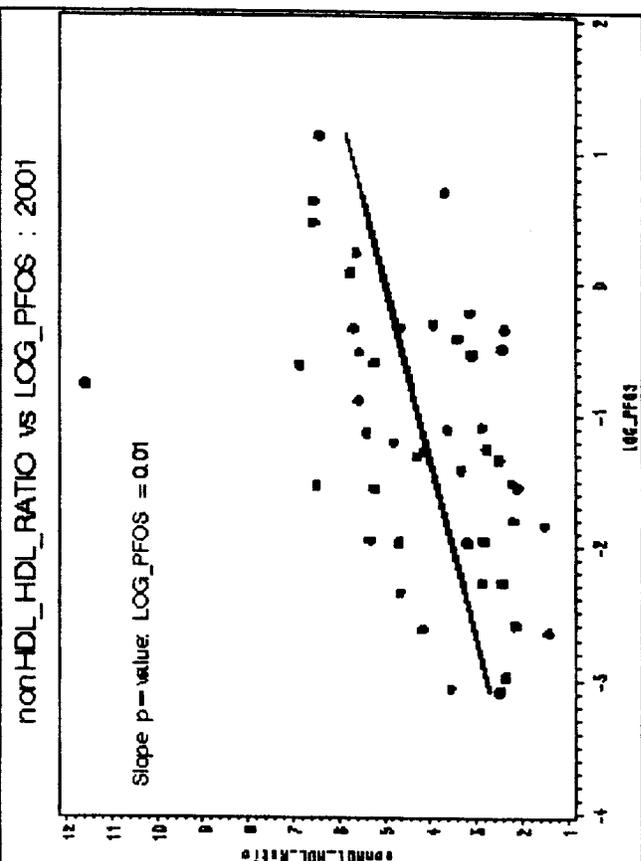
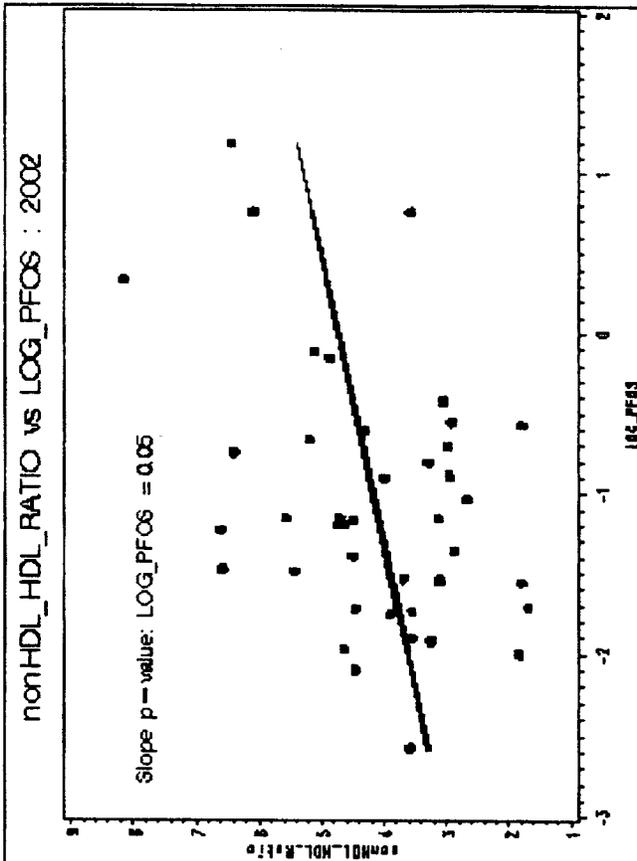


LOG_nonHDL_HDL_RATIO vs LOG_PFO5 : 2004



LOG_nonHDL_HDL_RATIO vs LOG_PFO5 : 2003







January 10, 2005

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Elkton Road, P.O. Box 50
Newark, DE 19714-0050

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Attention: 8(e) Coordinator
Office of Pollution Prevention and Toxics
U.S. Environmental Protection Agency
1201 Constitution Ave., NW
Washington, DC 20460

8EHQ-0105-0394

CONTAINS NO CBI

Dear 8(e) Coordinator:

8EHQ-0381-0394
Ammonium Perfluorooctanoate

This letter is to inform you of the results of the analyses completed to date of the comparison of the serum PFOA levels with the results of the blood and urine medical analysis. Approximately 60 parameters have been analyzed. This sampling is part of an ongoing study, "Ammonium Perfluorooctanoate: Cross-Sectional Surveillance Of Clinical Measures of General Health Status Related to a Serum Biomarker of Exposure and Retrospective Cohort Mortality Analyses in a Polymer Production Plant" of over 1,000 employees at our Washington Works plant. All participants have received their individual exposure levels for serum PFOA and their personal test results. What we are reporting are the results of the grouped analyses of the health outcomes that are completed.

2005 JAN 24 AM 10:37

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Median serum PFOA level of employees who work with PFOA was approximately 0.5 ppm with a maximum of approximately 10 ppm. Median serum PFOA level of employees that do not work with PFOA was approximately 0.1 ppm. The vast majority of parameters measured were within normal reference ranges and were not associated with serum PFOA levels. There were statistically significant but modest increases in some cholesterol fractions (total, and LDL) and triglycerides in the highest serum PFOA exposure group (> 1000 ppb). Serum PFOA levels did not affect HDL cholesterol or C-reactive protein (CRP) levels. As expected, age, body mass index (obesity), and alcohol consumption were also contributors to increases in cholesterol fractions and triglycerides. Other factors, such as genetics and lifestyle, also play a role, but have not been taken into account. There were statistically significant but slight increases in serum uric acid and iron with the highest concentrations of serum PFOA. These and other sporadic changes in clinical laboratory parameters may be spurious and unrelated to serum PFOA. The study, based on about 60 blood and urine tests, found no correlation between liver function and exposure to PFOA, no correlation between blood counts and exposure to PFOA, and no correlation between any cancer markers measured and exposure to PFOA with respect to prostate cancer, leukemia, or multiple myeloma.



In a study that examines as many data points as this one, it is not unusual to find statistically significant associations given the normal variations observed in the general population. Because the data are a one time "snapshot" of both the clinical laboratory and the exposure level data, it is unclear what factor(s) may account for the observed statistical associations. Therefore, both the cause and biological significance of these observed changes are unclear and require further analysis.

A copy of the final report of the larger ongoing study referred to above will be submitted to the Agency when available.

Sincerely,



A. Michael Kaplan, Ph.D.
Director – Regulatory Affairs and Occupational Health

AMK/RWR/RCL:clp
(302) 366-5260

**Results to Date from the
PFOA Worker Health Study
January 11, 2005**

Paul J. Bossert, Jr., Plant Manager

**Robert W. Rickard, Ph.D., Science Director
of Haskell Laboratory for Health and Environmental
Sciences**

Sol E. Sax, M.D., Chief Medical Officer

Results to Date from the PFOA Worker Health Study

Ammonium Perfluorooctanoate:
Cross-Sectional Surveillance of Clinical
Measures of General Health Status
Related to a Serum Biomarker of
Exposure and Retrospective Cohort
Analyses in a Polymer Production
Plant

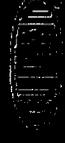
10/10/11

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Summary

To date, there are no human health effects known to be caused by PFOA; several statistical observations merit further study.

- Statistically significant associations are seen with serum PFOA levels and some serum lipid fractions, uric acid, and iron.
- These associations were only seen in those study participants with the highest serum PFOA levels, which were equal to or greater than 1000 ppb.
- DuPont, in collaboration with outside experts, is committed to conducting the studies that are necessary to understand the significance of these observations.



The miracles of science™

Purpose of Study

Phase 1 Cross- Sectional Survey:

Determine if there is a relationship of serum PFOA levels in current employees with respect to medical test results

Phase 2 Historical Mortality Study:

Determine if there is a relationship of past exposures to PFOA and any changes in frequencies and causes of mortality

288 071

General Methods—Phase 1

Cross-Sectional Survey

Voluntary participation across all areas of the plant

Cross-sectional design, that is, “snapshot” of both the exposure marker and the health outcome variables based on complete physical exam and clinical chemistries

Exposure groups, by deciles, used to compare lowest to highest groups based on serum PFOA levels

Statistical analyses for modeling relationships

Status—Phase 1 Cross-Sectional Survey

1,024 employees participated in the cross-sectional health survey

- **All participants have received their individual serum PFOA levels and medical test results.**
- **About 60 parameters have been analyzed to date.**

Not all of the questionnaire data have been analyzed, but these analyses are underway.



General Methods—Phase 2 Historical Mortality Study

Historical study of all causes of death among employees of Washington Works

- **Covers period of about 50 years**
- **Compares frequencies and causes to U.S. national rates, West Virginia populations and the rest of U.S. DuPont**
- **Takes into account estimates of past exposures to PFOA**

Study is in progress.



The miracles of science

Serum PFOA Levels By Work Assignment

Work Assignment	Serum PFOA (ppb)			
	Number in Group	Median	Min	Max
Works in PFOA areas	259	490	17	9550
Previously worked in PFOA areas	264	200	9	2590
Occasionally works in PFOA areas	160	180	8	2070
Never assigned to PFOA areas	342	110	5	963
Total Participants	1025			

General Findings

Nearly all of the parameters measured were within normal reference ranges and not associated with serum PFOA levels:

- No correlation with liver function tests**
- No correlation with blood counts**
- No correlation with any cancer markers measured—
prostate, leukemia, multiple myeloma**



General Findings

Statistically significant but modest increases in some cholesterol fractions (total, LDL) and triglycerides at the highest concentrations of serum PFOA.

Statistically significant but modest increases in uric acid and iron at the highest concentrations of serum PFOA.

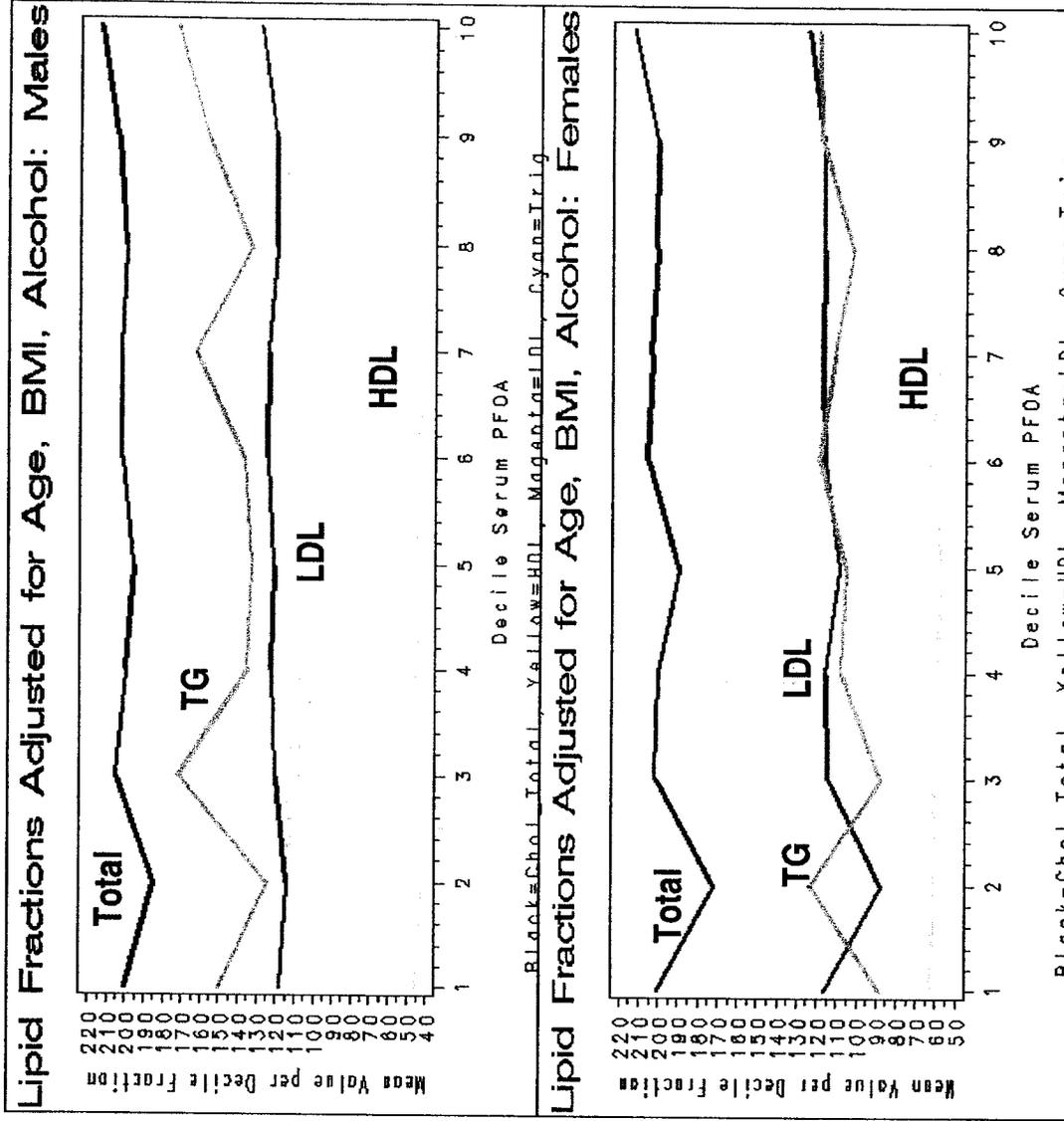
Notably, “good” cholesterol (HDL) was unaffected by serum PFOA levels.

CRP levels (C-reactive protein, a possible risk factor for heart disease) were unaffected by serum PFOA levels.





Mean adjusted lipid values for serum PFOA levels indicate a modest increase in highest decile (>1000 ppb)

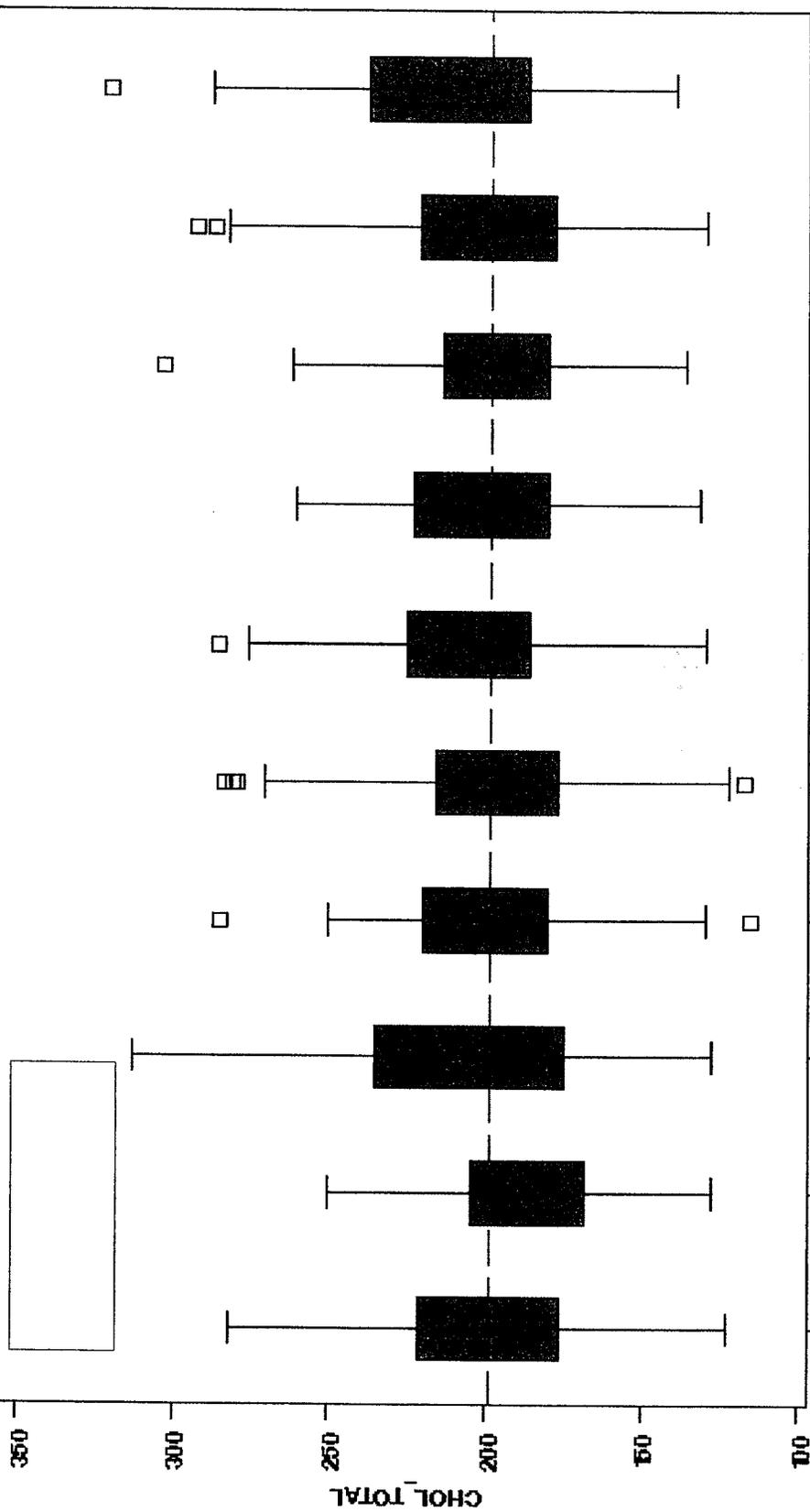




CHOL_TOTAL by PFOA Quantile

Sex=M, Heartmeds=N, MW 2004

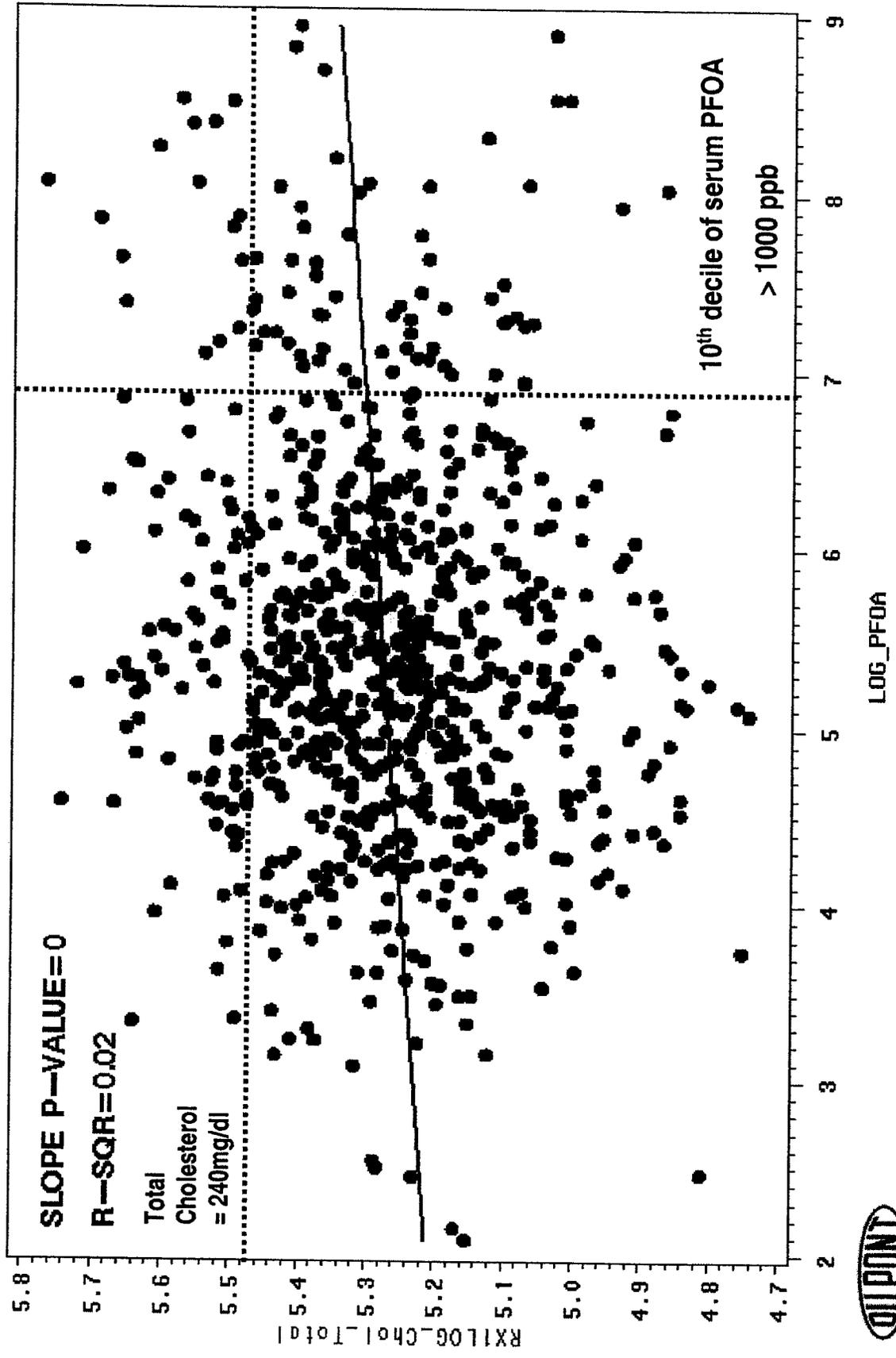
Mean	198.823	187.016	205.629	198.857	196.306	204.635	200.871	198.873	206.097	214.587
Std Dev	32.345	28.787	39.434	31.089	37.476	32.835	30.472	31.022	36.740	36.646



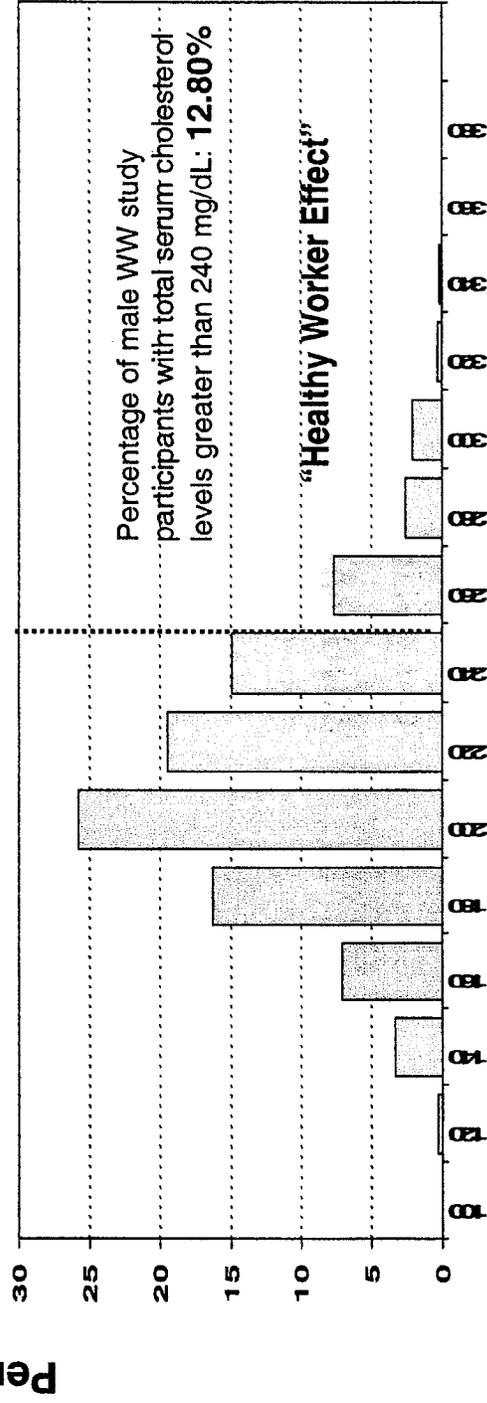
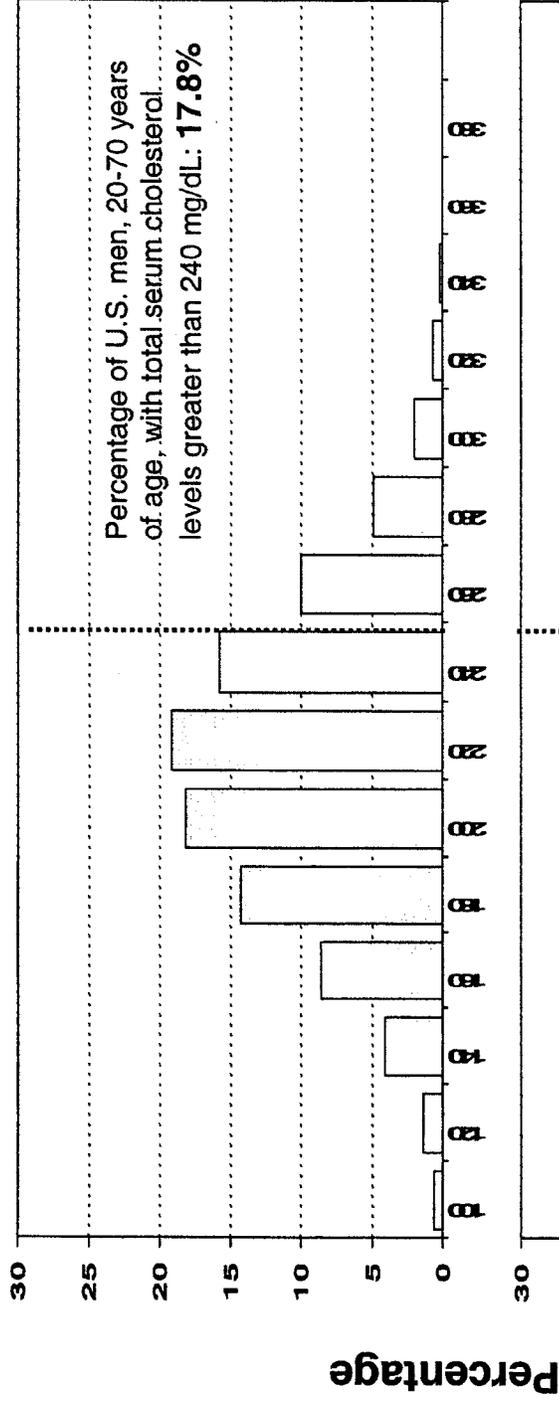
Deciles

LOG_Chol_Total vs LOG_PFOA : WW 2004, Terms = LogPFOA ALO6

Where Sex=M, Heartmeds=B



Overall Comparison of Male Washington Works Study Participants with the General US Population



Summary

To date, there are no human health effects known to be caused by PFOA; several statistical observations merit further study.

- Statistically significant associations are seen with serum PFOA levels and some serum lipid fractions, uric acid, and iron.
- These associations were only seen in those study participants with the highest serum PFOA levels, which were equal to or greater than 1000 ppb.
- DuPont, in collaboration with outside experts, is committed to conducting the studies that are necessary to understand the significance of these observations.



Plans for Further Work

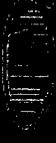
DuPont Medical, Epidemiology and Toxicology will work with medical and other scientific experts to design studies to answer remaining questions:

Are these observations reproducible?

Are similar associations seen in other worker populations?

Is there a cause and effect?

Is there a biological basis for these associations?



The miracles of science

Implications of Study Beyond Plant

Not a general public health issue:

- Associations were only seen in an occupational setting.
- PFOA levels in general public extremely low.

Not a consumer health issue:

- DuPont research has demonstrated that no detectable serum PFOA levels would result from the use of consumer articles made with DuPont products

11/11/00

The miracles of science.

2004 Progress Report Reduction of C8 Emissions & Discharges at DuPont Washington Works

Emissions Reduction

2004 Progress Report
Reduction of C8 Emissions & Discharges at
DuPont Washington Works

	1999	2004 YE	Reduction
Air	31209	185	99.4%
Water	55597	1542	97.2%
Total	86806	1727	98.0%

Emissions and Exposure Reduction

- **More than \$21.5 million invested since 1988; another \$6.2 million planned by 2006.**
- **DuPont is making recovery/recycle technology available to competitors.**

**Results to Date from the
PFOA Worker Health Study
January 11, 2005
Questions and Answers**

Paul J. Bossert, Jr., Plant Manager

Robert W. Rickard, Ph.D., Science Director
of Haskell Laboratories for Health and
Environmental Sciences

Sol E. Sax, MD, Chief Medical Officer

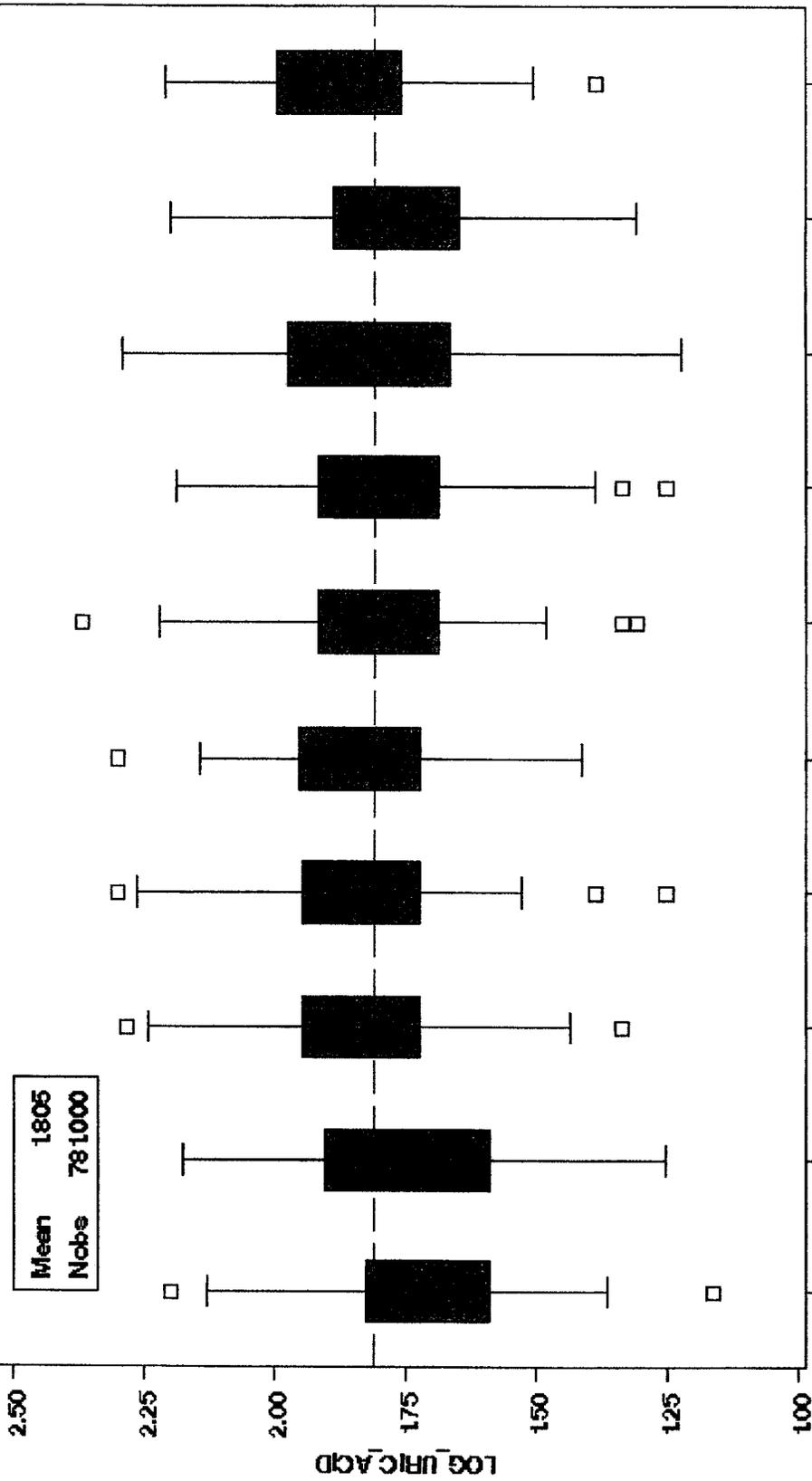


URIC_ACID by PFOA Quantile

Sex=M, Heartmeds=B, MW 2004

	1735	1755	1822	1828	1820	1805	1810	1816	1777	1875
Mean	1735	1755	1822	1828	1820	1805	1810	1816	1777	1875
Std Dev	0.198	0.209	0.179	0.183	0.177	0.189	0.205	0.205	0.180	0.178

Mean 1805
Nobs 781000



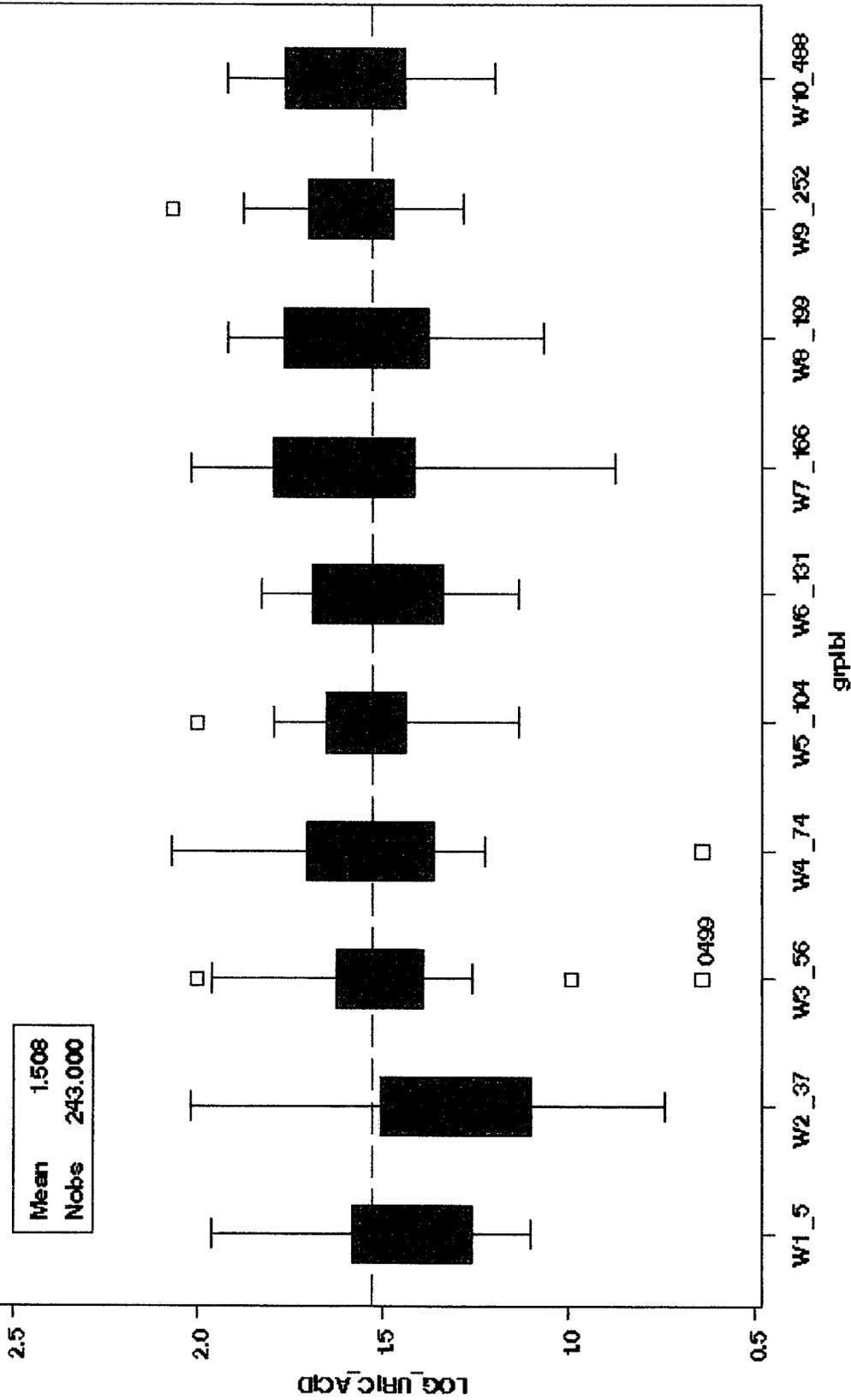
W1_8 W2_67 W3_97 W4_133 W5_169 W6_210 W7_266 W8_367 W9_537 W10_1040
PFOA Quantiles



URIC_ACID by PFOA Quantile

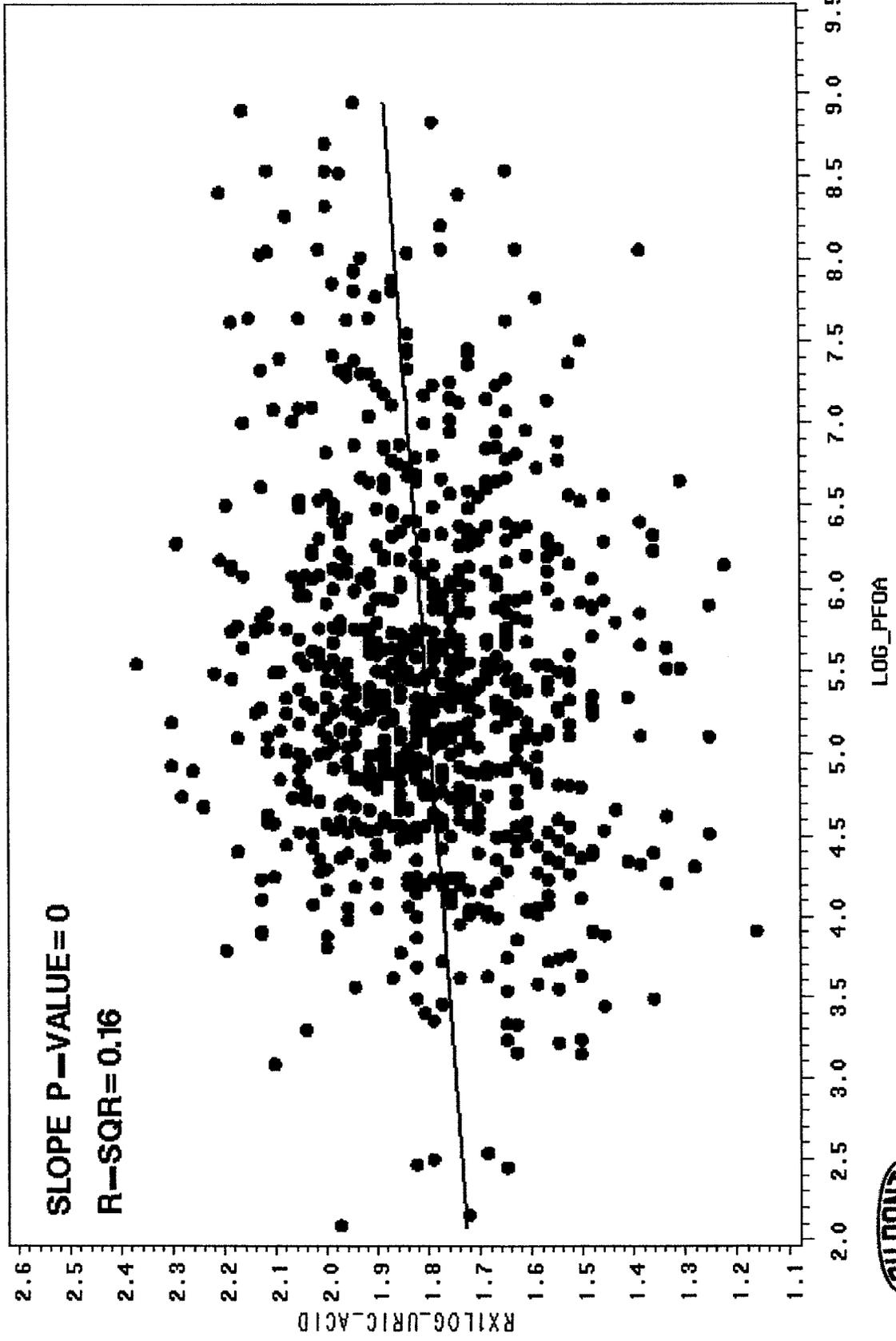
Sex=F, Heartmeds=B, MW 2004

	1410	1314	1495	1486	1542	1533	1568	1552	1594	1582
Mean										
Std Dev	0.213	0.309	0.285	0.287	0.195	0.192	0.275	0.242	0.175	0.222



LOG_URIC_ACID vs LOG_PFOA: WW 2004, Terms= LogPFOA BMI ALC4

Where Sex=M, Heartmeds=B





Results to Date from the PFOA Worker Study

Ammonium Perfluorooctanoate: Cross-Sectional Surveillance of Clinical Measures of General Health Status Related to a Serum Biomarker of Exposure and Retrospective Cohort Analyses in a Polymer Production Plant

Robin C. Leonard, Ph.D.

Principal Epidemiologist, DuPont Haskell Laboratory for
Health and Environmental Sciences



Agenda

- Purpose of Study
- General methodology
- Results to date (clinical pathology parameters)
- Summary
- Timeline
- Further Work



Purpose of Study

- Primary objectives :
 - Develop statistical models that *describe the relationship of serum PFOA to health outcome variables* suggested by previous animal and worker studies, taking into account potential confounders and effect modifiers.
 - Conduct retrospective cohort mortality analyses using appropriate stratification based on estimated past exposures to PFOA.



General Methodology

- Voluntary participation across all areas of the plant
- Cross-sectional design, that is, “snapshot” of both the exposure marker and the health outcome variables
 - Cross-sectional studies address “person and place”, but not “time”—the descriptive triad of epidemiology
 - No data collected over time, no baseline
 - Cannot determine causality, only identify associations
- Logistic, linear, and quadratic regression analyses for modeling
- Deciles of exposure used for internal comparisons



General Findings

- Most of the parameters measured were within normal reference ranges and not associated with serum PFOA levels.
- There were statistically significant ($p \leq 0.05$) modest increases in some cholesterol fractions (total, LDL) and triglycerides with higher concentrations of serum PFOA.
- HDL cholesterol was not associated with serum PFOA levels.
 - As expected, age, body mass index, and alcohol consumption were also contributors to increases in lipids.
- CRP levels (C-reactive protein) were not associated with **serum PFOA levels.**



Results from Clinical Chemistries

- 1,024 employees participated in the cross-sectional health surveillance
 - All participants have received their individual serum PFOA levels and medical test results
 - 62 parameters have been analyzed
- Not all of the questionnaire data have been analyzed, but these analyses are underway
- Restrospective cohort analyses not completed
 - NDI data received Dec. 21, 2004



General Findings

- There were no consistent relationships between results of liver tests and serum PFOA concentrations.
 - Different responses in males and females
 - Alcohol consumption was a factor, but inconsistent as to category and between genders
- There were statistically significant, but slight, increases in uric acid and iron with higher concentrations of serum PFOA.
 - Increased uric acid has been associated with increased lipids.



Serum PFOA Levels By Work Assignment

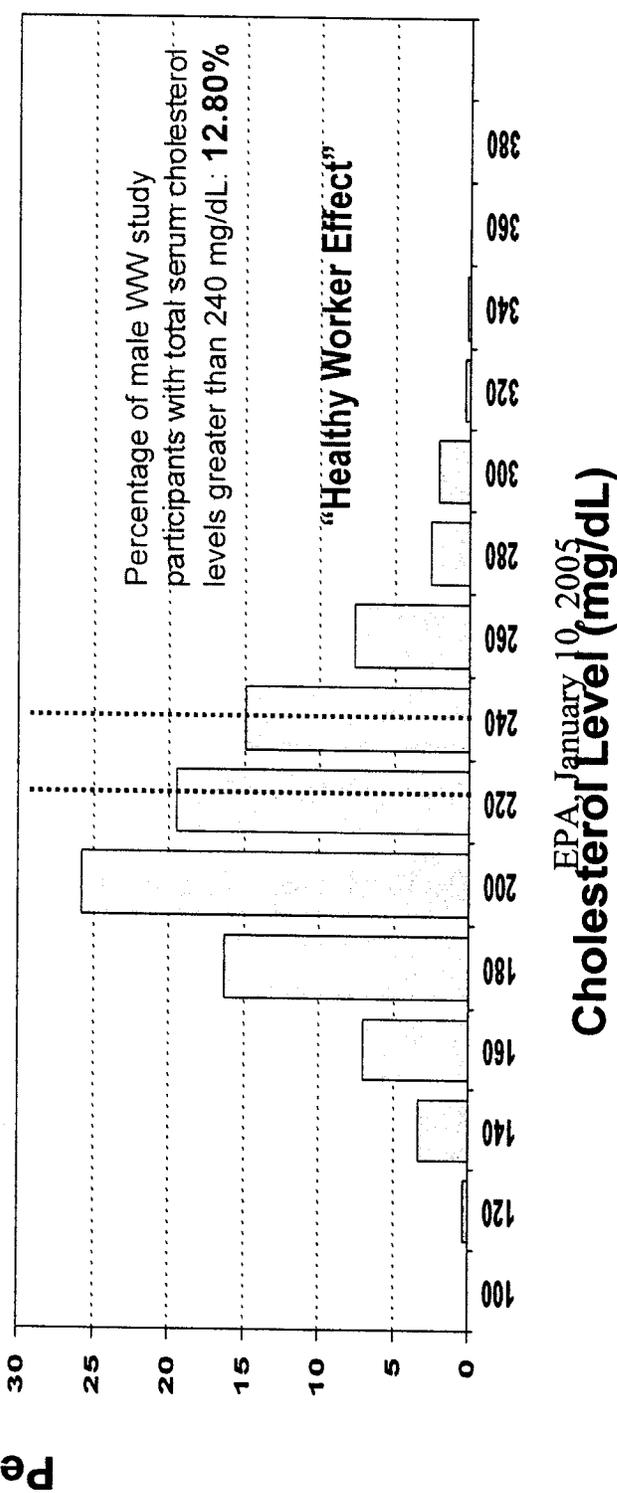
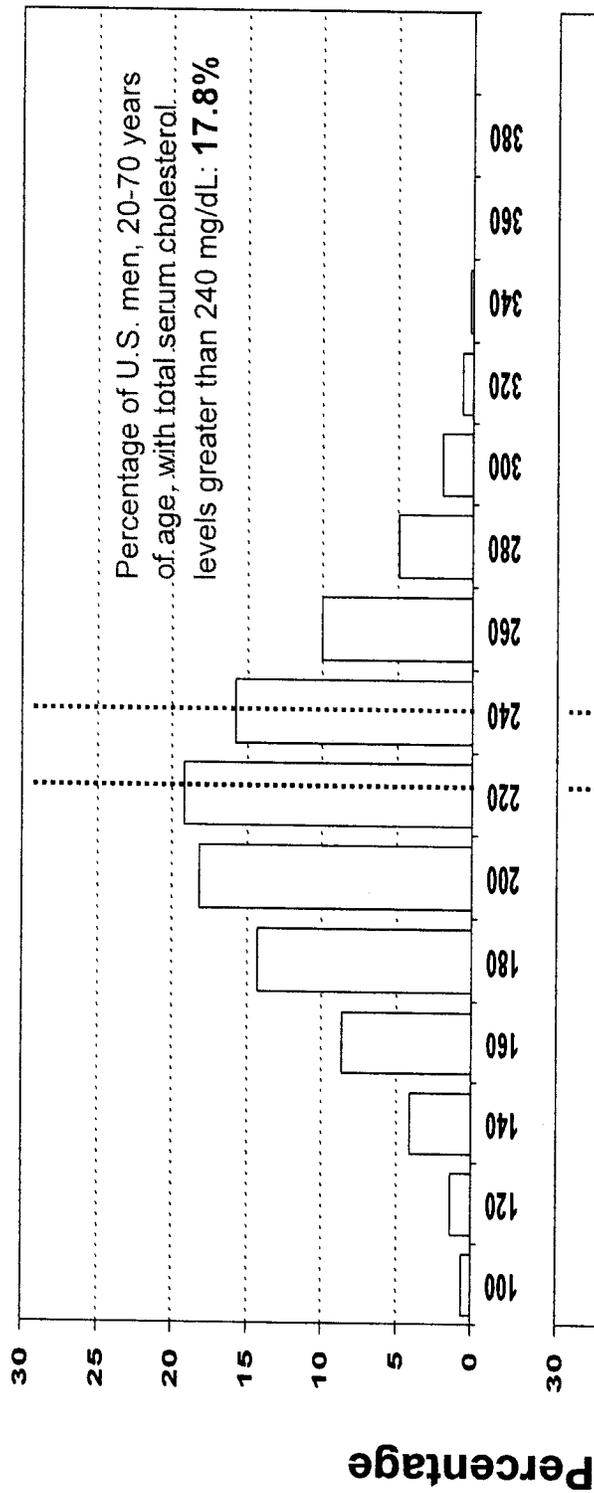
Work Assignment	Serum PFOA (ppb)			
	Number in Group	Median	Min	Max
Works in PFOA areas	259	490	17	9550
Previously worked in PFOA areas	264	200	9	2590
Occasionally works in PFOA areas	160	180	8	2070
Never assigned to PFOA areas	342	110	5	963
Total Participants	1025			



Context for Cholesterol Levels

- Male study participants' total cholesterol and LDL levels were compared to U.S. males using NHANES data.
- Study participants had a smaller percentage of males with high cholesterol than the U.S. population, except for the top decile of serum PFOA.

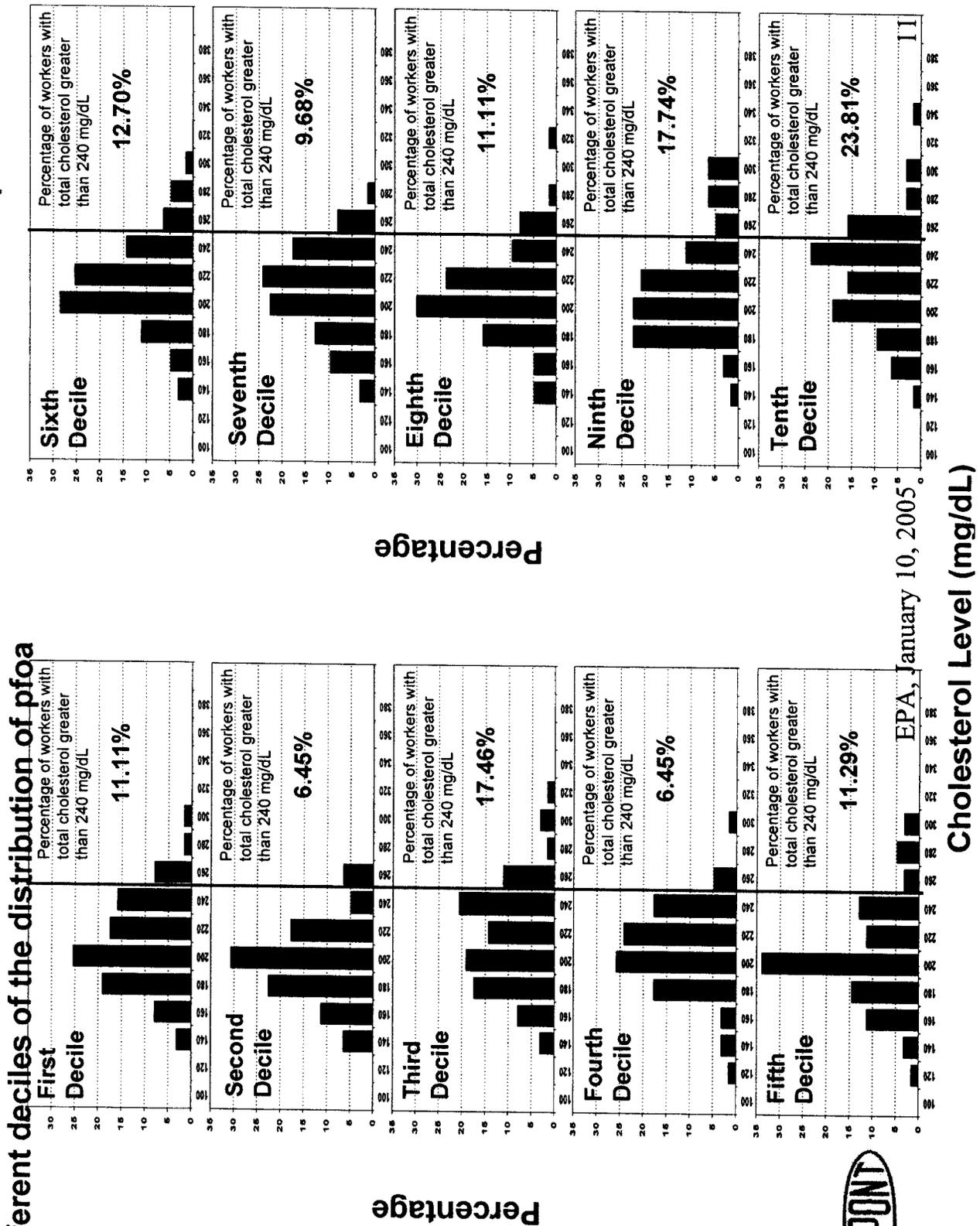
Overall Comparison of Male Washington Works Study Participants with the General US Population



EPA, January 10, 2005

Comparison of Washington Works Workers Compared to US Population

Distribution of total cholesterol level of non-medicated male workers exposed to different deciles of the distribution of pfoa



EPA, January 10, 2005

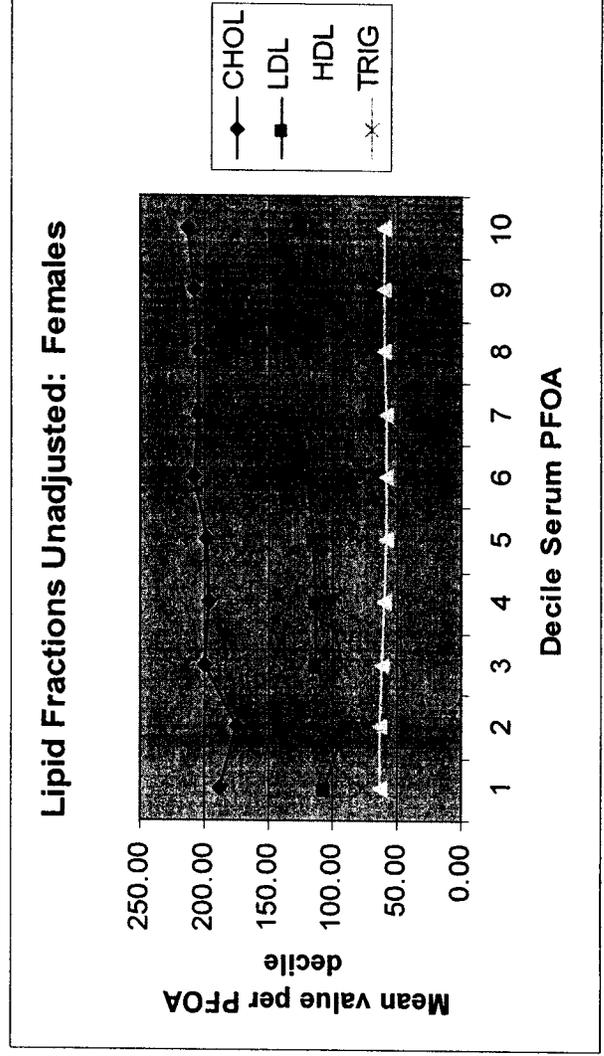
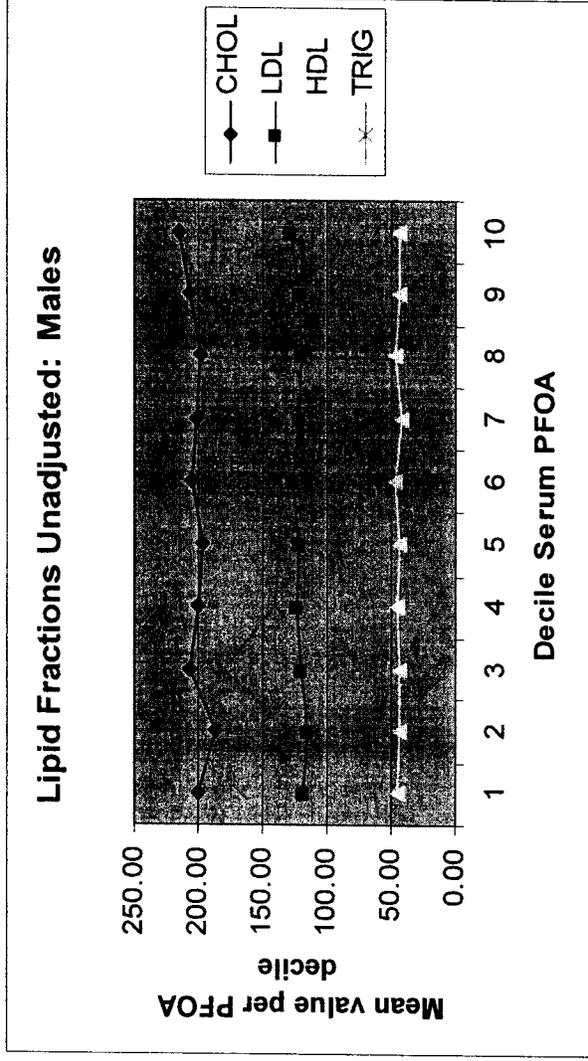
Cholesterol Level (mg/dL)



Excludes those on lipid-lowering meds

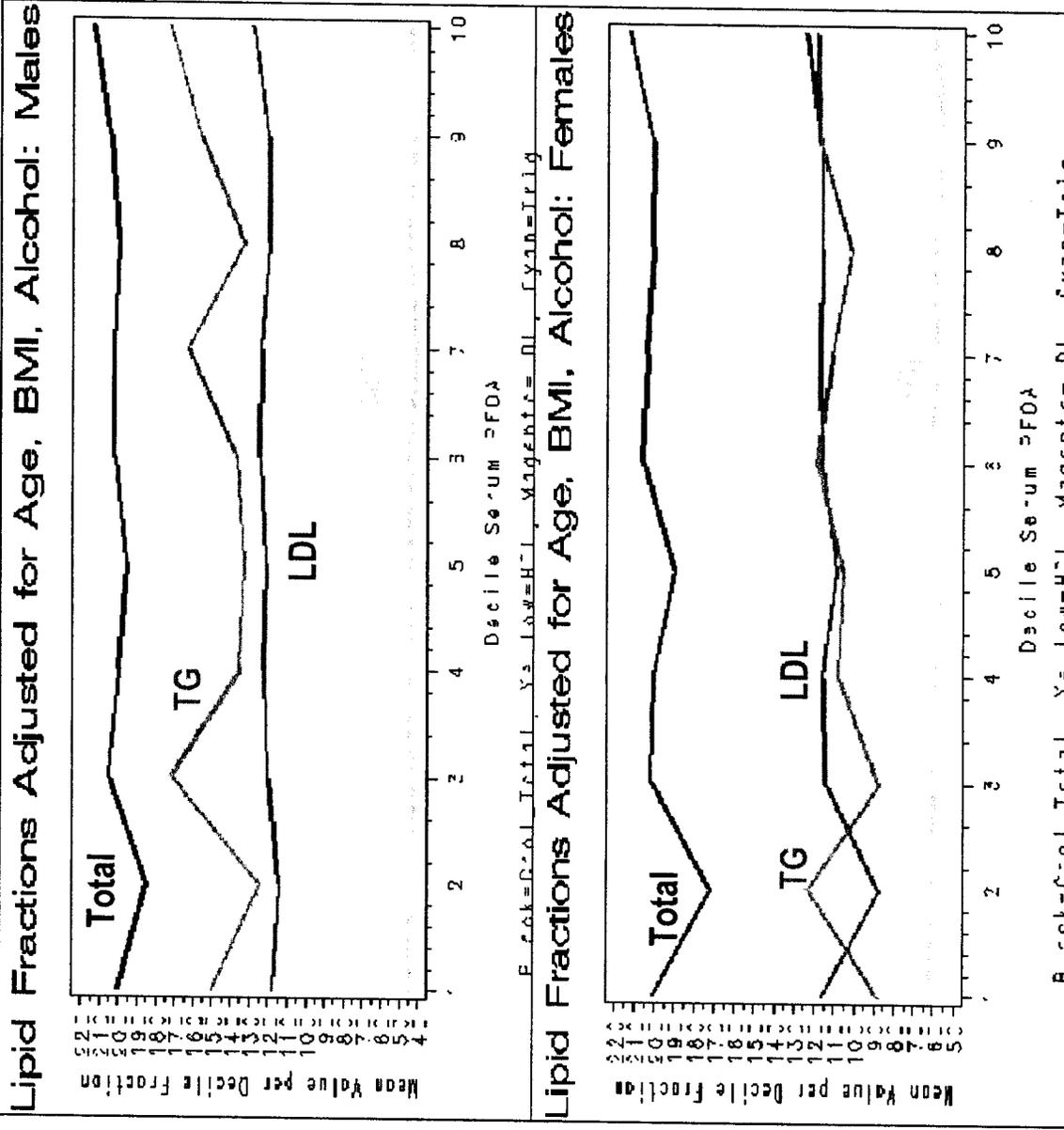
Simple charts of mean unadjusted lipid values for exposure decile indicate a small increase in last decile.

Modeling *that accounts for known risk factors* provides more information.





Mean adjusted lipid values for serum PFOA levels indicate a modest increase in highest decile (>1000 ppb)

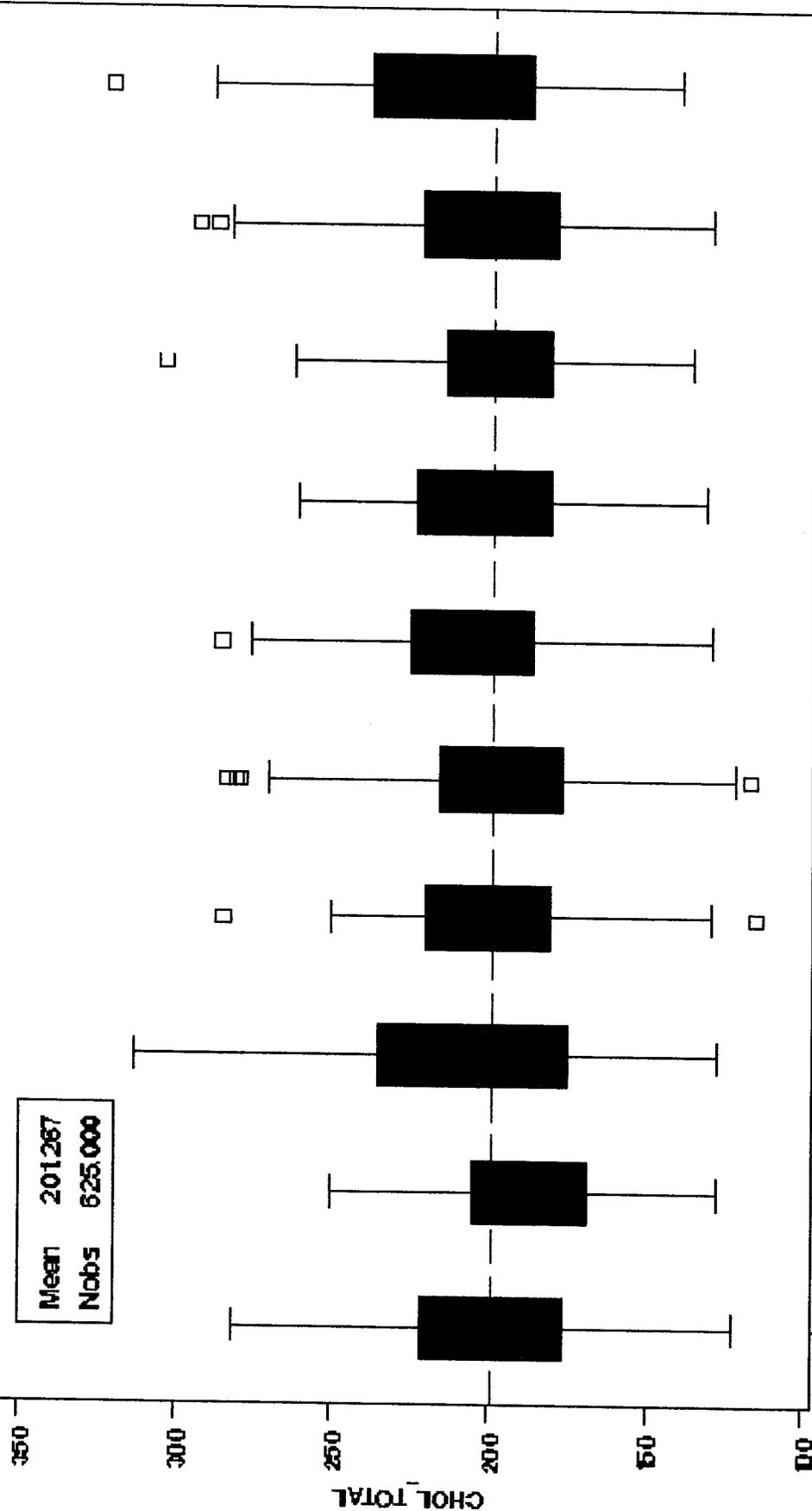




CHOL_TOTAL by PFOA Quantile

Sex=M, Heartmeds=N, MIH 2004

Mean	198.823	187.016	205.829	199.857	198.306	204.635	200.871	198.873	206.097	214.587
Std Dev	32.345	28.787	39.434	31.099	37.476	32.835	30.472	31.022	36.740	36.646



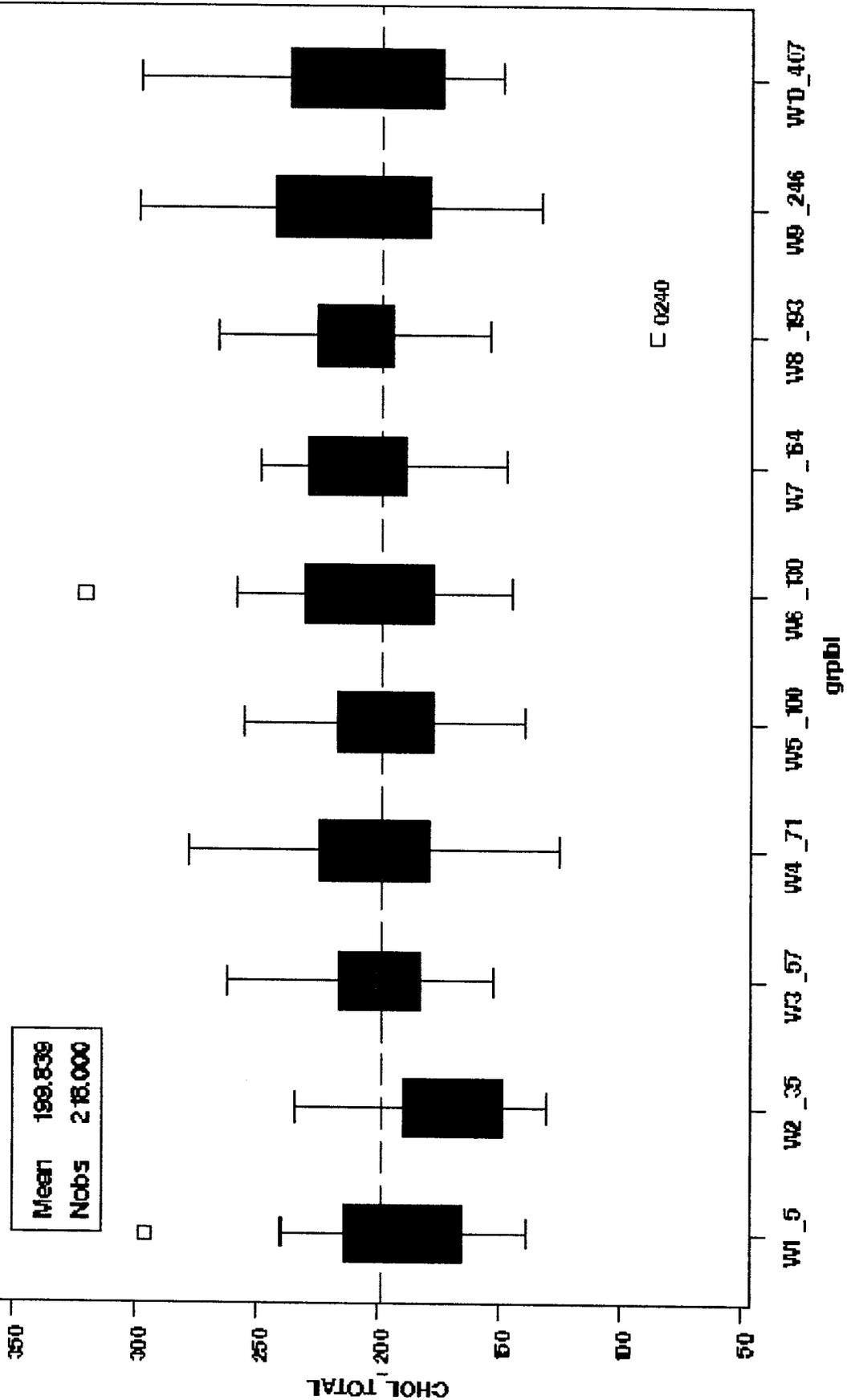
graph



CHOL_TOTAL by PFOA Quantile

Sex=F, Heartmeds=N, MI 2004

Mean	190.687	171.045	201727	197.318	195.150	208.478	205.636	206.045	208.000	213.091
Std Dev	38.111	28.550	28.944	40.554	27.609	39.160	31.709	38.367	43.470	41.657





LOG_Chol_Total: MALES, WW 2004

Results of Regression Modeling

Where Sex=M, Heartmeds=NO

Source	DF	SS	MS	FValue	ProbF
Model	4	0.78955	0.19739	6.88	<.0001
Error	620	17.78688	0.02869		
Corrected Total	624	18.57643			

Parameter Estimates

Variable	DF	Estimate	StdErr	tValue	ProbT	Sex=M
Intercept	1	4.99553	0.06366	78.47	<.0001	Meds=NO
LOG_PFOA	1	0.02307	0.00623	3.70	0.0002	
BMI	1	0.00357	0.00158	2.27	0.0238	
AGE	1	0.00153	0.00077841	1.97	0.0491	
ALC6	1	-0.07070	0.03469	-2.04	0.0420	
R-Square		0.042503				

Where Sex=M, Heartmeds=B (B=all subjects)

Source	DF	SS	MS	FValue	ProbF
Model	2	0.43865	0.21932	7.11	0.0009
Error	778	23.98564	0.03083		
Corrected Total	780	24.42429			

Parameter Estimates

Variable	DF	Estimate	StdErr	tValue	ProbT	Sex=M
Intercept	1	5.17480	0.03196	161.93	<.0001	Meds=B
LOG_PFOA	1	0.01921	0.00574	3.34	0.0009	
ALC6	1	-0.05789	0.03170	-1.83	0.0683	
R-Square		0.017960				

EPA, January 10, 2005



LOG_Chol_Total : FEMALES, WW 2004

Results of Regression Modeling

Source	DF	SS	MS	FValue	ProbF
Model	4	1.00313	0.25078	7.73	<.0001
Error	213	6.91462	0.03246		
Corrected Total	217	7.91775			

Parameter Estimates: Sex=F, Heartmeds=NO

Variable	DF	Estimate	StdErr	tValue	ProbT
Intercept	1	4.84428	0.08752	55.35	<.0001
LOG_PFOA	1	0.02381	0.01161	2.05	0.0415
BMI	1	0.00413	0.00195	2.11	0.0358
AGE	1	0.00474	0.00141	3.36	0.0009
ALC2	1	0.39119	0.18144	2.16	0.0322

R-Square 0.126694

Source	DF	SS	MS	FValue	ProbF
Model	4	1.04587	0.26147	8.36	<.0001
Error	238	7.43958	0.03126		
Corrected Total	242	8.48546			

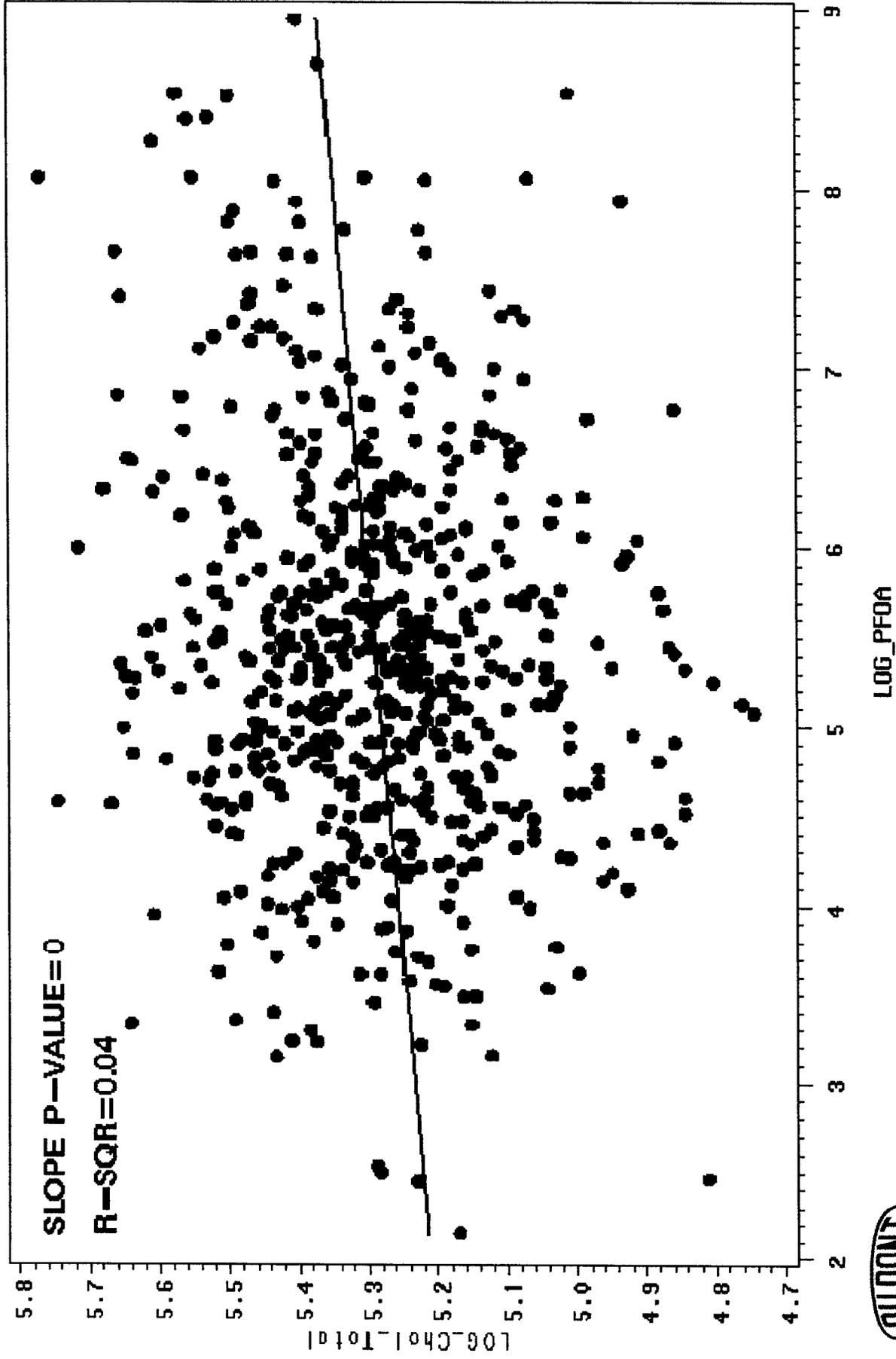
Parameter Estimates: Sex=F, Heartmeds=B

Variable	DF	Estimate	StdErr	tValue	ProbT
Intercept	1	4.86657	0.07935	61.33	<.0001
LOG_PFOA	1	0.02283	0.01067	2.14	0.0334
BMI	1	0.00475	0.00178	2.68	0.0080
AGE	1	0.00383	0.00127	3.01	0.0029
ALC2	1	0.40589	0.178705	2.28	0.0234

R-Square 0.123255

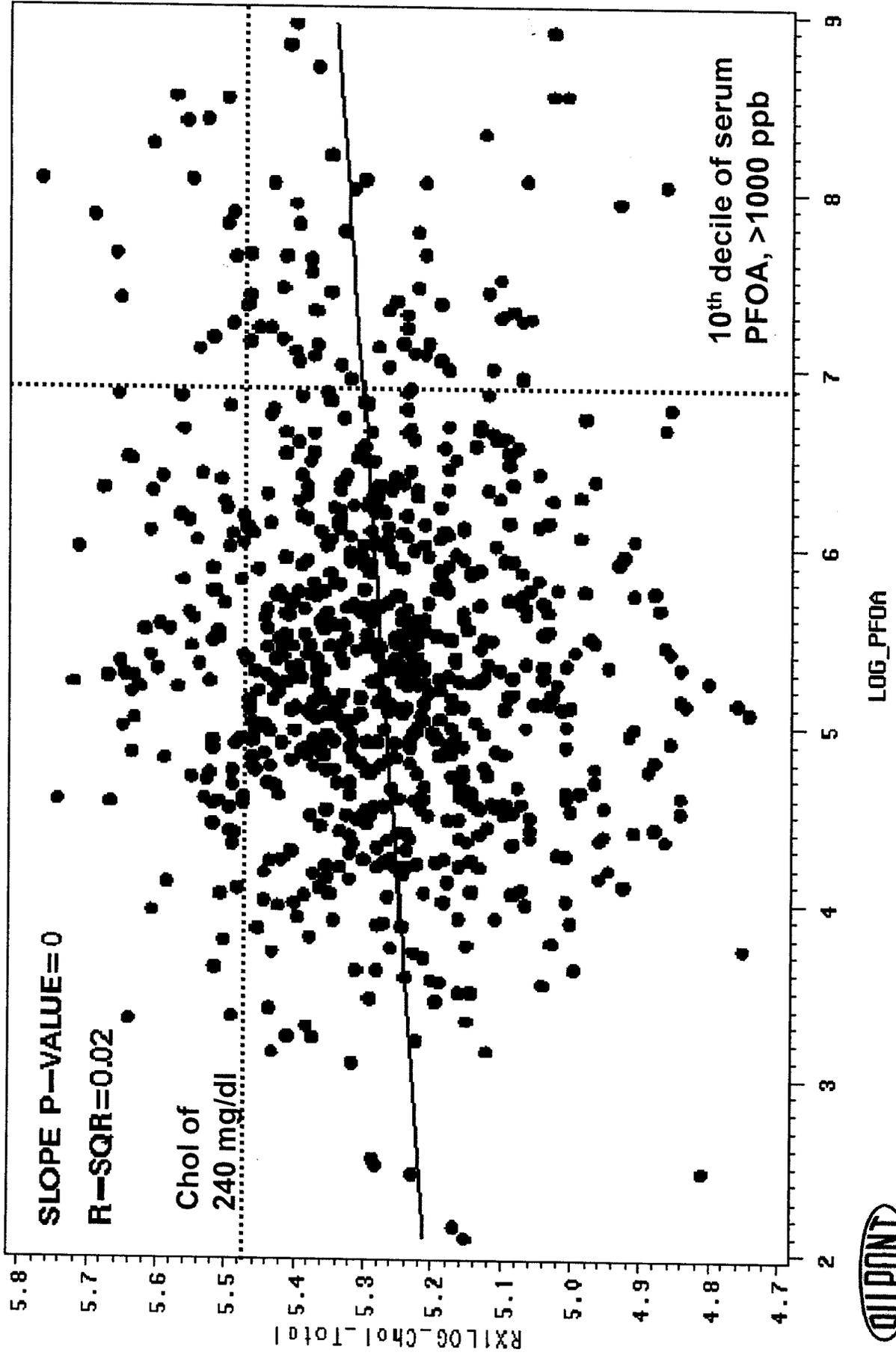
LOG_Chol_Total vs LOG_PFOA : VWV 2004, Terms=LogPFOA BMI AGE ALO6

Where Sex=M, Heartmeds=NO



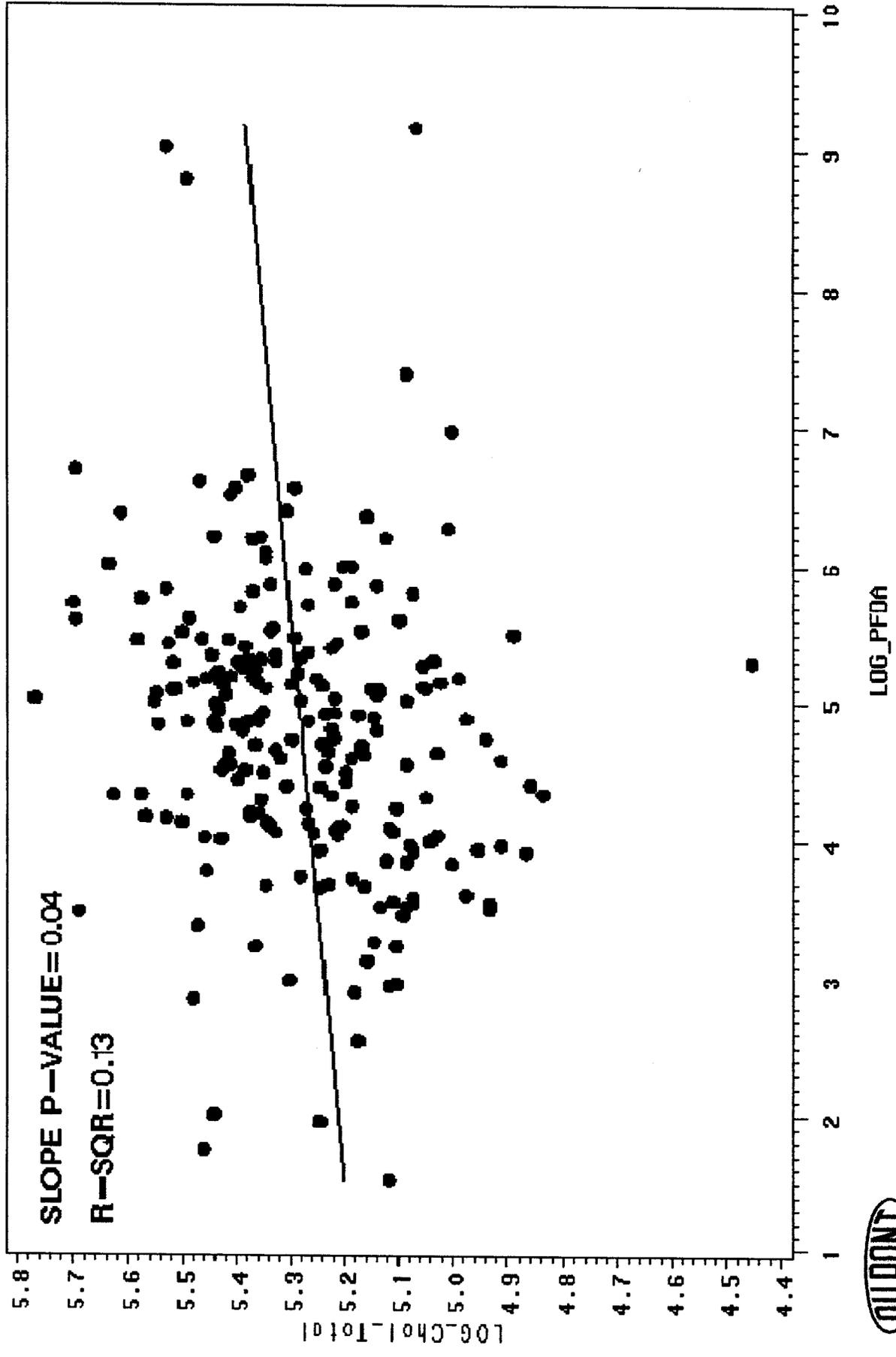
LOG_Chol_Total vs LOG_PFOA : WW 2004, Terms = LogPFOA ALC6

Where Sex=M, Heartmeds=B



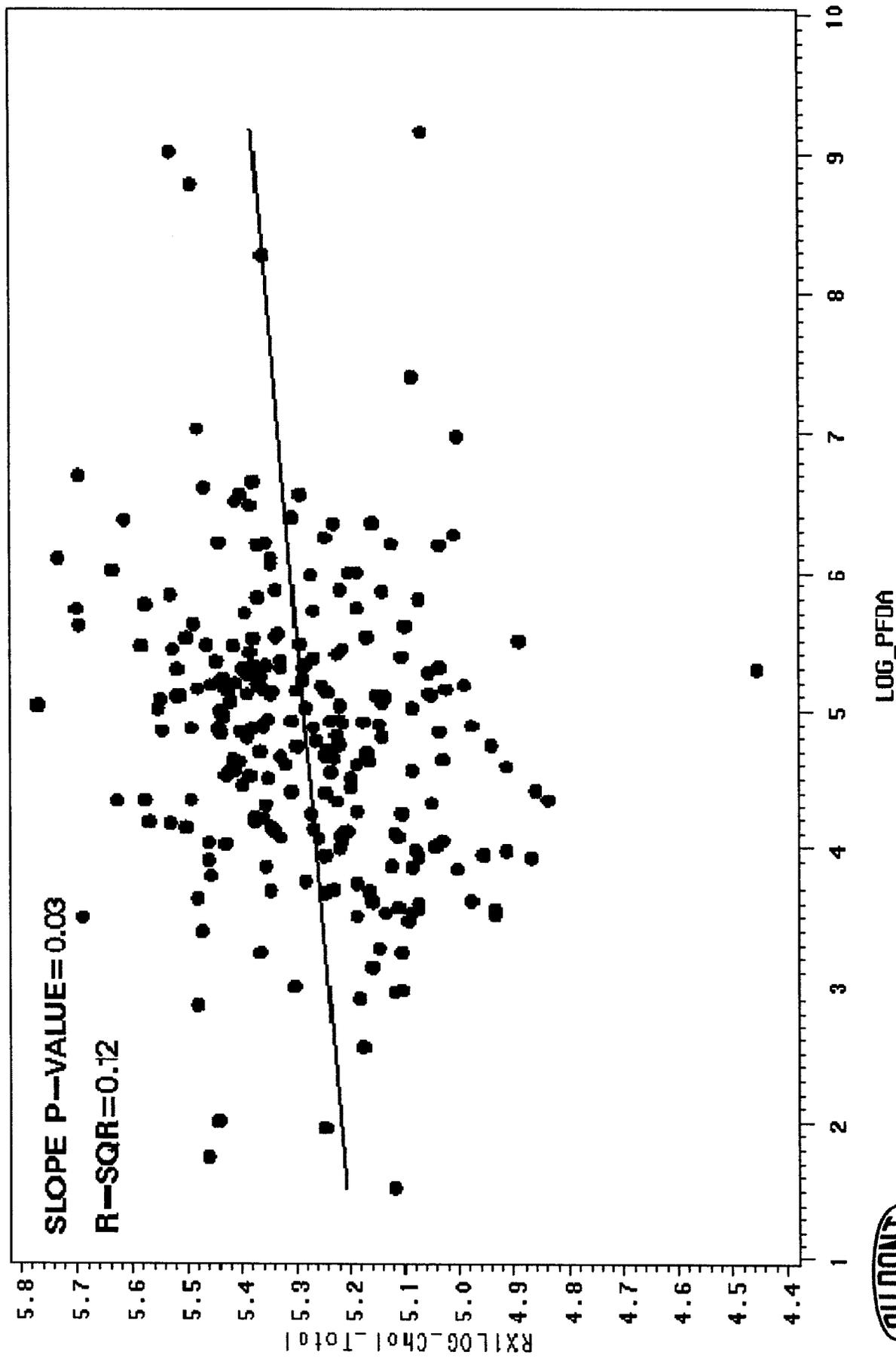
LOG_Chol_Total vs LOG_PFOA : WW 2004, Terms= LogPFOA BMI AGE ALC2

Where Sex=F, Heartmeds=N0,



LOG_Chol_Total vs LOG_PFOA : WW 2004, Terms= LogPFOA BMI AGE ALC2

Where Sex=F, Heartmeds=B



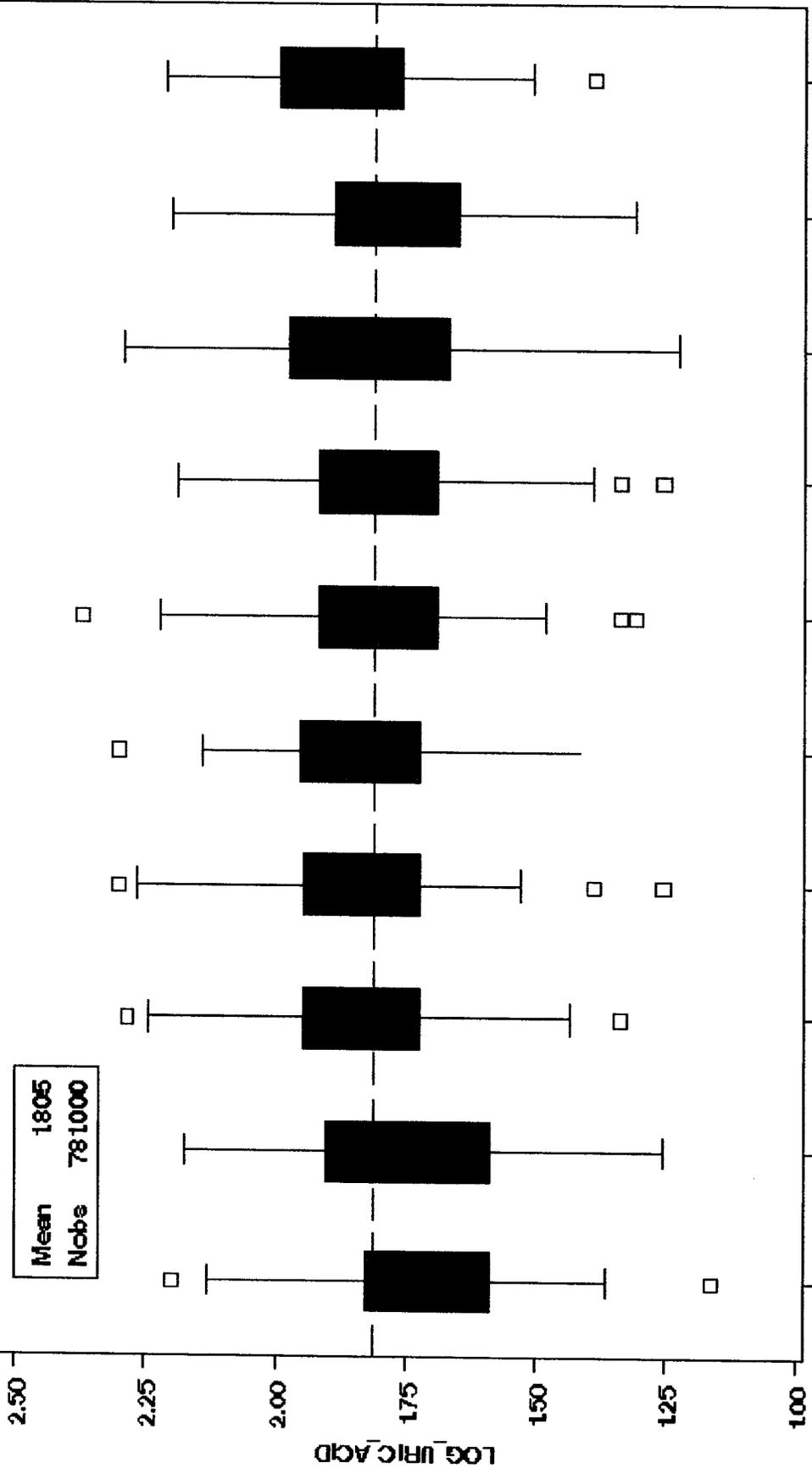


URIC ACID by PFOA Quantile

Sex=M, Heartmeds=B, W10 2004

Mean	1.735	1.755	1.822	1.828	1.820	1.805	1.810	1.816	1.777	1.875
Std Dev	0.198	0.209	0.179	0.183	0.177	0.189	0.205	0.205	0.180	0.178

Mean	1.805
Nobs	781000



W1_8 W2_67 W3_97 W4_133 W5_169 W6_210 W7_266 W8_367 W9_537 W10_1040

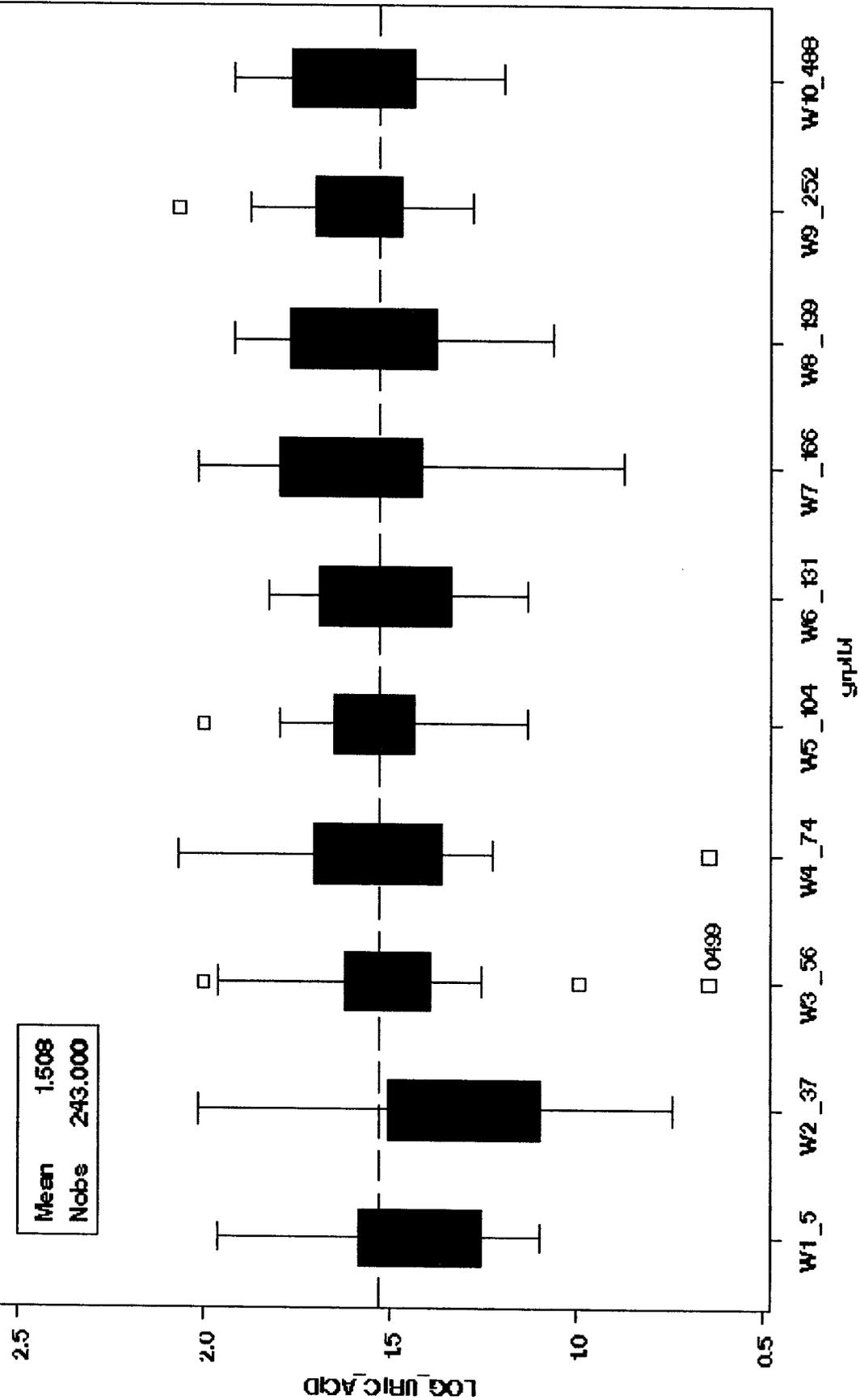


URIC_ACID by PFOA Quantile

Sex=F, Hear tmeds=B, WM 2004

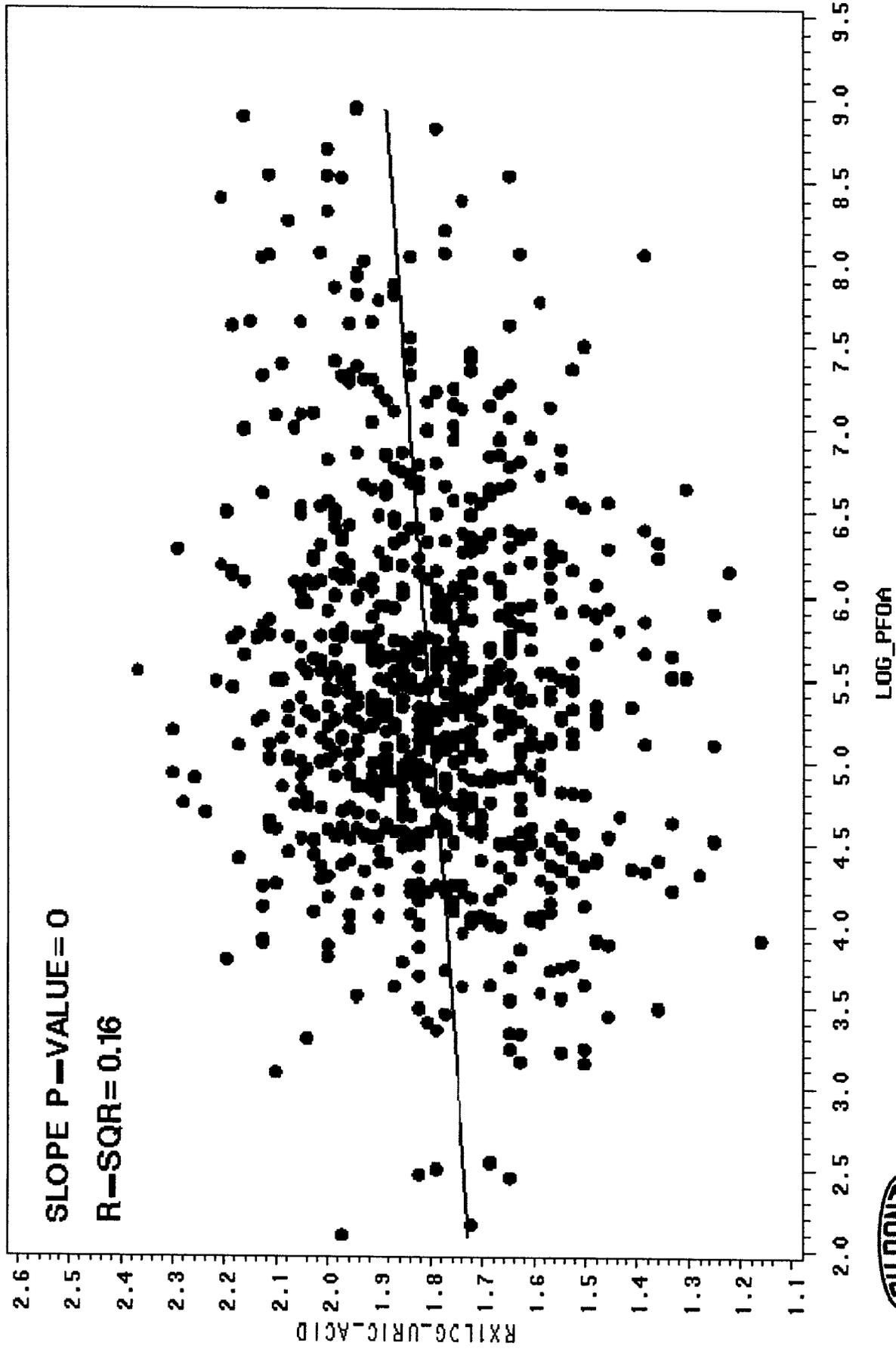
Mean	1410	1314	1486	1542	1533	1588	1552	1594	1582
Std Dev	0.213	0.309	0.287	0.195	0.192	0.275	0.242	0.175	0.222

Mean	1.508
Nobs	243.000



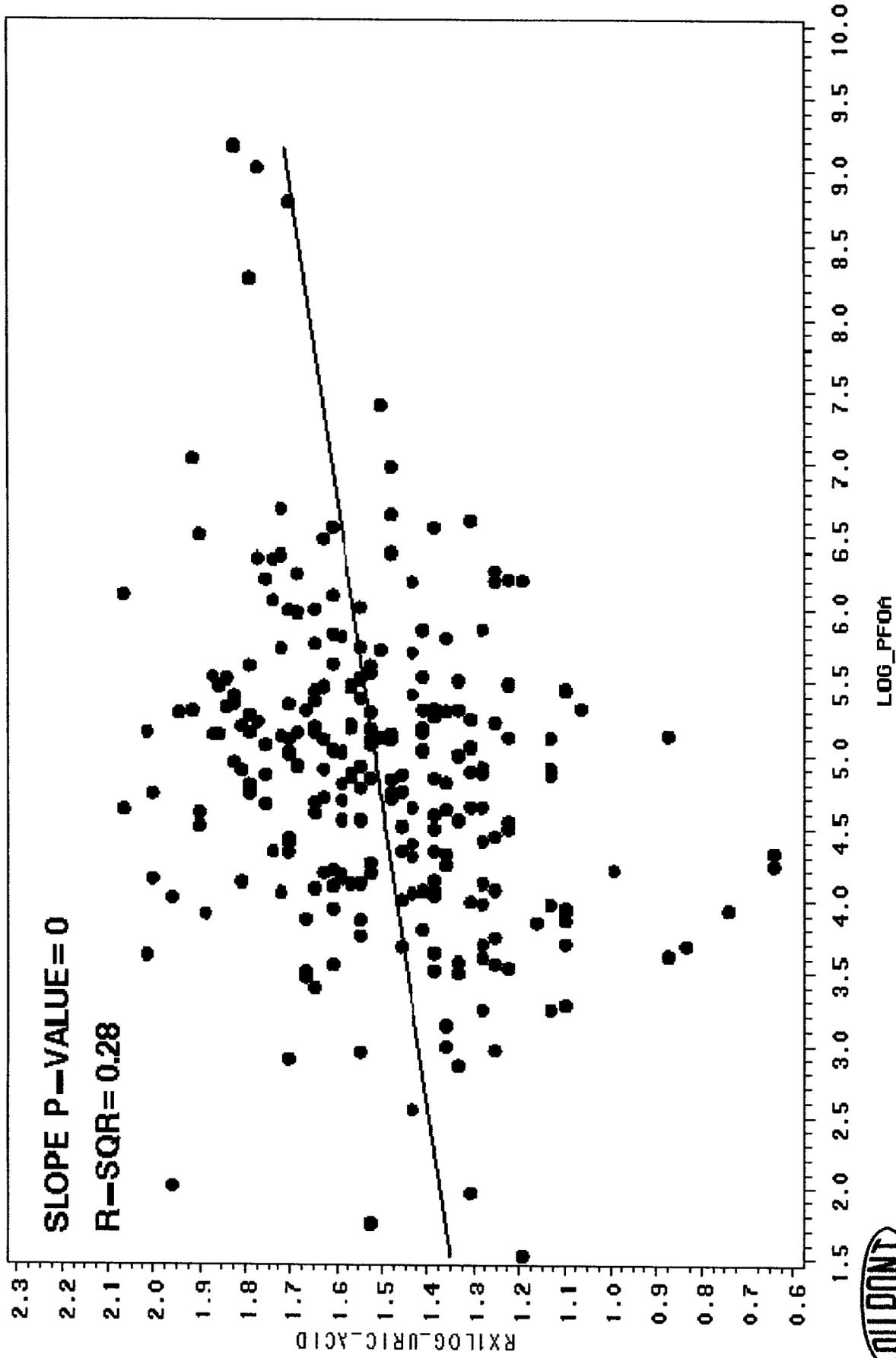
LOG_URIC_ACID VS LOG_PFOA: WWV 2004, terms= LOGPFOA BMI ALC4

Where Sex=M, Heartmarks=R



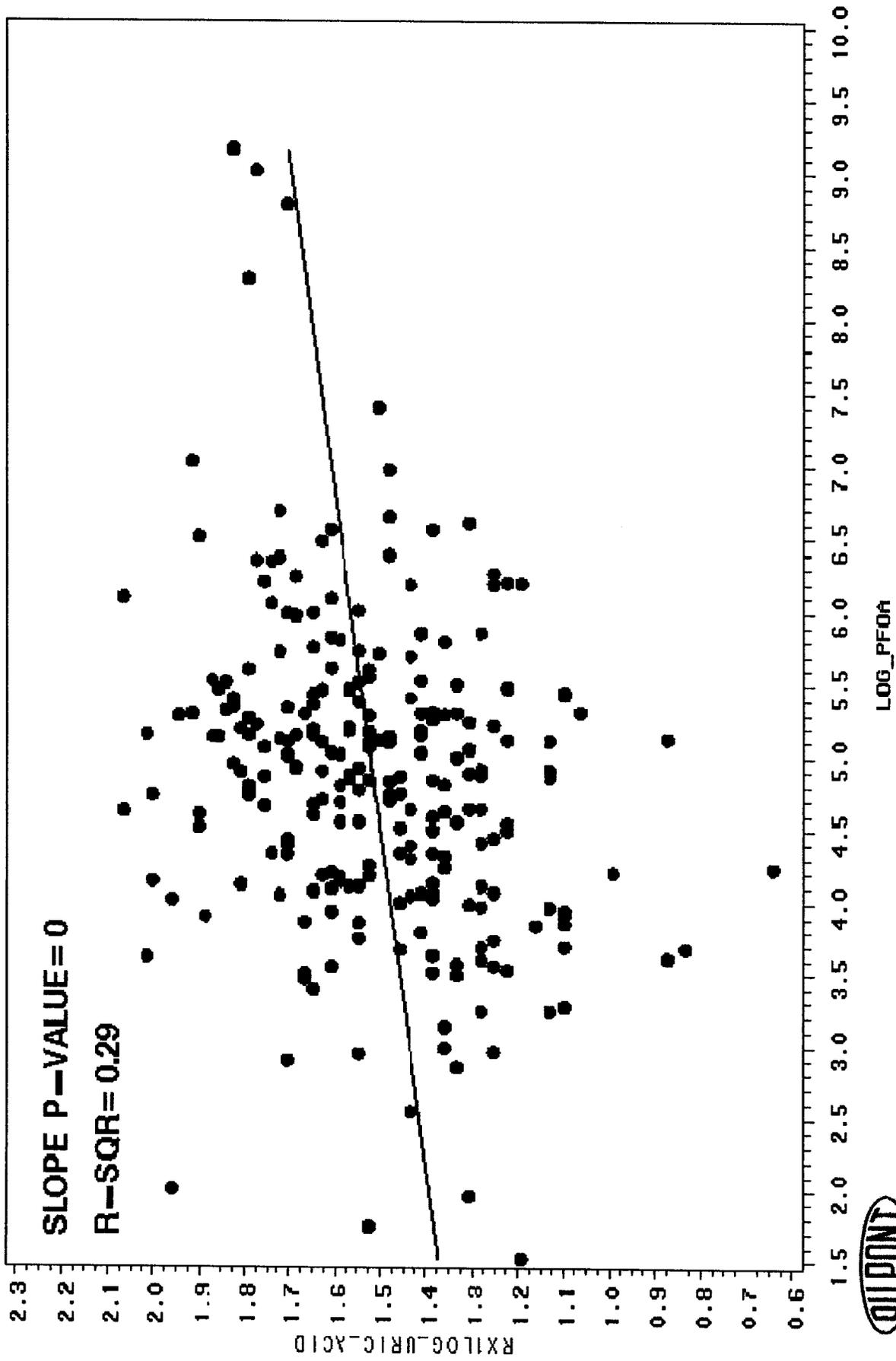
LOG_URIC_ACID vs LOG_PFOA: WW 2004, Terms= LogPFOA BMI AGE

Where Sex=F, Heartmeds=B



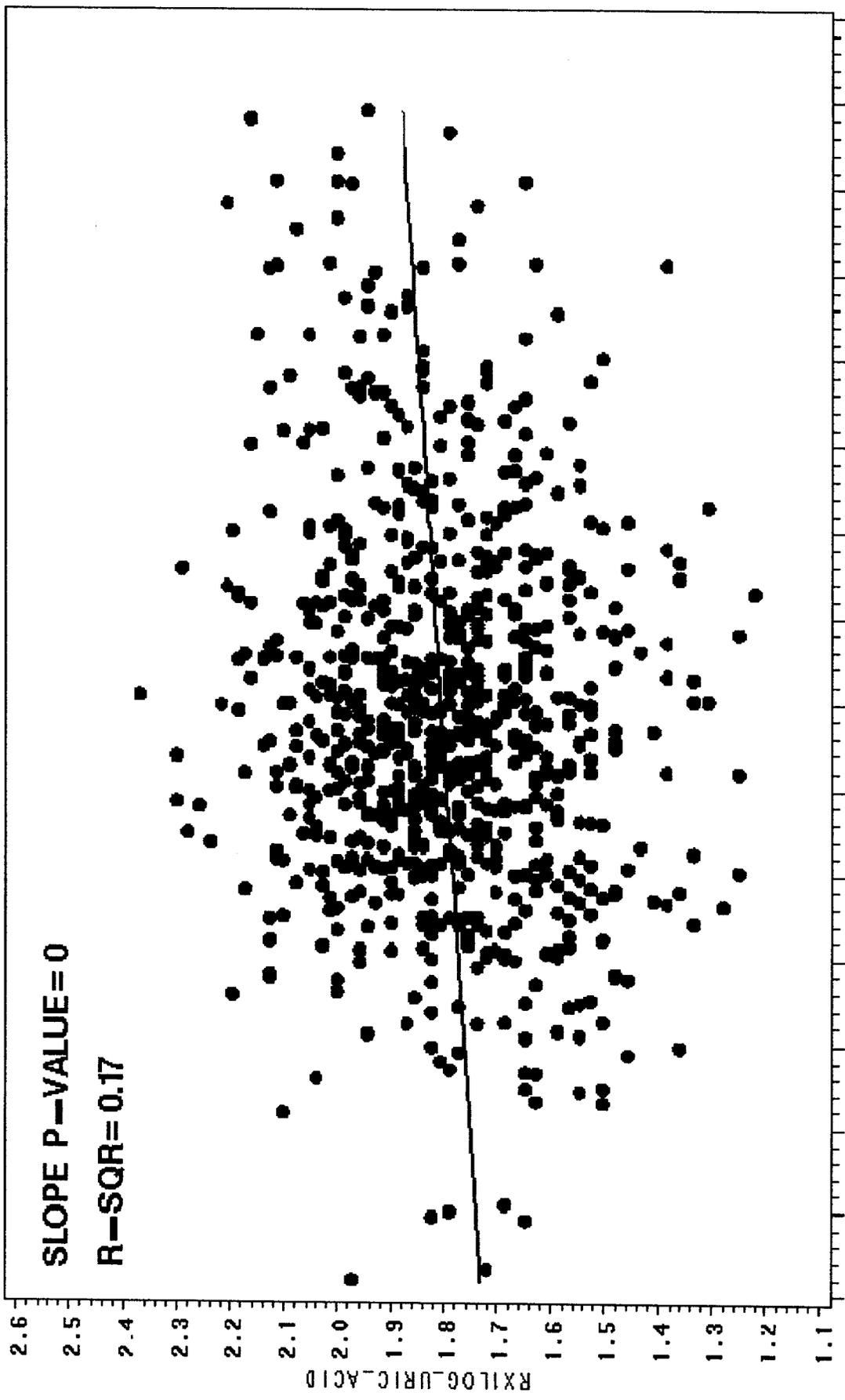
LOG_URIC_ACID vs LOG_PFOA: CLEANED, Terms= LogPFOA BMI AGE

Where Sex=F, Hear tmeds=B



LOG_URIC_ACID vs LOG_PFOA: CLEANED, Terms= LogPFOA BMI ALC4

Where Sex=M, Heartmeds=B

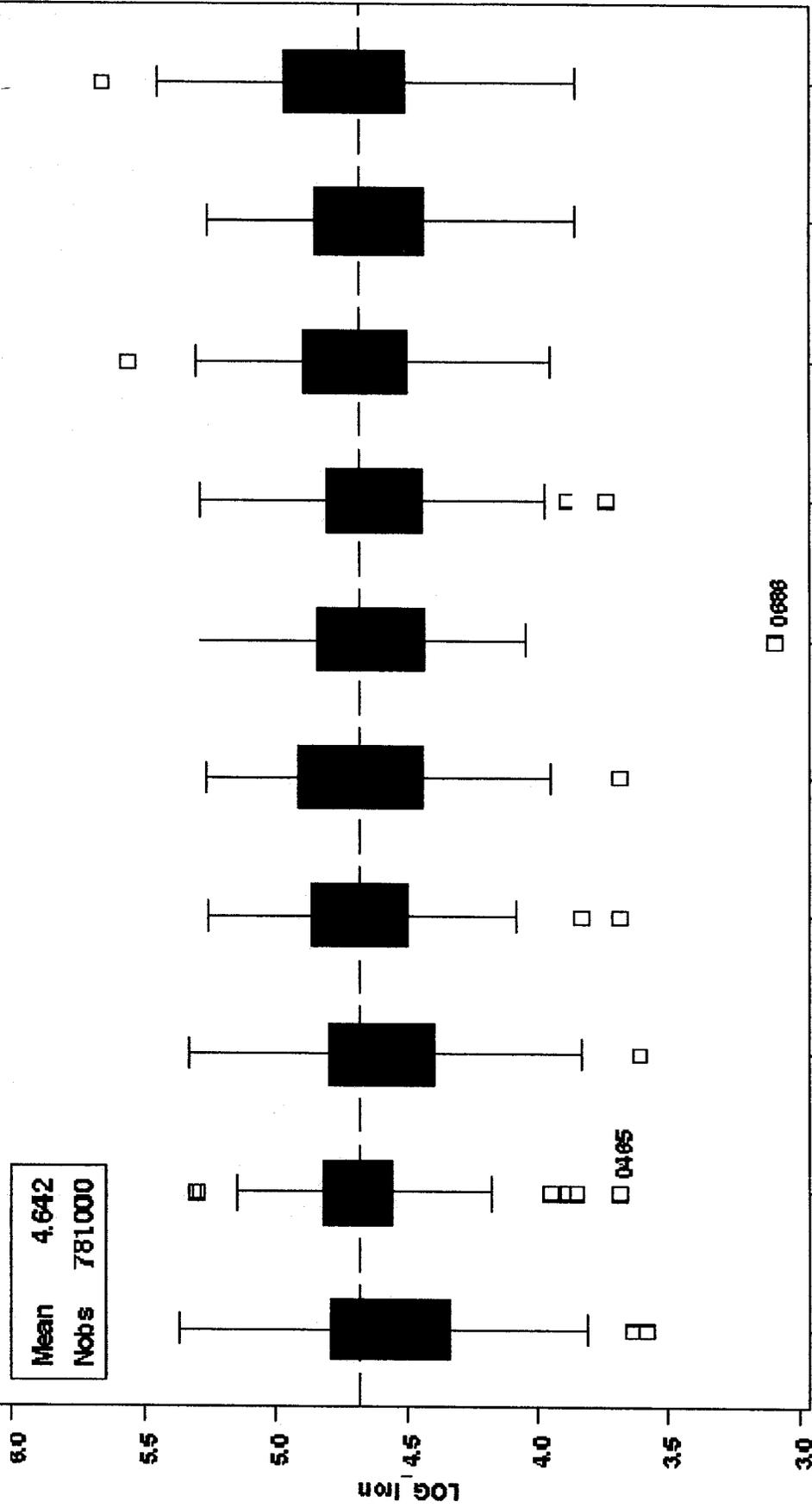


LOG_PFOA

IRON by PFOA Quantile

Sex=M, Heartmeds=B, MH 2004

Mean	4.545	4.670	4.568	4.672	4.668	4.621	4.616	4.699	4.622	4.737
Std Dev	0.364	0.304	0.349	0.307	0.335	0.331	0.314	0.315	0.327	0.355



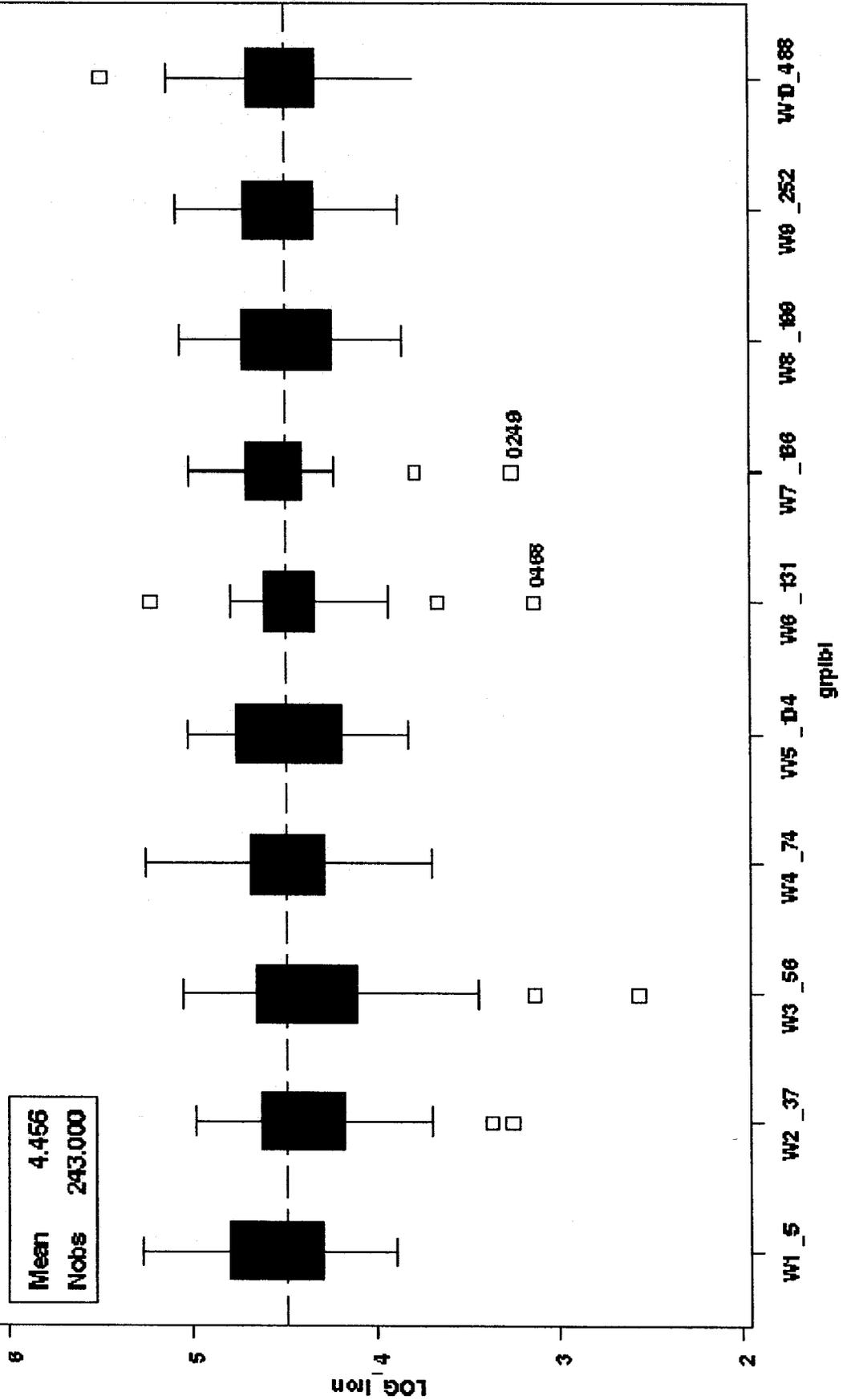
W1_8 W2_67 W3_97 W4_133 W5_169 W6_210 W7_268 W8_367 W9_537 W10_1040
gripbi

IRON by PFOA Quantile

Sex=F, Hear tmeds=B, MW 2004

	4.566	4.362	4.269	4.474	4.471	4.393	4.454	4.467	4.520	4.592
Mean	4.566	4.362	4.269	4.474	4.471	4.393	4.454	4.467	4.520	4.592
Std Dev	0.359	0.445	0.583	0.401	0.345	0.395	0.389	0.334	0.278	0.354

Mean 4.456
Nobs 293.000

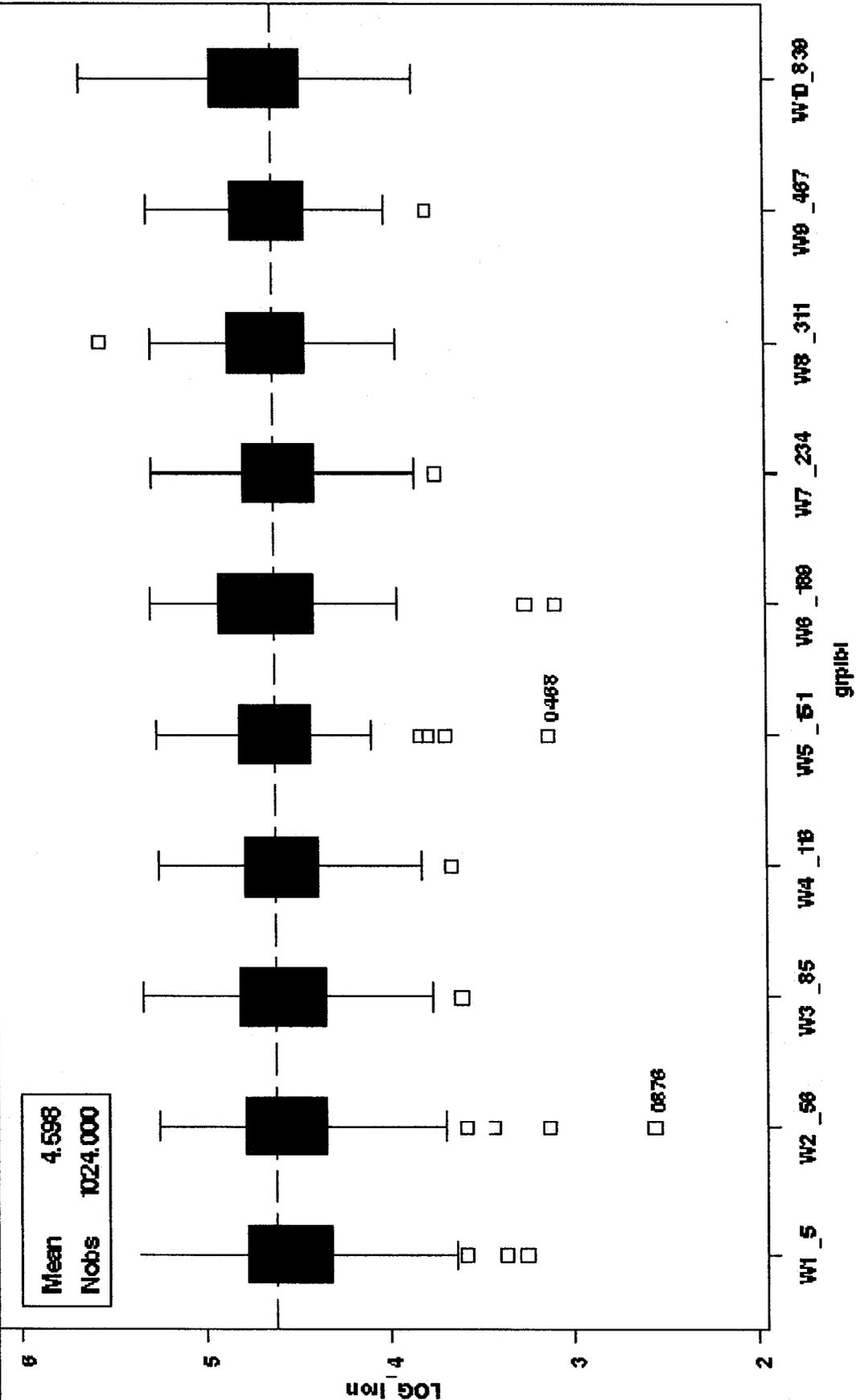


gip/ibi

IRON by PFOA Quantile

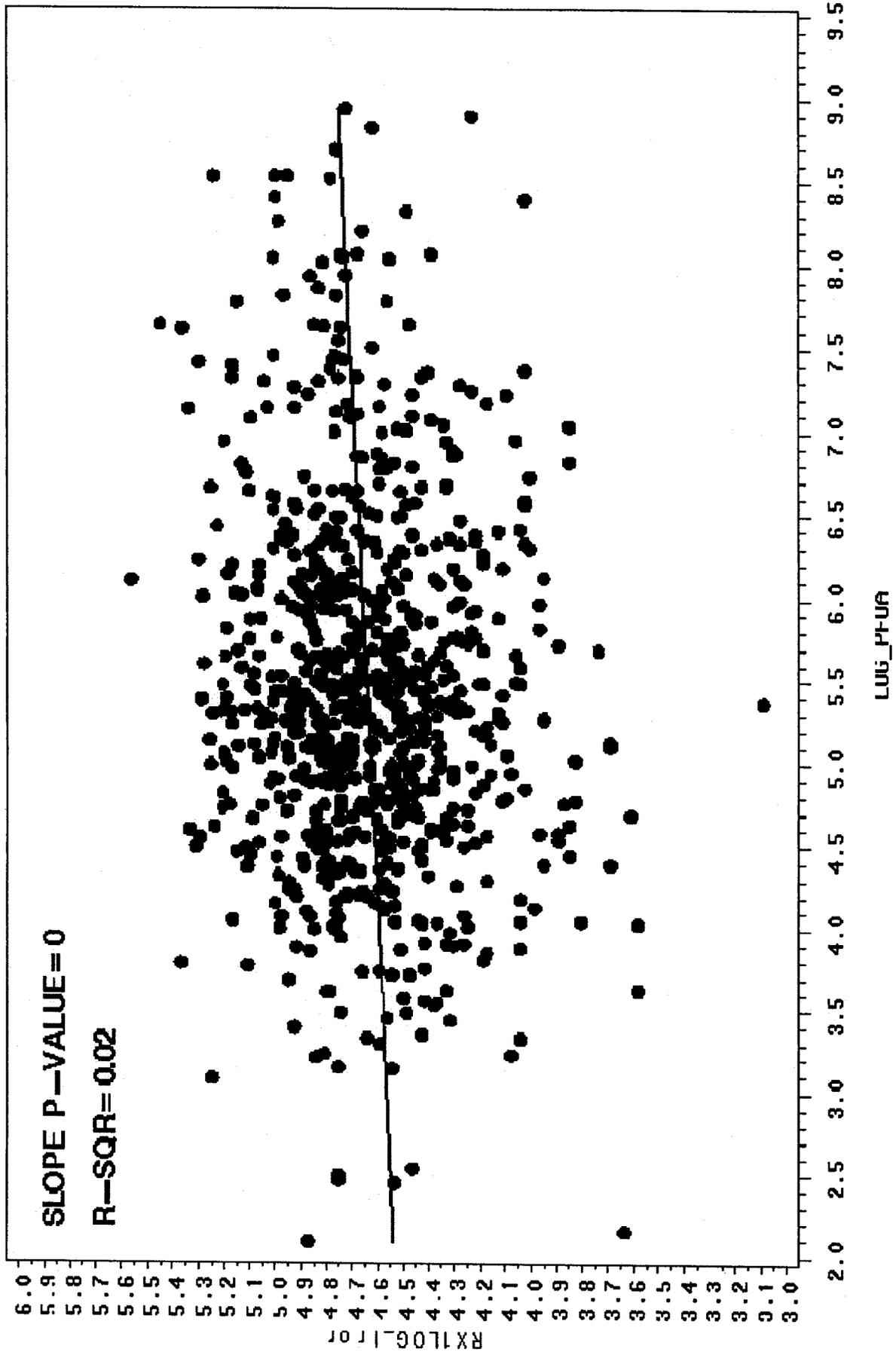
Sex=B, Heartmeds=B, WH 2004

	4.513	4.524	4.577	4.572	4.582	4.631	4.647	4.641	4.707
Mean	4.513	4.524	4.577	4.572	4.582	4.631	4.647	4.641	4.707
Std Dev	0.380	0.434	0.373	0.312	0.351	0.385	0.303	0.317	0.367



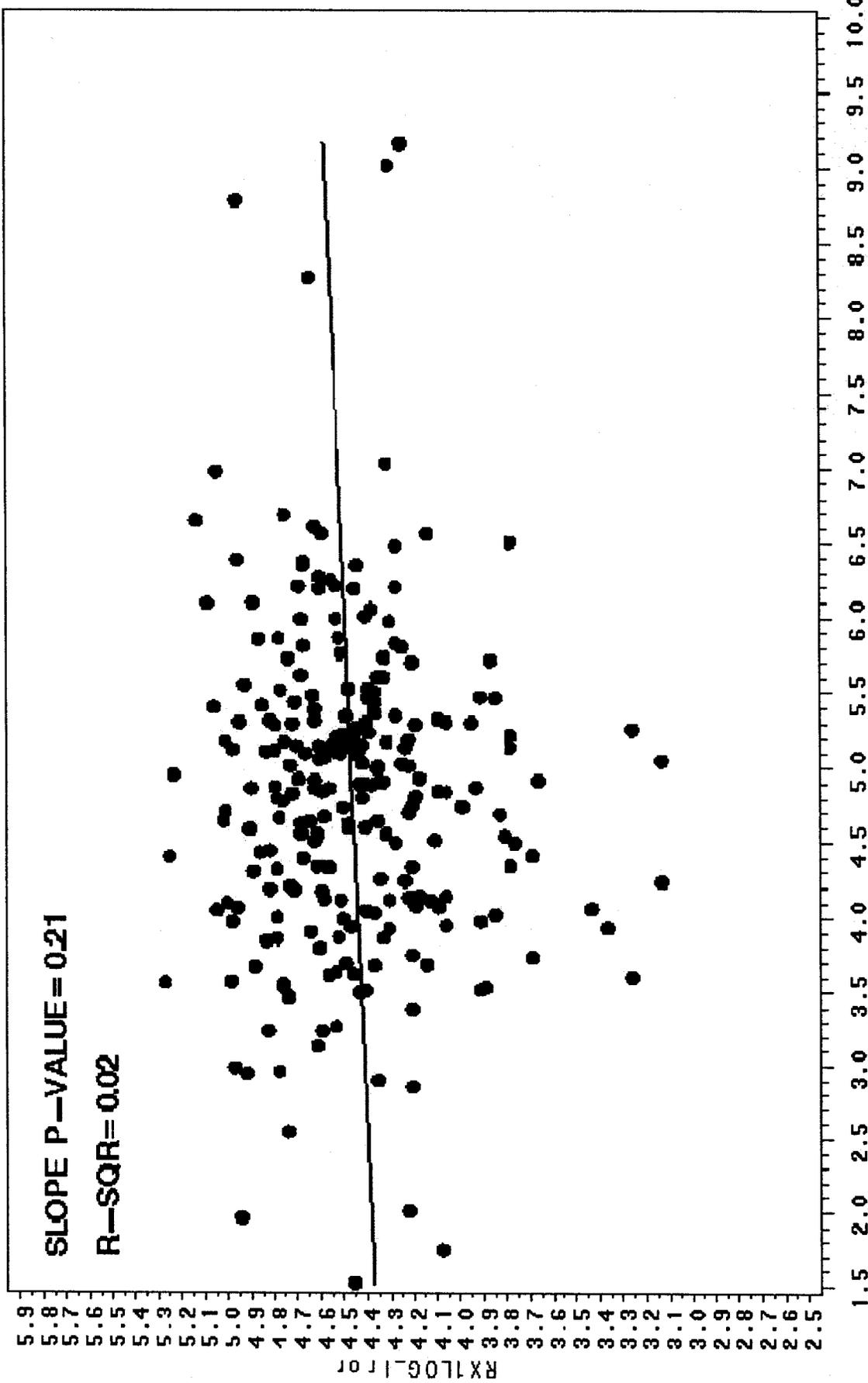
LOG_Iron vs LOG_PFOA: CLEANED, Terms= LogPFOA BMI

Where Sex=M, Heartmeds=B



LOG_Iron vs LOG_PFOA: CLEANED, Terms= LogPFOA BMI

Where Sex=F, Heartmeds=B





Summary

- *To date, there are no human health effects known to be caused by PFOA; several statistical observations merit further study.*
 - Statistically significant associations are seen with serum PFOA levels and some serum lipid fractions, uric acid, and iron.
 - These associations were only seen in those study participants with the highest serum PFOA levels, which were equal to or greater than 1000 ppb.
 - DuPont, in collaboration with outside experts, is committed to conducting the studies that are necessary to understand the significance of these observations.



Timeline

2004

<u>Epidemiology Study Timeline</u>	A	M	J	J	A	S	O	N	D	1Q	2Q
	A	M	J	J	A	S	O	N	D	1Q	2Q
	p	a	u	u	u	e	c	o	e	05	05
	r	y	n	l	g	p	t	v	c		
Design, identification of contractors, approvals from review boards											
Operations and logistics planning, employee communications											
Clinical examinations, blood draw											
Electronic data capture/transfer/QA											
Initiate statistical analyses, draft methods, outline results section, meet with ERB											
Draft Final Report (Cross-sectional analyses)											
Draft Final Report (Cohort Mortality analyses)											



Plans for Further Work

DuPont Medical, Epidemiology, and Toxicology will work with medical and scientific advisors to design studies to answer remaining questions:

Are these observations reproducible?

Are similar associations seen in other worker populations?

Is there a cause and effect?

Is there a biological basis of these associations?

STUDY OF MYOCARDIAL INFARCTION
AT WASHINGTON WORKS PLANT

This study was conducted to evaluate the incidence of cases of myocardial infarction among male wage and salary roll employees at the Washington Works Plant from 1956 to 1973. Dr. Y. L. Power, Plant Physician, requested the investigation because some workers had complained that the occurrence of heart attacks among employees seemed excessive.

DESIGN AND METHODS

A computer search of the morbidity files in Wilmington produced a list of cases for this retrospective study. A case is defined as any male employee who suffered a first myocardial infarction (M.I.) between January 1, 1956, and December 31, 1973. This definition includes persons who recovered from an acute M.I. and those who died suddenly from coronary heart disease. Persons who had left work for any reason were considered potential propositions during only those years in the study period when they were active employees. Females were excluded from the study, as they represent a group too small in size for statistical analysis.

Additional data concerning plant population statistics and occupational profiles for each case were obtained from records at Washington Works.

Sixty-one cases of M.I. were observed during the eighteen-year period. A breakdown by age categorization within three-year periods for salary roll and for wage roll men is presented in Table I.

EXHIBIT 3

EID713127

Age-specific Du Pont Company rates for male wage roll and salaried personnel were used to compute the expected number of M.I.'s at Washington Works. Again, three-year time periods were selected for this determination. It would not have been sufficient to have referred to the 1973 figures, nor to have averaged or otherwise aggregated the rates over the eighteen years, because a trend toward lower incidence in the Du Pont Company has occurred in recent years. The Company rates are shown in Table II. The observed number of myocardial infarctions at Washington Works and the number of cases predicted according to Company statistics are presented in Table III for wage roll and for salary roll employees.

ANALYSIS

No excess incidence of M.I. is evident among the male wage roll employees. The expected number of M.I.'s is 34.0; 32 were observed. One notices that the number of cases has been increasing over the years at Washington Works. One suggested explanation is that with an increasing percentage of employees over the age of fifty, both at Washington Works Plant and in the Company (Table IV), more M.I.'s are to be expected.

Among salaried employees, the observed incidence of myocardial infarction is significantly higher than the expected number, under the assumption that cases follow a Poisson distribution: 29 M.I.'s were observed; 21.5 is the expected number ($P \approx .06$). The high overall incidence is largely the result of elevated rates in recent years. In the period from 1971 to 1973, the difference between observed and predicted numbers is great:

12 observed cases versus 5.3 expected cases ($P = 0.008$) whereas in the preceding years no differences between observed and expected numbers were significant. ($P > .10$ for all comparisons).

Further investigation of occupation reveals that the high frequency of M.I. cases among salaried employees is seen largely in foremen. Twenty of the twenty-nine cases occurred among the population of foremen at Washington Works: 8 were mechanical foremen, 9 were production foremen, 2 were laboratory foremen, and 1 was a yard and transportation foreman. Approximately thirty-five percent of the salaried men at the plant are foremen. Since the age distribution of foremen is comparable to that of all salaried employees at Washington Works, one would expect that only 10 of the observed M.I.'s would present in this occupational class (35% of 29), compared to the 20 which occurred among foremen.

The foremen studied range in age at onset of the attack from 35 to 65 years. Both the mean and the median number of years of experience as foremen prior to M.I. is 9.0. The range is 1 week to 25 years.

Patrolmen, a group representing three percent of the Washington Works population, also showed a somewhat elevated incidence of M.I. Four cases were observed among patrolmen, whereas the expected number is 1.4 (3% of 29). The age at onset for these patrolmen, however, ranges from 55-59 years; this is older than the median age at onset for the entire group, which is about 50 years. This consideration explains the increased incidence among patrolmen. Distribution of M.I. cases among employee work classifications is presented in Table V.

SUMMARY

This study examines incidence of myocardial infarction among male employees at Washington Works over an eighteen-year period. Using Du Pont Company rates as the referrent, it was found that no excess of M.I. cases occurred among wage roll persons, whereas among salaried men, elevated morbidity rates obtain. The target group among salaried employees is foremen. Increased incidence in this group cannot be explained by their age distribution. One asks, then, if some aspect of the work routine is a causal link in the development of coronary heart disease, or if some personal characteristic which predisposes one to become a foreman is a risk indicator for M.I. As a result of the findings of this study, the Biostatistics Group in Wilmington will explore the possibility of a Company-wide investigation of morbidity among foremen.

Maureen T. O'berg
MAUREEN T. O'BERG
Biostatistician

TABLE I

AGE DISTRIBUTION OF M.I. CASES BY THREE-YEAR PERIODS AT WASHINGTON WORKS, 1956-1973

Age	Male Wage (Number of Employees)						Total
	<u>1956-58</u>	<u>1959-61</u>	<u>1962-64</u>	<u>1965-67</u>	<u>1968-70</u>	<u>1971-73</u>	
20							0
20-24					1		1
25-29							0
30-34			2				2
35-39		2			3	1	6
40-44		2	1				3
45-49		1	1	2	3	1	8
50-54	1	1		3	1		6
55-59				1		3	4
60-64						2	2
Total	1	6	4	6	8	7	32

Age	Male Salary (Number of Employees)						Total
	<u>1956-58</u>	<u>1959-61</u>	<u>1962-64</u>	<u>1965-67</u>	<u>1968-70</u>	<u>1971-73</u>	
20							0
20-24							0
25-29							0
30-34			1				1
35-39			1				1
40-44					1	2	3
45-49		3			2	1	6
50-54		1	1	2	1	2	7
55-59		1		1	1	7	10
60-64				1			1
Total	0	5	3	4	5	12	29

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TABLE II

Age-Specific Du Pont Company Rates,
Per 100,000, of MI Cases

WAGE - MEN

Age	1956-58	1959-61	1962-64	1965-67	1968-70	1971-73
20	0.0	0.0	0.0	0.0	0.0	0.0
20-24	0.0	0.0	0.0	5.1	12.4	0.0
25-29	4.0	0.0	6.0	4.2	11.7	4.5
30-34	18.6	21.3	34.4	10.6	15.8	0.0
35-39	88.7	117.7	114.6	55.8	111.8	86.2
40-44	178.2	282.7	254.9	191.2	242.0	183.6
45-49	484.7	384.0	358.4	385.4	372.9	422.5
50-54	707.9	812.1	630.7	674.4	565.3	648.2
55-59	883.3	962.5	713.8	895.9	919.6	804.4
60-64	940.8	910.3	857.4	989.7	1,064.9	1,169.8
*TOTAL	280	357	259	267	275	271

X

222
401
673

SALARY - MEN

Age	1956-58	1959-61	1962-64	1965-67	1968-70	1971-73
20	0.0	0.0	0.0	0.0	0.0	0.0
20-24	0.0	0.0	0.0	0.0	0.0	0.0
25-29	0.0	0.0	0.0	0.0	7.0	0.0
30-34	6.2	13.8	56.4	8.3	7.6	20.5
35-39	57.9	84.0	68.8	119.9	43.5	64.9
40-44	247.2	184.4	237.7	175.2	206.4	180.0
45-49	458.7	451.7	450.7	342.7	312.4	343.4
50-54	703.9	683.1	552.3	645.5	459.9	476.2
55-59	1,001.0	1,007.1	936.7	849.8	776.5	707.0
60-64	1,327.9	903.3	1,165.6	1,024.2	1,141.0	972.5
*TOTAL	354	330	331	303	271	215

*Age-adjusted rate

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TABLE III

OBSERVED NUMBER OF M.I. CASES AT WASHINGTON
WORKS AND EXPECTED NUMBER OF M.I. CASES,
BASED ON DU PONT COMPANY MORBIDITY

	Male Wage						
	<u>1956-58</u>	<u>1959-61</u>	<u>1962-64</u>	<u>1965-67</u>	<u>1968-70</u>	<u>1971-73</u>	<u>Total</u>
Observed	1	6	4	6	8	7	32
Expected	2.2	3.8	4.9	6.0	8.2	8.9	34.0

	Male Salary						
	<u>1956-58</u>	<u>1959-61</u>	<u>1962-64</u>	<u>1965-67</u>	<u>1968-70</u>	<u>1971-73</u>	<u>Total</u>
Observed	0	5	3 6	4 10	5 13	12 12	29
Expected	1.5	2.5	3.5	3.9	4.8	5.3	21.5

TABLE IV

Age Categorization of Male Employees at
Du Pont Company and Washington Works, 1956-1973

DU PONT COMPANY
WAGE AND SALARY COMBINED*

Age	1956-58	1959-61	1962-64	1965-67	1968-70	1971-73
< 50	203,712	181,142	186,413	205,110	197,957	183,441
≥ 50	44,287	49,733	58,954	67,960	72,259	74,020
% ≥ 50	18%	22%	24%	25%	27%	29%

WASHINGTON WORKS
WAGE AND SALARY COMBINED*

Age	1956-58	1959-61	1962-64	1965-67	1968-70	1971-73
< 50	3,136	4,421	4,712	5,528	6,370	6,031
≥ 50	151	322	526	744	1,000	1,225
% ≥ 50	4%	7%	10%	12%	14%	17%

*Each number represents the cumulative mid-year population for the three-year period.

TABLE V

OBSERVED NUMBER OF M.I. CASES BY OCCUPATION
AND EXPECTED NUMBER OF M.I. CASES, BASED ON
OCCUPATIONAL DISTRIBUTION AT WASHINGTON
WORKS, MALE SALARIED EMPLOYEES

<u>OCCUPATION</u>	<u>% OF SALARIED EMPLOYEES AT WASHINGTON WORKS</u>	<u>OBSERVED NUMBER OF M.I.'S</u>	<u>EXPECTED NUMBER OF M.I.'S*</u>
Foremen	35%	20	10.2
Supervision through Management	23%	3	6.7
Specialists	2%	1	1.4
Analysts, Accountants	3%	0	.9
Engineers	22%	0	6.4
Clerical	10%	0	2.9
Patrolmen	3%	4	.9
Draftsmen	2%	1	1.4
TOTAL		<u>29</u>	

*expected number = % x 29

STUDY OF MYOCARDIAL INFARCTION INCIDENCE AND
CORONARY HEART DISEASE MORTALITY RATES AT
WASHINGTON WORKS

William E. Fayerweather

3/30/81

EID584159

Acknowledgements

I want to thank J. F. Doughty and Y. L. Power for the many days they spent consulting and assembling data for this project; P. Thistleton for his industrial hygiene consulting, especially as it related to C-8 exposure potential at the plant; S. Pell for his helpful advice and comments; and R. M. Shepherd for a critical review.

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SUMMARY

M. T. O'Berg studied myocardial infarction (MI) incidence at Washington Works from 1956 to 1973. Her study found a slight excess of MI among male salary roll employees. The excess was largest for the period 1971 to 1973 in which 11 MI's were observed compared to only 5.3 expected ($p < 0.04$). Salary roll's MI excess appeared to be largely confined to foremen (see O'Berg's August 1975 report: Study of Myocardial Infarction at Washington Works Plant).

The following report updates the earlier study so as to include the years 1974 through 1977. Expected numbers were based on the plant's population and on Company rates.

For the years 1956 through 1977, ninety-one MI's were observed compared to 77.8 expected among male Washington Works employees. Wage roll had 56 MI and expected 48.7, and salary roll had 35 MI and expected 29.1. These differences were not statistically significant.

There was a statistically significant excess of MI among male wage roll employees for the period 1974 to 1977. During this period, male wage roll had 24 MI's compared to only 14.7 expected ($p < 0.05$). However, for several reasons this excess was not thought to be occupationally related. For instance, wage roll showed no significant excesses prior to this period, and salary roll showed no excess during this period. In general, when data are examined over several relatively short time intervals, chance fluctuations in rates will often produce an excess in at least one of these intervals.

To further investigate coronary heart disease (CHD) risks at Washington Works, four additional studies were undertaken.

- Matched-pairs case-control study of MI, 1956-1978: exposure was defined by work area and salary level.
- Plant-wide cohort study of CHD mortality, 1957-1977: active and pensioned employees were studied.
- CHD death case-control study, 1957-1978: exposure was defined by work area and salary level; non-CHD deaths were used as controls.
- Cross-sectional study of current Teflon® area workers' blood pressures: non-Teflon® area workers were used as controls.

Overall, these studies suggest that:

- The risk of having an MI is significantly higher among Washington Works foremen than among the plant's other salaried employees. This result was to be expected, since Du Pont foremen in general have higher risks of MI than do other salaried employees. The MI excess among foremen may result from other risk factors such as smoking, stress, educational level, socioeconomic status, or coronary-prone behavior.
- The risk of CHD death is no higher among foremen than among other salaried employees.
- There were no excesses of CHD deaths among wage or salary roll workers.
- Teflon® area workers show no excess risks for MI, CHD death, or high blood pressure.
- Workers from other areas of the plant show no significant excesses of MI cases or CHD deaths.

BACKGROUND

In August of 1975, M. T. O'Berg studied the incidence of myocardial infarction (MI) at Washington Works. This was a plant-wide cohort study of active employees from 1956 through 1973. The study had been requested by Dr. Y. L. Power because some workers had expressed concern that too many workers were having heart attacks. The study revealed that male salary roll workers had experienced a slight excess of MI's. Expected numbers were based on Company-wide MI rates. The excess was the largest for the period 1971 through 1973. Furthermore, the excess among male salaried workers appeared to be largely confined to foremen.

The earlier study had several limitations. It did not examine MI incidence by specific areas within the plant; and, being an MI incidence study, it did not follow workers beyond their pension date. The conclusions concerning excess risks in foremen were tenuous since the results were based on a crude method that did not lend itself to formal statistical hypothesis testing.

In 1979, PP&R Department asked that the earlier study be updated. Some people at Washington Works still were concerned that the plant's MI incidence might be excessive, particularly among Teflon® area workers. Consequently, case-control studies of MI cases and coronary heart disease (CHD) deaths were also initiated.

During the MI study update, data were concurrently being collected for a liver function study of the plant's Teflon® area workers. Blood pressures and work histories collected in the liver function study were used in the present CHD study as a further check on Teflon® area workers' risks of coronary heart disease.

OBJECTIVES

- (1) To update an earlier plant-wide cohort study of MI at Washington Works.
- (2) To study MI incidence and CHD mortality by specific work area within the plant. Teflon® was to be the work area of special interest.
- (3) To compare blood pressures of workers currently working in the Teflon® area to blood pressures of workers who never had any Teflon® area experience.

METHODS

1. Overall design

An historical cohort design was used to study MI incidence and CHD mortality rates at Washington Works. Observed numbers of cases/deaths were compared against expected numbers based on overall Company rates.

MI cases occurring within certain work areas or salary levels were studied by matched-pairs case-control methods. CHD deaths by work area or salary level were studied by unmatched case-control methods in which controls were all deaths from causes other than CHD.

Blood pressures of workers currently working in the Teflon® area were compared to blood pressures of workers having no Teflon® area experience. Blood pressures were also compared by specific Teflon® area job and by blood organic fluoride level.

2. Cohort study of MI incidence and CHD mortality

a. Cohort definition

The Washington Works cohort included all male employees who were on the Plant's June 30 payroll rosters during any year between 1956 (1957 for the CHD mortality study) and 1977. The cohort excluded employees from the Engineering Department.

b. Company population counts

Population counts by plant, sex, age, pay class, and year are kept for the whole Company in Wilmington. These counts are derived from each plant's payroll roster as of June 30 of each year.

c. Company MI file: source of MI cases

Ever since 1956, Medical Division has kept a company-wide file of first MI's. The Company-sponsored accident and health insurance (A&H) and life insurance plans are the mechanisms by which MI cases are reported. All employees are covered by the life insurance plan, and about 97 percent are covered by A&H.

Only workers with a confirmed MI are admitted to this file. Case confirmation is based on reviewing clinical histories, electrocardiograms, laboratory findings, and autopsy reports. Sudden deaths are also admitted to the

file, unless there is evidence that the cause of death was not an MI. The file contains only first MI's that have occurred among active employees.

From counts of MI's and yearly populations, Company MI rates can be computed. Lists of MI cases by plant, pay class, sex, and year can be easily extracted from this file.

d. Company mortality file: source of CHD deaths

Ever since 1957, Medical Division has kept a file of all deaths among active and pensioned employees. The Company-sponsored life insurance plan is the mechanism by which deaths are reported to this file.

Cause of death is ascertained from death certificates or from the Company's "proof of death" statement that must accompany each beneficiary's claim. A death certificate's cause of death is sometimes corrected when additional data indicate that the certificate is in error.

From this file company-wide mortality rates by cause of death can be computed. Lists of deaths by plant, pay class, sex, year, and cause of death are easily extracted from this file.

e. Statistical methods

(1) Comparison groups

The observed numbers of MI cases and CHD deaths at the plant were compared to the expected numbers based on overall company rates for male employees. Expected numbers were adjusted for Company-plant differences in age and pay class distributions.

(2) Person-years and follow-up

Person-years of observation for active employees were computed from mid-year counts of the plant's population. Lists of pensioned employees were used to compute person-years for the plant's pensioners.

For the MI study, follow-up began in 1956 and ended in 1977. It included only the plant's active employees.

The CHD study's follow-up began in 1957 and ended in 1977. It included both active and pensioned employees.

Both MI and CHD studies dropped Washington Works' employees from follow-up once they transferred out of the plant or once they quit prior to qualifying for a pension.

(3) Expected numbers and age adjustment

The study group's expected numbers of MI's and CHD deaths were based on overall Company rates. The Company's pay class-, age-, and time period-specific rates were multiplied by the corresponding person-years for the study group. This multiplication gave an expected number for a particular pay class, age, and time period. Expected numbers from all pay classes, ages, and time periods were summed to arrive at an overall expected number of MI's and CHD deaths for the study group.

This method weights the overall expected number by the number of person-years in each pay class, age, and time period. It is called the indirect method of adjustment. It adjusts for between-group differences in pay class, age, and time periods.

(4) Testing for statistical significance

Standardized incidence ratios (SIR) were computed for MI's as the ratio of the observed to the expected number of MI's. Standardized mortality ratios (SMR) were computed for CHD deaths as the ratio of the observed to expected number of CHD deaths. Tables of the cumulative Poisson probability distribution were used to test for significant differences between observed and expected numbers. Significance tests were two-tailed, and significance was judged at the 0.05 probability level.

3. Case-control studies of MI cases and CHD deaths

a. Definition of MI cases and matched controls

The Company's computerized MI file was searched for all confirmed MI cases occurring among the Plant's active male employees from 1957 through 1978. MI's occurring among the Engineering Department employees were excluded from the study.

For each MI case, one control was randomly selected from lists of employees who were working at the plant during the case's attack year. The control selected had to match the case's sex, age (\pm 2 years), and pay class.

b. Definition of CHD deaths and their controls

CHD deaths are all CHD deaths occurring among the plant's active and pensioned employees during the years 1957 through 1978. Engineering Department employees dying of any cause while assigned to the plant were excluded from the study.

Controls for the CHD deaths were all plant deaths from causes other than coronary heart disease.

CHD deaths and their controls were extracted from the Company's computerized mortality file.

c. Work history

The plant was divided into eleven work areas/divisions:

- Lucite®
- Zytel®
- Filaments
- Power and Services
- Technical-Research
- Utility Pool
- Teflon®
- Butacite®
- Delrin®
- Color and Processing
- Mechanical

Salary roll employees assigned to an area/division were divided into four categories:

- foreman
- supervision above foreman
- technical
- laboratory

In addition, the category of unassigned supervision (supervision not assigned to a product area/division) was formed.

Work histories were then assembled from individual employee records and transferred to code sheets (appendices A & B). Most records showed the plant area and the dates in which the employee had worked in the area. Very few records were detailed enough to show the worker's exact job within an area. All Washington Works jobs that appeared on the plant's records since the worker was first hired were transferred to code sheets.

Personnel records of employees who had quit, pensioned, transferred to other locations, or who had died many years ago often could not be found at the plant. Out of the MI study's 103 matched pairs, 9 of the MI cases and 14 of the matched controls had no work records. For the CHD death study, 13 of the 54 CHD deaths and 26 of the 80 non-CHD deaths had no work records.

A small committee of the plant's long-service employees and retirees met to reconstruct from memory the work histories for those study subjects with missing records. The committee consisted of four current employees and one pensioner, all of whom had long service at the plant. They were able to supply the remaining work histories (without dates) for all of the remaining MI matched-pairs, for 12 of the 13 CHD deaths, and for 23 of the 26 non-CHD deaths. One CHD death and four non-CHD deaths could not be remembered as ever having worked at the plant.

d. Definition of exposure

To study MI/CHD risks for a given work area, such as Teflon®, exposure was defined as ever having worked in that area prior to the MI/CHD attack date. Likewise, when MI/CHD risks for a specific salary level such as foreman were studied, exposure was redefined as ever having worked at that level prior to the MI/CHD attack date. A separate analysis was done for each work area and salary level listed above (section 3.c).

e. Statistical methods

For the MI and CHD case-control studies, odds ratios and chi-square tests were computed.

The odds ratio for the matched-pairs MI study was computed by taking the ratio of the two discordant pairs. That is, the number of pairs in which the case was exposed and the control was not exposed was divided by the number of pairs in which the control was exposed and the case was not. Under the null hypothesis, the expected value of the odds ratio is 1. The significance of this odds ratio's departure from 1 was tested by McNemar's chi-square test.

In the CHD case-control study, CHD deaths and non-CHD deaths were first categorized by 10-year age groups. An age-adjusted odds ratio and summary chi-square were then computed by the Mantel-Haenszel method. The odds ratio is defined as the odds of a CHD death having been

exposed divided by the odds of a control having been exposed. For a given age group, the odds of a CHD death having been exposed is the number of exposed CHD deaths divided by the number of never exposed CHD deaths.

Significance tests were two-tailed, and significance was judged at the 0.05 probability level.

4. Blood pressure study of Teflon® area workers

Blood pressures of Teflon® area workers were available from an earlier liver function study of these workers.

a. Selection of Teflon® area workers

The initial group consisted of 96 Washington Works employees who were in one of the following Teflon® area jobs as of October, 1979:

- TFE process operator
- FEP process operator
- TFE service operator
- FEP service operator
- Laboratorian; monomer operator; Teflon® area engineer, chemist, or foreman.

This group included 78 workers who had been tested earlier in the year for blood fluoride levels.

Only TFE/FEP process and service operators were considered to have had significant potential for exposure to ammonium perfluorooctanoate (C-8). Monomer operators, semi-works laboratorians, and Teflon® area foremen were kept as a separate comparison group since they worked in the Teflon® area but had only limited C-8 exposure potential.

The number in this group was later dropped to 88 since eight workers had not worked in the Teflon® area prior to their most recent blood pressure readings. These eight workers were added to the nonexposed group (i.e., the control group).

For these 88 employees, J. F. Doughty gathered detailed Teflon® area work histories from plant records and from personal interviews. Work histories were copied to code sheets (Appendix C).

b. Selection of non-Teflon® area control group

The control group consisted of a 10% systematic sample of all active Washington Works employees who, as

of August, 1979, had never worked in the Teflon® area. Mechanics and laboratorians were excluded from the controls since their exposure potentials could not be well documented.

The group was selected in the following manner. Dr. Y. L. Power pulled every tenth record from the plant's alphabetized medical files for active employees. These workers' names were then given to J. F. Doughty. From plant records and through personal interviews, Doughty obtained these workers' work histories. Workers who had ever worked in the Teflon® area or who had worked as mechanics or laboratorians were then dropped from the list. The remaining workers constituted the control group. Eight more workers were later transferred from the exposed to the control group because these eight had had no potential C-8 exposure prior to their most recent blood test.

c. Medical data

As a part of routine physical examinations, each worker's blood pressure had been recorded. From plant medical records, each study subject's first recorded blood pressure, plus any other blood pressures taken at the time that SMA-12 tests were done, were copied to code sheets (Appendix D). The subject's current height, weight, age, smoking status, and whether he was taking antihypertensive drugs were also recorded.

d. Blood fluōride levels

Prior to this study, blood organic fluoride levels had been measured on 78 of the plants's Teflon® area workers. Most of the workers tested had had potential C-8 exposure. Blood pressure readings were grouped and analyzed according to blood organic fluoride decile.

e. Statistical methods

Workers' most recent blood pressures were studied by exposure status, by specific Teflon® area job, and by blood organic fluoride decile. Analyses were based on (1) test means and (2) the proportion taking antihypertensive drugs or falling into the highest blood pressure decile. The highest decile was defined as the range in which the top ten percent of all control and exposed groups' test values lay. On the average, then, one would expect that ten percent of the control group's and ten percent of each exposed group's values would fall into this decile.

Group differences in proportions and in mean blood pressures were studied by analysis of covariance and least significant difference tests. This analysis adjusted for any group differences in age, sex or the Quetelet index (weight \div height²). Two-tail tests were performed, and significance was judged at the 0.05 probability level.

RESULTS

Plant-wide incidence of MI: cohort approach

Expected numbers of MI cases were based on the person-years distribution among the plant's male, active employees (table 1) and on the Company's MI incidence rates for the years 1956-77.

The 91 observed MI's at Washington Works were slightly greater than the 77.8 expected for the period 1956-77 (table 2). Although this difference was not statistically significant (two-tail $p = 0.16$), the difference was large enough to warrant further investigation. Table 3 indicates that the excesses were primarily limited to the period 1974-77 for wage roll and to the period 1971-73 for salary roll employees. These two excesses were statistically significant ($p < 0.04$).

Notice that although the male salary group showed an excess for the 1971-73 period, it showed no excess for the succeeding four-year period (table 3). This up-and-down pattern suggests that salary roll's MI excess during the 1971-73 period was a chance event that was not causally related to occupational factors.

Furthermore, since wage roll showed a significant excess for the most recent period but not for earlier periods, the circumstances suggest that this excess was also a chance event.

Case-control analysis of MI: exposure defined by salary level

When exposure was defined as ever having worked as a foreman, a significantly ($p < 0.01$) elevated odds ratio of 5.33 was found (table 4). The foremen's odds ratio was highest for the periods 1964-70 and 1971-78 (table 5).

A significantly ($p < 0.01$) decreased odds ratio of 0.28 was found when exposure was defined as ever having worked as a salaried technical person (table 4). When exposure was defined as ever having worked as unassigned supervision, a significantly ($p < 0.01$) decreased odds ratio of 0.28 was found. The odds ratio for supervision above foreman was less than 1 (0.5), but not significantly ($p > 0.20$).

Case-control analysis of MI: exposure defined by work area

When exposure was defined by work areas, none of the work areas showed odds ratios that were significantly different from 1 (table 6). Only the Technical-Research area had a high odds ratio (1.91) that approached statistical significance ($0.05 < p < 0.10$). The odds ratio for the Technical-Research area was high only for the periods 1957 to 1963 and 1971 to 1978 (table 7).

Case-control analysis of MI: exposure defined as being a foreman in a given area

The odds ratio of 7.00 for Mechanical area foremen was significantly ($p < 0.05$) greater than 1.00 (table 8). The odds ratio of 6.00 for Zytel® foremen approached statistical significance ($0.05 < p < 0.10$). When workers with mechanical foreman experience were excluded from the analysis, the odds ratio of 4.50 for non-Mechanical area foremen was statistically significant ($p < 0.05$).

Plant-wide mortality from coronary heart disease: cohort approach

The 48 CHD deaths observed were slightly less than the 56.5 expected for the period 1957-77 (table 10). Both wage and salary rolls experienced less than the expected number of CHD deaths.

The expected numbers of CHD deaths were based on the person-year distribution among the plant's active and pensioned male employees (table 9) and on the Company's CHD mortality rates.

Case-control analysis of CHD mortality: exposure defined by salary level

The odds ratio of 1.34 for foremen was not significantly ($p > 0.25$) greater than 1 (table 11). All other salary level definitions of exposure gave odds ratios that were less than but were not significantly ($p > 0.25$) different from 1.

Case-control analysis of CHD: exposure defined by work area

When exposure was defined by work area, none of the resulting odds ratios were significantly different from 1 (table 12). Delrin®, with a ratio of 2.56, had the highest odds ratio of all areas, but it was not statistically significant ($p > 0.10$). Teflon®, Lucite®, and Technical-Research areas had the lowest odds ratios (0.86, 0.87 and 0.63, respectively).

The odds ratio for Mechanical foremen (table 12, last entry) was 2.63 but was not statistically significant ($p > 0.25$).

Blood pressures in Teflon® area workers

There were no significant ($p > 0.10$) differences between Teflon® area workers and controls, or among the blood organic fluoride deciles, with respect to any of the blood pressure indices studied. These indices included mean systolic and diastolic blood pressures (tables 13 and 15) and the proportion of workers either taking antihypertensive drugs or falling into the highest blood pressure decile (tables 14 and 16).

DISCUSSION

There was no evidence that working in the Teflon® area increased the worker's risk of coronary heart disease. The MI incidence and CHD mortality odds ratios for Teflon® (1.20 and 0.86, respectively) were well within the bounds of random variation. There were no significant differences in blood pressures between Teflon® area workers and their controls. Nor were there significant blood pressure differences between jobs within the Teflon® area.

Statistically significant excesses of MI were observed among male wage roll workers for the period 1974 - 1977 and among male salary roll workers for the period 1971 - 1973. These excesses are thought to be due to the multiple comparison problem. That is, any epidemiologic study of this size presents the investigator with a large number of potential comparisons. A long series of comparisons will, with high probability, result in some comparisons testing significant, even in the absence of any causal associations. The significant excesses observed in wage and salary workers occurred only in isolated time intervals and showed no consistent trends with time. Consequently, these excesses provide no evidence of being occupationally related.

The high MI odds ratios for foremen and Mechanical area foremen are probably not related to occupational exposures. The patterns of disease that were actually observed contrast sharply with the patterns expected if a toxic agent in the workplace were responsible. For instance, one would expect the toxic agent to be confined to a few specific areas of the plant. Its effects would be felt only among employees from these areas. Furthermore, such an agent would not be expected to affect foremen without also affecting other workers below foreman. To find plant-wide excesses among foremen but not among other wage roll workers suggests that the excesses are not occupationally related.

Plant-wide both wage and salary roll workers experienced less than the expected number of CHD deaths. And unlike the MI odds ratio for foreman, the CHD death odds ratio for foreman was only slightly elevated and was not statistically significant.

The differences in results between the MI case and the CHD death analyses could have been due to several factors:

- Foremen may have shown unusually good survival following an MI.
- There may have been differences in CHD risks between active and pensioned foremen. For instance, pensioned foremen were no longer under the stresses of their jobs. Relatively lower CHD risks among pensioned foremen could have diluted the higher risks among active employees.
- Foremen may have had unusually high mortality rates from causes other than CHD. The use of non-CHD deaths as controls for the CHD case-control study may have biased the estimates of risk.

The high MI odds ratio for foremen and the low odds ratio for supervision above foremen were consistent with results from four previous studies:

- (1) Pell and D'Alonzo (1963) studied acute myocardial infarctions among male Du Pont employees for the years 1956 through 1961. They divided employees into five groups based on economic level and job responsibility. Having done this, they found that the group primarily representing foremen and clerical supervisors had the highest MI rates in the Company (4.0 per 1000/year). The lowest MI rates were found in two groups. The first group (2.5 per 1000/year) represented higher level salesmen, nonsupervisory professional personnel, administrators, and supervisors above foremen. The second group (2.2 per 1000/year) represented executives such as plant managers, district sales managers, laboratory directors, and division managers. Wage roll employees, who comprised skilled, semi-skilled, and unskilled production workers, had MI rates (3.2 per 1000/year) that fell between the rates for first line supervision and higher level supervision.
- (2) Hinkle (1968) of Cornell University Medical College studied the seven-year MI experience of 270,000 male employees of the Bell Telephone System. He found that foremen had a higher MI rate (4.52 per 1000/year) than workmen (4.33 per 1000/year), supervisors (3.91 per 1000/year), general managers (2.85 per 1000/year), and executives (1.85 per 1000/year).
- (3) Lee and Schneider (1958) found that cardiovascular disease prevalence rates were lower among executives than nonexecutives of comparable age.
- (4) Hoar (1980) has recently issued a preliminary report on MI incidence, cancer incidence, and mortality among foremen in the Du Pont Company. Her study found that a foreman's risk of having an MI was 2 to 3 times higher than expected based on a group of non-supervisory, non-sales salaried employees. This excess was restricted to foremen with high school

educations or less. Foremen also experienced significant excesses of lung cancer, of CHD mortality, and of overall mortality.

The MI odds ratio for foremen may be high because MI risk factors were not distributed equally among all segments of the workforce. These risk factors might include:

- cigarette smoking
- diet
- blood pressure
- serum lipids
- family history of coronary heart disease
- prior history of diabetes.
- psychologic and social variables

Recently, considerable attention has been given to psychologic and social risk factors for CHD. Jenkins (1976) has broken down these factors into several broad categories:

(1) Sociologic indices

Many sociologic indices have at one time or another been correlated with coronary heart disease risks. These include education, income, marital status, sex, race, obesity, physical activity, and social class. The effects of these factors should have been partially neutralized by the process of selecting matched controls. However, education may still have been a confounding factor. Hinkel (1968) found that MI incidence was higher among Bell System employees without college degrees than among employees with college degrees. If Washington Works foremen were less likely to have been to college than were other salaried employees, then maybe the MI rate should have been higher among foremen than among other salaried employees.

(2) Stress

Using different indices of stress, many studies have suggested that stress is related to the risk of coronary heart disease. Sources of stress that have been previously implicated include work overload, role conflict, job ambiguity, job responsibility for people as opposed to responsibility for things, life changes, social mobility, and status incongruity.

Role conflict occurs when a person is caught between two groups of people who demand different kinds of behavior or who expect that the job should entail different kinds of behavior or function (Kahn, 1974).

Status incongruity is defined as the condition of possessing simultaneously the identifying markings of different social classes. It can be identified by discrepancies among levels of education, income, occupation, quality of housing, and organizational membership. "Status incongruity is evidence that certain aspects, but not all, of a person's situation have moved upward (or downward) in status level during his own lifetime." (Jenkins, 1976).

Foremen may be more apt to experience role conflict, status incongruity, or other sources of stress than are other workers. Thus, excess stress may be one reason why foremen have a higher coronary heart disease risk than other workers do. Since cigarette smoking is one way of coping with stress, cigarette smoking may be more common among highly stressed workers and may contribute to high CHD risks in foremen.

(3) Coronary-prone behavior

Persons with certain behavior patterns have been found to have higher coronary heart disease risks than persons not exhibiting these patterns. These coronary-prone behavior patterns have been designated as type A. Type A behavior is a behavior style characterized by an excessive degree of aggressiveness, ambitiousness, competitiveness, and time urgency. The reciprocal of type A behavior is type B. Depending on the age group, coronary heart disease risks are 2- to 7-fold higher in type A than in type B individuals.

To the extent that foremen are more likely to exhibit type A than B behavior, they may be more prone to developing coronary heart disease.

(4) Anxiety and neuroticism

Coronary heart disease has been associated with a variety of expressions of anxiety, depression, psychophysiologic complaints, general nervousness, and with symptoms such as sleep disturbance, fatigue, and emotional drain.

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TABLE 1: PERSON - YEARS DISTRIBUTION
 AMONG WASHINGTON WORKS' MALE ACTIVE EMPLOYEES, 1956-77

<u>Age</u>	<u>Male Wage</u>	<u>Male Salary</u>
< 20	57	1
20-24	2,844	658
25-29	6,517	1,649
30-34	6,931	1,812
35-39	5,314	1,984
40-44	3,622	1,948
45-49	2,493	1,769
50-54	1,721	1,342
55-59	1,029	852
60-64	465	382
TOTAL	30,993	12,397

TABLE 2: OBSERVED AND EXPECTED NUMBERS (a) OF FIRST M.I.'S
 AMONG WASHINGTON WORKS' ACTIVE EMPLOYEES, 1956-77

	<u>Observed</u>	<u>Expected</u>	<u>SIR</u>
Male wage	56	48.7	1.1
Male salary	<u>35</u>	<u>29.1</u>	<u>1.2</u>
Total	91	77.8	1.2

(a) Expected numbers were based on the Du Pont Company's myocardial infarction incidence rates for the years 1956-77. Plant populations were determined from yearly payroll rates.

TABLE 3: OBSERVED AND EXPECTED NUMBERS (a) OF FIRST M.I.'S
 AMONG WASHINGTON WORKS' ACTIVE EMPLOYEES, 1956-77:
 BREAKDOWN BY PAYCLASS AND CASE YEAR

	<u>Male Wage</u>							<u>Total</u>
	<u>1956-58</u>	<u>1959-61</u>	<u>1962-64</u>	<u>1965-67</u>	<u>1968-70</u>	<u>1971-73</u>	<u>1974-77</u>	
Observed	1	6	4	6	8	7	24	56
Expected	2.2	3.8	4.9	6.0	8.2	8.9	14.7	48.7
SIR	0.5	1.6	0.8	1.0	1.0	0.8	1.6*	1.1
	<u>Male Salary</u>							<u>Total</u>
	<u>1956-58</u>	<u>1959-61</u>	<u>1962-64</u>	<u>1965-67</u>	<u>1968-70</u>	<u>1971-73</u>	<u>1974-77</u>	
Observed	0	5	3	4	5	11	7	35
Expected	1.5	2.5	3.5	3.9	4.8	5.3	7.6	29.1
SIR	0	2.0	0.9	1.0	1.0	2.1*	0.9	1.2
	<u>Total</u>							<u>Total</u>
	<u>1956-58</u>	<u>1959-61</u>	<u>1962-64</u>	<u>1965-67</u>	<u>1968-70</u>	<u>1971-73</u>	<u>1974-77</u>	
Observed	1	11	7	10	13	18	31	91
Expected	3.7	6.3	8.4	9.9	13.0	14.2	22.3	77.8
SIR	0.3	1.7	0.8	1.0	1.0	1.3	1.4	1.2

(a) Expected numbers were based on the Du Pont Company's myocardial infarction rates for the respective years. Plant populations were determined from yearly payroll figures.

TABLE 4: MATCHED PAIRS (a) CASE-CONTROL ANALYSIS OF
 FIRST MYOCARDIAL INFARCTION DURING THE YEARS 1957-78
 EXPOSURE IS DEFINED BY SALARY LEVEL (b) AT WASHINGTON WORKS

Definition of Exposures (Salary Level) (c)	Case		Odds Ratio	Chi-square
	+	-		
Foreman	Control + 10 - 16	3 67	5.33*	8.89
Supervision above Foreman	Control + 1 - 3	7 85	0.43	1.60
Technical	Control + 2 - 0	8 86	0.0	8.00
Laboratory	Control + 0 - 0	0 96	Not defined	-
Unassigned Supervision	Control + 4 - 5	18 69	0.28	7.35

(a) Controls were matched for age, sex, payclass, and year of attack.
 (b) Exposure was defined as ever having worked at the given salary level prior to the MI attack date.
 (c) + = exposed; - = not exposed
 * Significantly ($p < 0.05$) greater than 1.0 by McNemar's Chi-Square Test.

TABLE 5: MATCHED PAIRS (a) CASE-CONTROL ANALYSIS OF FIRST MYOCARDIAL INFARCTION DURING THE YEARS 1956-63, 1964-70, 1971-78. EXPOSURE IS DEFINED AS EVER HAVING WORKED AS A WASHINGTON WORKS FOREMAN PRIOR TO THE MI ATTACK DATE

Period (c)	Case		Odds Ratio	Chi-Square						
	+	-								
1956-63	Control	<table border="1"> <tr><td>+</td><td>2</td><td>1</td></tr> <tr><td>-</td><td>3</td><td>11</td></tr> </table>	+	2	1	-	3	11	3.00	1.00
+	2	1								
-	3	11								
1964-70	Control	<table border="1"> <tr><td>+</td><td>4</td><td>0</td></tr> <tr><td>-</td><td>4</td><td>17</td></tr> </table>	+	4	0	-	4	17	9.00 (b) *	4.00
+	4	0								
-	4	17								
1971-78	Control	<table border="1"> <tr><td>+</td><td>4</td><td>2</td></tr> <tr><td>-</td><td>9</td><td>39</td></tr> </table>	+	4	2	-	9	39	4.50*	4.45
+	4	2								
-	9	39								
Overall	Control	<table border="1"> <tr><td>+</td><td>10</td><td>3</td></tr> <tr><td>-</td><td>16</td><td>67</td></tr> </table>	+	10	3	-	16	67	5.33*	8.89
+	10	3								
-	16	67								

(a) Controls were matched for age, sex, payclass, and year of attack.
 (b) 0.5 was added to each cell before computing the odds ratio.
 (c) + = exposed; - = not exposed

* Significantly (p<0.05) greater than 1.0 by McNemar's Chi-Square Test.

TABLE 6: MATCHED PAIRS (a) CASE-CONTROL ANALYSIS OF
 FIRST MYOCARDIAL INFARCTION DURING THE YEARS 1957-78,
 EXPOSURE IS DEFINED BY WORK AREA (b) AT WASHINGTON WORKS

Definition of Exposure (Work Area) (c)	Case		Odds ratio	Chi-square	Definition of Exposure (Work Area) (c)	Case		Odds ratio	Chi-square
	+	-				+	-		
Teflon®	4	10	1.20	0.18	Utility Pool	16	13	1.38	0.81
	Control	12 70			Control	18 49			
Lucite®	2	11	1.55	1.29	Butacite®	3	11	0.91	0.05
	Control	17 66			Control	10 72			
Zytel®	0	19	1.16	0.22	Delrin®	2	4	2.50	2.57
	Control	22 55			Control	10 80			
Filaments	11	19	1.11	0.10	Color and Processing	0	4	1.75	0.82
	Control	21 45			Control	7 85			
Power and Services	2	10	0.40	2.57	Mechanical	12	19	0.84	0.26
	Control	4 80			Control	16 49			
Technical-Research	1	11	1.91	3.13					
	Control	21 63							

(a) Controls were matched for age, sex, payclass, and year of attack.
 (b) Exposure was defined as ever having worked in the given area prior to the MI attack date.
 (c) + = exposed; - = not exposed

Table 7: MATCHED - PAIRS (a) CASE-CONTROL ANALYSIS OF FIRST MYOCARDIAL INFARCTION DURING THE YEARS 1957-63, 1964-70, AND 1971-78: EXPOSURE IS DEFINED AS EVER HAVING WORKED IN TECHNICAL-RESEARCH AREA PRIOR TO THE MI ATTACK DATE.

<u>Period (t)</u>	<u>Case</u>	<u>Odds Ratio</u>	<u>Chi-Square</u>						
1957-63	<table border="1"> <tr><td>+</td><td>0</td><td>1</td></tr> <tr><td>-</td><td>4</td><td>12</td></tr> </table>	+	0	1	-	4	12	4.00	1.80
+	0	1							
-	4	12							
1964-70	<table border="1"> <tr><td>+</td><td>0</td><td>5</td></tr> <tr><td>-</td><td>2</td><td>18</td></tr> </table>	+	0	5	-	2	18	0.40	1.29
+	0	5							
-	2	18							
1971-78	<table border="1"> <tr><td>+</td><td>1</td><td>5</td></tr> <tr><td>-</td><td>15</td><td>33</td></tr> </table>	+	1	5	-	15	33	3.00*	5.00
+	1	5							
-	15	33							

Overall	<table border="1"> <tr><td>+</td><td>1</td><td>11</td></tr> <tr><td>-</td><td>21</td><td>63</td></tr> </table>	+	1	11	-	21	63	1.91	3.13
+	1	11							
-	21	63							

(a) Controls were matched for age, sex, payclass, and year of attack.
 (b) + = exposed; - not exposed.

* Significantly (p<0.05) greater than 1.0 by McNemar's Chi-Square Test.

Table 8: MATCHED-PAIRS (a) CASE-CONTROL ANALYSIS OF FIRST MYOCARDIAL INFARCTION DURING THE YEARS 1957-78. EXPOSURE (b) IS DEFINED AS BEING A FOREMAN IN THE GIVEN WORK AREAS AT WASHINGTON WORKS

Definition of Exposure (Work Area) (c)	Case		Odds ratio	Chi-square	Definition of Exposure (Work Area) (c)	Case		Odds ratio	Chi-square
	+	-				+	-		
Teflon [®]	+ 1	3	2.33	1.60	Utility Pool	+ 0	0	3.00 ^(d)	1.00
	Control - 7	85			Control	- 1	95		
Lucite [®]	+ 0	1	3.00	1.00	Butacite [®]	+ 0	3	0.67	0.20
	Control - 3	92			Control	- 2	91		
Zytel [®]	+ 0	1	6.00	3.57	Delrin [®]	+ 0	1	2.00	0.33
	Control - 6	89			Control	- 2	93		
Filaments	+ 0	2	0.50	0.33	Color and Processing	+ 0	1	2.00	0.33
	Control - 1	93			Control	- 2	93		
Power and Services	+ 0	2	0.00	2.00	Mechanical	+ 0	1	7.00*	4.50
	Control - 0	94			Control	- 7	88		
Technical Research	+ 0	0	3.00 ^(d)	1.00	Areas other than the Mechanical area (no Mechanical area experience)	+ 10	2	4.50*	4.45
	Control - 1	95			Control	- 9	67		

(a) Controls were matched for age, sex, payroll, and year of attack.
 (b) Exposure was defined as ever having worked as a foreman in the given area prior to the MI attack date.
 (c) + = exposed; - = Not exposed.
 (d) 0.5 was added to each cell before computing the odds ratio.
 * Significantly (p<0.05) greater than 1 by McNemar's Chi-Square Test.

Table 9: PERSON-YEARS DISTRIBUTION AMONG WASHINGTON WORKS'
MALE ACTIVE AND PENSIONED EMPLOYEES, 1957-77

<u>Age</u>	<u>Male Wage</u>	<u>Male Salary</u>
1-20	54	1
20-24	2728	626
25-29	6309	1599
30-34	6766	1761
35-39	5205	1923
40-44	3544	1905
45-49	2457	1746
50-54	1753	1347
55-59	1083	872
60-64	619	486
65-69	190	202
70-74	42	76
75-79	10	26
80-84	0	2
85+	0	0
Total	30,760	12,572

Table 10: OBSERVED AND EXPECTED NUMBERS (a) OF CORONARY HEART DISEASE DEATHS AMONG WASHINGTON WORK'S ACTIVE AND PENSIONED EMPLOYEES, 1957-77.

	<u>Observed</u>	<u>Expected</u>	<u>SMR</u>
Male Wage	26	33.2	0.8
Male Salary	22	23.2	0.9
Total	48	56.5	0.9

(a) Expected numbers were based on the Du Pont Company's coronary heart disease mortality rates for the years 1957-76. Plant populations were determined from yearly payroll rosters for active employees and from lists of pensioned employees.

Table 11: CASE-CONTROL (a) ANALYSIS OF CORONARY HEART DISEASE DEATHS DURING THE YEARS 1957-78:
EXPOSURE IS DEFINED BY SALARY LEVEL (D) AT WASHINGTON WORKS

Definition of Exposure (Salary Level)	Case		Control		Mantel-Haenszel Summary Odds Ratio (c)	Mantel-Haenszel Chi-Square
	Exposed	Not Exposed	Exposed	Not Exposed		
Foreman	12	41	12	65	1.34	0.33
Supervision Above Foreman	1	52	3	74	0.41	0.81
Technical	0	53	3	74	0.00	2.00
Laboratory	0	53	0	77	---	---
Unassigned Supervision	11	42	21	56	0.68	0.67

(a) Cases are all coronary heart disease deaths at Washington Works; controls are all deaths at Washington Works from causes other than coronary heart disease.
 (b) Exposure was defined as ever having worked at the given salary level prior to death.
 (c) The Mantel-Haenszel Odds Ratio is adjusted for age at death. Deaths were stratified by 10-year age groups prior to analysis.

Table 12: CASE-CONTROL. (a) ANALYSIS OF CORONARY HEART DISEASE DEATHS DURING THE YEARS 1957-78; EXPOSURE IS DEFINED BY WORK AREA (b) AT WASHINGTON WORKS.

Definition of Exposure (Work Area) (c)	Case		Control		Mantel-Haenszel Odds Ratio (c)	Mantel-Chi-Square	Definition of Exposure (Work Area)	Case		Control		Mantel-Haenszel Odds Ratio	Mantel-Chi-Square
	Exposed	Not Exposed	Exposed	Not Exposed				Exposed	Not Exposed	Exposed	Not Exposed		
Teflon®	4	49	11	66	0.86	0.05	Utility Pool	13	40	23	54	1.08	0.02
Lucite®	9	44	15	62	0.87	0.07	Butacite®	5	48	7	70	1.54	0.51
Zytel®	10	43	18	59	0.92	0.04	Deirin®	8	45	6	71	2.56	2.44
Filaments	12	41	15	62	1.15	0.10	Color and Processing	1	52	2	75	1.71	0.14
Power and Services	6	47	5	72	2.21	1.23	Mechanical	16	37	20	57	1.00	0.00
Technical-Research	6	47	12	65	0.63	0.90	Mechanical Foreman	6	47	3	74	2.63	1.61

(a) Cases are all coronary heart diseases deaths at Washington Works; controls are all deaths at Washington works from causes other than coronary heart disease.
 (b) Exposure was defined as ever having worked in the given area prior to death.
 (c) The Mantel-Haenszel Odds Ratio is adjusted for age at death. Deaths were stratified by 10-year age groups prior to analysis.

Table 13: MEAN SYSTOLIC AND DIASTOLIC BLOOD PRESSURES (a) BY OCCUPATIONAL GROUP (b)

Group	Group Size	Mean Age	Adjusted Weight (d)	Smoking (% smokers)	Adjusted Systolic Pressure (e)	Adjusted Diastolic Pressure (e)
Control (no Teflon, mechanic or laboratory work) (c)	80	38	3.6	39	120	75
FEP Process	13	49	4.0	77	122	76
FEP Service	3	37	3.6	33	126	78
TFE Process	24	46	3.7	38	120	74
TFE Service	26	37	3.5	27	118	75

Monomer Operator,
Semi-Works
Laboratorian,
Foreman 22 47 3.6 36 116 74

- (a) Based on most recent blood pressure reading as of October, 1979.
- (b) Based on job title at the time of the worker's most recent blood pressure reading.
- (c) Ten percent sample of current wage roll employees plus eight workers currently exposed to C-8 but who had never worked in Teflon at the time of their most recent blood pressure readings.
- (d) Adjusted weight = $\text{weight}/(\text{height})^2 \times 100$ (Quetelet index)
- (e) Adjusted for age, smoking habits, and adjusted weight.

Table 14: BLOOD PRESSURES (a) BY OCCUPATIONAL GROUP (b): PROPORTION OF WORKERS TAKING ANTIHYPERTENSIVE DRUGS OR FALLING INTO THE HIGHEST BLOOD PRESSURE DECILE

Group	Group Size	Mean Age	Adjusted Weight	Smoking (% Smokers)	Proportion on Antihypertensive Drugs or in Highest BP Decile	
					Systolic	Diastolic
Control (no Teflon, mechanic or laboratory work) (c)	80	38	3.6	39	0.10	0.10
FEP Process	13	49	4.0	77	0.39	0.23
FEP Service	3	37	3.6	33	0.33	0.33
TFE Process	24	46	3.7	38	0.25	0.25
TFE Service	26	37	3.5	27	0.08	0.08
Monomer Operator, Semi-Works Laboratorian, Foreman	22	47	3.6	36	0.14	0.18

(a) Based on most recent blood pressure reading as of October 1979.

(b) Based on job title at the time of the worker's most recent blood pressure reading.

(c) Ten percent sample of current wage roll employees plus eight workers currently exposed to C-8 but who had never worked in Teflon at the time of their most recent blood pressure readings.

(d) Adjusted weight = $\text{weight}/(\text{height})^2 \times 100$ (Quetelet index)

TABLE 15: WORKERS GROUPED BY ORGANIC FLUORIDE DECILES:
MEAN SYSTOLIC AND DIASTOLIC BLOOD PRESSURES

Of (a) Decile	Group Size	OF (a) Limits (ppm)	Mean No. of Years in C-8	Mean No. of Years in Teflon®	Mean Age	Adjusted Weight (b)	Smoking (% smoking)	Adjusted Systolic Blood Pressure (c)	Adjusted Diastolic Blood Pressure (c)
1	6	0.08-0.30	5	12	40	3.7	52	116	69
2	8	0.35-0.45	2	17	49	3.4	52	119	77
3	9	0.47-0.69	9	18	47	3.4	50	122	80
4	7	0.70-1.17	5	7	41	3.7	53	123	81
5	8	1.31-1.80	7	12	41	3.7	46	125	74
6	8	1.81-2.30	6	11	40	3.9	52	119	77
7	8	2.33-3.55	10	14	45	3.7	52	118	74
8	8	3.70-4.64	9	16	44	3.7	46	114	70
9	8	4.84-6.66	15	18	47	3.9	46	126	83
10	8	6.84-21.69	14	18	47	3.6	46	123	78

(a) OF = organic fluoride

(b) Adjusted weight = $\text{weight}/(\text{height})^2 \times 100$ (Quetelet index)

(c) Blood pressures adjusted for age, smoking habits (cigarette smoker vs. nonsmoker) and adjusted weight.

TABLE 16: WORKERS GROUPED BY ORGANIC FLUORIDE DECILES - PROPORTION OF WORKERS TAKING ANTIHYPERTENSIVE DRUGS OR FALLING INTO THE HIGHEST BLOOD PRESSURE DECILE

OF (a) Decile	Group Size	OF (a) Limits (ppm)	Mean No. of Years in C-8	Mean No. of Years in Teflon	Mean Age	Adjusted Weight (b)	Smoking (%)	Proportion on	
								Systolic	Diastolic
1	6	0.08-0.30	5	12	40	3.7	52	0.0	0.0
2	8	0.35-0.45	2	17	49	3.4	52	0.25	0.38
3	9	0.47-0.69	9	18	47	3.4	50	0.22	0.22
4	7	0.70-1.17	5	7	41	3.7	53	0.14	0.14
5	8	1.31-1.80	7	12	41	3.7	46	0.25	0.13
6	8	1.81-2.30	6	11	40	3.9	52	0.13	0.13
7	8	2.33-3.55	10	14	45	3.7	52	0.13	0.13
8	8	3.70-4.64	9	16	44	3.7	46	0.13	0.13
9	8	4.84-6.66	15	18	47	3.9	46	0.38	0.25
10	8	6.84-21.69	14	18	47	3.6	46	0.38	0.38

(a) OF = Organic Fluoride

(b) Adjusted weight = $\text{Weight}/(\text{Height})^2 \times 100$ (Quetelet index)

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LIVER TOXICITY
IN
FLUORO-CHEMICAL WORKERS

November 2003

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TETRA TECH, INC.

EXHIBIT 4

Liver Toxicity in Fluorochemical Workers

An analysis of medical surveillance data on employees exposed to perfluorooctanoate (PFOA) and perfluoro octane sulfonyl (POFS)-based chemicals in the workplace has been published by 3M Company (Olsen et al. 2001). The study is in the U.S. Environmental Protection Agency (EPA) PFOA administrative record as AR226-1047.

POFS-based chemicals may transform, to an undetermined degree, to perfluorooctanesulfonate (PFOS) in the body. The study reports that the more heavily exposed workers, as indicated by elevated PFOA and PFOS serum levels, have higher serum liver enzyme concentrations, suggesting liver damage. The study also performs a multivariate analysis that includes lifestyle and demographic factors that are known to be associated with elevated liver enzyme levels and concludes that it is these factors that are responsible for the indications of liver damage rather than PFOA/PFOS exposure. Unfortunately, Olsen et al. (2001) included independent variables in the analysis that are correlated and, therefore, are not truly independent. Thus, the multivariate analysis violates the assumptions of the statistical method used and, as will be demonstrated herein, the analysis cannot be used to support the conclusion that elevated liver enzyme levels in these workers are attributable to factors other than PFOA/PFOS exposure.

In this cross-sectional study, serum PFOA, PFOS, and total organic fluoride (TOF) levels were measured as indicators of exposure to fluorinated chemicals. The study reports the results of testing for clinical chemistry, thyroid hormone, hematology, and urinalysis parameters for male and female employees working production and nonproduction jobs in the 3M Antwerp and Decatur fluorochemical plants. Since PFOA and PFOS are known liver toxins, measurements of serum concentration for four liver enzymes plus total and indirect bilirubin measurements, were measured as indicators of liver damage. Data on lifestyle and demographic parameters, commonly associated with liver and kidney effects, were also included for each worker, e.g. alcoholic drinks per day, a known risk factor for liver disease. The data were stratified by plant, sex, and production versus nonproduction jobs. These three category variables are correlated with PFOA/PFOS serum levels since PFOA/PFOS exposure is higher in the Decatur versus the

Antwerp plant, in men versus women, and in production versus nonproduction jobs. These categories are used in the study to separate workers according to the magnitude of their exposure and will be referred to as “exposure categories” in this report.

Univariate Analysis – Comparisons Across Exposure Groups

The Olsen et al. (2001) study first presents several univariate analyses directly comparing data across the exposure categories described above. The data show that workers have high liver enzyme levels if they work in the Decatur versus the Antwerp plant, if they are production versus nonproduction workers, and if they are men rather than women. In each case, the group with higher PFOA/PFOS exposure has higher levels of each liver enzyme suggesting liver damage associated with PFOA/PFOS exposure. Statistical testing of the data was done only between plants with the Decatur workers significantly higher than the less heavily exposed Antwerp workers for liver enzymes in men and for three of four enzymes in women. Since the individual data were not provided in the study report, statistical testing of data across sex and job type cannot be done for the present report.

Significant differences between these exposure groups were also observed for variables related to thyroid function. For example, the highest value for TSH, a indicator of possible thyroid impairment, is in Decatur male production workers, the most heavily exposed group.

There were other notable differences between groups including those relating to lipid metabolism and several lifestyle and demographic parameters. For example, employees of the Antwerp plant consumed significantly more alcohol than those in the Decatur plant (1.1 versus 0.1 and 0.5 versus 0.1 drinks/day) for male production workers and female workers, respectively. Thus, alcohol intake runs counter to the usual association with liver damage, indicating the observed differences in liver enzymes between plants could not be explained by this lifestyle factor.

The data presented in Olsen et al. (2001) consisting of direct comparisons across exposure groups indicates the likely presence of liver and thyroid effects associated with PFOA/PFOS exposure in these workers.

Univariate Analysis – Comparison by Quartiles

An additional univariate analysis was conducted by separating the data based on serum PFOA/PFOS quartiles. The quartiles were defined based on PFOS levels, but that designation effectively separates the data by serum PFOA level as well since serum PFOA and PFOS levels are highly correlated in these workers. The quartile PFOA, PFOS, TOF, and ALT data for Decatur male production workers, the most heavily exposed group, are shown in Table 1 below. For these workers, quartile 4, the most heavily exposed quartile, is significantly elevated relative to the other three quartiles for the liver enzyme ALT. Other than the three exposure parameters (PFOA, PFOS, and TOF), and the liver enzyme ALT, no other measured variable was significantly different between any of the quartiles. Moreover, inspection of all the study data including clinical chemistry, thyroid, hematology, and demographic/lifestyle data provides no hint of any differences between quartiles that could account for an elevated liver enzyme value other than the differences in the exposure parameters—PFOA, PFOS, and TOF serum levels.

Table 1
Decatur Male Production Workers
Mean Serum PFOA/PFOS/TOF Concentration (ppm) and Serum ALT

	Quartile 1 (N=40)	Quartile 2 (N=40)	Quartile 3 (N=41)	Quartile 4 (N=40)
PFOA	1.24 ^{3,4}	1.82 ⁴	2.42 ^{1,4}	3.88 ^{1,2,3}
PFOS	0.55 ^{2,3,4}	1.01 ^{1,3,4}	1.74 ^{1,2,4}	3.22 ^{1,2,3}
TOF	1.34 ^{2,3,4}	2.20 ^{1,3,4}	3.43 ^{1,2,4}	5.75 ^{1,2,3}
ALT ⁵	33 ⁴	32 ⁴	33 ⁴	44 ^{1,2,3}

¹Mean is significantly different (P<0.05, Bonferroni (Dunn) t test) from the mean of the 1st Quartile

²Mean is significantly different (P<0.05, Bonferroni (Dunn) t test) from the mean of the 2nd Quartile

³Mean is significantly different (P<0.05, Bonferroni (Dunn) t test) from the mean of the 3rd Quartile

⁴Mean is significantly different (P<0.05, Bonferroni (Dunn) t test) from the mean of the 4th Quartile

⁵Concentration unit is IU/L

Quartile analysis for the groups exposed to lower PFOA and PFOS levels, such as the Antwerp plant workers, nonproduction workers, and female workers, did not produce statistically significant indications of a dose response for liver effects, possibly because splitting the worker groups into quartiles reduced the number of subjects per group by four, making the statistical detection of group differences more difficult.

When all the workers from both plants were included in a quartile analysis, the indications of liver toxicity in the most heavily exposed quartile persisted with an additional liver enzyme, alkaline phosphatase, significantly elevated in the highest two quartiles. The data are shown in Table 2 below. Since the number of subjects in each quartile is considerably larger, the statistical power of the analysis is improved. Alcohol consumption was significantly lower in quartile 3 and 4 compared to quartile 1 and was again not associated with liver toxicity.

Table 2
Decatur and Antwerp Male Production and Nonproduction Workers
Mean Serum PFOA/PFOS/TOF Concentration (ppm) and Serum ALT/Alk Phos

	Quartile 1 (N=105)	Quartile 2 (N=105)	Quartile 3 (N=106)	Quartile 4 (N=105)
PFOA	0.54 ^{2,3,4}	1.21 ^{1,3,4}	1.45 ^{1,4}	2.70 ^{1,2,3}
PFOS	0.27 ^{2,3,4}	0.60 ^{1,4}	1.19 ^{1,2,4}	2.69 ^{1,2,3}
TOF	0.62 ^{2,3,4}	1.40 ^{1,3,4}	2.12 ^{1,2,4}	4.41 ^{1,2,3}
ALT ⁵	26 ⁴	28	28	33 ¹
Alk Phos ⁵	61 ^{3,4}	67	69 ¹	70 ¹

¹Mean is significantly different (P<0.05, Bonferroni (Dunn) t test) from the mean of the 1st Quartile

²Mean is significantly different (P<0.05, Bonferroni (Dunn) t test) from the mean of the 2nd Quartile

³Mean is significantly different (P<0.05, Bonferroni (Dunn) t test) from the mean of the 3rd Quartile

⁴Mean is significantly different (P<0.05, Bonferroni (Dunn) t test) from the mean of the 4th Quartile

⁵Concentration unit is IU/L

Univariate analysis—Values Exceeding Normal Range by PFOA/PFOS Quartiles

An additional quartile analysis on all workers was performed reporting the number of workers in each exposure quartile with serum liver enzyme concentrations above the reference range

(normal value range). This value is an indicator of how many workers are manifesting clinically recognizable liver damage as opposed to those who may be experiencing more subtle effects. As with the previous univariate analyses the results were stratified by exposure category, i.e. by plant, by sex, and by production versus nonproduction job (Table 3).

For male production workers in the Decatur plant, the most highly exposed Q4 group, a greater number of workers had elevated serum levels than the other three quartiles for AST, ALT, GGT, and total liver panel. In the less heavily exposed Antwerp plant, Q4 male production workers were elevated relative to the other quartiles only for GGT. Looking across all four liver enzymes in Table 3, the number of workers exceeding reference range values is higher for men than women, for production versus nonproduction jobs, and for workers in the Decatur plant versus the Antwerp plant.



Table 3
Number of Workers Exceeding Normal Values for Hepatic Clinical Chemistry
by PFOA/PFOS Quartile and Exposure Category

	Alkaline Phosphatase				AST				ALT				GGT				Total Liver Panel*			
	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4
	Decatur																			
Males/Production	1	2	2	1	0	1	0	4	3	5	3	11	4	3	2	6	7	8	7	14
Males/Nonproduction	0	0	0	0	0	2	0	0	1	1	2	0	0	3	0	1	1	5	2	1
Females/Prod+Nonprod	0	0	0	0	1	0	0	0	1	0	0	0	0	0	2	0	1	0	2	0
	Antwerp																			
Males/Prod	0	0	0	0	1	0	0	0	1	0	0	0	1	1	2	4	3	5	4	5
Males/Nonproduction	0	0	0	0	0	0	0	1	0	1	0	0	1	1	2	1	2	2	3	1
Females/Prod+Nonprod	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0

*Number exceeding normal range for any enzyme or total or direct bilirubin.



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In summary, the quartile analysis presented in the 3M report clearly indicates a dose response for serum liver enzyme concentrations across serum PFOA/PFOS quartiles and between the exposure categories (sex, job type, and plant location) that are surrogates for exposure to PFOA/PFOS. Furthermore, the liver enzymes levels for a number of study subjects exceeded the upper bound of the normal range, indicating clinical liver toxicity. Even considering only the values exceeding the normal range, a dose response for the across exposure variables and categories is apparent. The statistical analyses presented by Olsen et al. (2001) and an inspection of demographic and lifestyle variables that might explain the elevated liver enzyme levels fails to provide any potential cause for the observed dose response other than exposure to PFOA/PFOS. Therefore, based on these univariate analyses one would conclude that exposure to PFOA/PFOS is likely to be causally associated with elevated serum liver enzyme levels resulting from liver toxicity in these worker populations.

Multivariate analysis

The Olsen et al. (2001) study dismisses the findings of the univariate analysis based on the results of a multivariate study that purports to demonstrate that the univariate results are due to "confounding factors" and not to PFOA/PFOS exposure. Olsen et al. (2001) states the following.

"However, after adjusting to the employees' individual liver function values by potential confounding factors including age, BMI, number of alcoholic drinks per day, cigarettes per day and serum triglyceride values, we found no association between liver function values and PFOS or PFOA. We therefore suspect the univariate associations were influenced by known confounders of liver function analyses."

In a subsequently published journal paper reporting on the same data reported in the Olsen et al. (2001), Olsen et al. (2003) also dismisses the elevated liver enzymes in a similar fashion. "Adjusting for potential confounding factors, there were no substantial associations between

hepatic enzymes and the employees' serum PFOS concentrations." The more recent paper does not show the details of the multivariate analysis on which it bases these conclusions.

Olsen et al. (2001) reported using multivariate regression to test for associations between various markers of liver toxicity, such as liver enzyme levels in serum, and various potential causal factors. The potential causal factors included PFOA, PFOS, or TOF serum level, and various lifestyle and demographic factors. The markers of liver toxicity, such as an individual serum liver enzyme, were the dependent variables and the potential causal factors were the independent variables in the analysis. The primary problem with this analysis is that the independent variables included in the regression equations are not independent. Each of the multivariate regression analyses that are presented in Olsen et al. (2001) contains independent variables that are highly correlated and not independent.

The multivariate analysis reported in Olsen et al. (2001) is a linear model fit by the "least square" method. The least square method makes strong assumptions about the structure of the data under study. When these assumptions are violated, the least squares method may completely misrepresent the data and the conclusion suggested by the results may not be correct. Regression diagnostics can be used that reveal these violations of the assumptions. Olsen et al. (2001) did not report the use of regression diagnostics and their analysis obviously violated many of the requirements of the statistical procedure employed.

A well-known requirement in conducting a multivariate linear regression is that the independent variables be truly independent of one another. This means that there must be no intercorrelation between any two explanatory variables (the technical term for this is collinearity). If this intercorrelation exists, computations are inaccurate, coefficients are unstable and their standard error is large. When there is a strong linear relation between predictors in a regression analysis, the precision of the estimated regression coefficients declines and conclusions are inaccurate (Fox and Monett 1997). If this occurs, as in this case, the regression analysis is faulty and cannot be used to draw conclusions.



Unfortunately, the Olsen et al. (2001) study apparently paid little attention to the requirement that independent variables be independent. For example, the study used the following nine independent variables, many of which would be expected to be highly correlated, in the regression analysis for serum ALT, one of the serum liver enzymes reflecting liver damage.

PFOA

Production/nonproduction job

Antwerp/Decatur plant

Age

BMI

Cigarettes/day

Alcoholic drinks/day

Years worked

Triglycerides

For this regression on ALT, the primary purpose of the analysis is to determine whether the variability in serum PFOA levels explains some of the variability in serum ALT levels and may, therefore, be causally related to it. This is done by testing the coefficient of serum PFOA in the regression equation to determine if it is significantly different from zero. The P value for the coefficient of the serum PFOA obtained by Olsen et al. (2001) was 0.13. Since a P value of less than 0.05 is generally required for such a variable to be considered as significantly different for zero, the analysis was judged to have not yielded a significant association between serum PFOA and ALT levels. However, that conclusion is flawed.

The inclusion of another variable in the analysis that is correlated with serum PFOA violates the assumptions of the analysis and invalidates any conclusion regarding the significance of an association between PFOA and ALT. The variable that is correlated with serum PFOA is the plant location variable (Decatur or Antwerp). The PFOA serum levels in Decatur workers are roughly twice that of Antwerp workers because the exposure is higher in the Decatur plant. Since the Antwerp/Decatur variable was intercorrelated with the PFOA level, the coefficients on



which the Olsen et al. (2001) study based its conclusions can be expected to be unstable, have large standard errors, and be inaccurate. Intercorrelated variables such as the Antwerp/Decatur variable should not have been included in the analysis. This could have been avoided if standard diagnostic methods that are used to run regression models when colinearity may exist should have been used. If these precautions had been taken, the PFOA variable might well have been found to be a predictor of serum ALT.

The same problem exists for the multivariate regression on GGT. If standard methods had been used, PFOA or PFOS may have been judged to explain a significant portion of the variability for this enzyme as well. The problems with the ALT and GGT regressions are the most significant as far as determining whether perfluorinated chemical exposure to the Antwerp and Decatur worker populations have resulted in significant liver toxicity. There are other examples of intercorrelated independent variables in the many multivariate regressions that were done for this study. For example, years worked and age will also be to highly correlated variables

When there are many potential predictors, standard variable selection techniques can be used that reduce the predictors to an optimal subset. They can include interaction terms (e.g. alcoholic drinks/day*age) as predictors and they assess their significance in the model.

Also, if the insignificant predictors are kept in the model along with the significant predictors, this will decrease the model fit i.e. artificially compromise (decrease) the effect of the significant variables in explaining the data and lead to incorrect conclusions. A way to avoid chance findings in regression analysis is to run a "cross validation" check. This is done by splitting the data in half to validate the conclusions made from one half to the other or, in this case, by fitting a model to the data for workers from each plant location separately.

Conclusion

The major finding of both the Olsen et al. (2001) and (2003) studies is that there are no indications in the Decatur and Antwerp 3M worker populations of hepatotoxicity. This finding depends entirely on the results of multivariate regressions to invalidate the positive statistical findings of the univariate analysis described above. Had the standard statistical procedures been

properly used in conducting the regression analysis, the assessment of the results of the univariate analysis likely would not have dismissed the unavoidable conclusion that liver toxicity exists in these workers.

References

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Olsen GW, Burris JM, Burlew MM, Mandel JH. 2003. Epidemiological study of Worker Serum Perfluorooctanesulfonate (PFOS) and Perfluorooctanate (PFOA) Concentrations and Medical Surveillance Examinations. J Occup Environ MED. 45:260-270.



September 20, 1978

PERSONAL AND CONFIDENTIAL

TO: W. A. BOWER
FROM: Y. L. POWER, M.D.

A review of the medical records of eleven operators and eighteen laboratorians who have had long-term exposure to C-8 was undertaken.

As you would anticipate, a great variety of illnesses and physical findings were found; but I do not believe any of these are caused by exposure to C-8. Some of the illnesses found are two heart attacks and five employees with high blood pressure. One questionable case of skin cancer was found during an employee's physical examination in 1976. No further mention of this possible tumor could be found.

Minor elevations of many blood tests did occur in larger-than-anticipated numbers and are listed separately. With the exception of one person, all of the elevations were borderline and not indicative of disease. One of the liver function tests (SGOT) is most frequently elevated in the operator group. However, no liver diseases were found. Many of the laboratorians also work with Perclene, which is a known hepatatoxin.

In conclusion, I could find no unusual health problems occurring in the group of people studied, with the exception of borderline elevation of liver function tests. Since it has been previously determined that C-8 is an hepatatoxin, it is possible that C-8 may be causing very minimal, and certainly not clinically apparent, toxic effects to the liver. Because the total number of records reviewed is small (31), I do not believe any findings of this study are statistically valid.

Y. L. P
YLP:vf

AJP001418

EXHIBIT 5

EID080233

PERSONAL AND CONFIDENTIAL

<u>LAB TEST</u>	<u>% OPERATORS WITH ABNORMAL TESTS **</u>	<u>% LABORATORIANS WITH ABNORMAL TESTS</u>	<u>ANTICIPATED LEVEL FROM STUDY 1976 (% WITH ABNORMAL TESTS)</u>
* SGOT	60	11.2	14.21
* Alkaline Phosphatase	30	16.7	6.84
Albumin	10	16.7	1.58
Uric Acid	10	5.6	4.21
Cholesterol	30	0	1.05
BUN	30	11.2	3.68
Glucose	10	27.8	1.58
Calcium	10	0	0
Total Protein	10	0	0
* Bilirubin	0	11.2	1.05
LDH	0	11.2	1.58

* Liver function tests.

** Only 10 operators had liver function test done.

YLP:vf
9/20/78

EID080234

AJP001419

CC: B. W. Karrh, M.D.



E. I. DU PONT DE NEMOURS & COMPANY
WILMINGTON, DELAWARE

EMPLOYEE RELATIONS DEPARTMENT

March 15, 1979

PERSONAL AND CONFIDENTIAL

P. G. GILBY
CDEP
B-13265

CHAMBERS WORKS
FLUOROSULFACTANT STUDY
(Ref. Letter from RDR to PGG, 1/23/79)

In response to our request to the plant for additional information to analyze the data statistically, we received a tabulation of the Dispensary Visits and Disability Wage incidents in the exposed and control groups (Attachment V). These data were broken down by body systems. We were also informed of the number of employees in each group who had abnormal liver function tests.

We performed a "chi-square" test to test the significance of differences between the exposed and control groups. The attached table shows only those differences that were found to be statistically significant.

In the category, "Allergic, Endocrine, and Metabolic" disorders, a significantly higher incidence was found in the exposed group for both Dispensary Visits and Disability Wage incidents. This was attributed in the report to a higher number of diabetics in the exposed group.

The exposed group also showed significantly higher numbers for "mental and psychoneurotic" disorders and for disorders of "skin and cellular tissues."

The control group, on the other hand, had considerably more Disability Wage incidents for circulatory diseases, 25 compared to 5. This difference is highly significant ($P < 0.001$).

Explanations for these differences cannot be found from the available data. It would be helpful to find out what specific diagnosis within these general categories accounted for the differences between the two groups.

GK000378

Although the number of employees with abnormal liver function tests was notably higher in the exposed group (6 compared to 1), the difference is not statistically significant ($P < 0.05$). Nevertheless, the data do suggest that the exposed group may be at an excess risk of developing liver disease, so continued surveillance would be advisable.

MEDICAL DIVISION

Sidney Pell

Sidney Pell
Manager
Epidemiology Section

SP:msd
Attach.

GK000379

GK000379



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EMPLOYEE RELATIONS DEPARTMENT

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R. M. Shepherd-PP&R-D-120
JOHN DOUGHTY-B-1

PERSONAL & CONFIDENTIAL

August 28, 1979

Y. L. POWER, M.D.
MEDICAL SUPERINTENDENT
PP&R DEPARTMENT
PARKERSBURG, W. VA.

STATUS REPORT ON WASHINGTON WORKS LIVER FUNCTION SURVEY AND
CORONARY HEART DISEASE MORTALITY STUDY

B. W. Karrh asked me to look into the liver function test results for workers with C-8 exposure, and Y. L. Power asked me to examine myocardial infarction cases and deaths at the Plant. S. Pell and R. M. Shepherd agreed that these items should be investigated.

End of October
My preliminary results suggest that C-8 exposed workers may possibly have positive liver function tests more often than the plant population as a whole, and that the number of active wage roll employees having myocardial infarctions from 1974 through 1977 was somewhat higher than was expected based on Company-wide experience. As a consequence of these preliminary findings, the following steps are being taken:

(1) Liver function survey

Report by [unclear] 8/28/79

- Y. L. Power is having every tenth active employee's most recent SMA-12 test results photocopied and sent to me. Included on each worker's SMA-12 sheet will be name, the date the blood chemistries were done and the worker's age. *OK*

copy [unclear]

- G. A. Ploeger is gathering exposure history records for every worker selected by Y. L. Power above (over 220 workers). These exposure histories will contain the worker's name, social security number, birth date, sex, payroll class, date hired, dates in and out of the Teflon area, and the job titles held during each period spent in Teflon area.

AIPO01399

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- Ploeger and Power will construct a list of everyone who is currently potentially exposed to C-8. SMA-12's and exposure histories for these workers will be sent to me. Ploeger and Power estimate that it will be two to three weeks before all SMA-12's and exposure histories can be supplied.

(2) Coronary heart disease mortality

- R. Dyer is seeing if it is possible to construct a list of pensioners who were receiving a pension in 1957. If he can make such a list, he should also be able to make lists for the years 1958-1978, in which case we could study coronary heart disease mortality among active and pensioned employees.

*week of
9/24*

MEDICAL DIVISION

W. E. Fayerweather

W. E. Fayerweather
Epidemiologist

WEF:msd

AJP001400

EID080215

LIVER FUNCTION STUDY OF WASHINGTON WORKS EMPLOYEES EXPOSED TO C-8

WILLIAM E. FAYERWEATHER

JANUARY 28, 1981

WIEF000076

EID102509

Acknowledgements

I want to thank J. F. Doughty and Y. L. Power for the many days they spent consulting and assembling data for this project; P. Thistleton for his industrial hygiene consulting, especially as it related to C-8 exposure potential at the plant; and S. Pell, W. L. Sprout, R. M. Shepherd, and V.A. Brewster for their helpful advice and comments.

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Summary

Dr. Y. L. Power assembled biochemical data on some recent Washington Works employees. Based on a crude analysis of these data, the results suggested that certain workers with potential ammonium perfluorooctanoate (C-8) exposure might be showing liver effects. Also, several unpublished animal studies have shown that C-8 produces liver damage when it is given at moderate or high doses. As a consequence of these findings, a more detailed assessment of C-8's health effects in Washington Works employees was undertaken.

Data from routine blood tests were collected and compared among groups of Teflon® area and non-Teflon® area workers. SGOT, LDH, AP, and bilirubin were studied, since these tests are generally good for detecting liver disease. Within the Teflon® area, C-8 exposure groups were defined by work history and by blood organic fluoride level.

These data provided no conclusive evidence of an occupationally related health problem among workers exposed to C-8. Although initial analyses suggested that there might be liver effects attributable to C-8 exposure, further analyses did not support this position.

Background

The Teflon® area consists of two divisions: the Teflon® Polymers Division and the Teflon® Copolymers Division. The Teflon® Polymers Division produces tetrafluoroethylene (TFE) and hexafluoropropylene (HFP) monomers and Teflon® polymers. These polymers are made by batch processes. Ammonium perfluorooctanoate (C-8) is a dispersing agent added to nearly all of the polymer processes. The monomers do not contain C-8.

The Teflon® Polymers Division makes three types of polymer products: fine powder, dispersion, and granular. More C-8 is used for dispersion than for fine powder products. Granular products use less C-8 than do dispersion products. Two continuous driers remove nearly all the C-8 from fine powder, and washing and drying processes remove essentially all of the C-8 from granular products. Dispersion products contain roughly 0.3 percent C-8 based on solids.

The Teflon® Copolymers Division produces four copolymers, all of which contain TFE. Three of these copolymers are made by batch processes. The fourth, Tefzel®, is made by a continuous process. C-8 is added as a dispersing agent for all of the copolymers except Tefzel®. Fluorinated ethylene propylene (FEP), the major copolymer, makes up about 60 percent of the copolymer produced. FEP consists of TFE and hexafluoropropylene (HFP).

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The FEP polymerization process also generates an in situ dispersing agent. In June, 1976 the plant began adding C-8 dispersing agent to increase the reaction rate. This change reduced the amount of time needed for the process and also reduced the amount of in situ dispersing agent that was formed. However, some in situ dispersing agent is still formed in all FEP batches.

Until the FEP polymer reaches the humid heat treating ovens, it contains in situ as well as C-8 dispersing agent. FEP polymer is very dusty. So, in the processing steps between the FEP polymerizers and the ovens, there is significant potential for exposure to C-8 and in situ dispersing agents. FEP dispersion products contain in situ dispersing agent and about 0.1 percent C-8 based on solids.

In situ dispersing agent is not well characterized. It is believed to be a mixture of homologs of low molecular weight TFE - HFP compounds, some with acid end groups. On a weight basis it is less surface active than C-8.

Several unpublished animal toxicity studies done at 3M Corporation and at Du Pont have found that moderate and high dose levels of C-8 produced liver damage. Both reversible and irreversible liver damage, elevated liver enzyme tests, and enlarged livers were found. Study results depended on the dose level, exposure route, sex and species tested.

Dr. Y. L. Power assembled biochemical data on some current Washington Works employees who had had company physical examinations in 1978. Based on a preliminary analysis of these data, the results suggested that certain workers with potential C-8 exposure might be showing liver effects.

As a consequence of the previous animal studies of C-8 and of Dr. Power's preliminary findings, a more detailed assessment of C-8's health effects in Washington Works employees was undertaken.

Study Objective

The objective was to determine whether occupational exposure to C-8 adversely affects liver functions as measured by blood levels of glutamic oxaloacetic transaminase (SGOT), lactic dehydrogenase (LDH), alkaline phosphatase (AP), and bilirubin.

Note: These blood tests are neither 100% sensitive nor 100% specific for detecting liver disease. There are a number of circumstances under which the test may give false positive or false negative results. These circumstances are discussed at the end of the paper under Liver function tests: limitations.

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Methods

1. General design

Recent blood test results for SGOT, AP, LDH, and bilirubin were studied by specific Teflon® area job and by blood fluoride level.

2. Selection of study groups

The initial group consisted of 96 Washington Works employees who were in one of the following Teflon® area jobs as of October, 1979:

- TFE process operator
- FEP process operator
- TFE service operator
- FEP service operator
- Laboratorian; monomer operator; Teflon® area engineer, chemist, or foreman.

This group included 78 workers who had been tested earlier in the year for blood fluoride levels.

Only TFE/FEP process and service operators were considered to have had significant potential for exposure to C-8. Monomer operators, semi-works laboratorians, and Teflon® area foremen were kept as a separate comparison group, since they worked in the Teflon area but had only limited C-8 exposure potential.

The number in this group was later dropped to 88, since 8 workers had not worked in the Teflon® area prior to their most recent blood test. These 8 workers were added to the nonexposed group.

For these 88 employees, J. F. Doughty gathered detailed Teflon® area work histories from plant records and from personal interviews. Work histories were copied to code sheets (table 1).

3. Selection of a nonexposed control group

The control group consisted of a 10% systematic sample of all active Washington Works employees who, as of August, 1979, had never worked in the Teflon® area. Mechanics and laboratorians were excluded from the controls, since their exposure potentials could not be well documented.

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The group was selected in the following manner: Dr. Y. L. Power pulled every tenth record from the plant's alphabetized medical files for active employees. These workers' names were then given to J. F. Doughty. From plant records and through personal interviews, Doughty obtained these workers' work histories. Workers who had worked in the Teflon® area or who had worked as mechanics or laboratorians were then dropped from the list. The remaining workers constituted the control group. Eight more workers were later transferred from the exposed to the control group, because these 8 had had no potential C-8 exposure prior to their most recent blood test.

4. Biochemical blood tests

As a part of routine physical examinations, each worker's blood is tested for 12 biochemical markers. These 12 tests are called the SMA-12.

From plant medical records, every SMA-12 on the exposed and control workers was copied to code sheets (table 2). All SMA-12 tests had been performed by the same laboratory and by the same methods. Very few SMA-12's had been done before 1974-75. Every worker's most recent SMA-12 had been done since 1977. Only tests pertaining to the liver were studied. These included the SGOT, AP, LDH, and bilirubin.

5. Blood fluoride levels

Prior to this study, blood fluoride levels had been measured on 78 of the plant's Teflon® area workers and on 25 Wilmington office workers. Blood fluoride measurements had been made at Jackson Laboratory by the 3M (bomb) method. Most of the workers tested at the plant had had potential C-8 exposure. Liver function test results were analyzed according to blood fluoride levels.

6. Statistical methods

SMA-12 results were studied by exposure status, by specific Teflon® area job, and by blood fluoride decile. Analyses were based on (1) test means and (2) the proportion falling into the highest liver function test decile. The highest decile was defined as the range in which the top 10 percent of all control and exposed groups' test values lie. On the average, then, one would expect that 10 percent of the control group's values would fall into this decile. Unless stated otherwise, test values were from the worker's most recent SMA-12.

Group differences in biochemistry test means were studied by analysis of covariance and least significant difference tests (LSD). This analysis adjusted for any group differences in age or sex. The statistical significance of differences in proportions was assessed by Fisher's exact test. Two-tail tests were performed, and p-values less than 0.10 were reported.

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Results

1. Test validation

Dr. Y. L. Power provided preliminary data on the SMA-12 results for 1978 (table 3). These data showed that the plant population as a whole had an unusually large percentage of elevated SGOT's. SGOT's were elevated in 19 percent of the workers whereas elevations would only have been expected in about 5% based on random statistical variation. AP, bilirubin, and LDH tests showed plant-wide elevations in 8, 4, and 3 percent of the workers, respectively.

The large, plant-wide elevations in SGOT's suggested one of two things. Either workers in many different areas were affected, or the plant's SGOT test was in error.

Dr. Power took two steps to validate the SGOT test. First, he took blood samples from about 100 workers and sent half of each blood sample to the standard laboratory (General Consultants, Inc.) and the other half to an Upjohn Laboratory to be tested. When the results of the standard laboratory were plotted against the results of Upjohn (figure 1), the two laboratories were correlated. High SGOT's at the standard laboratory were high at Upjohn, and low SGOT's at the standard were low at Upjohn.

However, at all SGOT levels the standard laboratory's value was higher than Upjohn's. Furthermore, about 16 percent of the standard laboratory's values were "abnormal," whereas none from Upjohn fell in the "abnormal" range.

Dr. Power also had the standard laboratory use a second method (manual enzymatic) to reanalyze samples that showed elevated SGOT's by the first method (automated colorimetric). In the 22 retested samples, only one sample was found to be elevated by the second method (table 4). When the results of the first method were plotted against the second, the results were correlated (figure 2).

The interlaboratory and intermethod comparisons suggested that

- SGOT's measured at the standard laboratory by the standard method were systematically higher than the true blood levels.
- By the standard method the standard laboratory's observed range for "normal" SGOT values was considerably higher than the stated normal range.
- Valid SGOT level comparisons can be made between exposed and nonexposed groups, provided that test

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means or the proportion falling into the highest test decile are used. Since SGOT levels were correlated between laboratories and between methods, valid between-group comparisons are possible.

2. Liver tests by job

TFE process workers' mean SGOT of 45 was higher than the control group's mean of 39. FEP service and process workers' mean AP's of 101 and 81, respectively, were higher than the control group's mean of 64. These differences were statistically significant at the 0.05 probability level (table 5). Similarly, FEP process and FEP service workers had significantly ($p < 0.05$) larger proportions of the AP values falling into the highest AP decile (table 6).

There were no other significant differences between Teflon® area workers and controls with respect to SGOT, AP, bilirubin, or LDH.

3. Liver tests by blood fluoride level

The mean SGOT for the highest blood organic fluoride decile was significantly higher than the mean for the lower nine deciles (52 vs 40, respectively). However, when the data were broken down into individual organic fluoride deciles, the data did not show a typical, steadily rising dose-response curve (tables 7 and 8). In fact, the second and third highest mean SGOT's were found in the first and third deciles.

AP, LDH, and bilirubin showed no unusual elevations when compared by organic fluoride decile. Likewise SGOT, AP, LDH, and bilirubin showed no relationship to inorganic fluoride levels.

4. Blood fluoride level by job

TFE process operators were overrepresented in the two highest organic fluoride deciles. TFE process operators made up about one third of the 78 workers tested for blood fluorides. But when the 16 workers from the two top organic fluoride deciles were listed by Teflon® area, 12 of these workers had been TFE process operators at the time they were tested (table 9).

Four others had worked as TFE process operators within 1 to 2 years prior to the time they were tested. The number of years of working with C-8 or of working in the Teflon® area did not appear to be related to organic fluoride level (table 9). In fact, the third highest organic fluoride level was measured in a worker having less than 3 years experience with C-8.

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These data suggest that TFE process operators have the highest potential for exposure, and that only 3 years of C-8 contact may be sufficient to elevate blood organic fluoride levels. TFE process operators usually have more service than TFE service operators.

Blood inorganic fluoride level and Teflon® area assignment appeared to be unrelated. The highest inorganic fluoride levels occurred in TFE and FEP process operators, monomer operators, and semiworks laboratorians (table 10). Wilmington office workers' blood fluoride levels have been included for comparison; their levels should represent the norm for workers who are not occupationally exposed to fluorides (table 11).

5. Liver tests by job: differences between before and after exposure

Very few workers had liver tests that were done before and after exposure began. Since the workers having both before and after tests may have been a select group, the results of these comparisons should be treated with caution.

The before and after C-8 exposure comparisons weakly suggest that FEP process and FEP service workers' AP levels may have risen following C-8 exposure (table 12). This result supports the earlier observation that FEP workers' most recent AP levels were higher than the control mean. However, these two observations are not independent.

TFE process and TFE service operators showed no unusual before and after differences with respect to SGOT, AP, LDH, or bilirubin. The result does not support the earlier observation that SGOT was elevated in TFE process workers.

All "after" tests were based on the worker's most recent physical examination. For exposed workers, the "before" tests were based on the worker's most recent physical examination prior to moving into the C-8 exposed job. In the control group, the "before" tests were based on the worker's physical examination immediately prior to his 1979 physical.

Discussion

Based on the data above, there is no conclusive evidence of an occupationally related health problem among workers exposed to C-8.

Some of the SGOT data suggested that there might be a liver effect among certain C-8 exposed workers. The mean SGOT for the TFE process operators was significantly ($p < 0.05$) higher than the non-Teflon® area control mean. TFE process operators as a group had considerably higher organic fluoride blood levels than other Teflon® area workers. Workers in the highest organic fluoride decile had a significantly higher mean SGOT than workers in the lower nine deciles.

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However, in other respects SGOT showed poor correlation with organic fluoride level and with C-8 exposure.

- Teflon® area workers with little or no C-8 exposure had a mean SGOT that was nearly as high as the TFE process operators' mean. Since Teflon® area workers with little or no C-8 exposure also had the lowest blood organic fluoride levels, their elevated SGOT could not realistically be caused by C-8 exposure.
- Workers from the third lowest blood organic fluoride decile had an SGOT mean that was nearly as high as the top decile's mean.

Other puzzling findings were that neither AP, LDH, nor bilirubin means were elevated among TFE operators. If a patient truly had a chemically induced liver disease, one would expect one or more of these other blood tests to be elevated.

Mean AP was significantly ($p < 0.05$) higher among FEP service and FEP process operators. Yet none of the other blood tests were elevated among these workers, and AP did not correlate with blood organic fluoride levels.

It seems very unlikely that a single material would raise only SGOT levels in one worker group and raise only AP levels in another worker group. More likely explanations for the SGOT and AP elevations are:

- The elevations resulted from chance events and were not causally related to C-8 exposure.
- Certain unmeasured confounding factors such as alcohol consumption or drug use may have influenced the blood test results.

It is also possible, however remote, that occupational exposures to other toxic materials were responsible for the observed elevations. For instance, acute and chronic exposure to inorganic fluorides can produce osteomalacia, a bone disease. This bone disease is often associated with elevated levels of serum AP.

Liver function tests: limitations

Bilirubin, SGOT, AP, and LDH assess different components of a liver's health and function. Only serum bilirubin is a true liver function test. SGOT, AP, and LDH are actually enzymes that are normally present at moderate levels in the serum. They may attain higher levels after various types of liver damage have occurred. SGOT and LDH leak out of damaged liver cells and into the blood stream. Elevated AP levels, on the other hand, appear to result from damaged liver cells synthesizing and releasing more enzyme.

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When assessing positive and negative test results, several points should be kept in mind:

- The liver has a large functional reserve and a great capacity to regenerate itself after it has been damaged. Studies have shown that within about a week after having removed over 80 percent of a rat's liver, one can find a liver of essentially normal weight and function. Consequently, mild and sometimes moderate liver injury often may not be accurately reflected by changes in liver function tests.
- Some liver functions are much more sensitive to injury than others. Thus, some liver functions (and function tests) may show changes while others do not.
- There is no one single test or procedure that effectively measures the total function of the liver.
- There is no direct quantitative correlation between the amount of liver cell injury and the height of serum enzyme levels. However, higher levels are generally found with more severe injury.
- If the serum enzymes are measured sometime after the acute insult or injury, the initial rise may have been missed. Thus, normal or low serum enzyme levels may be found as a consequence of a decreased functioning liver cell mass. Similarly, certain types of cirrhosis are associated with only slightly elevated or even normal SGOT levels.
- SGOT, AP, and LDH may be elevated from causes other than liver damage. For instance, most of the AP present in normal serum is derived from the bone. High levels of AP occur in patients with bone diseases characterized by osteoblastic activity. These include rickets, osteomalacia, and healing fractures. Growing children and pregnant women in the third trimester have elevated serum AP levels.

SGOT and LDH may also be elevated in patients during episodes of acute myocardial infarction, cardiac arrhythmias, congestive heart failure, pericarditis, and pulmonary infarction.

- There are other enzyme tests that are more sensitive to certain types of liver disease than are SGOT, AP, and LDH. One of these is gammaglutamyl transpeptidase (GGT). This enzyme is elevated in

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the serum of almost all patients with hepatobiliary disorders. It is the most sensitive test for alcoholic liver disease.

- A liver test's sensitivity can be defined as the ability to correctly identify persons who have liver disease. Specificity can be defined as the ability to correctly identify persons who do not have liver disease. Sensitivity and specificity have not been adequately studied for liver function tests.

"While a large amount of information is available concerning biochemical measures of acute hepatic injury, we have limited data about the effects of chronic lesions on the biochemical tests and on the sensitivity of these tests in detecting chronic injury or the sequelae of acute injury" (Guidelines for the Detection of Hepatotoxicity Due to Drugs and Chemicals. NIH Publication No. 79-313. Oct. 1979. pp. 33-34).

- Liver function tests are most useful if they can be used serially to assess health before, during, and after exposure. So-called "abnormal" values for one individual may be "normal" for another.

Normal/abnormal dichotomy vs the continuous approach

The basis for classifying a liver test value as normal or abnormal can be either functional or statistical. On a functional basis, any value could be considered normal if there were no increased risk associated with it. On a statistical basis, a normal value could be any one that fell within the limits in which X percent (e.g., 95%) of the population fell.

There is a major disadvantage to classifying continuous measurements as normal or abnormal: it oversimplifies a complex problem. Disease and health lie along a continuum. For instance, even within the central 95% of the total range of blood pressures, there is a gradient such that persons at the upper end are at a greater risk of coronary heart disease or stroke than those at the lower end. A similar situation may also hold for liver function tests. Thus, analyses based on group means most often use the data more efficiently than analyses based on the percent "abnormal".

A possible theoretical advantage to the dichotomous approach is that it might be more sensitive to "outliers", values on the high side of normal, than is an analysis of means. However, in animal toxicity studies practically all statistical analyses of biochemical tests are based on means rather than on the proportion above or below a certain value. Furthermore, the number of experimental observations needed to detect a real effect is considerably less when the analysis is based on means than when it is based on proportions (all else being equal and assuming an underlying continuous variable).

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TABLE 1:
C-8 STUDY CODE SHEET FOR WORK HISTORIES

Payclass (1 = wage, 2 = salary): _____

Date hired (month/year): _____ / _____

Name (last, first initial, middle initial): _____

Sex (1 = male, 2 = female): _____

Current C-8 exposure
 (0 = no; 1 = yes): _____
 Org. F = _____
 Inorg. F = _____

SS #: _____

Birth date (month/year): _____ / _____

Present or past Teflon area jobs or mechanic-type jobs (0 = no; 1 = yes): _____

Potential present or past C-8 exposure (0 = no, 1 = yes): _____

Number of jobs listed below (list all Teflon area and/or mechanic jobs): _____

<u>Job</u>	<u>C-8 Potential (0=none; 1=some)</u>	<u>Job code</u>	<u>Date in (mo./yr.)</u>	<u>Date out (mo./yr.)</u>	<u>Comments</u>
1					
2					
3					
4					
5					
6					
7					
8					
9					
10					
11					
12					
13					
14					
15					
16					
17					
18					
19					

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TABLE 3: WASHINGTON WORKS

1978 Blood Test Results

Group	No. of Tests 1978	SCOT	ALK. PHOS.	Bili	LDH
		(Normal = 10-50) Total > 50	(Normal = 30-85) Total > 85	(Normal = 0-1.0) Total > 1.0	(Normal = 100-225) Total > 225
Butacite*	119	30 (25%)	14 (12%)	6 (5%)	2 (3%)
C&P	78	16 (21%)	6 (8%)	4 (5%)	6 (8%)
Delrin*	82	15 (18%)	7 (9%)	1 (1%)	1 (1%)
Filaments	131	23 (18%)	11 (8%)	7 (5%)	4 (3%)
Lucite*	71	14 (20%)	7 (10%)	3 (4%)	2 (3%)
Teflon*	212	34 (16%)	23 (11%)	5 (2%)	5 (2%)
Zytel*	241	29 (12%)	8 (3%)	11 (5%)	6 (2%)
Mechanical	380	79 (21%)	27 (7%)	16 (4%)	10 (3%)
Research	77	16 (21%)	4 (5%)	4 (5%)	0 (0%)
Technical	251	50 (20%)	15 (6%)	11 (4%)	6 (2%)
Bus. Ser.	103	14 (14%)	8 (8%)	4 (4%)	1 (1%)
Emp'l. Rel.	32	7 (22%)	3 (9%)	3 (9%)	0 (0%)
Power & Ser.	63	14 (22%)	7 (11%)	2 (3%)	5 (8%)
Total Plant	1840	341 (19%)	140 (8%)	77 (4%)	48 (3%)
Total Plant					
Less Teflon*					
Area	1628	307 (19%)	117 (7%)	72 (4%)	43 (3%)

TABLE 4: SGOT RESULTS FROM TWO DIFFERENT METHODS
 PERFORMED AT THE SAME LABORATORY (GENERAL CONSULTANTS, INC.)

<u>Subject</u>	<u>Date</u>	<u>Standard SMA-12 (1)</u> <u>SGOT (normal = 10-50)</u>	<u>Alternate Method (2)</u> <u>SGOT (normal = 0-27)</u>
1	11/12/79	60*	19
2	11/14/79	58*	17
3	11/26/79	150*	42*
4	11/27/79	60*	18
5	12/10/79	55*	21
6	12/10/79	54*	14
7	12/10/79	51*	15
8	12/10/79	60*	15
9	12/10/79	62*	19
10	12/10/79	55*	13
11	12/11/79	54*	14
12	12/11/79	55*	17
13	12/11/79	63*	19
14	12/11/79	57*	23
15	12/11/79	85*	23
16	12/12/79	73*	21
17	12/18/79	52*	15
18	12/20/79	82*	24
19	12/26/79	57*	15
20	12/28/79	89*	24
21	12/31/79	60*	19
22	12/31/79	75*	27

(1) Automated colorimetric method

(2) Manual enzymatic method

*Abnormally high based on limits set by the laboratory

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TABLE 5: AGE AND BLOOD CHEMISTRY^(a) MEANS BY OCCUPATIONAL GROUP^(b)

<u>Group</u>	<u>Group Size</u>	<u>Age</u>	<u>SGOT</u>	<u>AP</u>	<u>Bili</u>	<u>LDH</u>
Control (no Teflon [®] , mechanic or laboratory work) ^(c)	80	38	39	64	0.7	156
FEP process	13	49	37	81*	0.5	154
FEP service	3	37	41	101*	0.6	146
TFE process	25	45	45*	64	0.5	158
TFE service	25	37	35	59	0.5	160
Monomer operator, semi-works laboratorian, foreman	22	47	44	69	0.7	151

(a) Based on most recent SMA-12 as of October, 1979

(b) Based on job title at the time of the worker's most recent SMA-12

(c) Ten percent sample of current wage roll employees plus eight workers currently exposed to C-8 but who had never worked in Teflon[®] at the time of their most recent physicals.

* Significantly ($p < 0.05$) higher than the control group after adjusting (by analysis of covariance) for age.

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TABLE 6: BLOOD CHEMISTRY (a) BY OCCUPATIONAL GROUP (b);
 PROPORTION OF TEST VALUES FALLING INTO THE HIGHEST DECILE

<u>Group</u>	<u>Group Size</u>	<u>Mean Age</u>	<u>Proportion in Highest Decile</u>			
			<u>SGOT</u>	<u>AP</u>	<u>Bili</u>	<u>LDH</u>
Control (no Teflon®, mechanic or laboratory work) ^(c)	80	38	0.10	0.05	0.18	0.10
FEP Process	13	49	0.08	0.31*	0.0	0.08
FEP Service	3	37	0.0	0.67*	0.0	0.0
TFE Process	25	45	0.20	0.12	0.08	0.12
TFE Service	25	37	0.04	0.12	0.0	0.16
Monomer operator, semi-works laboratorian, foreman	22	47	0.23	0.14	0.18	0.09

(a) Based on most recent SMA-12 as of October, 1979.

(b) Based on job title at the time of the worker's most recent SMA-12.

(c) Ten percent sample of current wage roll employees plus eight workers currently exposed to C-8 but who had never worked in Teflon® at the time of their most recent physicals.

* Significantly ($p < 0.05$) higher than the control group by Fisher's exact test (two tail).

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TABLE 7: WORKERS GROUPED BY ORGANIC FLUORIDE DECILES - BIOCHEMISTRY TEST MEANS

<u>OF^(a) Decile</u>	<u>Group Size</u>	<u>OF^(a) Limits</u>	<u>Mean No. of Years in C-8</u>	<u>Mean No. of Yrs. in Teflon</u>	<u>Mean Age</u>	<u>Mean SGOT</u>	<u>Mean AP</u>	<u>Mean Bili</u>	<u>Mean LDH</u>
1	6	0.08-0.30	5	12	40	46	69	0.7	184
2	8	0.35-0.45	2	17	49	40	67	0.7	131
3	9	0.47-0.69	9	18	47	49	67	0.6	166
4	7	0.70-1.17	5	7	41	34	73	0.5	161
5	8	1.31-1.80	7	12	41	40	71	0.5	165
6	8	1.81-2.30	6	11	40	36	68	0.6	154
7	8	2.33-3.55	10	14	45	37	64	0.5	152
8	8	3.70-4.64	9	16	44	36	72	0.5	152
9	8	4.84-6.66	15	18	47	39	62	0.5	149
10	8	6.84-21.69	14	18	47	52*	63	0.5	169

*Significantly ($p < 0.05$) higher than the mean of the lower 9 deciles. The data were age-adjusted by analysis of covariance before comparisons were made.

(a) OF = organic fluoride

EID102527

WEF000094

TABLE 8: WORKERS GROUPED BY ORGANIC FLUORIDE DECILES - PROPORTION OF TEST VALUES FALLING INTO THE HIGHEST LIVER FUNCTION TEST DECILE

OF Decile	Group Size	OF Limits	Mean No. of Years in C-8	Mean No. of Years in Teflon ^o	Mean Age	Proportion in Highest Decile			
						SCOT	AP	Bill	IDH
1	6	0.08- 0.30	5	12	40	0.17	0.17	0.33	0.33
2	8	0.35- 0.45	2	17	49	0.0	0.13	0.25	0.0
3	9	0.47- 0.69	9	18	47	0.33	0.0	0.22	0.11
4	7	0.70- 1.17	5	7	41	0.0	0.14	0.0	0.14
5	8	1.31- 1.80	7	12	41	0.0	0.25	0.13	0.13
6	8	1.81- 2.30	6	11	40	0.0	0.25	0.13	0.13
7	8	2.33- 3.55	10	14	45	0.0	0.13	0.13	0.13
8	8	3.70- 4.64	9	16	44	0.0	0.0	0.0	0.0
9	8	4.84- 6.66	15	18	47	0.13	0.0	0.25	0.0
10	8	6.84-21.69	14	18	47	0.38*	0.13	0.13	0.13

* Significantly (p<0.06) higher than the lower 9 deciles by Fisher's exact test (two tail).

OF = organic fluoride

TABLE 9: TEFLON AREA WORKERS WITH THE 16 HIGHEST ORGANIC FLUORIDE LEVELS

<u>Worker</u>	<u>Age</u>	<u>Years in C-8</u>	<u>Years in Teflon®</u>	<u>Blood Organic Fluoride Level</u>	<u>Job</u>
A	50	20.5	23.4	21.69	TFE process
B	59	23.8	25.8	20.81	TFE process
C	36	2.8	4.1	16.89	TFE process
D	60	23.2	23.9	14.38	TFE process
E	53	4.0	22.3	9.63	TFE process
F	48	23.4	23.4	8.89	TFE process
G	42	2.6	4.8	6.91	TFE process
H	35	13.4	14.6	6.04	TFE process
I	49	21.7	23.9	6.66	TFE process till 10/78
J	53	20.3	20.3	5.90	TFE process
K	44	16.1	17.2	5.64	TFE process till 11/77
L	56	24.5	24.5	5.61	TFE process
M	42	14.8	17.5	5.29	TFE process till 5/77
N	37	5.6	13.6	4.97	TFE process till 10/78
O	42	11.8	20.4	4.96	FEP process
P	55	3.2	3.2	4.84	TFE service

WEF000096

EID102529

TABLE 10: TEFLON AREA WORKERS WITH THE 16 HIGHEST
BLOOD INORGANIC FLUORIDE LEVELS

<u>Worker</u>	<u>Age</u>	<u>Years in C-8</u>	<u>Years in Teflon®</u>	<u>Blood Inorganic Fluoride Level</u>	<u>Job</u>
A	35	4.0	12.5	0.42	TFE process
B	48	19.9	23.1	0.41	TFE process
C	51	7.8	25.8	0.40	Monomer
D	58	11.3	26.3	0.39	FEP process
E	49	3.5	3.5	0.39	Semiworks laboratorian
F	53	1.8	24.0	0.38	Monomer
G	53	20.3	20.3	0.37	TFE process
H	61	11.8	22.3	0.37	FEP process
I	42	11.5	13.8	0.34	TFE process
J	26	3.2	3.2	0.31	TFE service
K	30	0.7	3.0	0.29	TFE process
L	56	0.4	29.7	0.28	Monomer
M	35	4.8	4.8	0.27	TFE service
N	24	3.1	3.1	0.26	TFE service
O	35	4.3	11.7	0.25	TFE service
P	51	2.6	2.6	0.24	Semiworks laboratorian

EID102530

WIEF000097

TABLE 11:

TABULATION
OF
BLOOD SAMPLES FROM WILMINGTON
PERSONNEL (25 TOTAL)

Sample #	Total F ppm	Inorganic F ppm	Organic F ppm (by difference)
60	0.28	0.19	0.09
61	0.31	0.09	0.22
66	0.23	0.16	0.07
72	0.20	0.10	0.10
73	0.23	0.12	0.11
76	0.23	0.17	0.06
77	0.33	0.15	0.08
78	0.24	0.25	-0.01
79	0.30	0.24	0.06
80	0.19	0.14	0.05
81	0.21	0.15	0.06
82	0.18	0.27	-0.09
92*	10.6	0	10.6
93	0.18	0.12	0.06
94	0.18	0.03	0.15
95	0.49	0.11	0.38
96	0.25	0.05	0.20
97	0.18	0.16	0.02
101	0.26	0.16	0.10
102	0.30	0.16	0.14
103	0.26	0.10	0.16
106	0.23	0.17	0.06
107	0.31	0.22	0.09
109	0.12	0.11	0.01
111	1.13	0.35	0.78

*Values obtained 3/15/79. Resample and recheck of this person's blood on 6/13/79 showed the following:

Recheck #92

Total F ppm	Inorganic F ppm	Organic F ppm
0.33	0.09	0.24

WEF000098

EID102531

TABLE 12: MEAN DIFFERENCES IN SGOT AND AP RESULTS WHEN THE FIRST TEST IS BEFORE ^(a) MOVING INTO A C-8 EXPOSURE JOB AND THE SECOND TEST IS AFTER ^(b) EXPOSURE ^(c)

<u>Group</u>	<u>Group size</u>	<u>AP</u> ^(d)	<u>SGOT</u> ^(d)
Control	45	- 3.3	- 4.7
FEP process operator	3	+ 11.7	- 4.0
FEP service operator	2	+ 8.0	+ 7.5
TFE process operator	2	- 3.0	- 11.5
TFE service operator	7	- 0.4	- 8.1

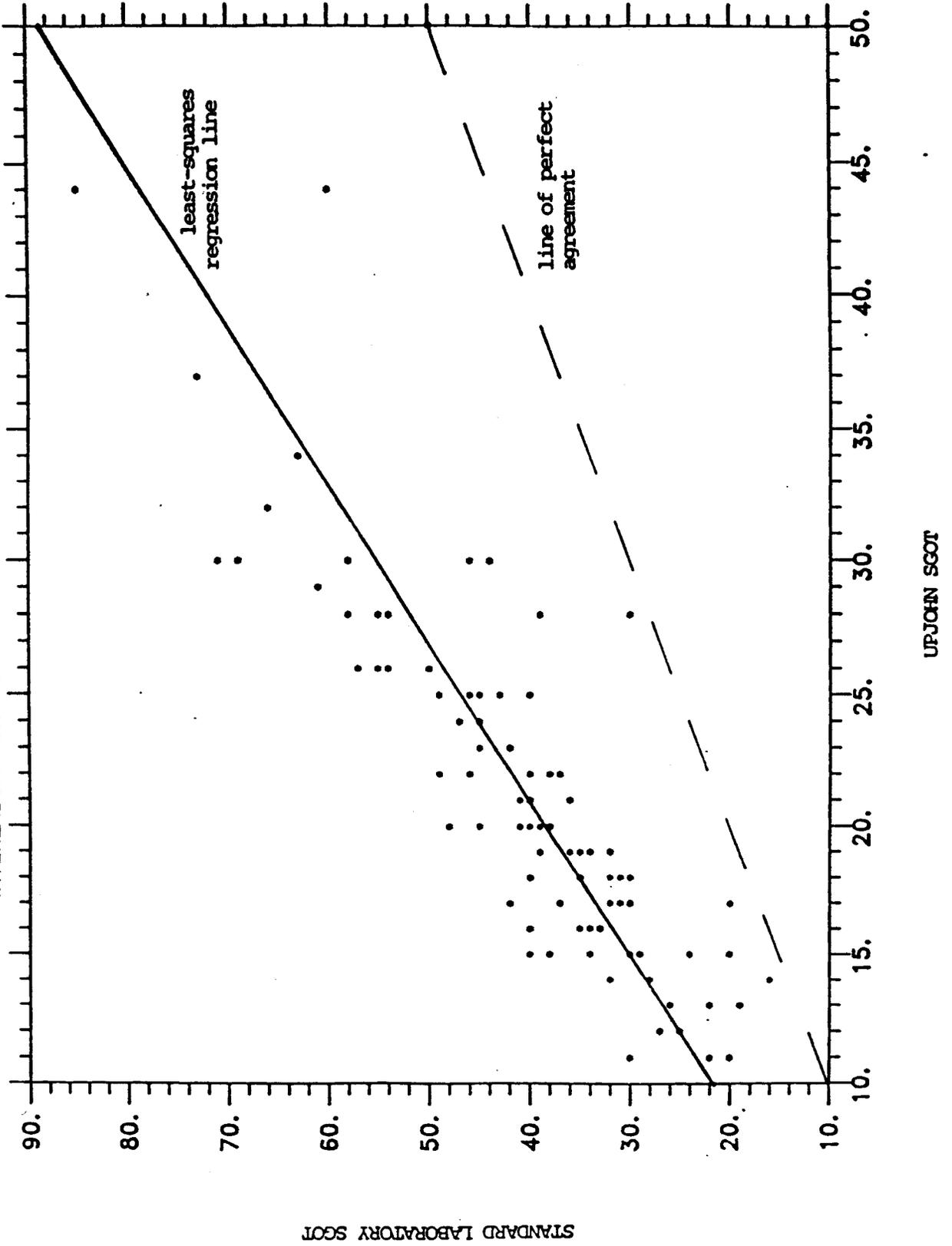
- (a) Most recent SMA-12 prior to starting C-8 exposure job
- (b) Most recent (primarily 1979) SMA-12
- (c) C-8 exposures ranged from 5 months to five years between tests
- (d) Second test minus first test

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FIGURE 1:
INTERLABORATORY COMPARISON OF SGOT DETERMINATIONS

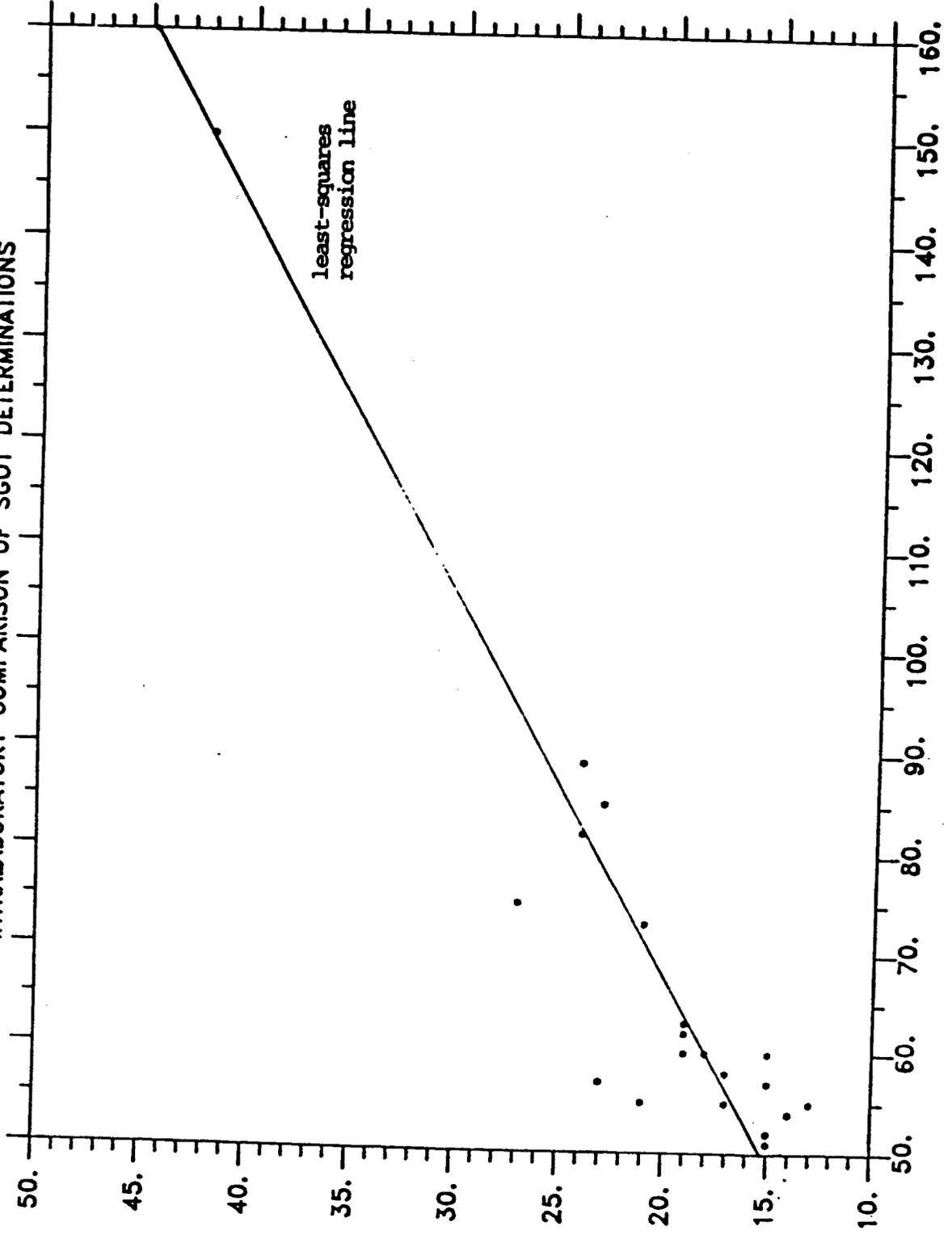




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FIGURE 2:

INTRALABORATORY COMPARISON OF SGOT DETERMINATIONS



SGOT LEVEL BY STANDARD METHOD AT STANDARD LAB

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SGOT LEVEL BY ALTERNATE METHOD AT STANDARD LAB



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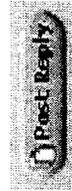
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1329. Cancer prevalence in subjects exposed to alkylated substances (PFAS)

Author : James Dahlgren

Perfluorooctanoic Acid (PFOA) is a man-made perfluorooctaned chemical, which is also referred to as C8. PFOA is a key ingredient agent in the production of Teflon® and other fluorochemical products and is biopersistent and bioaccumulates. There has been documented chronic exposure to PFOA of the residents near a plant using PFOA and other fluorinated contaminants. Our aim was to compare cancer distribution and cancer prevalence rates in a PFOA-exposed population (residents) to that of the industry cancer registry data from an occupational exposed population and finally to the general population (SEER). We performed a questionnaire on 599 residents living near a PFOA using manufacturing plant operated by Dupont. Cancer registry data from the PFOA Dupont workers was made available through discovery in pending litigation against Dupont and was not previously made available to the scientific community for review. The SEER data was used for comparison to the general population. The overall cancer prevalence rate is higher in the PFOA-exposed population when compared to the general population. Comparison of cancer prevalence rates for the industry cancer registry data, resident data and SEER is shown in Table 1. Our findings indicate that the PFOA exposed residential population and PFOA-exposed workers have elevated cancer prevalence (Table 1). Prostate cancer in the residents was proportionately elevated among young age males. Cervix and uterine cancer rates in women were also higher in the resident population compared to the U.S. general population. The distribution of cancers in the residents and workers is different than expected. Of note are the findings of elevated prevalence rates of atypical cancers such as Non-Hodgkin's, Leukemia, and Multiple Myeloma. This data suggests that exposure to PFOA may alter cancer distribution in exposed populations (worker and residents) and may be an important risk factor for an excess of cancer cases.



Author



Message

webmaster : Kamil Strakos

CANCER PREVALENCE IN SUBJECTS EXPOSED TO PERFLUOROOCTANONIC ACID (PFOA)

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2. Comprehensive Health Screening Services.

3. Dahlgren Medical Group

ABSTRACT

Perfluorooctanoic Acid (PFOA) is a man-made perfluorinated chemical also referred to as C8. PFOA is a key ingredient agent in the production of Teflon and other fluorocarbon products and is biopersistent and bioaccumulative. There has been documented chronic exposure to PFOA of the residents near a plant using PFOA and other fluorinated compounds.

Our aim was to compare cancer distribution and cancer prevalence rates in a PFOA-exposed population (residents) to that of the industry cancer registry data from an occupational exposed population and finally to the general population (SEER). We performed a questionnaire on 599 residents living near a PFOA using manufacturing plant operated by Dupont. Cancer registry data from the PFOA exposed Dupont workers was made available through discovery in pending litigation against Dupont and was not previously made available to the scientific community for review. The SEER data was used for comparison to the general population.

The overall cancer prevalence rate is higher in the PFOA-exposed population when compared to the general population. Comparison of cancer prevalence rates for the industry cancer registry data, resident data and SEER is shown in Table 1.

Our findings indicate that the PFOA exposed residential population and PFOA-exposed workers have elevated cancer prevalence (Table 1). Prostate cancer in the residents was proportionately elevated among young males. Cervix and uterine cancer rates in women were also higher in the resident population compared to the U.S. general population.

The distribution of cancers in the residents and workers is different than expected. Of note are the findings of elevated prevalence rates of atypical cancers such as Non-Hodgkin's lymphoma, Leukemia, and Multiple Myeloma.

This data suggests that exposure to PFOA may alter cancer distribution in exposed populations (workers and residents) and may be an important risk factor for an excess of cancer cases.

INTRODUCTION

- PFOA is a man-made perfluorinated chemical
 - Referred to as C8
 - Key ingredient agent in the production of Teflon
 - Biopersistent and bioaccumulates
- Documented chronic exposure to PFOA of residents near C8 plant
 - Table 1
- Evidence in animal studies that PFOA are responsible for multiple adverse health effects
- Human epidemiological studies reported statistically significant prevalence of cancer in the prostate, kidney, bladder, colon, and others
- Report results of self-reported cancers in residents near a C8 plant
 - SEER U.S. cancer rates
 - Industry cancer registry data

Discussion

- Resident cancer prevalence rate significantly higher than U.S. rate
- PFOA exposed worker cancer prevalence rate higher than U.S. rate
- Similar cancer rates and cancer distribution
 - Residents
 - Workers
- Review of cancer registry forms used in Dupont cancer study reveals additional 9 breast cancers, 7 cervix/uterine cancers, & 25 melanomas
 - Extra cancers not added to Dupont Worker data in Table 2
 - Numbers are conservative
- Recall Bias?
 - Unlikely with cancer, all confirmed
- Selection bias
 - Cohort was not selected on basis of illness
 - Cohort was recruited from the entire community
- Litigant Bias?
 - No statistically significant difference in litigant's versus nonlitigants (Allred 1995)

Materials and Methods

- Residents were recruited by invitation via radio, TV and newspaper advertisements to participate in the study
- Questionnaires were performed on 599 subjects
- All subjects are members of a class action lawsuit filed against a local company that uses PFOA in their production process
- Class members have C8 water levels of 0.05 ug/L or greater
- Inclusion: subjects exposed to PFOA contaminated water for at least one year
- Cancer data on PFOA exposed workers obtained through discovery in litigation against Dupont
- Cancer prevalence rates for the general U.S. population obtained from SEER website

Conclusions

- PFOA significantly alters cancer distribution and prevalence in exposed populations (workers and residents)
- PFOA appears to be a risk factor responsible for an excess of cancer in the near neighbor of a PFOA using plant
- PFOA increases cancer risk in humans

Results

TABLE 1.

Area	PFOA Levels (ppb)	Location	Households	Population*
1	1.7-4.3	Little Hocking, Ohio	4200	11760
2	0.4-3.9	Lubeck, WV	3700	10360
3	0.25-0.37	Tuppers Plains, Ohio	4800	13440
4	0.08-0.13	Beipre, Ohio	6000	16800
5	0.06-0.1	Mason, WV	4200	11760
6	0.06-0.07	Pomeroy, Ohio	1000	2800
7	1.0-5.0	Dupont, Washington Works	N/A	2200
8	1.75-1.87	GE Plastics	N/A	700
9	0.05-8.6	66 Private Wells WVA & Ohio	68	190

* Estimated as 2.8 people per household
N/A Not Applicable

TABLE 2.

Type of Cancer	Dupont Worker Study		Resident Data		U.S. Worker		Resident		
	Cancers	Population	Cancers	Population	Rate	Rate	Rate	Rate	
Bladder	18	5523	0.328%	4	599	0.668%	0.110%	2.98	8.07
Bone	2	5523	0.036%	0	599	0.000%	0.010%	3.62	
Breast	9	5523	0.145%	0	599	0.000%	0.240%	7.24	
Breast	8	1034	0.774%	6	301	1.993%	0.870%	0.88	2.28
Cervix/Uterine	1	1034	0.097%	13	701	3.844%	0.280%	1.81	10.9
Colon Rectum	32	5523	0.579%	4	599	0.668%	0.340%	1.70	1.98
Esophagus	3	5523	0.054%	0	599	0.000%	0.004%	13.88	
Gastric	4	5523	0.072%	1	599	0.167%	0.020%	3.92	8.15
Kidney	18	5523	0.326%	2	599	0.334%	0.070%	4.68	4.77
Lung	9	5523	0.163%	0	599	0.000%	0.020%	5.43	
Oral Cavity	11	5523	0.199%	2	599	0.334%	0.060%	3.98	8.98
Leukemia	16	5523	0.290%	1	599	0.167%	0.040%	7.24	4.31
Liver	4	5523	0.072%	1	599	0.167%	0.006%	14.07	1.17
Lung	64	5523	1.158%	7	599	1.169%	0.090%	12.88	12.98
Melanoma	17	5523	0.308%	4	599	0.668%	0.120%	2.57	5.98
Myeloma	9	5523	0.163%	2	599	0.334%	0.010%	18.30	14.95
Non Hodgkins	9	5523	0.163%	5	599	0.835%	0.080%	2.04	10.43
Prostate	9	5523	0.163%	0	599	0.000%	0.010%	18.30	
Prostate	19	4489	0.423%	9	285	3.158%	0.160%	0.387	11.1
Stomach	2	5523	0.036%	0	599	0.000%	0.014%	2.58	
Testicular	5	4489	0.111%	0	285	0.000%	0.060%	2.23	
Thyroid	3	5523	0.054%	1	599	0.167%	0.050%	1.09	3.34
TOTAL	309	5523	5.595%	60	599	10.017%	2.080%	2.99	4.82

* Based on Total Cancer Counts

* Based on SEER First Malignant Primary

Figure 1.



Figure 2. Overview of Washington Works Plant



Figure 3. Washington Works



**DISTRIBUTION OF CANCER IN SUBJECTS EXPOSED TO
PERFLUOROCTANATE (PFOA) COMPARED TO THE GENERAL
POPULATION**

James Dahlgren MD¹, Ayman B Ibrahim MD^{1,2},

Raphael Warshaw², Harpreet Takhar MPH³

1.UCLA School of Medicine 2 James Dahlgren Medical 3.Comprehensive Health Screening Services

Abstract:

Introduction:

Perfluorooctanate (PFOA) is a man made chemical used in many products.

Objective: To compare cancer distribution and cancer prevalence rates in a PFOA-exposed population to that of the general population, of a non-exposed population and to identify risk factors.

Methods: Data was collected from 301 females and 296 males with a documented PFOA-Exposure (C8) ≥ 0.05 ug/L in drinking water using standard questionnaire. Data from an historical control group (337 subjects) were used for comparison purposes. SEER prevalence data also was used for comparison with General Population (GP). Categorical data were compared using Fischer Exact test and a logistic regression model was used to identify risk factors.

Results: Overall cancer prevalence is higher in the PFOA-exposed population when compared to the general or the control population. There were 60 cancer diagnoses in 54/599 (9.02 %) subjects in the PFOA-exposed population, whereas there was 11/337 (3.26%) in the non-exposed control group respectively, Exact test, [OR = 4.04, 95 % CI 2.18- 7.49, (P = 0.000)] whereas in the US general population the overall cancer rate was (2.08%). There is a proportionate prostate cancer increase, [9/296 (3.04%), (1.16%)] in the PFOA- exposed population vs. the GP and a proportionate increase in lung cancer [7/599 (1.17%) and (0.09 %)], and bladder cancer [4/599 (0.67%), (0.11%)]. The logistic regression model indicates that age [(Odds Ratio [OR] 1.07 ± 0.008, [95 % [CI] 1.04 to 1.08, P = 0.0001], exposure status (PFOA exposed- population vs. control population) [(OR 6.12 ± 2.43, P = 0.0001, [CI] 2.81 to 13.31], are statistically significant independent predictors of having cancer. (Overall final model R²= 0.16, P = 0.0001).

Conclusions: The overall cancer prevalence rate is higher in the PFOA-exposed population when compared to either the historical control or to the general population, with a six-fold increase in the likelihood of having cancer in the PFOA-exposed residents' population. The overall distribution of cancers in this cohort remarkably deviated from expected. This data suggests that PFOA exposure alters cancer distribution and is an important risk factor for excess of cancer cases and cancer proportionate increase.

**PERFLUOROOCCTANONIC ACID (PFOA) EXPOSURE IS ASSOCIATED WITH
HIGHER RATES OF REPRODUCTIVE SYSTEM NEOPLASIA.**

James Dahlgren MD¹, Ayman B Ibrahim MD^{1,2},

Raphael Warshaw², Harpreet Takhar MPH³

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Abstract:

Introduction: Persistent environmental endocrine disruptive chemicals like PFOA are suspected to increase estradiol functions.

Objective: To describe reproductive system cancer distribution/cancer prevalence rates in female and male populations exposed to PFOA and compare it to female/male general population (GP).

Methods: Data was collected from 301 females and 296 males with a documented PFOA–Exposure (C8) ≥ 0.05 ug/L in drinking water using standardized questionnaire. SEER prevalence data were used for comparison. Two additional subjects had incomplete questionnaires.

Results: The most prevalent cancer sites in females were uterine/cervix (11/301, 3.64%), breast (6/301, 1.99%) and colon/rectum (4/599, 0.67%). When compared to the GP, there is a proportionate excess of uterine/cervical cancer [11/301 (3.64%), and (0.06 %)] and breast cancer [6/301 (1.99%), and (0.87 %)] in study population vs. GP. The prevalence rates for all sites combined were 0.15 for females between 45-54 years of age compared

to 0.04 in the same age females in the GP. Overall age-specific prevalence rates of all cancer sites combined among the female population in the study group was (0.13 vs. 0.09 in GP). There were (9/96, 3.04%) prostate cancer cases in the PFOA-exposed population. whereas in the GP, prostate cancer rate was (1.16%).

Conclusions: Reproductive system neoplasm's prevalence rates are higher in the PFOA-exposed population when compared to the general population. Overall age-specific prevalence rates of all cancer sites combined among the PFOA exposed female population is higher than its corresponding rates in GP as well as in males. This data suggests that PFOA increases the risk of reproductive organ cancer.

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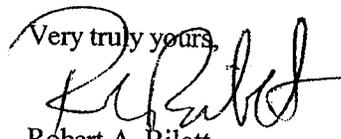
Re: PFOA Human Health Effects Study: Cancer Data

Ladies and Gentlemen:

In response to USEPA's request for available information regarding the potential threat to human health or the environment from PFOA, we previously forwarded to you preliminary abstracts/summaries of data generated in connection with a survey of adverse health effects among individuals exposed to PFOA-contaminated drinking water in communities near E.I. duPont de Nemours and Company's Washington Work Plant in Wood County, West Virginia (see, e.g., OPPT-2003-0012-607, OPPT-2003-0012-677, and AR-226-1714-16). As a supplement to those previous submissions, we have enclosed a copy of several slides from a presentation that was made to the public in Hong Kong during the November 2004 International

Dr. Charles M. Auer
Oscar Hernandez
Jennifer Seed
Mary Ellen Weber
Mary Dominiak
January 7, 2005
Page 2

Conference on Environmental and Public Health Management: Persistent Toxic Substances.
The slides summarize some of the reproductive effects data generated from the survey of the community near DuPont's Washington Works Plant in West Virginia. As with the prior study data, we request that you include this information in AR-226, OPT-2003-0012, and the appropriate IRIS database for PFOA.

Very truly yours,

Robert A. Bilott

RAB/mdm
Enclosures

cc: IRIS Submission Desk (w/ encls.)
Mark J. Garvey, Esq. (USEPA) (w/ encls.)
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Reproductive Effects of C8

James Dahlgren, MD
Pamela Anderson-Mahoney, PhD
November 2004

Reproductive Hormones

Male C8 Workers

■ Increased PFOA Levels

■ Lower

- Testosterone
- Prolactin

■ Higher

- Estrogen
- Prolactin if high alcohol intake

Health Study in Residents Near a Large C8 Source

- Methods
 - 301 females and 296 males with documented PFOA-Exposure ≥ 0.05 ug/L in drinking water
 - standardized questionnaire
 - SEER prevalence data were used for comparison

Demographics for Residential Study Participants

Age Category	No.	%
20 - 34	105	18.17
35 - 44	104	17.99
45 - 54	135	23.36
55 - 64	154	26.64
65 - 80	80	13.84
Gender		
Male	284	49.13
Female	294	50.87
Race/Ethnicity		
White	558	97.38
African American	6	1.05
Others	9	1.57
Education Level		
Less than 9 th grade	17	2.98
9 - 11 th grade	59	10.33
12 th /Vocational/Some College	430	75.31
College Graduate	65	11.38

Demographics for Residential Study Participants

	No.	%
Body Mass Index		
Underweight (<23)	106	18.53
Average (23 – 28)	190	33.22
Overweight (>28)	276	48.25
Smoking Status		
Never smoked	252	60.58
Smoked less than 15 years	72	17.31
Smoked more than 15 years	92	22.12
Work History		
Plant 1	54	9.42
Plant 2	19	3.32
No plant work	500	87.26

Reproductive Cancers in Residents Near a C8 Source

CANCER TYPE	Number of Cancers	Observed Rates ^a (per 100,000)	Expected Rates ^b (per 100,000)	PR	CI ^c
Uterine/Cervical	9	3,061	96	33.12	17.03 – 64.41*
Breast	5	1,701	1,579	1.12	0.46 – 2.71
Prostate	9	3,169	1633	1.96	0.98 – 3.92

Uterine/Cervical Cancer in Residents Near a C8 Source

Age Category	# of Cancers	N	PR	P-value
20-29	0	34	0	-
30-39	2	38	56.40	0.0002
40-49	2	64	25.02	0.0008
50-59	3	84	34.18	0.00001
60+	2	74	31.83	0.0005
Total	9	294		

Age at Menopause in Residents Near a C8 Source

Age Category	By 38	By 43	By 49	By 54
U.S. Population*	10%	20%	50%	90%
PFOA Exposed	31%	44%	66%	93%

* http://www.cdc.gov/nchs/data/series/sr_11/sr11_019.pdf

Prostate Cancer in Workers at the C8 Source

Cancer	OR	95% CI	P-Value ^b
Prostate			0.0002
<21 years employed ^a	1.0	-	-
21 - 29	2.68	0.82 - 8.79	0.10
30 - 50	8.71	2.63 - 28.83	0.0004

Conclusions

- These data indicate a strong effect of C8 on uterine/cervical cancer in women
- Together with previous research, these findings strongly implicate a causal relationship between C8 and prostate cancer in men

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February 7, 2005

FEDERAL EXPRESS

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Re: PFOA Human Health Effects Study: Cancer Data

Ladies and Gentlemen:

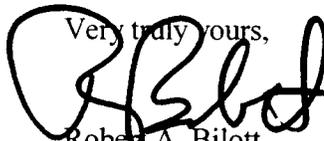
In response to USEPA's request for available information regarding the potential threat to human health or the environment from PFOA, we previously forwarded to you preliminary abstracts/summaries of data generated in connection with a survey of adverse health effects self-reported among individuals exposed to PFOA-contaminated drinking water in communities near E.I. duPont de Nemours and Company's Washington Works Plant in Wood County, West Virginia (*see, e.g.*, OPPT-2003-0012-607, OPPT-2003-0012-677, OPPT-2003-0012-836, AR-226-1714-16, and AR-226-1893-94). As a supplement to those previous submissions, we have enclosed a copy of several tables providing more detailed summaries of the age-adjusted, self-

W0350205.1

EXHIBIT 7

Dr. Charles M. Auer
Oscar Hernandez
Jennifer Seed
Mary Ellen Weber
Mary Dominiak
February 7, 2005
Page 2

reported cancer data from the PFOA community health study. (Exhibit 1) An article explaining the study and the cancer results in more detail has been peer reviewed and accepted for publication. The article is expected to be published this summer. Also enclosed are charts summarizing some of the other adverse health effects reported in the same community study. (Exhibit 2). An article explaining these results has recently been completed and is being submitted for peer review and publication. In addition, we have enclosed documents recently released by one of the public water suppliers to the community at issue, which discuss the increasing levels of PFOA being detected in that particular public water supply. (Exhibit 3) As with the prior PFOA community study data, we request that you include this information in AR-226, OPT-2003-0012, and the appropriate IRIS database for PFOA.

Very truly yours,

Robert A. Bilott

RAB/mdm
Enclosures

cc: IRIS Submission Desk (w/ encls.)
Mark J. Garvey, Esq. (USEPA) (w/ encls.)
R. Edison Hill, Esq. (w/ encls.)
Larry A. Winter, Esq. (w/ encls.)
Gerald J. Rapien, Esq. (w/ encls.)

Table A. PFOA levels by water district/source.

PFOA Levels (ppb)	Location	Households
1.7-4.3	Little Hocking, Ohio	4200
0.4-3.9	Lubeck, WV	3700
0.25-0.37	Tuppers Plains, Ohio	4800
0.08-0.13	Belpre, Ohio	6000
0.06-0.1	Mason, WV	4200
0.06-0.07	Pomeroy, Ohio	1000
0.165	Blennerhassett	71
1.0-5.0	Dupont, Washington Works	N/A
1.75-1.87	GE Plastic	N/A
0.05-8.6	68 Private Wells WVA & Ohio	68

Abbreviations:
 N/A Not Applicable

Table B. Demographics in a residentially PFOA-exposed population.

Variable	Categories	n	Percentage
Age	20 – 34	105	18.17
	35 – 44	104	17.99
	45 – 54	135	23.36
	55 – 64	154	26.64
	65 – 80	80	13.84
Gender	Male	284	49.13
	Female	294	50.87
Race/Ethnicity	White	558	97.38
	African American	6	1.05
	Others	9	1.57
Education	Less than 9 th grade	17	2.98
	9 – 11 th grade	59	10.33
	12 th /Vocational/Some College	430	75.31
	College Graduate	65	11.38
Body Mass Index (BMI)	Underweight (<23)	106	18.53
	Average (23 – 28)	190	33.22
	Overweight (>28)	276	48.25

Smoking Habit	Never smoked	252	60.58
	Smoked less than 15 years	72	17.31
	Smoked more than 15 years	92	22.12
Work History	Plant 1	54	9.42
	Plant 2	19	3.32
	No plant work	500	87.26

Table C. Unadjusted odds ratios of cancer and 95% confidence interval for demographic variables of a population residentially exposed to PFOA.

Variable	Categories	# Cancer Obs. Used in Logistic Regression	Percentage with Cancer	Odds Ratio	95% CI	P-value
Gender	Male	25	8.80%	1.04	(0.58 - 1.86)	0.9
	Female **	25	8.50%	1	-	-
Age						0.0002*
	20 -- 34 **	5	4.76%	1	-	-
	35 -- 44	4	3.88%	0.8	(0.21 -- 3.07)	0.74
	45 -- 54	9	6.67%	1.43	(0.46 -- 4.40)	0.53
	55 -- 64	16	10.39%	2.32	(0.82 -- 6.54)	0.11
	65 -- 80	16	20.00%	5	(1.75 -- 14.32)	0.003
Education	Less than 9 th grade	8	47.06%	10.84	3.97-29.53	<0.0001
	9 th grade or higher **	42	7.58%	1	-	-
Body Mass Index (BMI)						0.93*
	Underweight (<23)	8	7.55%	0.78	(0.33 -- 1.86)	0.58
	Average (23 -- 28) **	18	9.47%	1	-	-
	Overweight (>28)	23	8.33%	0.87	(0.46 -- 1.66)	0.67
Smoking Habit						0.16*

	Never smoked **	20	7.94%	1	-	-
	Smoked less than 15 years	7	9.72%	1.25	(0.51 3.08)	0.63
	Smoked more than 15 years	12	13.04%	1.74	(0.81 3.72)	0.15
Work Site	Plant 1	8	14.81%	1.87	(0.83 4.22)	0.13
	Plant 2	1	5.26%	0.48	(0.06 3.62)	0.47
	No plant employment **	41	8.20%	1	-	-

* p-value refers to the p-value for a test for trend

** Denotes the reference value of each variable for the logistic regression

Table D. Comparison of total cancer prevalence rates (per 100,000) between PFOA-exposed resident population and the US population (Whites only) by age and gender.

Age Group	US Population				Exposed Population				Prevalence Ratio			
	Age Specific Rates		Age Specific Rates		Age Specific Rates		Rates ratio of Exposed/US		Rates ratio of Exposed/US		P-value	
	Male	Female	Male	Female	Male	Female	Males	Females	Males	Females	P-value	P-value
20-34	338	451	1,923	7,547	5.69	16.75	0.16	0.0001	0.16	0.0001	0.0001	0.0001
35-44	799	1,447	-	7,547	-	5.21	-	0.008	-	0.008	0.008	0.008
45-54	1,722	3,167	4,839	8,219	2.81	2.59	0.09	0.03	0.09	0.03	0.03	0.03
55-64	5,080	5,390	9,211	11,538	1.81	2.14	0.1	0.03	0.1	0.03	0.03	0.03
65+	15,661	9,173	32,558	5,405	2.08	0.59	0.009	0.85	0.009	0.85	0.85	0.85

Table E. Standardized Morbidity Prevalence ratio comparing age-adjusted observed cancer rates (per 100,000) to expected cancer rates

CANCER TYPE	Number of Cases	Observed Rates (per 100,000)	Age Adjusted Expected Rates (per 100,000)	Prevalence Ratio	Confidence Interval
All Cancer	50	8,651	3,426	2.58	1.91 - 3.47*
Bladder	5	865	163	5.3	2.19 - 12.87*
Breast	5	1,701	1,579	1.12	0.46 - 2.71
Colon/Rectal	4	692	261	2.65	0.99 - 7.11
Kidney	1	173	79	2.2	0.31 - 15.63
Lung	7	1,211	153	7.89	3.72 - 16.74*
M. Myeloma	2	346	22	15.71	3.91 - 63.14*
Melanoma	3	519	214	2.42	0.78 - 7.54
Non-Hodgkins	5	865	130	6.67	2.76 - 16.13*
Prostate	9	3,169	1633	1.96	0.98 - 3.92
Uterine and/or Cervical	9	3,061	96	33.12	17.03 - 64.41*

* Excludes the null value

Table F. Demographics in an occupationally PFOA-exposed population

Variables	Categories	n	Percentage
Birth Year	1900 -- 1919	160	3.76
	1920 -- 1939	1209	28.42
	1940 -- 1959	2203	51.79
	1960 -- 1989	682	16.03
Gender	Male	3583	84.23
	Female	671	15.77
Years of Occupational Exposure	<21 years	1266	30.92
	21 -- 29	1462	35.71
	30 -- 50	1366	33.37
Working Condition	No direct PFOA exposure	2157	60.85
	Direct PFOA exposure	1388	39.15

Table G. Age-adjusted Proportional Hazard ratios of certain types of cancers among workers hired between 1950 and 1990, between those working in departments with direct PFOA exposure and those with no direct exposure.

Cancer Type	Department Environment	Number of Cancer Incidents	Percentage with Cancer	Hazard Ratio	CI	P-value
Pancreatic Cancer	No direct exposure	2	0.09%	1		
	Direct PFOA exposure	6	0.48%	4.46	(0.87,22.91)	0.07
Respiratory Cancer	No direct exposure	11	0.51%	1		
	Direct PFOA exposure	26	2.10%	4.41	(2.13,9.13)	<0.0001
Kidney Cancer	No direct exposure	6	0.28%	1		
	Direct PFOA exposure	11	0.89%	3.14	(1.10,8.95)	0.03
Colon/Rectal Cancer	No direct exposure	9	0.42%	1		
	Direct PFOA exposure	11	0.89%	2.96	(1.15,7.64)	0.02
Prostate Cancer	No direct exposure	14	0.65%	1		
	Direct PFOA exposure	23	1.86%	2.51	(1.24,5.08)	0.01
Non-Hodgkin's Lymph	No direct exposure	3	0.14%	1		
	Direct PFOA exposure	3	0.24%	2.44	(0.47, 12.73)	0.29
Bladder Cancer	No direct exposure	10	0.46%	1		

	Direct PFOA exposure	10	0.81%	1.46	(0.59, 3.54)	0.41
Liver Cancer	No direct exposure	1	0.05%	1		
	Direct PFOA exposure	1	0.08%	1.13	(0.06, 23.07)	0.94
Breast Cancer	No direct exposure	5	0.23%	1		
	Direct PFOA exposure	1	0.08%	0.21	(0.02, 1.88)	0.16

Table H. Logistic regression analysis controlling for age and work environment

Cancer Type	Years of Exposure	Adjusted Odds Ratio	95% CI	P-Value
Prostate	<21 years	1	-	0.0002*
	21 - 29	2.68	0.82 - 8.79	0.1
	30 - 50	8.71	2.63 - 28.83	0.0004
Kidney	<21 years	1	-	0.03*
	21 - 29	6.28	0.75 - 52.89	0.09
	30 - 50	11.57	1.38 - 97.32	0.02
Respiratory	<21 years	1	-	0.07*
	21 - 29	1.42	0.61 - 3.30	0.42
	30 - 50	1.47	0.63 - 3.43	0.37
Bladder	<21 years	1	-	0.17*
	21 - 29	1.3	0.40 - 4.24	0.66
	30 - 50	2.09	0.59 - 7.40	0.29
Colon/Rectal				0.24*

	<21 years	1	-	-
	21 - 29	0.38	0.10 - 1.50	0.17
	30 - 50	1.41	0.50 - 4.00	0.52
Pancreatic				0.35*
	<21 years	1	-	-
	21 - 29	1.71	0.28 - 10.49	0.56
	30 - 50	1.92	0.28 - 13.20	0.51

* p-value refers to the p-value for a test for trend

Table A. Standardized Prevalence Ratio (SPR) comparing observed disease rate per 100,000 among a residentially PFOA-exposed population to the expected disease rate of the general U.S. population controlling for age and gender.

Disease or Symptom Type	Number diseased in exposed group	Observed Rates (per 100,000)	Expected Rates ^a (per 100,000)	SPR	CI ^b
Cardiovascular problems ^c	170	30,088	7,019	4.29	3.47 - 5.29*
Chronic bronchitis	113	22,114	6,145	3.60	2.92 - 4.44*
Kidney disease	21	3,757	1,665	2.26	1.45 - 3.51*
Shortness of breath on stairs	323	57,270	27,994	2.05	1.70 - 2.46*
Asthma	105	20,669	11,369	1.82	1.47 - 2.25*
Thyroid problems	82	15,589	10,019	1.56	1.22 - 1.98*
Diabetes	56	9,947	6,457	1.54	1.16 - 2.05*
High blood pressure	186	33,096	28,077	1.18	0.97 - 1.43
Liver problems	19	3,754	3,728	1.01	0.64 - 1.59

^aExpected rates are from NHANES 2001 - 2002 using sampling weights to calculate an unbiased estimate of national rates while adjusting for non-response, survey design and sampling technique while giving an accurate estimate of sampling error.

^bConfidence Interval

^cIncludes MI, Stroke, Angina

*Statistically significant ($p \leq 0.05$)

Table B. Prevalence Ratios (PR) comparing observed disease rate per 100,000 among a residentially PFOA-exposed population to the expected disease rate of the general U.S. population by age group and gender for various disease outcomes.

Age Group	Males			Females			Prevalence Ratio		
	Age Specific Rates (US ^a)	Age Specific Rates (EP ^b)	Age Specific Rates (US)	Age Specific Rates (EP)	EP/US Males	P	EP/US females	P	
Asthma									
18-34	12543.8 7	37209.30	15209.92	30000	2.97	<0.0001	1.97	<0.0001	
35-49	7895.13	14705.88	15149.32	21052.63	1.86	0.0005	1.39	0.0003	
50-64	9363.58	12903.23	13065.51	21568.63	1.38	0.002	1.65	<0.0001	
65+	5694.06	19047.62	10790.07	18181.82	3.35	<0.0001	1.69	0.01	
Chronic Bronchitis									
18-34	4136.27	23255.81	5867.84	18000	5.62	<0.0001	3.07	<0.0001	
35-49	4716.72	20000	8192.81	25333.33	4.24	<0.0001	3.09	<0.0001	
50-64	2870.57	18750	8022.41	27884.62	6.53	<0.0001	3.48	<0.0001	
65+	5000.83	15000	11843.53	25000	2.99	0.0006	2.11	0.0008	
High Blood Pressure									
18-34	9799.81	22000	7359.86	9090.91	2.24	<0.0001	1.24	0.05	
35-49	18366.5 9	21250	17218.61	13414.63	1.16	0.002	0.78	0.10	
50-64	32115.1 5	37623.76	38440.91	50877.19	1.17	<0.0001	1.32	<0.0001	

Age Group	Males			Females			Prevalence Ratio		
	Age Specific Rates (US ^a)	Age Specific Rates (EP ^b)	Age Specific Rates (US)	Age Specific Rates (EP)	EP/US Males	P	EP/US females	P	
65+	48057.7	59090.91	60185.45	57142.86	1.23	<0.0001	0.95	0.006	
Short of breath climbing stairs									
18-34	--	45098.04	--	58181.82	--	--	--	--	
35-49	18804.0	44444.44	32506.6	56790.12	2.36	<0.0001	1.75	<0.0001	
50-64	33173.6	51960.78	42327.8	73684.21	1.57	<0.0001	1.74	<0.0001	
65+	37010.2	54545.45	49553.3	71428.57	1.47	<0.0001	1.44	<0.0001	
Cardiovascular problems^c									
18-34	647.54	21568.63	746.23	21818.18	33.31	<0.0001	29.24	<0.0001	
35-49	3273.62	28395.06	1775.02	21951.22	8.67	<0.0001	12.37	<0.0001	
50-64	8524.01	41176.47	7616.51	32456.14	4.83	<0.0001	4.26	<0.0001	
65+	26458.91	40909.09	18080.3	25714.29	1.55	<0.0001	1.42	0.005	
Liver									
18-34	424.68	2325.58	1696.30	6122.45	5.48	0.09	3.61	0.009	
35-49	6240.89	28,98.55	2642.29	4000	0.46	0.63	1.51	0.08	
50-64	5221.11	5376.34	3983.46	3921.57	1.03	0.10	0.98	0.15	
65+	3400.71	2439.02	3026.29	--	0.72	0.50	--	--	

Age Group	Males			Females			Prevalence Ratio		
	Age Specific Rates (US ^a)	Age Specific Rates (EP ^b)	Age Specific Rates (US)	Age Specific Rates (EP)	EP/US Males	P	EP/US females	P	
Kidney Disease									
18-34	342.84	2000.00	--	3636.36	5.83	0.08	--	--	
35-49	965.12	2500.00	267.94	1234.57	2.59	0.06	4.61	0.10	
50-64	1497.24	6930.69	2369.68	1785.71	4.63	<0.0001	0.75	0.38	
65+	6177.16	4545.45	4083.52	11428.57	0.74	0.39	2.80	0.006	
Thyroid Disease									
18-34	--	--	5761.79	13725.49	--	--	2.38	0.0008	
35-49	3551.87	5555.56	10420.1 9	20512.82	1.56	0.04	1.97	<0.0001	
50-64	4169.26	7216.49	18424.4 3	30188.68	1.73	0.005	1.64	<0.0001	
65+	12164.48	11904.76	28167.6 6	32352.94	0.98	0.11	1.15	0.01	

^aExpected rates are from NHANES 2001 – 2002 using sampling weights to calculate an unbiased estimate of national rates while

adjusting for non-response, survey design and sampling technique while giving an accurate estimate of sampling error.

^bpFOA-exposed population (EP)

^cMI, Stroke, Angina

January 2005 Supplemental Notice of Contamination

In June, 2004, the Little Hocking Water Association (“Little Hocking”) sent out a Notice reminding our members that drinking or otherwise using water contaminated with C8 may pose health risks. **Consistent with our efforts to keep our members apprised of C8 developments, we want to share some important recent information.**

Little Hocking’s November 2004 Sampling Results

The most recent sampling results of Little Hocking’s water (collected on November 29, 2004, which Little Hocking received on January 12, 2005) show that levels of C8 in our water supply continue to rise. Levels of C8 in samples taken from Little Hocking’s production wells are as high as:

- 18.6 parts per billion (ppb)** in production well no. 5;
- 3.90 ppb** in production well no. 3;
- 9.89 ppb** in production well no. 2; and
- 9.03 ppb** in production well no. 1.

By comparison, the highest level reported in our June 2004 Notice of Contamination was **10.10 ppb** in well no. 5. Please remember that Little Hocking has not used well no. 5 since 2002. However, due to sunken barges at the Belleville Locks and Dam, the Ohio River is dropping to abnormally low levels. If the low river level causes Little Hocking’s production capacity to diminish, it may be necessary to activate well no. 5 in order to meet minimum water demands. Should using well no. 5 become necessary for any reason, Little Hocking will provide a public notification so you have the option of taking additional precautions.

The level of C8 in water entering our distribution system has been measured as high as **7.2 ppb**.

Little Hocking’s current C8 levels are either very close to or exceed C8 “safe levels” used by at least one state – Minnesota.

Minnesota’s Safe Level for C8

Minnesota currently regards 7.0 parts per billion (ppb) as the maximum concentration of C8 in water that poses little or no risk to health. Unlike West Virginia’s CATT-established protective screening level of 150 ppb, Minnesota’s value takes into consideration exposure routes other than drinking water.

Even though Minnesota’s level is more protective than the West Virginia-established screening level, Minnesota’s value does not address higher exposures during childhood and effects on the elderly. For example, **if childhood exposures are considered, Minnesota’s “safe level” would drop below 7 ppb.**

The U.S. Environmental Protection Agency (“EPA”) Draft Risk Assessment for C8

In another current development, on January 12, 2005, EPA released its “Draft Risk Assessment of the Potential Human Health Effects Associated With Exposure to Perfluorooctanoic Acid and Its Salts [C8]” (“Draft Risk Assessment”). While the Draft Risk Assessment does not establish a safe level for

EXHIBIT 3

C8, at least one organization – the Environmental Working Group (“EWG”) – has taken the position that the Draft Risk Assessment dramatically underestimates human health risks associated with C8 exposure. As one example, EWG points out that the Draft Risk Assessment discounts cancer risks by ignoring data linking C8 to various cancers (i.e. mammary, testicular, pancreatic, and liver).

Little Hocking wants to be sure you are aware of both the Draft Risk Assessment and EWG’s questions about its protectiveness. The Draft Risk Assessment can be found on the Internet at: <http://www.epa.gov/opptintr/pfoa/pfoarisk.htm>. EWG’s analysis can be found at: <http://ewg.org/issues/PFCs/20050112/scienceanalysis.php>.

DuPont’s Worker Study

On January 11, 2005, DuPont announced results of a recent health study it conducted of more than 1,000 DuPont Washington Works employees. In the study, DuPont observed an approximate 10 percent increase in “bad cholesterol” (LDL) and a rise in triglycerides among some of the highest C8-exposed individuals. According to the EWG website, the DuPont cholesterol finding “is the fourth in a string of studies conducted since 1994 pointing to excess risks for stroke and heart attack among workers exposed to [C8].” DuPont’s press release states that “[t]he study data did not indicate that PFOA was or was not the cause of the increases in serum cholesterol and triglycerides.”

Little Hocking’s Current Actions

Considering the above information and the rising levels of C8 in our water, Little Hocking will seek immediate – within weeks, not months – action by DuPont to address these risks and uncertainties. **Little Hocking maintains its longstanding position that C8 does not belong in its water.**

Little Hocking remains committed to securing a resolution to the C8 issue. Until the issue is resolved, Little Hocking believes that the information in this Notice will help our members to make more informed decisions about C8.

To keep you apprised of the status of the issue, we will continue to post updated information on our website at www.littlehockingwater.org. You can also contact us for additional information:

Little Hocking Water Association, Inc
Attn: Robert L. Griffin
3998 State Route 124
P.O. Box 188
Little Hocking, OH 45742
(740) 989-2181

Please share this information with your medical advisors or other public health advisors and with all other people who drink Little Hocking’s water, especially those who may not have received this notice directly (for example, people in apartments, nursing homes, schools, and businesses). You can do this by posting this notice in a public place or distributing copies by hand or mail.

Little Hocking thanks you for your patience as we work toward a resolution of this issue.

Very Truly Yours,
Little Hocking Water Association, Inc.

By _____
Robert L. Griffin, PE
General Manager

January 31, 2005

NEWS MEDIA RELEASE

IMPORTANT NOTICE:

WATER USE REDUCTION ADVISORY

ALL CUSTOMERS OF THE LITTLE HOCKING WATER ASSOCIATION ARE ASKED TO VOLUNTARILY REDUCE THEIR WATER USE ON A TEMPORARY BASIS IN ORDER TO REDUCE THE WATER DEMAND ON THE SYSTEM.

THE SUNKEN BARGES AT THE BELLEVILLE LOCKS AND DAM HAVE CAUSED THE LEVEL OF THE OHIO RIVER TO DROP DRAMATICALLY. THE RIVER LEVEL IS LOWERING THE WATER TABLE AND REDUCING OUR WELLFIELD'S CAPACITY TO PRODUCE WATER. CONSEQUENTLY, WE ARE HAVING PROBLEMS MEETING THE WATER DEMANDS OF THE SYSTEM. **UNLESS THE WATER DEMAND IS SUFFICIENTLY REDUCED, WE WILL NEED TO ACTIVATE WELL NO. 5 TO MEET OUR CUSTOMERS' CURRENT DEMAND FOR WATER.**

WE HAVE AVOIDED PUMPING WATER FROM WELL NO. 5 INTO THE DISTRIBUTION SYSTEM BECAUSE OF WELL NO. 5'S HIGHER LEVEL OF C-8 .AS DISCUSSED DURING OUR PUBLIC MEETING IN FEBRUARY 2002; ON OUR WEBSITE; IN OUR CONSUMER CONFIDENCE REPORTS; AND IN RECENT NOTICES TO OUR MEMBERS, C-8 WAS DISCOVERED IN OUR WELLS IN JANUARY, 2002. WELL NO. 5 HAS THE HIGHEST C-8 LEVELS OUT OF ALL OF OUR PRODUCTION WELLS. OUR LATEST NOTICE IS ATTACHED FOR YOUR CONVENIENCE.

WE WANT TO AVOID USING WELL NO.5 SO WE ARE ASKING ALL CUSTOMERS OF THE LITTLE HOCKING WATER ASSOCIATION TO VOLUNTARILY REDUCE THEIR WATER USE ON A TEMPORARY BASIS. IF WATER DEMAND IS NOT SUFFICIENTLY REDUCED AND RIVER LEVELS CONTINUE TO DROP, WELL NO. 5 WILL HAVE TO BE USED. HOWEVER, WE WILL USE WELL NO. 5 AS SPARINGLY AS POSSIBLE AND ONLY UNTIL OUR WELLFIELD CAN RETURN TO NORMAL OPERATION.

THANK YOU FOR YOUR COOPERATION.

C-8 Results for Little Hocking Distribution System Little Hocking Water Association Washington County, Ohio			
Sample Location	Sample Date	PFOA ug/L	C-8 ug/L
SR 339 Booster Station	1/22/02		1.81
Bartlett County Corner	1/22/02		1.94
Torch Booster Station	1/22/02		1.850
Porterfield Community Building	1/22/02		1.690
Porterfield Community Building	3/26/02		2.62
Porterfield Community Building	4/23/02		1.93
Porterfield Community Building	4/23/02		1.55
Porterfield Community Building	10/16/02		4.29
Porterfield Community Building	2/26/03		2.33
Porterfield Community Building	5/28/03		2.54
Porterfield Community Building	8/29/03		3.73
Porterfield Community Building	12/17/03		1.5
Porterfield Community Building	2/24/04		4.33
Porterfield Community Building	5/28/04		3.64
Porterfield Community Building	9/16/04		5.39
Porterfield Community Building	11/29/04	6.92	7.20

U.S. EPA SPLIT

=Highest Level Detected

REDACTED

ALLEGATION OF SIGNIFICANT ADVERSE REACTION
TOXIC SUBSTANCES CONTROL ACT (TSCA) SECTION 8(c)

1.

Name of allegor	Year of Birth *	Date of receipt of allegation at site
	1959	6-81 → 8/84
Job title or description	Sex *	Location of plant receiving allegation
ENGINEER	(M) or (F)	PARKERSBURG WV
Signature	Date of Occurance	Street address, city, state, zip
	6/81-8/84	Box 127 PARKERSBURG WV 26102

2. Type of alleged significant adverse reaction:

Human Health Effect Environmental Effect

3. Describe the probable cause or situation associated with the alleged significant adverse reaction (see "Instructions 1").

WORKING IN THE FLUOROPOLYMERS AREA AND POSSIBLY
AT WASHINGTON (JOPIC)

4. Summarize the alleged significant adverse reaction(s). For allegations of human effects, describe the nature and extent of the effects, including how the effects became known and route of presumed exposure (if known). For allegations of environmental effects, describe the alleged environmental effects, identify the affected plant and/or animal species or contaminated portion of the physical environment.

VARIOUS MALE OPERATORS HAVE COMPLAINED ABOUT DIFFICULTY
IN HAVING THEIR WIVES CONCEIVE CHILDREN AFTER THEY HAVE
WORKED AT THE PLANT FOR A FEW YEARS ALL OPERATORS
WERE WORKING IN THE TEFLOW[®] AREA

5. Results of any self-initiated investigation with respect to an allegation filed under Section 8(c). Attach copies of other required records or reports relating to allegation under Section 8(c), e.g., OSHA form 101.

*Required only for health effects.

(OVER)

SL001108

3 EID523009

EXHIBIT 8

REDACTED

6.

Person submitting allegation info.	Job Title	Telephone Number Extension
Signature	Street address, city, state, zip	
	ENGINEER	() 4815
	81217 PARKERSBURG WV 26102	

7. Allegation Reviewed by:

Name	Title	Location	Date
Y.L. Power, M.D.	Medical Superintendent	Washington Works	8-13-84
J.G. Loschiavo	Occupational Health Coordinator	Washington Works	8-13-84
<i>[Signature]</i>	Legal	Wilmington	9/17/84

8. Disposition of Section 8(c) significant adverse reaction allegation:

Recorded:

Not recorded

9/17/84
Date recorded

Information is a known human effect as described in scientific literature, product labeling or in material safety data sheets

[Signature]
Recorded by

Allegation does not meet definition of significant adverse reaction

[Signature]
PPD Coordinator

Information has been submitted under other mandatory reporting requirements to the federal government and meet requirements of Section 8(c) of TSCA

Other _____

TALKING POINTS
8(C) ALLEGATION
(COVER ORALLY WITH EMPLOYEE)

- We have reviewed your recent allegation.
- It is being recorded according to TSCA 8(C) guidelines.
- Under the conditions of use in the Teflon® area, the chemicals present are not known to cause male infertility.
- Since we always have an interest in the ongoing well being of people, we welcome your comments to further our understanding of the facts in your expressed concern. These would be of value in any future considerations we may undertake.

Give employee a copy of his allegation which indicates that it has been reviewed by those listed on page 2 and recorded by the PPD coordinator.

HES/clt.
9/18/84

SL001110

EID5230**



E. I. DU PONT DE NEMOURS & COMPANY
 WILMINGTON, DELAWARE 19898

POLYMER PRODUCTS DEPARTMENT

CC: B. W. CULPEPPER, ER
 J. T. SMITH
 C. F. RIDDICK
 G. A. HAPKA, LEGAL
 M. H. CHRISTMAN, LEGAL
 L. F. PERCIVAL
 H. E. SERENBETZ/J. W. RAINES
 H. V. BRADLEY, WASH. WKS.

December 18, 1984

TO: PPD 8(c) FILE (R. E. STAHL)

FROM: R. D. INGALLS *RD*

REC'D
 P&R

DEC 20 1984

TSCA 8(c) ALLEGATION
 WASHINGTON WORKS
 PPD 84-1-E

ASA	ASB	JSP
AKA	AKB	WCP
AWB	AWC	DNB
WFB	WFC	ASB
FILE	FILE	WHT

Allegation

An employee assigned to the "Teflon" area alleged that several male operators had complained that their marriages had become less fertile after they had worked in the "Teflon" area for a few years.

Disposition

- The allegation was recorded and the employee was so advised.
- He was asked for any further elaboration he cared to share. He claimed a half dozen people had complained, but he named only two (memo to file by T. L. Schrenk).

Follow-up Action

- The plant support team was polled and were not cognizant of any concern about infertility in the "Teflon" area or elsewhere on the plant.
- The plant physician had not heard complaints similar to that alleged.
- Haskell Laboratory made a quick literature search and did not find scientific evidence linking a list of "Teflon" area chemicals to effects on the male reproductive system (letter by R. R. Montgomery, 10/18/84).
- Haskell also reviewed information on the major area chemicals including hexafluoroacetone formerly used. With input from L. F. Percival, who had knowledge of conditions of use of the chemicals at Washington Works, it was concluded that under the conditions of use, these chemicals should not impair male reproductive function (letter from W. L. Sprout, 10/23/84).

Better Things For Better Living... from Du Pont

SL001111

EID523012

December 18, 1984

- Because of the absence of evidence of significant employee concern, and the negative findings from Haskell's review, further followup (including contact with the two named employees) at this time was not judged to be warranted.

RDI/s

SL001112

EID523013

cc: J. B. Armitage, PPD, CHS-314
L. F. Percival, Haskell



E. I. DU PONT DE NEMOURS & COMPANY
INCORPORATED

HASKELL LABORATORY FOR TOXICOLOGY
AND INDUSTRIAL MEDICINE
P. O. BOX 50, ELKTON ROAD
NEWARK, DELAWARE 19711

CENTRAL RESEARCH AND DEVELOPMENT DEPARTMENT

October 18, 1984

R. D. INGALLS
PPD
M-5625

CHEMICALLY INDUCED REPRODUCTIVE
EFFECTS IN MALES

Under separate cover L. F. Percival has sent you MEDLINE, TOXLINE, and CHEMLINE (plus pertinent backfile) printouts generated in connection with your recent inquiry on male reproductive effects associated with a series of chemicals that were identified by J. G. Loschiavo. The list of this series of chemicals plus the checksheets on Haskell correspondence files prepared by V. Berryhill were also enclosed in L. F. Percival's package.

This letter will confirm that in a very quick scan of the MEDLINE citations* I did not observe any direct connection to the chemicals on J. G. Loschiavo's list. No attempt was made to check details or original sources.

Please do not hesitate to call if you should have any question when examining these printouts or other related data.

R. R. Montgomery
R. R. MONTGOMERY
INFORMATION ANALYST

RRM:ldp

* See attached sheet for details of strategy.

SL001113

EID523014

C12 - DISEASES-UROLOGIC AND MALE GENITAL

UROLOGIC AND MALE GENITAL DISEASES (NON MESH)

UROLOGIC AND MALE GENITAL DISEASES (NON ESH)

GENITAL DISEASES, MALE	C12		
EPIDIDYMITIS	C12.294		
GENITAL NEOPLASMS, MALE	C12.294.199		
HEMATOCELE	C12.294.260	CA.588.948.	
HERPES GENITALIS	C12.294.287	C23.542.349	
HYDROCELE	C12.294.300	C1.294.464.	C13.371.330
INFERTILITY	C12.294.340		
INFERTILITY, MALE	C12.294.365	C13.371.348	
OLIGOSPERMIA	C12.294.365.700		
PENILE DISEASES	C12.294.365.700.508		
BALANITIS	C12.294.494		
KRAUROSIS PENIS -	C12.294.494.136		
PENILE INDURATION	C12.294.494.136.505		
PENILE NEOPLASMS	C12.294.494.508	C23.208.714	
PHIMOSIS	C12.294.494.591	CA.588.948.	
PARAPHIMOSIS -	C12.294.494.684		
PRIAPISM	C12.294.494.684.587		
PROSTATIC DISEASES	C12.294.494.786		
PROSTATIC HYPERTROPHY	C12.294.565		
PROSTATIC NEOPLASMS	C12.294.565.500		
PROSTATITIS	C12.294.565.625	CA.588.948.	
SEX DISORDERS	C12.294.565.750		
IMPOTENCE	C12.294.644	C13.371.648	
SPERMATIC CORD TORSION	C12.294.644.486	F2.124.496	F3.708.897.
SPERMATOCELE	C12.294.693		
TESTICULAR DISEASES	C12.294.731		
ORCHITIS	C12.294.829		
TESTICULAR NEOPLASMS	C12.294.829.493		
TUBERCULOSIS, MALE GENITAL	C12.294.829.782	CA.588.948.	CA.588.948.
VARICOCELE	C12.294.889	C1.252.40.	C12.672.721
TUBERCULOSIS, UROGENITAL	C12.294.936	C14.907.903	
TUBERCULOSIS, MALE GENITAL	C12.672	C1.252.40.	C13.371.803
TUBERCULOSIS, RENAL	C12.672.721	C1.252.40.	C12.294.889
UROLOGIC DISEASES	C12.672.847	C1.252.40.	C12.777.419.
BLADDER DISEASES	C12.777		
BLADDER CALCULI	C12.777.103		
BLADDER FISTULA	C12.777.103.124	C12.777.889.	
VESICOVAGINAL FISTULA	C12.777.103.187	C12.777.898.	
BLADDER NECK OBSTRUCTION	C12.777.103.187.733	C12.777.898.	C13.371.894.
BLADDER NEOPLASMS	C12.777.103.249	C12.777.767.	
BLADDER, NEUROGENIC	C12.777.103.312	CA.588.948.	
CYSTITIS	C12.777.103.374	C18.597.182	C23.888.592.
VESICO-URETERAL REFLUX	C12.777.103.495		
HEMATURIA	C12.777.103.920		
HEMOGLOBINURIA	C12.777.295	C23.542.484	
KIDNEY DISEASES	C12.777.337	C18.378.71.	
ANURIA	C12.777.419		
OLIGURIA -	C12.777.419.78		
DIABETIC NEPHROPATHIES	C12.777.419.78.574		
FANCONI SYNDROME	C12.777.419.192	C18.452.297.	C19.246.482
HYDRONEPHROSIS	C12.777.419.250	C5.116.198.	C18.452.174.
HYPERTENSION, RENAL	C12.777.419.307		C18.452.448.
HYPERTENSION, RENOVASCULAR	C12.777.419.331		
KIDNEY CALCULI	C12.777.419.331.490	C14.907.489.	
KIDNEY CORTEX NECROSIS	C12.777.419.373	C12.777.889.	
KIDNEY, CYSTIC	C12.777.419.393		
KIDNEY, POLYCYSTIC	C12.777.419.413	CA.182.394	
KIDNEY, SPONGE	C12.777.419.413.420	CA.182.394.	C16.131.539.
KIDNEY FAILURE, ACUTE	C12.777.419.413.586	CA.182.394.	
KIDNEY TUBULAR NECROSIS, ACUTE -	C12.777.419.433		
KIDNEY FAILURE, CHRONIC	C12.777.419.433.503		
	C12.777.419.453		

* INDICATES MINOR DESCRIPTOR



E. I. DU PONT DE NEMOURS & COMPANY
INCORPORATED

HASKELL LABORATORY FOR TOXICOLOGY
AND INDUSTRIAL MEDICINE
P.O. BOX 50, ELKTON ROAD
NEWARK, DELAWARE 19711

cc: B. W. Karrh - ER - N-11543
G. W. Lovett
L. F. Percival
E. D. Riehl

CENTRAL RESEARCH AND DEVELOPMENT DEPARTMENT

October 23, 1984

R. D. INGALLS
POLYMER PRODUCTS DEPARTMENT
M-5625

REPRODUCTIVE EFFECTS: TEFLON® CHEMICALS

I have reviewed toxicological information, including summaries of reproductive effects, on fluorocarbon 22, tetrafluoroethylene, hexafluoropropylene, and other compounds used in the manufacture of Teflon®. I conclude that under the conditions of use in Du Pont's manufacturing sites, none of these materials presents a hazard of impairment to reproductive function in either sex.

A handwritten signature in cursive script, appearing to read "William L. Sprout".

WILLIAM L. SPROUT, M.D.
MEDICAL CONSULTANT

WLS/egg

SL001115

EID523016

FACSIMILE TRANSMISSION COVER SHEET

DATE: 12/18/84

TO:

NAME Bob Ingalls
DEPT. PPD
LOCATION M 5625

FROM:

NAME Thomas L. Schrank
DEPT. PPD
LOCATION Washington Works

TOTAL PAGES 5 (INCLUDING COVER SHEET)

PLEASE COPY _____

2287A:0015A

12/19/84

11:49

NO. 006

001

SI001116

EID523017

Personal & Confidential

12/15/84

To: File
From: TL Schrank

- Allegation of Significant Adverse
Health Effect - Bruce A. Deuts

REDACTED

During August of 1984, [redacted], an
engineer in the Tetflu[®] Polymers Division at
Washington Works issued a TSCA 8(c) - Allegation
of Significant Adverse Reaction. During September
I had a conversation with Bruce covering the
following points:

- Telling him we had received the allegation
- That it had been duly recorded according to TSCA 8(c) guidelines
- That the chemicals present in the Tetflu[®] resin are not known to cause male infertility under the conditions of use.
- Asking him what basis he had for the allegation
- Discussing what further information might be helpful to him.

12/13/84

11:49

NO. 066

022

SL001117

EID523018

(2)

said his basis was only what others working in the area (operators) had told him and that he had not substantiated any of it. Several operators (two by name) claimed to that many men working in Tetlin[®] were infertile and they suspected their childlessness was caused by chemicals. He also related a rumor about a nurse from plant Medical who had been reprimanded (some time in the past) for conducting her own independent epidemiology study on Tetlin[®] male infertility.

REDACTED

I pointed out to that a few cases of childless marriages he had heard about may not be a cause for concern in a group this large even if confirmed infertile.

I indicated that I had recalled 2-10% of marriages are infertile. He was interested in that and asked if I could get this data.

REDACTED

In a later meeting with [redacted] in November I showed [redacted] a 1978 Haskell lab paper which indicated about 7-10% of all married couples are involuntarily infertile, and an October 12, 1984 Wall Street Journal article that ~~first~~ referenced a 1982 government study that showed 17% of married couples were involuntarily infertile. This information interested and he thanked me for it.

I then asked if he felt his own childlessness may be due to his infertility not which

(4)

point he smiled and said, no, his wife
was now pregnant.

* Put together package on Confidentiality Protection *

: Study 3 teratogens instead of 1 [C-8, DMF, HFA] ? F22?

STUDY OF PREGNANCY OUTCOME IN WASHINGTON WORKS EMPLOYEES:

RESEARCH PROPOSAL

- Company Wide

WEF000116

Why not define ^{exposure by} Blood Levels

1. Don't know blood levels during pregnancy
2. Don't know what signif. of OF level is in relation to pregnancy.

As blood data became available, we will include in analyses.

William E. Fayerweather
Employee Relations Department
Medical Division
Epidemiology Section
April 13, 1981

Why not add Circleville?
Asi " is lower exposure therefore might dilute effect

- Want to discuss question: potential C-8 exposure
- M.D. meets with circles; handle study
- Keep I.O. information separate from questions on pregnancy outcome.

EID106191

I. Objectives

The study's objectives are to determine whether

- a. Pregnancy outcome among female Washington Works employees is causally related to their occupational exposure to C-8.
- b. Pregnancy outcome among wives of Washington Works employees is causally related to their husbands' exposure to C-8.

WEF00117

II. Background and Rationale

There have been five toxicologic experiments in which C-8 was administered repeatedly to experimental animals and in which the male reproductive system was examined. In none of the studies were treatment-related testicular changes observed.

Recently 3M conducted an oral rangefinder study of C-8. The purpose of this study was to determine the upper dose level of C-8 for a subsequent oral teratology study in rats. Suspensions of C-8 and corn oil were given by oral intubation to 5 groups of time-mated female rats (Charles River Sprague-Dawley derived). The doses received were 150, 100, 75, 50, or 25 mg/kg/day of C-8. These doses were given on days 6 through 15 of gestation (i.e., the period of organogenesis). There was one control group that received only corn oil by intubation on these same days. Each dosed and control group consisted of 6 time-mated female rats.

At day 20 of gestation the rats from the 3M study were sacrificed. Four fetuses were examined from each of four dams in the 150 and 25 mg/kg/day dose groups. All of the readable fetuses

EID106192

sectioned had eye lens abnormalities. The authors noted that two previous teratology studies with chemically related compounds resulted in fetuses with similar abnormal changes in the lens of the eye.

At Washington Works significant occupational exposure to C-8 is limited to the Teflon area. C-8 is a dispersing agent that is used in nearly all Teflon polymer and copolymer processes. The monomers do not contain C-8. Based on previous analyses of blood organic fluoride levels of workers, the greatest potential for C-8 exposure occurs in four jobs: TFE process operator, FEP process operator, TFE service operator, and FEP service operator.

In the proposed study of pregnancy outcome, exposed female employees and wives of exposed male employees will be studied. Female workers are studied because they may have been exposed to C-8 during or immediately prior to their pregnancies. Wives of male workers are studied because the husbands may somehow bring C-8 home with them and expose their wives at home. There is no evidence at present to suggest that C-8 exposure affects the husband's reproductive system.

III. Specific Aims

Histories of pregnancy outcome and of potential exposure to C-8 will be ascertained for

- a. Washington Works active female employees, and
- b. Wives of Washington Works active male employees.

Potential exposure to C-8 will be determined from personal records, medical records, and employee interviews. Pregnancy outcome will be determined via self-administered questionnaires given to female employees and wives of male employees.

EID106193

WEF000118

If an association is observed between pregnancy outcome and having had potential exposure to C-8, the association will be assessed as to whether it is causal or whether it is due to other confounding factors.

IV. Methods

A. Study Groups

1. Workers with potential C-8 exposure

a. Definition of exposure: Teflon area

All Teflon area jobs will be defined as having potential exposure to C-8. These jobs will be further categorized as having either high or low potential for exposure.

Table I shows the exposure categorization scheme used in the previous liver function study of C-8 workers. Notice that several job titles appear in both the high and low exposure potential columns. This happens because exposure potentials for most Teflon area jobs depend on the particular time period and task considered. Within the high potential category, current TFE/FEP service and process operators have the highest potential for exposure based on blood organic fluoride levels.

Some mechanics, non-semiworks laboratorians, and chemists/engineers occasionally come in contact with C-8. However, the natures of their jobs and of the personnel record keeping system make it very difficult to determine these workers' exposure to

WEF000119

EID106194

C-8 or to other chemicals. For this reason, mechanics, non-semiworks laboratorians, and chemists/engineers will be defined as having unknown exposure potential.

b. Selection of exposed workers

All active male and female workers who have ever worked in a C-8 exposure job (as defined above) will be identified. Brief questionnaires will be given to these workers to determine who has ever been married. All ever married workers will be included and all never married will be excluded from the study.

2. Workers with no potential C-8 exposure

a. Definition of non-exposure

All non-Teflon area jobs, with the exception of the jobs with unknown exposure potential (e.g., mechanic), will be defined as having no potential for C-8 exposure.

b. Selection of non-exposed workers (controls)

All of the plant's non-exposed active female workers will be selected as controls for the exposed female workers.

For each C-8 exposed active male employee, one matched non-exposed male employee will be chosen as a control. Matching will be on payclass, birth date (± 3 years), and adjusted service date (± 3 years). The control for each exposed worker will

EID106195

WEF000120

be the first eligible employee appearing in the yearly employee roster after the exposed worker's name.

Each male and female control will be given a questionnaire to determine whether he/she has ever been married. All never married controls will be dropped from the study. For the male subjects, new controls will be chosen to replace those controls who either were never married or who refused to participate in the study.

B. Sources of Data

1. Exposure histories

Plant personnel will be responsible for:

- determining which active employees have ever had potential exposure to C-8.
- collecting detailed exposure histories on the study subjects.

These histories will be assembled from personnel records, medical records, and employee interviews. The work histories should contain:

- name
- color (white/non-white)
- birth date
- payclass
- date hired
- all jobs having C-8 exposure potential
- month and year the worker moved in and out of C-8 jobs

EID106196

WEF000121

- each job's exposure potential (high or low)
- blood organic fluoride level and date taken

Exposure histories will be recorded on code sheets that will be designed and supplied by Medical Division.

2. Pregnancy outcome data

All female study subjects will be asked to complete a self-administered questionnaire on pregnancy outcome.

All male subjects will be given an initial questionnaire to determine whether they have ever been married and whether they are now living with their wives. Males who have been married but who no longer live with their wives (e.g., because of divorce, separation, or death) will be asked to complete the pregnancy outcome questionnaire themselves. Males who are now living with their wives will be asked to give the questionnaire to their wives to complete. Never married workers will be dropped from the study.

C. Major Response Variables

The major measures of pregnancy outcome, which are to be ascertained via a self-administered questionnaire, include:

1. # Pregnancies
2. # Spontaneous abortions/miscarriages
3. # Stillbirths
4. # Induced abortions (for medical or personal reasons)
5. # Live-born children
6. # Live-born children with birth defects or other problems at birth

EID106197

WJEF000122

7. Types of birth defects or problems observed at birth
8. Birth weights

D. Potentially Confounding Variables

Information on a number of potentially confounding factors will be ascertained via the pregnancy outcome questionnaire. These include:

1. Maternal age
2. Paternal age
3. Infectious diseases (e.g., rubella)
4. Family history of malformations/miscarriages/stillbirths
5. Medications/drugs
6. Ionizing radiation
7. Smoking
8. Chemical exposures outside the plant (e.g., other occupations)
9. Alcohol
10. Number of previous marriages
11. Birth control/desire for more children
12. Color/ethnicity (to be determined by plant personnel).

E. Quality Control

If the final product of this study is to fair well against peer review from outside of the Company, steps must be taken to assure, measure, and document the quality of the data collected.

1. Validation of pregnancy outcome supplied by female workers

The responses on 100% of the female workers' questionnaires should be validated. A worker's responses could be validated by checking existing Du Pont medical records and by contacting the worker's personal physician. This

EID106198

WJEF00123

last step would only be done after having obtained the worker's informed consent to do so.

2. Validation of pregnancy outcome supplied by husbands

The responses on 10% of the questionnaires given to workers' wives should also be compared with the responses given independently by their working husbands. This comparison will help document the quality of the responses given by husbands.

3. Validation of work histories supplied by the plant

After work histories for exposed and nonexposed subjects have been sent to Medical Division, data from a 10% sample of these subjects will be auditted. For this audit the plant will be asked to supply the records from which these work histories have been assembled.

F. Pilot Study

Prior to giving questionnaires to all study subjects, a pilot study should be done. This pilot study should include about 5 male and 5 female workers who have had no potential C-8 exposure. It will allow us to pre-test the pregnancy outcome questionnaire and other study procedures.

V. Sample Size

A. Female Employees

Currently there are 32 exempt, 130 non-exempt, and 159 wage roll females actively employed at the plant. As of April 1, about 50 of these women worked in the Teflon

EID106199

WEF000124

area. Only about one dozen of these women were in jobs having a high potential for C-8 exposure.

From 1965 through 1980 there were 103 leaves of absence due to pregnancy (table II). Thirteen of these leaves were among wage roll employees.

B. Male Employees

Over 300 men, or about ten percent of the plant's workforce currently work in the Teflon area. Within the Teflon area, 60 to 70 workers are in jobs that have high potential for C-8 exposure. Since each exposed male will be matched with one non-exposed male, the total number of males included in the study will be over 600. The number of active workers who no longer work in the Teflon area is unknown. The number of births to wives of male employees is also unknown.

C. Statistically Significant Excesses

The national incidence rate for craniofacial malformations is about 2 per 1000 live births, and the rate for malformations of all types is about 20 per 1000. Given these background rates, table III shows the minimum number of births with malformations that must be observed in the study group to say that there is a statistically significant excess ($p < 0.05$). For instance, 2 malformations in 10 exposed live births is a significantly higher rate than a national rate of 2 per 1000. Two malformations per 10 exposed live births is also significantly higher than a plant rate of 0 per 50 nonexposed births.

WFEF000125

EID106200

VI. Analyses

1. Data on C-8 exposed female workers will be analyzed separately from data on wives of exposed male workers.
2. C-8 exposed female workers and wives of exposed male workers will be compared with four control groups:
 - Female W.W. workers never exposed to C-8
 - Wives of male workers at W.W. never exposed to C-8
 - Non-W.W. female employees at another Du Pont plant
 - Wives of non-W.W. employees at another Du Pont plant.
3. All of the measures of pregnancy outcome mentioned in the earlier section on major response variables will be analyzed.
4. The analyses will be adjusted for the effects of the potentially confounding variables mentioned earlier. Binary regression and Mantel-Haenszel methods will be used for these adjustments.
5. Analyses will take into account that only exposures occurring immediately prior to conception or during the first trimester of the pregnancy are likely to produce malformations.
6. Hypothesis testing will be two-tailed, and significance will be judged at the 0.05 probability level.

WFE000126

VII. Confidentiality and Informed Consent

Any female employees, male employees, or wives of male employees who are asked to participate in this study will be

EID106201

asked to first read, understand, and then sign an informed consent statement. This informed consent statement will clearly describe:

- The study's purpose and design.
- Potential risks and benefits to individuals who decide to participate in the study.
- How the data will be used.
- The individual's right to refuse to participate at any time in the study without prejudice to him/her.
- How the study's results will be reported back to the individual.

All completed questionnaires, data forms, and raw data will be stored under lock and key or in limited-access computer files. Only the principal investigators will have unlimited access to these data.

When the study is finished, the collected data will be stored in Du Pont's Hall of Records.

All results will be published in aggregate or group forms only. Individual workers will not be identified.

EID106202

WEP000127

TABLE I: EXPOSURE CATEGORIZATION SCHEME USED IN LIVER FUNCTION STUDY OF C-8 WORKERS AT WASHINGTON WORKS

<u>HIGH EXPOSURE POTENTIAL</u>		<u>LOW EXPOSURE POTENTIAL</u>	
<u>NO.</u>		<u>NO.</u>	
4-1	TFE Service Operator	4-2	TFE Service Operator
6-2	TFE Process Operator	6-4	TFE Process Operator
4-5	FEP Service Operator	4-6	FEP Service Operator
6-7	FEP Process Operator	6-8	FEP Process Operator
6-9	Semiworks Laboratorian	6-10	Semiworks Laboratorian
8-11	Mechanic (good possible)	6-12	TEFZEL-TELOMER A Operator
8-13	Mechanic (possible)	7-14	MONOMER Operator
6-15	Laboratorian (Tech Assistant)	8-16	Mechanic (unlikely)
WS-17	Engineer or Chemist	WS-18	TFE Production Foreman
		6-20	Laboratorian
		WS-20	Chemist or engineer

EID106203

WEF000128

TABLE II: NUMBER OF PREGNANCIES BY YEAR (OF LEAVE OF ABSENCE) AND BY PAYCLASS:
 WASHINGTON WORKS FEMALE EMPLOYEES 1965 - 1980

WEF000129

Year of leave of absence

	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80	65 - 80
PAGE	0	0	0	0	0	1	0	0	0	1	1	2	1	2	3	2	13
SALARY	6	7	7	4	7	10	12	8	7	4	4	3	2	3	3	3	90
TOTAL	6	7	7	4	7	11	12	8	7	5	5	5	3	5	6	5	103

EID106204

TABLE III: MINIMUM NUMBER OF MALFORMATIONS NEEDED TO SHOW STATISTICAL SIGNIFICANCE

Type of malformation	Malformation incidence nation-wide	Minimum number of births with malformations that must be observed in the study group to be significantly higher than the national incidence, given a study group with N live births:			WEF000130
		N=5	N=10	N=50	
craniofacial	2 per 1000	1	2	2	
all malformations	20 per 1000	2	2	4	

EID106205



Minimum number of births with malformations that must be observed in the study group to be significantly higher than the control group's incidence, given a study group with N live births:

Number of live births in the plant control group	Minimum number of births with malformations that must be observed in the study group to be significantly higher than the control group's incidence, given a study group with N live births:		
	N=5	N=10	N=50
50	2	2	6
50	2	3	8
50	3	4	10

births with malformations in the control group

0
1
2



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INCORPORATED
WILMINGTON, DELAWARE 19898

J. T. SMITH
N. J. IRSCH
C. F. REINHARDT - CR&D
B. W. KARRH - ER
H. E. SERENBETZ
J. W. RAINES

POLYMER PRODUCTS DEPARTMENT

May 26, 1981

PERSONAL & CONFIDENTIAL

J. H. TODD
POLYMER PRODUCTS DEPARTMENT
WASHINGTON WORKS

C-8 PROGRAM STATUS

It has been several weeks since the announcement of 3M's findings of the teratogenic potential of C-8 and the subsequent reassignment and relocation of affected female employees from the "Teflon" area. Communications to employees at that time indicated that we planned further animal testing, further blood sampling, and some follow-up to see if birth defects may have resulted from exposure to C-8.

Although these programs are either just under way or still in the discussion stage, a status report is in order.

You may choose to share some of the more sensitive information with your immediate staff. Other parts of the program, such as the Haskell activities, may be of more widespread interest.

If you wish to prepare a general communication, we will be glad to assist with Medical or Haskell review.

RISK ASSESSMENT (Attachment I)

The latest risk assessment letter of May 6 from Drs. C. F. Reinhardt and B. W. Karrh is included for your information. It refers to an earlier letter of April 10, and this is also attached.

HASKELL LABORATORY STUDIES (Attachment II)

E. D. Champney's memo of 4/13/81 summarizes the extensive program being undertaken at Haskell Laboratory.

EID090076

AJP002917

HASKELL LABORATORY STUDIES (Cont'd.)

- The inhalation teratology study aimed at determining a no-effect exposure level in female rats is proceeding on schedule. Facilities at the Experimental Station are being used beginning this week for blood analyses to support this study during a two-week period. Although there will be some results at the end of June, the full-term test will not be complete until year end.
- Screening studies for an alternate dispersing agent have started. In about three months we should know if we have a promising candidate. Full-scale testing of several months would then be required to confirm absence of teratogenic potential.
- Because of the rapid elimination of C-8 by female rats, it is difficult to relate a no-effect dosage and blood level in rats to an acceptable exposure level and blood level of C-8 in humans. A second species, more closely related to humans, will be chosen shortly. The radio-active C-8 is now available. Information about how it is accumulated and held in the body will come from experiments using it.
- A reproduction study is still in the planning stage.

BLOOD SAMPLING RESULTS (Attachment III)

Attached is a summary of sampling results available through May 14.

As expected from previous sampling, sites where only the dispersion is being used are indicating low blood levels.

Samples from Dordrecht are just being received. When results are available, we will be able to compare this plant, where direct exposure to C-8 is possible, with Washington Works' experience.

We understand that strategies for further sampling at Washington Works are being discussed.



R. D. INGALLS
ENERGY & ENVIRONMENTAL AFFAIRS
MANUFACTURING DIVISION

RDI:tps
Attachments

EID090077

AJP002918



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INCORPORATED

WILMINGTON, DELAWARE 19898
CENTRAL RESEARCH & DEVELOPMENT DEPARTMENT

HASKELL LABORATORY
FOR
TOXICOLOGY AND INDUSTRIAL MEDICINE

May 6, 1981

PERSONAL & CONFIDENTIAL

MEMO TO: H. E. SERENBETZ
PPD, MONTCHANIN 642

FROM : C. F. REINHARDT, MD, CR&D, HASKELL *CFR*
B. W. KARRH, MD, ERD, N-11400 *BWK*

FC-143

(Ammonium perfluorooctanoate; C-8; CAS-3825-26-1)
Ref.: CFReinhardt & BWKarrh to HESerenbetz,
"FC-143," dated 4/10/81.

The reference memo describes a pilot study by 3M in which FC-143 caused abnormal eye lenses in rat fetuses. The memo recommends "that women of childbearing capacity be removed from jobs where it has been demonstrated that there is potential for exposure to FC-143 and blood levels of FC-143 are above defined background levels (0-0.4 ppm). Areas where the employees have blood levels of organic fluorine in the background range and where the airborne concentration of FC-143 is in compliance with our provisional acceptable exposure limit of 0.01 mg/m³ should present no significant risk to the fetus."

Originally we estimated blood concentrations of FC-143 by an imprecise measurement of total organic fluorine. The background concentration of organic fluorine, determined by measuring it in the blood of Wilmington office workers, was 0-0.4 ppm (as fluorine). Subsequently a method for measuring the blood level of FC-143 itself was developed. It is sensitive to about 0.004 ppm (4 ppb), as fluorine. It was presumed that background levels by either method would give values in the same range. However, initial measurements of Wilmington office workers indicate that the background level of blood FC-143 is below the level of detection, that is, less than 0.004 ppm. The question has arisen whether the acceptable blood level for female employees (0.4 ppm) should be lowered to the detection level of FC-143 (0.004 ppm).

EID090078

AJP002919

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H. E. SERENBETZ

-2-

May 6, 1981

We advise against this step because our information is limited.

1. *The evidence that FC-143 is a teratogen in the rat is inconclusive. Teratogenic tests meeting current standards are being carried out by 3M and Du Pont and results should be available by Q3-81.*
2. *Even if the preliminary 3M study is assumed to demonstrate teratogenicity, it is inadequate for setting acceptable exposure standards. The current animal studies should provide a basis for establishment of acceptable workplace standards. The human data now being collected should also help in setting standards.*
3. *Because of the unusual difference between male and female rats in their rate of excreting FC-143, the rat may not be the best model for man. A better model is being sought.*
4. *We need many more measurements before we can say that the background level of FC-143 in the population of the U.S. women is less than 0.004 ppm.*
5. *FC-143 has been in use for decades without apparent adverse effects in humans.*

We recommend that our acceptable blood level of 0.4 ppm not be changed until we have more definitive information. We should have enough information for a decision in a few months. The departments have already taken significant steps to lower exposure to FC-143. A few months, particularly with lowered exposure, should not significantly extend the hazard of a substance that has been in use for many years.

J. R. Gibson, Director of Health and Safety, concurs with our conclusions.

CFR/BWK/bjd

EID090079

AJP002920

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H. E. SERENBETZ

-3-

May 6, 1981

- cc's to: J. R. Gibson, Admn, D-9058
- W. E. Tatum, Admn, D-9064
- F. E. French, Jr., C&P, B-17249
- A. L. Dade, F&F, B-2202
- A. C. Haven, Intl, D-3047
- G. A. Hapka, Legal, B-13373
- C. C. Griffith, Photo, RSQ-210
- J. T. Smith, PPD, D-12008
- J. L. Stowell, PA, D-8112
- R. L. Rhodes, Fibr, N-4448
- H. E. Simmons, Jr., CR&D, D-6036
- B. C. McKusick, CR&D, Haskell
- J. G. Aftosmis, CR&D, Haskell

EID090080

AJP002921



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INCORPORATED
WILMINGTON, DELAWARE 19898
CENTRAL RESEARCH & DEVELOPMENT DEPARTMENT

HASKELL LABORATORY
FOR
TOXICOLOGY AND INDUSTRIAL MEDICINE

April 10, 1981

PERSONAL & CONFIDENTIAL

EID090081

MEMO TO: H. E. SERENBETZ
PPD, M-642

FROM : C. F. REINHARDT, M.D., CR&D, HASKELL *CFR*
B. W. KARRH, M.D., ERD, N-11400 *BWK*

FC-143

(Ammonium perfluorooctanoate; C-8; CAS-3825-26-1)

At your request, we have reviewed the information pertinent to whether FC-143 is a teratogen.

During the many years that Du Pont has used FC-143, there has been no known evidence of adverse health effects from employee exposure. However, our supplier of FC-143 (3M) informed Du Pont on March 20, 1981, that FC-143 caused defects (abnormal eye lenses) in rat fetuses when fed daily (days 6-15) to pregnant rats by stomach tube at doses of 25 or 150 mg/kg body weight. This observation was from a pilot study designed to determine the maximum dosage rate that pregnant females could tolerate in preparation for a full-scale study to assess FC-143's teratogenic potential.

On March 27 two Haskell scientists, Dr. R. E. Staples, Staff Teratologist, and Dr. T. Chiu, Senior Research Pathologist, visited 3M and reviewed the data with several 3M scientists. Staples and Chiu concurred with 3M that the lens defects were probably caused by FC-143.

Both Du Pont and 3M plan to start full-scale teratogenicity studies promptly. A major goal will be to determine a dosage or exposure concentration of FC-143 that does not cause birth defects and to relate this dosage to blood levels of FC-143. Until we have these data, we have no good basis for setting an acceptable exposure limit (AEL) for women of childbearing capacity. We recommend that women of childbearing capacity be removed from jobs where it has been demonstrated that there is potential for exposure to FC-143 and blood levels of FC-143 are above defined background levels (0-0.4 ppm). Areas where the employees have blood levels of organic fluorine in the background range and where the airborne concentration of FC-143 is in compliance with our provisional allowable exposure limit of 0.01 mg/m³ should present no significant risk to the fetus.

PERSONAL & CONFIDENTIAL

MEMO TO: H. E. SERENBETZ -2-

April 10, 1981

J. R. Gibson, Director of Health and Safety, concurs with our conclusions.

CFR/BWK/bjd

cc's to:

J. R. GIBSON, ADMN, D-9058
W. E. TATUM, ADMN, D-9064
F. E. FRENCH, JR., C&P, B-17249
A. L. DADE, F&F, B-2202
A. C. HAVEN, INTL, D-3047
G. A. HAPKA, LEGAL, B-13373
C. C. GRIFFITH, PHOTO, RSQ-210
J. T. SMITH, PPD, D-12008
J. L. STOWELL, PA, D-8112
R. L. RHODES, FIBR, N-4448
H. E. SIMMONS, JR., CR&D, D-6036
B. C. MCKUSICK, CR&D, Haskell
J. G. AFTOSMIS, CR&D, HASKELL

EID090082

AJP002923

PERSONAL & CONFIDENTIAL

C-8 BLOOD SAMPLING RESULTS

● Births and Pregnancies

<u>PPM C-8 in Blood</u>	<u>Status</u>
0.45	Normal child - born June 1980. Transferred out of Fluorocarbons 4/79.
0.28	Normal child - born April 1981.
0.078	Normal child - born April 1981. Umbilical cord blood 0.055 ppm.
1.5	Five months pregnant.
0.013	Five months pregnant.
2.5*	Child - 2 plus years. Unconfirmed eye and tear duct defect.
0.048	Child - 4 months. One nostril and eye defect.

*Current blood level - in fluorocarbons area only one month before pregnancy.

RDI:ldr

EID090083

AJP002926

● C-8 Level - Current Washington Works Female Employees

Number of Samples 56

Range .0.013 - 5.1

Average 0.92 ppm C-8

Number Above	0.05	ppm	C-8	53
"	"	0.10	"	46
"	"	0.20	"	35
"	"	0.30	"	29
"	"	0.40	"	28

RDI:ldr

EID090084

AJP002927

● C-8 Level Locations Other than Washington Works

<u>Location</u>	<u>No. of Samples</u>	<u>PPM C-8 Range</u>	<u>PPM C-8 Average</u>
Wilmington	32	ND	ND
Haskell	9	ND - 0.030	0.007
Chestnut Run	15	ND - 0.043	0.006
Spruance	27	ND - 0.070	0.027
Fairfield	5	ND - 0.048	0.014
Toledo	7	ND - 0.014	0.003
Circleville	10	ND - 0.030	0.014

RDI:ldr

EID090085

AJP002928

FROM: .

PAUL THISTLETON
Polymer Products Dept.
Teflon® Division
Washington Works

863-2387

PAC

Date:

9/16

TO:

John Doughty

Here's revised draft
Thanks for your
comments

D



EID079371



E. I. DU PONT DE NEMOURS & COMPANY
INCORPORATED
P. O. Box 1217
PARKERSBURG, W. VA. 26101

CC: C. G. McGlone-Tokyo
S. Hayashi-Tokyo
D. K. Duncan - Wilm.

POLYMER PRODUCTS DEPARTMENT

September 15, 1981

MR. S. TAKADA
MITSUI FLUOROchemicals CO. LTD.
MIHO 3600
SHIMIZU
SHIZUOKA PREFECTURE
JAPAN

PROPOSED EMPLOYEE BLOOD SAMPLING PROGRAM

We would like to obtain blood samples from a representative group of employees at Shimizu Works to determine if there is a significant difference between C-8 APFC dispersing agent values at Shimizu and Washington Works. About twelve samples should be enough. They should include several people who work around the TEFLON® fine powder dryers because we believe that they are a major source of exposure. Your plant has batch dryers whereas Dordrecht and Washington Works have continuous dryers. We will analyze the samples at Du Pont's Experimental Station and return the results to you so that they can be given to the employees as confidential medical information.

0139W

EID079372

AJP002509

BACKGROUND

We have used C-8 at Washington Works for more than 25 years and in earlier years it was handled less carefully than in recent years. Limited data indicates that C-8 is persistent in the human body and we have established a program to monitor selected employees regularly. We have established engineering controls to reduce potential exposure to C-8 and required the use of protective equipment for some jobs.

Significant additional control effort began in 1979 after 3M Company(our supplier of C-8 APFC dispersing agent) advised us of accumulation of organic fluorine in the blood of some of their workers. In March, 1981, 3M Company advised us that tests indicated that oral doses of C-8 caused birth defects in rats. As a result, we transferred all females of child bearing potential from jobs with significant potential for C-8 exposure and increased our efforts to prevent exposure.

Du Pont's Haskell Laboratory is making tests to determine if exposure by inhalation of C-8 causes birth defects and also is making tests with oral doses similar to the 3M tests. We expect results of these tests in about a month. 3M Company is repeating their original study and we expect to receive some information in October, 1981.

EID079373

0139W

AJP002510

Samples of blood taken at Washington Works showed that polymerization operators had an average of about 5 ppm organic fluorine and the maximum value was about 29 ppm. Monomer operators and professionals generally had much lower values. We sampled some employees in the TEFLON® Division at Dordrecht Works in May, 1981, and we found that the C-8 content of their blood samples was very similar to results at Washington Works. There appears to be no background level of naturally occurring C-8 in blood samples. A thorough study of the employees health records showed no conclusive evidence of effects resulting from exposure to C-8.

We have asked Haskell Laboratory to establish an acceptable level for C-8 in workers blood that will be the basis for managing our blood monitoring programs.

We will be glad to answer any questions and provide more information that you may need.

Paul Thistleton
Senior Engineer
Technical Department

PT/nsw
0139W

EID079374

AJP002511

11

*prints type only
employee number
not name
california*

~~PERSONAL & CONFIDENTIAL~~

C-8 BLOOD SAMPLING RESULTS

Births and Pregnancies

Employee
Number

Current (v)
PPM C-8
in Blood
(April 1981)
0.45

Status

Normal child - born June 1980.
Transferred out of Fluorocarbons 4/79.

0.28

Normal child - born April 1981..

0.078

Normal child - born April 1981.
Umbilical cord blood 0.055 ppm.

1.5

~~Five months pregnant.~~ *on pregnancy leave*

0.013

~~Five months pregnant.~~ *Normal child - born August 1981*

2.5*

Child - 2 plus years.
Unconfirmed eye and tear duct defect.

0.048

Child - 4 months.
One nostril and eye defect.
Babies blood 0.012 ppm

2.007

Normal child - born July 1981

*Current blood level - in fluorocarbons area only one month before pregnancy.

REDACTED

RDI:ldr

EID079379

March 5, 2004

MEMORANDUM

SUBJECT: Transmittal of Meeting Minutes of the FIFRA Scientific Advisory Panel Meeting Held December 9, 2003

TO: James J. Jones, Director
Office of Pesticide Programs

Charles M. Auer, Director
Office of Pollution Prevention and Toxics

FROM: Steven M. Knott, Designated Federal Official
FIFRA Scientific Advisory Panel
Office of Science Coordination and Policy

THRU: Larry C. Dorsey, Executive Secretary
FIFRA Scientific Advisory Panel
Office of Science Coordination and Policy

Joseph J. Merenda, Jr., Director
Office of Science Coordination and Policy

Attached, please find the meeting minutes of the FIFRA Scientific Advisory Panel open meeting held in Arlington, Virginia on December 9, 2003. This report addresses a set of scientific issues being considered by the Environmental Protection Agency regarding the proposed science policy: PPAR- α agonist-mediated hepatocarcinogenesis in rodents and relevance to human health risk assessment.

Attachment

cc:

Susan Hazen
Adam Sharp
Anne Lindsay
Janet Andersen
Steven Bradbury

cc:

EXHIBIT 10

William Diamond
Debbie Edwards
Arnold Layne
Tina Levine
Lois Rossi
Frank Sanders
Margaret Stasikowski
Randolph Perfetti
Karl Baetcke
Vicki Dellarco
Oscar Hernandez
David Lai
Elizabeth Mendez
Esther Rinde
Jennifer Seed
William Jordan
Douglas Parsons
Daniel Rosenblatt
David Deegan
Vanessa Vu (SAB)
OPP Docket

FIFRA Scientific Advisory Panel Members

Gary E. Isom, Ph.D. (Session Chair)
Stephen M. Roberts, Ph.D. (FIFRA SAP Chair)
Christopher J. Portier, Ph.D.

FQPA Science Review Board Members

George B. Corcoran, Ph.D.
Yvonne P. Dragan, Ph.D.
Ronald N. Hines, Ph.D.
Randy L. Jirtle, Ph.D.
Lisa M. Kamendulis, Ph.D.
James P. Kehrer, Ph.D.
Lois D. Lehman-Mckeeman, Ph.D.
David E. Moody, Ph.D.
Daniel J. Noonan, Ph.D.
Carmen E. Perrone, Ph.D.
Martha S. Sandy, Ph.D.
Michael D. Wheeler, Ph.D.

SAP Minutes No. 2003-05

**A Set of Scientific Issues Being Considered by the
Environmental Protection Agency Regarding:**

**Proposed Science Policy: PPAR- α Agonist-
Mediated Hepatocarcinogenesis in Rodents and
Relevance to Human Health Risk Assessment**

December 9, 2003

**FIFRA Scientific Advisory Panel Meeting,
Held at the Holiday Inn National Airport Hotel,
Arlington, Virginia**

NOTICE

These meeting minutes have been written as part of the activities of the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA), Scientific Advisory Panel (SAP). These meeting minutes represent the views and recommendations of the FIFRA SAP, not the United States Environmental Protection Agency (Agency). The content of these meeting minutes does not represent information approved or disseminated by the Agency. These meeting minutes have not been reviewed for approval by the Agency and, hence, the contents of this report do not necessarily represent the views and policies of the Agency, nor of other agencies in the Executive Branch of the Federal government, nor does mention of trade names or commercial products constitute a recommendation for use.

The FIFRA SAP is a Federal advisory committee operating in accordance with the Federal Advisory Committee Act and was established under the provisions of FIFRA, as amended by the Food Quality Protection Act FQPA of 1996. The FIFRA SAP provides advice, information, and recommendations to the Agency Administrator on pesticides and pesticide-related issues regarding the impact of regulatory actions on health and the environment. The Panel serves as the primary scientific peer review mechanism of the EPA, Office of Pesticide Programs (OPP) and is structured to provide balanced expert assessment of pesticide and pesticide-related matters facing the Agency. Food Quality Protection Act Science Review Board members serve the FIFRA SAP on an ad hoc basis to assist in reviews conducted by the FIFRA SAP. Further information about FIFRA SAP reports and activities can be obtained from its website at <http://www.epa.gov/scipoly/sap/> or the OPP Docket at (703) 305-5805. Interested persons are invited to contact Steven Knott, SAP Designated Federal Official, via e-mail at knott.steven@epa.gov.

In preparing these meeting minutes, the Panel carefully considered all information provided and presented by the Agency presenters, as well as information presented by public commenters. This document addresses the information provided and presented within the structure of the charge by the Agency.

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**A Set of Scientific Issues Being Considered by the
Environmental Protection Agency Regarding:**

**Proposed Science Policy: PPAR- α Agonist-
Mediated Hepatocarcinogenesis in Rodents and
Relevance to Human Health Risk Assessment**

December 9, 2003

**FIFRA Scientific Advisory Panel Meeting,
Held at the Holiday Inn National Airport Hotel,
Arlington, Virginia**

**Steven M. Knott, M.S.
Designated Federal Official
FIFRA Scientific Advisory Panel
Panel
Date: March 5, 2004**

**Gary E. Isom, Ph.D.
FIFRA SAP, Session Chair
FIFRA Scientific Advisory
Date: March 5, 2004**

**Federal Insecticide, Fungicide, and Rodenticide Act
Scientific Advisory Panel Meeting
December 9, 2003**

**Proposed Science Policy: PPAR- α Agonist Mediated Hepatocarcinogenesis in
Rodents and Relevance to Human Health Risk Assessment**

PARTICIPANTS

FIFRA SAP, Session Chair

Gary E. Isom, Ph.D., Professor of Toxicology, School of Pharmacy and Pharmacal Sciences, Purdue University, West Lafayette, IN

Designated Federal Official

Mr. Steven M. Knott, FIFRA Scientific Advisory Panel Staff, Office of Science Coordination and Policy, EPA

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INTRODUCTION

The Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA), Scientific Advisory Panel (SAP) has completed its review of the set of scientific issues being considered by the Agency pertaining to the Proposed Science Policy: Peroxisome Proliferator Activated Receptor-alpha (PPAR- α) Agonist-Mediated Hepatocarcinogenesis in Rodents and Relevance to Human Health Risk Assessment. Advance notice of the meeting was published in the *Federal Register* on October 24, 2003. The review was conducted in an open Panel meeting held in Arlington, Virginia, on December 9, 2003. Dr. Gary Isom chaired the meeting. Mr. Steven Knott served as the Designated Federal Official.

Dr. Elizabeth Mendez (Health Effects Division, Office of Pesticide Programs, EPA) provided the Agency presentation on the proposed science policy regarding PPAR- α agonist-mediated hepatocarcinogenesis in rodents and relevance to human health risk

assessment. Dr. Jeff Peters (Penn State University) provided a presentation on the paper "PPAR- α Agonist-Induced Rodent Tumors: Modes of Action and Human Relevance" (Klaunig et al., 2003). The paper and presentation summarized the evaluation of a working group convened by the International Life Sciences Institute, Risk Science Institute. This evaluation, along with the pertinent scientific literature, was considered by EPA's Office of Prevention, Pesticides, and Toxic Substances in developing its proposed science policy. Ms. Margaret Stasikowski (Director, Health Effects Division, Office of Pesticide Programs, EPA) provided an introduction to the session and also participated in the meeting. In addition, Dr. Karl Baetcke (Health Effects Division, Office of Pesticide Programs, EPA), Dr. Jennifer Seed and Dr. David Lai (both from the Risk Assessment Division, Office of Pollution Prevention and Toxics, EPA) participated in the session.

In preparing these meeting minutes, the Panel carefully considered all information provided and presented by the Agency presenters, as well as information presented by public commenters. These meeting minutes address the information provided and presented at the meeting, especially the response to the charge by the Agency.

PUBLIC COMMENTERS

Oral statements were presented as follows:

Jennifer B. Sass, Ph.D., Natural Resources Defense Council

Written statements were provided as follows:

Robert A. Bilott, Taft, Stettinius, Hollister, LLP

CHARGE

Developments in the area of research on peroxisome proliferating chemicals have led to a reevaluation of the state of the science to characterize the mode(s) of action (*i.e.*, PPAR- α agonism) and the human relevance of rodent tumors induced by PPAR- α agonists. Recently, the ILSI Risk Science Institute (ILSI RSI) convened a large expert technical group to evaluate new information on the association between PPAR- α agonism and the induction of tumors by peroxisome proliferating chemicals. OPPTS considered the 2003 ILSI report as well as the pertinent scientific literature in developing its proposed science policy.

Please provide comment and advice on the following questions. In addressing these questions consider the completeness of the data sets evaluated.

Issue 1: Rodent PPAR- α Mode of Action (MOA) for Hepatocarcinogenesis

OPPTS has concluded that there is sufficient weight of evidence to establish the mode of action (MOA) for PPAR- α agonist-induced rodent hepatocarcinogenesis. It is proposed in the OPPTS document that PPAR- α agonists activate PPAR- α leading to an increase in cell proliferation and a decrease in apoptosis, and eventually further clonal expansion of preneoplastic cells and formation of liver tumors. The key events in PPAR- α agonist-induced hepatocarcinogenesis may be classified as either causal (required for this MOA) or associative (marker of PPAR- α agonism).

Question 1 - Please comment on the weight of evidence and key events for the proposed MOA for the PPAR- α agonist-induced rodent hepatocarcinogenesis. Please comment on the adequacy of the data available to identify the key events in the PPAR- α MOA. Discuss whether the uncertainties and limitations of these data have been adequately characterized.

Issue 2: Relative Sensitivity of Fetal, Neonatal, and Adult Rodent

OPPTS has provided a review of the ontogeny of PPAR- α expression and peroxisomal assemblage during fetal and postnatal development in rodents as well as an analysis of the available data evaluating effects on peroxisomal proliferation, peroxisomal enzyme activity, and liver weights following exposure to PPAR- α agonists during fetal and postnatal development in rats and mice (see Section V of the OPPTS Document). Based on this analysis, OPPTS concluded that fetal and neonatal rats do not exhibit an increased sensitivity to PPAR- α agonist-induced hepatocarcinogenicity relative to the adult rodent. Therefore, any conclusions regarding this MOA in adult rodents would also apply to young rodents, and similarly any conclusions regarding the relevance of this MOA for human hepatocarcinogenesis would apply to the young, as well as the adults.

Question 2 - Please comment on the weight of the evidence approach and mechanistic data used to support this conclusion.

Issue 3: Human Relevance

OPPTS has provided an analysis of a variety of *in vitro* and *in vivo* studies on the key events pertaining to PPAR- α agonist-induced hepatocarcinogenesis with hamsters, guinea pigs, non-human primates, and humans. Based on the weight of the evidence, OPPTS concludes that although PPAR- α agonists can induce liver tumors in rodents and while PPAR- α is functional in humans, quantitatively, humans and nonhuman primates are refractory to the hepatic effects of PPAR- α agonists.

Therefore, OPPTS is proposing the following scientific policy:

When liver tumors are observed in long term studies in rats and mice, and
1) the data are sufficient to establish that the liver tumors are a result of a

PPAR- α agonist MOA and 2) other potential MOAs have been evaluated and found not operative, the evidence of liver tumor formation in rodents should not be used to characterize potential human hazard.

Question 3 - Please comment on the data and weight of evidence regarding the hepatic effects of PPAR- α agonists in humans, and please comment on the proposed OPPTS's science policy regarding human relevance.

Issue 4: Data Requirements

OPPTS has proposed a data set that would be sufficient to demonstrate that PPAR- α agonism is the MOA for the induction of rodent liver tumors. The data set includes evidence of PPAR- α agonism (*i.e.*, from an *in vitro* reporter gene assay), *in vivo* evidence of an increase in number and size of peroxisomes, increases in the activity of acyl CoA oxidase, and hepatic cell proliferation. The *in vivo* evidence should be collected from studies designed to provide the data needed to show dose-response and temporal concordance between precursor events and liver tumor formation.

Question 4 - Please comment in general on the proposed data set and particularly on its adequacy to demonstrate that a PPAR- α agonist-mediated MOA is operating in rodent hepatocarcinogenesis.

Issue 5: Other Tumors Induced by PPAR- α Agonists

Some PPAR- α agonists may also induce pancreatic acinar cell and Leydig cell tumors in rats and modes of action involving agonism of PPAR- α have been proposed. An in depth analysis of these tumors is provided in the 2003 ILSI technical panel report. Based on this analysis, OPPTS agrees that the data available to date are insufficient to support the proposed MOAs.

Thus, OPPTS is proposing the following science policy:

Given the limited evidence available to support that a chemical may induce pancreatic and Leydig cell tumors through a PPAR- α agonist MOA, the evidence is inadequate at this time to support a linkage between PPAR- α agonism and formation of these tumor types. Thus, it is presumed that chemicals that induce pancreatic or Leydig cell tumors may pose a carcinogenic hazard for humans.

Question 5 - Please comment on OPPTS's conclusion that there is limited evidence that a chemical may induce pancreatic and Leydig cell tumors through a PPAR- α agonist MOA, and OPPTS's proposed science policy regarding other tumors induced by PPAR- α agonists.

SUMMARY OF PANEL DISCUSSION AND RECOMMENDATIONS

Rodent PPAR- α Mode of Action (MOA) for Hepatocarcinogenesis

Overall, the majority of the Panel felt the evidence in support of the proposed MOA for PPAR- α agonist induced rodent hepatocarcinogenesis was adequate, though the opinions of individual Panel members ranged from full agreement to complete disagreement. The key event in the MOA is PPAR- α activation. PPAR- α activation triggers multiple events leading to tumorigenesis but the PPAR- α -altered genes in the causal pathway for tumor induction have not been identified. While some of the key events that occur after PPAR α activation, such as increased cell proliferation, inhibition of apoptosis, and the clonal expansion of preneoplastic lesions are known, the PPAR- α dependent mechanism for the perturbation of these key events is less well established. Specifically, mechanisms and steps linking key events downstream of PPAR- α activation are not known. The data are sufficient to demonstrate a PPAR- α activation dependence to the MOA, but are inadequate to provide the quantitative linkages associated with a more defined mechanism of action. The Panel members agreed that additional evidence of specific alterations associated with PPAR- α activation would greatly strengthen the proposed MOA.

There was agreement among most, but not all, of the Panel that data from the PPAR- α $-/-$ (null or knockout) mouse indicate the requirement for the activation of PPAR- α in the MOA of the hepatocarcinogenic effect of these agents. That the PPAR- α null mouse fails to exhibit the key and associated events when challenged with 11 months exposure to a potent PPAR- α agonist at a dose that induces 100% incidence of multiple liver adenomas in concurrently exposed control (wildtype) mice demonstrated to most, but not all, Panel members the underlying basis of the MOA statement. A few Panel members expressed concern over the short duration of the studies in the PPAR- α $-/-$ mouse (i.e., 11 months vs. 24 months in standard cancer bioassays), which rendered the studies incapable of assessing the lifetime liver cancer risk of PPAR- α agonists in this knockout mouse model, and thus, inadequate to conclusively demonstrate that PPAR- α activation is required for hepatocarcinogenesis. One Panel member did not find the weight of evidence for the proposed MOA to be sufficient based on the current absence of scientific understanding or identification of any of the intermediate critical events on the causal pathway which link PPAR- α activation with increased proliferation, decreased apoptosis, clonal expansion of preneoplastic lesions, or liver tumor formation. In addition, this Panel member observed that there is a large body of data demonstrating that PPAR- α agonists activate Kupffer cells through a PPAR- α independent mechanism, resulting in the release of cytokines capable of stimulating parenchymal cell mitosis and suppressing apoptosis.

Relative Sensitivity of Fetal, Neonatal, and Adult Rodent

The Panel does not support the OPPTS conclusions that the PPAR- α agonist MOA in adult rodents would also apply to young rodents, and similarly any conclusions regarding the relevance of this MOA for human hepatocarcinogenesis would apply to the young, as well as the adults. Differences in peroxisome biogenesis have been reported during the ontogenic development of rodents and humans. While the assembly of peroxisomes in rats and mice, including the insertion of β -oxidation enzymes into the peroxisomes, occurs near birth, the assembly of human peroxisomes has been observed as early as 8 weeks of gestation (Espeel, et al, 1997). The number and density of peroxisomes plateau by 17 weeks of gestation in humans. Moreover, acyl-CoA oxidase and 3-ketoacyl CoA thiolase are immunodetectable in the peroxisomes by 10 and 9 weeks of gestation, respectively. Thus, this suggests differences in β -oxidation capabilities in developing rodents and humans. It was also considered that differences in cell proliferation, xenobiotic metabolism, and other factors in the developing rodent (or human) could affect sensitivity to PPAR- α hepatocarcinogenesis. Therefore, information on the expression of the PPAR- α during ontogeny as well as responses of embryonic and fetal human hepatocytes to PPAR- α agonists should be evaluated before concluding that the developing human conceptus is unresponsive to PPAR- α agonist exposures.

Human Relevance

Overall, the majority of the Panel agreed that there are relevant data indicating that humans are less sensitive than rodents to the hepatic effects of PPAR- α agonists. However, the opinions of individual Panel members ranged from full agreement with the proposed OPPTS policy statement, as currently written, to complete disagreement. The majority of the Panel recognized weaknesses in the data that supported the policy noting in particular that the case for lack of human relevance was deficient in the human data. In addition, the Panel members agreed that the MOA and its application to addressing human relevance would be greatly strengthened by additional evidence of the specific alterations associated with PPAR- α activation that lead to the more general steps of hepatocellular proliferation, clonal expansion of initiated hepatocytes and tumor development. However, the Panel was divided regarding whether such additional evidence is necessary before accepting the MOA and its application to human relevance. Some Panel members believed that the data failed to demonstrate that the effect could only occur in liver and that, therefore, the policy statement should be limited to hepatocarcinogenic effects (see number 2 below). Other Panel members believed that the overall data limitations were significant enough to disagree with the MOA and its application to addressing human relevance.

As noted previously, there was agreement among most, but not all of the Panel that data from PPAR- α null mice showing that, in the absence of the receptor, there were no ensuing changes in cell proliferation and hepatic tumor formation, was strong evidence that activation of PPAR- α is necessary for all subsequent steps in the MOA. It also was noted previously that a few Panel members expressed concern over the short duration of the studies in the PPAR- α null mice (i.e., 11 months vs. 24 months in

standard cancer bioassays), which rendered the studies incapable of assessing the lifetime liver cancer risk of PPAR- α agonists in this knockout mouse model, and thus, inadequate to conclusively demonstrate that PPAR- α activation is required for hepatocarcinogenesis. Considering the proposed MOA, there was agreement that PPAR- α is present in humans and that the receptor is activated in human liver following exposure to known agonists. Accordingly, the proposed MOA for PPAR- α agonist-induced hepatocellular carcinogenesis in rodents is plausible for humans. There was also agreement that the nature of gene expression associated with hepatocellular PPAR- α activation is qualitatively different between humans and rodents. This difference may result from species differences in peroxisome proliferator response elements (PPREs), but there are few data available that identify these potentially important differences, particularly in humans. Humans are at least as sensitive to activation end-points that lead to hypolipidemia but are much less sensitive to other end-points normally associated with peroxisome proliferation. This qualitative difference will be what is referred to in subsequent references as human sensitivity.

One overall concern with the proposed MOA and the application of the MOA to addressing human relevance was that, whereas PPAR- α activation is a very specific component of the MOA, the other steps deemed to be causally-related, namely increased hepatocellular proliferation and clonal expansion of initiated hepatocytes leading to tumor development, were very general and non-specific. Overall, the Panel members agreed that additional evidence of specific alterations associated with PPAR- α activation in primates and especially humans would greatly strengthen the proposed MOA.

The Panel discussed three other issues relative to assessing the weight of evidence regarding the hepatic effects of PPAR- α agonists in humans, and the proposed science policy regarding human relevance. These included:

1. The use of the word "refractory" to describe the human response to PPAR- α activation is too absolute. The Panel agreed that "less sensitive" is a more appropriate description of the nature of the human response relative to that observed in rats and mice.
2. The policy statement drafted by OPPTS concludes with the phrase "evidence of liver tumor formation in rodents should not be used to characterize potential human hazard." After some discussion, it was suggested by one member of the Panel, and supported by several other Panel members, that this phrase should be modified to read, "evidence of liver tumor formation in rodents should not be used to characterize potential human hepatocarcinogenic hazard."
3. One member of the Panel expressed a concern, which was shared by some other Panel members, that the MOA and evaluation of human relevance was lacking in its assessment of altered gene expression that could be associated with altered methylation of DNA. There is evidence that DNA methylation is modified in rodents following exposure to PPAR- α agonists (Ge et al., 2001, Ge et al., 2002, and Pereira, et al., 2004).

Given the accepted role for DNA methylation in gene imprinting and the loss of imprinting in cancer etiology (see for example McClachlan et al., 2001), such a role for PPAR- α agonists in causing similar alterations in humans should be explored before human relevance can be appropriately evaluated, particularly for exposure during early life stages and for questions regarding site concordance.

Data Requirements

There was general consensus among the Panel that the proposed data set was adequate and provided a straight forward approach to classify a chemical as a PPAR- α agonist. The Panel also concurred that the use of PPAR- α knockout mice would provide definitive evidence to classify a chemical as a PPAR- α agonist, but that the proposed data set would be sufficient in lieu of the use of this rather costly tool.

In the course of the Panel's discussion, questions for clarification were posed to the Agency as to when (i.e., before or after a positive liver tumor finding in rodents) this set of assays testing for PPAR- α agonist activity would be conducted. The Agency indicated that data demonstrating PPAR- α agonist activity could be submitted in the absence of testing in long-term carcinogenesis studies. In response to this, a Panel member observed that in the absence of testing in standard long-term rodent carcinogenicity studies, it is not possible to determine whether the chemical would operate through a PPAR- α agonist MOA producing rodent liver tumors. A chemical with PPAR- α agonist activity may either: 1) not cause cancer in rodents, 2) cause liver cancer in rodents by the proposed PPAR- α agonist MOA, 3) cause liver cancer by a MOA other than the proposed PPAR- α agonist MOA (e.g., cytotoxicity), or 4) cause cancer at sites other than the liver (with or without liver cancer). The Panel concurred that an overriding requirement is that other MOAs have been excluded. For example, rigorous tests must be performed to exclude mutagenicity, other forms of DNA damage (clastogenicity), or overt cytotoxicity directly produced by the test compound, or its metabolic products.

Other Tumors Induced by PPAR- α Agonists

In addition to the hepatic tumors that appear to be a general occurrence in rats and mice, nine PPAR- α agonists have been reported to induce Leydig cell tumors (LCTs) and pancreatic acinar cell tumors (PACTs) in rats. Together with the hepatic tumors, this is referred to as the tumor triad. The Panel was in agreement with the OPPTS conclusion that chemicals that induce pancreatic or Leydig cell tumors may pose a carcinogenic hazard for humans.

Given the limited amount of data available on the true MOA for LCTs or PACTs, including the possibility raised by some Panel members that epigenetic effects of the PPAR- α agonists may occur, it is not possible to determine whether PPAR- α agonists pose a carcinogenic hazard to humans. Thus, the conclusion by the OPPTS that the

available data for the induction of rat LCTs and PACTs by PPAR- α agonists are insufficient to conclude that the sole MOA involves the PPAR- α receptor is considered by the Panel to be appropriate. Further, the Panel concurs that it should be presumed that chemicals that induce pancreatic or Leydig cell tumors may pose a carcinogenic hazard for humans.

PANEL DELIBERATIONS AND RESPONSE TO CHARGE

The specific issues addressed by the Panel are keyed to the Agency's background documents, and the Agency's charge questions.

Response to Charge

Question 1 - Rodent PPAR- α Mode of Action (MOA) for Hepatocarcinogenesis

OPPTS has concluded that there is sufficient weight of evidence to establish the MOA for PPAR- α agonist-induced rodent hepatocarcinogenesis. It is proposed in the OPPTS document that PPAR- α agonists activate PPAR- α leading to an increase in cell proliferation and a decrease in apoptosis, and eventually further clonal expansion of preneoplastic cells and formation of liver tumors. The key events in PPAR- α agonist-induced hepatocarcinogenesis may be classified as either causal (required for this MOA) or associative (marker of PPAR- α agonism).

Please comment on the weight of evidence and key events for the proposed MOA for the PPAR- α agonist-induced rodent hepatocarcinogenesis. Please comment on the adequacy of the data available to identify the key events in the PPAR- α MOA. Discuss whether the uncertainties and limitations of these data have been adequately characterized.

Response

Weight of the Evidence for Proposed MOA

Overall, the majority of the Panel felt the evidence in support of the proposed MOA for PPAR- α agonist induced rodent hepatocarcinogenesis was adequate, though the opinions of individual Panel members ranged from full agreement to complete disagreement. The majority of the Panel felt the weight of evidence in support of the proposed MOA in rodents is adequate for PPAR- α agonists in which hepatic activation of PPAR- α results in the key downstream events of increased proliferation, decreased apoptosis, and clonal expansion of preneoplastic lesions resulting in hepatocarcinogenesis. Associated events (indicators of PPAR- α activation) include induction of peroxisome proliferation and altered expression of related genes. One Panel member did not find the weight of evidence for the proposed MOA to be sufficient, based

on the current absence of scientific understanding or identification of any of the intermediate critical events on the causal pathway which link PPAR- α activation with increased proliferation, decreased apoptosis, clonal expansion of preneoplastic lesions, or liver tumor formation. In addition, this Panel member observed that there is a large body of data demonstrating that PPAR- α agonists activate Kupffer cells through a PPAR- α independent mechanism, resulting in the release of cytokines capable of stimulating parenchymal cell mitosis and suppressing apoptosis (Rolfe et al., 1997; Rusyn et al., 2001; Parzefall et al., 2001; Hassmall et al., 2001).

The proposed MOA for PPAR- α agonist induced rodent hepatocarcinogenesis is based on a considerable body of evidence that has accrued over the past 3 decades, and particularly on the more recent demonstration of a lack of a tumorigenic response in the PPAR- α $-/-$ mouse after 11 months of PPAR- α agonist administration at a dose that induces 100% incidence of liver adenomas in concurrent studies in the PPAR- α $+/+$ mouse with the same genetic background. This PPAR- α null mouse is devoid of responses indicative of PPAR- α agonism. There was agreement among most, but not all, of the Panel that data from the PPAR- α $-/-$ mouse indicate the requirement for the activation of PPAR- α in the MOA of the hepatocarcinogenic effect of these agents. A few Panel members expressed concern over the short duration of the studies in the PPAR- α $-/-$ mouse (i.e., 11 months vs. 24 months in standard cancer bioassays), which rendered the studies incapable of assessing the lifetime liver cancer risk of PPAR- α agonists in this knockout mouse model, and thus, inadequate to conclusively demonstrate that PPAR- α activation is required for hepatocarcinogenesis.

Additional supporting evidence for the MOA, as discussed in the review by Klaunig et al. (2003) comes from the concordance of this MOA for several PPAR- α agonists, dose dependence of the effect, with both consistency and biological plausibility for the key events. One Panel member noted several inconsistencies in the supporting data however. These include observations from long-term carcinogenicity studies of the PPAR- α agonist gemfibrozil, where a dose-related increase in liver tumors was observed in male rats, while in females, a dose-dependent decrease in liver tumors was seen (IARC, 1996). In another example, studies in rats with two PPAR- α agonists, WY-14,463 and DEHP, demonstrated that doses that produced equivalent levels of hepatic peroxisome proliferation, measured as peroxisome number and peroxisomal enzyme activity, produced markedly different liver tumor incidences (Marsman et al., 1988). Another Panel member noted that these differences may be due to sex, species, and strain differences in pharmacokinetics.

In addition to the above, a Panel member expressed concern with the lack of understanding of key causal events in the proposed MOA intermediate between PPAR- α activation and cell proliferation, suppression of apoptosis and clonal expansion, given that activation of PPAR- α results in regulation of a multitude of genes involved in a variety of cellular functions, including lipid metabolism and transport, amino acid

metabolism, signaling molecules, transcription factors, and cell cycle and growth regulatory proteins.

The Panel agreed that data in the wild type and the PPAR- α knockout mouse would be strengthened if it were determined that the null mice generated on a 129 genetic background are not resistant to liver tumorigenesis in general, as opposed to specifically resistant to PPAR- α agonists (see Drinkwater and Bennett, 1991). In addition, the PPAR- α knockout mouse data would be strengthened by a demonstration of gene dose sensitivity. The Panel members also agreed that additional evidence of specific alterations associated with PPAR- α activation would greatly strengthen the proposed MOA.

Adequacy of the Data

Though the opinions of individual Panel members ranged from full agreement to complete disagreement, overall, the majority of the Panel felt the data supporting the key events associated with the proposed MOA in rats and mice are adequate, but recognized areas where the data could be strengthened. One overall concern with the proposed MOA was that, whereas PPAR- α activation is a very specific component of the MOA, the other steps deemed to be causally related, namely increased hepatocellular proliferation and clonal expansion of the initiated hepatocytes leading to tumor development, were very general and non-specific.

In support of the adequacy of the data, the key events and the associated events have been demonstrated to occur following administration of PPAR- α agonists. These data have been derived from many laboratories over the course of the last 30 years. Many of the associative events are highly correlated markers of PPAR- α agonist exposure and potential contributors to the causal events in the proposed MOA. The mechanistic linkage between the required step of PPAR- α activation and the key events (increased cell proliferation, decreased apoptosis, and clonal expansion of preneoplastic hepatic lesions) has not been determined. Although having these steps in the mechanism of PPAR- α induced rat and mouse hepatocarcinogenesis would strengthen the MOA, the majority of the Panel agreed that the current dataset is adequate to support the MOA. That the PPAR- α null mouse fails to exhibit the key and associated events when challenged with 11 months exposure to a potent PPAR- α agonist at a dose that induces 100% incidence of multiple liver adenomas in concurrently exposed control (wildtype) mice demonstrated to most, but not all, Panel members the underlying basis of the MOA statement.

Several concerns regarding the adequacy of the data also were discussed. As previously noted, a few Panel members expressed concern over the short duration of the studies in PPAR- α null mice which rendered the studies inadequate to conclusively demonstrate that PPAR- α activation is required for hepatocarcinogenesis. One member of

the Panel was concerned that the data were not adequate to identify the key events in the MOA for PPAR- α agonist induced rodent hepatocarcinogenesis, stating that although PPAR- α activation is believed to be the earliest key event, none of the many genes whose expression is regulated by PPAR- α has been identified as being in the causal pathway for liver tumorigenesis. More data are needed to establish and link the events that have been proposed as key causal events in the proposed MOA. In addition, a number of studies provide compelling data that suggest that a PPAR- α independent event, namely Kupffer cell activation, is required for increased hepatocyte proliferation by PPAR- α agonists. The Panel member felt that more data characterizing the relationship between Kupffer cell activation, and the cytokines that are released upon activation in hepatocarcinogenesis, and PPAR- α activation were needed before the identification of key events in the MOA could be properly evaluated. Another member of the Panel expressed concern, which was shared by some other Panel members, that data were lacking on the potential roles alterations in DNA methylation and chromatin structure play in the hepatocarcinogenic MOA of PPAR- α agonists.

Uncertainties and Inadequacies of the Data

Limitations of the available data have been detailed in the Klaunig et al. (2003) review. As noted above, the mechanism for the induction of cell proliferation and apoptosis suppression induced by PPAR- α agonists is not known. One significant factor to consider is the role of nonparenchymal hepatic cells in these processes. For example, Kupffer cells release cytokines, some of which are mitogenic to parenchymal cells and some that affect parenchymal cell apoptosis. In addition, many of the enzymes used as indicators of PPAR- α activation are regulated through a well defined mechanism of action that involves altered transcription of PPRE containing genes. Because this pathway of PPAR- α -dependent alteration of gene regulation is only associated with PPAR- α activation and not with the regulation of key events in the MOA, other mechanisms for induction of the key events need to be considered. Specific uncertainties may include whether agents must be metabolized from a pro-form to an active-form to be able to modulate the PPAR- α pathway, the induction of PPREs, or other indirect events.

Many, but not all, agents that demonstrate an ability to induce peroxisomes in rats and mice also induce a neoplastic response in the liver of rats and mice. Morphologic and biochemical evidence of peroxisome proliferation in rat and mouse liver is supportive evidence of the proposed MOA. It should be noted that these remain associated key events that are not proposed at this time to be causally related to tumor formation. The Panel agreed that there were considerable uncertainties as to the significance of associated key events, such as hepatic acyl CoA oxidase induction, with regard to the tumor forming potential of PPAR- α agonists in rats and mice. PPAR- α agonists can bind directly to PPAR- α , but may also perturb interactions with the RXR binding partner, the binding of co-activators and co-repressors to the receptor, or the availability and action of endogenous ligands or inhibitors.

Question 2 - Relative Sensitivity of Fetal, Neonatal, and Adult Rodents

OPPTS has provided a review of the ontogeny of PPAR- α expression and peroxisomal assemblage during fetal and postnatal development in rodents as well as an analysis of the available data evaluating effects on peroxisomal proliferation, peroxisomal enzyme activity, and liver weights following exposure to PPAR- α agonists during fetal and postnatal development in rats and mice (see Section V of the OPPTS Document). Based on this analysis, OPPTS concluded that fetal and neonatal rats do not exhibit an increased sensitivity to PPAR- α agonist-induced hepatocarcinogenicity relative to the adult rodent. Therefore, any conclusions regarding this MOA in adult rodents would also apply to young rodents, and similarly any conclusions regarding the relevance of this MOA for human hepatocarcinogenesis would apply to the young, as well as the adults.

Please comment on the weight of the evidence approach and mechanistic data used to support this conclusion.

Response

The Panel does not support the OPPTS conclusions. Although fetal and embryonic rats and mice respond to PPAR- α agonists as demonstrated by changes in peroxisomal enzyme activities, strong evidence demonstrating that fetal and neonatal rats do not exhibit an increased sensitivity to PPAR- α agonist-induced hepatocarcinogenesis is lacking. Moreover, conclusions regarding this MOA for human hepatocarcinogenesis should not be applied to developing humans.

As discussed in the response to question 1, the proposed MOA involves activation of PPAR- α , which regulates the expression of numerous genes, including several that encode for peroxisomal enzymes, and identifies as key causal events increases in cell proliferation, inhibition of apoptosis, and clonal expansion of preneoplastic lesions, which result in the formation of liver tumors. Published reports have shown that both the expression of PPAR- α and the assembly of peroxisomes occur late in the development of rats and mice. Furthermore, it has been shown that, as in adult livers, embryonic, fetal and neonatal livers of rats and mice respond to PPAR- α agonists by increasing peroxisome number, peroxisome volume density, liver weight, and the expression of the peroxisomal enzyme palmitoyl CoA oxidase. This suggests that at least some of the cellular macromolecules involved in the proposed PPAR- α agonist MOA are functional and responsive to PPAR- α agonists in rat and mouse embryonic, fetal, and neonatal livers. However, data on the hepatocarcinogenic response of rat and mouse embryonic, fetal, and neonatal livers to PPAR- α agonists are lacking and, therefore, no conclusions can be made at this time as to the relative sensitivity of these early life stages to PPAR- α agonist induced hepatocarcinogenicity.

Although the exposure of pregnant rats and mice led to increases in peroxisomal enzyme activities and increases in liver weight in embryonic, fetal, and neonatal liver tissues, other parameters involved in the proposed MOA, such as cell proliferation, inhibition of apoptosis and clonal expansion of preneoplastic cells, were not examined in these studies. In addition, responses to PPAR- α agonists in the fetal and neonatal rat and mouse, as measured by the peroxisomal enzyme expression levels, suggest that there are differences in young animals relative to adults. It is unclear how these differences in enzyme expression levels might translate into differences in sensitivity to hepatocarcinogenesis. Regarding the comparison of changes in liver weights across early and later life stages, it is inappropriate to assume that a given proliferative response seen at one stage of life is equivalent to a similar proliferative response at another stage of life. For example, an increase in liver weight during the neonatal period might result in a much greater lifetime risk of cancer than an equivalent increase occurring during adulthood, because a larger number of cells in the neonatal liver will undergo multiple cell divisions than in the adult. Finally, none of the studies examining the response of the rodent in utero or during early life stages were carried out with the late onset of tumors as a specific endpoint. A two-generation study conducted in mice was designed as a reproductive study and not as a cancer study. Thus, no liver pathology was documented from F1 male and female mice after approximately 4 and 6 months of exposure, respectively (one Panel member noted that complete pathology was not evaluated in this study). The available data pertain to effects that have not been demonstrated as causally linked to the carcinogenic MOA of these agents. The relevance of the induction of peroxisomes or peroxisomal enzymes to the carcinogenic process has not been established. As stated above, there is the possibility that developing organs and tissues may respond differently to peroxisome proliferators compared to adult organs and tissues. There may also be PPAR- α independent effects occurring in the young animal that result in an increased cancer risk. In the absence of this information, conclusions regarding the sensitivity of developing rodents to PPAR- α agonists cannot be formulated. Chemical exposures early in development could increase the sensitivity to cancer risk. It is known that PPAR- α modulates metabolic pathways other than β -oxidation of fatty acids, such as glucose and amino acid metabolism. Moreover, PPAR- α is a transcription factor involved in the modulation of gene expression. PPAR- α agonists not only modulate the expression of genes with PPREs but they may also regulate gene expression by altering levels of gene methylation (Ge, et al., 2001). Such DNA methylation is known to be involved in imprinting and alterations or loss of imprinting can directly or indirectly impact disease risk at later life stages (Cui, H. et al., 2003).

Conclusions regarding the relevance of the PPAR- α agonist MOA for human hepatocarcinogenesis applied to adults may not apply to the young. In contrast to adult human liver, there are no data establishing PPAR- α expression levels in embryonic, fetal and neonatal human liver. To date, there is only one publication reporting the effects of one PPAR- α agonist in lactating non-human primates (Cappon et al. 2002). In this report, the exposure of four Rhesus monkey females to HCFC-123 for short periods of time decreased the activities of cytochrome P450 enzymes and acyl CoA oxidase in

maternal monkey liver, as well as induced centrilobular hepatocyte vacuolation, necrosis and mild to moderate inflammation; however, no histological or biochemical data were reported from the infant monkeys. Non-human primate studies investigating preneoplastic and neoplastic effects of fetal or neonatal exposure to PPAR- α agonists would be desirable.

In contrast to embryonic and fetal rodent liver in which cytochrome P450 enzymes are expressed near, during and after birth (Ring et al. 1999), embryonic and fetal human livers possess metabolic activation capabilities resulting from the early developmental expression of cytochrome P450 enzymes. Moreover, the expression profiles of xenobiotic metabolizing enzymes and isozymes are different in embryonic, fetal, neonatal and adult human livers. Like the gene expression profile of xenobiotic metabolizing enzymes, it is difficult to disregard the possibility that there could be differences between the expression of PPAR- α and its transcriptional co-factors in the human conceptus and adult human liver. In addition, metabolic differences in rats and mice play an important role in determining the degree of response to some PPAR- α agonists (Lake, 1995) and that could also apply to the human conceptus.

Differences in peroxisome biogenesis have been reported during the ontogenic development of rodents and humans. While the assembly of peroxisomes in rats and mice, including the insertion of β -oxidation enzymes into the peroxisomes, occurs near birth, the assembly of human peroxisomes has been observed as early as 8 weeks of gestation (Espeel, et al, 1997). The number and density of peroxisomes plateau by 17 weeks of gestation in humans. Moreover, acyl-CoA oxidase and 3-ketoacyl CoA thiolase are immunodetectable in the peroxisomes by 10 and 9 weeks of gestation, respectively. These observations suggest differences in β -oxidation capabilities in developing rodents and humans and therefore information on the expression of the PPAR- α during ontogeny, as well as responses to PPAR- α agonists in embryonic and fetal human hepatocytes should be evaluated before concluding that the developing human conceptus is unresponsive to PPAR- α agonist exposures.

There are numerous uncertainties concerning the relevance of the PPAR- α agonist MOA for human hepatocarcinogenesis in the young. These uncertainties stem largely from our incomplete understanding of the species-specific differences in sensitivity. Although numerous mechanisms have been posited (see Klauning et al., 2003), none have adequate data supporting their validity. Some of these include differences in the PPREs in specific critical genes, species-specific co-factors that suppress transactivation ability of the ligand activated PPAR- α , sequence differences that result in the prevalence of inactive, splice variants and/or dominant negative PPAR- α gene products, perturbation of RXR binding partner interactions with other nuclear receptors, and polymorphisms that result in a less efficient transcription factor. Most importantly, there is no reason to eliminate the possibility that one or more of these scenarios would function differently in the human fetus, neonate or infant relative to the adult, impacting both MOA and sensitivity at these different life stages.

Question 3 – Human Relevance

OPPTS has provided an analysis of a variety of *in vitro* and *in vivo* studies on the key events pertaining to PPAR- α agonist-induced hepatocarcinogenesis with hamsters, guinea pigs, non-human primates, and humans. Based on the weight of the evidence, OPPTS concludes that although PPAR- α agonists can induce liver tumors in rodents and while PPAR- α is functional in humans, quantitatively, humans and nonhuman primates are refractory to the hepatic effects of PPAR- α agonists.

Therefore, OPPTS is proposing the following scientific policy:

When liver tumors are observed in long term studies in rats and mice, and 1) the data are sufficient to establish that the liver tumors are a result of a PPAR- α agonist MOA and 2) other potential MOAs have been evaluated and found not operative, the evidence of liver tumor formation in rodents should not be used to characterize potential human hazard.

Please comment on the data and weight of evidence regarding the hepatic effects of PPAR- α agonists in humans, and please comment on the proposed OPPTS's science policy regarding human relevance.

Response

Overall, the majority of the Panel agreed that there are relevant data indicating that humans are less sensitive than rodents to the hepatic effects of PPAR- α agonists. However, the opinions of individual Panel members ranged from full agreement with the proposed OPPTS policy statement, as currently written, to complete disagreement. The majority of the Panel recognized weaknesses in the data that supported the policy noting in particular that the case for lack of human relevance was deficient in the human data. In addition, the Panel members agreed that the MOA and its application to addressing human relevance would be greatly strengthened by additional evidence of the specific alterations associated with PPAR- α activation that lead to the more general steps of hepatocellular proliferation, clonal expansion of initiated hepatocytes and tumor development. However, the Panel was divided regarding whether such additional evidence is necessary before accepting the MOA and its application to human relevance. Some Panel members believed that the data failed to demonstrate that the effect could only occur in liver and that, therefore, the policy statement should be limited to hepatocarcinogenic effects (see number 2 below). Other Panel members believed that the overall data limitations were significant enough to disagree with the MOA and its application to addressing human relevance.

Over the past 30 years, a variety of data have been accumulated that demonstrate species-specific sensitivities to agonist activation of PPAR- α , PPAR- α agonist-induced

liver peroxisome proliferation and PPAR- α agonist-induced hepatocarcinogenesis. As noted in the response to question 1, there was agreement among most, but not all of the Panel that data from PPAR- α null mice, showing that in the absence of the receptor, there were no ensuing changes in cell proliferation and hepatic tumor formation, was strong evidence that activation of PPAR- α is necessary for all subsequent steps in the MOA. It also was noted in the response to question 1 that a few Panel members expressed concern over the short duration of the studies in the PPAR- α null mice (i.e., 11 months vs. 24 months in standard cancer bioassays), which rendered the studies incapable of assessing the lifetime liver cancer risk of PPAR- α agonists in this knockout mouse model, and thus, inadequate to conclusively demonstrate that PPAR- α activation is required for hepatocarcinogenesis. Considering the proposed MOA, there was agreement that PPAR- α is present in humans and that the receptor is activated in human liver following exposure to known agonists. Accordingly, the proposed MOA for PPAR- α agonist-induced hepatocellular carcinogenesis in rodents is plausible for humans. There was also agreement that the nature of gene expression associated with hepatocellular PPAR- α activation is qualitatively different between humans and rodents. This difference may result from species differences in PPREs, but there are few data available that identify these potentially important differences, particularly in humans. Humans are at least as sensitive to activation end-points that lead to hypolipidemia but are much less sensitive to other end-points normally associated with peroxisome proliferation. This qualitative difference will be what is referred to in subsequent references as human sensitivity.

One overall concern with the proposed MOA was noted in the response to question 1 and is also a concern regarding the application of the MOA to addressing human relevance. Whereas PPAR- α activation is a very specific component of the MOA, the other steps deemed to be causally-related, namely increased hepatocellular proliferation and clonal expansion of initiated hepatocytes leading to tumor development were very general and non-specific. Overall, the Panel members agreed that additional evidence of specific alterations associated with PPAR- α activation in primates and especially humans would greatly strengthen the proposed MOA.

Although much of the data cumulatively support the hypothesis that agonist-induced human PPAR- α (hPPAR- α) activation fails to follow the MOA seen in rodent livers, namely, increased liver cell proliferation, decreased apoptosis, formation of preneoplastic foci and clonal expansion of these foci into liver tumors, the weight of evidence for this MOA and consequences of agonist-induced PPAR- α activation events in humans is less well defined than in rodents. Human liver biopsy data, while limited, indicate that clinical administration of PPAR- α agonists results in increases in the number and volume density of hepatic peroxisomes. The Panel agreed that the available cancer epidemiological data on pharmacologic PPAR- α agonists are too limited in study size and duration to provide any relevant information to evaluate human relevance. As such, data from other animals, including non-human primates, along with in vitro studies in human hepatocytes, or cell lines, provide the basis for evaluating the relevance of the proposed MOA in humans.

The available data from other animals includes guinea pigs, hamsters, dogs and non-human primates. In all cases, these animals demonstrate reduced liver sensitivities to PPAR- α agonists. Hamsters have a functional PPAR- α receptor but are intermediate in response between rats (and mice) and humans, and no increased cell proliferation or liver tumors have been observed in hamsters (Lake et al., 1993). Similarly, PPAR- α is constitutively present in guinea pigs, albeit at lower levels than rats or mice, and guinea pigs are also less sensitive than rats and mice to PPAR- α activation (Roberts et al., 2000). Data from non-human primates are limited, but generally indicate that PPAR- α agonists do not elicit the typical pattern of histopathological and biochemical changes associated with peroxisome proliferation in rats and mice, as the non-human primate responses to PPAR- α agonists have involved changes of lesser magnitude in fewer of the histopathological and biochemical markers (Reddy et al., 1984; Lalwani et al., 1985; Lake et al., 1989; Graham et al., 1994; and Kurata et al., 1998). Collectively, the Panel was split on the applicability of data from other animals to contribute to a weight of evidence regarding the hepatocarcinogenic effects of PPAR- α agonists in humans. All Panel members recognized that the data on non-rodent, non-human species provided relevant information on the reduced activity of PPAR- α agonists and contributed to the MOA. Also, while all Panel members recognized the limitations of these data (number of compounds studied, study sizes, and study durations), some believed that the data were sufficient to conclude the MOA was working, whereas others were concerned that the limitations were significant enough to disagree with the MOA.

There was a general consensus that the data linking PPAR- α activation to increased cell proliferation in all species was relatively weak. The strongest evidence in support of the importance of this step in subsequent tumor development is derived from the PPAR- α knockout mouse studies in which no increase in hepatic cell proliferation and no tumors are observed after 11 months of treatment (Peters et al., 1997). The Panel was again divided on the conclusions that can be reached from studies in the knockout mouse, as some were convinced by such data, whereas others felt that the overall susceptibility of this mouse model to hepatocarcinogenesis in 11 months had not been defined.

The strength of the hypothesis that humans are less sensitive to agonist-induced PPAR- α -mediated hepatocarcinogenesis lies in the human primary hepatocyte data. The Panel was again divided on the interpretation and utility of these data. First, there was a difference of opinion on the applicability of the in vitro studies used to assess the ability of human hepatocytes to proliferate in response to treatment with a PPAR- α agonist. Although limited in total sample size, these studies have shown that in vitro cultured human hepatocytes respond differently to PPAR- α agonists when compared to in vitro cultured rodent hepatocytes. As discussed in more detail below, whether these differences are attributable to true interspecies differences or reflect differences in human and rodent hepatocyte culture preparations remains an open question. In parallel experiments with in vitro cultured rodent hepatocytes, in vitro cultured human

hepatocytes fail to display several of the key responses deemed essential for the MOA in agonist-induced PPAR- α -mediated rodent hepatocarcinogenesis, those being increased cell proliferation and decreased apoptosis. Furthermore, in vitro cultured human hepatocytes appear to be less responsive to upregulation of peroxisomal genes and proliferation of peroxisomes, two key associative events of agonist-induced PPAR- α -mediated rodent hepatocarcinogenesis. Several Panel members suggested that further experiments in human primary hepatocytes (co-cultured with and without Kupffer cells; see comments below) would be useful if they provide additional biochemical data that demonstrate reduced levels of PPAR- α expression in human liver and an inability for agonist-induced PPAR- α to modulate the gene expression for several key peroxisomal enzymes. Such experiments would strongly support the hypothesis that human liver cells are less sensitive to agonist-induced PPAR- α -mediated hepatocarcinogenesis. Positive controls for known hPPAR- α responsive gene products should be included in such experiments (see, for example, Lawrence et al. 2001).

Those who disagreed with the conclusions noted above based their opinion largely on data that suggest that Kupffer cells are required to elicit a proliferative response in cultured hepatocytes. Specifically, evidence is emerging that supports a role for Kupffer cell activation on the induction of DNA synthesis, and subsequent neoplastic development following PPAR- α agonist treatment. In vivo studies have shown that depletion of Kupffer cells or inhibition of Kupffer cell activation prevents the induction of DNA synthesis by several PPAR- α agonists. These findings suggest that the lack of response from PPAR- α agonist exposure in human hepatocytes in vitro, may be due to the lack of nonparenchymal cells in the hepatocyte preparations. For example, the growth permissive factors released from activated Kupffer cells following PPAR- α agonist exposure are absent and may explain the lack of induction of DNA synthesis seen in cultured human hepatocytes. Support for this possibility has been demonstrated in rodent cultures in vitro (Rose, et al., 1999). In these studies, PPAR- α agonists were unable to induce DNA synthesis in purified preparations of rodent hepatocytes (devoid of nonparenchymal cells), while PPAR- α agonist-induced DNA synthesis was restored upon the addition of nonparenchymal cells, or medium derived from activated Kupffer cells, to the purified hepatocyte cultures.

It was noted that arguments against the involvement of the Kupffer cells comes from studies in the PPAR- α null mice. In these mice, agonists failed to elicit a DNA synthetic response. Since this model is replete with Kupffer cells, the lack of DNA synthesis has been interpreted as indicating that the Kupffer cell is not required. On the other hand, some members of the Panel felt that the communication and/or interplay between PPAR- α agonism and Kupffer cells has not been fully characterized and as such, the null mouse, lacking PPAR- α , is not directly applicable to the human situation in which PPAR- α is present and can be activated.

With regard to the human data, the Panel noted deficiencies arising from studies in which the duration of exposures to PPAR- α agonists were significantly less than

lifetime, the exposure levels were at therapeutic doses, and the populations of exposed individuals were fairly small. As stated previously, the Panel agreed that the available cancer epidemiological data on pharmacologic PPAR- α agonists are too limited in study size and duration to provide sufficient information to evaluate human risk potential. Although the human data are limited, the existing data do provide some important information for consideration. Human liver contains functional PPAR- α receptors and the fibrate class of drugs is able to activate this receptor to alter the expression of genes involved in lipid metabolism that induce hypolipidemia. Chronic exposure data reported in humans for two different PPAR- α agonists suggest that humans do not respond to PPAR- α agonists by an increase of the associated key events (such as cell proliferation, suppressed apoptosis, and clonal expansion of preneoplastic hepatic lesions) observed during PPAR- α activation in rats and mice exposed to these agonists. In addition to the short duration of exposure and the use of therapeutic doses (lower than the doses used in studies with rats and mice), the limitations of these studies include the use of weak agonists. The human epidemiology data from short duration follow up (5 year time period) indicated an early increase in GI tract tumors, although liver cancer was not reported independently. However, no differences were noted after 8 years of follow up. Evidence for peroxisome proliferation and increased cell proliferation was lacking in human liver biopsies. Problems with these observations include the high variability in assessing peroxisome increases in biopsy material that are not representative of all zones of the liver, and whether the timing of biopsy sample acquisition was appropriate for detecting an increase in cell proliferation. A slight increase in the number and density of peroxisomes is observed in humans with chronic exposure to therapeutic levels of a PPAR- α agonist. This level is indicative of normal physiologic or metabolic changes and is lower than the approximately three fold level defined by Ashby et al. (1994) as the threshold level of peroxisome induction associated with liver cancer risk in rats and mice. These observations in humans are strengthened by the studies of chronic exposure of non-human primates to PPAR- α agonists for 5 or more years. Again, the number of non-human primates exposed was limited and the duration of exposure was less than lifetime. Assessment of the presence or absence of PPAR- α regulated gene expression and of preneoplastic lesions needs to be detailed in primates compared to rats and mice following exposure to PPAR- α agonists. The non-human primate appears to have a markedly attenuated response to fairly potent PPAR- α agonists (e.g., ciprofibrate) compared with rats and mice, although, as with the human data, the PPAR- α agonist challenge has been at lower doses of shorter duration. Studies by Pugh et al., (2000) wherein numerous PPAR α agonists were administered to nonhuman primates support this contention in that a lack of increase in liver weights indicates a lack of cell proliferation as verified by replicative DNA synthesis.

The Panel discussed three other issues relative to assessing weight of evidence regarding the hepatic effects of PPAR- α agonists in humans, and the proposed science policy regarding human relevance. These included:

1. The use of the word "refractory" to describe the human response to PPAR- α activation is too absolute. The Panel agreed that "less sensitive" is a more appropriate description of the nature of the human response relative to that observed in rats and mice.
2. The policy statement drafted by OPPTS concludes with the phrase "evidence of liver tumor formation in rodents should not be used to characterize potential human hazard." After some discussion, it was suggested by one member of the Panel, and supported by several other Panel members, that this phrase should be modified to read, "evidence of liver tumor formation in rodents should not be used to characterize potential human hepatocarcinogenic hazard."
3. One member of the Panel expressed concern, which was shared by some other Panel members, that the MOA and evaluation of human relevance was lacking in its assessment of altered gene expression that could be associated with altered methylation of DNA. There is evidence that DNA methylation is modified in rodents following exposure to PPAR- α agonists (Ge et al., 2001, Ge et al., 2002, and Pereira, et al., 2004). Given the accepted role for DNA methylation in gene imprinting and the loss of imprinting in cancer etiology (see for example McClachlan et al., 2001), such a role for PPAR- α agonists in causing similar alterations in humans should be explored before human relevance can be appropriately evaluated, particularly for exposure during early life stages and for questions regarding site concordance.

Question 4 – Data Requirements

OPPTS has proposed a data set that would be sufficient to demonstrate that PPAR- α agonism is the MOA for the induction of rodent liver tumors. The data set includes evidence of PPAR- α agonism (*i.e.*, from an *in vitro* reporter gene assay), *in vivo* evidence of an increase in number and size of peroxisomes, increases in the activity of acyl CoA oxidase, and hepatic cell proliferation. The *in vivo* evidence should be collected from studies designed to provide the data needed to show dose-response and temporal concordance between precursor events and liver tumor formation.

Please comment in general on the proposed data set and particularly on its adequacy to demonstrate that a PPAR- α agonist-mediated MOA is operating in rodent hepatocarcinogenesis.

Response

Data requirements refer to the experimental data needed to demonstrate that a compound acts through a PPAR- α agonist MOA. These data may be used subsequent to a bioassay that finds induction of hepatic tumors to demonstrate such tumors arose from a PPAR- α agonist MOA, or subsequent to initial (sub)acute experiments to assist in the subsequent experimental design of long-term experiments for submission to the Agency. This use of the data may dictate some differences in the data requirements needed. The

following discussion focuses on requirements after a positive bioassay, with suggestions provided for the converse situation.

There was general consensus among the Panel that the proposed data set was adequate and provided a straight forward approach to classifying a chemical as a PPAR- α agonist. The Panel also concurred that the use of PPAR- α knockout mice would be definitive evidence to ascribe a chemical as a PPAR- α agonist, but that the proposed data set would be sufficient in lieu of the use of this rather costly tool. While the Panel agreed with these data needs, they suggested some clarifications and additional supportive approaches.

The clarifications indicated were as follows: the term 'direct DNA reactivity' may need to be clarified as 'direct' may be interpreted by some to mean "without metabolism"; in keeping with the ILSI document (Klaunig et al., 2003), rather than using the term 'mutagenicity' alone, the terms 'mutagenicity and/or clastogenicity' may be more appropriate; palmitoyl CoA activity is simply a substrate-specific name for acyl CoA oxidase activity; and microsomal fatty acid oxidation (as opposed to microsomal fatty acid omega-oxidation) is not specific enough to designate CYP4A activity.

In the course of the Panel's discussion, questions for clarification were posed to the Agency as to when (i.e., before or after a positive liver tumor finding in rodents) this set of assays testing for PPAR- α agonist activity would be conducted. The Agency indicated that data demonstrating PPAR- α agonist activity could be submitted in the absence of testing in long-term carcinogenesis studies. In response to this, a Panel member observed that in the absence of testing in standard long-term rodent carcinogenicity studies, it is not possible to determine whether the chemical would operate through a PPAR- α agonist MOA producing rodent liver tumors. A chemical with PPAR- α agonist activity may either: 1) not cause cancer in rodents, 2) cause liver cancer in rodents by the proposed PPAR- α agonist MOA, 3) cause liver cancer by a MOA other than the proposed PPAR- α agonist MOA (e.g., cytotoxicity), or 4) cause cancer at sites other than the liver (with or without liver cancer). The Panel concurred that an overriding requirement is that other MOAs have been excluded. For example, rigorous tests must be performed to exclude mutagenicity, other forms of DNA damage (clastogenicity), or overt cytotoxicity directly produced by the test compound, or its metabolic products.

The Panel also concurred that direct evidence of the activation of PPAR- α is required to show that complementary in vivo results do not result from activation of other PPARs or from an unknown mechanism as exemplified by dehydroepiandrosterone (DHEA) (Isseman and Green, 1990, Peters, et al., 1996 and Waxman, 1996). The activation of PPAR- α is often demonstrated using chimeric systems that include an expression system for the PPAR- α receptor and a reporting system that includes the PPRE in the promoting region. It was recommended by one Panel member that this study could be supplemented by gene-dosage experiments in knockout mice or transgenic mice

overexpressing the receptor with respective loss or exacerbation of responsiveness. These experiments would demonstrate a direct effect of the receptor on a true genomic PPRE, rather than a construct. It was also recommended that it be acknowledged that in some cases a metabolite of the test compound may be a more suitable substrate to use in these experiments. Direct involvement of PPAR- α can alternatively be assessed using in vivo experiments with wild type (PPAR α +/+) and knockout mice (PPAR α -/-); endpoints for these in vivo experiments are discussed below. Compounds with positive bioassays in rats but not mice would not be suitable for this alternative approach.

In vivo experiments should be conducted using doses that produce positive bioassays; as they are normally (sub)acute they will meet temporal requirements that they occur prior to tumor formation. Of highest priority, they must demonstrate an increase in hepatocyte cell replication/reduced apoptosis, induction of peroxisomal acylCoA oxidase and an increase in number and volume percent of peroxisomes. Demonstration of induction of other enzymes with PPRE sequences in the promoter region (CYP4A, carnitine acetyl transferase, fatty acid binding protein, etc.) or catalase provides supportive evidence. It was recommended that at least one 'supportive example of enzyme induction' be included. Induction of enzymes can be demonstrated from increased enzyme activity and/or increased expression of mRNA. It was also noted that the need to show both increases in peroxisome volume percent and density would require morphometric analysis of liver sections examined by electron microscopy (demonstration of increased density, but not volume percent, could be approached using light microscopic methods).

One Panel member suggested that when acute evidence of a PPAR- α agonist MOA has been found prior to long-term dosing studies, the evidence of the MOA can be further enhanced by inclusion of an initiation/promotion test system where the test compound is administered as the promoter after suitable initiation. These experiments demonstrate the key event of clonal expansion. In addition, there are some (immuno)histochemical stains that can be used to show a greater degree of specificity for this MOA. It was acknowledged that while such experiments would further support the MOA, they were fairly time- and cost-inefficient with regard to the main objective of demonstrating that the compound is a PPAR- α agonist.

Question 5 – Other Tumors Induced by PPAR- α Agonists

Some PPAR- α agonists may also induce pancreatic acinar cell and Leydig cell tumors in rats and modes of action involving agonism of PPAR- α have been proposed. An in depth analysis of these tumors is provided in the 2003 ILSI technical panel report. Based on this analysis, OPPTS agrees that the data available to date are insufficient to support the proposed MOAs.

Thus, OPPTS is proposing the following science policy:

Given the limited evidence available to support that a chemical may induce pancreatic and Leydig cell tumors through a PPAR- α agonist MOA, the evidence is inadequate at this time to support a linkage between PPAR- α agonism and formation of these tumor types. Thus, it is presumed that chemicals that induce pancreatic or Leydig cell tumors may pose a carcinogenic hazard for humans.

Please comment on OPPTS's conclusion that there is limited evidence that a chemical may induce pancreatic and Leydig cell tumors through a PPAR- α agonist MOA, and OPPTS's proposed science policy regarding other tumors induced by PPAR- α agonists.

Response

In addition to the hepatic tumors that appear to be a general occurrence in rats and mice, nine PPAR- α agonists have been reported to induce Leydig cell tumors (LCTs) and pancreatic acinar cell tumors (PACTs) in rats. Together with the hepatic tumors, this is referred to as the tumor triad. The Panel was in agreement with the OPPTS conclusion that chemicals that induce pancreatic or Leydig cell tumors may pose a carcinogenic hazard for humans.

LCTs were most apparent when PPAR- α agonists were tested in non-F344 male rats, likely because by 2 years of age, the F344 rat has virtually a 100% incidence of spontaneously occurring LCTs. This will obscure any ability to detect a xenobiotic-induced testicular tumor in this strain. The finding that a relationship appears to exist between PPAR- α agonists and LCT formation has led to speculation that many, if not all, such agonists would induce this tumor if tested adequately in a rat strain other than F344. This speculation has been supported by limited studies in other strains (Biegel, et al., 2001, Maltoni, et al., 1988 and Mennear, 1988).

It was originally hypothesized that PPAR- α agonists cause LCTs by a PPAR- α -dependent mechanism similar to that of the liver. However, evidence exists using PPAR- α null mice (Ward et al., 1998) to suggest that the PPAR- α agonist DEHP can induce toxic lesions in kidney and testis independently of this receptor. In addition, although Leydig and pancreatic acinar cells contain PPAR- α , agonists do not appear to induce peroxisome proliferation in these cells. This suggests that tumors developing in these tissues in rats do so via different mechanisms than in the liver where peroxisome proliferation is always observed. A prevailing hypothesis is that PPAR- α agonists cause an increase in estradiol that promotes the secretion of transforming growth factor (TGF- α). Evidence in support of this hypothesis is that PPAR- α agonists increase the expression of aromatase, an enzyme that under normal conditions maintains serum estradiol concentrations by converting testosterone to estradiol (Biegel, et al., 1995). Estradiol stimulates TGF- α production which induces Leydig cell proliferation (Khan, Teerds, and Dorrington, 1992).

Another proposed MOA of PPAR- α agonist-induced LCTs is that they cause testicular hypertrophy by decreasing testosterone biosynthesis, leading to an imbalance of androgen/estrogen levels. This leads to an increase in leutinizing hormone (LH) which promotes LCTs. However, it is not known whether steroid synthesis pathways in testis are regulated by PPAR- α , and in Cook et al. (2001) no changes in LH were observed.

The Panel agreed that although some data suggest LCTs may involve PPAR- α , additional research will be required to confirm this role. In addition, the link to PPAR- α activation is considered tenuous because limited studies of PPAR- α agonists in other animal species, such as the mouse, hamster and nonhuman primates, did not show extrahepatic carcinogenic responses, including PACTs and LCTs. As noted previously, the Panel agreed that the available cancer epidemiological data for pharmaceutical PPAR- α agonists are too limited in study size and duration to be informative as to cancer risk at any site. While LCT data in mice remain limited, this species difference from rats is certainly indicative of some unique feature either in rats which causes the tumors, or in mice which are resistant. Further data are needed to determine which is the case. It is also noteworthy that the spontaneous rate of LCTs is much lower in humans than in rats suggesting innate resistance to this type of cancer, and that rat and human Leydig cells respond differently to human chorionic gonadotropin (human cells undergo hypertrophy while rat cells proliferate). Finally, a human condition with constant LH receptor activation does not lead to LCTs, even though this is one of the major proposed MOAs in rats.

Key events in the postulated MOA for PACTs in rats are considered to begin with PPAR- α activation in the liver, followed by changes in bile composition and a decrease in its synthesis. This results in cholestasis and a sustained increase in cholecystokinin. This stimulates acinar cell proliferation and promotes the development of PACTs. If this is true, then the rat PACTs are secondary to the liver effects of PPAR- α agonists. Some data indicate that many of the non-hepatocarcinogenic parameters and symptoms manifested in rodents upon long-term administration of PPAR- α agonists are also manifested in humans. This is particularly true since it has been observed in rodents that long-term administration of PPAR- α agonists results in marked changes in bile acid secretion and composition. In human studies it is also established, by multiple investigators, that fibric acid drug treatment increases biliary cholesterol and induces supersaturation of bile. Studies demonstrating that hPPAR- α is functional in the regulation of a variety of enzymes associated with bile acid metabolism in human liver cells would suggest that the risks of PACTs in humans exposed to PPAR- α agonists could involve a PPAR- α mechanism. However, the data are not sufficient to firmly conclude that this MOA is operative. Furthermore, the difference between rodents and humans in the cellular origin of pancreatic tumors (acinar in rat, ductal in humans) suggests that these animal data are of limited relevance to humans. Again, although data in other species are limited, only rats have shown these tumors.

Finally, in addition to PPAR- α agonism as a potential MOA of extrahepatic tumors, as noted previously, one member of the Panel expressed concern, which was shared by some other Panel members, that consideration needs to be given to epigenetic phenomena that may be activated by these chemicals. DNA methylation and chromatin structure alterations are significant nongenotoxic mechanisms involved in deregulating gene function. Furthermore, PPAR- α agonists inhibit methylation during DNA replication (Ge et al., 2001), thereby altering the cellular epigenome. This is important since the earliest change identified in tumor cells compared to their normal counterpart is genome-wide hypomethylation (Feinberg and Vogelstein, 1983). These changes can be particularly critical during development, including puberty, but even adults vary dramatically in their susceptibility to cancer because of marked differences in the epigenome. For example, there is now evidence that approximately 10% of the human population is at high risk, at least for colon cancer, because of either an inability to maintain imprinting at the IGF2 locus or exposure early in development to some environmental factor resulted in IGF2 loss of imprinting (Cui et al., 2003). It is conceivable that these "preneoplastic" individuals are more susceptible to PPAR- α agonists than the general population.

In summary, given the limited amount of data available on the true MOA for LCTs or PACTs, including the possibility raised by some Panel members that epigenetic effects of the PPAR- α agonists may occur, it is not possible to determine whether PPAR- α agonists pose a carcinogenic hazard to humans. Thus, the conclusion by the OPPTS that the available data for the induction of rat LCTs and PACTs by PPAR- α agonists are insufficient to conclude that the sole MOA involves the PPAR- α receptor is considered by the Panel to be appropriate. Further, the Panel concurs that it should be presumed that chemicals that induce pancreatic or Leydig cell tumors may pose a carcinogenic hazard for humans.

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Re: PFOA-Exposed Community Blood Sample Results
(For AR-226 And OPPT-2003-0012)

Ladies and Gentlemen:

In response to USEPA's previous requests for information relating to the threat to human health and the environment involving PFOA/C-8, we have enclosed the results of PFOA serum testing performed through DuPont and its contractor, Exygen, for twelve members of the general population living near DuPont's Washington Works facility in Wood County, WV. All twelve of the individuals tested have been exposed to PFOA/C-8 through drinking water provided by the Lubeck Public Service District where, according to DuPont, the level of PFOA/C-8 in the drinking water has averaged approximately 0.5 ppb over the last several years. These individuals also claim to have stopped using the contaminated public drinking water as their primary source of drinking water approximately three years ago, and switched to alternative sources, such as bottled water. Only one of the individuals (with a PFOA serum result of 90.4 ppb) ever worked

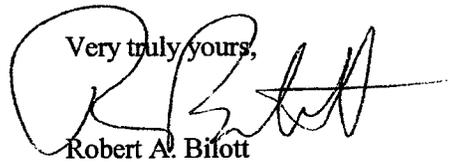
W0223075.1

EXHIBIT 11

Dr. Charles M. Auer
Oscar Hernandez
Jennifer Seed
Mary Ellen Weber
Mary Dominiak
September 15, 2004
Page 2

at the DuPont Washington Works Plant. A chart summarizing the results from the enclosed lab report (EID871401-10) is presented below. Please include this information in AR-226 and OPPT-2003-0012.

<u>SEX</u>	<u>AGE</u>	<u>PFOA SERUM (PPB)</u>	<u>APPROX. YEARS ON WATER</u>
F	55	128	>20
M	52	103/103	>20
M	70	90.4	>20
F	80	83.1	10-20
M	63	78.5/73.7	10-20
F	46	65	5-10
M	69	61.3	<5
M	36	56.4/50.8	>20
M	57	51.2	10-20
F	55	42.6	10-20
F	48	40.1	10-20
M	57	15.7	<5

Very truly yours,

Robert A. Bilott

RAB/mdm
Enclosure

Dr. Charles M. Auer
Oscar Hernandez
Jennifer Seed
Mary Ellen Weber
Mary Dominiak
September 15, 2004
Page 3

cc: R. Edison Hill, Esq. (w/o encl.)
Larry A. Winter, Esq. (w/o encl.)
Gerald J. Rapien, Esq. (w/o encl.)

Analytical Report

Occupational and Environmental Health

Analysis of Perfluorooctanoic Acid (PFOA) in Human Serum Samples

Exygen Report No. L0003000

Testing Laboratory

**Exygen Research
3058 Research Drive
State College, PA 16801**

Requester

**Dr. Marsha Bailey
Occupational and Environmental Health
4 Rosemar Circle
Parkersburg, WV 26101
(304)-767-0270**

EID871401

1 Introduction

Results are reported for the analysis of perfluorooctanoic acid (PFOA) in human serum samples received at Exogen from Dr. Marsha Bailey at Occupational and Environmental Health. The Exogen project number assigned to the samples is P959.

2 Sample Receipt

A total of twelve samples were received at Exogen in 5 mL screw-cap vials labeled with permanent marker. A copy of all sample log-in information is presented in Attachment A.

The twelve samples were received on 07/23/04. The samples were shipped frozen on dry ice via FedEx. The samples were stored frozen from time of receipt until analysis.

3 Methods - Analytical and Preparation

3.1 Sample Preparation

The samples were extracted and analyzed according to the current revision of method EdM-008-211. Fifty microliters of sample was used for the extraction procedure. Using the Multiprobe apparatus, 500 μ L of acetonitrile was added to the sample and then passed through a protein precipitation column.

3.2 Sample Analysis by LC/MS and LC/MS/MS

In High Pressure Liquid Chromatography (HPLC), an aliquot of extract is injected and passed through a liquid-phase chromatographic column. Based on the affinity of the analyte for the stationary phase in the column relative to the liquid mobile phase, the analyte is retained for a characteristic amount of time. Following HPLC separation, mass spectrometry provides a rapid and accurate means for analyzing a wide range of organic compounds. Molecules are ionized, fragmented, and detected. The ions characteristic of the compounds are observed and quantitated against extracted standards.

An HP1100 system interfaced to a PE Sciex API 4000 system was used to analyze the sample extracts. A gradient elution through a Jones Chromatography Genesis C-8 50 x 2.1 mm x 4 μ m column was used for separation.

The following gradient was performed:

Mobile Phase (A):	2mM Ammonium Acetate in Type I Water
Mobile Phase (B):	Methanol

Time	%A	%B	Flow Rate (mL/min)
0.0	40	60	0.3
3.0	40	60	0.3
3.5	0	100	0.3
3.7	0	100	0.5
7.0	0	100	0.5
7.5	40	60	0.5
9.0	40	60	0.5
9.5	40	60	0.3
12.0	40	60	0.3

The following parameters were used for operation of the mass spectrometer:

Parameter	Setting
Ionization Mode	Electrospray
Polarity	Negative
Transitions Monitored	413->369 (PFOA), 415->370 (¹³ C-PFOA)
Gas Temperature	350°C
Drying Gas (N2)	7.0 L/min

2. Analysis

4.1 Calibration

A 6-point calibration curve was analyzed throughout the analytical sequence for the fluorocompounds. The calibration points were prepared at 0.5, 1, 5, 10, 20, 50 ng/mL for PFOA. The instrument response versus the concentration was plotted for each point. Using linear regression with 1/x weighting, the slope, y-intercept and coefficient of determination (r^2) were determined. A calibration curve is acceptable if $r^2 \geq 0.985$.

For the results reported here, calibration criteria were met.

4.2 Surrogates

Surrogate spikes were not a part of this analysis.

4.3 Laboratory Control Spikes

Laboratory control spikes in the analytical set were prepared by adding a known concentration of the analytes to control human serum. Laboratory control spikes are used to assess method accuracy. The laboratory control spikes must show recoveries between 80-120% for levels at the LOQ and 85-115% for levels greater than the LOQ or the data is rejected. For the results reported here, the spikes were within the acceptable range.

4.4 Matrix Spikes

Two matrix spikes in the analytical set were prepared by adding a known concentration of the target analyte to separate human serum samples. Matrix spikes are used to assess method accuracy in the matrix. The matrix spikes should show recoveries between 85-115%. For the results reported here, the matrix spikes were within the acceptable range.

4.5 Sample Related Comments

Three samples in the analytical set were extracted and analyzed in duplicate. Duplicate sample results are reported along with the sample results in Attachment B.

5 Data Summary

Please see Attachment B for a detailed listing of the analytical results. Results are reported in parts per billion (ng/ml.) for the analytes on an as-received basis.

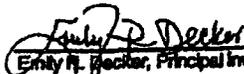
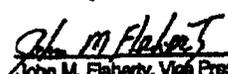
6 Data Sample Retention

Samples are disposed of one month after the report is issued unless otherwise specified. All electronic data is archived on retrievable media and hard copy reports are stored in data folders maintained by Erygen. Hardcopy data is stored for a minimum of five years. Occupational and Environmental Health will be notified 30 days prior to the disposal of hardcopy data.

7 Attachments

- 7.1 Attachment A: Chain of Custody
- 7.2 Attachment B: Analytical Results

8 Signatures

 Emily H. Becker, Principal Investigator	<u>7/29/04</u> Date
 John M. Flaherty, Vice President	<u>7/29/04</u> Date



3058 Research Drive
State College, PA 16801

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Fax: 814-231-1580

Login

Login Group: L0003000

Login #: 3110
 Project: P0000959
 Company Name: DuPont de Nemours & Co, Inc.
 Submitted By: Charles R. Powley
 Login Type: Immediate Receipt of Samples
 Started: T
 Date Start: 07/23/2004
 Due Date: 08/06/2004
 Received Date: 07/23/2004
 Received By: Ammerman, Mark
 Spread Sample:
 Label:
 Exygen SD/PI: Decker, Emily
 Project Title/Type: Analysis for PFOA in Human Serum Samples by LC/MS/MS / ROUTINE
 Login Notes:
 Conform Notes:

Conform COC Sample: True
 Conform COC: True
 Conform Sample: True
 Conform Request: True

Packages / Containers

Package	Canon	Mail Date / Condition	Shipper / ID	Temp. Control/Temp.	Direction / Handled By	
PK0003722		7/23/04 3:38:30PM Package & Contents Uncompromised	FEDEX 8458 8093 4889	Dry Ice 0.0	RECEIVED Ammerman, Mark	
Container #	Gross Weight	pH	Container Type	Preservative	Mfg. Lot	Mfg. ID
C0040828	4.00 g		5 ml serum tube	NONE		
C0040828	4.40 g		5 ml serum tube	NONE		
C0040829	4.70 g		5 ml serum tube	NONE		
C0040831	4.80 g		5 ml serum tube	NONE		
C0040834	4.80 g		5 ml serum tube	NONE		
C0040838	4.30 g		5 ml serum tube	NONE		
C0040832	4.80 g		5 ml serum tube	NONE		
C0040841	4.80 g		5 ml serum tube	NONE		
C0040843	5.00 g		5 ml serum tube	NONE		
C0040844	4.80 g		5 ml serum tube	NONE		
C0040845	5.30 g		5 ml serum tube	NONE		
C0040846	4.00 g		5 ml serum tube	NONE		

EID871405



Login

<u>Samples</u>					<u>Date Sampled</u>	<u>Date Received</u>	<u>Date Due</u>
L0003000-0001	Container C0040826	Matrix LIQUID	Fraction Human Serum	Sample	07/22/2004	07/23/2004	08/06/2004
L0003000-0002	C0040828	LIQUID	Human Serum	CONFIDENTIAL INFORMATION REDACTED	07/22/2004	07/23/2004	08/06/2004
L0003000-0003	C0040829	LIQUID	Human Serum		07/22/2004	07/23/2004	08/06/2004
L0003000-0004	C0040831	LIQUID	Human Serum		07/22/2004	07/23/2004	08/06/2004
L0003000-0005	C0040834	LIQUID	Human Serum		07/22/2004	07/23/2004	08/06/2004
L0003000-0006	C0040836	LIQUID	Human Serum		07/22/2004	07/23/2004	08/06/2004
L0003000-0007	C0040838	LIQUID	Human Serum		07/22/2004	07/23/2004	08/06/2004
L0003000-0008	C0040841	LIQUID	Human Serum		07/22/2004	07/23/2004	08/06/2004
L0003000-0009	C0040843	LIQUID	Human Serum		07/22/2004	07/23/2004	08/06/2004
L0003000-0010	C0040844	LIQUID	Human Serum		07/22/2004	07/23/2004	08/06/2004
L0003000-0011	C0040845	LIQUID	Human Serum		07/22/2004	07/23/2004	08/06/2004
L0003000-0012	C0040846	LIQUID	Human Serum		07/22/2004	07/23/2004	08/06/2004

EID871406



EID871408

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 27. Restricted Tastes
 28. Restricted Touches
 29. Restricted Feels
 30. Restricted Thoughts
 31. Restricted Emotions
 32. Restricted Intentions
 33. Restricted Actions
 34. Restricted Reactions
 35. Restricted Responses
 36. Restricted Outcomes
 37. Restricted Consequences
 38. Restricted Implications
 39. Restricted Inferences
 40. Restricted Assumptions
 41. Restricted Beliefs
 42. Restricted Attitudes
 43. Restricted Values
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 45. Restricted Norms
 46. Restricted Standards
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 85. Restricted Swaps
 86. Restricted Exchanges
 87. Restricted Trades
 88. Restricted Swaps
 89. Restricted Exchanges
 90. Restricted Trades
 91. Restricted Swaps
 92. Restricted Exchanges
 93. Restricted Trades
 94. Restricted Swaps
 95. Restricted Exchanges
 96. Restricted Trades
 97. Restricted Swaps
 98. Restricted Exchanges
 99. Restricted Trades
 100. Restricted Swaps

102017
 6984660995488

Summary of Residue Found (ng/mL) for PFOA
in Human Serum Samples

Sponsor ID	PFOA (ppb)
	103
	103
CONFIDENTIAL INFORMATION	85.0
	18.7
	81.3
REDACTED	90.4
	88.4
	88.8
	48.1
	128
	81.3
	83.1
	73.7
	78.8
	42.8

EID871409

X
3058 Research Drive
State College, PA 16801, USA
T: 814.272.1899
F: 814.281.1550
oxygen.com

Summary of Recoveries (%) for PFOA
in Human Serum Samples

Sponsor ID	PFOA (ppb)	Amount Fortified (ppb)	Recovery (%)
CONFIDENTIAL INFORMATION REDACTED	8.19	8.0	102
	18.2	10	107
	1040	1000	103
	1000	1000	98
	4500	5000	90
AVERAGE:			100
STD DEV:			8.4
% RSD:			8.4

Summary of PFOA (ng/mL)
in Human Serum Control Samples

Sponsor ID	PFOA (ng/mL)
Control A	8.47

EID871410



2550 M Street, NW
Washington, DC 20037-1350
202-457-6000

Facsimile 202-457-6315
www.pattonboggs.com

February 1, 2005

Peter D. Robertson
(202) 457-6320
probertson@pattonboggs.com

Via Hand Delivery

Mr. Charles M. Auer
Director
Office of Pollution Prevention and Toxics
U.S. Environmental Protection Agency
EPA East
1201 Constitution Avenue, NW
Washington, DC 20460

Re: Further DuPont Voluntary Response to EPA's November 4, 2004, Request
Submission to AR-226 and TSCA 8(e) Office
Supplement to December 20, 2004 Submission

Dear Charlie:

I write in response to your request for additional information about the blood sample results provided to EPA on December 20, 2004. As we have informed you, the samples were taken as part of a lawsuit styled: *Tennant, et al, v. E. I. du Pont de Nemours & Co., Inc.* C.A. 6:99 0488. The information provided in this letter was obtained from review of discovery responses and from discussions with counsel for plaintiffs in that lawsuit. The accuracy of this information has not been verified. In conjunction with collection of this information, counsel for plaintiffs informed DuPont on January 19, 2005, of the existence and results of one additional round of blood sampling for some of these individuals. DuPont was not involved with the collection, analysis or reporting of those results; however the results are listed in the table below under the column "NMS 2001".¹

We have been informed that all of the individuals referenced below consumed water for varying lengths of time from private water wells and/or cisterns located in the vicinity of the Dry Run Landfill and Dry Run Creek. Analysis of some of this private well water by Pacific Analytical, Inc. in late 2000 indicated the presence of PFOA in the private well water between 0.12 ppb and

¹ As you know, the 2002 results were generated by National Medical Services and the 2004 results were generated through Exygen. We have been informed by counsel for Plaintiffs that the 2001 results were also generated by National Medical Services.

Mr. Charles M. Auer

February 1, 2005

Page 2

0.17 ppb. This well water data was submitted to USEPA in 2001 as an attachment to a letter from Mr. Robert Bilott, counsel for plaintiffs in the lawsuit. As indicated below, we have been informed that several of the individuals tested also were exposed prior to testing to drinking water from the Lubeck Public Service District in Wood County, West Virginia, which water had been purchased and stored in their private cisterns for domestic use over a 3-4 year period of time. The average level of PFOA in the Lubeck Public Service District water supply during the time period in question was approximately 0.5 ppb. In addition to exposure to drinking water containing PFOA, each of the individuals tested reportedly also may have sporadically come into contact with Dry Run Creek water containing PFOA. It is our current understanding that only one of the individuals tested (as indicated below), ever worked at the DuPont Washington Works facility.

The following table was prepared by plaintiffs' Counsel in a format that both plaintiffs and DuPont agreed could be submitted to EPA with no confidentiality restrictions.

<u>SEX</u>	<u>CURRENT AGE</u>	<u>NMS 2001</u>	<u>NMS 2002</u>	<u>EXYGEN 2004</u>	<u>YEARS ON WELL WATER</u>
M	60-70	13 ppb	29 ppb	54.2 ppb	>50*
F	50-60	55 ppb	42 ppb	51.6 ppb	20-30*
M	60-70	37 ppb	41 ppb	40.8 ppb	>50**
F	50-60	18 ppb	30 ppb	38.4 ppb	10-20
M	60-70	63 ppb	58 ppb	71.6 ppb	>50
F	30-40	Not Tested	12 ppb	20 ppb	10-20
F	20-30	Not Tested	10 ppb	35.7 ppb	10-20*
F	20-30	Not Tested	21 ppb	15.2 ppb	10-20*
F	30-40	Not Tested	22 ppb	40.4 ppb	20-30*
M	30-40	Not Tested	85 ppb	126 ppb	30-40

Mr. Charles M. Auer
February 1, 2005
Page 3

- * Also sporadically drank water purchased from Lubeck Public Service District and stored in a cistern for <4 years.
- ** Worked sporadically at DuPont's Washington Works facility in Wood County, West Virginia through a contractor/union during the 1960s-early 1980s.

You have also requested information on the test methods identified in the above Table. No comment is provided on the test method used in 2001 as that testing was conducted independent of DuPont involvement. The test method identified as NMS 2002 was developed and validated in November 2001. This test method was provided to EPA by DuPont on December 10, 2004; it is in volume 35, tab 4. Other documents in volume 35 related to this method development are found under tabs 3 and 6. The 2002 NMS method has a reporting limit of 10 ng/mL.

The method used by Exygen in 2004 could be one of three versions. Because DuPont does not have information on exactly what date the testing was done, it cannot be determined exactly which method was used. However, all three versions are provided with this letter and are as follows:

- Method number ExM-008-211 revision 1, dated December 2, 2003; limit of quantitation is 0.5 ppb;
- Method number ExM-008-276 revision 2, dated January 8, 2004; limit of quantitation is 10 ppb;
- Method number ExM-008-211 revision 3, dated August 2, 2004; limit of quantitation is 0.5 ppb.

TSCA 8(e) Office Submission

DuPont does not believe that the information submitted with this letter triggers reporting obligations under TSCA section 8(e). However, in the course of discussions with OPPT and the Office of Regulatory Enforcement (ORE), it has become clear to DuPont that those two offices are applying standards of reporting under TSCA section 8(e) that DuPont cannot anticipate. Accordingly, DuPont initially informed EPA that DuPont intended to submit its response to the November 4, 2004, EPA request to both the AR-226 docket and the TSCA 8(e) office, as was done with the submission of November 15, 2004. EPA has, however, informed counsel for DuPont that it is not necessary to make a duplicate, formal submission to the 8(e) office in order to discharge any reporting obligations that EPA might otherwise assert. Instead, EPA has asked that DuPont make only a single submission and advised that DuPont should indicate in this cover letter that the submission is intended for both the AR-226 docket and the TSCA 8(e) office.



Mr. Charles M. Auer
February 1, 2005
Page 4

As such, DuPont states that the enclosed documents are intended to be a submission to both the AR-226 docket and, as a precaution, to the TSCA 8(e) office, notwithstanding DuPont's firm belief that the information does not trigger reporting obligations under that section of TSCA. This submission should not be construed as a direct or indirect admission that DuPont believes that any of the enclosed information triggers such reporting obligations. We understand that ORE has agreed that DuPont's submission shall not prevent DuPont from asserting, in any proceeding, that section 8(e) did not require submission of this information.

If you have further questions on the information provided in this letter, please do not hesitate to contact me.

Sincerely,

A handwritten signature in black ink, appearing to read 'Peter D. Robertson', written over a light blue horizontal line.

Peter D. Robertson

Attachments

- Method number ExM-008-211 revision 1, dated December 2, 2003 – 27 pages
- Method number ExM-008-276 revision 2, dated January 8, 2004 – 20 pages
- Method number ExM-008-211 revision 3, dated August 2, 2004 – 27 pages

MR# 281116



AR226-1884

RECEIVED
OPPT CBIC

2004 NOV 24 AM 11:43

DuPont Haskell Laboratory
for Health and Environmental Sciences
Elkton Road, P.O. Box 50
Newark, DE 19714-0050

November 23, 2004

Via Federal Express

8EHQ-1104-00394

2p.

Document Processing Center (Mail Code 7407M)
Room 6428
Attention: 8(e) Coordinator
Office of Pollution Prevention and Toxics
U.S. Environmental Protection Agency
1201 Constitution Ave., NW
Washington, DC 20460

8EHP-81-394

CONTAINS NO CBI

Dear 8(e) Coordinator:

8EHQ-0381-0394
Ammonium Perfluorooctanoate

This letter is to inform you of the summary results (Attachment I) of a recently conducted blood sampling for the above referenced material in employees from our Washington Works facility in West Virginia. This exposure sampling is part of a larger ongoing study, "Ammonium Perfluorooctanoate: Cross-Sectional Surveillance Of Clinical Measures of General Health Status Related to a Serum Biomarker of Exposure and Retrospective Cohort Mortality Analyses in a Polymer Production Plant" of over 1,000 employees at our plant. This information has already been presented to EPA OPPT on November 10, 2004. These results are consistent with previously reported blood levels in workers. Individual blood sampling results, redacted for privacy reasons, will be provided to EPA on or before December 6, 2004.

In the course of discussions with OPPT and with the Office of Regulatory Enforcement, it has become clear to DuPont that those two offices are applying standards of reporting under TSCA Section 8(e) that DuPont cannot anticipate. Therefore, as a precaution DuPont is submitting the enclosed information to the Section 8(e) office, notwithstanding DuPont's firm belief that the information does not trigger reporting obligations under that section of TSCA. This submission should not be construed as a direct or indirect admission that DuPont believes that any of the enclosed information triggers such reporting obligations. We understand that ORE has agreed that DuPont's submission shall not prevent DuPont from asserting, in any proceeding, that Section 8(e) did not require submission of this material.

A copy of the final report of the larger ongoing study referred to above will be submitted to the Agency when available.

Sincerely,

A. Michael Kaplan, Ph.D.
Director - Regulatory Affairs and Occupational Health

AMK/RWR/RCL:clp
(302) 366-5260

2004 DEC -6 AM 9:04

RECEIVED
OPPT CBIC



8 9 0 5 0 0 0 0 7 1



AR 226-1885

**Distribution of Serum PFOA Levels
By Current Work Assignment**

Work Assignment	Serum PFOA (ppm)			
	Number in Group	Median	Min	Max
Teflon®	259	0.494	0.0174	9.55
Research/Technical	160	0.176	0.0081	2.07
Never assigned to Teflon®	342	0.114	0.0046	0.963
Assigned to Teflon® at some point	264	0.195	0.0086	2.59
Total Participants	1025			

**A Simple, Conservative Compartmental Model to Relate
Ammonium Perfluorooctanoate (APFO) Exposure to
Estimates of Perfluorooctanoate (PFO) Blood Levels in
Humans**

Paul M. Hinderliter, Ph.D.

Gary W. Jepson, Ph.D.

**Biochemical Toxicology
DuPont Haskell Laboratory for Health and Environmental Sciences**

10 October, 2001

DRAFT

Page 1 of 10

EID166599

GK004797

EXHIBIT 14

Abstract

A simple and conservative compartmental model was developed to relate ammonium perfluorooctanoate (APFO) exposures to estimates of perfluorooctanoate (PFO) concentrations in human blood. The model was based on kinetic principles, but it did not include mechanistic or physiological descriptions. Further, the model was not intended to replace the need for more robust models that include mechanistic and appropriate physiological descriptions. The model included zero-order mathematical descriptions of oral and inhalation input and a first order elimination description. Standard estimates of the volumes of daily water consumption and air breathed were used to relate daily intake of APFO to concentrations of APFO in air and drinking water. The model was exercised under a variety of exposure conditions and used to create a table relating APFO intake via drinking water and/or air to PFO blood concentrations. The simplicity and utility of this model provide decision-makers with an easily applied tool to relate APFO exposures to estimates of resulting PFO concentrations in human blood.

Introduction

A simple compartmental model was developed and used to estimate the concentration of perfluorooctanoate (PFO) in blood following inhalation or ingestion of ammonium perfluorooctanoate (APFO). The model presented is intended to complement various consequence analysis and planning activities and is not intended to be a substitute for a robust, mechanism based physiological model. In order to realize both the strengths and limitations of the model, it is important to carefully consider the assumptions and caveats relevant to the model development and application.

Approach

Model Development:

The model developed for this application was a two-compartment open model with one compartment defined as the blood compartment and the other as the body compartment. While the model is constructed as a two-compartment model, transfer of PFO is confined to only one compartment (blood compartment) in order to provide a conservative estimate of PFO concentrations in blood following APFO exposure. Functionally, this reduces to a one-compartment open model with two zero-order-input processes and one first-order elimination process. In other words, PFO is confined to the blood compartment and the PFO concentration in blood cannot be reduced by the distribution of PFO into other body tissues. In order to contribute to the conservative estimates produced by this model, any APFO that is ingested or inhaled is not subject to diffusional resistance and is assumed to be completely and instantly absorbed into the blood compartment. Since PFO is not metabolized, elimination from the blood is via renal excretion. In this model the elimination is described as a pseudo first-order process. A schematic of the model is shown in Figure 1.

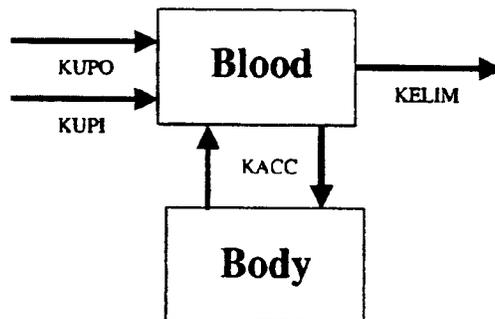


Figure 1. Schematic of PFO Compartmental Model.

In Figure 1, KACC is the distribution coefficient for transfer of PFO between the blood and body compartments. It has the units of day^{-1} , but as discussed earlier, it is set to zero

in order to create a conservative one-compartment model. KUPO is a zero-order term to describe PFO input into the blood compartment (ug/day) via the oral route. KUPI is a zero-order term to describe PFO input into the blood compartment (ug/day) via the inhalation route. KELIM is a pseudo first-order elimination coefficient (day⁻¹) that describes removal of PFO from the blood compartment via renal excretion. Differential rate equations were developed from the schematic in Figure 1 and the equations were solved using Advanced Continuous Simulation Language (ACSL, Aegis Corp.). The mathematical equations used to describe the concentration of PFO in the blood compartment (CBLOOD) are shown in the series of equations below.

$$\frac{dAB}{dt} = KUPO + KUPI - KELIM * CBLOOD * VOL - RAF \quad (1)$$

$$dAB = (KUPO + KUPI - KELIM * CBLOOD * VOL - RAF)dt \quad (2)$$

$$\int_{AB=0}^{AB} dAB = \int_{t=0}^t (KUPO + KUPI - KELIM * CBLOOD * VOL - RAF)dt \quad (3)$$

$$AB = \int_{t=0}^t (KUPO + KUPI - KELIM * CBLOOD * VOL - RAF)dt \quad (4)$$

$$CBLOOD = AB / VOL \quad (5)$$

In the equations above, AB is the amount (ug) of PFO in blood, t is time (days), VOL is the volume (ml) of the blood compartment and RAF (ug/day) is the rate of PFO movement between the blood and body compartments (RAF=0 in this model). The ACSL coding of the above equations is given immediately below and in Appendix 1. The corresponding ACSL command file is provided in Appendix 2.

$$RA = KUPO + KUPI - KELIM * CBLOOD * VOL - RAF \quad (6)$$

$$CBLOOD = INTEG(RA, 0) / VOL \quad (7)$$

Model Input Assumptions/Descriptions:

Blood Compartment Volume: The blood volume of 3.5 L used in the model was that of a 50-Kg human (average human female weight). The female weight was selected to maintain the conservative approach desired for this model. Obviously, blood volume is a function of body weight so larger body weights will equate to larger blood volumes. PFO concentrations in blood will therefore decrease for a given APFO exposure as body weights increase.

Elimination Rate Constant: The elimination rate constant, KELIM, was assigned a value of 0.0019/day. This was derived assuming a PFO half-life ($t_{1/2}$) in humans of 365 days and that first order kinetics apply. While current human half-life estimates are placed in the 200-300 day range, the 365-day half-life is a conservative value for initial model conditions. The actual value for KELIM was derived using the relationship between the half-life and the elimination rate constant where first order kinetics are obeyed.

$$KELIM = \frac{\ln 2}{t_{1/2}} \quad (8)$$

Input of APFO via Drinking Water: Drinking water concentrations of APFO were converted to micrograms (ug) of APFO ingested per day using the assumption that approximately 2L of the water are consumed per day. An example follows where drinking water containing 1 part per billion (ppb) APFO was consumed:

$$1 \text{ ppb} = \frac{1 \text{ ug}}{L} \quad \text{so} \quad \frac{1 \text{ ug}}{L} \times \frac{2L}{\text{day}} = \frac{2 \text{ ug}}{\text{day}} \quad (9)$$

Input of APFO via Inhalation: Inhaled concentrations of APFO were converted to micrograms of APFO absorbed into the blood using the assumption that approximately 20 m³ of air are breathed per day. An example follows where air containing 1 ug/m³ APFO was inhaled.

$$\frac{1 \text{ ug}}{\text{m}^3} \times \frac{20 \text{ m}^3}{\text{day}} = \frac{20 \text{ ug}}{\text{day}} \quad (10)$$

General Assumptions:

The simple model described here is designed to be conservative and is not intended to be a substitute for a more robust, mechanism based physiological model. Consistent with the design of this model, several general assumptions have been made.

- ~~No~~ (1) The PFO is distributed only in the human blood compartment. *Conservative*
- ~~No~~ (2) There is no metabolism of PFO.
- ~~No~~ (3) No binding or mechanistic descriptions are included in the model. *Conservative*
- ~~ok~~ (4) Elimination occurs by a single first-order pathway. It is likely that elimination actually displays biphasic elimination with an initial rapid elimination phase followed by a slower or terminal phase elimination. In order to be consistent with the conservative nature of the model, only the slow (terminal) phase elimination is included in the model. *± Effect*
- ~~probably~~ ? (5) All APFO inhaled or ingested in drinking water is instantly and completely absorbed into the blood compartment. *Assumed **
- ~~real~~ ? (6) APFO exposures occur every day throughout the exposure period modeled. *Assumed*

Results

The simulated PFO levels in human blood resulting from repeated ingestion of 6 ug/day APFO are shown in Figure 2. As would be expected based on the estimated half-life of PFO in the human body, the simulation illustrates that steady-state PFO blood levels are reached only after repeated exposure for over 6 years. Figure 3 is a simulation of the elimination of PFO from the blood once PFO levels are at steady state and PFO exposure is terminated.

Figure 2. Simulated PFO Concentration in Human Blood Following Continuous Intake of 6 ug/day

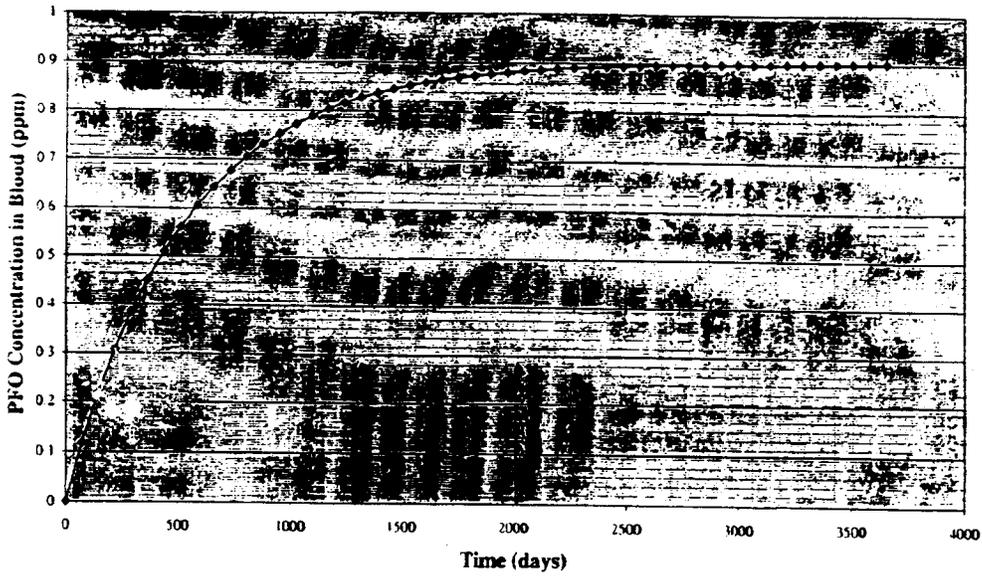
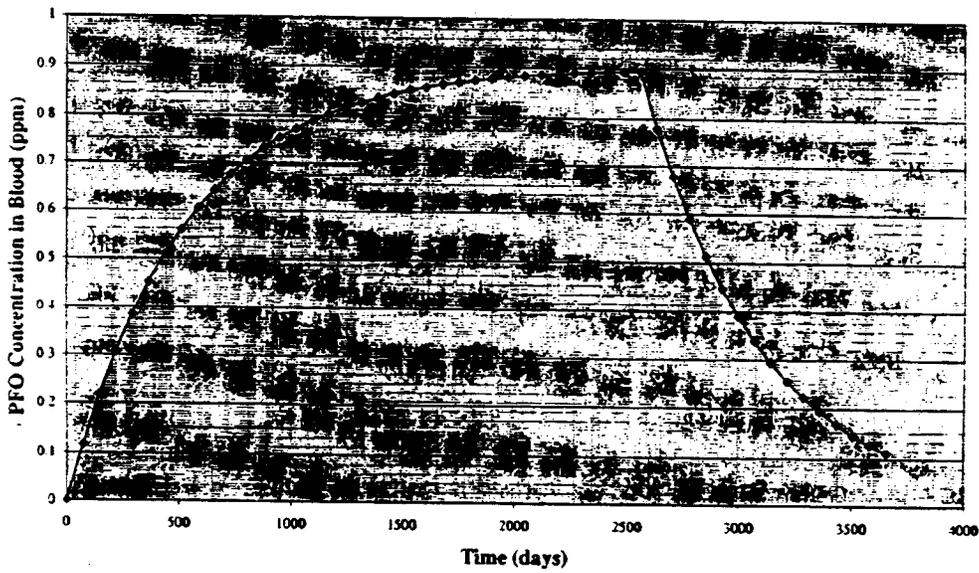


Figure 3. Simulated PFO Concentration in Human Blood During and After 2600 Days of Exposure to 6 ug/day APFO



A series of model simulations were run to estimate the steady-state human PFO blood levels resulting from drinking water containing APFO, breathing air containing APFO or combinations of the two. The resulting estimates of PFO concentrations in human blood are shown in Table 1. Table 1 can be used under the conditions described in the text, to assign a PFO blood concentration to a particular exposure. Example 1: If drinking water containing 1 ppb APFO was consumed and no APFO was present in the inhaled air, the resulting steady-state PFO concentration estimate in human blood would be 0.30 ppm. Example 2: If no APFO was present in the drinking water and 0.05 ug/m³ APFO was in the inhaled air, the resulting steady-state PFO concentration estimate in human blood would be 0.15 ppm. Example 3: If APFO was present in the drinking water at 1ppb and in the air at 0.3 ug/m³, the resulting steady-state PFO concentration estimate in human blood would be 1.20 ppm.

Table 1. Estimated human steady-state PFO blood levels (ppm) following exposure to APFO via air and/or drinking water.

		Parts per billion APFO in drinking water																
		0	1	2	3	4	5	6	7	8	9	10	15	30	40			
µg/m ³ APFO in air	0.00	0.00	0.30	0.60	0.90	1.20	1.50	1.80	2.10	2.40	2.70	3.00	3.31	3.61	3.91	12.02		
	0.05	0.15	0.45	0.75	1.05	1.35	1.65	1.95	2.25	2.55	2.85	3.16	3.46	3.76	4.06	12.17		
	0.10	0.30	0.60	0.90	1.20	1.50	1.80	2.10	2.40	2.70	3.00	3.31	3.61	3.91	4.21	12.32		
	0.15	0.45	0.75	1.05	1.35	1.65	1.95	2.25	2.55	2.85	3.16	3.46	3.76	4.06	4.36	12.47		
	0.20	0.60	0.90	1.20	1.50	1.80	2.10	2.40	2.70	3.00	3.31	3.61	3.91	4.21	4.51	12.62		
	0.30	0.90	1.20	1.50	1.80	2.10	2.40	2.70	3.00	3.31	3.61	3.91	4.21	4.51	4.81	12.92		
	0.40	1.20	1.50	1.80	2.10	2.40	2.70	3.00	3.31	3.61	3.91	4.21	4.51	4.81	5.11	10.22	13.22	
	0.50	1.50	1.80	2.10	2.40	2.70	3.00	3.31	3.61	3.91	4.21	4.51	4.81	5.11	5.41	10.52	13.52	
	1.00	3.00	3.31	3.61	3.91	4.21	4.51	4.81	5.11	5.41	5.71	6.01	6.31	6.61	6.91	12.02	15.02	
	2.00	6.00	6.31	6.61	6.91	7.21	7.51	7.81	8.11	8.41	8.71	9.01	9.31	9.61	9.91	10.22	15.02	18.03
	3.00	9.00	9.31	9.61	9.91	10.22	10.52	10.82	11.12	11.42	11.72	12.02	12.32	12.62	12.92	13.22	18.03	21.03
	4.00	12.02	12.32	12.62	12.92	13.22	13.52	13.82	14.12	14.42	14.72	15.02	15.32	15.62	15.92	16.22	21.03	24.04

 PFO Blood levels less than or equal to 5 ppm
 PFO Blood levels greater than 5 ppm but less than or equal to 10 ppm

* Use of this table requires careful consideration of assumptions and limitations described in the text.

Discussion

A relatively simple and conservative compartmental model was developed and exercised to create an estimate of the PFO concentration in human blood following exposure to APFO in drinking water and/or inhaled air. The model was then used to create a table relating APFO exposures to estimates of steady-state PFO blood concentrations. Within the constraints of the assumptions and descriptions provided in this report, a variety of

exposure combinations could be evaluated using the model. Given a specific PFO concentration in blood, the model could also be used to create a plausible exposure scenario that could produce the observed PFO blood level. For example, if one had a hypothetical steady-state PFO concentration of 5 ppb in blood, the corresponding APFO exposure estimate using the model would be approximately 16 parts per trillion (ppt).

The model and approach presented in this report may be valuable for consequence analysis or planning activities, however, it should not serve as a substitute for more robust mechanistic, physiologically based models as they become available. The model presented here is based on sound compartmental analysis principles and is exclusive of mechanistic or physiological descriptions. As discussed earlier, this model is based on conservative assumptions and therefore is likely to provide high estimates of PFO concentrations in blood following ingestion or inhalation of PFO. Nevertheless, the simplicity and utility of this model provide decision-makers an easily applied tool to relate APFO exposures to estimates of resulting PFO concentrations in human blood.

Appendix 1: ACSL Model Code

```
PROGRAM
!MODEL TO SIMULATE PFO BLOOD LEVELS FOLLOWING ORAL AND
!INHALATION OF APFO
VARIABLE TIME

INITIAL

!CONSTANTS CAN BE GIVEN VALUES TO SIMULATE EXPOSURE AND
!SYSTEM OF INTEREST

CONSTANT KUPI      = 0.    !ZERO ORDER INHALATION UPTAKE (ug/day)
CONSTANT KUPO      = 0.    !ZERO ORDER ORAL UPTAKE (ug/day)
CONSTANT KELIM     = 0.    !FIRST-ORDER ELIMINATION (/day)
CONSTANT KACC      = 0.    !FIRST-ORDER DISTRIBUTION TO BODY (/day)
CONSTANT VOL       = 1.    !BLOOD VOLUME (ml)
CONSTANT VF        = 1.    !BODY VOLUME (ml)

!TIMING COMMANDS

CONSTANT TSTOP     =3650.   !LENGTH OF EXPOSURE (days)
CONSTANT POINTS    =3650.   !NO. OF POINTS IN PLOT
CONSTANT TOFF      =3650.   !END OF EXPOSURE TIME (DAYS)

CINT=TSTOP/POINTS  !COMMUNICATION INTERVAL
END                !END INITIAL

DYNAMIC

ALGORITHM IALG=2

DERIVATIVE
IF (TIME .GT. TOFF) THEN
KUPI = 0.
KUPO=0.
END

IF TERMT(TIME.GE.TSTOP)

!CONCENTRATION OF PFO IN THE BLOOD COMPARTMENT (ug/day)
RA=KUPO + KUPI - KELIM*CBLOOD*VOL - RAF
CBLOOD=INTEG(RA,0.)/VOL

!CONCENTRATION OF PFO IN THE BODY
RAF = KACC*(CBLOOD*VOL-CF*VF)
CF = INTEG(RAF,0.0)/VF

END !END DERIVATIVE
END !END DYNAMIC
END
```

DRAFT

Page 9 of 10

EID166607

GK004805

Appendix 2: ACSL Command File for Assigning Appropriate Parameter Values

```
TSTOP=10*365;  
POINTS=50;  
TOFF=TSTOP+1;  
VOL=3500;
```

```
KACC=0.0;  
KELIM=0.0019;  
KUPO=2;  
KUPI=6;
```

```
keyboard  
figure;  
!!START  
line(_time, _cblood, @linestyle="+");  
_cblood(POINTS)
```

```
xlabel('Time (Days)');  
ylabel('Conc. in blood (ug/mL)');  
title('BLOOD CONCENTRATION');
```

226

33 PP

Screening level cumulative risk assessment perfluorinated alkyl acids on human health

Rich Purdy

- **Guidance on Cumulative Risk Assessment of Pesticide Chemicals That Have a Common Mechanism of Toxicity.** Office of Pesticide Programs, US EPA. EPA 2002.
- **Guidance for Identifying Pesticide Chemicals and Other Substances that have a Common Mechanism of action.** EPA 1999.

Cumulative chemicals should have same “mechanism of toxic action” and “site of toxic effect” .

Or in other words:

Cumulative chemicals have a common mechanism of toxicity.

Common mechanism of toxicity pertains to two or more chemicals that cause a common toxic effect(s) by the same or essentially the same, sequence of major biochemical events (i.e., mode of action)

Proposed Cumulative Class:

Perfluorinated Alkyls

Currently including:

PFOS and its homologs:



PFOA and its homologs:



Justification 1

Common mechanism of action

Uncoupling of oxidative phosphorylation is apparently the primary molecular mechanism of toxicity.

References:

- Langley AE, Pilcher GD. 1985. Thyroid, bradycardic and hypothermic effects of perfluoro-n-decanoic acid in rats. *Journal of Toxicology and Environmental Health* 15:485-491.
- Schnellmann G. 1990. The Cellular Effects of unique pesticide Sulfluramid (N-ethylperfluorooctanesulphonamide) on rabbit renal proximal tubules. *Toxic. In vitro* 4:71-76.
- Wallace KB, Starkov A. 1998. The effect of perfluorinated arylalkylsulfonamides on bioenergetics of rat liver mitochondria. US EPA OPPT AR226-0167
- Starkov AA, Wallace KB. 2002. Structural determinants of fluorochemical-induced mitochondrial dysfunction. *Tox Sci* 66:244-252.

PFAs shown to be uncouplers

- **PFOS**
- **FOSA (perfluorooctane sulfamide)**
- **PFHS (perfluorohexane sulfonate)**
- **PFOA**
- **PFDA (perfluorodecanoic acid)**

Justification 1b:

**Data supporting uncoupling
as a mechanism**

Poor body mass gain efficiency is symptom of the uncoupling of oxidative phosphorylation.

Most PFA toxicity studies report loss of weight, poor growth, or poor food conversion efficiency can be calculated from data.

References:

- Covance. 2000. 104-week dietary chronic study and carcinogenicity study with perfluorooctane sulfonic acid potassium salt (PFOS: T-6295) in rats. US EPA OPPT AR226-0956.
- Case MT. 1999. Oral (stomach tube) Developmental toxicity of PFOS in rabbits. US EPA OPPT AR226-0949*. York et al 1999;
- 3M 1987. Two year oral (diet) toxicity / carcinogenicity study of fluorochemical FC-143 in rats. US EPA OPPT AR225-0437
- Campbell SM, Lynn SP, Beavers JB. 1993a. Lithium Perfluorooctane Sulfonate [6861D11] A dietary LC50 study with the Northern Bobwhite, Wildlife International Ltd. Project No.: 319-101. Sponsor: SC. Johnson & Son, Inc.
- ---. 1993b. Lithium Perfluorooctane Sulfonate [6861D11] A dietary LC50 study with the Mallard, Wildlife International Ltd. Project No.: 319-102. Sponsor: SC. Johnson & Son, Inc.
- Cook JC, Murray SM, Frame SR, Hurtt ME. 1992. Induction of Leydig Cell Adenomas by Ammonium Perfluorooctanoate: A Possible Endocrine-Related Mechanism. Toxicology and Applied Pharm 113:209-317.
- Borges T, Robertson LW, Peterson RE, Glauert H P. 1992. Dose-related effects of perfluorodecanoic acid on growth, feed intake and hepatic peroxisomal beta-oxidation. Archives of Toxicology 66:18-22.
- George ME, Andersen ME. 1986. Toxic Effects of nonadecafluoro-n-decanoic acid in rats. Toxicology and Applied Pharmacology 85: 169-180.

References continued

- Haugthom B, Spydevold Ø. 1992. The Mechanism Underlying the Hypolipemic Effect of Perfluorooctanoic acid (PFOA), Perfluorooctane sulphonic acid (PFOSA) and Clofibrilic Acid. *Biochemica et Biophysica Acta* 112:65-72.
- Goldenthal EI, Jessup DC, Geil RG, Mehring JS. 1978a. Ninety-day subacute Rhesus monkey toxicity study, with Fluorad fluorochemical surfactant FC95. US EPA OPPT AR226-0137
- Goldenthal EI, Jessup DC, Geil RG, Jefferson ND, Arceo RJ. 1978b. Ninety-day subacute rat toxicity study, with Fluorad fluorochemical surfactant FC-95. US EPA OPPT AR226-0141
- Hansen K. 1999a. Laboratory report: analysis of fluorochemicals in wild bird livers. US EPA OPPT AR226-0079
- Langley AE. 1990. Effects of perfluoro-n-decanoic acid on the respiratory activity of isolated rat liver mitochondria. *J. Toxicol Environ Health* 29:329-336.
- Thomford PJ. 1998. 4-Week capsule toxicity studies with perfluorooctane sulfonic acid potassium salt (PFOS; T-6295) in Cynomolgus monkeys [includes draft final report, cell proliferation report, protocol and memorandum from Marvin Case re histopathology review of liver tissue. US EPA OPPT AR226-0144.

Justification 2

Common sites of toxic effects seen

Similar effects on tissues are
observed across the class

Thymus atrophy

Thyroid hormone decrease

Thyroid mass increase

Liver enlargement

References

- Goldenthal EI, Jessup DC, Geil RG, Mehring JS. 1978a. Ninety-day subacute Rhesus monkey toxicity study, with Fluorad fluorochemical surfactant FC95. US EPA OPPT AR226-0137*.
- Goldenthal EI, Jessup DC, Geil RG, Jefferson ND, Arceo RJ. 1978b. Ninety-day subacute rat toxicity study, with Fluorad fluorochemical surfactant FC-95. US EPA OPPT AR226-0141*.
- Covance. 2000. 104-week dietary chronic study and carcinogenicity study with perfluorooctane sulfonic acid potassium salt (PFOS: T-6295) in rats. US EPA OPPT AR226-0956*.
- Van Rafelghem MC, Baskin MJ, Bruner RH, Andersen ME. 1987. A time-course study of perfluoro-n-decanoic acid pathology in male Fischer-344 rats. *Fund & Appl Toxicol* 11:503.
- Van Rafelghem MC, Inhorn SL, Peterson RE. 1987. Effects of perfluorodecanoic acid on thyroid status in rats. *Toxicology and Applied Pharmacology* 87:430-439.
- Van Rafelghem MC, Mattie DR, Bruner RH, Andersen ME. 1987. Pathological and hepatic ultrastructural effects of a single dose of perfluoro-n-decanoic acid in the rat hamster, mouse, and guinea pig. *Fund and Appl Tox* 9:522-540.

References, continued

- Langley AE, Pilcher GD. 1985. Thyroid, bradycardic and hypothermic effects of perfluoro-n-decanoic acid in rats. *Journal of Toxicology and Environmental Health* 15:485-491.29-336.
- Case MT. 1999. Summary PFOS rat two-generation reproduction study. US EPA OPPT AR226-0569
- Metrick M, Marias AJ. 1977. 28-day oral toxicity study with FC-143 in albino rats. US EPA OPPT AR225-0445*
- Thomford PJ. 1998. 4-Week capsule toxicity studies with perfluorooctane sulfonic acid potassium salt (PFOS; T-6295) in Cynomolgus monkeys [includes draft final report, cell proliferation report, protocol and memorandum from Marvin Case re histopathology review of liver tissue. US EPA OPPT AR226-0144
- Thomford PJ. 2000. 26-week capsule toxicity study with perfluorooctane sulfonic acid potassium salt (PFOS: T-6295) in Cynomolgus monkeys. US EPA OPPT AR226-0957
- Seacat AM, Hansen K. 2000. Analytical laboratory report from the 26-week capsule toxicity study with Perfluorooctanesulfonic acid potassium salt (T-6295) in Cynomolgus monkeys. US EPA OPPT AR226-0981.
- Thomford PJ 2001. 26-week capsule toxicity study with ammonium perfluorooctanoate in Cynomolgus monkeys. US EPA OPPT AR226-1052a

Structurally Similar



Reasons for Thymus

- Thymus effects seen in every study in which it was evaluated.
- No NOEL yet established, so could be most sensitive toxic response.
- In early development thymus health is extremely important.

References for Study Used

- Thomford P.J. 2000. 26-week capsule toxicity study with perfluorooctane sulfonic acid potassium salt (PFOS: T-6295) in Cynomolgus monkeys. US EPA OPPT AR226-0957
- Seacat AM, Hansen K. 2000. Analytical laboratory report from the 26-week capsule toxicity study with Perfluorooctanesulfonic acid potassium salt (T-6295) in Cynomolgus monkeys. US EPA OPPT AR226-0981

Observations of Atrophied Thymus

	<u>Control</u>	<u>Low</u>	<u>Mid</u>	<u>High</u>
Females	0 of 4	4 of 4	4 of 4	3 of 4
Males	1 of 4	2 of 4	3 of 4	2 of 2

- Concentration PFOS in sera of low dose females at end of study: 11.4 – 17 ppm

Identification of fluorochemicals in human sera.

III. Pediatric participants in a group A
Streptococci clinical trial investigation.

Olsen GW, Burris JM, Lundberg JK, Hansen KJ,
Mandel JH et al. 2002.

US EPA OPPT AR226-1085.

Conc. ppm

Chemical

Perfluoro sulfonates

C6

C7

PFOS

C9

C10

Perfluoro fatty acids

C6

C7

C8, PFOA

C9

C10

C11

C12

C13

C14

C15

C16

Sum Perfluorinated acids:

Female rat sera NOELS for PFOS and PFOA

PFOA: 0.15 mg/l

PFOS: 5.3mg/l

Reference:

- Metrick M, Marias AJ. 1977. 28-day oral toxicity study with FC-143 in albino rats. US EPA OPPT AR225-0445
- Belisle J.1978. FC-143 analysis of serum samples from rats from 28 and 90-day studies. US EPA OPPT AR225-0442
- Case 1999. Summary PFOS rat two-generation reproduction study. US EPA OPPT AR226-0569

<u>Chemical</u>	<u>High Conc. ppm</u>	<u>Median Conc. ppm</u>
C6 sulfonate	0.50	0.004
C7 sulfonate	?	?
PFOS	0.22	0.03
C9 sulfonate	?	?
C10 sulfonate	?	?
C6 fatty acid	?	?
C7 fatty acid	?	?
C8, PFOA	0.03	0.004
C9 fatty acid	?	?
C10 fatty acid	?	?
C11 fatty acid	?	?
C12 fatty acid	?	?
C13 fatty acid	?	?
C14 fatty acid	?	?
C15 fatty acid	?	?
C16 fatty acid	?	?

Perfluorodecanesulfonate (PFDS) estimated to be equal to PFOS

Rational: Detected in samples where
PFOS levels are close to limits of
detection.

Reference:

- Hansen K. 1999. Laboratory report: analysis of fluorochemicals in wild bird livers. US EPA OPPT AR226-0079.
- Hansen K. 1999. Analysis of FCs in samples of children's sera. US EPA OPPT AR226-0961

Perfluorodecanoic acid (PFDA) estimated from PFOA level

- Based on bioaccumulation factor (>500 times that of PFOA) and the relative level in a dominate product, Zonyl BA.
-
- References:
 - Hansen K. 1999. Report of data for exploratory 28-day oral toxicity study in rats: telomer alcohol, telomer acrylate, PFBS, PFHS, PFOS. US EPA OPPT AR226-0951
 - duPont material safety data sheet on Zonyl BA

Concentration (ppm) in rat liver

	<u>Day 1</u>	<u>Day 14</u>	<u>Day 28</u>
PFOA	0.16	0.016	<.003
PFDA	1.69 1.20	1.36 1.20	2.24 0.81
C12	0.117 0.029	0.085 0.035	0.101 0.036

*Gavage telomer alcohol mixture for 28 days. Stopped dosing on day1

Half-lives of PFOS and PFOS in Sera

Rats Humans

PFOA	4.8 days	4.4 years
PFOS	7.5 days	8.7 years

Reference:

- Gibson SJ, Johnson JD. 1979. Absorption of FC-143-14C in rats after a single oral dose. US EPA OPPT AR226-0455
- Gibson SJ, Johnson JD. 1979. Absorption of FC-95-14C in rats after a single oral dose. US EPA OPPT AR226-0007
- Burris JM, Lundberg JK, Olsen G, Simpson C, Mandel J. 2002. Determination of serum half-lives of several fluorochemicals. US EPA OPPT AR226-1086

C12 and C14 perfluoro fatty acids estimated from PFDA

Reference:

- Mabury, S. 2002. Fascinating fluoro facts of perfluorinated alkyl carboxylates and sulfonates. SETAC 2002 meeting presentation.

C9, C11, C13 and C15 estimated
to be same as homolog with one
less carbon.

Reference:

- Mabury, S. 2002. Fascinating fluoro facts of perfluorinated alkyl carboxylates and sulfonates. SETAC 2002 meeting presentation.

Estimated Concentration of Perfluorinated Acids in Sera of 2-year-old Girls.

<u>Chemical</u>	Highest	Estimated	Median	Estimated
	Measured	Highest	Measured	Median
	<u>Conc. ppm</u>	<u>Conc. ppm</u>	<u>Conc. ppm</u>	<u>Conc. ppm</u>
<i>Perfluoro sulfonates</i>				
C6	0.50	0.62	0.004	0.005
PFOS	0.22	0.22	0.03	0.03
C10	ND	0.18	ND	0.02
<i>Perfluoro fatty acids</i>				
C8, PFOA	0.03	0.04	0.004	0.005
C9	ND	0.04	ND	0.005
C10	ND	12	ND	1.4
C11	ND	10	ND	1.3
C12	ND	19	ND	2.3
C13	ND	15	ND	1.8
C14	ND	25	ND	3.0
C15	ND	20	ND	2.0

<u>Chemical</u>	<u>Highest Measured Conc. ppm</u>	<u>Estimated Highest Conc. ppm</u>	<u>Median Measured Conc. ppm</u>	<u>Estimated Median Conc. ppm</u>
<i>Perfluoro sulfonates</i>				
C6	0.50	0.62	0.004	0.005
PFOS	0.22	0.22	0.03	0.03
C10	ND	0.18	ND	0.02
<i>Perfluoro fatty acids</i>				
C8, PFOA	0.03	0.04	0.004	0.005
C9	ND	0.04	ND	0.005
C10	ND	12	ND	1.4
C11	ND	10	ND	1.3
C12	ND	19	ND	2.3
C13	ND	15	ND	1.8
C14	ND	25	ND	3.0
C15	ND	20	ND	2.0
Sums:		102		12

Level causing thymus atrophy < 11.4



UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

AUG 7 2003

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

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Environmental Toxicology Program
National Institute of Environmental Health Sciences
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111 T.W. Alexander Drive, Room A326
Research Triangle Park, NC, USA 27709

Rec'd 8/15/03 SPN

Dear Dr. Masten:

On behalf of the U.S. EPA Office of Pollution Prevention and Toxics (OPPT), I am submitting a nomination for consideration by the National Toxicology Program (NTP) to conduct studies on a series of perfluorinated chemicals.

OPPT has been assessing perfluorinated compounds since 1999. This interest was prompted by reports submitted to the agency describing the toxic properties and widespread presence in the environment, including in human populations, of some of these chemicals. Initial efforts focused on perfluorooctane sulfonate (PFOS), and the assessment of ecological and human health hazards was recently published under the auspices of the Organization for Economic Cooperation and Development (OECD, 2002). In general, PFOS is persistent and does not biodegrade in the environment. It bioaccumulates in fish, and the half-life in humans is estimated to be on the order of years. Biomonitoring studies have shown that PFOS is present in humans and wildlife around the world. The toxicity profile of PFOS is consistent in mice, rats and monkeys, and includes liver toxicity, hypolipidemia, liver tumors, and developmental toxicity. In all species and life stages, there is a very steep dose-response curve for mortality which appears to be related to the cumulative internal dose. The U.S. EPA Office of Research and Development has been investigating the mode of action of the neonatal mortality associated with prenatal exposure to PFOS for several years.

EXHIBIT 16

Subsequent efforts have focused on perfluorooctanoic acid (PFOA). The ecological and human health hazards of PFOA have been summarized (OPPT, 2002), and a preliminary risk assessment of the developmental effects has been released (OPPT, 2003). PFOA is also persistent and does not biodegrade. In contrast to PFOS, PFOA does not bioaccumulate in fish. However, it also has a half-life of years in humans, and domestic biomonitoring studies have shown that it is present in the general population, as well as in wildlife. The toxicity of PFOA has been studied in rats, mice and monkeys, and includes liver toxicity, immunotoxicity, cancer (liver, pancreatic, and leydig cell tumors), and developmental toxicity. The uncertainty in the quantitation of potential human risks is compounded by a substantial gender difference in the elimination rate of PFOA in rats. Since the release of the hazard assessment and the preliminary risk assessment, industry has conducted detailed pharmacokinetic studies in adult rats and monkeys. In addition, studies to elucidate the ontogeny of the gender difference in elimination are currently underway.

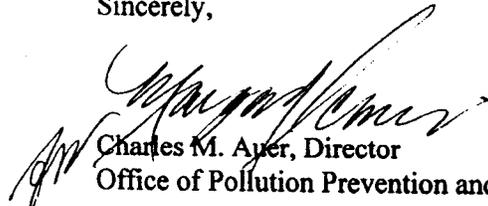
Biomonitoring studies have shown that PFOS, PFOA and several other perfluorinated compounds are present in the serum of the general domestic population. To further understand the distribution of exposure across the country, the U.S. EPA recently nominated several perfluorinated chemicals to CDC for inclusion in the next NHANES survey. A decision by CDC is expected sometime soon. Unfortunately, the sources and pathways of exposure are unknown. This is problematic for any exposure reduction activities in this multi-billion dollar industry. To address this large area of uncertainty, OPPT is currently negotiating Enforceable Consent Agreements (ECAs) with industry groups (FR Notice, April, 2003). This particular ECA process focuses on PFOA and the C10 telomer which may degrade to PFOA, and will, if successful, provide some information on environmental fate, exposure pathways, and some environmental biomonitoring data.

To date, extensive toxicity information is only available for PFOS and PFOA. Yet, the class of perfluorinated compounds is quite large, and includes straight chain, as well as branched chain compounds. Some of these compounds have already been shown to be present in human serum, and the production of some of these compounds may increase if they prove suitable as replacements for PFOS in the marketplace. Therefore, OPPT is nominating a class study of the perfluorosulfonates and carboxylic acids, as well as the telomer derivatives. The latter are potential precursors of perfluorinated acids. This category would include C4 and higher compounds. Due to the unique kinetic properties of the C8 compounds and the substantial gender differences in elimination of PFOA in rats, it is recommended that pharmacokinetic studies on representative members of the different classes of chemicals be conducted first to help inform decisions about which chemicals to focus on and the appropriate animal model for toxicology studies. Initial efforts would focus on a subset that would include even and odd chain length compounds of defined isomeric composition. The inclusion of appropriate mechanistic endpoints in the kinetic studies such as protein binding, PPAR α activation, etc., would optimize utilization of resources. Such mechanistic information would also better inform decisions on specific compounds that would move forward for toxicology studies or for

evaluation of specific organ systems. In addition, these efforts will benefit from concurrent collaborative research between NTP and the U. S. EPA Office of Research and Development (NHEERL/RTP) laboratories on the mechanisms of toxicity of perfluorinated organic chemicals. This collaboration offers the opportunity to further extend the value of NTP's research investment by sharing information (generated from the NTP effort) that will greatly promote the understanding how these compounds may act and overall increase the effectiveness of the perfluorinated chemicals research program.

Thank you for your consideration of this class study. If you have any questions or need additional information, please contact Oscar Hernandez at 202-564-7641 or Jennifer Seed at 202-564-7634.

Sincerely,



Charles M. Auer, Director
Office of Pollution Prevention and Toxics

Attachments: OECD, 2002 - Hazard Assessment of Perfluorooctane Sulfonate (PFOS) and its Salts
OPPT, 2002 - Draft Hazard Assessment of Perfluorooctanoic Acid (PFOA) and its Salts
OPPT, 2003 - Preliminary Risk Assessment of the Developmental Toxicity Associated with Perfluorooctanoic Acid and its Salts
FR notice - Perfluorooctanoic Acid (PFOA), Fluorinated Telomers; Request for Comment, Solicitation of Interested Parties for Enforceable Consent Agreement Development, and Notice of Public Meeting

cc: Oscar Hernandez
Jennifer Seed
William Farland
Margaret Schneider

Attachments to EPA/OPPT Nomination of Perfluorinated Compounds

OECD, 2002. Hazard Assessment of Perfluorooctane Sulfonate (PFOS) and its Salts. Available at <http://www.oecd.org/dataoecd/23/18/2382880.pdf>

OPPT, 2002. Revised Draft Hazard Assessment of Perfluorooctanoic Acid (PFOA) and its Salts. Available at http://cascade.epa.gov/RightSite/getcontent/Tempfile.pdf?DMW_OBJECTID=090007d4801572b2&DMW_FORMAT=pdf

OPPT, 2003. Preliminary Risk Assessment of the Developmental Toxicity Associated with Perfluorooctanoic Acid and its Salts. Available at <http://www.epa.gov/opptintr/pfoa/pfoara.pdf>

EPA, 2003. Federal Register Notice: Perfluorooctanoic Acid (PFOA), Fluorinated Telomers; Request for Comment, Solicitation of Interested Parties for Enforceable Consent Agreement Development, and Notice of Public Meeting. Available at <http://www.epa.gov/opptintr/pfoa/pfoatr.pdf>

Proposal for Perfluorinated Compounds Class Study

Introduction

EPA proposes a class study that would comprise a series of chain lengths ranging from C6 to C12 of fluoroalkyl sulfonates, fluorocarboxylic acids, and fluorotelomeric alcohols. The chain lengths selected are based on existing knowledge of the uses, exposures and toxicology of these compounds. The fluoroalkyl sulfonates (C6 - C12) and their derivatives were predominantly intermediates for the polymeric products used as water, oil, and stain treatments for carpet, paper, and textiles. A few product lines were developed to use the surfactant properties including metal working fluids, fire fighting foams, and photographic additives. The C6 and C8 product lines were the dominant materials manufactured. Some higher homologs as well as other residual organic fluorides have no commercial use but are components of the formulated final products. Ultimately the sulfonates may be the degradation chemicals from the corresponding polymeric products. The fluorocarboxylic acids have chemical properties that make them useful as additives and surfactants, the chief use being as a processing aid for the polymerization of fluoropolymers (polytetrafluoroethylene, polyvinylidene fluoride). The fluorocarboxylic acids or their simple derivatives have also been used as additives in fire fighting foams, electronic circuit and photographic film manufacturing. The C6 and C8 are the predominant commercial products, although others, including the C9, are also commercially used. The 8:2 and 10:2 fluorotelomeric alcohols (perfluoroalkyl ethanols) are the building blocks (and the anticipated abiotic degradation products) for most of the polymeric products used as carpet, paper and textile coatings to impart water, oil, and soil repellency. The alcohols have no substantial market apart from chemical intermediates.

Toxicology studies of the C4 fluoroalkyl sulfonate (PFBS), the C8 fluoroalkyl sulfonate (PFOS) and the C8 fluorocarboxylic acid (PFOA) indicate that chain length is an important factor in toxicity, perhaps partially due to pharmacokinetic factors; toxicity and persistence appears to increase with increasing chain length. Human biomonitoring studies have shown that the C6 perfluorosulfonate, PFOS and PFOA are present in the serum of the general US population, and that the levels of the C6 perfluorosulfonate are quite high in children. Other chain lengths have not yet been monitored in the general population. Environmental monitoring studies have demonstrated the presence of the entire series of perfluorinated compounds, as well as some higher homologs. Current hazard and risk assessments have addressed individual chemicals. If there is a common mode of action for these chemicals the latter efforts are likely to underestimate potential hazard and risk to exposed populations.

Rationale for Specific Studies

A summary of the recommended chemicals and studies is provided in Table 1. The recommended sequence is to begin with the lower chain lengths followed by the higher homologs. The rationale for these studies is provided below.

Table 1. Summary of Proposed Studies^{1,2}

Chemical	Pre-chronic range finding	Pharmacokinetics	Modified one-generation reproductive toxicity study	2-year bioassay with in utero exposure
C6 sulfonate	XXX	XXX	XXX	
C9 sulfonate	XX	XX	XX	
C10 sulfonate	XX	XX	XX	
C12 sulfonate	X	X	X	
C6 carboxylic acid	XXX	XXX	XXX	
C8 carboxylic acid				XXX
C9 carboxylic acid	XX	XX	XX	
C10 carboxylic acid	XX	XX	XX	
C12 carboxylic acid	X	X	X	
Telomer alcohol 8+2	XXX	XXX	XXX	
Telomer alcohol 10+2	X	X	X	

- 1- The number of X's denotes the recommended sequence - XXX denotes the highest priority.
 2- Selection of appropriate animal model to be determined during study design but it is anticipated that studies in both sexes of rats and mice will not be necessary for each "cell" in table.

Extensive pharmacokinetic information is available for PFOS and PFOA and more limited information is available for PFBS. The existing information for PFOS and PFOA suggest the following issues may be critical determinants of pharmacokinetics and blood dosimetry for other members of the class:

- These compounds are well absorbed.
- The carboxylic acids and sulfonates are cleared by urinary and biliary elimination in rodents with no evidence of metabolism, while the telomer alcohols are metabolized apparently to carboxylic acid derivatives. Extensive enterohepatic recirculation has been demonstrated for both PFOA and PFOS.
- Species and sex differences in clearance are most dramatic for PFOA (female rat (hrs)>>male rat (days)>mouse>monkey (weeks)>human (years)). PFOS demonstrates higher blood levels in female rats than males following repeated exposures and the clearance during week 105 was faster than older intravenous and oral studies would predict. PFBS is rapidly eliminated in several hours in rats. The mechanism(s) for species and sex variation in clearance is ill-defined but may in part be related to differential expression of renal transport proteins.
- Serum protein binding (generally albumin) is extensive resulting in high concentrations in serum. Liver has very high concentrations as well, followed by kidney. Other tissues have generally low concentrations (and the fluorination make this a non-lipophilic compound), but account for a significant fraction of the mass in the body.

Blood concentrations of perfluorinated hydrocarbon compounds play a critical role in the interpretation of animal toxicity data with respect to differences among members of this class and cross-species comparisons with measured levels in human blood. As a broader range of

compounds in this class are addressed, EPA considers it essential to develop adequate data to support risk assessment activities based upon blood concentrations. This reflects, in part, the increasing ability to measure these compounds in human blood (e.g. NHANES), as well as the difficulty in reconstructing the exposures leading to these blood levels. In addition, it reflects the observations with PFOA indicating dramatic differences in urinary elimination in male and female rats, while early indications are that mice clear the compound much more slowly; humans are apparently even slower still. Thus, default methods using exposure doses and $BW^{0.75}$ scaling (or $UFa=10$) currently appear particularly uninformative and inappropriate for this class of compounds making the focus on blood dosimetry critical.

Blood dosimetry is a valuable surrogate for target organ dosimetry because the target organs for the perfluorinated compounds are only partially identified, particularly for developmental effects in contrast to adult liver and kidney toxicity and because it is measurable in humans. It is generally measured as plasma levels. Data required to support analyses based upon blood dosimetry (and potentially target organ dosimetry) are of two overall kinds. First, measurements of blood and tissue levels in animals in the various repeated exposure toxicity studies (or satellite groups, particularly for mice) provide direct data on the exposures in the toxicity studies, at a minimum at terminal sacrifice. Such data should be routinely a part of all study designs for this class of chemicals. Second, pharmacokinetic studies provide time course data following controlled exposures. Pharmacokinetic issues related to different species, repeated dosing, and life stages in the relevant toxicity studies will need to be addressed. The combination of these two kinds of data provide the information needed to construct classical or physiologically-based pharmacokinetic models for use in interspecies extrapolation for data interpretation and risk assessment.

There is extensive toxicology information available on PFBS, PFOS and PFOA. Studies of PFOS and PFOA have shown that the developing organism is a primary target. A two-generation reproductive toxicity study of PFOS in rats, and several subsequent studies in rats and mice, have shown a very high incidence of mortality in the F1 offspring in the first few days following birth. A two-generation reproductive toxicity study of PFOA in rats has demonstrated mortality in the F1 offspring in the first few days following weaning, as well as a delay in sexual maturation. Preliminary studies of PFOA in mice have shown a mortality pattern very similar to that observed following exposure to PFOS in that mortality occurs in the first few days after birth. In contrast, these effects were not noted in a two-generation reproductive toxicity study of PFBS in rats or a limited one-generation toxicity study of C6 perfluorosulfonate in rats. To date, there is no information on developmental effects following exposure to chain lengths greater than C8.

Given that the postnatal developmental outcomes are a key feature of PFOS and PFOA, it is crucial that we understand the impact of chain length on developmental endpoints. The two-generation reproductive toxicity studies of PFOS and PFOA have shown that there are no unique effects in the F2 generation. Therefore, we are proposing a modified one-generation toxicity study which would include following the F1 generation up to a minimum of 70 days and assessing the endpoints that are typically assessed in the two-generation reproductive toxicity study, including full histopathology assessments. Assessments of developmental neurotoxicity and immunotoxicity should also be considered for inclusion in the one-generation toxicity

studies. In addition, serum and tissue levels of the administered chemical need to be determined at appropriate timepoints as described above.

In addition, several of the compounds in the proposed series are peroxisome proliferators, and PFOS and PFOA have been shown to be carcinogenic in rats. Chronic studies of PFOA in rats have shown the presence of hepatocellular, Leydig cell and pancreatic acinar cell tumors. PFOA is a demonstrated PPAR- α agonist and this has been hypothesized to be the mode of action for the hepatocellular adenomas. Chronic exposure of PFOS in rats is also associated with hepatocellular adenomas. PFOS is also a peroxisome proliferator, but studies have not been conducted to firmly establish the role of PPAR- α agonism in the induction of the liver adenomas. Chronic exposure studies of PFBS have not yet been conducted. Although preliminary studies indicate that PFBS is a weak peroxisome proliferator at comparatively high doses, only very limited liver toxicity has been noted in a two-generation reproductive toxicity study, and no liver toxicity was noted in 28-day and 90-day studies at comparable doses. Limited studies of the C9 and C10 perfluorocarboxylic acids indicate that both compounds are peroxisome proliferators. Thus, it is likely that liver adenomas may be expected following chronic exposures to many of the compounds in the proposed series. However, several scientific groups have concluded that PPAR- α agonist induced liver tumors in adult rodents are of questionable relevance to humans. Others have questioned whether chronic exposure to peroxisome proliferators would result in a different outcome if exposures were initiated prenatally rather than in adulthood. To resolve this issue, we are proposing a 2-year bioassay that commences with *in utero* exposure of PFOA. We are not proposing chronic studies of the other compounds at this time.

The EPA is also interested in pursuing opportunities to share tissues from animals in selected toxicity studies. This would facilitate follow-up on mechanistic and other endpoints, which are the subject of ongoing studies within EPA laboratories with selected members of this class.

Finally, there are several ongoing efforts to utilize “omics” to help better define the target organ effects and potential common modes of action for this class of compounds. Use of “omics” will improve our predictive ability for this class of compounds. Where appropriate, adjunct comparative “omics” and mechanistic studies should be considered for the compounds in Table 1. These studies should also include the C4 and C8 sulfonates, and others as appropriate.

Table 2. Chemical identification for perfluorinated compounds for EPA class study nomination

CASRN links to EPA Substance Registry System record (<http://www.epa.gov/srs>)

Chemical shorthand name	CASRN	Chemical name (acronym)	Systematic chemical name (9CI)
C4 sulfonate	<u>375-73-5</u>	Perfluorobutanesulfonic acid (PFBS)	1-Butanesulfonic acid, 1,1,2,2,3,3,4,4,4-nonafluoro-
C6 sulfonate	<u>355-46-4</u>	Perfluorohexanesulfonic acid (PFHxS)	1-Hexanesulfonic acid, 1,1,2,2,3,3,4,4,5,5,6,6,6-tridecafluoro-
C8 sulfonate	<u>1763-23-1</u>	Perfluorooctanesulfonic acid (PFOS)	1-Octanesulfonic acid, 1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-heptadecafluoro-
C9 sulfonate	<u>474511-07-4*</u>	Perfluorononanesulfonic acid (PFNS)	1-Nonanesulfonic acid, 1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,9,9,9-nonadecafluoro-
C10 sulfonate	<u>335-77-3</u>	Perfluorodecane sulfonic acid (PFDS)	1-Decanesulfonic acid, 1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,9,10,10,10-heneicosafuoro-
C12 sulfonate	<u>79780-39-5*</u>	Perfluorododecane sulfonic acid (PFDoS)	1-Dodecane sulfonic acid, 1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,9,10,10,11,11,12,12,12-pentacosafuoro-
C6 carboxylic acid	<u>307-24-4</u>	Perfluorohexanoic acid (PFHxA)	Hexanoic acid, undecafluoro-
C8 carboxylic acid	<u>335-67-1</u>	Perfluorooctanoic acid (PFOA)	Octanoic acid, pentadecafluoro-
C9 carboxylic acid	<u>375-95-1</u>	Perfluorononanoic acid (PFNA)	Nonanoic acid, heptadecafluoro-
C10 carboxylic acid	<u>335-76-2</u>	Perfluorodecanoic acid (PFDA)	Decanoic acid, nonadecafluoro-
C12 carboxylic acid	<u>307-55-1</u>	Perfluorododecanoic acid (PFDoA)	Dodecanoic acid, tricosafuoro-
Telomer alcohol 8+2	<u>678-39-7</u>	1,1,2,2-Tetrahydroperfluoro-1-decanol	1-Decanol, 3,3,4,4,5,5,6,6,7,7,8,8,9,10,10,10-heptadecafluoro
Telomer alcohol 10+2	<u>865-86-1</u>	1,1,2,2-Tetrahydroperfluoro-1-dodecanol	1-Dodecanol, 3,3,4,4,5,5,6,6,7,7,8,8,9,10,10,11,11,12,12,12-heneicosafuoro-

*From CAS Registry record; not in EPA SRS, ChemFinder; PFDoS in ChemIDplus (on EINECS)

DEPARTMENT OF HEALTH AND HUMAN SERVICES**Public Health Service****National Toxicology Program; Announcement of and Request for Public Comment on Substances Nominated to the National Toxicology Program (NTP) for Toxicological Studies and Study Recommendations Made by the NTP Interagency Committee for Chemical Evaluation and Coordination (ICCEC)**

SUMMARY: The National Toxicology Program (NTP) continuously solicits and accepts nominations for toxicological studies to be undertaken by the program. Nominations of substances of potential human health concern are received from Federal agencies, the public, and other interested parties. These nominations are subject to several levels of review before selections for testing are made and toxicological studies are designed and implemented. Evaluation by the NTP Interagency Committee for Chemical Evaluation and Coordination (ICCEC) is the initial external review step in the NTP's formal selection process for NTP study nominations. On June 24, 2004, the ICCEC met to review 10 new nominations and make study recommendations. This announcement (1) provides brief background information regarding the substances nominated to the NTP for study, (2) presents the ICCEC's study recommendations from its June 24, 2004 meeting, (3) solicits public comment on the nominations and study recommendations, and (4) requests the submission of additional relevant information for consideration by the NTP in its continued evaluation of these nominations. An electronic copy of this announcement, Internet links to electronic versions of supporting documents for each nomination, and further information on the NTP and the NTP Chemical Nomination and Selection Process can be accessed through the NTP Web site: <http://ntp-server.niehs.nih.gov>.

Review of Study Nominations

Evaluation by the ICCEC is the initial external step in the NTP's formal selection process for NTP study nominations. At its meeting on June 24, 2004, the ICCEC reviewed 10 new nominations for NTP studies. For 7 of these nominations, the ICCEC recommended one or more types of toxicological studies, and for 3 nominations, the ICCEC deferred making specific study recommendations

pending review of additional information. The nominated substances with Chemical Abstract Service (CAS) Registry numbers, nomination source, nomination rationale, and specific study recommendations are given in the accompanying tables.

The ICCEC is composed of representatives from the U.S. Consumer Product Safety Commission, U.S. Department of Defense, U.S. Environmental Protection Agency (U.S. EPA), U.S. Food and Drug Administration's National Center for Toxicological Research, National Center for Environmental Health/Agency for Toxic Substances and Disease Registry, National Institutes of Health's (NIH) National Cancer Institute, NIH's National Institute of Environmental Health Sciences (NIEHS), National Institute for Occupational Safety and Health, NIH's National Library of Medicine, and the Occupational Safety and Health Administration. The ICCEC meets once or twice annually to evaluate groups of new study nominations and to make recommendations with respect to both specific types of studies and testing priorities.

Request for Public Comment

Interested parties are invited to submit written comments or supplementary information on the nominated substances and study recommendations that appear in the accompanying tables. The NTP welcomes toxicology and carcinogenesis study information from completed, ongoing, or anticipated studies, as well as information on current U.S. production levels, use or consumption patterns, human exposure, environmental occurrence, or public health concerns for any of the nominated substances. The NTP is also interested in identifying appropriate new animal and non-animal models for mechanistic-based research, and as such, solicits comments regarding the use of specific *in vivo* and *in vitro* experimental models to address scientific questions relevant to the nominated substances or issues under consideration. All information received will be considered by the NTP in its continued review of these nominations. Comments or information should be sent to Dr. Scott Masten (contact information below) by October 19, 2004. Persons responding to this request should include their name, affiliation, mailing address, phone, fax, e-mail address and sponsoring organization (if any) with the submission. Written submissions will be made available

electronically on the NTP Web site as they are received.

Send comments or information to Dr. Scott A. Masten, Office of Chemical Nomination and Selection, NIEHS/NTP, P.O. Box 12233, MD A3-07, Research Triangle Park, North Carolina 27709; telephone: (919) 541-5710; FAX: (919) 541-3647; e-mail: masten@niehs.nih.gov.

Background

The NTP actively seeks to identify and select for study chemicals and other agents for which sufficient information is not available to adequately evaluate potential human health hazards. The NTP accomplishes this goal through a formal open nomination and selection process. Substances considered appropriate for study generally fall into two broad yet overlapping categories: (1) Substances judged to have high concern as a possible public health hazard based on the extent of human exposure and/or suspicion of toxicity and (2) substances for which toxicological data gaps exist and additional studies would aid in assessing potential human health risks, e.g. by facilitating cross-species extrapolation or evaluating dose-response relationships. Input is also solicited regarding the nomination of studies that permit the testing of hypotheses to enhance the predictive ability of future NTP studies, address mechanisms of toxicity, or fill significant gaps in the knowledge of the toxicity of classes of chemical, biological, or physical substances. Substances may be studied to evaluate a variety of health-related effects, including but not limited to reproductive and developmental toxicity, genotoxicity, immunotoxicity, neurotoxicity, metabolism and pharmacokinetics, and carcinogenicity. In reviewing and selecting nominated substances, the NTP also considers legislative mandates that require responsible private sector commercial organizations to evaluate their products for health and environmental effects. The possible human health consequences of anticipated or known human exposure, however, remain the over-riding factor in the NTP's decision to study a particular substance.

The review and selection of substances nominated for study is a multi-step process. A broad range of concerns are addressed during this process through the participation of representatives from the NIEHS, Federal agencies represented on the ICCEC, the NTP Board of Scientific Counselors—an external scientific advisory body, the NTP Executive Committee—the NTP Federal interagency policy body, and

the public. This process is described in further detail in a March 2, 2000 Federal Register announcement (Volume 65, Number 42, pages 11329–11331). This multi-step evaluative process provides the NTP with direction and guidance to ensure that its testing program addresses toxicological concerns relative to all areas of public health, and furthermore, that there is balance among the types of substances selected for study (e.g., industrial

chemicals, consumer products, therapeutic agents). As such, it should be recognized that at any given time, the new study nominations under consideration do not necessarily reflect the overall balance of substances historically or currently being evaluated by the NTP in its toxicology testing program. For further information on NTP toxicology studies (previous or in progress) visit the NTP Web site at <http://ntp-server.niehs.nih.gov>.

Dated: August 10, 2004.

Samuel Wilson,

Deputy Director, National Institute of Environmental Health Sciences.

Substances Nominated to the NTP for Toxicological Studies and Recommendations Made by the NTP Interagency Committee for Chemical Evaluation and Coordination on June 24, 2004

TABLE 1.—SUBSTANCES RECOMMENDED FOR STUDY *

Substance [CAS number]	Nominated by	Nomination rationale	Recommendations for toxicological studies
Bitter orange extract [No CAS No.].	Private Individual	Consumer exposure through increasing dietary supplement use; suspicion of toxicity; lack of adequate toxicity data.	Toxicological studies: —Developmental toxicity —Physiological responses (e.g., cardiovascular and cerebrovascular) —Subchronic toxicity —Toxicokinetics (of constituents) —Studies alone and in combination with caffeine —Studies in rats and possibly miniature pigs.
n-Butyl glycidyl ether [2426–08–6].	National Institute of Environmental Health Sciences.	Suspicion of toxicity based on structural features; positive results in genetic toxicity studies; substantial potential for human exposure and a lack of chronic toxicity data.	Toxicological studies: —Toxicological characterization including reproductive toxicity, carcinogenicity, and analysis of urinary metabolites —Coordinate with voluntary data development activities of the U.S. EPA.
Di-(2-ethylhexyl)phthalate (DEHP) [118–71–7].	U.S. Food and Drug Administration.	Long-term risks associated with medical exposures of infants have not been clearly elucidated; significant knowledge gaps on the toxicokinetics and effects in fetal and neonatal primates of intravenous exposure; further studies will better define risks and benefits of utilizing non-DEHP-containing products.	Toxicological studies: Tiered research programs to address: —Quantitative studies of toxicokinetics and biotransformation following intravenous exposure in neonatal male non-human primates —Assessment of toxicokinetics, reproductive and immune endpoints following acute and subchronic intravenous exposure to neonatal male rats and nonhuman primates.
Ionic liquids 1-Butyl-3-methylimidazolium chloride [79917–90–1] 1-Butyl-1-methylpyrrolidinium chloride [479500–35–1] N-Butylpyridinium chloride [1124–64–7].	University of Alabama Center for Green Manufacturing.	Widespread interest as replacements for volatile organic compounds (VOCs) in various applications; lack of toxicity data.	Toxicological studies: —Toxicological characterization —Coordinate research program with the U.S. EPA.
Perfluorinated compounds class study [Multiple CAS Nos.].	U.S. Environmental Protection Agency.	Presumed widespread human exposure; known toxicity of certain class members; insufficient information to assess hazard/risk across entire structural class.	Toxicological studies: —Tiered research program to include pharmacokinetics, mechanistic, reproductive toxicity, and carcinogenicity studies (for specific compounds, see supporting document available at http://ntp-server.niehs.nih.gov/NomPage/noms.html)
<i>Stachybotrys chartarum</i> [67892–26–2].	Private Individual National Institute of Environmental Health Sciences.	Public concern regarding potential non-infectious adverse health effects of fungal exposures in indoor environments; inadequate toxicological data available evaluating potential systemic toxicity from long-term exposure to this organism under relevant exposure scenarios.	Toxicological studies: —Toxicological characterization including immunotoxicity.
Tungsten trioxide [1314–35–8] and fibrous tungsten sub-oxides.	National Cancer Institute.	Important industrial raw materials; one of several metals that may form toxic fibrous "whiskers"; carcinogenic potential of tungsten (vs. cemented tungsten carbide) is not adequately characterized.	Toxicological studies: —Toxicological characterization —Genotoxicity —Characterize fiber stability and biopersistence — <i>In vitro</i> toxicity to lung cells —Comparative intratracheal toxicity studies with a known hazardous fiber

TABLE 1.—SUBSTANCES RECOMMENDED FOR STUDY*—Continued

Substance [CAS number]	Nominated by	Nomination rationale	Recommendations for toxicological studies
			—Further studies including carcinogenicity will be considered following completion of above.

* Note: A recommendation for "toxicological characterization" in this table includes studies for genotoxicity, subchronic toxicity, and chronic toxicity/ carcinogenicity, as determined to be

appropriate during the conceptualization and design of a research program to address toxicological data needs. Though other types of studies (e.g., metabolism, pharmacokinetics, immunotoxicity,

reproductive/developmental toxicity) may be conducted as part of a complete toxicological characterization, these types of studies are not listed unless they were specifically recommended.

TABLE 2.—SUBSTANCE FOR WHICH SPECIFIC STUDY RECOMMENDATIONS WERE DEFERRED

Substance [CAS number]	Nominated by	Nominated for	Nomination rationale	Rationale for deferral/further information needed
Butylparaben [94-26-8].	National Institute of Environmental Health Sciences.	—Toxicological characterization including reproductive toxicity studies.	Widespread use in foods, cosmetics, and pharmaceuticals; potential reproductive toxicant; lack of adequate toxicity data.	Further review of data on estrogen receptor binding, pharmacokinetics, dose-response of male reproductive effects, and human exposure.
Decane [124-18-5].	National Cancer Institute.	—Carcinogenicity studies.	Widespread industrial use and environmental occurrence as air pollutant; suspicion of carcinogenicity but no adequate carcinogenicity study available.	Review of industry voluntary data development activities coordinated by the U.S. EPA.
Undecane [1120-21-4].	National Cancer Institute.	—Carcinogenicity studies.	Widespread industrial use and environmental occurrence as air pollutant; suspicion of carcinogenicity but no adequate carcinogenicity study available.	Review of industry voluntary data development activities coordinated by the U.S. EPA.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES

Administration for Children and Families

[Program Announcement No. HHS-2004-ACF-ORR-RE-0004 CFDA 93.576]

ORR Announcement for Services to Recently Arrived Refugees

AGENCY: Office of Refugee Resettlement (ORR), Administration for Children and Families, HHS.

ACTION: Modification to the Standing Announcement published in the *Federal Register* on April 23, 2004 (69 FR 22276). Notice of additional deadline for Priority Area 2—Unanticipated Arrivals, in the Standing Announcement for Services to Recently Arrived Refugees.

SUMMARY: The Office of Refugee Resettlement Standing Announcement for Services to Recently Arrived Refugees, Volume 69, *Federal Register* page number 22276, April 23, 2004, is hereby modified to reflect an additional deadline for the Priority Area 2—

Unanticipated Arrivals for FY 2005. This additional deadline encourages applicants to respond to the needs of newly arriving populations.

DATES: October 8, 2004, is the closing date. Please note that all applications must be postmarked by October 8, 2004. Mailed applications postmarked after the closing date will be classified as late. Due to delays in mail delivery to Federal offices, we encourage applicants to use overnight courier service to ensure prompt delivery and receipt.

Announcement Availability: The program announcement and the application materials are available from Sue Benjamin, Office of Refugee Resettlement (ORR), 370 L'Enfant Promenade, SW., 8th Floor West, Washington, DC 20447 and from the ORR Web site at: <http://www.acf.hhs.gov/programs/orr/funding> or <http://www.acf.hhs.gov/grants/open/HHS-2004-ACF-ORR-RE-0004.html>.

Funding Availability: ORR expects to award \$1 million in discretionary social service funds.

FOR FURTHER INFORMATION CONTACT: Sue Benjamin, Office of Refugee Resettlement, telephone number 202-401-4851.

Dated: August 11, 2004.

Nguyen Van Hanh,

Director, Office of Refugee Resettlement.

[FR Doc. 04-19174 Filed 8-19-04; 8:45 am]

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DEPARTMENT OF HEALTH AND HUMAN SERVICES

Administration for Children and Families

Notice of Correction for the Modified Standing Announcement for Services to Recently Arrived Refugees

AGENCY: Administration for Children and Families, ACF, DHHS.

Funding Opportunity Title: Modified Standing Announcement for Services to Recently Arrived Refugees.

ACTION: Notice of Correction.

Funding Opportunity Number: HHS-2004-ACF-ORR-RE-0004.

SUMMARY: This notice is to inform interested parties of a clarification made to the Modified Standing Announcement for Services to Recently Arrived Refugees published on Friday, April 23, 2004. The following clarification should be noted:

Clarification of Eligibility for Priority Area 1—Preferred Communities.