

# **For 50 Years, Polluters Knew PFAS Chemicals Were Dangerous But Hid Risks From Public**

As far back as 1950, studies conducted by 3M showed that the family of toxic fluorinated chemicals now known as PFAS could build up in our blood. By the 1960s, animal studies conducted by 3M and DuPont revealed that PFAS chemicals could pose health risks. But the companies kept the studies secret from their employees and the public for decades.

**Here is a timeline of internal memos, studies and other company documents detailing the two companies' history of deception.**



Know your environment.  
Protect your health.

1950 – 3M mice study reveals that PFAS builds up in blood.

1956 – Stanford University study finds that PFAS binds to proteins in human blood.

1961 – DuPont toxicologist warns that PFAS chemicals enlarge rat and rabbit livers.

1962 – Volunteers who smoke PFAS-laced cigarettes get “polymer fume fever.”

1963 – 3M technical manual deems PFAS toxic.

1965 – DuPont rat study shows liver damage and increased spleen size.

1966 – The Food and Drug Administration rejects a DuPont petition to use PFAS chemicals as a food additive, citing liver studies.

1966 – 3M study finds that PFAS causes “acute oral toxicity” in rats.

1970 – 3M warns Fire Journal, the magazine of the National Fire Protection Association, that PFAS is toxic to fish.

1970 – DuPont scientists say PFAS is “highly toxic when inhaled.”

1973 – DuPont finds there is no safe level of exposure to PFAS in food packaging.

1975 – 3M is informed that PFAS builds up in human blood samples.

1975 – DuPont warns 3M about “toxic effects” of PFAS in food packaging.

1977 – 3M tests workers and animals to measure PFAS in blood.

1977 – 3M finds PFOS, the PFAS chemical in the company’s Scotchgard fabric treatment, “more toxic than anticipated.”

1978 – 3M animal tests find lesions on spleen, lymph nodes and bone marrow.

1978 – 3M concludes that PFOS and PFOA, a PFAS chemical used to make DuPont’s Teflon, “should be regarded as toxic.”

1979 – DuPont survey of employees in its Parkersburg, W.Va., Teflon plant finds possible evidence of liver damage.

1981 – 3M and DuPont reassign female workers after animal studies reveal PFAS damages the eyes of the developing fetus.

1983 – 3M identifies PFAS’ potential harm to the immune system as a cause for concern.

1984 – 3M documents rising fluorine levels in workers’ blood.

1984 – DuPont detects PFAS in the tap water in Little Hocking, Ohio, but does not alert the local water utility.

1987 – 3M PFOA animal study finds tumors.

1989 – 3M study finds elevated cancer rates among PFAS workers.

1990 - 3M study finds risk of testicular cancer from exposure to PFOA, also known as C8.

1992 - DuPont study finds elevated cancer rates among workers.

1992 - Former 3M scientist finds male PFOA workers more likely to die from prostate cancer.

1995 - DuPont scientist expresses concern over long-term PFAS health effects.

1997 - DuPont study finds heightened cancer rates among workers at the Parkersburg plant.

1998 - 3M scientists report that PFAS moves through the food chain.

1998 - 3M provides EPA evidence that PFAS accumulates in blood.

1998 - 3M animal study finds liver damage from PFAS exposure.

1999 - 3M scientist describes PFOS as "the most insidious pollutant since PCB."

2000 - 3M animal study finds liver damage from PFOS exposure.

EMPIRICAL FORMULA:  $C_4F_7HO_2$   
STRUCTURAL FORMULA:  $C_3F_7COOH$   
TEST: toxicity  
TEST ORGANISM: mice  
DOSE OR CONCENTRATION:

NAME: Perfluorobutyric  
Acid

DESCRIPTION OF TEST oral, intraperitoneal, and intravenous  
LD<sub>50</sub>: mg./Kg.

	<u>Oral</u>	<u>ip</u>	<u>iv</u>	
RESULT OF TEST	$\frac{72 \text{ hrs.}}{1001}$	$\frac{1 \text{ wk}}{804}$	$\frac{435}{(24 \text{ hrs.})}$	$\frac{755}{(24 \text{ hours})}$

REFERENCE: Masterton Lab Report 1/10/50 DATE  
SCREENING AGENCY: SUBMITTED:

ROCKET ANALYSIS CARD CHARLES F. GUNDEL CO. PATENTERS - PRINTED IN U.S.A.

Additional data in card file  
DOR

Exhibit  
1009

State of Minnesota v. 3M Co.,  
Court File No. 27-CV-10-28862



# PERFLUOROOCCTANOIC ACID INTERACTIONS WITH HUMAN SERUM ALBUMIN

By GORDON L. NORDBY\* AND J. MURRAY LUCK

(From the Department of Chemistry, Stanford University, Stanford, California)

(Received for publication, August 1, 1955)

For several years perfluorooctanoic acid (1) ( $\text{CF}_3 \cdot (\text{CF}_2)_6 \cdot \text{COOH}$ ) has been used in this laboratory as a protein precipitant (2). Qualitatively it has been observed to bind so completely to proteins that little of the precipitant remains in the filtrate; this is an obvious advantage in chromatography and in various analytical procedures.

The purpose of the present study is to define the conditions under which perfluorooctanoic acid will effect a precipitation of human serum albumin and to describe in detail the interaction between these two reactants. Human serum albumin was used because of its unusual properties in binding a variety of ions.

## Materials

A solution of human serum albumin (decanol procedure) was electro-dialyzed at 40 volts per cm. against conductivity water and clarified by pressure filtration through a sterilizing filter pad. The product was lyophilized and stored at 3°; appropriate amounts of the powder were removed as needed for the preparation of albumin solutions.

Sodium perfluorooctanoate (PF8) solutions were prepared by carefully neutralizing a 0.5 per cent aqueous solution of the acid with a minimal volume of sodium hydroxide.

## Procedure

*Precipitation*—A number of buffered 0.3 per cent solutions of serum albumin in PF8 were prepared. Each solution was distinctive with respect either to its PF8 concentration or to its pH, the latter being determined by 0.1 M McIlvaine buffers (3). The pH of each solution was between 4.25 and 5.25. The PF8 concentration of each solution was such that the mole ratio (PF8 to albumin) ranged between 0 and 200.<sup>1</sup> The solutions were thoroughly mixed, allowed to stand for 15 minutes, and centrifuged in a clinical centrifuge at room temperature for 20 minutes. For conditions under which the protein in a given solution was only partially precipitated, opaque supernatant solutions were sometimes formed; in all other cases the

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<sup>1</sup> A value of 61,500 was used for the molecular weight of human serum albumin (4).

supernatant solutions were clear. An aliquot of each supernatant liquid, or solution if no precipitate existed, was then analyzed for protein by the Lowry *et al.* modification of the Folin test (5). PF8 does not interfere with this analysis. The percentage of albumin that was precipitated from each solution under the stated conditions of pH and PF8 concentration was calculated. The results appear in Fig. 1.

*Anion Binding*—A number of 0.3 per cent albumin solutions were prepared in radioactive PF8. The ratio (PF8 to albumin) in each solution was between 80 and 200. The solutions were then titrated at 25° to about pH 3 with hydrochloric acid. In the course of the titration between pH

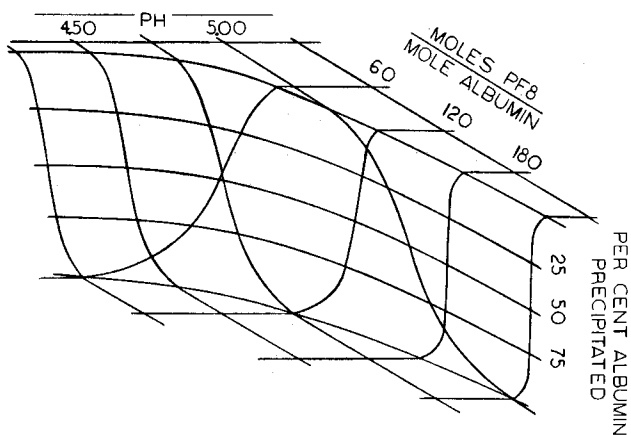


FIG. 1. The percentage of human serum albumin precipitated from aqueous solution is a function of the pH of the solution and the molar ratio (PF8 to albumin) in the system. For example, at pH 4.75 and a molar ratio (PF8 to albumin) of 60, about 48 per cent of the albumin is precipitated.

4 and 3, the protein precipitated completely; several 0.005 ml. aliquots were removed from each supernatant liquid, placed on aluminum disks, and immediately dried under an infra-red lamp. The disks were then placed on an automatic sample changer which operated into a gas flow counter (6). The time required for an arbitrary number of counts was recorded for the sample on each disk. After making suitable corrections for efficiency and background interference in the counting, the relative activities of the samples were determined and compared with the original activities of their respective solutions before the titration was started. The molar ratio (bound PF8 to albumin) in each solution was then calculated as a function of the molar ratio (PF8 to albumin). The data are given in Fig. 2.

*Hydrogen Ion Binding*—A number of 0.3 per cent albumin solutions were

prepared. Half of the solutions contained that concentration of PF8 for which the molar ratio (PF8 to albumin) was 196; the remaining solutions contained an equivalent concentration of sodium chloride. Each solution

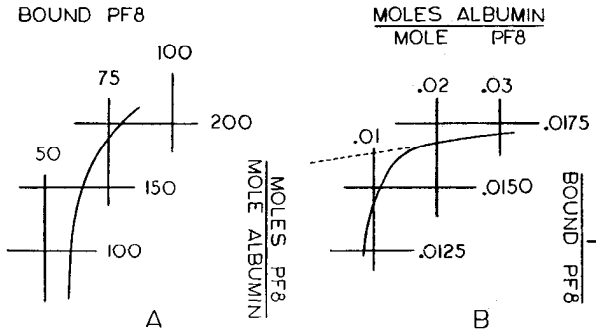


FIG. 2. Curve A, the molar ratio (PF8 to albumin) in a system determines the number of PF8 anions that bind to each albumin molecule. Curve B, the reciprocal of the number of PF8 anions bound to each albumin molecule is plotted against the molar ratio (albumin to free PF8).

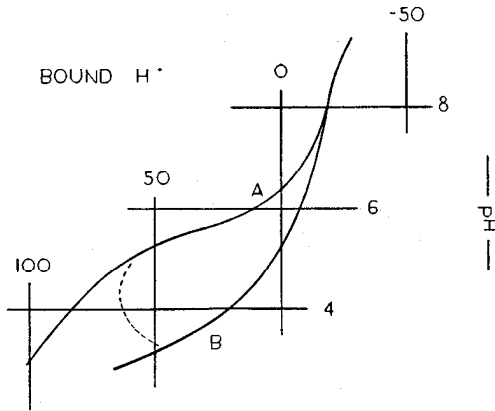


FIG. 3. The number of hydrogen ions bound to an albumin molecule is a function of the pH of the solution. Curve A represents 1 mole of albumin in the presence of sodium PF8; Curve B represents 1 mole of albumin in the presence of sodium chloride.

was then titrated at 25° with either hydrochloric acid or sodium hydroxide. The amount of hydrogen ion bound by the albumin, or dissociated from it, was calculated quite directly from the total acid or base added to a given solution and the amount of acid or base actually present in the solution as reflected by its pH. Subsequently, the charge on the albumin molecule was calculated as a function of pH. The data are presented in Fig. 3.

## DISCUSSION

*Precipitation*—Although Fig. 1 indicates a certain critical range of pH and PF8 concentration which must prevail for the precipitation of human serum albumin, it also indicates in a qualitative manner the conditions under which most other proteins would be expected to precipitate. Three rather striking features are noted for attention. (1) The conditions of pH and ionic strength under which complete albumin precipitation occurs are very mild. (2) The precipitation of albumin is completely reversible with respect to pH. An albumin precipitate formed in a PF8 solution can be completely dissolved by making the solution somewhat alkaline (pH 6 to 7) to the pH at which precipitation occurred. The minimal pH to which the solution must be raised will depend, of course, upon the PF8 concentration of the solution. (3) In acidic solutions, the precipitation of albumin is irreversible with respect to the PF8 concentration. Provided that an albumin precipitate is dialyzed against an appropriately acidic solution, it will not dissolve appreciably as the concentration of PF8 in equilibrium with the precipitate decreases. However, the PF8 precipitant can readily be dialyzed from albumin in neutral or slightly alkaline solutions.

*Anion Binding*—The scope of Curve A in Fig. 2 is restricted at the upper limit by the rather low solubility of PF8 in aqueous solution and at the lower limit by the concentration of PF8 which would adequately precipitate albumin under the conditions of the experiments. However, it is clear from the graph that a considerable number of PF8 anions bind to each albumin molecule. The molar ratio (bound PF8 to albumin) increases quite rapidly as the PF8 concentration is increased. In contrast, the molar ratio (bound PF8 to albumin) decreases very slowly as the molar ratio (PF8 to albumin) is decreased below about 120. In solutions containing the minimal concentration of PF8 which is effective in completely precipitating a given concentration of albumin, the PF8 is almost completely removed from the solution as part of the precipitating complex.

The data of Curve A are treated by an expression derived by Klotz (7); the results appear as Curve B of Fig. 2.

$$\frac{1}{r} = \frac{1}{Kn} \times \frac{1}{c} + \frac{1}{n}$$

In the above equation,  $r$  is the molar ratio (bound PF8 to albumin),  $n$  is the molar ratio (maximal bound PF8 to albumin),  $c$  is the molar ratio (free PF8 to albumin) in the solution, and  $K$  is a constant proportional to the equilibrium constant for the PF8 anion-binding reaction. The parameters in the above equation are  $1/r$  and  $1/c$ . Although there is no *a priori* evidence that the equation does apply to PF8 anion binding, it can be as-

sumed, by virtue of the linearity of a portion of Curve B, that the equation does apply to the linear portion. The indicated extrapolation to infinite PF8 concentration reveals that a maximum of 63 PF8 anions bind to an albumin molecule by reactions having nearly the same equilibrium constant. Additional anions are bound to albumin at high PF8 concentrations by some less easily described series of reactions. At very high PF8 concentrations, Curve B of Fig. 2 becomes nearly vertical; therefore the maximal number of PF8 anions that can possibly bind to an albumin molecule cannot be calculated by the evidence available. In contrast, a study of octanoic acid anion binding by Teresi and Luck (8) reveals that a total of 36 octanoate ions binds to two types of sites on the albumin molecule. Preliminary isotope dilution studies indicate that the PF8 anions are reversibly bound to the albumin molecule.

*Hydrogen Ion Binding*—Two hydrogen ion binding curves for human serum albumin are depicted in Fig. 3. These binding curves are completely applicable to reversible titrations over the pH range illustrated. It is readily apparent from these curves that PF8 strongly influences the albumin molecule charge in acid solution. A similar effect has been observed by Steinhardt (9) for the titration of wool protein in the presence of 2,4,6-trinitroresorcinol, picric acid, or flavianic acid.

By means of the equation described above, the extrapolation of Curves A and B to infinite hydrogen ion concentration indicates that the maximal number of bound hydrogen ions per albumin molecule is 107 in each case. This number agrees with that found by Tanford (10). Thus, the charge on the albumin molecule in extremely acid solutions in the presence of that concentration of PF8 represented by a molar ratio (PF8 to albumin) of 196 does not exceed 28; 79 PF8 anions are bound under these conditions. The dotted line in Fig. 3 represents the difference between Curves A and B as a function of pH. The maximal difference between the two curves is near pH 4.25 at which an extra 64 hydrogen ions are bound by the albumin in the presence of PF8. The significance of the coincidence between the 64 extra hydrogen ions and the 63 strongly bound PF8 anions which are bound to the albumin is not known at this time; but the coincidence is so striking that it is brought to attention. Finally, it is apparent that Curves A and B coincide above pH 8. This is additional, although inconclusive, evidence that the PF8 completely dissociates from the albumin molecule in alkaline solutions.

#### SUMMARY

It has been shown that human serum albumin can be reversibly precipitated from aqueous solution under mildly acid conditions in the presence of low concentrations of perfluorooctanoic acid. In the formation of the

precipitate, the albumin molecule binds both hydrogen ions and perfluorooctanoate ions to form, under the conditions of the experiments described, a complex that has a net charge of almost zero.

Acknowledgment is made to the Cutter Laboratories for a sample of Fraction V (11), decanol human serum albumin, and to the Minnesota Mining and Manufacturing Company for a sample of perfluorooctanoic acid. Carboxyl-labeled ( $C^{14}$ ) perfluorooctanoic acid was obtained through the Atomic Energy Commission from the Minnesota Mining and Manufacturing Company.

*Addendum*—Klevens and Ellenbogen have recently published their research on the van der Waals association of bovine serum albumin in the presence of perfluoro acids (12).

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Gordon L. Nordby and J. Murray Luck

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November 9, 1961

GERALD J. ARENSON  
POLYCHEMICALS DEPARTMENT  
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TOXICITY OF TEFLON DISPERSING AGENTS

A brief summary of our toxicity work on AHT and other Teflon dispersing agents with emphasis on liver enlargement which seems to be the most sensitive sign of toxicity is given below. The detailed reports of work completed to date will be available within a few days.

AHT - (Ammonium 3,6 dioxo 2,5 di(trifluoro methyl undecafluorononanoate)

The oral ALD for rats was found to be 60 mg/kg. Survivors showed definite liver enlargement in doses down to 1.5 mg/kg and with possible changes at 0.45 and 0.13 mg/kg. Single doses of 12 mg/kg produced liver enlargement which tended to increase during the two months following the dose. One one-hundredth of the lethal dose or 0.6 mg/kg given daily 5 times a week for 2 weeks produced enlargement which was significant in those rats killed on the day of final treatment and in those killed 14 days later. Histological examination of the livers indicated that the enlargement was due to increase in cell size rather than an increase in the number of cells.

The lethal dose by skin absorption in rabbits was 130 mg/kg. Although the changes in liver weight in these rabbits are more difficult to evaluate, there was a tendency toward enlargement and similar signs of liver injury.

A 25% aqueous solution in contact with the eye caused damage which persisted through 8 days. Washing with water 20 seconds after instillation prevented permanent damage. Ten and twenty-five percent solutions were also irritating to guinea pig skin but did not cause skin sensitization.

C<sub>8</sub>-AFTC - (Ammonium perfluorocaprylate)

JAZ

The oral ALD for rats was 670 mg/kg. Liver enlargement was definite down to a dose of 200 mg/kg with possible early signs down to 1.5 mg/kg.



- 2 -

C<sub>9</sub>-AFC - (Ammonium *n*-hydrohexa decafluorooctanoate)

The oral LD<sub>50</sub> was 1500 mg/kg. Survivors showed enlargement which appears evident in doses as low as 12 mg/kg.

"Teflon" Feeding Tests with "Teflon" 7, "Teflon" 6 made with C<sub>9</sub>-APFC, "Teflon" 6C made with C<sub>9</sub> and "Teflon" 6C made with AHT.

The compounds were fed at a level of 25% in the diet of rats for 3 weeks. Rats were sacrificed 2, 3 and 5 weeks after feeding of test materials started.

Livers of rats sacrificed after two and three weeks of continuous feeding showed slight enlargement only in the group fed "Teflon" 6C with AHT. After a two-week rest period the remaining rats were killed and those fed "Teflon" 6C with AHT and "Teflon" C<sub>9</sub> APFC showed liver weights significantly different from the controls and those fed "Teflon" 7. The values of those fed "Teflon" 6C with C<sub>9</sub>-AFC fall midway between the controls and the others. Although the numbers of animals used were small and the time of feeding relatively short, the trend observed confirms the earlier liver enlargement observed in rats fed 25% "Teflon" 6 resin in the diet for 90 days (H. Report No. 49-60). A direct comparison among these compounds is difficult to make in these feeding tests because we do not know the concentrations of the fluoro acid dispersing agents present.

Conclusions:

AHT is a very toxic compound. Not only does it have a low lethal dose but a single dose of 1/5 the lethal dose produced liver enlargement which increased with time. And 1/100 of the lethal dose fed 10 times produced definite liver enlargement. In addition, it was easily absorbed through the skin and produced liver damage in a second species. When "Teflon" containing less than 5 ppm AHT was fed to rats, it still produced enlargement which was apparent after 2 weeks.

The C<sub>9</sub> and C<sub>8</sub> acids have much lower acute toxicity, but they too have the ability to increase the size of the liver of rats at low doses. These short experiments may indicate differences in rate of development rather than qualitative differences but completion of microscopic examination of animals in the current series as well as dosing of greater numbers of rats at the critical levels and holding them for longer periods would be needed to establish the lowest effect level for each compound.

It is recommended that all of these materials, especially AHT, be handled with extreme care. Contact with the skin should be strictly avoided. Tests on a third species, e.g. dogs, should be carried out where changes in liver function could be studied over a long period of time. The results of such tests might also throw some light on any possible species differences in JAZ susceptibility.

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# An Epidemic of Polymer-Fume Fever

Charles E. Lewis, MD, and Gerald R. Kerby, MD

An "epidemic" of polymer-fume fever involved 36 of 61 employees in one industry over a 90-day period. All of those involved demonstrated the classic history of an influenza-like syndrome, with fever and chills occurring several hours after exposure to the products of pyrolysis of polytetrafluoroethylene (Teflon). The majority of cases resulted from the smoking of cigarettes which were contaminated with a fine dust of this material. A study of pulmonary function of all workers involved demonstrated changes that could be accounted for only on the basis of smoking habits. Three persons experienced changes in pulmonary function consistent with mild obstruction of the airways, in association with the onset of symptoms. While no serious consequences were observed, the effects of these illnesses upon the health and productivity of the group could have been prevented.

Within ten years after the first description of the properties of polymers of tetrafluoroethylene (Teflon, Fluon) the first account of their effects on man appeared in the medical literature. In 1951, Harris described four cases of "polymer-fume fever."<sup>1</sup> The signs, symptoms, and natural history of this malady were similar to those of "metal-fume fever," which was described by Thackerah in 1831. In the interval since 1951, there have been several reports of illness resulting from exposure to the products of pyrolysis when these polymers are heated to temperatures in excess of 300 C.<sup>2,3</sup> Also, false reports of fatal illnesses resulting from such exposures have appeared in the medical literature.<sup>4</sup>

During a 90-day period in the summer of 1964, an "epidemic" of polymer-fume fever occurred in a large industrial plant. Thirty-six out of 61 workers in a single department were affected. This study reports an epidemiologic investigation of this outbreak, as well as the results of pulmonary-function studies performed on the men in this environment.

## Process and Events Preceding Investigation

The department involved manufactured small, light-weight sub-assemblies which required metal bonding, using epoxy resins. These parts were assembled on a "tool" or assembly block. The unit then was autoclaved at increased pressure and

temperature to cure the resin. A parting compound was applied to the tool or assembly unit to permit separation of the sub-assembly from the tool after autoclaving. This process was originally carried out in a large, open space and had not been attended by any medical difficulties.

For editorial comment see page 406.

In April 1964 the sub-assembly operation was moved to a balcony location in the same general plant area. On May 13 it became necessary to enclose and air-condition the area to improve the environmental conditions for a more efficient assembly of the unit. During the same week, a different parting compound was substituted for the original silicone-base material.

Approximately 40 of the men in this department work on a day or an evening shift in the subassembly enclosed area (A). The other personnel in the department (25 men) are employed on the balcony, which is an open area (B) in the same general plant. These men handle the "tools" or assembly blocks and prepare them for reuse after they come from the autoclave.

Symptoms of polymer-fume fever first appeared among the group in area A as early as May 14 (the day after the air conditioning was installed). Symptoms also appeared in the group working in the same department, but outside the enclosed air conditioning (area B) during May. The symptoms noted by the workers in area B were milder than in those in area A.

## Methods

A history form was used to collect data from all employees working in areas A and B. All members of group A and those with complaints of polymer-

Table 1.—Frequency of Various Complaints Among 36 Workers With Symptoms of Polymer-Fume Fever

Complaint	No.	% of Total of 61 Workers
Tightness of chest	31	51
Malaise	30	49
Shortness of breath	26	43
Headache	24	39
Cough	22	36
Chills	22	36
Temperature, 100-104 F (37.8-40 C)	20	33
Sore throat	6	10
Sputum	1	1.6

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Table 2.—Relationship of Smoking to Symptoms of Polymer-Fume Fever

	Group A		Total
	Symptoms	No Symptoms	
Smoke	14	6	20
Do not smoke	7	13	20
<b>Totals</b>	<b>21</b>	<b>19</b>	<b>40</b>
	Group B		
Smoke	13	1	14
Do not smoke	2	5	7
<b>Totals</b>	<b>15</b>	<b>6</b>	<b>21</b>
	Both Groups		
Smoke	27	7	34
Do not smoke	9	18	27
<b>Totals</b>	<b>36</b>	<b>25</b>	<b>61</b>

fume fever symptoms in group B were subjected to ventilatory-function studies. Forced expiratory capacity (FEC), forced expiratory volume in one second (FEV<sub>1</sub>), and maximum midexpiratory flow rate between 25% and 75% of the forced expiratory capacity (MMF) were measured on a 6-liter recording vitalometer, using the best of two or more efforts. The peak-flow rate (PFR) was measured with a peak-flow meter as the best of three efforts. Histories and spirometric measurements were done at the beginning of the day shift (between 7 and 9 AM), and were repeated at the end of the shift (between 2 and 3:30 PM). The same procedure was followed for the second, or evening, shift. Special attention was paid to eliciting any past history of hay fever, asthma, or other respiratory disease, and a quantitative estimate of smoking was made.

### Results

In area A symptoms developed in 12 workers during May; two became symptomatic in June, five in July, and one in August. In area B, the onset of symptoms was as follows: six in May, two in June, six in July, and one in August.

Table 1 demonstrates the frequency with which various symptoms were observed in the 61 employees interviewed. Symptoms developed in 59% of the group, or 36 workers. The most frequent complaint was a tightness of the chest, which was described with some difficulty by many of the workers. Descriptions such as "difficult to get a breath" and "a sort of squeezing feeling" were among the

Table 3.—Physical Measurements in Area A

Temperature	72 F
Relative humidity	62%-50%
Room volume	21,600 cu ft
Fresh air changes	5.5/hour
Total air changes	20/hour

most common given. General malaise and fatigue, particularly involving the lower extremities, were the next most common complaints. Only one sixth of those who were symptomatic claimed to have irritation of the throat, and only one worker had a cough productive of sputum.

A characteristic history of the illness was recorded. With two exceptions, the workers noticed symptoms after being at work four to five hours, and usually immediately after the afternoon smoke break. Chills and fever occurred approximately 12 hours after the onset of exposure and approximately 5 to 6 hours after the onset of the first symptoms. Attacks occurred at least once per week in most workers. Some stated that some symptoms developed every day at work in area A or B.

Table 2 presents data on the relationship of symptoms and smoking habits. Among those with symptoms, only nine of the 36 persons did not smoke. Among those without symptoms, seven gave a positive history of smoking. Of this group, two had worked in the area only four days; one worked only outside areas A and B; one was in area A only two minutes every second or third day; and one spent three days per week doing a time-and-motion study in areas A and B.

Table 3 gives data regarding ventilation and physical measurements of the environment in area A.

The results of the spirometric studies are demonstrated in Tables 4-7. FEC, MMF, and PFR are expressed as percentage of predicted normal for age, or age and height, according to standard tables.<sup>5-7</sup> FEV<sub>1</sub> is expressed as a percent of FEC. There was a general reduction of all of these values in the group of smokers as contrasted to the non-smokers. Since these two groups are almost identical to those with symptoms of polymer-fume fever and those without, there are very few data available for use in separating the effects of smoking and the effects of environmental exposure. However, Table 6 shows the means of the two small

Table 4.—Mean Values of Pulmonary Function Studies\*

Group	No.	Baseline†						Following Exposure†									
		FEC, % Predicted Normal	SD	FEV <sub>1</sub> , % FEC	SD	MMF, % Predicted Normal	SD	PFR, % Predicted Normal	SD	FEC, % Predicted Normal	SD	FEV <sub>1</sub> , % FEC	SD	MMF, % Predicted Normal	SD	PFR, % Predicted Normal	SD
All subjects	47	93	18.2	86.7	12.2	87.5	25.1	94.3	14.5	94.2	14.9	86.8	9.0	86.5	26.3	95.2	16.6
Subjects with respiratory symptoms	24	90.1	20.3	85.3	15.6	87.1	28.7	90.7	14.6	90.3	13.6	87.0	9.6	79.0	20.3	90.2	20.9
Subjects without respiratory symptoms	23	96.0	15.9	88.1	8.5	87.9	21.7	98.0	14.1	98.4	15.9	86.5	16.1	94.7	30	100.6	13.5
t value		1.11		0.75		0.11		1.75		1.98		0.13		2.05		1.95	
Probability		<0.3		<0.5		>0.5		<0.1		<0.1		>0.5		<0.05		<0.1	

\* t tests for all four function tests—before vs after exposure showed  $P > 0.05$ .

† Forced expiratory capacity, FEC; forced expiratory volume in 1 second, FEV<sub>1</sub>; maximum midexpiratory flow rate between 25% and 75% of the forced expiratory capacity, MMF; peak-flow rate, PFR; and standard deviation, SD.

Table 5.—Mean Values of Pulmonary Function Studies\*

Group	No.	Baseline†								Following Exposure†							
		FEC, % Predicted Normal	SD	FEV <sub>1</sub> , % FEC	SD	MMF, % Predicted Normal	SD	PFR, % Predicted Normal	SD	FEC, % Predicted Normal	SD	FEV <sub>1</sub> , % FEC	SD	MMF, % Predicted Normal	SD	PFR, % Predicted Normal	SD
Smokers	29	90.4	14.6	86.9	3.1	86.9	31.5	91.0	15	91.3	14.5	86.9	2.5	82.8	23.7	90.7	18.2
Nonsmokers	18	97.1	22.3	86.2	16.2	88.4	9.5	99.6	12.4	98.6	15.4	86.5	11.7	92.2	29	102.1	11.7
t value		1.24		0.18		0.19		2.04		1.59		0.14		1.16		2.31	
Probability		<0.3		>0.5		>0.5		<0.05		<0.2		>0.5		<0.3		<0.05	

\* t tests for all four function tests—before vs after exposure showed  $P > 0.05$ .

† Forced expiratory capacity, FEC; forced expiratory volume in 1 second, FEV<sub>1</sub>; maximum midexpiratory flow rate between 25% and 75% of the forced expiratory capacity, MMF; peak-flow rate, PFR; and standard deviation, SD.

groups—those with symptoms who do *not* smoke, and those who do not have symptoms and who *do* smoke. These suggest that the difference may be attributed completely to the effects of cigarette smoking.

As indicated in the tables, there was no significant change in the results of the tests of function at the beginning and at the end of the work shift. This was true in both groups—smokers and nonsmokers.

As demonstrated in Table 7, only three patients showed a significant change in their pulmonary-function studies during the course of the day at work. One of these gave a history of asthma; one had a history of hay fever. The changes noted during the day in these individuals are suggestive of the development of obstructive changes in the airways. One of these men had some improvement in pulmonary function after the inhalation of isoproterenol.

**Comment**

This “epidemic” of polymer-fume fever illustrates very well the interaction of agent, host, and environment in the causation of illness. The present outbreak would seem to be explained according to the following sequence of events: The new parting compound was a telomere of polytetrafluoroethylene with a molecular weight of 3,700-5,000, which existed as a fine dust on the tools. Cigarettes became indirectly contaminated with small particles of this material which had been deposited on the workers’ hands. Inhalation of the products of pyrolysis of polytetrafluoroethylene produced the syndrome in those who smoked contaminated cigarettes. The occurrence of symptoms in a few men who did not smoke would seem to be related to the fact that a small hot-air gun was used in the application of the epoxy resin in the subassembly. This gun has a heating element that reaches a temper-

ature of 750 F. In all probability, the air currents generated by this gun resulted in dispersion of the particles of polymer, which subsequently reached the heating element. With two possible sources of thermal degradation of the polymer—the heating element of the hot-air gun and the cigarettes—it would seem that an adequate supply of pyrolysis products of the polymer was available.

The epidemic pursued a rather lengthy course because of two factors: (1) management had confidence in the innocuous properties of the new parting compound, and (2) changes in ventilation had been associated with the onset. As complaints of symptoms began to accumulate, the ventilation was changed so that in area A, air-conditioners brought in 25% outside air rather than providing 100% recirculated air. This change in ventilation seemed to reduce the severity of symptoms, but it did not completely eliminate them. The spread of the complaints of symptoms in area B was also somewhat baffling initially. The increase in ventilation would have decreased somewhat the ambient-air concentration of these products of pyrolysis. This would not have helped those who were smoking contaminated cigarettes.

In retrospect, the history given by these employees was classical for polymer-fume fever. It is rather interesting that seven men specifically identified the new parting compound as the agent which they felt was causing the problem. This is significant with regard to the Oslerian aphorism about “listening to the patient.”

In 1955, Sherwood reported seven cases of polymer-fume fever and related it to a history of smoking in the workers.\* In the literature there have appeared second-hand reports of deaths resulting from inhalation of the pyrolysis products of polymers of tetrafluoroethylene. These have proved to be difficult to exterminate and have had at least one rebirth in the past two years. There

Table 6.—Mean Values of Pulmonary Function Studies

Group	No.	Baseline*								Following Exposure*							
		FEC, % Predicted Normal	SD	FEV <sub>1</sub> , % FEC	SD	MMF, % Predicted Normal	SD	PFR, % Predicted Normal	SD	FEC, % Predicted Normal	SD	FEV <sub>1</sub> , % FEC	SD	MMF, % Predicted Normal	SD	PFR, % Predicted Normal	SD
Nonsmokers with symptoms	4	91.6	14.5	85.0	4.4	87.3	5.5	99.6	2.5	94.2	10.6	84.2	2.2	86.0	5.4	100	5.6
Smokers without symptoms	6	91.1	12.7	92.6	4.9	93.0	25.3	94.5	14.9	93.0	8.1	91.2	3.0	104.8	23.0	93.4	12.4

\* Forced expiratory capacity, FEC; forced expiratory volume in 1 second, FEV<sub>1</sub>; maximum midexpiratory flow rate between 25% and 75% of the forced expiratory capacity, MMF; peak-flow rate, PFR; and standard deviation, SD.

Table 7.—Patients Demonstrating Significant Reduction in Pulmonary Function During and After 8-Hour Exposure

	Patient 1	Patient 2	Patient 3
*FEC—before	3.5 liters	3.4 liters	4.4 liters
after	3.1 liters	3.3 liters	4.4 liters
FEV <sub>1</sub> —before	2.3 liters	3.4 liters	4.0 liters
after	2.1 liters	2.6 liters	3.8 liters
MMF—before	2.2 liters/sec	2.8 liters/sec	5.5 liters/sec
after	1.9 liters/sec	2.7 liters/sec	3.7 liters/sec
PFR—before	480 liters/min	250 liters/min	680 liters/min
after	310 liters/min	220 liters/min	720 liters/min

\* Forced expiratory capacity, FEC; forced expiratory volume in 1 second, FEV<sub>1</sub>; maximum midexpiratory flow rate between 25% and 75% of the forced expiratory capacity, MMF; peak-flow rate, PFR; and standard deviation, SD.

seems to be little doubt that teflon itself has rather remarkable properties, including a physiologic inertness. However, when heated to above 300 C, the products of its thermal degradation are capable of producing a short-lived "influenza-like syndrome" in almost all those inhaling these by-products.

Numerous studies have attempted to characterize these breakdown products. They consist of higher-chain fluorocarbons, the most toxic of which is isooctofluorobutylene.<sup>9</sup>

Capodaglio studied four cases of polymer-fume fever and stated that three of four showed abnormal pulmonary function for six weeks to six months after exposure.<sup>10</sup> Data derived in this study would indicate that changes in respiratory functions which occur while at work are minimal and consistent with mild obstructive disease in the airway. Gandevia studied the pulmonary function of workers exposed to toluene di-isocyanate (TDI) vapor.<sup>11</sup> A decrease of 180 cc in FEV<sub>1</sub> on successive days was noted and said to be significant. One half of all the subjects (all smokers) showed an increased sensitivity to inhalation of histamine aerosol.

The decrease in pulmonary function in the group with symptoms of polymer-fume fever is consistent with differences noted between smoking and nonsmoking populations, as reported in previous studies.<sup>12-15</sup>

One of the most severely affected workers was on two occasions admitted to a hospital, with severe respiratory distress and x-ray findings suggestive of pulmonary edema. This patient's symptoms and x-ray changes responded rapidly to corticotropin (ACTH). Such a case was recently reported.<sup>16</sup>

The mechanism by which pyrolysis products produce fever is not known. Cavagna et al,<sup>17</sup> and Pernis et al<sup>18</sup> have showed a degranulation of polymorphonuclear leukocytes after their exposure to teflon. They suggest that release of endogenous pyrogen is the mechanism for the production of the syndrome.

It is apparent from this study that, despite adequate warning in the manufacturer's brochure, and reports in the medical literature, polymer-fume fever may not be recognized. It is also apparent that workers handling the dust of such polymers cannot smoke in the work area. A past history of hay fever, asthma, or other pulmonary disease also is probably sufficient cause to exclude such a worker from exposure to these materials. While the disease process is short and self-limited, it can significantly reduce the operational effectiveness of a department, as well as produce unnecessary illness in man. Sufficient knowledge is available to classify this as a preventable disease.

#### Generic and Trade Names of Drug

Corticotropin (ACTH)—*Acth, Acthar, Corticotropin.*

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# TECHNICAL INFORMATION

Chemical Division

**3M**  
COMPANY

## 3M BRAND FLUOROCHEMICAL SURFACTANTS

FC-95, FC-98, FC-128, FC-134, FX-161,  
FC-170, FX-172

Exhibit  
1042

State of Minnesota v. 3M Co.,  
Court File No. 27-CV-10-28862

3MA01201629

1042.0001

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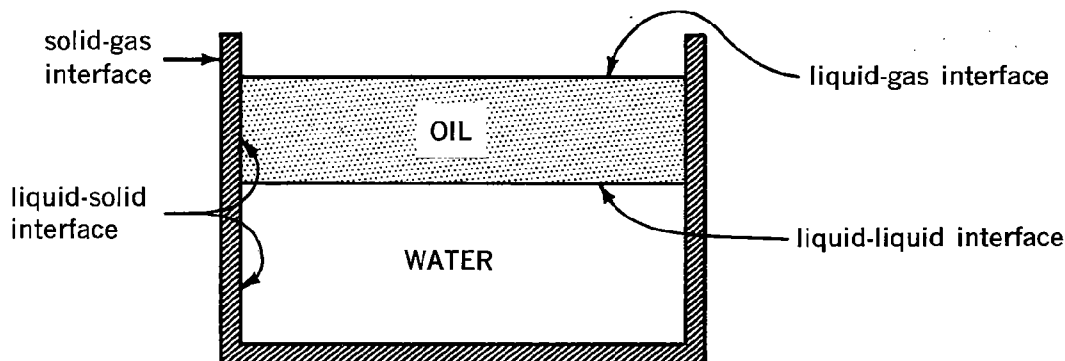


## TECHNICAL INFORMATION

### INTRODUCTION

#### Surfaces or Interfaces

Oil and water do not mix, hence, there is a boundary between them. These boundaries, known as surfaces or interfaces, exist between liquids and liquids, liquids and solids, solids and solids, solids and gases, and liquids and gases.



#### Surface Tension

Liquids often behave as though there is an elastic skin stretched on their surface. A water glass, for example, can be filled to a point where the water level is above the rim of the glass, or a needle can be floated on the surface of the water. The strong "elastic membrane" or high surface tension of the water makes these effects possible. If you were to add a detergent to

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Seller's and manufacturer's only obligation shall be to replace such quantity of the product proved to be defective. Neither seller nor manufacturer shall be liable for any injury, loss or damage, direct or consequential, arising out of the use of or the inability to use the product. Before using, user shall determine the suitability of the product for his intended use, and user assumes all risk and liability whatsoever in connection therewith.

No statement or recommendation not contained herein shall have any force or effect unless in an agreement signed by officers of seller and manufacturer.

Since the manufacturer of the product described in this technical data sheet has no means of controlling the final use of the product by the consumer or user, it is the responsibility of the immediate purchaser and any intermediate seller or sellers to inform the user of the purposes for which the product may be fit and suitable and of the properties of the product, including the precautionary measures which must be taken in order to ensure the safety of the user and of other third persons and property.

**WARNING!** Do not take internally. Avoid breathing dust. Wash thoroughly after handling.



## Surface Tension (continued)

the water, the glass could not be filled above the rim and the needle would not float. The elastic skin loses its strength; the water becomes wetter; its surface tension is reduced. Measurements of surface tension are, therefore, able to compare the effectiveness of compounds to concentrate at the surface and so effect the properties.

## Surfactants

Any material capable of reducing the surface tension of a liquid is a surfactant, or surface active agent. In the illustration above, a detergent reduced the surface tension of water. Hydrocarbon detergents are excellent surfactants and are capable of reducing the surface tension of water from 72 dynes/cm. to 30-35 dynes/cm. These materials are commonly used in many processes such as washing clothes and dishes, emulsifying components of paint, industrial cleaning and etching, textile dyeing, and countless other applications.

## Fluorochemical Surfactants

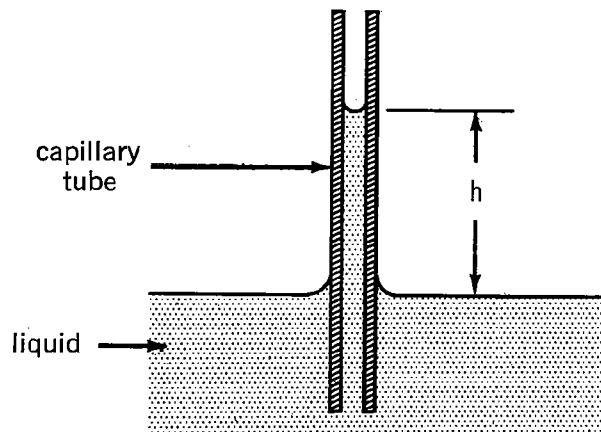
A fluorochemical surfactant contains a completely fluorinated tail and solubilizing group. These compounds are capable of reducing the surface tension of water from 72 dynes/cm. to 15-20 dynes/cm. Like the hydrocarbon surfactants, they are also active at all interfaces.

The difference between hydrocarbon and fluorochemical surfactants is further illustrated by concentration requirements. Minimum surface tension reduction of hydrocarbon surfactants (30-35 dynes/cm.) is generally achieved at concentrations of 0.1-1% by weight; while fluorochemical surfactants can achieve a minimum surface tension (15-20 dynes/cm.) at approximately 0.01% by weight (100 parts per million).

## Determination of Surface Tension

Surface tensions can be measured in any one of a number of ways.

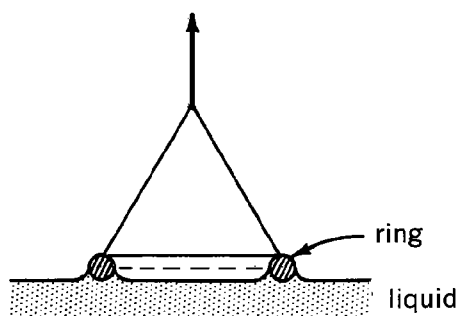
Capillary Rise: This method is the oldest, going back to Leonardo de Vinci. When a vertical capillary is immersed in a liquid which wets it, the liquid rises to a height,  $h$ , which is inversely proportional to the surface tension.



## Determination of Surface Tension (continued)

The internal radius of the capillary tube, density of the liquid, and the acceleration due to gravity must also be known. Exact measurements of height and radius of the capillary are extremely difficult; hence, accuracy of the method is limited.

2. Drop Weight: The weight of a drop which falls from a capillary tube is proportional to the surface tension of the liquid and the radius of the capillary. Instruments currently available, called drop-counters and stalagmometers, are based on this principle. A serious disadvantage of the method exists, however, in that drops must be formed very slowly, only one in every several minutes. This measurement is neither accurate nor rapid.
3. Rupture of the Surface. "Tensiometer": Probably the fastest and most reliable method of surface tension measurement is the pull-ring method by duNouy. The procedure measures the "tension" or pull required to cause a ring to break free from the liquid surface. The tensile force is proportional to the surface tension.



The accuracy of the duNouy Tensiometer is especially great if liquids having similar properties are compared. It is also a fast procedure, capable of measuring surface tension of rapidly aging systems. Platinum rings are used; hence, clean-up between measurements is simplified over the capillary methods.

Additional information on surface tension measurements is found in the bibliography references (1, 2). The extremely low surface energy of the fluorocarbon tail is also discussed (3, 4).

## CHARACTERISTICS OF 3M BRAND FLUORO-CHEMICAL SURFACTANTS

The main features which distinguish these materials from conventional surfactants are as follows:

1. Stability
  - a. Chemical - Several are stable in 90% H<sub>2</sub>O<sub>2</sub>, anhydrous hydrazine, hot concentrated mineral acids, and alkalis.
  - b. Thermal - Certain members of this family are stable up to 750°F.
  - c. Biological - Some are completely resistant to biological attack.
  
2. Surface Activity
  - a. Aqueous Systems - Capable of surface tension reduction, 10-15 dynes/cm. lower than conventional surfactants.
  - b. Organic Systems - Many 3M surfactants are active in organic systems, causing reductions in the surface tensions of organic solvents.
  - c. Wetting - Wetting of materials can be greatly improved. Or, a variety of substrates can be made hydrophobic and/or oleophobic.
  - d. Foams - Stable, long-lasting foams can be produced in media which would be destructive to conventional surface active agents. Low foaming materials are also available.
  
3. Efficiency
  - a. Economical - Effective at extremely low concentrations, frequently at 50-100 parts per million or less.

SOLUBILITY OF FLUOROCARBON SURFACTANTS

<u>SOLVENT</u>	<u>SOLUBILITY</u>						
	Grams Surfactant per 1000 Grams of Solvent, 25°C.						
	<u>FC-95</u>	<u>FC-98</u>	<u>FC-128</u>	<u>FC-134</u>	<u>FX-161</u>	<u>FC-170</u>	<u>FX-172</u>
Acetone	100	20	2	> 10	> 1000	600	1000
Benzene	0.8	0.2	0.2	0.6	2	500	1
Carbon Tetra- chloride	0.04	0.04	0.1	Nil	0.8	470	2
Ethyl Alcohol	2	20	10	100	80	> 1000	> 1000
Heptane	0.03	0.09	0.03	Nil	0.7	7	1
Isopropyl Alcohol	3	30	8	10	> 1000	> 1000	> 1000
Isopropyl Ether	0.1	0.3	0.2	0.04	20	250	15
Methyl Alcohol	60	40	60	80	70	> 1000	> 1000
Perchloro- ethylene	0.07	0.09	0.2	0.04	0.7	690	100
Toluene	1	0.2	0.1	0.1	1	550	10
Water	2	10	200 (gel)	> 5 (gel)	10	> 300 (gel)	> 500 (Foam)

Procedure Used Above: Saturated surfactant solutions were equilibrated and filtered. Solubilities were determined after evaporation of the solvent.

SOLUBILITIES IN ACIDS AND ALKALI

	<u>FC-95</u>	<u>FC-98</u>	<u>FX-161</u>
Hydrochloric acid, 12½%	> 1	< 10	< 0.01
Hydrochloric acid, 37%	0.1		
Nitric acid, 12½%	> 1	< 20	< 0.01
Nitric acid, 70%	> 5		
Phosphoric acid, 12½%	> 1		
Phosphoric acid, 85%	> 1		
Sulfuric acid, 12½%	10	< 10	< 0.01
Sulfuric acid, 97%	0.5		
Potassium hydroxide, 20%	1		

## HANDLING AND USE

The solubilities of the 3M Brand Fluorochemical Surfactants in aqueous and organic media are usually lower than those of conventional hydrocarbon surfactants. Although the solubility limits of a given 3M surfactant in water may be 0.1% or less, this is not necessarily restrictive of its capacity to function effectively. However, the surfactant must have some solubility in a system if it is to be effective. Surface tension should be checked when solubility is in question, to determine whether or not the material is actually present in adequate levels to affect surface properties of the system significantly. Examination for color change and foaming can also be used as guides to indicate adequate solubility. In the case of surfactant use as a primary emulsifier, addition levels higher than 0.1% are usually required. Their use as secondary emulsifiers or stabilizers may not require use levels greater than this.

Where solubilities are not sufficient to achieve results in a particular media, it is recommended that the use of cosolvents be investigated. In many cases this will mean the addition of only a few percent of a cosolvent (or cosolvents) to form a binary or ternary system. For example, the addition of 5% acetone or isopropanol greatly increases the solubility of many fluorochemical surfactants in water. Similar examples occur with organic systems.

Because of low solubilities, 3M Brand Surfactants may be slow to dissolve. The use of heat will greatly increase solution rates and is recommended whenever possible. In some cases, pre-dissolving the surfactant in a small amount of a compatible solvent in which its solubility is large will greatly facilitate its solution. In such cases, very little of the pre-solvent will be needed; in fact, simple wetting out of a solid surfactant will often serve to increase its rate and degree of solution. It should be emphasized that:

Wherever possible, the surfactant should be added to a liquid system in a compatible liquid form. (This will insure that all of the surfactant which has been added is being utilized.)

The following is intended as a guide to helpful cosolvents for various 3M Brand Fluorochemical Surfactants.

<u>SURFACTANT</u>	<u>COSOLVENTS FOR WATER SOLUTIONS</u>	<u>COSOLVENTS FOR ORGANIC SOLUTIONS</u>
FC-95	Acetone or	50:50 CCl <sub>4</sub> :methanol or
FC-98	Methanol	50:50 acetone:isopropanol
FC-128	Acetone or Isopropanol	Same as above
FC-134	Acetone Methyl cellosolve or Dimethyl formamide	Carbon tetrachloride
FX-161	Acetone or Isopropanol	Same as FC-95
FC-170	None required	Toluene Alcohol
FX-172	Acetone or Isopropanol	Acetone Isopropanol

## SURFACE PROPERTIES

Surface tension measurements are included to show the effect of 3M Brand Fluorochemical Surfactants in water, various acids, alkalis, and other media.

Generally speaking, lowered surface tension values imply better wetting properties of the surfactant solution. This is true for many of them in many media. However, some of these surfactants are very capable of preferential absorption on many surfaces which, in effect, would decrease the wettability of that surfactant solution to that particular surface. An example of this diverse effect is the use of Fluorochemical Surfactants in hydrochloric acid. FC-95 will cause a great increase in the rate of attack of HCl on aluminum, while FX-161 can actually stop the corrosion of aluminum by formation of a tenacious monomolecular corrosion-resistant barrier film on the aluminum. It should be emphasized that the surfactant effects just described are very selective. The solvent, surfactant, and substrate are dependent upon one another. Our laboratories will be pleased to assist in the selection of the proper fluorochemical surfactant for problem applications upon receipt of complete information on the system and the effects desired.

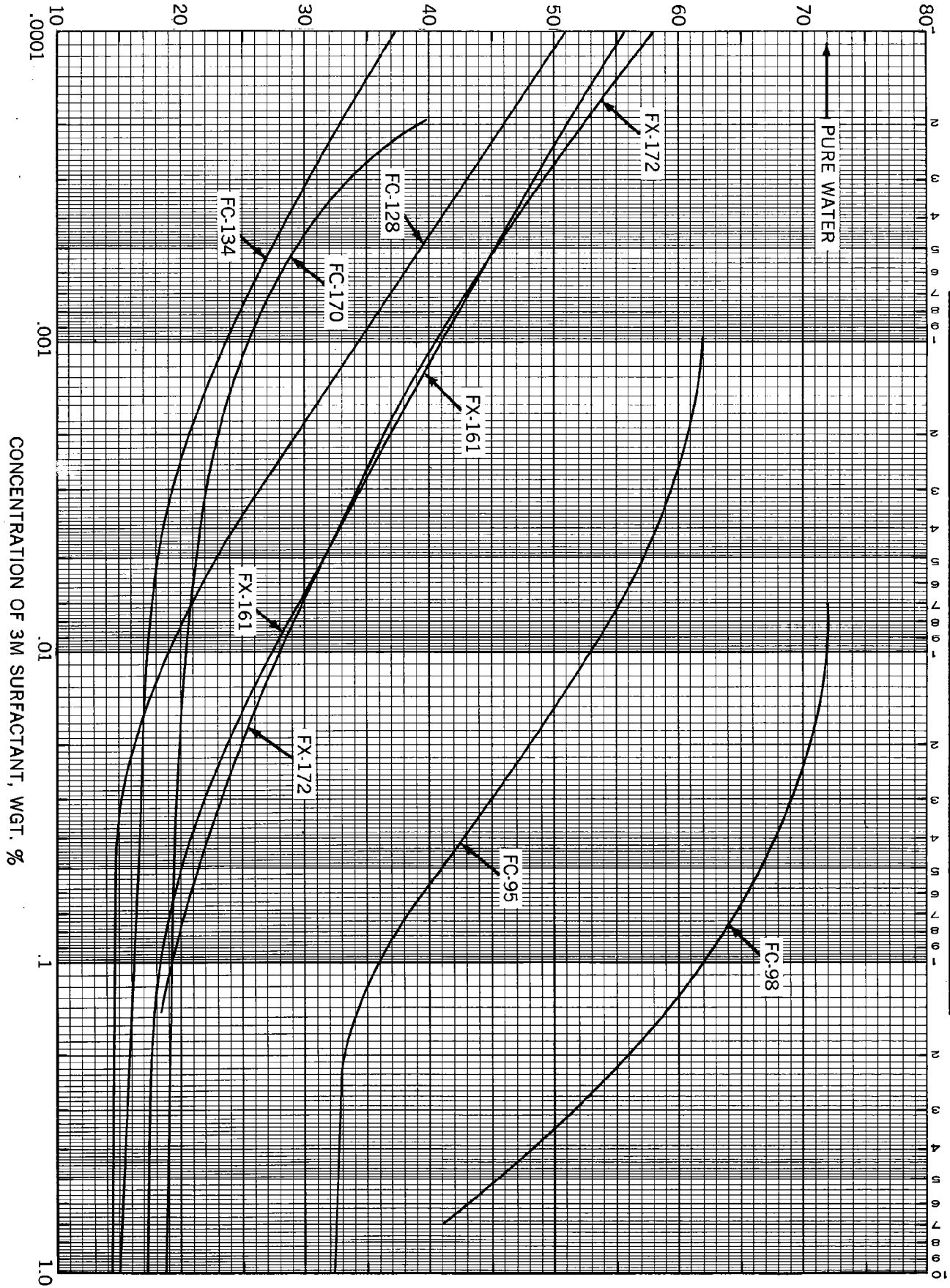
Unless stated to the contrary, all surface tension data presented were obtained with a duNouy tensiometer on solutions which had aged 16-24 hours prior to measurement. (See introduction)

### SURFACE TENSIONS IN WATER

Figure 1 compares surface tension vs. concentration of 3M Brand Fluorochemical Surfactants in water. It should be noted that extremely low concentrations of FC-134 and FC-170 give remarkably low surface tensions in water. For example, 0.001% (parts per million) FC-134 reduces the surface tension of water from about 72 dynes/centimeter to about 24 dynes/cm.

Figure 2 represents the effect of temperature on the surface tensions of FC-128 and FC-134 solutions; each at concentrations of 0.1, 0.01, and 0.001 (1,000, 100, and 10 ppm respectively).

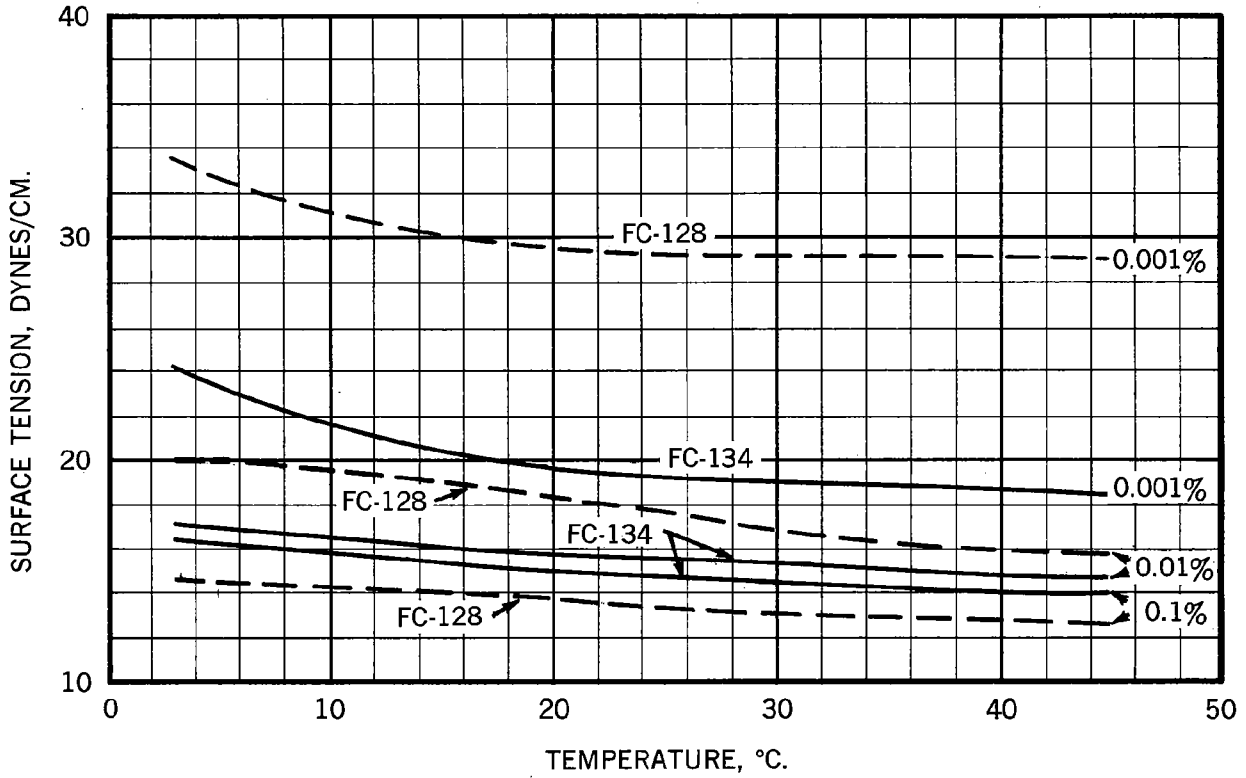
SURFACE TENSION, 25°C., DYNES/CM.



SURFACE TENSIONS OF 3M BRAND FLUOROCHEMICAL SURFACTANTS IN WATER



SURFACE TENSION vs. TEMPERATURE OF FC-128, FC-134  
IN WATER



## SURFACE TENSIONS IN ACIDS

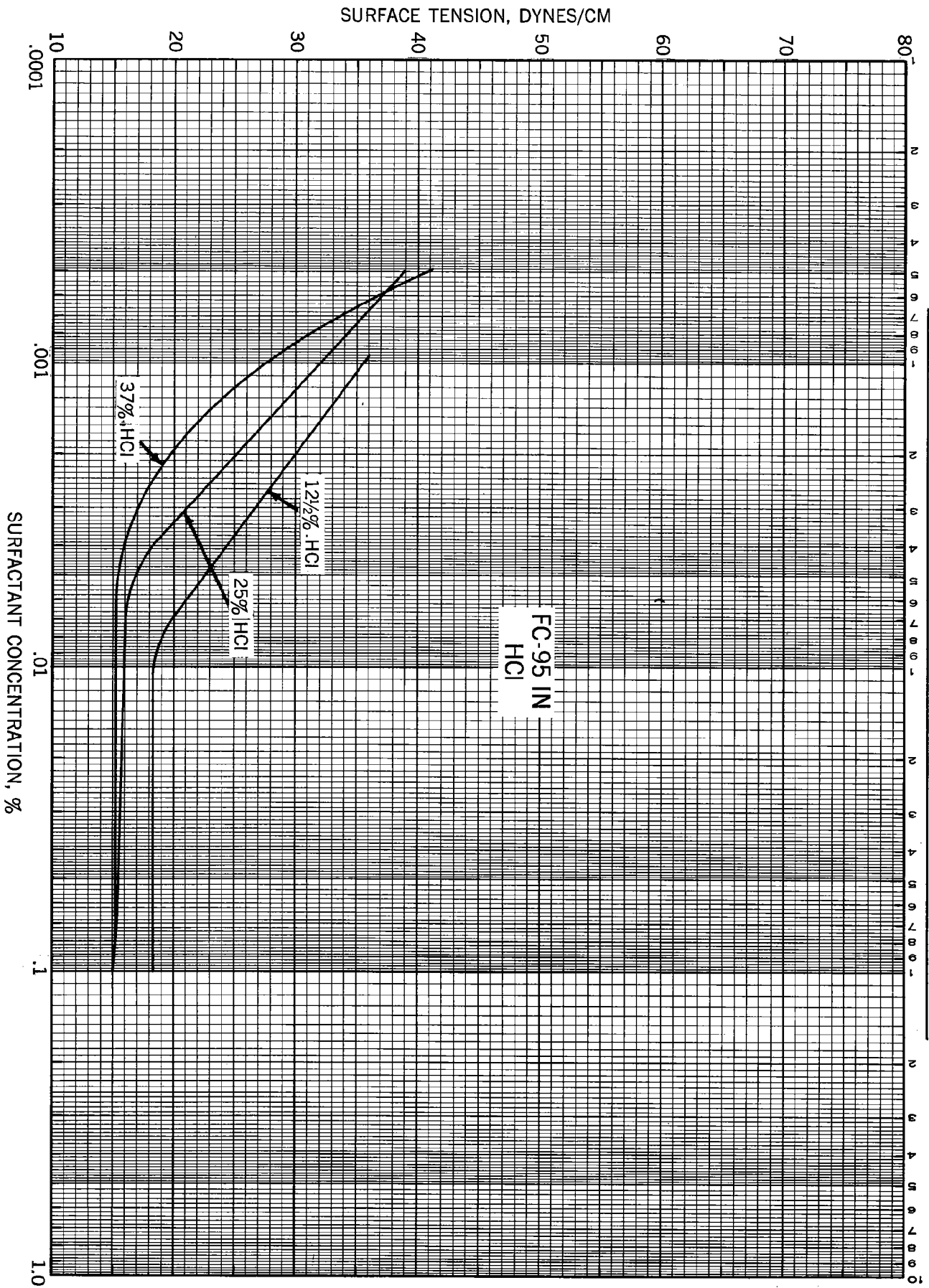
The stability of certain 3M Brand Fluorochemical Surfactants to strong mineral acids provides a unique means of reducing surface tensions of these highly corrosive chemicals. Certain of the fluorochemical surfactants are extremely stable in these acids even at elevated temperatures, and are often the only materials which provide surface activity in these media.

The effectiveness of materials such as FC-95 and FC-98 in reducing surface tension of acidic media is in marked contrast to their effect in water. The importance of solution pH is, therefore, not to be overlooked. Both solubility and activity are dependent on pH, the presence of other dissolved salts, etc.

### 1. Hydrochloric Acid

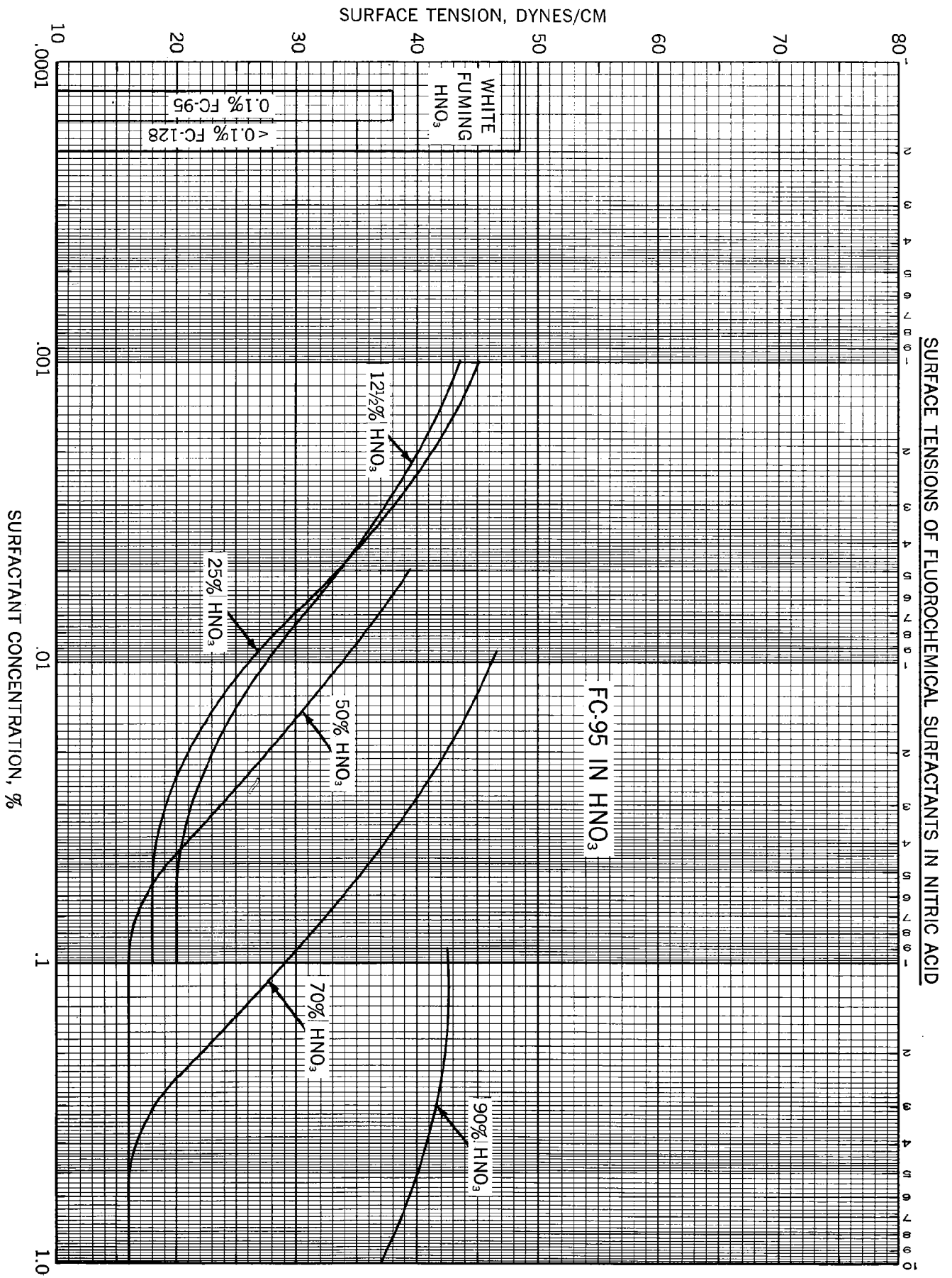
Curves for FC-95 in 12½%, 25%, and 37% hydrochloric acid are found in Figure 3. Note that minimum surface tension is attained at from 50 to 100 ppm (.005-0.01%) FC-95.

SURFACE TENSIONS OF FLUOROCHEMICAL SURFACTANTS IN HYDROCHLORIC ACID



2. Nitric Acid

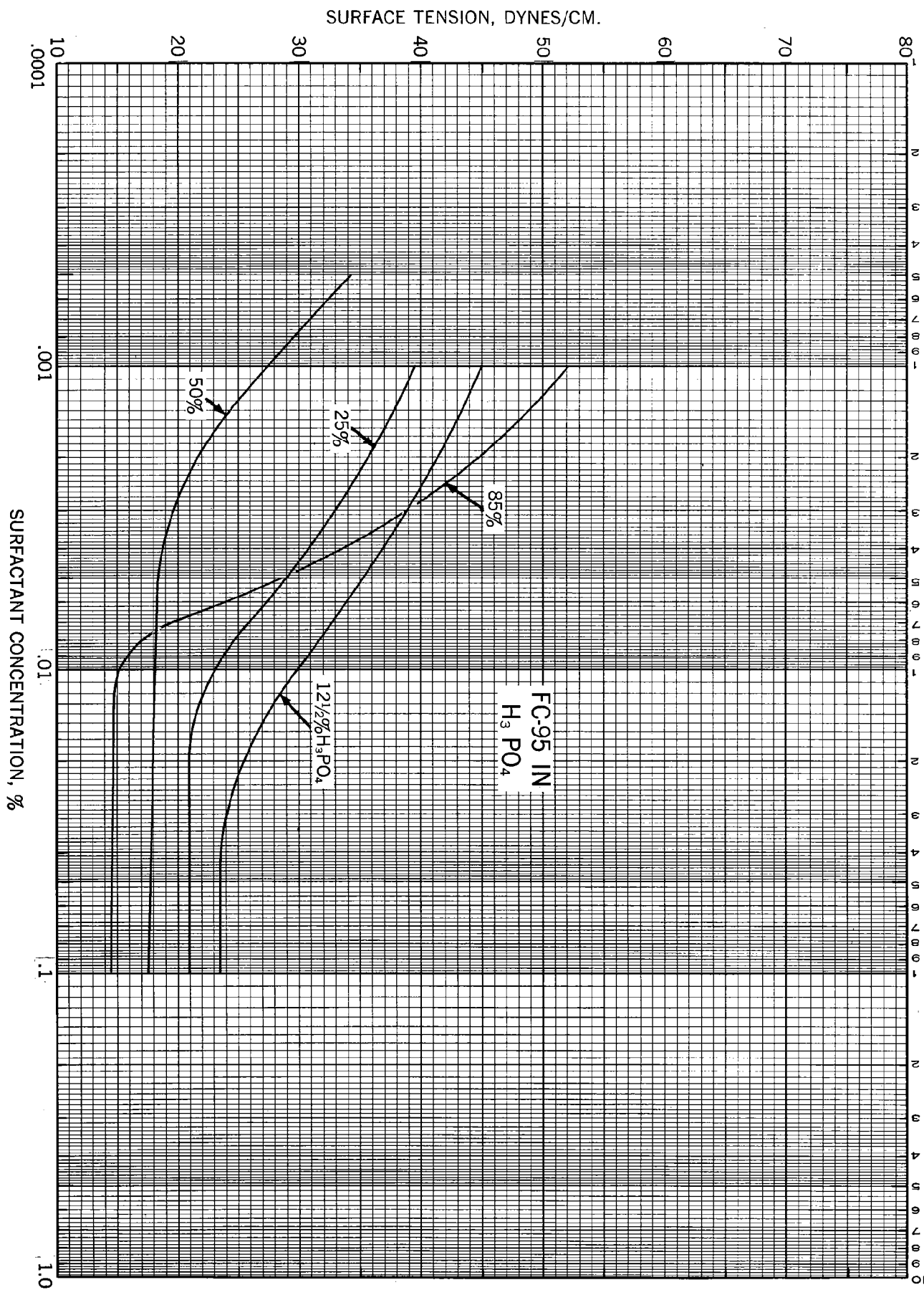
Figure 4 presents surface tension vs. concentration curves for FC-95 in 12½, 25, 50, 70, and 90% HNO<sub>3</sub>. Bar charts are included showing the effect of FC-95 and FC-128 in white fuming nitric acid. The area at the left of the bar chart indicates the surface tension of the pure acid.



3. Phosphoric Acid

Curves indicating the effect of FC-95 in 12½, 25, 50, and 85% H<sub>3</sub>PO<sub>4</sub> are given in Figure 5. Note that minimum surface tensions are achieved at concentrations of 50-500 ppm (0.005-0.05%) FC-95.

SURFACE TENSIONS OF 3M FLUORO-CHEMICAL SURFACTANTS IN PHOSPHORIC ACID

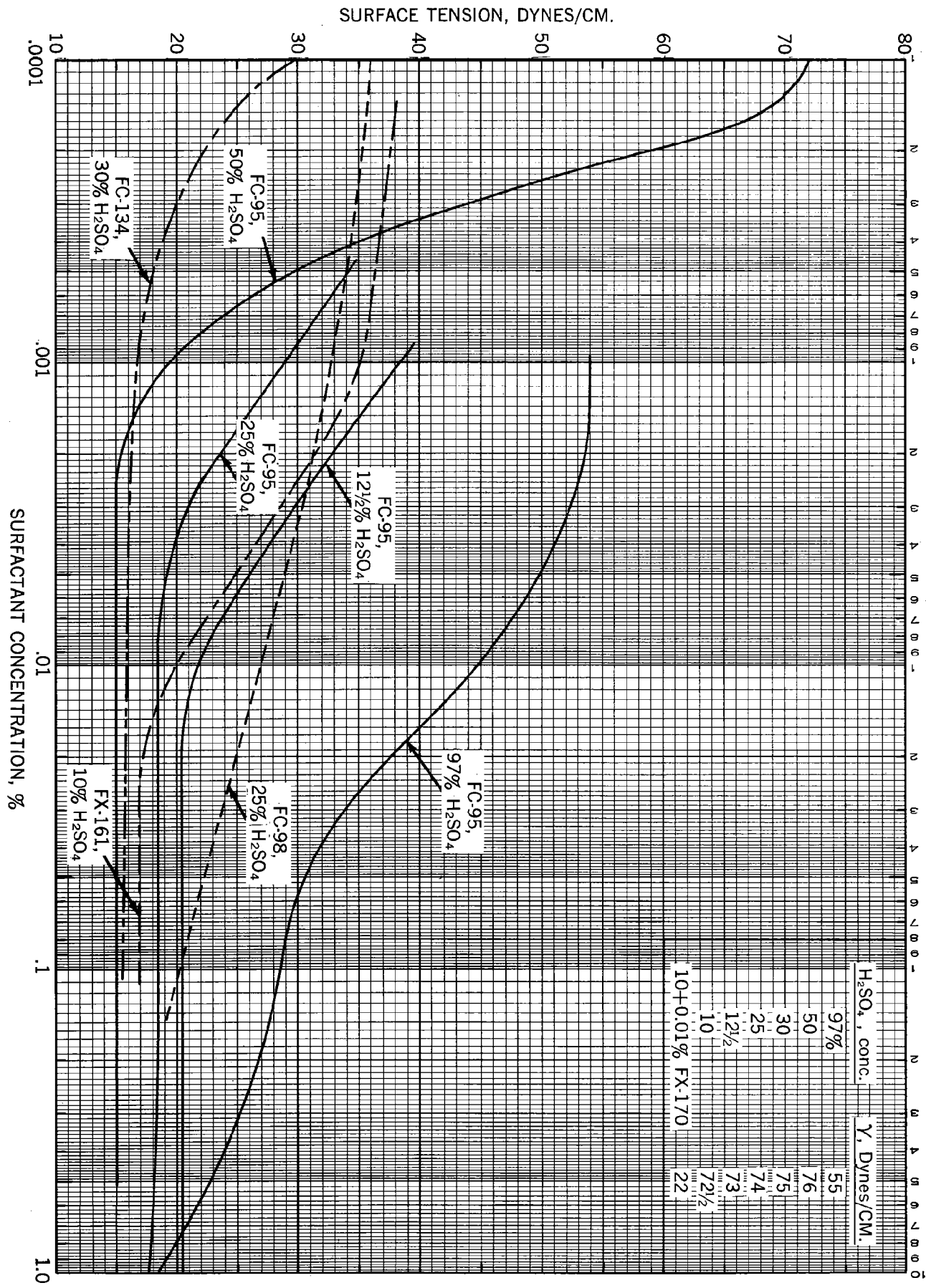


#### 4. Sulfuric Acid

Many 3M Brand Fluorochemical Surfactants show excellent surface activity in sulfuric acid as shown in Figure 6. Curves of surface tension vs. concentration are given for FC-95 in 12½, 25, 50, and 97% H<sub>2</sub>SO<sub>4</sub>, for FC-98 in 25% H<sub>2</sub>SO<sub>4</sub>, for FC-134 in 30% H<sub>2</sub>SO<sub>4</sub>, and for FX-161 in 10% H<sub>2</sub>SO<sub>4</sub>. By way of comparison, the surface tensions of the pure sulfuric acid solutions are given in the upper right-hand corner.



SURFACE TENSIONS OF FLUORO-CHEMICAL SURFACTANTS IN SULFURIC ACID



In acid media it is often possible to observe an even lower surface tension with time. The following table illustrates this effect.

FC-134 in 30% Sulfuric Acid vs. Time

<u>Concentration of FC-134%</u>	<u>0.1</u>	<u>0.01</u>	<u>0.001</u>	<u>0.0001</u>
Freshly mixed	15.2	16.4	24.4	71.9
After 48 hrs. standing	15.5	16.0	16.8	29.4
After 48 hrs. standing plus heating 16 hrs. @78°C.		14.1		24.5

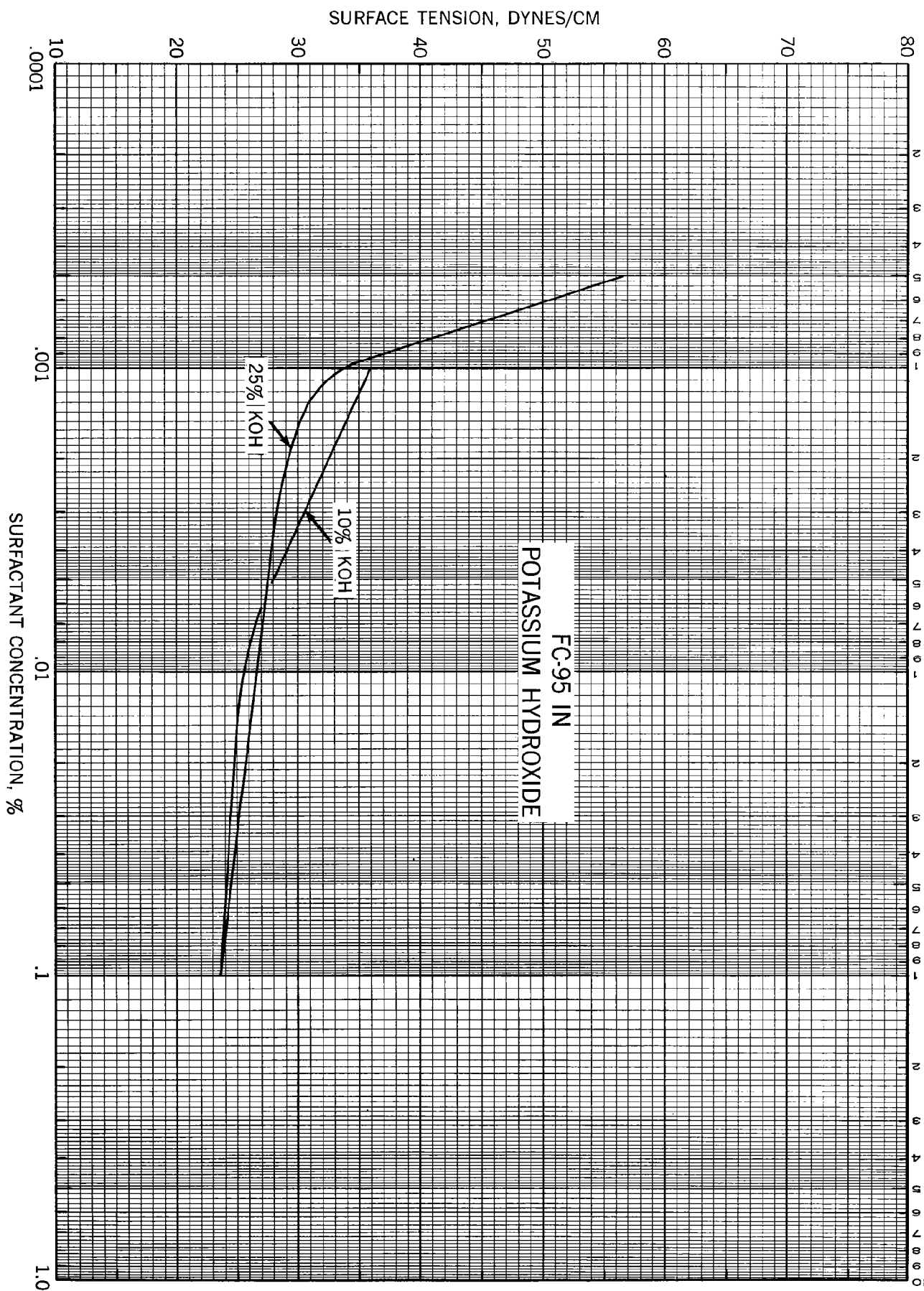
SURFACE TENSIONS IN ALKALIS

3M Brand Fluorochemical Surfactants show excellent surface tension reduction at low concentrations in alkaline systems.

1. Potassium Hydroxide

Curves for the effect of FC-95 in 10 and 25% KOH are given in Figure 7.

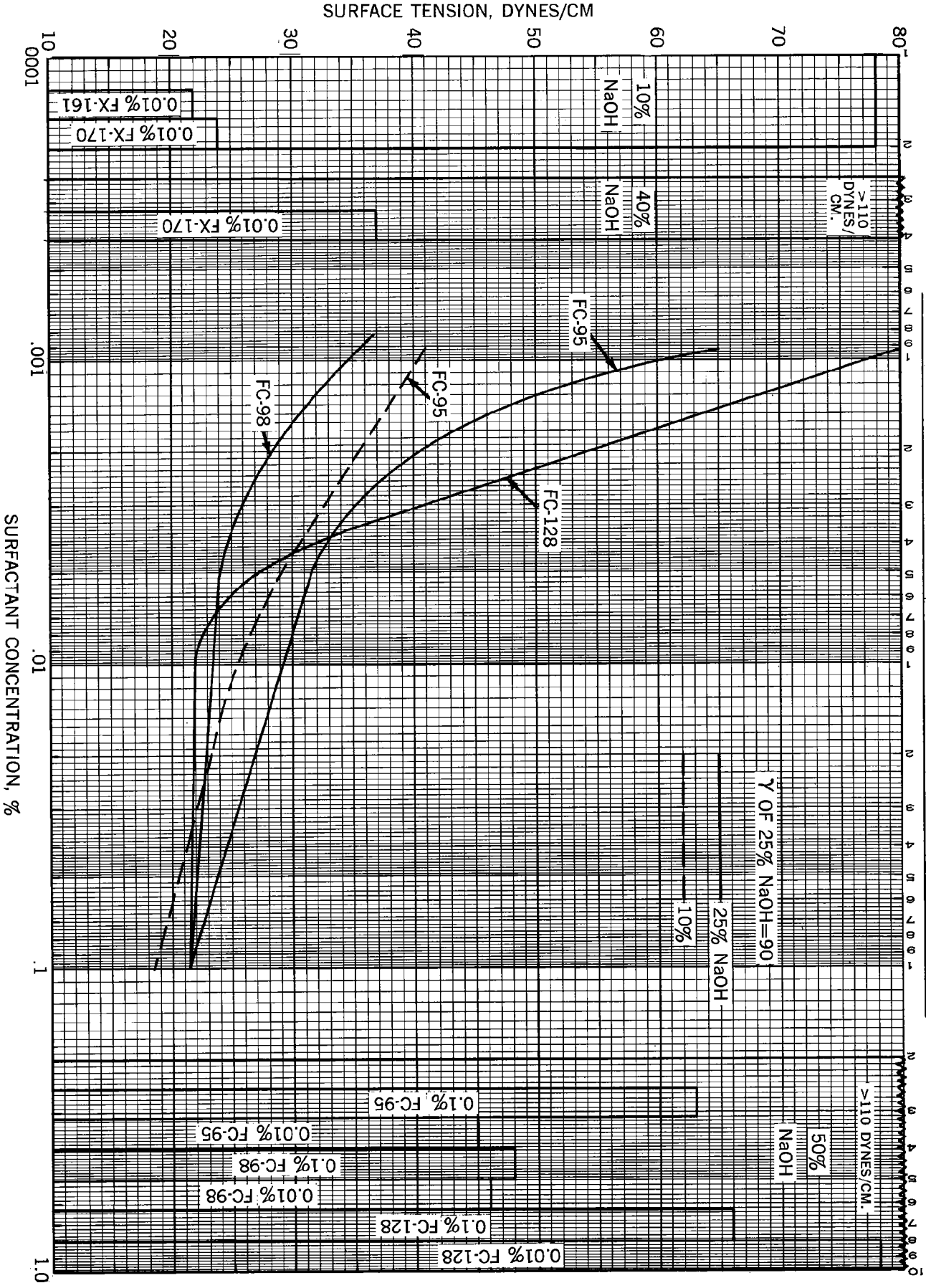
SURFACE TENSIONS OF FLUORO-CHEMICAL SURFACTANTS IN POTASSIUM HYDROXIDE



2. Sodium Hydroxide

Surface tension vs. concentration curves are presented in Figure 8 for FC-95 in 10 and 25% NaOH and for FC-98 and FC-128 in 25% NaOH. Bar charts illustrate the effect of other surfactants in 10, 40 and 50% NaOH. The bars at the left indicate the surface tension of the solution without surfactant.

SURFACE TENSIONS OF FLUOROCHEMICAL SURFACTANTS IN SODIUM HYDROXIDE



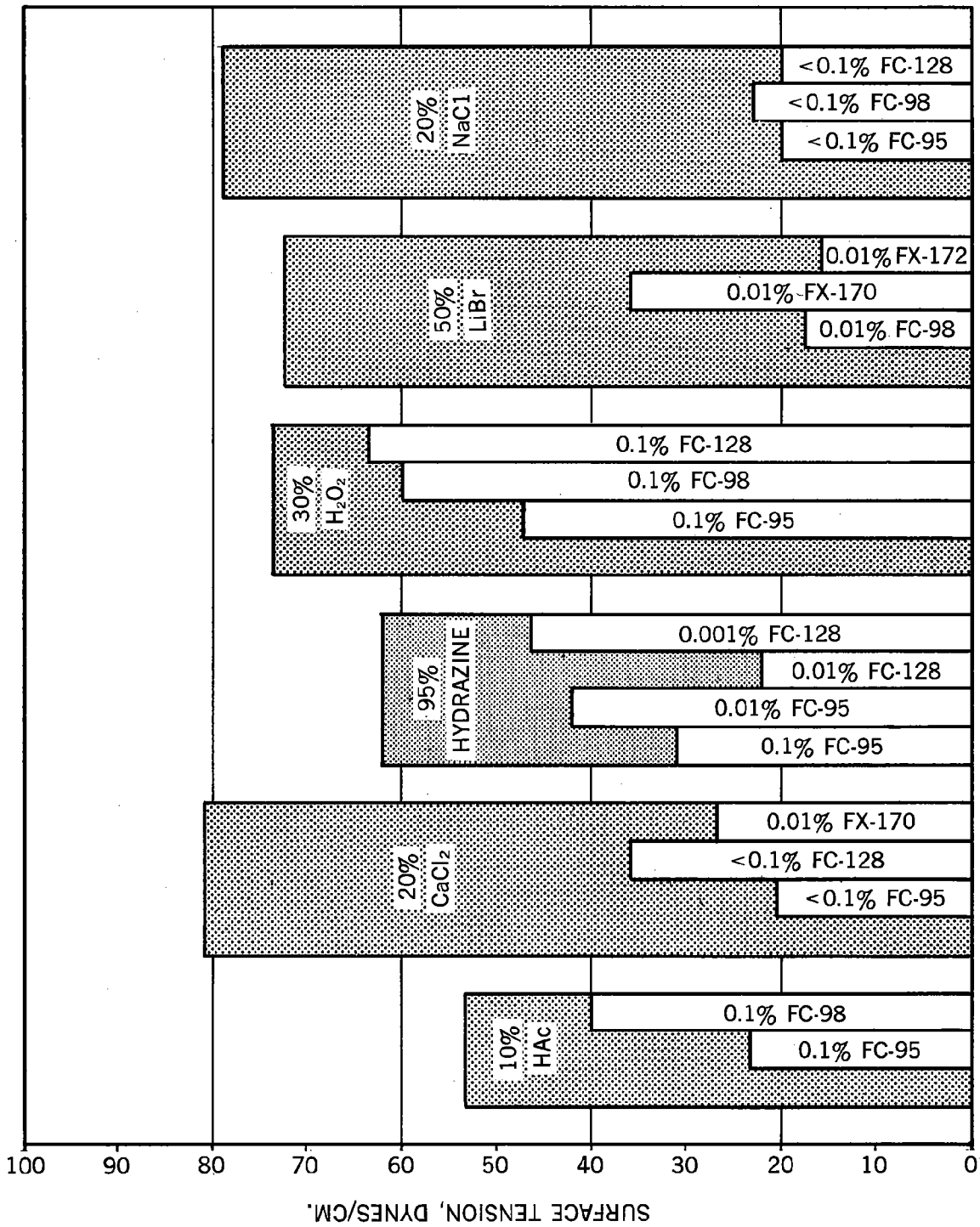
## SURFACE TENSIONS IN OTHER MEDIA

The following bar chart, Figure 9 presents data on surface tensions of a variety of media containing fluorochemical surfactants:

1. 10% Acetic acid
2. 20% Calcium chloride
3. 95% Hydrazine
4. 30% Hydrogen Peroxide (at 18°C.)
5. 50% Lithium Bromide
6. 20% Sodium Chloride

Effectiveness and stability in such rigorous media as hydrazine and hydrogen peroxide is an important characteristic of these versatile surfactants.

SURFACE TENSIONS IN OTHER MEDIA



## SURFACE TENSION REDUCTION IN DETERGENTS

FC-134 effectively reduces the surface tension of a commercial dishwashing compound (0.8% in water at 145°F.) as noted below:

<u>FC-134 CONCENTRATION</u> %	<u>SURFACE TENSION,</u> dynes/cm.
0 (control)	30.3
0.002	21.0
0.003	16.2
0.005	14.2

A comparison of these values with points on the curve of FC-134 in water, Figure 1, indicates a slight synergistic effect between the two compounds.

## INTERFACIAL TENSION

The interfacial tensions between acids and organic liquids can be greatly reduced with FC-95. For example, the interfacial tension between concentrated hydrochloric acid and an organic ester, tributyl aconitate, was lowered from 12.6 to 1.8 dynes/cm by the addition of 0.5% FC-95 to the acid phase. Figure 10 illustrates the effect of FC-95 on the interfacial tension between n-decane and dilute sulfuric acid solutions of various strengths:

FIGURE 10

### INTERFACIAL TENSION OF DECANE-DILUTE SULFURIC ACID SOLUTIONS CONTAINING FC-95

MOLAR CONCENTRATION OF SULFURIC ACID SOLUTION



## EMULSIFICATION

Fluorochemical surfactants are effective emulsifiers. However, it is usually necessary to use concentrations equal to those required for hydrocarbon surfactants which often imposes an economic restriction on their use. Those applications which are destructive to conventional surfactants by heat or chemical attack are excellent areas for the use of the fluorochemicals as emulsifiers.

Synergistic effects are often encountered when fluorochemical surfactants are used in addition to conventional hydrocarbon emulsifier blends. Effectiveness at considerably lower total surfactant concentration is often realized.

Many vinyl polymers have been prepared by emulsion polymerization utilizing fluorochemical surfactants as the emulsifiers (see U. S. Patent 2, 559, 752). It has been demonstrated that significantly lower emulsifier concentrations may be used when fluorocarbons are used instead of hydrocarbon emulsifiers.

FC-128 and FC-170 are used to advantage for emulsifying liquids of low surface energy (silicones and fluorocarbons). An homogenizer is generally required.

FX-172 is an excellent stabilizer for latexes.

In applications where wetting is required, but where emulsification is not desired, fluorochemical surfactants should receive first consideration. Here, the normal, very low concentrations are used to provide excellent wetting with a low degree of emulsification.

FC-170 is also used to break certain emulsions.

FOAMING POWER IN WATER

(Ross-Miles Test, ASTM Method D 1173-53).

SURFACTANT	CONCENTRATION, %	TEMPERATURE, °C	FOAM HEIGHT, mm.	
			Immediate	After 5 Min.
FC-95	0.5	25	126	65
	0.5	50	169	97
	0.1	25	10	4
	0.1	50	103	17
FC-128	0.5	25	213	207
	0.5	50	259	233
	0.1	25	188	185
	0.1	50	232	210
	0.01	25	12	6
	0.01	50	15	7
FC-134	0.5	25	164	164
	0.5	50	232	222
	0.1	25	87	86
	0.1	50	159	149
	0.01	25	9	9
	0.01	50	14	12

FC-134 forms remarkably stable foams which, in some cases, will last for several hours. FC-95 gives foams especially stable in basic media. FC-95 and FC-98 give stable foams in acids. Foams in certain organic solvents can be formed by FC-134. FX-172 is excellent in strong acids (producing very stable "dry" foams) and in neutral aqueous media.

Although in many cases the foams produced by fluorochemical surfactants do not appear as good as foams produced by conventional surfactants, they have a demonstrated stability to heat and chemicals which makes them invaluable in these applications. Users of fluorochemical surfactants in foams have noted that many "unhealthy" looking foams were outstandingly effective in their applications.

Defoaming of certain organic systems is possible through the use of FX-161 and FC-170.

## FOAMING POWER IN OTHER MEDIA

Foams produced by many surfactants are sensitive to water hardness and pH of the solution. These conditions have been tested with FC-128 where it was found that FC-128 produces its maximum foam at a pH of about 10. As the pH decreases, the foam does also. At a pH of 7 or below, little or no foam is produced.

Hardness in water also reduces the foams in FC-128 solutions. This factor is easily overcome by the use of water softening or sequestering agents.

## WETTING POWER IN WATER

(Draves-Clarkson Test - Reference: American Association of Textile Chemists and Colorists Technical Manual and Yearbook).

<u>SURFACTANT</u>	<u>CONCENTRATION, %</u>	<u>TEMPERATURE, °C.</u>	<u>SINKING TIME sec.</u>
FC-95	0.1	25	over 300
	0.1	50	24
	0.1	70	35
FC-128	0.1	25	52
	0.1	50	13
	0.1	70	7
	0.05	25	223
	0.05	50	30
	0.05	70	19
FC-134	0.05	25	over 300
	0.05	70	25

FX-172 and FC-170 also show excellent wetting power. FX-172 is superior to FC-128 and FC-134 and considerably better than FX-161 and FC-170 in the wetting of fabrics at the 0.05-0.1% level.

## APPLICATIONS OF 3M BRAND FLUORO-CHEMICAL SURFACTANTS

New uses for this class of industrial surfactants are continually being found. Examples of their diverse applications are presented below. These commercial products will continue to find additional problems which they can solve effectively and economically. In this respect they are augmented by a growing list of experimental laboratory fluorochemical surfactants whose structures are specifically designed for an even wider area of consumer applications. A request to our laboratories with full particulars as to the system involved and effects desired will result in recommendations of the experimental or production fluorochemical surfactants most likely suited to your application.

The highly specific nature of these materials makes any generalizations regarding their use extremely difficult. The "Guide to the Use of 3M Brand Fluorochemical Surfactants" on the following page lists the most important properties of each commercial product. The major outstanding characteristic of each product is underlined for emphasis.

It should be added that in comparative evaluations of these materials, it is frequently observed that one surfactant performs best in one test while another is definitely superior in a different effect. It is then very advisable to evaluate surfactant blends. These blends provide the major effects required and, synergistically, can often show performance vs. concentration characteristics superior to either surfactant alone. These effects are available with blends of fluorochemical surfactants as well as with hydrocarbon/fluorochemical surfactant blends.

GUIDE TO THE SELECTION OF 3M BRAND FLUORO-CHEMICAL SURFACTANTS

TYPE FORM & APPEARANCE	FC-95	FC-98	FC-128	FC-134	FX-161	FC-170	FX-172
STABILITY to heat, chemicals others	anionic white, free flowing pd.	anionic white, free flowing pd.	anionic light tan, free flowing powder	cationic brown, waxy solid	anionic light colored solid	nonionic amber liquid	amphoteric viscous liq.
	390°C., excellent	350°C., excellent	good, good light sensitive	good, good	good, good	good, avoid strong bases	good, good
SUBSTANTIVE TO:	-----essentially nothing-----						
WETTING PENETRATION	fair	fair-good	good	poor	poor	very good	excellent
FOAMING	very good in acids & bases	good *	good in water, bases	good in water *	very low	good to poor	excellent in water, acids
METAL TREATMENTS; CORROSION	Descaling assist; increase acid attack on metals; cooling tower waters.	Aluminum bright dip	Alkaline cleaners - reduces smutting and decreases immersion time.	Inhibitor for aluminum in acid; pick- ling inh. for SS in H <sub>2</sub> SO <sub>4</sub> ; acid copper strike.	Inhibitor for metals; reduces smut in bases.	Pickle in- hibitor in 15% H <sub>2</sub> SO <sub>4</sub> with FC-134	Fair descal- ing assist.
OTHER USES	Increases acid activity in hetero- geneous rea- ctions; imp- roves flow of powders.	Improves flow of powders; similar to FC-95, but more solu- ble.	Leaf wetting; remove buffing compounds; dis- perse solids; concrete anti- foam; floor waxes; excellent leveling; wets plastics without stress cracking; emulsion stabili- zer.	Improves flow of solid part- icles; floor waxes; good leveling.	Monomolecu- lar films; reduces ice adhesion on metals, oil and chemical barrier film	Wets plastics without stress crac- king; fair antistat; extrusion aid; glass clean- ing; excell- ent surface tension depre- ssant; emulsion breaker.	Latex stabilizer; hydroscopic; evaporation inhibitor; excellent wetting agent.

\* FC-98 and FC-134, 50/50, good antifoam in organics

## CLEANERS

FC-98 possesses outstanding surface activity in highly alkaline systems. Concentrations as low as 0.002% (20 parts per million) in 25% caustic at 50°C. result in surface tensions of 30 dynes per cm. FC-95 and FC-128 are similarly effective.

### 1. ALKALINE CLEANERS

Commercial alkaline cleaners are highly selective and specific in their cleaning action. The high chemical stability and surface activity of the fluorochemical surfactants offer a new approach to improving the efficiency and broadening the selectivity range of these alkaline cleaners.

The following formulation is typical of an effective alkaline cleaner used to remove paint and other deposits from steel:

5% sodium hydroxide  
0.01% FC-128  
Temperature: 180°F.

The use of FC-128 in the cleaner requires half the immersion time and doubles its effective life.

An alkaline cleaning bath used on missile products uses 12 ppm FC-128. Cleaning speed is increased substantially at lower cost than the previous systems.

### 2. BOTTLE CLEANERS

These are used primarily where removal of aluminum labels is necessary. FC-98 at 5 ppm is superior to FC-95 primarily due to the requirement of supplying the cleaner in a concentrate form containing 50% NaOH + 5% gluconic acid. FC-95 is not sufficiently soluble to be used in this concentrate.

### 3. CONCRETE CLEANERS

Phosphoric-hydrochloric acid concrete cleaners show improved wetting of oily surfaces when FC-134 is used at levels of 25-100 ppm.

CORROSION INHIBITORS -  
CORROSION RESISTANT FILMS

It has been found that 3M Brand Fluorochemical Surfactants act as corrosion inhibitors in various metal-acid systems. They may be used as additives to the acid media or they may be applied as a thin, protective film on the metal prior to contact with the acid. In some cases, they exhibit a pronounced synergistic effect with organic corrosion inhibitors.

1. CORROSION INHIBITORS - GENERAL

Examples of corrosion inhibiting power of fluorochemical surfactants as shown in following data:

<u>SYSTEM</u>	<u>CORROSION RATE, MILS PER YEAR</u>
304 Stainless steel in 10% hydrochloric acid at 150°F.	175
Same, with 0.01% FC-134	21
Aluminum in 2N (7%) hydrochloric acid at 75°F.	1410
Same, with 0.01 FC-134	190

From the above, the effectiveness of FC-134 in reducing corrosion of aluminum and stainless steel in hydrochloric acid is evident.

The importance of inhibitor concentration and its means of addition is illustrated in the following data:

<u>SYSTEM</u>	<u>CORROSION RATE, MILS PER YEAR</u>
1100 Aluminum in 10% hydrochloric acid at 75°F.	2320
Same, with 0.02% FX-161 *	710
Same, with 0.2% FX-161 *	204
Same, with 2.0% FX-161 **	264

2. STEEL

A very smooth etch is obtained when FC-95 is used in conjunction with thio-urea or ethyl mercaptan as a corrosion inhibitor for cold rolled steel in 5% H<sub>2</sub>SO<sub>4</sub>. A synergistic effect is observed with ethyl mercaptan and FC-95.

The following table lists corrosion rates, in mils per year, for the various systems.

- \* FX-161 added as 10% solution in acetone
- \*\* No cosolvent used. FX-161 was not all being utilized

## 2. STEEL (continued)

### INHIBITION OF CORROSION ON COLD ROLLED STEEL BY 5% H<sub>2</sub>SO<sub>4</sub> AT ROOM TEMPERATURE

Inhibitor	CORROSION RATE		MILS PER YEAR
	0-4 hrs.	0-146 hrs.	146-1035 hrs.
None	456		
0.5% FC-95	275		
0.011% Thiourea		8.8	15.6
0.011% Thiourea + 0.5% FC-95		8.1	12.5
0.093% Ethyl mercaptan		4.9	13.4
0.093% Ethyl mercaptan + 0.5% FC-95		5.0	1.1

## 3. PROTECTIVE COATINGS

Properly applied, FX-161 and FC-134 will improve the tarnish resistance of many metals exposed to corrosive environments as long as flushing conditions do not prevail. These films have oil and water repellency and resistance to fingerprinting. On sealed, anodized aluminum, increased salt spray resistance results, and 3% hydrochloric acid will not attack the surface. Similar effects have been noted on other metals including steel, brass, and copper. An effective procedure for preparation of these thin films is described below:

### FX-161 on Aluminum

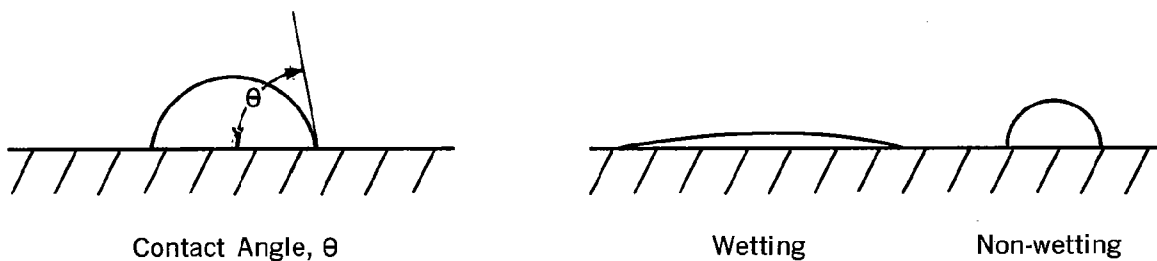
FX-161 is best applied to aluminum from acetone, alcohol, alcohol:water, or acetone:water solutions. Usually 0.5-2.0% solutions of FX-161 in isopropanol, ethanol, or acetone suffice to assure uniform coatings in single immersion dips of 10-30 seconds.

The aluminum to be dipped should first be cleaned and degreased completely (so that no water break is noted). Common degreasing solvents followed by a dip in dilute base and a complete flushing rinse will properly prepare the surface. Abrasive cleaning with "SCOTCHBRITE" following the degreasing is also recommended.

Slow removal of treated pieces from the bath will insure almost complete drainage. It is often possible to observe during withdrawal that the bath no longer "wets" the piece. Pieces should immediately be given a quick rinse in distilled water to remove any excess solution which, upon drying, might cause spotting. Heating the metal after treatment often improves the permanence and tenacity of the film.

The presence of the film is easily detected because of the auto-repellency of the bath (it no longer "wets" the metal) or by placing a drop of mineral oil on the surface. The drop should "bead up" showing a contact angle of 45° or more.





Note that where wetting occurs, the contact angle,  $\theta$ , is very small. A drop exhibiting non-wetting has a large contact angle and there is no tendency for the drop to spread.

FC-134 is applied in the same manner from the same solvents or from acidified water. It provides good tarnish resistance for copper and brass even in the presence of high humidity and  $H_2S$  or  $SO_2$ . Fingerprint and soiling resistance are also improved.

## DIPS, ETCHES, AND STRIKES

Unusual effects are frequently observed when the fluorochemical surfactants are included in etches, strikes, stripping solutions, and dips utilized to achieve matte, or bright finishes, and the like. Such effects may include either accelerated reaction rate, inhibition of reaction, improved appearance and uniformity of attack, protection from staining after dipping, and development of water repellent surfaces.

### 1. ALUMINUM BRIGHT DIPS

FC-95 in a concentration of 0.005% (50 parts per million) causes an increase in the reaction rate of conventional phosphoric-nitric acid-based aluminum bright dips.

<u>TREATMENT</u>	<u>REACTION RATES</u> mg per sq. in. per 15 sec.
Aluminum in 90% phosphoric acid + 10% nitric acid at 170°F.	5.2
Same, with 0.005% (50 ppm) FC-95	11.7

Improved brightening is imparted. Due to the formation of a layer of foam at the bath surface, the mist and spray normally associated with these baths is eliminated.

FC-98 has a similar effect in these dips in the 25-50 ppm range. Increased reaction rate, improved brightening, and reduced drag-out losses occur.

FC-134 and FX-161 are useful in 10-15% hydrochloric acid bright dips for aluminum. Here, however, reaction rate is sharply reduced allowing very smooth, blue-bright finishes on aluminum alloys which would normally be spotty after such a dip. (See Corrosion Inhibitors.)

### 2. COPPER AND BRASS BRIGHT DIPS

FC-134 and FX-161 produce brighter finishes with reduced drag-out and improved stain resistance when incorporated into a 50% sulfuric 25% nitric, trace hydrochloric acid, copper bright dip solution. Superior fingerprint resistance and a lower degree of staining in nitrous oxide fumes is imparted when the final rinses contain 0.05-0.1% of the same surfactant, plus 10-20% alcohol.

Similar advantageous effects are obtained with brass:

<u>TREATMENT</u>	<u>REACTION RATE</u> mg per sq. in. per 30 sec.
Brass in 50% Sulfuric acid + 25% Nitric acid at 75°F.	7.8
Same, with 0.02% FX-161	9.5

## 2. COPPER AND BRASS BRIGHT DIPS (continued)

Drag out losses were reduced 25% and the brass was brighter after treatment with the bright dip containing 0.02% (200 ppm) FX-161.

## 3. ALUMINUM ACID ETCH

Smoother etches are obtained in a 15% hydrochloric acid system at 150-180°F. when 0.01% FC-134 or FX-161 is used. The reaction rate is reduced. (See Corrosion Inhibitors). The same quantities of FC-95 or FC-98 will sharply raise the rate of etch. If this is desired, FC-98 is preferred since FC-95 can cause blackening.

## 4. ALUMINUM BASE ETCH

FX-161 and FC-134 can be utilized in 2% sodium hydroxide to alter etching rates. For example, at 75°F 0.01% FC-134 will reduce the rate of attack about 10% while 0.02% FX-161 can increase this rate by 0-20%. Due to the speed of reaction, little or no increase in rate is observed during a short etch with FX-161. A longer immersion is required to show the 20% rate increase. The use of either surfactant will reduce surface tension of the bath which should result in reduced losses due to drag out.

## 5. MAGNESIUM ETCHES

In a 20% chromic acid or in an 8% nitric - 2% sulfuric acid etch. 50 ppm FC-95 sharply raises the rate of attack.

FC-95 and FC-98 are also effective in mist suppression, elimination of gray powder, and in producing a more desirable microscopically pebbled coating in chrome pickle for magnesium. This is a sodium dichromate, nitric acid solution at 70-90°F. FC-95 is suggested at the 0.02% level; FC-98 at 0.08%.

## 6. COPPER STRIKES

FC-134 and FX-161 are effective in a 3% copper sulfate, 3% sulfuric acid, 0.5% Carbowax 20M formulation. Used at the 0.01-0.1% level, these surfactants allow a several-fold increase in immersion time for the same deposition of copper. This results in a plating which is considerably more durable.

## ELECTROLYTIC PROCESSES

### 1. CHROME PLATING (5, 6)

FC-95 and FC-98 possess extreme chemical resistance and surface activity in acid media. They are used to eliminate misting of decorative chrome plating baths, due to their ability to develop stable, fine foam blankets on top of the bath. In addition, drag out losses are reduced considerably and hazards to operating personnel are minimized. Decreased exhaust velocities and reductions in corrosion of exhaust systems is also attained.

These surfactants generally leave some pin holes in hard chrome plates but can be used to advantage in those applications where such an effect is tenable.

### 2. NICKEL PLATING

FX-161, 20 ppm, is useful as a non-foaming surfactant in the Watts type bright nickel plating bath. A significant reduction in surface tension of the bath is effected even at this low concentration.

### 3. ELECTROCHEMICAL MILLING

FC-128 has been shown effective in increasing the rate of metal removal in these applications at 10-100 ppm concentrations. Surface tension of a 25% sulfuric acid drilling electrolyte for a nickel alloy was reduced from 75 to 23 dynes/cm.

FX-161 at 10-100 ppm increases the rate of milling by a factor of about 2 without foaming. FC-128 causes foaming at these concentrations.

## EVAPORATION INHIBITION

Reducing losses from evaporation is readily accomplished through the use of fluorochemical surfactants. The following illustrates the types of effects obtained in this application.

1. 1,1,1-Trichloroethane - Tests were conducted in open beakers, not in a draft, at room temperature

<u>SAMPLE</u>	<u>% SOLVENT RETENTION after 114 hours</u>
Control	19
0.01% FX-172	90
0.1% FX-172	92

2. Methylene Chloride - Tests were conducted in wide-mouth jars at room temperature in the draft of a laboratory hood. Approximating commercial practice, a layer of water was floated on the top of the solvent. The height of each phase remaining after 2 days is recorded.

	<u>Surfactant Used</u>			
	<u>None</u>	<u>FC-128</u>	<u>FC-134</u>	<u>FX-172</u>
<u>Water Phase</u>				
Original thickness, mm.	11.5	14.0	14.0	6.5
Retention, %	0	21	11	0
<u>Methylene Chloride Phase</u>				
Original thickness, mm	57.5	75.5	76.0	75.5
Retention, %	22	98.7	98.7	91.4

A sample with no water layer and no surfactant evaporated to dryness within six hours. FC-128 and FC-134 were used at 100 ppm in the water phase; FX-172 at 100 ppm in the methylene chloride phase.

3. JP-1 Aviation Kerosene - This jet fuel was evaluated at 140°F in a laboratory hood for 84 hours.

<u>SAMPLE</u>	<u>% FUEL RETENTION after 84 hours</u>
Control	52%
100 ppm FX-172	63%

Inhibition of solvent evaporation with fluorochemical surfactants is highly specific as to the solvent type. Our laboratories will be pleased to assist in selecting the best surfactant for a particular solvent.

## PICKLING OR DESCALING OF STAINLESS STEEL

A 10% hydrochloric acid bath is effectively inhibited by 0.01% FC-134. Corrosion is essentially stopped 30-60 seconds after immersion.

## RESINS AND PLASTICS

The addition of Fluorochemical Surfactants to liquid resins reduces their surface tensions considerably. For example, the addition of 0.1% FC-134 to a liquid epoxy resin, reduced the surface tension to 34 dynes/cm. after 1 hour (from 48 dynes/cm. without surfactant). Such effects can be used to advantage to speed up the wetting of glass fibers or other fillers for the resin, to increase the rate of release of trapped air bubbles from these viscous resin systems, and to improve wetting of concrete, metal or other substrates to which they may be applied.

FC-170 in 0.1% concentration added to a phenolic resin (55% solids) from a 50:50 cellosolve:water solution, will reduce surface tension from 46.5 dynes/cm. to 24.2 dynes/cm. Improved speed and thoroughness of wetting asbestos or other fillers will result.

FC-128 and FC-170 may be used as wetting agents for water on polyethylene or other plastics. Unlike conventional surface active agents, they do not cause stress-cracking.

In many resin systems, water, oil, or soil resistance can be upgraded by the incorporation of small quantities of 3M Brand Fluorochemical Surfactants.

## SOLVENTS

FC-128 and FX-161 speed up the rate of removal of epoxy resin based paints from magnesium and aluminum by methylene chloride paint strippers. Solvent evaporation may be reduced by certain fluorochemical surfactants. This effect is highly selective depending on the particular solvent involved since the solubility of the surfactant in the solvent is an extremely important factor. See the Evaporation Inhibition Section.

## WATER TREATMENTS

### 1. Open Cooling Water Systems (8)

Laboratory studies indicated low corrosion rates would be obtained on mild steel in synthetic cooling water if FC-95 were used in conjunction with the noraml chromatic inhibitor. A summary of these 6-day test results of corrosion rate in mils per year follows:

#### CORROSION RATES OF MILD STEEL CORROSOMETER PROBES IN SYNTHETIC COOLING WATER

<u>INHIBITOR</u>	<u>CORROSION RATE, Mils Per Year</u>
None	60+
15 ppm Chromate*	7.5
25 ppm Chromate	4.6
40 ppm Chromate	3.3
5 ppm FC-95 + 15 ppm Chromate*	3.2
5 ppm FC-95 + 40 ppm Chromate	0.6
10 ppm FC-95 + 15 ppm Chromate*	2.9
10 ppm FC-95 + 25 ppm Chromate	0.4
10 ppm FC-95 + 40 ppm Chromate	0.4

\*pH 4.0-4.5; all others pH 6.5-7.5

As a result of this laboratory work, this effect was verified in a forced draft cooling tower for 300 ton air conditioning condenser. The cooling system operated twelve hours a day and had a capacity of 3000 gallons. The circulating water temperature averaged 85° F., and the heat exchanger surface temperature was about 105° F. Circulation rate was 900 gpm, and blow-down averaged 4 gpm initially. After the first 49 days of the 115-day test, blowdown was discontinued to increase the scaling potential of the water.

Results were monitored with mild steel Corrosometer probes. With no inhibitor, a corrosion rate of 73 mpy was obtained. A level of 60 ppm chromate for filming the system, followed by 15 ppm chromate for film maintenance gave a 2.5 mpy corrosion rate. The addition of 10 ppm FC-95 to the 15 ppm chromate reduced the rate to 1.2 mpy in 3 weeks and to 0.9 mpy in 7 weeks. Duplication of the synergistic effect noted in the laboratory work was excellent.

### 2. Closed Heating System (8)

Another application of fluorochemical surfactants in the water treatment field has been in closed heating systems. Their use as a corrosion inhibitor in these systems evolved during an investigation of a somewhat unrelated problem, that of wear on pump seal rings in closed heating systems.

2. Closed Heating System (8) (continued)

The graphite seal rings were wearing to failure at a rapid rate in systems inhibited with 300 ppm chromate. It was believed that most of this wear was due to the evaporation of the high chromate water at the seal surface forming abrasive chromate crystals.

One of the systems in which a failure had taken place was charged with the combination 25 ppm chromate - 10 ppm FC-95. New graphite seal rings were measured by micrometer and installed in the pumps. Mild steel and Admiralty brass probes also were installed in the system. Results of this evaluation are shown below:

CORROSION AND SEAL WEAR EVALUATION

<u>PERIOD</u>	<u>INHIBITOR</u>	<u>CORROSION RATE, mpy</u>		<u>SEAL RING WEAR</u>
		<u>Steel</u>	<u>Admiralty</u>	<u>mils/month</u>
8.5 months	None	1.1	-	1.6
6 weeks	300 ppm Chromate	0.4	-	28.6
8 months	25 ppm chromate + 10 ppm FC-95	0.1	0.1	0.4

It is seen that besides maintaining or reducing the low corrosion rate, the FC-95 - low chromate combination inhibitor markedly reduced seal wear rates. It is possible that the surfactant is also acting as a surface lubricant on the seal rings.



## WAXES

### 1. Alkaline Systems

Self-polishing floor waxes, based on polystyrene or acrylic emulsions are an excellent application for fluorochemical surfactants (7). These products require a high gloss, excellent durability to acid or neutral media, ease of application, stain and abrasion resistance, re-buffability, re-coatability, and ready removal with alkaline cleaners.

A typical formulation follows:

- 85 pbw - Emulsion polymer, 40% solids
- 5-10 pbw - Wax emulsion
- 5-10 pbw - Alkali soluble resin
- 2 pbw - Plasticizer
- 1 pbw - Fluorochemical surfactant, 1% solution

The emulsion polymer is the basic vehicle and can be acrylic, styrene, or a blend of the two. The wax emulsion gives buffability to the system. In the acrylic system, the alkali soluble resin acts as a leveling agent, while in the polystyrene system it acts as a carrier for the polymer and promotes ease of removal with alkaline cleaners. A plasticizer is required for the styrene systems - not for the acrylics. FC-128 or FC-134 are used to improve leveling, gloss, wetting power, and minimize streaking. At low concentrations (70 parts per million) no adverse effects on water resistance are noted.

### 2. "Lock and Key" Systems

The "Lock and Key" systems are those which employ resins which are insoluble in alkaline compounds, such as soaps and detergents, and which can be easily removed with acidic cleaners. The major advantages of these systems are:

- (1.) Scrubability with household cleaners without removing the finish.
- (2.) Higher gloss due to easy re-coatability, and
- (3.) Easy removal.

Formulation of these products is basically the same as the alkaline soluble systems, except that the resins are modified for acid solubility - alkali insolubility. Here, again, the fluorochemical surfactant does what normal surfactants could do in improving the compatibility and/or solubility of the various phases in each other, but at a level which will not cause sensitivity of the dried film to water or alkali

In applications of this type, it is recommended that both FC-128 and FC-134 be evaluated and compared.

## TOXICITY

Toxicity classifications of 3M Brand Fluorochemical Surfactants range from slightly toxic to moderately toxic. Listed below are LD<sub>50</sub> values obtained by determination of acute oral dosage to adult rats and mice.

<u>SURFACTANT</u>	<u>LD<sub>50</sub> gm/Kg of Body Wgt.</u>	<u>CLASSIFICATION</u>
FC-95	0.45	moderately toxic
FC-98	0.18	moderately toxic
FC-128	0.75	slightly toxic
FC-134	0.5	slightly toxic
FX-161	6.2	slightly toxic
FC-170	3.2	slightly toxic
FX-172	2.0	slightly toxic

Due care should be exercised in handling these materials until further information is available on their physiological properties.

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3. Ellison, A. H., and Zisman, W. A., J. Phys. Chem., 58:260 (1954)
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6. U. S. Patents 2,750,334 and 2,750,337.
7. U. S. Patent 2,937,098.
8. "Fluorochemical Surfactants Control Diverse Corrosion Problems," R. D. Burke, J. B. Kittredge, J. S. Spira and M. L. Vietor, presented at the 1963 Conference of the National Association of Corrosion Engineers, New York, N. Y., March 14, 1963.

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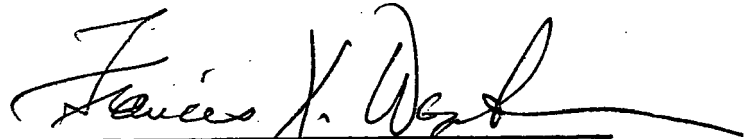
*International Research and Development Corporation*

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SPONSOR: E.I. duPont de Nemours and Company

MATERIAL: 

SUBJECT: Ninety-Day Feeding Study in the Rat.



Francis X. Wazeter, Ph.D.  
Director of Research  
International Research and  
Development Corporation

Collaborators:

R. H. Buller, Ph.D., Director of Pharmacology  
R. G. Geil, D.V.M., Director of Pathology

Date: November 30, 1965

  
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I. SYNOPSIS

In a 90-day feeding study, male and female albino rats were fed diets containing [REDACTED] at levels of 100, 500 or 2500 ppm. After 35 days of continuous feeding, the 500 and 2500 ppm. dietary levels were increased to 1000 and 5000 ppm., respectively for the remainder of the study. After the prescribed 90-day period of compound administration, representative animals were placed on a withdrawal study.

All rats appeared essentially normal with respect to behavior and appearance throughout the study.

No adverse effect on body weight gain was found at any dietary level employed in this study, both in the active compound administration phase and in the withdrawal period.

Average total weekly food consumption measured in grams/rat/week in those groups fed 100 and 500 - 1000 ppm. of [REDACTED] in the diet compared favorably with the control rats throughout the study. At the 2500 - 5000 ppm. dietary level, food consumption of the male rats ranged from 1.1 to 8.7 per cent less than control male rats, and food consumption of the female rats ranged from 5.2 to 16.4 per cent less than the female control rats. These differences were first noted in the 8th week for males and in the 4th week for females and continued throughout the treatment period.

No meaningful differences in food consumption were reflected by the treated groups of rats in comparison to the control group on the basis of grams of food consumed per day per kilogram of body weight.

No compound-related hematologic or biochemical changes were found

[REDACTED]

at the 100 and 500 - 1000 ppm. dietary levels of [REDACTED] However, slightly decreased values for erythrocyte counts, hematocrits and hemoglobin concentrations were found for males and females at the 2500 - 5000 ppm. level, particularly at the terminal (90-day) clinicopathology examination. Urinalyses were normal at all times.

Compound-related changes observed at the 90-day necropsy examination consisted of increased liver and kidney weight at the 1000 and 5000 ppm. dosage levels and pale yellowish livers in some male rats from the 500 - 1000 and 2500 - 5000 ppm. dosage levels. In histologic section, only livers from the 2500 - 5000 ppm. dosage level showed any change and this consisted of a slight hypertrophy of centrolobular hepatocytes. The increase in liver and kidney weights and centrolobular hepatocyte hypertrophy persisted with diminished magnitude through 21 days of compound withdrawal. Similar organ weight and histologic changes were observed at the 30 and 60-day interim sacrifices.

II. COMPOUND

The test compound was received from E. I. duPont de Nemours and Company, Wilmington, Delaware, on June 19, 1965. It was a brown amorphous solid in containers bearing the label [REDACTED] [REDACTED] Haskell No. 4212."

[REDACTED]



III. CLINICAL STUDIES:

A. METHODS:

1. General Procedure:

Eighty male (weighing from 45 to 64 grams) and eighty female (weighing from 47 to 63 grams) albino rats of the Charles River strain were used for this study.

The rats were housed individually in cages suspended above the droppings in an air-conditioned room throughout the study and were fed a diet of Purina Laboratory Chow for rats ad libitum. Water also was available at all times.

The animals were divided into one control group and three treated groups of 20 male and 20 female rats each.

The rats in each sex group were selected so that the average body weight of each group was similar to that of the other groups of the same sex.

2. Compound Administration:

[REDACTED] was incorporated into the standard powdered laboratory diet of Purina Laboratory Chow and offered to the treated groups of rats ad libitum. The test diet was freshly prepared each week and the compound-in-diet levels mixed so that the rats received [REDACTED] at dietary levels of 100, 500, or 2500 ppm. In the sixth week of compound administration those groups receiving 500 or 2500 ppm. were increased in concentration to dietary levels of 1000 or 5000 ppm., respectively. Those animals receiving 100 ppm. of [REDACTED] in the diet continued to receive this level throughout the 13-week study period.

The control groups of rats received the powdered diet of Purina Laboratory Chow, but without [REDACTED]

[REDACTED]

Following 13 weeks of compound administration rats in all groups were sacrificed and subjected to necropsy examination with the exception of certain selected animals from the control group and from the treated groups at the 1000 and 5000 ppm. dietary levels which were continued on study in a compound withdrawal phase. The withdrawal phase of this study will be reported in its entirety in a subsequent and separate report.

3. Observations:

The control and test animals were observed daily for mortality, alteration in general appearance and behavior, and signs of pharmacodynamic and/or toxic effects.

Body weights, food consumption, and food efficiency values were recorded for each rat weekly throughout the study.

4. Laboratory Tests:

a. Hematology:

Hematologic examination consisted of erythrocyte counts, total<sup>1</sup> and differential leucocyte counts, hematocrits<sup>2</sup>, and hemoglobin<sup>3</sup> concentrations. These studies were performed individually on 6 male and 6 female rats randomly selected in the control and each test group during the control period and again at 30, 60, and 90 days.

b. Urinalysis:

Urine samples were obtained from the same animals at the same time intervals used to obtain blood for hematology. Urinalysis

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<sup>1</sup> Coulter Particle Size Counter, Model A., Coulter Electronics, 590 W. 20th Street, Hialeah, Florida.

<sup>2</sup> Miller, S., Microcapillary Method, Textbook of Clinical Pathology, 1960, Williams and Wilkins Company, Philadelphia, Pa., p. 43.

<sup>3</sup> Miller, S., Cyanmethemoglobin Method, Textbook of Clinical Pathology, 1960, Williams and Wilkins Company, Philadelphia, Pa., p. 35.

consisted of qualitative tests for glucose,<sup>4,5,6</sup> bilirubin<sup>7</sup>, occult blood,<sup>8,9,10</sup> and albumin,<sup>4,11,12,13</sup> measurements of volume, pH<sup>14</sup> and specific gravity, and microscopic examination of the urinary sediments.

c. Biochemistry:

Biochemical examinations were conducted at the same intervals as for hematology. Serum transaminase (SGOT and SGPT)<sup>15</sup> and plasma alkaline phosphatase determinations<sup>16</sup> were performed on 6 male

- 
- 4 "Combistix" (Ames Reagent Strips).  
5 "Clinistix" (Ames Reagent Strips).  
6 "Clinitest" (Ames Reagent Tablets).  
7 "Ictotest" (Ames Reagent Tablets).  
8 "Hemastix" (Ames Reagent Strips).  
9 "Hematest" (Ames Reagent Tablets).  
10 "Occultest" (Ames Reagent Tablets).  
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13 Heller's Ring Test, Practical Physiologic Chemistry, Hawk, Oser and Summerson, 13th Ed., p. 830.  
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16 Marsh, W., Modified King-Armstrong Method, Clin. Chem. 5: 119, 1959.

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and 6 female rats randomly selected from the control and treated groups. The animals chosen for hematology values were not used for these biochemical determinations.

B. RESULTS:

1. General Behavior and Appearance:

No adverse changes in behavior or appearance were encountered that could be related to the administration of [REDACTED]

Animals in the control and all treated groups appeared essentially normal each day with the exception of an occasional rat in each group that exhibited slight nasal and/or ocular porphyrin discharge.

Other incidental findings, unrelated to compound administration, included one treated female animal (Rat #14374) at the 5000 ppm. dietary level which exhibited a swollen nose in the 13th week of study, one treated male (Rat #14298) at this dietary level which exhibited a mass on the flank from the 16th week (withdrawal period) until terminal necropsy examination, and one treated male (Rat #14324) at this same dietary level which exhibited destruction of the right eye, from the 15th week to the terminal (in the withdrawal period) necropsy examination.

2. Body Weights (Tables 1-8 and Figures 1 and 2):

a. Control:

The control animals maintained body weight curves which were consistent with those curves exhibited by control animals of the same age and strain maintained in these laboratories from time-to-time.

b. 100 and 500 ppm.\*:

Male and female rats at these dietary levels maintained body weights which paralleled closely those of their respective control groups.

\* 500 ppm. dietary level increased to 1000 ppm. in the 6th week of study.

c. 2500 ppm.\*\*:

No marked body weight changes occurred among male and female rats at this dietary level during the course of compound administration. Male animals in the 9th week exhibited a body weight gain 8.8 per cent less than control males. This difference in body weight gain persisted for the duration of the study period. During the withdrawal phase of this study the greatest decrease in body weight gain occurred. Even then, however, this difference was only about 10 per cent less than that of the male control animals.

Female treated animals in this group in the 7th week of study exhibited a weight gain which was 11.5 per cent less than that of the control female animals. This difference in body weight gain persisted for the duration of the treatment period. The greatest difference in body weight gain of the female group was noted in the 12th week of study at which time a difference of only 11.7 per cent occurred.

3. Food Consumption (Tables 10 and 11):

a. Grams/Rat/Week:

Average total weekly food consumption for male and female rats in those groups receiving 100 ppm. and 500 ppm.\* compared favorably with similar measurements obtained from the control group.

Treated rats receiving 2500 ppm.\*\* showed food consumption values less than those of control animals beginning in the 4th week for treated females and in the 8th week for treated males. This decrease in food consumption continued throughout the study period and ranged from 1.1 to 8.7 per cent for the males and 5.2 to 16.4 per cent for the females in this group. The decreased food consumption in this

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\* 500 ppm. dietary level increased to 1000 ppm. in the 6th week of study.  
\*\* 2500 ppm. dietary level increased to 5000 ppm. in the 6th week of study.

group continued in both sexes for the duration of the treatment period.

b. Grams/Kg./Day:

No biologically meaningful differences were observed on food consumption in the treated groups of rats when compared with the control group on a basis of grams/kg./day food consumed.

4. Survival (Table 9):

Other than for those animals subjected to interim necropsy examination at 30 and 60 days, all control and treated animals survived the course of study with two exceptions. One control female (Rat #14160) succumbed in the terminal (13th) week of study and one treated male (Rat #14212) at the 100 ppm. level of [REDACTED] succumbed in the 11th week of study.

5. Laboratory Tests:

a. Hematology:

No compound-related hematologic changes were found at the 100 and 500 ppm. dietary levels of [REDACTED]. At the 2500 ppm. level, group values for both sexes, with respect to erythrocyte count, hematocrit and hemoglobin concentration, generally were slightly lower than those for the control animals and rats at the 100 and 500 ppm. dietary levels of [REDACTED]. Although some changes in these parameters were seen at the 60-day interval of examination, they were overall more pronounced after 90 days of compound administration. It is of interest that inspection of these values for individual rats in the high dietary level groups failed to reveal marked changes for any given animal, that is, whereby that animal's value would tend to markedly lower the group average, but rather that lower values, with a relatively small spread from individual to individual, were found for most of these rats.

Group average values are summarized for male rats in Table 12 and for female rats in Table 13. Individual values for all male and female rats appear in Tables 14 through 17.

b. Plasma Biochemistry:

No compound-related changes were found at any period of examination with respect to serum alkaline phosphatase activity or serum glutamic pyruvic transaminase (SGPT) or serum glutamic oxalacetic transaminase (SGOT) activities.

Values obtained in these studies appear in Tables 18 through 21.

c. Urinalysis:

Urinalysis examinations failed to reveal changes which were considered to be related to treatment with the test compound. Results of these measurements appear in Tables 22 through 25.

IV. PATHOLOGICAL STUDIES

A. METHODS:

1. Gross Examination:

After 30 and 60 days of compound administration, 3 male and 3 female rats from the control and each treated group were sacrificed by exsanguination and subjected to necropsy examination. After 90 days of compound administration, 10 male and 10 female rats from the control, 1000, and 5000 ppm. dietary level groups and all surviving rats from the 100 ppm. group were sacrificed by exsanguination and subjected to necropsy examination. Three male and 3 female rats from the control, 1000 and 5000 ppm. groups were sacrificed and subjected to necropsy examination after a 21-day compound withdrawal period. (Other rats that remained on withdrawal beyond 21 days will be reported on in a separate report.)

At necropsy major organs were weighed and representative tissues from each rat were collected into 10 per cent neutral buffered formalin for subsequent histologic processing. At the 90-day sacrifice, specimens of brain, liver, kidneys, muscle, fat, spleen, testes and blood were pooled by sex and dietary group, frozen and forwarded to the sponsor. Specimens of liver from the interim and withdrawal sacrifice were also pooled by sex and dietary group, frozen and shipped to the sponsor.

Rats which died on study were also subjected to necropsy examination unless this was precluded by advanced autolysis.

2. Microscopic Examination:

The following tissues from each of 3 male and 3 female rats from the control and high dosage groups from the 30 and 60-day interim and 21-day withdrawal sacrifices and from each of 10 male and 10



female control and high dietary group rats from the 90-day terminal sacrifice were paraffin-embedded, sectioned, stained with hematoxylin and eosin and examined microscopically:

brain	heart	pancreas
spinal cord	spleen	liver
peripheral nerve	lymph node	kidneys
eye	thymus	urinary bladder
pituitary	bone marrow	testes or ovaries
thyroid	salivary gland	prostate or uterus
parathyroid	stomach	skeletal muscle
adrenal	small intestine	skin
lung	large intestine	bone

Sections of liver from 10 male and 10 female rats from the 1000 ppm. level - 90-day sacrifice rats were also processed as above and examined.

**B. RESULTS:**

1. Gross Pathology (Table 26) and Organ Weights (Tables 27 and 28):

Compound related gross changes observed at necropsy were limited to male rats from the 1000 and 5000 ppm. dietary level groups and consisted of pale, yellowish livers in some but not all male rats from the 5000 ppm. dietary level group and in a few rats from the 1000 ppm. dietary level group.

None of the rats dying on study died of compound related causes. Rat #14160 (Control) died of pneumonia. Autolysis precluded diagnostic necropsy of Rat #14212 (100 ppm.).

Compound related variations in organ weights were limited to the livers and kidneys of treated rats. At the 90-day sacrifice there was a moderate increase in actual and relative liver weights of the 1000 and 5000 ppm. dietary level rats. This increase was also seen in the 5000 ppm. dietary level rats at the 60-day interim

sacrifice and in the 2500 ppm. dietary level rats at the 30-day interim sacrifice. After 21 days of compound withdrawal, a slight increase in liver weight persisted at the 5000 ppm. dietary level.

Mean actual and relative kidney weights were slightly <sup>higher</sup> in the 1000 and 5000 ppm. dietary level rats at the 90-day sacrifice. Kidney weights were also slightly increased in the 1000 and 5000 ppm. level rats at the 60-day interim sacrifice and 21-day withdrawal sacrifice and in the 500 and 2500 ppm. level at the 30-day interim sacrifice. Although the values from the interim and withdrawal sacrifices represent only 3 rats per sex group, these variations in kidney weights always had a dietary-level relationship.

2. Histopathology (Tables 29 and 30):

Compound related histopathologic changes were found only in the livers of rats from the highest (2500-5000 ppm.) dietary level and consisted of slight hypertrophy of centrilobular hepatocytes. Affected liver cells had cytoplasm which was less coarsely granular and more homogeneous than the unaffected cells at the periphery of the liver lobules and in the livers of rats in the control and lower dietary levels. This change, to a slight degree was seen after 30 days at the 2500 ppm. level. After this group was raised to 5000 ppm., the change was more marked at the 60 and 90-day sacrifices. A very slight change persisted in the 5000 ppm. level male rats sacrificed after a 21-day compound withdrawal period. This liver change was always more marked in male rats and was seen only at the highest (2500-5000 ppm.) dietary level.

No lesions in other organs were considered to have been of compound related origin. No histologic basis was found for the slight increase in kidney weights in treated rats.

## Ninety-Day Feeding Study in the Rat.

TABLE 1. Individual Weekly Body Weights, Grams.

Rat Number	Control Period		Compound Administration Weeks									
	1	2	1	2	3	4	5	6	7	8	9	10
Control - Female:												
14142	55	93										
14143	54	68	129	149	175	185	195	Sacrificed				
14144	56	99	95	123	136	144	155	169	191	198	Sacrificed	
14145	56	83	134	163	187	207	249	260	285	296	306	314
14146	56	94	107	130	142	159	166	Sacrificed				
14147	55	91	136	161	184	196	217	Sacrificed				
14148	57	88	123	141	160	179	184	191	231	218	221	236
14149	60	101	124	149	170	178	197	214	249	241	252	268
14150	55	72	135	162	177	199	215	230	261	265	Sacrificed	
14151	58	83	103	126	149	164	191	213	243	243	252	271
14152	56	67	116	137	158	181	189	202	237	224	224	234
14153	50	52	114	133	154	171	189	203	235	226	Sacrificed	
14154	57	63	93	122	138	143	166	183	231	207	216	248
14155	57	71	82	117	141	156	172	195	237	225	233	246
14156	51	67	116	142	160	170	192	203	239	223	244	258
14157	58	64	115	139	161	161	196	210	243	241	250	261
14158	57	73	105	121	134	154	153	166	165	174	178	185
14159	58	54	106	132	153	149	172	190	232	244	215	236
14160	50	76	99	146	156	181	199	219	240	232	250	264
14161	54	48	124	147	162	157	189	206	221	215	239	256
			91	128	148	144	178	192	231	214	226	236
Mean	55	75	112	138	157	169	188	203	234	229	236	251

Ninety-Day Feeding Study in the Rat.

TABLE 1. Continued. Individual Weekly Body Weights, Grams.

Rat Number	Compound Administration Weeks								
	11	12	13	14*	15	16	17	18	19
<u>Control - Female:</u>									
14144	320	326	332						
14147	244	252	332						
14148	271	285	267						
14150	285	288	233						
14151	241	251	242						
14153	260	248	244						
14154	256	263	241						
14155	265	271	238						
14156	275	283	259						
14157	200	200	191						
14158	245	253	231	250	264	266	Sacrificed		
14159	277	281	282	289	299	310	Sacrificed		
14160	265	271	Died						
14161	241	251	257	261	268	276	Sacrificed		
14313				242	249	251	256	255	254
Mean	260	266	258	261	270	276	256	255	254

\* Initiation of withdrawal period (14th week).

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Ninety-Day Feeding Study in the Rat.

TABLE 2. Individual Weekly Body Weights, Grams.

Rat Number	Control Period			
	1	2	1	2
<u>Control - Male:</u>				
14162	64	99	143	185
14163	62	97	151	207
14164	60	100	89	169
14165	59	94	157	214
14166	55	92	133	175
14167	50	67	103	145
14168	61	98	147	189
14169	45	74	113	143
14170	58	95	136	185
14171	51	83	119	158
14172	60	83	152	205
14173	52	55	105	153
14174	51	64	113	155
14175	54	76	137	185
14176	57	76	136	180
14177	54	71	123	167
14178	62	64	117	153
14179	52	66	130	181
14180	53	67	120	172
14181	55	67	128	177
Mean	55	79	128	175

Company Sanitized. Does not contain TSCA CB1

Compound Administration Weeks

3	4	5	6	7	8	9	10
230	244	263	Sacrificed				
256	294	309	338	354	388	402	412
229	288	324	366	388	446	461	480
251	309	346	Sacrificed				
211	248	274	300	321	344	360	379
187	231	260	290	311	308	Sacrificed	
243	276	308	Sacrificed				
224	274	307	337	365	395	413	433
231	265	285	317	348	380	Sacrificed	
191	260	257	287	310	349	364	390
263	307	300	336	371	417	441	467
199	242	263	291	306	334	346	370
204	238	273	318	341	373	382	412
234	251	293	317	370	422	449	469
222	264	294	328	354	390	393	420
209	241	271	300	318	336	341	358
200	234	250	283	311	340	Sacrificed	
231	238	261	322	347	380	395	420
224	272	300	334	360	388	398	423
234	241	300	344	373	401	409	441
224	261	287	318	344	376	397	420

Ninety-Day Feeding Study in the Rat.

TABLE 2. Continued. Individual Weekly Body Weights, Grams.

Rat Number	Compound Administration Weeks								
	11	12	13	14*	15	16	17	18	19
<u>Control - Male:</u>									
14163	406	391	375						
14164	505	505	495						
14166	398	409	383						
14169	446	453	431						
14171	412	423	385						
14172	488	483	478						
14173	389	405	376						
14174	427	440	401						
14175	498	496	506						
14176	442	452	425						
14177	365	374	357	396	402	414	Sacrificed		
14179	435	455	461	479	489	500	Sacrificed		
14180	415	463	461	489	472	514	Sacrificed		
14181	451	463	472	476	466	497	506	515	522
14314				538	518	556	574	571	585
14315				512	503	523	539	544	559
Mean	434	444	429	482	475	501	540	543	555

\* Initiation of withdrawal period (14th week).

Company Sanitized. Does not contain TSCA CP!

Ninety-Day Feeding Study in the Rat.

TABLE 3. Individual Weekly Body Weight, Grams.

Rat Number	Control Period		Compound Administration Weeks									
	1	2	1	2	3	4	5	6	7	8	9	10
100 ppm. - Female:												
14182	56	86	112	131	151	167	175	Sacrificed				
14183	51	77	123	153	171	203	210	231	250	262	273	285
14184	59	82	113	126	145	165	182	194	211	218	Sacrificed	
14185	55	87	110	135	154	174	186	Sacrificed				
14186	57	101	144	176	210	252	251	275	290	303	307	331
14187	55	76	109	134	155	175	194	206	230	245	252	269
14188	55	80	111	124	139	149	162	Sacrificed				
14189	55	63	91	119	150	168	177	198	216	230	245	257
14190	55	88	116	137	154	176	180	200	211	225	225	242
14191	53	84	122	153	177	187	208	223	241	258	269	286
14192	54	60	106	127	141	172	174	185	193	207	215	234
14193	53	67	113	141	158	181	199	210	222	238	Sacrificed	
14194	61	73	90	124	148	160	179	203	221	234	Sacrificed	
14195	59	69	107	133	157	175	190	211	231	245	243	265
14196	57	65	105	147	159	179	183	199	213	227	236	267
14197	50	58	98	121	146	157	179	197	207	227	238	258
14198	62	76	123	141	167	195	202	220	237	253	259	270
14199	51	60	102	125	141	144	180	197	215	236	241	256
14200	53	70	123	147	165	184	197	212	224	244	246	264
14201	59	65	115	152	179	183	208	235	245	263	271	275
Mean	56	74	112	137	158	177	191	212	227	242	251	269



Ninety-Day Feeding Study in the Rat.

TABLE 3. Continued. Individual Weekly Body Weights, Grams.

Rat Number	Compound Administration Weeks								
	11	12	13	14	15	16	17	18	19
<u>100 ppm. : - Female:</u>									
14183	290	306	283						
14186	345	353	333						
14187	271	285	258						
14189	272	274	252						
14190	248	255	227						
14191	287	303	283						
14192	236	248	221						
14195	280	286	261						
14196	277	276	247						
14197	251	262	244						
14198	283	296	278						
14199	261	269	273						
14200	273	278	285						
14201	298	301	308						
Mean	277	285	268						

Company Sanitized. Does not contain TSCA CBI

Ninety-Day Feeding Study in the Rat.

TABLE 4. Individual Weekly Body Weight, Grams.

Rat Number	Control Period		Compound Administration Weeks									
	1	2	1	2	3	4	5	6	7	8	9	10
100 ppm. - Male:												
14202	58	79	119	168	214	246	270	Sacrificed				
14203	51	76	116	157	201	224	261	296	328	356	376	400
14204	62	87	149	203	256	310	343	371	407	448	467	495
14205	55	86	139	189	242	275	286	Sacrificed				
14206	63	103	160	215	255	298	320	346	373	403	405	434
14207	54	85	124	165	209	248	282	306	338	363	378	402
14208	55	81	120	159	199	211	228	Sacrificed				
14209	55	88	128	166	209	244	254	269	301	327	347	372
14210	60	86	142	197	252	303	330	354	388	398	417	454
14211	54	87	134	173	217	251	284	312	339	369	385	410
14212	50	60	108	122	130	168	211	250	256	296	317	300
14213	53	81	116	161	195	238	243	271	290	305	308	335
14214	61	83	149	205	267	284	326	352	377	399	407	441
14215	63	83	136	171	210	250	259	267	289	320	349	375
14216	53	70	126	164	217	271	308	247	377	407	420	457
14217	49	58	97	135	168	186	219	260	286	316	Sacrificed	
14218	58	64	100	142	199	247	286	330	369	398	321	454
14219	55	73	121	155	207	216	275	310	333	358	364	393
14220	61	68	110	136	196	213	264	287	307	329	Sacrificed	
14221	60	74	135	176	227	255	281	308	331	357	Sacrificed	
Mean	57	79	127	168	214	252	277	308	335	362	376	409

Company Sanitized. Does not contain TSCA CBI

Ninety-Day Feeding Study in the Rat.

TABLE 4. Continued. Individual Weekly Body Weight, Grams.

Rat Number	Compound Administration Weeks								
	11	12	13	14	15	16	17	18	19
100 ppm. - Male:									
14203	411	440	427						
14204	521	527	515						
14206	450	468	432						
14207	423	436	410						
14209	385	398	363						
14210	462	477	481						
14211	432	456	432						
14212	Died								
14213	353	368	348						
14214	446	456	448						
14215	385	405	383						
14216	482	501	500						
14218	473	496	507						
14219	406	421	430						
Mean	433	450	437						

Company Sanitized. Does not contain TSCA CBI

Ninety-Day Feeding Study in the Rat.

TABLE 5. Individual Weekly Body Weight, Grams.

Rat Number	Control Period		Compound Administration Weeks										
	1	2	1	2	3	4	5	6	7	8	9	10	
500 ppm. - Female:*													
14222	55	88	126	153	177	190	204	Sacrificed					
14223	60	83	116	134	154	172	181	195	202	213	Sacrificed		
14224	58	92	111	127	141	163	185	200	212	238	273	260	
14225	48	59	91	115	136	141	154	Sacrificed					
14226	57	73	115	140	161	182	188	204	215	223	235	247	
14227	57	92	130	157	178	201	210	230	244	256	264	276	
14228	55	86	130	154	166	178	197	Sacrificed					
14229	55	78	113	140	160	174	184	202	215	237	254	261	
14230	55	90	119	134	147	158	175	190	202	215	Sacrificed		
14231	52	76	114	153	184	195	219	237	254	272	287	303	
14232	50	57	100	134	147	174	186	204	216	234	241	253	
14233	52	64	105	125	130	148	160	170	181	191	201	204	
14234	59	73	110	129	140	140	164	176	187	195	Sacrificed		
14235	58	68	119	148	175	201	214	255	249	266	279	297	
14236	56	76	117	150	171	193	213	216	236	242	248	263	
14237	52	52	86	123	145	146	168	188	200	212	221	223	
14238	50	77	119	150	171	195	199	211	222	227	236	243	
14239	57	74	129	146	163	183	198	206	221	234	242	253	
14240	55	75	117	138	154	157	188	197	214	224	230	245	
14241	55	72	100	128	144	143	180	188	200	206	216	236	
Mean	55	75	113	139	157	172	188	204	216	229	243	255	

\* Dosage level increased in the 5th week of study to 1000 ppm.

Ninety-Day Feeding Study in the Rat.

TABLE 5. Continued. Individual Weekly Body Weights, Grams.

Rat Number	Compound Administration Weeks								
	11	12	13	14*	15	16	17	18	19
<b>500 ppm. - Female:</b>									
14224	267	279	263						
14226	246	257	239						
14227	244	282	269						
14229	265	277	270						
14231	310	314	268						
14232	255	269	251						
14233	212	216	207						
14235	304	312	299						
14236	262	272	254						
14237	232	244	234						
14238	253	256	242	263	276	278	Sacrificed		
14239	259	266	261	262	272	279	Sacrificed		
14240	256	263	259	274	278	280	Sacrificed		
14241	235	245	246	254	269	267	269	282	276
14306				254	266	270	272	284	284
14308				249	258	266	265	273	283
14320				234	236	241	246	250	253
Mean	257	268	254	256	265	269	263	272	274

\* Initiation of withdrawal period (14th week).

Company Sanitized. Does not contain TSCA CBI

Ninety-Day Feeding Study in the Rat.

TABLE 6. Individual Weekly Body Weight, Grams.

Rat Number	Control Period		Compound Administration Weeks									
	1	2	1	2	3	4	5	6	7	8	9	10
500 ppm. - Male:*												
14242	58	85	124	155	185	201	228	Sacrificed				
14243	57	92	136	178	226	269	297	325	351	374	387	410
14244	56	78	134	196	249	295	314	371	412	443	465	485
14245	63	96	136	172	213	251	265	Sacrificed				
14246	56	63	91	133	172	210	234	268	292	320	Sacrificed	
14247	58	72	120	162	209	266	305	342	365	386	407	430
14248	55	83	114	149	183	208	234	Sacrificed				
14249	62	90	134	176	224	270	291	325	356	381	378	415
14250	58	85	130	192	236	276	300	321	334	359	379	397
14251	52	75	124	165	207	239	272	297	328	357	Sacrificed	
14252	63	89	147	195	249	293	321	355	326	391	399	445
14253	62	78	144	189	230	238	299	327	355	379	Sacrificed	
14254	55	75	134	187	235	258	297	317	348	381	396	423
14255	57	70	123	162	188	221	250	281	306	329	345	368
14256	55	67	102	140	182	221	258	280	300	328	334	351
14257	53	81	117	154	192	219	268	306	339	369	365	390
14258	56	98	151	189	235	285	328	359	385	412	425	453
14259	52	63	105	145	191	225	249	274	303	330	346	372
14260	58	69	124	169	220	229	289	324	352	372	385	398
14261	59	79	151	203	271	285	324	377	408	436	446	467
Mean	57	79	127	170	215	248	281	321	345	373	390	415

\* Dosage level increased in the 5th week of study to 1000 ppm.

Company Sanitized. Does not contain TSCA CBI

Ninety-Day Feeding Study in the Rat.

TABLE 6. Continued. Individual Weekly Body Weights, Grams.

Rat Number	Compound Administration Weeks								
	11	12	13	14*	15	16	17	18	19
<u>500 ppm. - Male:</u>									
14243	445	435	422						
14244	509	525	499						
14247	449	471	447						
14249	434	451	441						
14250	412	431	411						
14252	453	463	434						
14254	452	437	428						
14255	382	396	380						
14256	369	377	348						
14257	408	415	390						
14258	478	499	477	517	496	545	Sacrificed		
14259	384	404	419	435	446	465	Sacrificed		
14260	400	417	434	452	458	476	Sacrificed		
14261	500	520	548	568	542	563	582	612	632
14321				507	487	532	538	559	544
14322				516	497	531	545	550	549
14325				549	525	562	569	586	577
Mean	434	446	434	506	493	525	559	577	576

\* Initiation of withdrawal period (14th week).

Company Sanitized. Does not contain TSCA CBI

Ninety-Day Feeding Study in the Rat.

TABLE 7. Individual Weekly Body Weights, Grams.

Rat Number	Control Period		Compound Administration Weeks									
	1	2	1	2	3	4	5	6	7	8	9	10
* 2500 ppm. - Female												
14262	52	87	120	138	164	174	181*	Sacrificed				
14263	53	79	119	145	166	182	200	230	246	264	Sacrificed	
14264	53	71	107	124	140	149	160	169	180	195	194	207
14265	55	72	104	127	143	154	165	Sacrificed				
14266	50	63	78	109	133	153	171	181	195	208	222	228
14267	55	76	113	134	147	163	175	188	202	216	223	232
14268	63	85	114	132	137	135	158	Sacrificed				
14269	56	86	115	130	148	163	173	182	192	200	202	216
14270	56	85	116	136	151	161	171	174	184	193	Sacrificed	
14271	60	95	134	161	180	187	212	225	240	246	256	260
14272	58	74	117	134	148	158	173	186	193	200	207	213
14273	51	66	116	140	161	169	185	197	202	210	220	226
14274	47	61	99	133	151	171	182	195	206	219	215	225
14275	57	75	122	144	168	174	198	207	222	233	Sacrificed	
14276	52	63	107	135	148	162	179	191	209	226	231	243
14277	52	66	106	134	152	151	168	188	196	208	205	223
14278	47	55	85	115	141	149	174	189	198	216	221	230
14279	61	76	123	144	160	155	185	206	211	222	230	236
14280	56	75	126	154	178	173	204	221	233	244	256	265
14281	53	66	108	132	146	142	171	180	189	193	207	207
Mean	55	75	113	136	154	161	179	196	207	218	221	229

\* Dosage level increased in the 5th week of study to 5000 ppm.

**Company Sanitized. Does not contain TSCA CBI**



Ninety-Day Feeding Study in the Rat.

TABLE 7. Continued. Individual Weekly Body Weights, Grams.

Rat Number	Compound Administration Weeks								
	11	12	13	14*	15	16	17	18	19
<b>2500 ppm. - Female:</b>									
14264	205	216	201						
14266	226	228	213						
14267	234	244	213						
14269	214	217	208						
14271	261	264	244						
14272	207	210	204						
14273	226	233	223						
14274	226	218	212						
14276	262	260	263						
14277	228	232	212						
14278	229	241	231	249	259	270	Sacrificed		
14279	236	239	237	257	254	269	Sacrificed		
14280	274	276	276	278	284	285	Sacrificed		
14281	214	216	218	222	239	245	252	258	257
14311				233	246	253	260	278	278
14326				233	236	239	246	258	261
14329				198	202	209	207	220	218
Mean	232	235	225	239	246	253	241	254	254

\* Initiation of withdrawal period. (14th week)

Company Sanitized. Does not contain TSCA CBI

Ninety-Day Feeding Study in the Rat.

TABLE 8. Individual Weekly Body Weights, Grams.

Rat Number	Control Period		Compound Administration Weeks										
	1	2	1	2	3	4	5	6	7	8	9	10	
* 2500 ppm. - Male													
14282	60	80	117	141	184	225	246	Sacrificed					
14283	53	84	124	151	193	237	268	299	321	343	354	374	
14284	55	76	129	182	219	277	310	351	382	416	440	454	
14285	55	84	133	178	226	260	283	Sacrificed					
14286	52	65	92	125	147	173	203	222	245	270	291	302	
14287	55	84	133	181	227	262	289	308	332	357	371	388	
14288	46	71	193	171	227	265	274	Sacrificed					
14289	60	98	151	194	249	298	322	373	404	437	Sacrificed		
14290	56	84	125	154	193	234	275	314	335	366	387	399	
14291	57	60	107	163	206	228	271	306	332	354	370	381	
14292	52	69	72	104	138	181	203	239	273	297	315	334	
14293	57	82	143	184	228	261	285	312	339	364	Sacrificed		
14294	58	82	133	165	207	231	266	291	315	337	339	355	
14295	64	92	149	189	228	265	294	341	378	416	Sacrificed		
14296	61	84	144	189	231	272	295	321	345	363	365	385	
14297	58	75	133	196	247	281	323	363	396	385	422	446	
14298	52	82	107	140	178	214	236	258	284	306	312	331	
14299	63	89	143	190	241	290	325	351	378	406	411	435	
14300	55	78	114	135	197	215	272	303	325	342	345	370	
14301	52	76	132	169	205	240	271	292	312	332	345	370	
Mean	56	80	129	165	209	245	276	308	335	358	362	380	

\* Dosage level increased in the 5th week of study to 5000 ppm.

Company Sanitized. Does not contain TSCA CBI

Ninety-Day Feeding Study in the Rat.

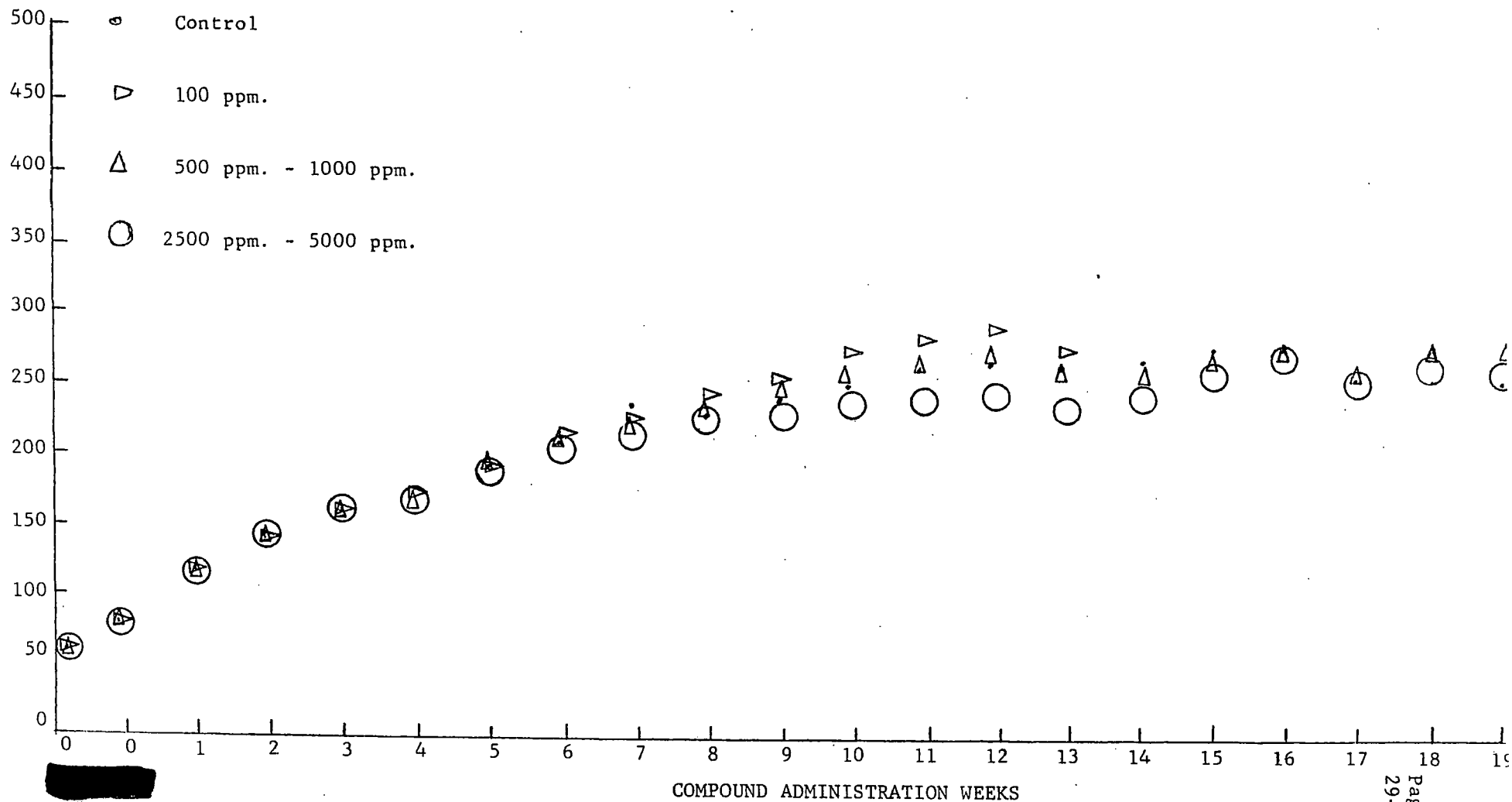
TABLE 8. Continued. Individual Weekly Body Weights, Grams.

Rat Number	Compound Administration Weeks								
	11	12	13	14*	15	16	17	18	19
<u>2500 ppm. - Male:</u>									
14283	394	410	393						
14284	478	507	497						
14286	318	328	316						
14287	400	414	401						
14290	415	425	413						
14291	401	410	385						
14292	344	373	357						
14294	367	388	364						
14296	405	418	412						
14297	468	480	456						
14298	349	465	327	341	339	342	Sacrificed		
14299	453	463	476	472	473	512	Sacrificed		
14300	381	395	407	416	429	442	Sacrificed		
14301	382	398	414	431	447	448	466	486	508
14327				362	377	390	497	427	433
14328				492	500	517	537	550	556
14324				461	478	489	500	516	531
Mean	397	420	401	425	435	449	500	495	507

\* Initiation of withdrawal period. (14th week)

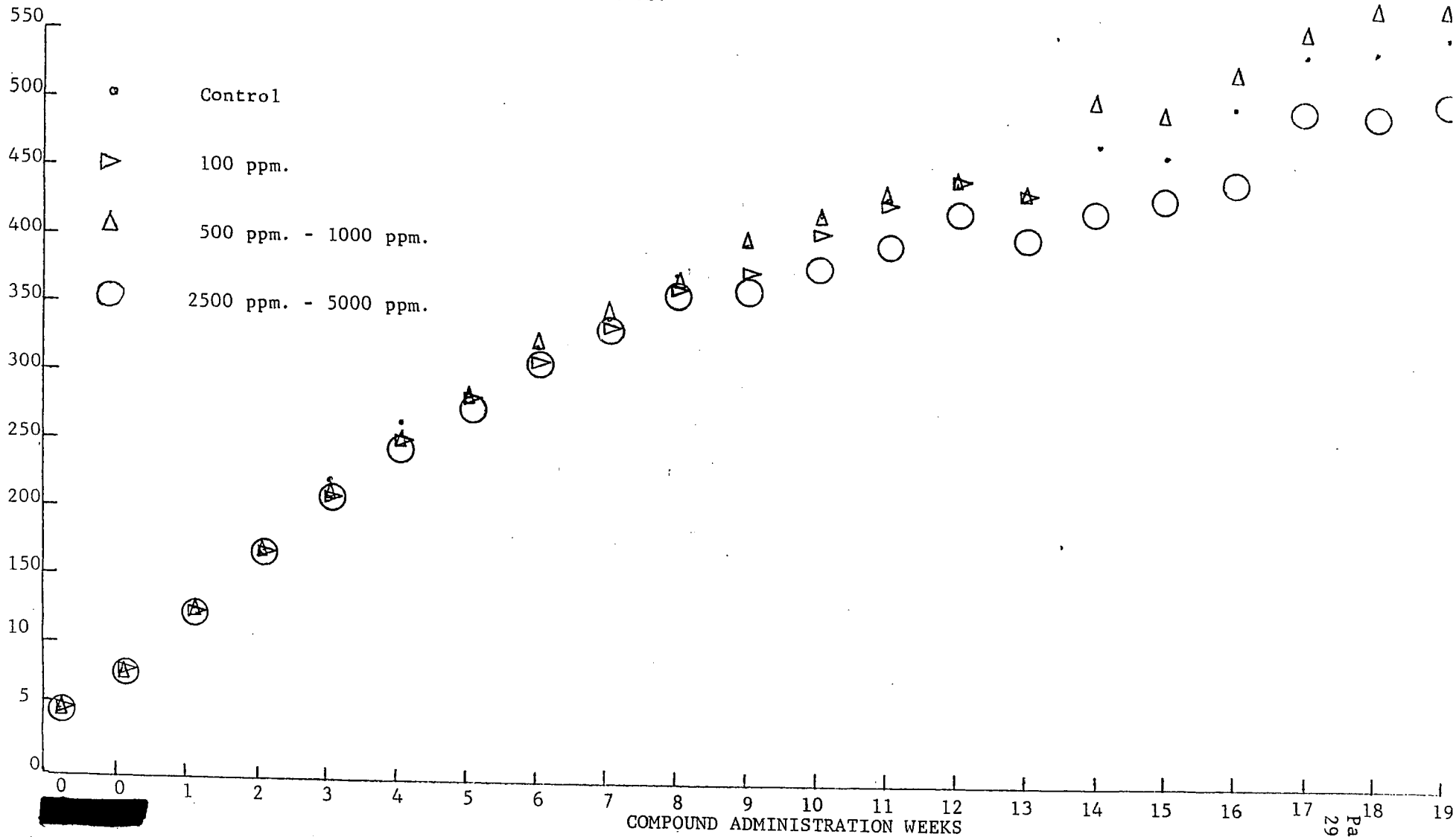
Company Sanitized. Does not contain TSCA CB1

FIGURE 1. Group Mean Body Weights. Female Rats



Company Sanitized. Does not contain TSCA CBI

FIGURE 2. Group Mean Body Weights. Male Rats.



Company Sanitized. Does not contain TSCA CBI

Ninety-Day Feeding Study in the Rat.

BLE 9. Mean Body Weights, Grams; Weight Ranges, Grams; and Survival: FEMALE RATS.

Compound Administration Weeks	Control			100 ppm.			500 ppm.*			2500 ppm.**		
	Mean Body Wt.	Weight Ranges	Survival	Mean Body Wt.	Weight Ranges	Survival	Mean Body Wt.	Weight Ranges	Survival	Mean Body Wt.	Weight Ranges	Survival
0	55	50-60	20/20	56	51-62	20/20	55	48-60	20/20	55	47-63	20/20
0	75	48-101	20/20	74	58-101	20/20	75	52-92	20/20	75	55-95	20/20
1	112	82-136	20/20	112	91-144	20/20	113	86-130	20/20	113	78-134	20/20
2	138	117-163	20/20	137	119-176	20/20	139	115-157	20/20	136	109-161	20/20
3	157	134-187	20/20	158	139-210	20/20	157	130-184	20/20	154	133-180	20/20
4	169	143-207	20/20	177	144-252	20/20	172	140-201	20/20	161	135-187	20/20
5	188	153-249	17/20	191	174-251	17/20	188	154-219	17/20	179	160-212	17/20
6	203	166-260	17/20	212	185-275	17/20	204	170-237	17/20	196	169-230	17/20
7	234	165-285	17/20	227	193-290	17/20	216	181-254	17/20	207	180-246	17/20
8	229	174-296	17/20	242	207-303	17/20	229	191-272	17/20	218	193-264	17/20
9	236	178-306	14/20	251	215-307	14/20	243	201-287	14/20	221	194-256	14/20
10	251	135-314	14/20	269	234-331	14/20	255	204-303	14/20	229	207-265	14/20
11	260	230-320	14/20	277	236-345	14/20	257	212-310	14/20	232	207-274	14/20
12	266	230-326	14/20	285	248-353	14/20	268	216-314	14/20	235	210-276	14/20
13	258	131-332	13/20	268	221-333	14/20	254	207-299	14/20	225	201-276	14/20
14	267	250-289	3/20			0/20	259	249-274	6/22	248	222-278	5/22
15	277	254-299	3/20				270	258-278	6/22	256	239-284	5/22
16	284	256-310	3/20				273	266-280	6/22	264	253-285	5/22
17			0/20				269	265-272	3/22	256	252-260	2/22
18							280	273-284	3/22	268	258-278	2/22
19							281	276-284	3/22	243	228-257	2/22

TE - Withdrawal period was initiated following 13-weeks of compound administration. Selected animals were continued into the period of withdrawal which continues at the writing of this report.

\* Dosage level in this group increased to 1000 ppm. in the 5th week of study.

\*\* Dosage level in this group increased to 5000 ppm. in the 5th week of study.

Company Sanitized. Does not contain TSCA CBI

ninety-Day Feeding Study in the Rat.

TABLE 9. Continued. Mean Body Weights, Grams; Weight Ranges, Grams; and Survival: MALE RATS.

Compound Administration Weeks	Control			100 ppm.			500 ppm.*			2500 ppm.**		
	Mean Body Wt.	Weight Ranges	Survival	Mean Body Wt.	Weight Ranges	Survival	Mean Body Wt.	Weight Ranges	Survival	Mean Body Wt.	Weight Ranges	Survival
0	55	45-64	20/20	57	49-63	20/20	58	52-63	20/20	56	45-64	20/20
0	79	55-100	20/20	79	58-103	20/20	80	63-98	20/20	80	60-98	20/20
1	128	89-157	20/20	127	97-160	20/20	127	91-151	20/20	129	72-193	20/20
2	175	143-214	20/20	168	122-215	20/20	169	125-203	20/20	165	104-196	20/20
3	224	187-263	20/20	214	130-267	20/20	215	172-271	20/20	209	138-249	20/20
4	261	231-309	20/20	252	168-310	20/20	248	201-293	20/20	245	173-298	20/20
5	287	250-346	17/20	277	211-343	17/20	281	234-324	17/20	276	203-325	17/20
6	318	283-366	17/20	308	250-371	17/20	321	268-377	17/20	308	222-373	17/20
7	344	306-388	17/20	335	256-407	17/20	345	292-412	17/20	335	245-404	17/20
8	376	308-446	17/20	362	296-448	17/20	373	320-443	17/20	358	270-437	17/20
9	397	341-461	14/20	376	317-467	14/20	390	334-465	14/20	362	291-440	14/20
10	420	358-480	14/20	409	300-495	14/20	415	351-485	14/20	380	302-454	14/20
11	434	365-505	14/20	429	353-521	13/20	434	369-509	14/20	397	318-478	14/20
12	444	374-505	14/20	446	368-527	13/20	446	377-525	14/20	420	328-507	14/20
13	429	357-506	14/20	437	363-515	13/20	434	348-548	14/20	401	316-497	14/20
14	447	242-538	7/23			0/20	472	234-568	8/24	378	198-492	9/25
15	443	249-518	7/23				461	236-542	8/24	387	202-500	9/25
16	465	251-556	7/23				489	241-563	8/24	399	209-517	9/25
17	469	256-574	4/23				496	246-582	5/24	394	207-537	6/25
18	471	255-571	4/23				511	250-612	5/24	410	220-550	6/25
19	480	254-585	4/23				511	253-632	5/24	418	218-556	6/25

NOTE - Withdrawal period was initiated following 13-weeks of compound administration. Selected animals were continued into the period of withdrawal which continues at the writing of this report.

\* Dosage level in this group increased to 1000 ppm. in the 5th week of study.

\*\* Dosage level in this group increased to 5000 ppm. in the 5th week of study.

Company Sanitized. Does not contain TSCA CBI

Ninety-Day Feeding Study in the Rat.

TABLE 10. FEMALE RATS: Mean Food Consumption, Grams/Rat/Week and Grams/Kilogram/Day; Compound Consumption as Miligrams/Kilograms/Day and Food Efficiency.

Compound Administration week	CONTROL FOOD			100 ppm. FOOD			CPD. mg/kg/d	500 ppm. * FOOD			CPD. mg/kg/d	2500 ppm. ** FOOD			CPD. mg/kg/d
	g/r/wk	g/kg/d	Eff.	g/r/wk	g/kg/d	Eff.		g/r/wk	g/kg/d	Eff.		g/r/wk	g/kg/d	Eff.	
0															
1	96.1	122.3		99.6	126.8		12.7	98.9	124.8		62.4	91.4	115.9		289.8
2	110.5	114.5	0.24	110.7	115.3	0.23	11.5	111.9	115.1	0.23	57.6	107.0	112.5	0.21	304.0
3	110.8	100.6	0.17	116.6	105.7	0.18	10.6	117.9	107.0	0.15	53.5	108.9	101.3	0.17	253.3
4	104.4	88.1	0.11	113.7	91.5	0.17	9.2	108.1	89.5	0.14	44.8	95.4	84.5	0.07	211.3
5	113.8	86.7	0.17	113.1	84.8	0.12	8.5	110.2	83.5	0.15	41.8	107.6	86.0	0.17	215.0
6	105.7	74.4	0.14	110.8	74.5	0.13	7.5	105.5	74.0	0.15	74.0	100.2	73.0	0.17	365.0
7	111.5	67.9	0.28	115.4	72.7	0.13	7.3	110.5	73.1	0.11	73.1	102.7	71.0	0.11	355.0
8	113.2	70.7	-.04	117.7	69.4	0.13	6.9	111.7	69.9	0.12	69.9	103.5	67.9	0.10	339.5
9	108.1	65.3	0.07	112.8	64.1	0.08	6.4	112.2	65.8	0.12	65.8	93.6	60.6	0.03	303.0
10	102.4	58.2	0.15	121.4	64.3	0.15	6.4	123.9	69.4	0.10	55.9	107.8	67.2	0.07	336.0
11	112.6	61.6	0.09	129.4	66.8	0.06	6.7	116.7	65.0	0.02	65.0	98.0	60.3	0.03	301.5
12	116.2	62.4	0.05	124.0	62.1	0.06	6.2	116.8	62.3	0.09	62.3	97.2	59.1	0.03	295.5
13	123.9	68.6	-.05	128.1	68.3	-.13	6.8	128.8	72.4	-.11	72.4	112.0	71.1	-.09	355.5
14	***58.0	54.3	+.09					***59.7	57.5	+.05		***66.8	63.7	+.20	
15	110.7	57.0	+.09					113.7	60.0	+.10		118.0	66.0	+.07	
16	114.0	57.4	+.06					111.0	58.2	+.03		115.2	61.4	+.07	
17								109.3	58.0	-.04		121.0	67.6	-.07	
18								111.0	56.8	+.01		119.5	63.8	+.10	
19								110.7	56.2	+.01		111.5	65.4	-.22	

NOTE - Withdrawal period was initiated following 13-weeks of compound administration. Selected animals were continued into the period of withdrawal which continues at the writing of this report.

\* Dosage level increased to 1000 ppm. in the 5th week of study.

\*\* Dosage level increased to 5000 ppm. in the 5th week of study.

\*\*\* Grams/rat/4 days.



Ninety-Day Feeding Study in the Rat.

TABLE 11. MALE RATS: Mean Food Consumption, Grams/Rat/Week and Grams/Kilograms/Day; Compound Consumption Mg./Kg./Day and Food Efficiency.

Compound Administration week	CONTROL			100 ppm.			CPD. mg/kg/d	500 ppm.*			2500 ppm. **				
	FOOD			FOOD				FOOD			FOOD			CPD. mg/kg/d	
	g/r/wk	g/kg/d	Eff.	g/r/wk	g/kg/d	Eff.		g/r/wk	g/kg/d	Eff.	g/r/wk	g/kg/d	Eff.		
0															
1	108.8	121.1		99.6	118.3		11.8	100.4	112.6		56.3	112.2	124.0		310.0
2	135.5	110.9		126.3	107.1	0.33	10.7	128.8	108.9	0.33	54.5	121.6	105.5	0.30	263.8
3	150.3	96.0		141.9	94.9	0.32	9.5	144.9	96.3	0.31	48.2	140.5	95.7	0.31	239.3
4	151.7	83.1		151.1	85.7	0.25	8.6	145.3	83.9	0.23	42.0	145.0	84.5	0.25	211.3
5	151.0	75.3	0.11	152.8	78.7	0.20	7.9	154.9	78.6	0.21	39.3	151.9	78.6	0.20	196.5
6	146.6	65.7	0.21	148.0	68.5	0.21	6.9	150.0	66.7	0.26	66.7	149.9	69.5	0.21	347.5
7	155.1	64.5	0.17	157.1	66.9	0.18	6.7	151.6	62.9	0.16	62.9	152.3	65.1	0.18	325.5
8	165.2	62.8	0.19	158.2	62.4	0.17	6.2	161.8	61.9	0.17	61.9	154.1	61.5	0.15	307.5
9	154.1	55.4	0.14	154.1	58.5	0.09	5.9	148.6	54.4	0.11	54.4	140.7	55.5	0.03	277.5
10	159.9	54.3	0.14	166.6	58.2	0.20	5.8	162.5	55.9	0.15	55.9	146.8	38.7	0.12	293.5
11	158.1	52.1	0.09	175.2	58.3	0.11	5.8	164.4	54.1	0.12	54.1	156.4	56.2	0.03	281.0
12	162.8	52.5	0.06	170.9	54.7	0.10	5.5	159.6	51.1	0.08	51.1	150.6	51.2	0.03	256.0
13	184.8	61.5	-.08	186.2	60.9	-.07	6.1	181.3	59.7	-.07	59.7	178.5	63.6	-.11	318.0
14	***80.4	45.0	+.13					***89.3	47.2	+.24		***79.6	52.6	-.17	
15	145.1	46.7	-.03					150.3	46.6	-.07		138.8	51.2	-.06	
16	159.0	48.8	+.14					169.0	49.3	+.17		138.4	49.6	+.09	
17	162.0	49.3	+.02					167.4	48.2	+.04		138.3	50.3	-.04	
18	149.3	45.2	+.01					145.0	40.5	+.10		143.5	50.0	+.11	
19	149.5	44.6	+.06					161.8	45.2	0		145.5	49.8	+.05	

NOTE - Withdrawal period was initiated following 13-weeks of compound administration. Selected animals were continued into the period of withdrawal which continues at the writing of this report.

\* Dosage level increased to 1000 ppm. in the 5th week of study.

\*\* Dosage level increased to 5000 ppm. in the 5th week of study.

\*\*\* Grams/rat/4 days.

Company Sanitized. Does not contain TSCA CB

Ninety-Day Feeding Study in the Rat.

TABLE 12. Summary of Hematologic Values for Male Rats.

Hematology	Compound Administration Month	Control	100 ppm.	500 ppm.*	2500 ppm.**
Hematocrit, %	0	47	45	45	44
	1	48	46	47	43
	2	48	45	45	38
	3	48	47	45	38
Hemoglobin gms./100 ml.	0	12.7	12.7	12.3	12.4
	1	14.6	14.1	14.5	13.3
	2	15.9	15.2	15.3	13.2
	3	15.7	15.0	14.7	12.0
Erythrocytes, $\times 10^6$ /cmm.	0	6.38	6.04	6.21	6.04
	1	6.60	6.74	6.43	6.31
	2	7.21	7.55	7.35	6.29
	3	7.16	7.05	7.06	5.90
Leucocytes, $\times 10^3$ /cmm.	0	10.75	9.90	12.48	11.30
	1	11.79	11.21	8.83	8.62
	2	11.22	9.40	11.24	11.93
	3	15.25	10.28	11.15	11.55
Neutrophils, % Seg., %	0	15	15	15	12
	1	7	17	14	11
	2	14	13	14	11
	3	17	20	16	8
Non-Seg., %	0	1	1	0	1
	1	0	0	0	0
	2	0	0	0	0
	3	0	0	0	0
Lymphocytes, %	0	83	82	83	85
	1	90	82	85	87
	2	83	84	84	87
	3	80	78	82	89
Monocytes, %	0	1	1	1	1
	1	2	0	1	1
	2	3	2	1	1
	3	2	0	1	1
Eosinophils, %	0	0	1	1	1
	1	1	1	0	1
	2	0	1	1	1
	3	1	2	1	2
Basophils, %	0	0	0	0	0
	1	0	0	0	0
	2	0	0	0	0
	3	0	0	0	0

\* Dosage level in this group increased to 1000 ppm. in the 5th week of study.

\*\* Dosage level in this group increased to 5000 ppm. in the 5th week of study.

Company Sanitized. Does not contain TSCA CBI

██████████ Ninety-Day Feeding Study in the Rat.

TABLE 13. Summary of Hematologic Values for Female Rats.

Hematology	Compound Administration Month	Control	100 ppm.	500 ppm. *	2500 ppm. **
Hematocrit, %	0	49	45	46	46
	1	49	46	45	43
	2	46	44	41	33
	3	45	47	40	33
Hemoglobin gms./100 ml.	0	13.3	12.3	12.4	12.9
	1	15.1	14.4	14.4	13.5
	2	15.8	14.8	14.3	11.7
	3	15.6	14.9	13.6	10.6
Erythrocytes, $\times 10^6$ /cmm.	0	6.63	6.38	6.23	6.51
	1	7.13	6.82	6.51	6.30
	2	6.74	6.81	6.57	5.44
	3	6.64	6.82	6.21	5.54
Leucocytes, $\times 10^3$ /cmm.	0	12.64	13.72	11.73	11.72
	1	9.41	7.40	9.08	11.53
	2	11.76	9.27	8.76	8.67
	3	10.96	9.04	10.14	11.38
Neutrophils, % Seg., %	0	14	11	14	11
	1	15	17	14	15
	2	15	13	17	12
	3	11	20	11	8
Non-Seg., %	0	0	0	0	0
	1	0	0	0	0
	2	0	0	0	0
	3	1	0	0	0
Lymphocytes, %	0	83	87	84	88
	1	81	81	84	84
	2	82	83	79	87
	3	85	77	88	90
Monocytes, %	0	1	2	1	1
	1	2	1	1	1
	2	2	2	2	1
	3	2	2	0	1
Eosinophils, %	0	1	0	1	0
	1	2	1	1	0
	2	1	2	2	0
	3	1	1	1	1
Basophils, %	0	0	0	0	0
	1	0	0	0	0
	2	0	0	0	0
	3	0	0	0	0

\* Dosage level in this group increased to 1000 ppm. in the 5th week of study.

\*\* Dosage level in this group increased to 5000 ppm. in the 5th week of study.

Company Sanitized. Does not contain TSCA CBI

Ninety-Day Feeding Study in the Rat.

TABLE 14. Individual Rat Hematologic Values during Control Period.

Rat No. & Sex	Hematocrit %	Hemoglobin gms./100 ml.	Erythrocytes (x10 <sup>6</sup> /cmm.)	Total Leucocytes (x10 <sup>3</sup> /cmm.)	Differential					
					Neutrophils		Lymphocytes	Monocytes	Eosinophils	Basophils
					Seg.%	Non-Seg.%	%	%	%	%
<b>Control:</b>										
14163M	46	12.6	6.09	7.29	7	1	92	0	0	0
14167M	50	14.0	6.90	14.00	13	0	84	3	0	0
14173M	45	12.3	6.42	12.82	31	0	69	0	0	0
14175M	46	12.7	6.01	10.32	9	2	85	4	0	0
14178M	48	12.6	6.94	11.52	21	1	78	0	0	0
14181M	45	12.0	5.91	8.57	9	0	91	0	0	0
Mean	47	12.7	6.38	10.75	15	1	83	1	0	0
14143F	48	13.2	6.14	16.18	9	0	90	1	0	0
14146F	48	12.5	6.14	12.34	19	0	80	1	0	0
14150F	48	13.2	6.89	14.18	20	0	75	3	2	0
14154F	50	13.9	6.98	13.03	8	0	91	1	0	0
14157F	49	13.1	6.40	9.74	7	0	92	0	1	0
14160F	49	13.6	7.22	10.38	21	0	78	0	1	0
Mean	49	13.3	6.63	12.64	14	0	84	1	1	0
<b>100 ppm.:</b>										
14204M	49	13.4	6.50	9.65	16	0	78	4	2	0
14208M	43	13.4	5.40	8.63	15	0	84	1	0	0
14210M	44	12.1	6.20	12.96	16	2	81	0	1	0
14205M	43	12.2	5.81	7.36	19	1	78	1	1	0
14216M	45	12.0	6.10	6.44	13	0	87	0	0	0
14220M	46	12.9	6.23	14.36	8	0	90	1	1	0
Mean	45	12.7	6.04	9.90	15	1	82	1	1	0
14184F	46	12.1	6.02	25.67	15	0	81	4	0	0
14189F	41	11.1	5.55	14.31	14	0	84	1	1	0
14191F	45	12.0	6.07	10.55	6	0	92	2	0	0
14194F	46	12.6	6.25	10.63	5	0	95	0	0	0
14198F	45	11.7	6.02	9.78	11	0	85	3	1	0
14201F	49	14.1	8.36	11.40	14	0	84	2	0	0
Mean	45	12.3	6.38	13.72	11	0	87	2	0	0

Company Sanitized. Does not contain TSCA CB1  
 Company Sanitized. Does not contain TSCA CB1

Ninety-Day Feeding Study in the Rat.

TABLE 14. Continued. Individual Rat Hematologic Values during Control Period.

Rat No. & Sex	Hematocrit %	Hemoglobin gms./100 ml.	Erythrocytes ( $\times 10^6$ /cmm.)	Total Leucocytes ( $\times 10^3$ /cmm.)	Differential					
					Neutrophils		Lymphocytes %	Monocytes %	Eosinophils %	Basophils %
				Seg.%	Non-Seg.%					
<u>500 ppm.:</u>										
14244M	45	11.5	6.33	10.25	22	0	77	0	1	0
14247M	44	12.3	6.66	13.42	21	0	78	1	0	0
14249M	45	12.3	6.02	12.58	5	0	94	0	1	0
14251M	47	13.0	6.22	10.76	15	0	82	3	0	0
14255M	43	12.1	6.04	10.19	16	0	81	2	1	0
14257M	46	12.6	5.99	17.68	10	0	88	1	1	0
Mean	45	12.3	6.21	12.48	15	0	83	1	1	0
14225F	48	13.3	6.74	23.52	24	0	75	1	0	0
14227F	47	11.3	5.83	9.47	10	0	88	1	0	0
14231F	44	12.8	6.51	10.19	10	0	89	1	1	0
14233F	45	12.5	6.15	10.35	8	0	90	1	0	0
14236F	45	12.4	6.13	8.58	19	0	79	2	0	0
14241F	45	12.0	6.01	8.26	15	0	82	0	2	0
Mean	46	12.4	6.23	11.73	14	0	84	2	1	0
<u>2500 ppm.:</u>										
14284M	44	12.5	6.03	15.14	18	1	79	2	0	0
14288M	46	12.9	6.48	10.32	12	0	88	0	0	0
14290M	44	12.4	6.01	13.88	10	1	87	1	0	0
14295M	43	12.3	5.72	8.92	11	0	86	2	1	0
14298M	42	12.0	6.22	10.30	13	1	85	0	1	0
14301M	44	12.0	5.77	9.21	8	0	90	2	1	0
Mean	44	12.4	6.04	11.30	12	1	85	2	0	0
14264F	46	13.0	6.25	11.62	13	1	85	1	1	0
14267F	46	12.9	6.58	11.10	7	0	91	2	0	0
14269F	45	12.2	6.13	8.96	11	0	87	1	1	0
14276F	51	13.7	6.76	11.35	9	1	89	0	1	0
14278F	43	12.2	7.16	17.47	8	0	90	2	0	0
14281F	45	13.4	6.20	9.84	18	0	81	1	0	0
Mean	46	12.9	6.51	11.72	11	0	88	1	0	0

Ninety-Day Feeding Study in the Rat.

TABLE 15. Individual Rat Hematologic Values at One Month.

Rat No. & Sex	Hematocrit %	Hemoglobin gms./100 ml.	Erythrocytes (x10 <sup>6</sup> /cmm.)	Total Leucocytes (x10 <sup>3</sup> /cmm.)	Differential					
					Neutrophils		Lymphocytes	Monocytes	Eosinophils	Basophils
					Seg.%	Non-Seg.%	%	%	%	%
<u>Control:</u>										
14162M	50	15.4	7.18	14.40	8	0	86	5	1	0
14165M	46	13.9	6.66	14.75	6	0	91	3	0	0
14168M	47	14.8	6.80	9.27	6	0	90	3	1	0
14171M	47	14.5	6.22	14.34	5	0	94	1	0	0
14174M	48	14.4	6.44	10.88	7	0	92	1	0	0
14177M	47	14.5	6.30	7.08	10	0	88	1	1	0
Mean	48	14.6	6.60	11.79	7	0	90	2	1	0
14142F	48	14.7	6.87	7.82	14	0	82	1	3	0
14144F	48	15.2	7.04	8.80	19	0	77	1	3	0
14146F	50	15.5	8.12	11.60	18	0	77	3	2	0
14148F	51	15.2	7.27	10.53	17	0	80	1	1	1
14150F	47	14.3	6.61	7.00	14	0	80	3	3	0
14153F	49	15.4	6.89	10.72	9	0	89	1	1	0
Mean	49	15.1	7.13	9.41	15	0	81	2	2	0
<u>100 ppm.:</u>										
14202M	44	13.6	6.32	19.70	7	0	90	1	2	0
14205M	48	14.8	6.73	7.10	25	0	74	0	1	0
14208M	47	14.6	7.08	8.22	30	0	69	0	1	0
14211M	45	13.7	6.54	6.78	9	0	88	1	2	0
14214M	49	13.8	7.03	11.69	23	0	76	0	1	0
14217M	45	14.1	6.72	13.77	7	0	92	1	0	0
Mean	46	14.1	6.74	11.21	17	0	82	0	1	0
14182F	47	14.2	6.69	9.15	6	0	91	1	2	0
14185F	44	13.6	6.68	3.97	46	3	49	1	1	0
14188F	46	14.3	6.91	8.64	11	0	87	0	2	0
14191F	48	14.8	7.10	6.98	18	0	81	1	0	0
14194F	45	13.9	7.28	6.53	11	0	86	2	1	0
14197F	46	14.4	6.30	9.15	8	0	91	1	0	0
Mean	46	14.4	6.82	7.40	17	0	81	1	1	0

Ninety-Day Feeding Study in the Rat.

TABLE 15. Continued. Individual Rat Hematologic Values at One Month.

Rat No. & Sex	Hematocrit %	Hemoglobin gms./100 ml.	Erythrocytes ( $\times 10^6$ /cmm.)	Total Leucocytes ( $\times 10^3$ /cmm.)	Differential					
					Neutrophils		Lymphocytes	Monocytes	Eosinophils	Basophils
					Seg.%	Non-Seg.%	%	%	%	%
<u>500 ppm.:</u>										
14242M	51	15.9	7.07	7.48	17	0				
14245M	45	13.6	6.03	7.84	14	1	82	0	1	0
14248M	47	14.6	6.77	9.75	9	0	84	1	0	0
14251M	46	13.9	6.17	11.62	12	0	90	1	0	0
14254M	47	14.7	5.99	8.16	10	0	87	1	0	0
14257M	48	14.5	6.55	8.13	20	0	90	0	0	0
Mean	47	14.5	6.43	8.83	14	0	79	0	1	0
14222F	45	14.4	6.55	11.49	8	0	85	1	0	0
14225F	46	14.3	6.83	9.65	10	0	90	2	0	0
14228F	45	14.4	6.20	7.68	11	0	89	1	0	0
14231F	45	14.2	6.42	9.95	21	1	87	0	2	0
14234F	46	14.4	6.69	5.79	20	0	77	1	0	0
14237F	45	14.5	6.35	9.90	14	0	79	1	0	0
Mean	45	14.4	6.51	9.08	14	0	83	1	2	0
						0	84	1	1	0
<u>2500 ppm.:</u>										
14282M	42	13.2	6.02	9.79	8	0				
14285M	44	13.9	6.57	11.49	11	0	91	0	1	0
14288M	42	12.6	5.93	8.82	9	0	88	1	0	0
14291M	45	13.7	6.68	6.71	18	0	90	1	0	0
14294M	41	12.5	6.14	6.54	11	0	82	0	0	0
14297M	45	13.9	6.50	8.34	10	0	85	3	1	0
Mean	43	13.3	6.31	8.62	11	0	89	0	1	0
14262F	44	13.6	6.12	19.29	9	0	87	1	1	0
14265F	42	13.3	6.07	9.08	16	0	88	3	0	0
14268F	46	14.6	7.19	6.28	14	0	83	1	0	0
14271F	41	13.0	5.96	13.43	18	0	85	0	1	0
14274F	40	12.9	5.95	8.84	16	0	80	1	1	0
14277F	44	13.8	6.53	12.23	16	1	83	1	0	0
Mean	43	13.5	6.30	11.53	15	0	81	2	0	0
						0	84	1	0	0

Ninety-Day Feeding Study in the Rat.

TABLE 16. Individual Rat Hematologic Values at Two Months.

Rat No. & Sex	Hematocrit %	Hemoglobin gms./100 ml.	Erythrocytes ( $\times 10^6$ /cmm.)	Total Leucocytes ( $\times 10^3$ /cmm.)	Differential					
					Neutrophils		Lymphocytes %	Monocytes %	Eosinophils %	Basophils %
				Seg.%	Non-Seg.%					
<u>Control:</u>										
14164M	47	15.4	7.02	11.44	7	0	90	2	1	0
14167M	49	16.5	7.43	13.95	12	0	83	5	0	0
14170M	52	16.6	7.47	11.11	5	0	93	1	1	0
14174M	47	15.6	7.07	9.73	31	0	64	5	0	0
14178M	47	16.0	7.00	11.22	19	0	81	0	0	0
14181M	48	15.4	7.24	9.88	8	0	90	2	0	0
Mean	48	15.9	7.21	11.22	14	0	83	3	0	0
14143F	47	15.4	6.67	11.86	32	0	67	1	0	0
14147F	48	16.2	6.78	15.15	9	0	87	3	1	0
14149F	49	16.4	7.26	10.64	9	0	88	1	2	0
14152F	41	15.2	6.36	10.92	22	0	78	0	0	0
14154F	50	15.8	7.03	12.83	7	0	90	3	0	0
14158F	42	16.0	6.31	9.16	8	0	90	2	0	0
Mean	46	15.8	6.74	11.76	15	0	82	2	1	0
<u>100 ppm.:</u>										
14204M	50	16.0	7.84	10.75	19	0	75	3	3	0
14210M	44	14.9	7.55	9.29	8	0	90	1	1	0
14213M	41	14.8	7.87	12.15	14	0	84	1	1	0
14217M	42	14.9	6.84	7.30	8	0	91	1	0	0
14220M	50	15.8	8.02	9.37	15	0	82	2	1	0
14221M	40	14.5	7.20	7.56	12	0	85	3	0	0
Mean	45	15.2	7.55	9.40	13	0	84	2	1	0
14184F	45	15.2	6.85	10.00	7	0	90	1	2	0
14190F	45	15.0	6.74	6.42	22	2	69	4	3	0
14193F	41	13.6	6.12	7.11	10	0	87	2	1	0
14194F	44	15.1	6.71	6.23	12	0	83	3	2	0
14196F	43	15.1	6.98	13.75	14	0	85	0	1	0
14200F	43	14.5	7.47	12.12	10	0	90	0	0	0
Mean	44	14.8	6.81	9.27	13	0	83	2	2	0



Ninety-Day Feeding Study in the Rat.

TABLE 16. Continued. Individual Rat Hematologic Values at Two Months.

Rat No. & Sex	Hematocrit %	Hemoglobin gms./100 ml.	Erythrocytes (x10 <sup>6</sup> /cmm.)	Total Leucocytes (x10 <sup>3</sup> /cmm.)	Differential					
					Neutrophils		Lymphocytes	Monocytes	Eosinophils	Basophils
					Seg.%	Non-Seg.%	%	%	%	%
<u>1000 ppm.:</u>										
14243M	42	15.4	7.71	9.97	23	0	75	1	1	0
14246M	46	15.2	7.11	12.04	13	0	84	1	2	0
14251M	47	15.4	7.42	12.47	11	0	87	1	1	0
14253M	46	14.8	6.94	8.86	15	1	82	1	1	0
14257M	46	15.9	8.03	10.67	6	0	93	0	1	0
14261M	42	15.1	6.89	13.43	13	1	82	2	2	0
Mean	45	15.3	7.35	11.24	14	0	84	1	1	0
14223F	42	15.5	7.28	8.34	16	1	81	0	2	0
14226F	43	14.2	6.63	6.64	18	0	72	5	5	0
14230F	41	14.3	6.28	10.28	8	0	92	0	0	0
14234F	37	13.6	6.63	6.17	24	0	74	1	1	0
14237F	43	15.8	6.85	13.43	28	0	62	8	2	0
14240F	38	12.4	5.77	7.68	6	0	94	0	0	0
Mean	41	14.3	6.57	8.76	17	0	79	2	2	0
<u>5000 ppm.:</u>										
14283M	42	13.3	6.33	11.96	7	0	92	0	1	0
14289M	34	12.0	6.12	17.50	23	0	76	1	0	0
14293M	35	12.3	6.09	11.20	17	0	81	1	1	0
14295M	36	12.7	6.13	6.97	8	0	91	0	1	0
14297M	38	13.6	6.08	14.49	4	0	94	1	1	0
14300M	44	15.0	6.96	9.46	9	1	87	3	0	0
Mean	38	13.2	6.29	11.93	11	0	87	1	1	0
14263F	37	13.0	5.60	11.57	10	1	87	2	0	0
14270F	29	11.2	5.31	5.16	14	0	84	2	0	0
14273F	35	12.1	5.64	9.55	8	0	91	1	0	0
14275F	33	11.8	5.47	8.90	8	0	92	0	0	0
14279F	30	10.4	4.93	6.78	17	0	81	1	1	0
14281F	33	11.7	5.68	10.07	17	0	82	0	1	0
Mean	33	11.7	5.44	8.67	12	0	87	1	0	0

Ninety-Day Feeding Study in the Rat.

TABLE 17. Individual Rat Hematologic Values at Three Months:

Rat No. & Sex	Hematocrit %	Hemoglobin gms./100 ml.	Erythrocytes (x10 <sup>6</sup> /cmm.)	Total Leucocytes (x10 <sup>3</sup> /cmm.)	Differential					
					Neutrophils		Lymphocytes	Monocytes	Eosinophils	Basophils
					Seg.%	Non-Seg.%	%	%	%	%
<u>Control:</u>										
14164M	53	16.1	7.52	17.93	15	0	82	3	0	0
14169M	46	16.0	6.68	21.59	6	0	94	0	0	0
14172M	47	15.6	7.14	14.62	23	0	70	5	2	0
14176M	48	15.6	6.79	13.86	15	0	84	1	0	0
14179M	47	15.2	7.53	10.06	17	0	80	1	2	0
14181M	50	15.6	7.10	13.45	24	0	74	2	1	0
Mean	48	15.7	7.16	15.25	17	0	80	2	1	0
14147F	46	16.9	6.64	13.71	7	1	89	2	1	0
14151F	43	15.1	6.52	7.16	18	2	80	0	0	0
14154F	45	15.2	6.66	12.03	15	1	79	1	4	0
14156F	41	15.1	6.33	7.12	9	1	88	1	1	0
14158F	47	16.0	7.02	11.53	5	0	91	2	2	0
14161F	45	15.2	6.69	14.18	13	0	84	2	1	0
Mean	45	15.6	6.64	10.96	11	1	85	1	2	0
<u>100 ppm.:</u>										
14203M	45	14.5	6.60	13.85	40	1	58	1	0	0
14206M	50	15.2	7.47	10.73	24	0	73	0	3	0
14210M	47	14.8	6.91	9.58	7	0	91	0	2	0
14215M	46	15.1	6.80	7.47	20	1	78	0	1	0
14218M	46	14.6	6.90	12.35	16	0	82	0	2	0
14219M	50	15.6	7.60	7.71	12	0	87	0	1	0
Mean	47	15.0	7.05	10.28	20	0	78	0	2	0
14183F	49	15.6	6.89	12.16	13	0	85	2	0	0
14186F	50	15.3	7.26	10.83	29	0	69	1	0	0
14190F	38	12.0	6.09	6.65	28	3	63	3	2	1
14195F	49	15.9	6.89	5.57	11	0	87	1	1	0
14197F	50	16.0	6.77	11.79	15	0	85	0	0	0
14200F	46	14.8	7.03	7.25	26	0	73	1	0	0
Mean	47	14.9	6.82	9.04	20	0	77	2	1	0

Company Sanitized. Does not contain TSCA CBI

Ninety-Day Feeding Study in the Rat.

TABLE 17. Continued. Individual Rat Hematologic Values at Three Months.

Rat No. & Sex	Hematocrit %	Hemoglobin gms./100 ml.	Erythrocytes (x10 <sup>6</sup> /cmm.)	Total Leucocytes (x10 <sup>3</sup> /cmm.)	Neutrophils		Differential			
					Seg.%	Non-Seg.%	Lymphocytes %	Monocytes %	Eosinophils %	Basophils %
<u>500 ppm.:</u>										
14244M	48	15.6	7.59	8.18	7	0	92	0	1	0
14247M	45	14.4	6.91	10.44	23	0	74	0	3	0
14252M	47	15.1	7.28	8.53	9	0	90	0	1	0
14255M	44	13.9	6.48	11.35	12	0	86	0	2	0
14257M	46	15.0	7.27	12.50	31	0	68	1	0	0
14260M	43	14.0	6.82	15.89	13	0	84	2	1	0
Mean	45	14.7	7.06	11.15	16	0	82	1	1	0
14224F	43	14.2	6.14	9.40	6	0	93	0	1	0
14227F	36	12.7	6.07	12.13	18	0	82	0	0	0
14232F	41	14.6	6.52	12.63	16	0	84	0	0	0
14236F	45	14.6	6.39	7.02	13	0	87	0	0	0
14238F	39	12.8	6.22	9.94	6	0	94	0	0	0
14240F	38	12.6	5.90	9.70	8	0	89	1	2	0
Mean	40	13.6	6.21	10.14	11	0	88	0	1	0
<u>2500 ppm.:</u>										
14283M	38	12.1	6.20	13.87	6	0	93	1	0	0
14287M	35	11.8	5.29	8.41	8	0	91	0	1	0
14291M	37	11.9	5.82	10.18	7	0	92	1	0	0
14294M	35	11.0	5.15	12.18	11	1	84	0	4	0
14297M	41	11.8	6.19	13.55	4	0	94	1	1	0
14300M	41	13.4	6.73	11.12	13	1	79	4	3	0
Mean	38	12.0	5.90	11.55	8	0	89	1	2	0
14266F	31	9.7	5.25	13.37	8	0	91	0	1	0
14269F	34	10.5	5.04	13.63	8	1	89	2	0	0
14272F	36	11.6	6.38	8.95	10	0	88	2	0	0
14276F	34	10.5	5.54	9.45	8	1	89	1	1	0
14278F	30	9.5	5.42	14.55	2	0	95	2	1	0
14281F	36	12.0	5.63	8.35	10	0	88	1	1	0
Mean	33	10.6	5.54	11.38	8	0	90	1	1	0

Company Sanitized. Does not contain TSCA CBI

**[REDACTED]** Ninety-Day Feeding Study in the Rat.

TABLE 18. Individual and Mean Results of Biochemical Studies During Control Period

Group	Rat No.	Alkaline Phosphatase	SGOT <sup>1</sup>	SGPT <sup>2</sup>
Control, Female	14142	78		
	14145	78		
	14149	75		
	14153	105		
	14156	93		
	14154	45		
	14144		34	24
	14147		27	24
	14152		29	23
	14155		28	23
	14158		28	23
	14160		30	25
	Mean		79	29
Control, Male	14162	99		
	14164	93		
	14166	57		
	14172	93		
	14177	93		
	14179	99		
	14170		29	23
	14168		30	22
	14174		31	24
	14176		27	22
	14180		32	23
	14171		29	26
	Mean		89	30
100 ppm., Female	14183	36		
	14188	48		
	14192	39		
	14199	48		
	14182	90		
	14196	45		
	14185		26	22
	14190		29	23
	14193		29	22
	14200		29	25
	14197		29	24
	14195		29	24
	Mean		51	29

<sup>1</sup> Serum Glutamic Oxalacetic Transaminase

<sup>2</sup> Serum Glutamic Pyruvic Transaminase

██████████ Ninety-Day Feeding Study in the Rat.

TABLE 18. Continued. Individual and Mean Results of Biochemical Studies During Control Period.

Group	Rat No.	Alkaline Phosphatase	SGOT <sup>1</sup>	SGPT <sup>2</sup>
100 ppm., Male	14202	57		
	14209	87		
	14211	63		
	14219	93		
	14214	96		
	14221	36		
	14203		29	23
	14206		27	23
	14212		30	23
	14215		29	23
	14218		26	24
	14217		30	26
	Mean		72	29
500 ppm., Female	14230	90		
	14224	87		
	14222	84		
	14240	96		
	14235	99		
	14232	90		
	14229		29	24
	14228		25	22
	14223		29	22
	14239		30	22
	14237		28	25
	14234		27	22
	Mean		91	28
500 ppm., Male	14242	51		
	14245	57		
	14248	54		
	14252	93		
	14254	39		
	14258	87		
	14243		27	22
	14246		29	22
	14250		26	24
	14253		29	22
	14256		27	22
	14259		25	23
	Mean		64	27

<sup>1</sup> Serum Glutamic Oxalacetic Transaminase

<sup>2</sup> Serum Glutamic Pyruvic Transaminase

██████████ Ninety-Day Feeding Study in the Rat.

TABLE 18. Continued. Individual and Mean Results of Biochemical Studies During Control Period

Group	Rat No.	Alkaline Phosphatase	SGOT <sup>1</sup>	SGPT <sup>2</sup>	
2500 ppm., Female	14271	39			
	14266	75			
	14262	57			
	14273	99			
	14274	78			
	14277	69			
	14268		27	22	
	14265		32	24	
	14263		28	21	
	14272		31	24	
	14275		28	23	
	14280		29	23	
	Mean		70	29	23
	2500 ppm., Male	14282	90		
14286		90			
14296		51			
14283		51			
14289		93			
14297		48			
14287			32	27	
14291			27	25	
14300			26	24	
14285			29	22	
14292			29	23	
14299			27	21	
Mean			71	28	24

<sup>1</sup> Serum Glutamic Oxalacetic Transaminase

<sup>2</sup> Serum Glutamic Pyruvic Transaminase

**[REDACTED]** Ninety-Day Feeding Study in the Rat.

TABLE 19. Individual and Mean Results of Biochemical Studies at One Month.

Group	Rat No.	Alkaline Phosphatase	SGOT <sup>1</sup>	SGPT <sup>2</sup>
Control, Female	14143	60	26	24
	14145	84	28	25
	14147	90	25	23
	14149	78	26	25
	14151	96	24	23
	14154	72	28	25
Mean		80	26	24
Control, Male	14163	72	25	24
	14166	53	24	21
	14169	129	25	24
	14172	63	28	23
	14175	114	27	24
	14178	129	24	24
Mean		93	26	23
100 ppm., Female	14183	45	26	23
	14186	75	26	24
	14189	117	25	24
	14192	54	25	22
	14195	108	25	23
	14198	114	25	24
Mean		86	25	23
100 ppm., Male	14203	84	25	24
	14206	129	27	25
	14209	132	27	24
	14212	105	26	23
	14215	105	26	24
	14218	126	25	23
Mean		114	26	24
500 ppm., Female	14223	52	28	25
	14226	99	28	25
	14229	84	26	23
	14232	117	27	23
	14235	123	28	26
	14238	102	26	25
Mean		96	27	25

<sup>1</sup> Serum Glutamic Oxalacetic Transaminase

<sup>2</sup> Serum Glutamic Pyruvic Transaminase

**[REDACTED]** Company Sanitized. Does not contain TSCA CB

██████████ Ninety-Day Feeding Study in the Rat.

TABLE 19. Continued. Individual and Mean Results of Biochemical Studies at One Month.

Group	Rat No.	Alkaline Phosphatase	SGOT <sup>1</sup>	SGPT <sup>2</sup>
500 ppm., Male	14243	72	27	23
	14246	132	27	25
	14249	132	26	24
	14252	96	26	24
	14255	87	25	23
	14258	132	25	25
	Mean		109	26
2500 ppm., Female	14263	84	28	24
	14266	51	27	24
	14269	60	26	23
	14272	72	28	24
	14275	84	28	24
	14278	45	28	25
	Mean		66	28
2500 ppm., Male	14283	72	28	23
	14286	117	27	24
	14289	117	24	23
	14292	69	25	23
	14295	105	26	23
	14298	81	25	24
	Mean		94	26

<sup>1</sup> Serum Glutamic Oxalacetic Transaminase

<sup>2</sup> Serum Glutamic Pyruvic Transaminase



██████████ Ninety-Day Feeding Study in the Rat.

TABLE 20. Individual and Mean Results of Biochemical Studies at Two Months.

Group	Rat No.	Alkaline Phosphatase	SGOT <sup>1</sup>	SGPT <sup>2</sup>
Control, Female	14144	60	27	21
	14148	73	26	24
	14150	59	25	22
	14151	56	19	22
	14153	67	25	22
	14155	67	26	22
Mean		64	25	22
Control, Male	14163	54	28	20
	14166	38	28	23
	14169	79	26	23
	14171	98	27	24
	14172	78	24	24
	14173	78	25	22
Mean		71	26	23
100 ppm., Female	14183	31	24	21
	14186	47	23	23
	14187	58	25	22
	14192	39	24	21
	14195	36	30	22
	14198	37	29	22
Mean		41	26	22
100 ppm., Male	14203	56	25	24
	14206	36	28	21
	14209	111	27	22
	14211	64	27	22
	14216	33	29	24
	14219	46	29	23
Mean		58	28	23
1000 ppm., Female	14224	62	26	21
	14227	58	27	22
	14229	64	29	24
	14232	63	26	22
	14235	46	31	22
	14236	27	25	21
Mean		53	27	22

<sup>1</sup> Serum Glutamic Oxalacetic Transaminase

<sup>2</sup> Serum Glutamic Pyruvic Transaminase

██████████ Company Sanitized. Does not contain TSCA CB

██████████ Ninety-Day Feeding Study in the Rat.

TABLE 20. Continued. Individual and Mean Results of Biochemical Studies at Two Months.

Group	Rat No.	Alkaline Phosphatase	SGOT <sup>1</sup>	SGPT <sup>2</sup>
1000 ppm., Male	14242	39	26	23
	14247	45	27	22
	14249	53	29	21
	14254	41	26	23
	14256	38	29	21
	14259	70	28	22
	Mean		48	28
5000 ppm., Female	14264	64	27	20
	14267	32	27	21
	14269	32	26	21
	14271	36	27	21
	14274	28	29	21
	14277	37	28	22
	Mean		40	27
5000 ppm., Male	14284	47	26	23
	14289	49	27	21
	14292	82	27	21
	14294	27	27	20
	14296	50	29	24
	14298	88	28	23
	Mean		57	27

<sup>1</sup> Serum Glutamic Oxalacetic Transaminase

<sup>2</sup> Serum Glutamic Pyruvic Transaminase

**[REDACTED]** Ninety-Day Feeding Study in the Rat.

TABLE 21. Individual and Mean Results of Biochemical Studies at Three Months.

Group	Rat No.	Alkaline Phosphatase	SGOT <sup>1</sup>	SGPT <sup>2</sup>
Control, Female	14144	26	30	23
	14248	59	23	24
	14155	21	26	22
	14157	20	28	22
	14159	31	27	23
	14160	28	28	26
Mean		31	27	23
Control, Male	14174	24	26	21
	14171	30	26	23
	14173	32	27	22
	14175	79	25	25
	14177	77	27	24
	14180	107	28	27
Mean		58	27	24
100 ppm., Female	14187	22	25	22
	14189	29	26	23
	14192	18	25	21
	14196	14	22	21
	14198	68	23	22
	14199	32	24	21
Mean		31	24	22
100 ppm., Male	14204	16	24	22
	14207	25	23	21
	14209	47	26	20
	14211	25	25	21
	14213	35	24	21
	14216	41	22	22
Mean		32	24	21
1000 ppm., Female	14226	39	23	24
	14229	53	26	24
	14233	73	25	23
	14235	55	25	25
	14237	50	25	22
	14239	22	23	20
Mean		49	25	23

<sup>1</sup> Serum Glutamic Oxalacetic Transaminase

<sup>2</sup> Serum Glutamic Pyruvic Transaminase

**[REDACTED]** Ninety-Day Feeding Study in the Rat.

TABLE 21. Continued. Individual and Mean Results of Biochemical Studies at Three Months.

Group	Rat No.	Alkaline Phosphatase	SGOT <sup>1</sup>	SGPT <sup>2</sup>
1000 ppm., Male	14243	41	29	27
	14250	43	25	22
	14254	15	26	19
	14256	26	23	20
	14259	87	23	23
	14261	34	22	22
	Mean		41	25
5000 ppm., Female	14264	26	25	22
	14277	19	25	21
	14271	18	25	20
	14274	18	26	22
	14280	26	28	22
	14279	16	25	19
	Mean		21	26
5000 ppm., Male	14284	49	27	24
	14286	66	25	24
	14290	37	24	21
	14292	75	24	22
	14296	45	24	22
	14298	33	24	21
	Mean		51	25

<sup>1</sup> Serum Glutamic Oxalacetic Transaminase

<sup>2</sup> Serum Glutamic Pyruvic Transaminase

Ninety-Day Feeding Study in the Rat.

BLE 22. Urinalysis Values for Male and Female Rats during Control Period.

Rat No.	Sex	Volume (ml.)	Appearance	pH	Specific Gravity	Albumin	Bilirubin	Glucose	Occ. Blood	Microscopic							Bladder Worn		
										WBC	RBC	Epi. Cells	Amor. Urates	Amm. Urates	Triple Phos.	Calcium Ox.		Bacteria	
Control:																			
43	F	5	S;C	7.0	1.028	N	N	N	N	occ									
46	F	12	LS;cl	6.9	1.030	N	N	N	N						F				F
50	F	3	S;cl	6.3	1.032	N	N	N	N					F	F				M
54	F	5	S;C	6.8	1.032	N	N	N	N			F			F				M
57	F	6	S;C	7.0	1.032	N	N	N	N				F		M				F
60	F	6	S;C	7.2	1.030	N	N	N	N						F				M
63	M	21	LS;C	6.5	1.030	N	N	N	N					F	M				F
67	M	1	Am;C	6.4	1.063	3+	N	N	N			occ		F					F
73	M	1	LAm;C	6.2	1.065	N	N	N	N	occ		1-3							F
75	M	4	S;cl	6.8	1.040	N	N	N	N				F			F			F
78	M	2	S;cl	6.4	1.045	N	N	N	N	occ					F	F			M
81	M	12	LS;C	9.0	1.030	N	N	N	N						F	F			M
															F				F
ppm.:																			
84	F	6	LS;cl	6.8	1.030	N	N	N	N	occ									F
89	F	7	LS;cl	8.8	1.030	N	N	N	N					F	F				F
91	F	4	S;C	6.5	1.030	N	N	N	N				F		F				F
94	F	3	S;cl	8.8	1.040	N	N	N	N	occ		F							M
98	F	13	LS;C	9.0	1.030	N	N	N	N			occ		M	F				F
01	F	3	LAm;C	6.8	1.045	N	N	N	N			occ			F				F
04	M	6	LS;cl	8.5	1.030	N	N	N	N					F	F				F
08	M	3	S;C	6.2	1.055	N	N	N	N			occ			F				M
10	M	2	S;C	6.5	1.042	N	N	N	N					F	M				F
05	M	4	S;cl	6.8	1.030	N	N	N	N				M		F				F
16	M	4	LS;C	7.0	1.032	N	N	N	N			occ	F		F				F
20	M	9	LS;cl	9.0	1.030	N	N	N	N						M				F
														F	M				F

S - Straw                      C - Clear                      N - Negative  
 LS - Light Straw              cl - Cloudy                    1+ - Trace-to-Slight              F - Few  
 Am - Amber                      2+ - Slight-to-Moderate          M - Many  
 LAm - Light Amber              3+ - Moderate                    occ - Occasional  
    4+ - Marked

Company Sanitized. Does not contain TSCA CB!

Ninety-Day Feeding Study in the Rat.

LE 22. Continued. Urinalysis Values for Male and Female Rats during Control Period.

t	Sex	Volume (ml.)	Appearance	pH	Specific Gravity	Albu- min	Bili- rubin	Glu- cose	Occ. Blood	Microscopic									
										WBC	RBC	Epi. Cells	Amor. Urates	Amm. Urates	Triple Phos.	Cal- cium Ox.	Bact- eria	Blad- der Worm	
ppm.:																			
25	F	1	S;C	6.3	1.045	N	N	N	N	occ									
27	F	3	S;cl	8.8	1.042	N	N	N	N				F			M		F	
31	F	3	S;C	6.3	1.045	N	N	N	N							F		M	
33	F	3	S;cl	8.0	1.040	N	N	N	N			1-2				F		F	
36	F	7	LS;cl	6.5	1.030	N	N	N	N				F		F	M		M	
41	F	3	LAm;C	6.0	1.045	N	N	N	N				F					F	
44*	M	4	S;C	6.2	1.040	N	N	N	N						F			F	
47	M	4	S;cl	9.0	1.035	N	N	N	N						F			F	
49	M	5	S;cl	8.3	1.032	N	N	N	N				F		M			M	
51	M	4	S;cl	6.7	1.045	N	N	N	N			occ			M			M	
55	M	6	LS;cl	6.7	1.030	N	N	N	N				F		F		occ	M	
57	M	4	S;cl	6.5	1.042	N	N	N	N					occ	M		occ	M	
) ppm.:																			
54	F	1	Am;C	6.5	1.065	N	N	N	N										
57	F	6	S;cl	6.9	1.030	N	N	N	N				F		M		M		F
59	F	21	LS;C	7.0	1.030	N	N	N	N	occ					F		occ		M
76	F	4	S;cl	7.3	1.030	N	N	N	N				F		occ		occ		F
78	F	4	S;C	7.4	1.033	N	N	N	N		occ				M		occ		M
31	F	2	DS;C	9.0	1.042	N	N	N	N				occ		M		M		F
34	M	2	S;C	7.0	1.048	N	N	N	N				F		F		F		F
38	M	2	S;cl	9.0	1.053	N	N	N	N						M		occ		M
10	M	5	S;cl	9.0	1.037	N	N	N	N				occ		M				M
15	M	10	LS;cl	9.0	1.030	N	N	N	N						M				F
18	M	12	S;cl	6.9	1.030	N	N	N	N	occ					F		M		M
11	M	1	Am;C	6.2	1.055	N	N	N	N	occ			occ		M		M		L

S - Straw                      C - Clear                      N - Negative                      F - Few                      \* Occasional uric acid  
 LS - Light Straw              cl - Cloudy                      1+ - Trace-to-Slight              M - Many  
 DS - Dark Straw                      2+ - Slight-to-Moderate              L - Loaded  
 Am - Amber                              3+ - Moderate                      occ - Occasional  
 LAm - Light Amber                      4+ - Marked

Company Sanitized. Does not contain TSCA CBI

Ninety-Day Feeding Study in the Rat.

LE 23. Urinalysis Values for Male and Female Rats at One Month.

t	Sex	Volume (ml.)	Appearance	pH	Specific Gravity	Albumin	Bilirubin	Glucose	Occ. Blood	Microscopic						Bladder Worms
										WBC	RBC	Epi. Cells	Amor. Urates	Amm. Urates	Triple Phos.	
<u>Control:</u>																
62	M	12	LS;c1	8.5	1.013	N	N	N	N							
65	M	12	LS;c1	7.9	1.016	N	N	N	N							
68	M	17	LS;c1	8.9	1.014	N	N	N	N							
71	M	4	LS;c1	7.2	1.048	N	N	N	N							
74	M	15	LS;c1	7.0	1.013	N	N	N	N							
77	M	9	LS;c1	6.9	1.020	N	N	N	N							
42	F	10	LS;c1	6.5	1.017	N	N	N	N							
45	F	7	S;c1	9.0	1.041	N	N	N	N							
46	F	15	LS;c1	7.1	1.012	N	N	N	N							
48	F	6	DAm;C	8.9	1.031	N	N	N	N							
50	F	12	LS;c1	6.2	1.011	N	N	N	N							
53	F	7	S;c1	6.9	1.035	N	N	N	N							
<u>ppm.:</u>																
102	M	7	S;c1	7.0	1.044	N	N	N	N							
105	M	26	LS;c1	7.3	1.008	N	N	N	N							
108	M	10	S;c1	6.9	1.021	N	N	N	N							
111	M	16	LS;c1	9.0	1.009	N	N	N	N							
114	M	14	S;c1	6.5	1.013	N	N	N	N							
117	M	10	S;c1	6.3	1.021	N	N	N	N							
182	F	4	S;c1	7.0	1.042	N	N	N	N							
185	F	13	LS;c1	7.1	1.011	N	N	N	N							
188	F	14	LS;C	9.0	1.007	N	N	N	N							
191	F	12	LS;c1	6.8	1.016	N	N	N	N							
194	F	7	S;c1	9.0	1.019	N	N	N	N							
197	F	9	S;c1	6.5	1.017	N	N	N	N							

S - Straw  
 LS - Light Straw  
 DAm - Dark Amber  
 C - Clear  
 cl - Cloudy  
 N - Negative  
 1+ - Trace-to-Slight  
 2+ - Slight-to-Moderate  
 3+ - Moderate  
 4+ - Marked

F - Few  
 M - Many  
 L - Loaded  
 occ - Occasional

Company Sanitized. Does not contain TSCA CB1

Ninety-Day Feeding Study in the Rat.

TABLE 23. Continued. Urinalysis Values for Male and Female Rats at One Month.

Rat No.	Sex	Volume (ml.)	Appearance	pH	Specific Gravity	Albumin	Bilirubin	Glucose	Occ. Blood	Microscopic							Bladder Worms
										WBC	RBC	Epi. Cells	Amor. Urates	Amm. Urates	Triple Phos.	Calcium Ox.	
<u>500 ppm.:</u>																	
14242	M	12	LS;c1	7.1	1.012	N	N	N	N								F
14245	M	10	LS;c1	9.0	1.023	N	N	N	N			1-2		F		M	F
14248	M	12	LS;c1	9.0	1.011	N	N	N	N							F	M
14251	M	13	LS;c1	6.1	1.015	N	N	N	N			1-2		F		F	M
14254	M	16	LS;c1	6.8	1.011	N	N	N	N				F			F	M
14257	M	17	LS;c1	8.9	1.012	N	N	N	N					F		M	M
14222	F	8	S;c1	6.5	1.024	N	N	N	N					F		F	M
14225	F	4	S;c1	6.5	1.048	N	N	N	N			occ				F	F
14228	F	6	S;c1	6.6	1.034	N	N	N	N	1-2						F	F
14231	F	14	S;c1	7.2	1.012	N	N	N	N			occ		F		M	F
14234	F	3	DS;c1	6.1	1.050	N	N	N	N	occ			F			F	F
14237	F	16	LS;c1	8.8	1.010	N	N	N	N					F		L	F
<u>2500 ppm.:</u>																	
14282	M	14	LS;C	7.1	1.023	N	N	N	N					F		M	F
14285	M	7	LS;c1	7.3	1.035	N	N	N	N			1-2		F		F	F
14288	M	17	LS;c1	8.8	1.015	N	N	N	N							L	F
14291	M	14	LS;c1	9.0	1.012	N	N	N	N					F		F	F
14294	M	4	DS;c1	8.9	1.042	N	N	N	N			occ				M	F
14297	M	17	S;c1	6.9	1.014	N	N	N	N				F			F	F
14262	F	4	S;c1	7.0	1.044	N	N	N	N			occ	F				F
14265	F	4	S;c1	6.1	1.040	N	N	N	N	2-3						F	M
14268	F	6	S;c1	6.5	1.025	N	N	N	N					F		F	F
14271	F	15	LS;c1	7.0	1.011	N	N	N	N				F			F	M
14274	F	14	S;c1	6.7	1.013	N	N	N	N			occ				F	M
14277	F	8	S;c1	7.1	1.017	N	N	N	N			occ		F		M	M

Code: S - Straw      C - Clear      N - Negative      F - Few  
 LS - Light Straw    cl - Cloudy      1+ - Trace-to-Slight      M - Many  
 DS - Dark Straw      2+ - Slight-to-Moderate      L - Loaded  
 3+ - Moderate      occ - Occasional  
 4+ - Marked



## Ninety-Day Feeding Study in the Rat.

TABLE 24. Urinalysis Values for Male and Female Rats at Two Months.

Rat No.	Sex	Volume (ml.)	Appearance	pH	Specific Gravity	Albu- min	Bili- rubin	Glu- cose	Occ. Blood	Microscopic						Blad- der Worms
										WBC	RBC	Epi. Cells	Amor. Urates	Amm. Urates	Triple Phos.	
<u>Control:</u>																
14164	M	28	LS;c1	7.0	1.015	N	N	N	N			M	M		M	F
14167	M	16	LS;c1	7.1	1.022	N	N	N	N			M	F		M	
14170	M	12	LS;c1	7.0	1.028	N	N	N	N			M	F		M	F
14174	M	25	LS;c1	9.0	1.014	N	N	N	N			F			F	
14178	M	24	LS;c1	7.5	1.012	N	N	N	N	occ		M	F		M	
14181	M	30	LS;c1	8.0	1.009	N	N	N	N			F		F	M	F
14143	F	6	LS;c1	7.5	1.030	N	N	N	N		occ		F	F	M	
14147	F	13	ES;C	7.7	1.022	N	N	N	N			F	M		M	
14149	F	6	S;c1	9.0	1.030	N	N	N	N			F	M		F	
14152	F	11	LS;c1	9.0	1.024	N	N	N	N			F	L		F	
14154	F	3	S;C	6.2	1.065	N	N	N	N	F		occ	M		F	
14158	F	4	S;C	6.0	1.047	N	N	N	N	F		F	F		F	
<u>100 ppm.:</u>																
14204	M	30	LS;C	7.8	1.016	N	N	N	N			M	M		F	F
14210	M	18	LS;c1	8.8	1.022	N	N	N	N		occ	F	F		M	F
14213	M	21	LS;c1	9.0	1.016	N	N	N	N			F	M		F	F
14217	M	17	S;c1	9.0	1.015	N	N	N	N			F			F	F
14220	M	30	LS;c1	8.0	1.008	N	N	N	N	occ		F	F		M	F
14221	M	23	LS;c1	9.0	1.011	N	N	N	N			F	F		F	
14184	F	14	S;c1	7.5	1.044	N	N	N	N	occ		M	M		F	
14190	F	20	LS;c1	8.4	1.008	N	N	N	N			F	M		F	
14193	F	24	LS;c1	6.4	1.006	N	N	N	N			M	occ		M	
14194	F	6	S;c1	9.0	1.028	N	N	N	N			M	F		M	
14196	F	5	S;c1	6.8	1.028	N	N	N	N			F	F		M	
14200	F	8	S;c1	6.2	1.030	N	N	N	N			F			F	

Code: S - Straw C - Clear N - Negative F - Few  
LS - Light Straw c1 - Cloudy 1+ - Trace-to-Slight M - Many  
2+ - Slight-to-Moderate L - Loaded  
3+ - Moderate occ - Occasional  
4+ - Marked

Company Sanitized. Does not contain TSCA CBI

Ninety-Day Feeding Study in the Rat.

TABLE 24. Continued. Urinaysis Values for Male and Female Rats at Two Months.

Rat No.	Sex	Volume (ml.)	Appearance	Specific pH	Gravity	Albu- min	Bili- rubin	Glu- cose	Occ. Blood	Microscopic							
										WBC	RBC	Epi- Cells	Amor. Urates	Amm. Urates	Triple Phos.	Cal- cium Ox.	Bact- eria
<u>1000 ppm.:</u>																	
14243	M	24	LS;c1	9.0	1.013	N	N	N	N				F	F	F		F
14246	M	16	LS;c1	6.8	1.017	N	N	N	N					F	M		F
14251	M	12	LS;c1	6.0	1.026	N	N	N	N	occ	F			F	F		F
14253	M	30	LS;C	7.8	1.010	N	N	N	1+					F	F		M
14257	M	26	LS;c1	7.2	1.013	N	N	N	N					F	F		M
14261	M	23	LS;c1	7.2	1.015	N	N	N	N					F	F		F
14223	F	11	S;c1	7.2	1.013	N	N	N	N				F	M	occ		M
14226	F	10	S;c1	9.0	1.030	N	N	N	N					F	M		F
14230	F	12	LS;c1	8.8	1.016	N	N	N	N				F	F	F		M
14234	F	7	S;c1	6.4	1.023	N	N	N	N			F		F	F		M
14237	F	14	LS;C	7.5	1.010	N	N	N	N					F	F		F
14240	F	15	LS;c1	7.8	1.009	N	N	N	N					F	M		M
<u>5000 ppm.:</u>																	
14283	M	14	LS;c1	9.0	1.021	N	N	N	N					M	F		F
14289	M	27	LS;c1	9.0	1.013	N	N	N	N					F			F
14293	M	10	S;c1	9.0	1.034	N	N	N	N			occ		F	F		F
14295	M	40	LS;c1	9.0	1.006	N	N	N	N					F	F		M
14297	M	27	LS;c1	9.0	1.012	N	N	N	N					F	F		F
14300	M	23	LS;c1	6.7	1.010	N	N	N	N					F	M		F
14263	F	12	S;c1	7.0	1.020	N	N	N	N					F	F		F
14270	F	10	S;c1	7.2	1.017	N	N	N	N				occ	F	F		F
14273	F	14	LS;c1	9.0	1.013	N	N	N	N					F	M		F
14275	F	5	Am;c1	8.5	1.024	N	N	N	N			F		M	M		M
14279	F	8	S;C	6.0	1.026	N	N	N	N					F	F		F
14281	F	4	S;C	8.2	1.028	N	N	N	N				F		F		F

Code: S - Straw      C - Clear      N - Negative      F - Few  
 LS - Light Straw    cl - Cloudy      1+ - Trace-to-Slight      M - Many  
 Am - Amber          2+ - Slight-to-Moderate      L - Loaded  
                          3+ - Moderate                      occ - Occasional  
                          4+ - Marked

Ninety-Day Feeding Study in the Rat.

TABLE 25. Urinalysis Values for Male and Female Rats at Three Months.

Rat No.	Sex	Volume (ml.)	Appearance	pH	Specific Gravity	Albumin	Bilirubin	Glucose	Occ. Blood	Microscopic						Bladder Worms	
										WBC	RBC	Epi. Cells	Amor. Urates	Amm. Urates	Triple Phos.		Calcium Ox.
<u>Control:</u>																	
14164	M	8	LS;cl	6.8	1.053	N	N	N	N					F	M		F
14169	M	7	S;cl	7.3	1.055	N	N	N	N				F	F	M		F
14172	M	11	LS;cl	9.0	1.035	N	N	N	N				F	F	F		F
14176	M	5	S;cl	6.5	1.066	N	N	N	N			1-2		F	M		F
14179	M	4	S;cl	7.8	1.058	N	N	N	N				F	F	M		F
14181	M	7	S;cl	6.9	1.052	N	N	N	N					F	M		F
14147	F	1	S;C	6.1	1.080	N	N	N	N					F	F		F
14151	F	2	S;C	6.3	1.069	N	N	N	N					F	M		F
14154	F	2	S;cl	7.4	1.065	N	N	N	N			occ		F	M		M
14156	F	2	S;cl	6.1	1.065	N	N	N	N				F	F	F		F
14158	F	1	S;C	6.0	1.080	N	N	N	N			1-2		F	F		F
14161	F	2	S;C	6.7	1.065	N	N	N	N	occ		occ		F	M		F
<u>100 ppm.:</u>																	
14203	M	7	S;cl	6.7	1.058	N	N	N	N					F	F		M
14206	M	7	LS;cl	7.0	1.048	N	N	N	N				F	F	F		M
14210	M	6	S;cl	9.0	1.055	N	N	N	N			2-3		F	F		F
14215	M	3	DS;cl	6.0	1.080	N	N	N	N					F	F		M
14218	M	9	S;cl	9.0	1.045	N	N	N	N					F	F		F
14219	M	6	LS;cl	6.3	1.049	N	N	N	N			occ			M		F
14183	F	1	S;C	6.0	1.080	N	N	N	N					F	F		F
14186	F	6	LS;cl	8.0	1.045	N	N	N	N					F	L		F
14190	F	6	S;cl	7.0	1.035	N	N	N	N			1-2	F	F	F		M
14195	F	6	LS;cl	6.2	1.035	N	N	N	N				F	F			M
14197	F	1	S;C	7.7	1.072	N	N	N	N					M	F		F
14200	F	2	S;cl	6.0	1.065	N	N	N	N	occ				F	M		F

Code: S - Straw      C - Clear      N - Negative      F - Few  
 LS - Light Straw    cl - Cloudy      1+ - Trace-to-Slight      M - Many  
 DS - Dark Straw      2+ - Slight-to-Moderate      L - Loaded  
 3+ - Moderate      occ - Occasional  
 4+ - Marked

Ninety-Day Feeding Study in the Rat.

TABLE 25. Continued. Urinalysis Values for Male and Female Rats at Three Months.

Rat No.	Sex	Volume (ml.)	Appearance	pH	Specific Gravity	Albu- min	Bili- rubin	Glu- cose	Occ. Blood	Microscopic								
										WBC	RBC	Epi. Cells	Amor. Urates	Amm. Urates	Triple Phos.	Cal- cium Ox.	Bact- eria	Blad- der Worms
1000 ppm.:																		
14244	M	10	LS;c1	7.0	1.035	N	N	N	3+*			occ		F	F		M	F
14247	M	6	S;c1	6.8	1.057	N	N	N	N						F		M	F
14252	M	5	S;c1	6.6	1.065	N	N	N	N		2-3			F	F		F	F
14255	M	4	S;C	6.0	1.065	N	N	N	N			occ	F	F	F		F	F
14257	M	10	LS;c1	9.0	1.037	N	N	N	N		2-3			F	M		M	M
14260	M	3	S;c1	6.7	1.073	N	N	N	N			1-2		F	M		F	F
14224	F	5	S;c1	9.0	1.045	N	N	N	N					F	F		M	F
14227	F	2	S;C	6.7	1.068	N	N	N	N				F		F		F	F
14232	F	3	S;c1	5.9	1.057	N	N	N	N	occ				F	F		M	F
14236	F	2	S;C	6.0	1.075	N	N	N	N	1-2			F	F			F	F
14238	F	2	S;c1	6.5	1.065	N	N	N	N					F	F		F	F
14240	F	1	S;C	6.2	1.078	N	N	N	N					F	F		F	F
5000 ppm.:																		
14283	M	2	Am;C1	6.2	1.075	N	N	N	N	occ			F	F	F		F	F
14287	M	4	S;c1	6.8	1.073	N	N	N	N					F	F		F	F
14291	M	6	S;c1	8.8	1.045	2+	N	N	N					F	M		M	F
14294	M	2	DS;c1	7.7	1.080	N	N	N	N					F	M		F	F
14297	M	4	DS;C	6.1	1.075	N	N	N	N			occ		F	F		F	F
14300	M	4	S;c1	6.8	1.065	N	N	N	N				F	F	F		F	M
14266	F	1	S;C	7.0	1.080	N	N	N	N					F	F		F	F
14269	F	2	S;c1	6.2	1.068	N	N	N	N	occ				F	M		F	F
14272	F	1	DS;C	5.8	1.065	N	N	N	N			occ	F	F	M		F	F
14276	F	1	DS;C	6.6	1.065	N	N	N	N			1-2		F	M		F	F
14278	F	2	S;C	6.3	1.065	N	N	N	N	1-2				F	M		F	F
14281	F	1	DS;C	6.0	1.080	N	N	N	N	1-2			F		F		M	F

Code: S - Straw C - Clear N - Negative F - Few  
 LS - Light Straw c1 - Cloudy 1+ - Trace-to-Slight M - Many  
 DS - Dark Straw 2+ - Slight-to-Moderate L - Loaded  
 Am - Amber 3+ - Moderate occ - Occasional  
 4+ - Marked \* Repeat 3+

( [REDACTED] )  
Ninety-Day Feeding Study in the Rat.

TABLE 26. Necropsy Observations. Thirty-Day Interim Sacrifice.

Animal Number	Sex	Organ	Comment
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Rats were normal except as noted below:

Control:

14165	M	thymus	Few petechial hemorrhages.
14146	F	uterus	Mild hydrouterus.

100 ppm.:

14202	M	lung	Moderate pneumonia.
14208	M	lung	Mild pneumonia.
14185	F	uterus	Mild hydrouterus.

2500 ppm.:

14288	M	spleen	Slightly enlarged.
14262	F	spleen	Slightly enlarged.

( [REDACTED] ) Ninety-Day Feeding Study in the Rat.

TABLE 26. Continued. Necropsy Observations. Sixty-Day Interim Sacrifice.

Animal Number	Sex	Organ	Comment
---------------	-----	-------	---------

Rats were normal except as noted below:

Control:

14149	F	lung	Few scattered gray areas.
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100 ppm.:

14221	M	liver lung	Pale. Gray pinpoint areas scattered throughout.
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5000 ppm.:

14289	M	liver testes	Slightly pale. Left testis approximately twice the size of right.
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14293	M	liver	Pale.
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14295	M	liver	Pale yellow in color.
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( [REDACTED] ) Ninety-Day Feeding Study in the Rat.

TABLE 26. Continued. Necropsy Observations. Ninety-Day Terminal Sacrifice.

Animal Number	Sex	Organ	Comment
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Rat were normal except as noted below:

Control:

14175	M	lung	Mild pneumonia.
14153	F	lung	Mild pneumonia.

100 ppm.:

14214	M	lung	Mild pneumonia.
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1000 ppm.:

14252	M	liver	Slightly yellowish.
14254	M	lung	Mild pneumonia.
14227	F	abdominal cavity	6 mm. firm hemorrhagic area in abdominal fat.
14233	F	lung	Mild pneumonia.
14237	F	lung	Mild pneumonia.

5000 ppm.:

14284	M	liver	Slight yellowish cast.
14286	M	lung	Mild pneumonia.
14291	M	liver	Slight yellowish cast.
14294	M	liver	Slight yellowish cast.
14297	M	liver	Slight yellowish cast.
14266	F	lung	Moderate pneumonia and bronchiectasis.
14274	F	kidney	Hydronephrosis, right kidney.

( [REDACTED] ) Ninety-Day Feeding Study in the Rat.

TABLE 26. Continued. Necropsy Observations. 21-Day Compound Withdrawal.

Animal Number	Sex	Organ	Comment
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Rats were normal except as noted below:

5000 ppm.:

14299	M	liver	Slightly pale.
14300	M	liver	Slightly pale.



( [REDACTED] ) Ninety-Day Feeding Study in the Rat.

TABLE 27. Mean Actual (Grams) and Relative (% Body Weight) Organ Weights.

Dietary Level	Sex	Terminal Body Wt. Gm.	Spleen		Liver		Adrenals		Kidneys		Testes/Ovaries	
			Gm.	%	Gm.	%	Gm.	%	Gm.	%	Gm.	%
<u>30-Day Interim Sacrifice:</u>												
0	M	315	0.97	0.308	14.11	4.479	0.039	0.010	2.66	0.844	2.99	0.949
0	F	202	0.70	0.347	8.74	4.327	0.069	0.034	1.73	0.856	0.089	0.040
100	M	268	0.70	0.261	11.65	4.347	0.044	0.020	2.22	0.828	2.69	1.003
100	F	212	0.69	0.326	7.28	3.434	0.054	0.025	1.56	0.736	0.092	0.040
500	M	243	0.62	0.255	10.06	4.136	0.037	0.015	2.30	0.946	2.74	1.127
500	F	188	0.47	0.250	8.26	4.394	0.054	0.029	1.86	0.990	0.097	0.050
2500	M	277	1.12	0.404	15.68	5.661	0.044	0.016	2.80	1.011	2.74	0.989
2500	F	173	0.59	0.341	8.65	5.000	0.068	0.039	1.85	1.069	0.123	0.070
<u>60-Day Interim Sacrifice:</u>												
0	M	355	0.76	0.214	13.02	3.668	0.062	0.017	2.62	0.738	3.07	0.865
0	F	233	0.52	0.22	8.25	3.541	0.070	0.030	1.72	0.738	0.133	0.060
100	M	338	0.79	0.234	10.91	3.228	0.048	0.014	2.53	0.749	3.30	0.976
100	F	230	0.52	0.226	8.00	3.478	0.065	0.028	1.70	0.739	0.132	0.060
1000	M	355	0.74	0.208	12.94	3.645	0.046	0.013	2.70	0.761	3.08	0.868
1000	F	210	0.54	0.257	7.89	3.757	0.075	0.036	1.92	0.914	0.162	0.080
5000	M	412	0.90	0.218	21.71	5.269	0.071	0.017	3.46	0.840	3.82	0.927
5000	F	232	0.62	0.267	12.04	5.190	0.067	0.029	2.36	1.017	0.145	0.060
<u>90-Day Terminal Sacrifice:</u>												
0	M	495	1.08	0.218	14.12	2.853	0.066	0.013	2.87	0.580	3.32	0.670
0	F	270	0.71	0.263	8.17	3.026	0.075	0.028	1.73	0.641	0.154	0.057
100	M	469	1.14	0.243	14.19	3.026	0.057	0.012	2.95	0.629	3.39	0.723
100	F	284	0.81	0.285	8.27	2.912	0.071	0.025	1.87	0.658	0.171	0.060
1000	M	452	0.97	0.215	15.02	3.323	0.060	0.013	3.05	0.675	3.39	0.750
1000	F	284	0.77	0.271	10.59	3.729	0.087	0.031	2.30	0.810	0.155	0.055
5000	M	425	0.98	0.231	20.38	4.795	0.057	0.013	3.33	0.783	3.37	0.793
5000	F	237	0.81	0.342	10.95	4.620	0.072	0.030	2.25	0.949	0.142	0.060
<u>21-Day Compound Withdrawal:</u>												
0	M	490	0.70	0.143	14.82	3.024	0.056	0.014	2.97	0.606	3.36	0.686
0	F	297	0.56	0.189	9.17	3.087	0.073	0.025	2.09	0.704	0.224	0.080
1000	M	507	0.91	0.179	17.41	3.434	0.065	0.013	3.36	0.663	3.59	0.709
1000	F	287	0.61	0.213	9.35	3.258	0.077	0.027	2.25	0.784	0.177	0.060
5000	M	440	0.61	0.139	16.55	3.761	0.058	0.013	3.18	0.723	3.32	0.755
5000	F	277	0.57	0.206	10.75	3.881	0.083	0.030	2.45	0.884	0.162	0.060

Thymus		Heart		Lung		Thyroid		Brain		Pituitary	
Gm.	%	Gm.	%	Gm.	%	Gm.	%	Gm.	%	Gm.	%
0.90	0.286	1.15	0.365	1.63	0.517	0.023	0.007	1.86	0.591	0.009	0.003
0.68	0.337	0.78	0.386	1.32	0.653	0.021	0.010	1.79	0.886	0.007	0.003
0.73	0.272	0.98	0.366	1.56	0.582	0.027	0.010	1.74	0.649	0.009	0.003
0.47	0.222	0.69	0.325	1.25	0.590	0.018	0.008	1.74	0.820	0.009	0.004
0.67	0.276	0.94	0.386	1.58	0.650	0.024	0.010	1.84	0.757	0.008	0.003
0.71	0.378	0.77	0.410	1.28	0.681	0.024	0.013	1.74	0.926	0.008	0.004
0.74	0.267	1.06	0.383	1.65	0.596	0.018	0.006	1.96	0.708	0.006	0.002
0.59	0.341	0.68	0.393	1.34	0.774	0.018	0.010	1.80	1.040	0.004	0.002
0.64	0.180	1.41	0.397	1.91	0.538	0.026	0.007	1.61	0.454	0.014	0.004
0.57	0.244	0.86	0.369	1.16	0.498	0.024	0.010	1.79	0.768	0.011	0.005
0.79	0.234	1.17	0.346	1.70	0.503	0.027	0.008	1.88	0.556	0.010	0.003
0.50	0.217	0.76	0.330	1.44	0.626	0.036	0.016	1.75	0.761	0.012	0.005
0.66	0.186	1.09	0.307	1.92	0.501	0.022	0.007	2.00	0.563	0.010	0.003
0.56	0.267	0.81	0.386	1.41	0.671	0.033	0.016	1.87	0.890	0.010	0.005
0.90	0.218	1.36	0.330	2.06	0.500	0.030	0.007	1.98	0.481	0.012	0.003
0.56	0.241	0.85	0.366	1.34	0.578	0.025	0.011	1.86	0.802	0.013	0.006
0.66	0.130	1.37	0.277	2.02	0.408	0.028	0.006	1.92	0.388	0.014	0.003
0.51	0.189	0.84	0.311	1.37	0.507	0.025	0.009	1.82	0.674	0.013	0.005
0.64	0.136	1.42	0.303	2.11	0.450	0.027	0.006	1.99	0.424	0.011	0.002
0.52	0.183	0.87	0.306	1.40	0.493	0.024	0.008	1.87	0.658	0.013	0.005
0.59	0.131	1.32	0.292	2.08	0.460	0.028	0.006	1.96	0.434	0.013	0.003
0.50	0.176	0.98	0.345	1.48	0.521	0.031	0.011	1.89	0.665	0.013	0.005
0.66	0.155	1.29	0.304	1.91	0.449	0.025	0.006	2.00	0.471	0.010	0.002
0.50	0.211	0.85	0.359	1.54	0.650	0.022	0.009	1.85	0.781	0.010	0.004
0.79	0.161	1.32	0.269	2.47	0.504	0.035	0.007	2.07	0.422	0.010	0.002
0.52	0.175	0.89	0.300	1.53	0.515	0.026	0.009	1.87	0.630	0.012	0.004
0.84	0.166	1.51	0.298	2.29	0.452	0.036	0.007	2.12	0.418	0.014	0.003
0.58	0.202	0.84	0.293	1.63	0.568	0.029	0.010	1.95	0.680	0.014	0.005
0.67	0.152	1.28	0.291	1.82	0.414	0.030	0.007	2.01	0.457	0.012	0.003
0.62	0.224	0.93	0.336	1.60	0.578	0.030	0.010	1.90	0.686	0.012	0.005

Ninety-Day Feeding Study in the Rat.

TABLE 28. Organ Weights, Grams

Animal No. & Group	Sex	Terminal Weight Grams	Spleen	Liver	Adrenals	Kidneys	Testes/Ovaries	Thymus	Heart	Lung	Thyroid	Brain	Pituitary
<u>30-DAY INTERIM SACRIFICE:</u>													
<u>Control:</u>													
14162	M	270	1.09	12.02	0.024	2.35	3.06	0.75	0.90	1.44	0.020	1.84	0.007
14165	M	360	0.92	17.02	0.051	3.11	2.81	0.93	1.43	1.68	0.024	1.83	0.010
14168	M	315	0.89	13.30	0.043	2.52	3.10	1.01	1.12	1.76	0.025	1.92	0.008
14142	F	205	0.52	8.63	0.079	1.79	0.084	0.79	0.79	1.30	0.022	8.80	0.008
14145	F	175	0.99	8.11	0.056	1.50	0.063	0.53	0.71	1.22	0.020	1.78	0.006
14146	F	225	0.60	9.49	0.071	1.91	0.120	0.71	0.85	1.45	0.021	1.80	0.007
<u>100 ppm.:</u>													
14202	M	275	0.94	12.51	0.044	2.39	2.76	0.81	0.93	1.84	0.025	1.90	0.009
14205	M	300	0.67	13.42	0.050	2.41	3.11	0.69	1.07	1.47	0.027	1.72	0.008
14208	M	230	0.50	9.02	0.038	1.86	2.19	0.70	0.93	1.37	0.029	1.61	0.010
14182	F	280	0.57	7.75	0.060	1.61	0.110	0.60	0.72	1.30	0.030	1.70	0.010
14185	F	190	1.10	7.81	0.053	1.67	0.092	0.41	0.65	1.36	0.012	1.72	0.010
14188	F	165	0.40	6.28	0.048	1.42	0.075	0.39	0.70	1.08	0.012	1.79	0.007
<u>500 ppm.:</u>													
14242	M	225	0.49	9.49	0.028	2.16	2.83	0.50	0.99	1.76	0.033	1.73	0.006
14245	M	270	0.79	11.02	0.046	2.52	2.82	0.93	1.03	1.60	0.023	2.00	0.010
14248	M	235	0.59	9.66	0.036	2.21	2.56	0.58	0.79	1.38	0.016	1.78	0.007
14222	F	205	0.50	9.20	0.052	1.96	0.084	0.80	0.81	1.31	0.028	1.71	0.007
14225	F	160	0.40	7.08	0.051	1.59	0.083	0.45	0.70	1.14	0.026	1.71	0.007
14228	F	200	0.50	8.51	0.060	2.04	0.123	0.87	0.80	1.38	0.018	1.79	0.009
<u>2500 ppm.:</u>													
14282	M	255	0.71	14.75	0.061	2.49	2.29	0.65	0.96	1.46	0.026	1.93	0.005
14285	M	290	0.90	15.29	0.051	2.90	2.83	0.88	1.02	1.40	0.019	2.01	0.006
14288	M	285	1.76	16.99	0.019	3.00	3.10	0.70	1.20	2.08	0.010	1.94	0.007
14262	F	195	0.99	10.35	0.079	2.12	0.100	0.67	0.76	1.41	0.021	1.81	0.003
14265	F	170	0.47	7.78	0.056	1.70	0.117	0.61	0.65	1.14	0.013	1.75	0.002
14268	F	155	0.32	7.82	0.068	1.74	0.153	0.50	0.63	1.47	0.020	1.83	0.006

( [REDACTED] ) Ninety-Day Feeding Study in the Rat.

TABLE 28. Continued. Organ Weights, Grams.

Animal No. & Group	Sex	Terminal Weight Grams	Spleen	Liver	Adrenals	Kidneys	Testes/Ovaries	Thymus	Heart	Lung	Thyroid	Brain	Pituitary
<u>21-DAY COMPOUND WITHDRAWAL:</u>													
<u>Control:</u>													
14177	M	420	0.60	11.80	0.050	2.67	3.30	0.58	1.18	2.43	0.030	2.21	0.010
14179	M	520	0.65	14.98	0.064	3.02	3.29	0.88	1.41	2.23	0.034	2.07	0.010
14180	M	530	0.85	17.68	0.055	3.22	3.50	0.90	1.38	2.75	0.042	1.93	0.010
14158	F	280	0.42	8.48	0.071	1.98	0.185	0.41	0.93	1.57	0.041	1.80	0.011
14159	F	320	0.53	9.34	0.072	2.15	0.248	0.64	0.90	1.50	0.018	1.92	0.010
14161	F	290	0.73	9.70	0.075	2.15	0.239	0.51	0.85	1.52	0.020	1.90	0.014
<u>1000 ppm.:</u>													
14258	M	560	1.05	18.65	0.064	3.65	3.80	0.90	1.70	2.23	0.035	2.15	0.014
14259	M	470	0.63	17.40	0.068	3.20	3.55	0.83	1.41	2.30	0.034	2.00	0.015
14260	M	490	1.05	16.17	0.062	3.23	3.41	0.80	1.41	2.34	0.038	2.22	0.012
14238	F	280	0.68	8.90	0.081	2.26	0.180	0.52	0.80	2.11	0.027	1.98	0.015
14239	F	290	0.57	9.63	0.069	2.40	0.192	0.61	0.91	1.42	0.023	1.81	0.010
14240	F	290	0.59	9.52	0.081	2.09	0.160	0.60	0.82	1.35	0.037	2.06	0.017
<u>5000 ppm.:</u>													
14298	M	340	0.55	11.10	0.051	2.80	3.32	0.58	1.05	1.64	0.018	2.01	0.012
14299	M	535	0.78	22.95	0.074	3.75	3.40	0.75	1.60	2.09	0.044	2.13	0.014
14300	M	445	0.50	15.61	0.048	2.98	3.25	0.68	1.20	1.72	0.028	1.90	0.010
14278	F	280	0.57	11.10	0.087	2.30	0.124	0.61	0.88	1.70	0.029	1.78	0.009
14279	F	260	0.53	10.13	0.085	2.52	0.160	0.60	0.92	1.32	0.034	1.93	0.014
14280	F	290	0.62	11.01	0.077	2.52	0.203	0.65	1.00	1.78	0.028	1.98	0.013

( [REDACTED] ) [REDACTED]  
 Company Sanitized. Does not contain TSCA CBI

Ninety-Day Feeding Study in the Rat.

TABLE 28. Continued. Organ Weights, Grams.

Animal No. & Group	Terminal Weight Grams	Sex	Spleen	Liver	Adrenals	Kidneys	Testes/Ovaries	Thymus	Heart	Lung	Thyroid	Brain	Pituitary
<u>60-DAY INTERIM SACRIFICE:</u>													
<u>Control:</u>													
14167	340	M	0.81	13.13	0.058	2.60	3.14	0.69	1.16	1.67	0.027	1.81	0.014
14170	385	M	0.71	14.70	0.069	2.90	2.89	0.70	1.48	2.03	0.027	1.20	0.017
14178	340	M	0.75	11.24	0.059	2.36	3.19	0.52	1.58	2.02	0.025	1.81	0.011
14143	200	F	0.49	7.28	0.059	1.55	0.170	0.49	0.75	1.07	0.015	1.87	0.01
14149	260	F	0.47	8.88	0.071	1.90	0.100	0.69	0.98	1.22	0.031	1.74	0.009
14152	240	F	0.59	8.58	0.080	1.71	0.130	0.53	0.84	1.19	0.027	1.75	0.014
<u>100 ppm.:</u>													
14217	325	M	0.79	11.78	0.051	2.69	3.32	0.83	1.13	1.60	0.037	2.01	0.010
14220	340	M	0.79	10.52	0.053	2.31	3.31	0.96	1.22	1.81	0.025	1.71	0.009
14221	350	M	0.79	10.44	0.041	2.59	3.27	0.59	1.15	1.69	0.019	1.92	-
14184	215	F	0.41		0.062	1.61	0.144	0.45	0.70	1.15	0.044	1.79	0.012
14193	235	F	0.54	8.29	0.064	1.66	0.135	0.47	0.80	1.78	0.023	1.73	0.013
14194	240	F	0.61	7.70	0.069	1.82	0.118	0.58	0.78	1.38	0.042	1.73	0.010
<u>1000 ppm.:</u>													
14246	320	M	0.62	11.75	0.050	2.57	2.70	0.60	1.12	1.95	0.024	1.91	0.010
14251	360	M	0.79	14.00	0.048	3.09	3.17	0.62	1.11	1.81	0.029	1.99	0.012
14253	385	M	0.81	13.08	0.041	2.45	3.37	0.77	1.05	2.01	0.014	2.09	0.008
14223	215	F	0.55	8.24	0.081	1.89	0.138	0.58	0.89	1.40	0.042	1.82	0.010
14230	220	F	0.65	8.01	0.076	2.01	0.200	0.50	0.88	1.62	0.038	1.90	0.009
14234	195	F	0.41	7.42	0.068	1.85	0.149	0.60	0.66	1.21	0.018	1.90	0.010
<u>5000 ppm.:</u>													
14289	450	M	0.71	26.05	0.070	3.49	1.69, 2.95	1.00	1.40	1.89	0.029	1.90	0.014
14293	355	M	1.34	16.75	0.067	3.33	3.32	0.91	1.38	2.19	0.036	1.96	0.013
14295	430	M	0.66	22.33	0.075	3.56	3.49	0.78	1.29	2.11	0.025	2.08	0.009
14263	265	F	0.50	13.71	0.074	2.46	0.168	0.68	0.87	1.29	0.021	1.80	0.019
14270	200	F	0.55	10.29	0.063	2.20	0.079	0.50	0.80	1.44	0.033	1.88	0.012
14275	230	F	0.80	12.11	0.065	2.41	0.188	0.50	0.89	1.30	0.022	1.90	0.007

Company Sanitized. Does not contain TSCA CBI

Ninety-Day Feeding Study in the Rat.

TABLE 28. Continued. Organ Weights, Grams.

Animal No. & Group	Terminal Weight Sex Grams	<u>Spleen</u>	<u>Liver</u>	<u>Adrenals</u>	<u>Kidneys</u>	<u>Testes/ Ovaries</u>	<u>Thymus</u>	<u>Heart</u>	<u>Lung</u>	<u>Thyroid</u>	<u>Brain</u>	<u>Pituitary</u>
<u>90-DAY TERMINAL SACRIFICE:</u>												
<u>Control:</u>												
14163	M 410	1.10	11.63	0.061	2.29	2.80	0.52	1.22	2.00	0.040	1.88	0.009
14164	M 545	1.22	17.92	0.059	3.71	3.58	1.09	1.90	2.38	0.051	2.02	0.017
14166	M 420	0.91	12.83	0.053	2.51	3.22	0.43	1.13	1.64	0.019	1.88	0.014
14169	M 460	1.11	14.18	0.063	3.10	3.65	0.71	1.23	1.78	0.036	2.01	0.012
14171	M 430	1.32	13.81	0.066	2.79	3.37	0.59	1.40	2.09	0.021	1.81	0.014
14172	M 490	1.10	15.47	0.078	3.10	3.19	0.95	1.38	2.07	0.024	1.91	0.013
14173	M 800	0.99	11.91	0.058	2.39	3.15	0.41	1.12	2.03	0.020	1.80	0.014
14174	M 450	1.00	15.12	0.080	3.02	3.31	0.69	1.38	1.60	0.019	1.85	0.015
14175	M 545	1.28	17.71	0.065	3.12	3.49	0.60	1.58	2.92	0.026	2.00	0.015
14176	M 400	0.75	10.48	0.081	2.65	3.42	0.58	1.39	1.68	0.025	2.08	0.012
14144	F 325	1.00	9.90	0.096	2.49	0.242	0.60	1.05	1.71	0.031	1.85	0.014
14147	F 255	0.51	7.11	0.065	1.52	0.134	0.52	0.80	1.00	0.015	1.70	0.013
14148	F 280	0.76	8.62	0.060	1.70	0.155	0.39	0.71	1.35	0.024	1.80	0.013
14150	F 300	0.75	9.80	0.084	1.92	0.167	0.51	0.98	1.52	0.031	1.92	0.013
14151	F 250	0.71	7.50	0.051	1.61	0.104	0.60	0.75	1.21	0.031	1.65	
14153	F 255	0.69	7.88	0.080	1.58	0.147	0.60	0.83	1.61	0.023	1.85	
14154	F 275	0.72	8.39	0.078	1.72	0.140	0.51	0.78	1.48	0.030	1.82	0.014
14155	F 255	0.81	8.32	0.090	1.60	0.170	0.53	0.85	1.37	0.024	1.80	0.016
14156	F 290	0.78	8.63	0.090	1.89	0.154	0.38	0.89	1.40	0.023	1.89	0.012
14157	F 210	0.39	5.58	0.055	1.23	0.123	0.41	0.73	1.05	0.018	1.89	0.008

Ninety-Day Feeding Study in the Rat.

TABLE 28. Continued. Organ Weights, Grams.

Animal No. & Group	Sex	Terminal Weight Grams	Spleen	Liver	Adrenals	Kidneys	Testes/Ovaries	Thymus	Heart	Lung	Thyroid	Brain	Pituitary
<u>30-DAY TERMINAL SACRIFICE:</u>													
<u>100 ppm.:</u>													
14203	M	455	1.49	14.08	0.051	2.50	3.05	0.41	1.43	2.28	0.019	1.85	0.007
14204	M	560	0.99	17.48	0.061	3.38	3.70	0.60	1.73	2.10	0.025	1.93	0.014
14206	M	465	1.20	14.60	0.067	3.25	3.50	0.60	1.38	2.72	0.024	2.08	0.011
14207	M	435	1.20	12.02	0.060	3.05	3.93	0.82	1.22	1.74	0.023	2.08	0.009
14209	M	400	1.21	12.07	0.054	2.72	3.19	0.44	1.28	1.90	0.025	1.82	0.009
14210	M	520	1.24	15.48	0.053	2.79	3.10	1.11	1.39	2.20	0.021	1.90	0.010
14211	M	470	1.48	13.78	0.035	3.10	3.42	0.36	1.43	1.95	0.039	2.19	0.011
14213	M	380	1.03	11.20	0.051	2.61	3.43	0.58	1.20	1.62		2.09	0.009
14214	M	485	1.02	14.03	0.061	2.89	3.50	0.60	1.46	2.71	0.027	2.09	0.013
14215	M	420	0.70	12.49	0.056	2.80	3.33	0.90	1.30	2.08	0.028	2.00	0.011
14216	M	535	0.90	15.90	0.055	3.29	3.32	0.59	1.50	2.02	0.036	1.97	0.013
14218	M	520	1.41	17.65	0.081	3.02	3.74	0.72	1.71	2.21	0.022	1.83	0.015
14219	M	450	1.00	13.65	0.055	2.95	2.85	0.54	1.40	1.90	0.029	2.08	0.010
14183	F	310	0.64	8.78	0.076	1.93	0.210	0.50	0.80	1.52	0.028	1.78	0.014
14186	F	345	0.81	9.62	0.093	2.15	0.203	0.58	1.14	1.34	0.034	2.02	0.014
14187	F	280	0.81	8.00	0.074	1.77	0.186	0.44	0.79	1.21	0.026	1.90	0.018
14189	F	265	1.10	8.40	0.081	1.91	0.183	0.81	0.83	1.42	0.027	1.73	0.015
14190	F	255	0.70	7.58	0.067	1.58	0.165	0.33	0.71	1.29	0.023	1.75	0.011
14191	F	290	1.22	7.51	0.061	1.96	0.168	0.56	0.92	1.43	0.016	1.89	0.012
14192	F	245	0.68	7.58	0.069	1.80	0.161	0.30	0.72	1.43	0.023	1.91	0.011
14195	F	280	0.71	8.50	0.093	2.01	0.161	0.60	0.93	1.30	0.026	1.88	0.014
14196	F	270	0.89	8.59	0.064	1.80	0.177	0.48	0.89	1.53	0.028	1.92	0.008
14197	F	260	0.54	7.32	0.049	1.72	0.116	0.49	0.88	1.28	0.022	1.77	0.012
14198	F	295	1.00	8.40	0.055	1.79	0.181	0.42	0.79	1.60	0.014	1.92	0.013
14199	F	280	0.76	8.83	0.050	1.81	0.170	0.61	1.01	1.41	0.019	1.81	0.015
14200	F	285	0.81	8.80	0.077	1.98	0.119	0.71	0.81	1.37	0.023	1.87	0.014
14201	F	310	0.68	7.88	0.084	1.99	0.198	0.48	0.93	1.50	0.020	2.09	0.013

Ninety-Day Feeding Study in the Rat.

TABLE 28. Continued. Organ Weights, Grams.

Animal No. & Group	Sex	Terminal Weight Grams	Spleen	Liver	Adrenals	Kidneys	Testes/Ovaries	Thymus	Heart	Lung	Thyroid	Brain	Pituitary
<u>90-DAY TERMINAL SACRIFICE:</u>													
<u>1000 ppm.:</u>													
14243	M	455	1.09	12.68	0.054	2.87	3.39	0.27	1.30	1.92	0.019	1.82	0.030
14244	M	535	0.97	17.13	0.065	3.51	3.60	0.98	1.50	2.94	0.026	1.98	0.012
14247	M	475	0.99	16.20	0.053	3.00	3.40	0.41	1.31	2.21	0.031	2.25	0.010
14249	M	470	1.17	15.88	0.082	3.21	3.58	0.68	1.51	1.65	0.034	1.93	0.012
14250	M	440	0.89	16.13	0.057	3.01	3.30	0.42	1.32	1.98	0.031	1.91	0.013
14252	M	470	0.79	16.61	0.067	3.42	3.80	0.60	1.39	1.93	0.029	1.89	Missed
14254	M	460	1.18	15.50	0.058	3.23	3.41	0.59	1.31	2.75	0.017	1.81	0.012
14255	M	410	1.00	12.80	0.053	2.60	3.30	0.79	1.21	2.00	0.026	2.01	0.012
14256	M	385	1.01	12.39	0.055	2.58	2.81	0.35	1.10	1.71	0.024	1.98	0.008
14257	M	415	0.63	14.90	0.055	3.03	3.29	0.78	1.21	1.68	0.040	1.99	0.009
14224	F	290	0.69	12.38	0.107	2.60	0.172	0.40	0.90	1.49	0.027	1.81	0.011
14226	F	270	0.51	8.91	0.066	2.21	0.097	0.22	1.40	1.39	0.037	1.89	0.005
14227	F	295	0.50	9.40	0.073	2.49	0.127	0.35	0.85	1.52	Missed	1.90	0.009
14229	F	305	0.89	10.75	0.075	2.10	0.186	0.61	0.98	1.35	Missed	1.91	0.012
14231	F	320	0.77	11.55	0.097	2.55	0.206	0.37	1.09	1.83	0.026	2.08	0.010
14232	F	280	0.77	11.00	0.096	2.17	0.201	0.80	0.88	1.24	0.040	1.90	0.014
14233	F	230	1.22	14.39	0.116	2.89	0.171	0.68	1.12	1.65	0.031	1.99	0.019
14235	F	325	0.84	8.14	Missed	1.73	0.130	0.31	0.70	1.20	0.022	1.68	0.014
14236	F	275	0.61	10.52	0.074	2.21	0.144	0.50	0.91	1.33	0.028	1.92	0.017
14237	F	250	0.88	8.81	0.077	2.08	0.117	0.75	1.00	1.79	0.034	1.85	0.016



Ninety-Day Feeding Study in the Rat.

TABLE 28. Continued. Organ Weights, Grams.

Animal No. & Group	Terminal Sex	Terminal Weight Grams	<u>Spleen</u>	<u>Liver</u>	<u>Adrenals</u>	<u>Kidneys</u>	<u>Testes/ Ovaries</u>	<u>Thymus</u>	<u>Heart</u>	<u>Lung</u>	<u>Thyroid</u>	<u>Brain</u>	<u>Pituitary</u>
<u>90-DAY TERMINAL SACRIFICE:</u>													
<u>1000 ppm.:</u>													
14243	M	455	1.09	12.68	0.054	2.87	3.39	0.27	1.30	1.92	0.019	1.82	0.030
14244	M	535	0.97	17.13	0.065	3.51	3.60	0.98	1.50	2.94	0.026	1.98	0.012
14247	M	475	0.99	16.20	0.053	3.00	3.40	0.41	1.31	2.21	0.031	2.25	0.010
14249	M	470	1.17	15.88	0.082	3.21	3.58	0.68	1.51	1.65	0.034	1.93	0.012
14250	M	440	0.89	16.13	0.057	3.01	3.30	0.42	1.32	1.98	0.031	1.91	0.013
14252	M	470	0.79	16.61	0.067	3.42	3.80	0.60	1.39	1.93	0.029	1.89	Missed
14254	M	460	1.18	15.50	0.058	3.23	3.41	0.59	1.31	2.75	0.017	1.81	0.012
14255	M	410	1.00	12.80	0.053	2.60	3.30	0.79	1.21	2.00	0.026	2.01	0.012
14256	M	385	1.01	12.39	0.055	2.58	2.81	0.35	1.10	1.71	0.024	1.98	0.008
14257	M	415	0.63	14.90	0.055	3.03	3.29	0.78	1.21	1.68	0.040	1.99	0.009
14224	F	290	0.69	12.38	0.107	2.60	0.172	0.40	0.90	1.49	0.027	1.81	0.011
14226	F	270	0.51	8.91	0.066	2.21	0.097	0.22	1.40	1.39	0.037	1.89	0.005
14227	F	295	0.50	9.40	0.073	2.49	0.127	0.35	0.85	1.52	Missed	1.90	0.009
14229	F	305	0.89	10.75	0.075	2.10	0.186	0.61	0.98	1.35	Missed	1.91	0.012
14231	F	320	0.77	11.55	0.097	2.55	0.206	0.37	1.09	1.83	0.026	2.08	0.010
14232	F	280	0.77	11.00	0.096	2.17	0.201	0.80	0.88	1.24	0.040	1.90	0.014
14233	F	230	1.22	14.39	0.116	2.89	0.171	0.68	1.12	1.65	0.031	1.99	0.019
14235	F	325	0.84	8.14	Missed	1.73	0.130	0.31	0.70	1.20	0.022	1.68	0.014
14236	F	275	0.61	10.52	0.074	2.21	0.144	0.50	0.91	1.33	0.028	1.92	0.017
14237	F	250	0.88	8.81	0.077	2.08	0.117	0.75	1.00	1.79	0.034	1.85	0.016

Ninety-Day Feeding Study in the Rat.

TABLE 28. Continued. Organ Weights, Grams.

Animal No. & Group	Sex	Terminal Weight Grams	<u>Spleen</u>	<u>Liver</u>	<u>Adrenals</u>	<u>Kidneys</u>	<u>Testes/ Ovaries</u>	<u>Thymus</u>	<u>Heart</u>	<u>Lung</u>	<u>Thyroid</u>	<u>Brain</u>	<u>Pituitary</u>
<b>90-DAY TERMINAL SACRIFICE:</b>													
<b>5000 ppm.:</b>													
14283	M	420	1.30	19.57	0.058	3.30	3.30	0.58	1.21	1.88	0.024	2.04	0.010
14284	M	525	1.02	25.68	0.065	3.91	2.87	1.32	1.43	2.35	0.035	2.09	0.015
14286	M	345	0.98	15.13	0.059	2.62	3.21	0.69	0.91	2.18	0.023	2.08	0.008
14287	M	425	1.11	20.11	0.050	3.30	3.39	0.80	1.30	1.98	0.029	2.10	0.011
14290	M	430	0.90	21.49	0.059	3.38	3.60	0.42	1.58	1.61	0.027	1.92	0.012
14291	M	410	0.51	21.65	0.060	3.18	3.20	0.59	1.17	2.02	0.031	1.83	0.010
14292	M	390	1.19	19.82	0.050	3.20	3.49	0.63	1.20	1.42	0.019	2.02	0.009
14294	M	395	0.77	18.30	0.054	3.30	3.40	0.30	1.38	2.09	0.021	1.90	0.008
14296	M	430	1.12	19.82	0.054	3.31	3.60	0.79	1.42	1.60	0.023	1.92	0.010
14297	M	480	0.91	22.19	0.058	3.80	3.61	0.51	1.28	1.92	0.017	2.05	0.011
14264	F	220	0.90	9.84	0.063	2.00	0.146	0.41	0.78	1.38	0.020	1.78	0.010
14266	F	225	0.83	11.40	0.077	2.03	0.122		0.80	2.93	0.018	1.88	0.011
14267	F	250	0.48	10.85	0.067	2.28	0.178	0.58	0.72	1.28	0.019	1.80	0.012
14269	F	220	0.58	11.01	0.068	2.25	0.092	0.53	0.83	1.13	0.024	1.91	0.011
14271	F	270	1.08	11.91	0.072	2.78		0.57	0.98	1.40	0.018	1.79	
14272	F	215	0.67	9.57	0.083	1.80	0.130	0.58	0.70	1.32	0.032	1.91	0.010
14273	F	240	0.70	11.75	0.062	2.38	0.180	0.42	0.90	1.21	0.018	1.98	0.007
14274	F	240	1.51	10.41	0.059	2.45	0.107	0.51	0.83	1.60	0.015	1.81	0.008
14276	F	250	0.48	11.30	0.085	2.12	0.144	0.43	1.03	1.61	0.031	1.81	0.013
14277	F	235	0.83	11.41	0.079	2.40	0.182	0.48	0.95	1.51	0.025	1.78	

Company Sanitized. Does not contain TSCA CBI

( [REDACTED] ) Ninety-Day Feeding Study in the Rat.

TABLE 29. Incidence of Histopathologic Lesions.

Tissue and Lesion	30 Day				60 Day			
	Interim Sacrifice		Interim Sacrifice		Interim Sacrifice		Interim Sacrifice	
	Control	2500 ppm.	Control	5000 ppm.	Control	5000 ppm.	Control	5000 ppm.
	M	F	M	F	M	F	M	F
brain - no lesion	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3
- glial nodules								
spinal cord - no lesion	3/3	3/3	2/2	3/3	3/3	3/3	3/3	3/3
peripheral nerve - no lesion	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3
eye - no lesion	3/3	3/3	3/3	3/3	3/3	3/3	3/3	2/2
pituitary - no lesion	2/2	3/3	2/2	1/1	3/3	2/2	3/3	2/2
thyroid - no lesion	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3
parathyroid - no lesion			1/1		3/3	3/3	2/2	1/1
adrenal - no lesion	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3
- ossification								
lung - no lesion	3/3	2/3	3/3	2/3	3/3	2/3	3/3	3/3
- chronic respiratory disease		1/3		1/3		1/3		
heart - no lesion	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3
- arteritis								
spleen - no lesion	3/3	3/3	2/3	3/3	3/3	3/3	3/3	3/3
- hematopoësis			1/3					
lymph node - no lesion	3/3	3/3	3/3	3/3	2/2	3/3	3/3	3/3
thymus - no lesion	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3
bone marrow - no lesion	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3
salivary gland - no lesion	3/3	3/3	3/3	3/3	3/3	2/2	3/3	3/3
stomach - no lesion	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3
small intestine - no lesion	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3
large intestine - no lesion	3/3	3/3	3/3	3/3	3/3	3/3	2/3	3/3
- nematodes							1/3	

90 Day Terminal Sacrifice						21 Day Withdrawal			
Control		1000 ppm.		5000 ppm.		Control		5000 ppm.	
M	F	M	F	M	F	M	F	M	F
10/10	10/10			9/10	10/10	3/3	3/3	3/3	3/3
				1/10					
8/8	9/9			9/9	9/9	3/3	3/3	3/3	3/3
9/9	9/9			9/9	8/8	3/3	3/3	3/3	3/3
10/10	9/9			9/9	9/9	3/3	3/3	3/3	3/3
9/9	3/3			8/8	1/1	3/3	3/3	3/3	3/3
10/10	9/9			10/10	9/9	3/3	3/3	3/3	3/3
5/5	6/6			3/3	2/2	1/1	1/1	3/3	1/1
10/10	10/10			9/10	10/10	3/3	3/3	3/3	3/3
				1/10					
5/10	9/10			7/10	7/10	2/3	1/3	2/3	1/3
5/10	1/10			3/10	3/10	1/3	2/3	1/3	2/3
9/10	10/10			10/10	10/10	3/3	3/3	3/3	3/3
1/10									
10/10	10/10			9/9	10/10	3/3	3/3	3/3	3/3
9/9	8/8			8/8	10/10	3/3	2/2	3/3	3/3
9/9	9/9			9/9	8/8	3/3	3/3	3/3	3/3
10/10	10/10			9/9	10/10	3/3	3/3	3/3	3/3
9/9	9/9			9/9	8/8	3/3	3/3	3/3	3/3
10/10	10/10			10/10	10/10	3/3	3/3	3/3	3/3
10/10	10/10			10/10	10/10	3/3	3/3	3/3	3/3
10/10	10/10			10/10	9/10	3/3	3/3	3/3	3/3
				1/10					

Ninety-Day Feeding Study in the Rat.

TABLE 29. Continued. Incidence of Histopathologic Lesions.

Tissue and Lesion	30 Day				60 Day			
	Interim Sacrifice		Interim Sacrifice		Interim Sacrifice		Interim Sacrifice	
	Control	2500 ppm.	Control	5000 ppm.	Control	5000 ppm.	Control	5000 ppm.
	M	F	M	F	M	F	M	F
pancreas - no lesion	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3
pancreatitis, focal								
liver - no lesion	2/3	3/3	0/3	2/3	2/3	2/3	0/3	0/3
- portal inflammatory infiltrate	1/3			1/3	1/3	1/3	1/3	1/3
- centrilobular change			3/3				3/3	2/2
kidney - no lesion	3/3	3/3	3/3	2/3	3/3	3/3	3/3	3/3
- focal nephritis				1/3				
- hyaline droplets								
urinary bladder - no lesion	2/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3
- seminal plug	1/3							
testes or ovaries - no lesion	3/3	3/3	3/3	3/3	3/3	3/3	2/3	3/3
- edema							1/3	
prostate or uterus - no lesion	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3
- hydrometra								
skeletal muscle - no lesion	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3
skin - no lesion	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3
bone - no lesion	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3

90 Day Terminal Sacrifice						21 Day Withdrawal			
Control		1000 ppm.		5000 ppm.		Control		5000 ppm.	
M	F	M	F	M	F	M	F	M	F
10/10	10/10			10/10	10/10	2/3	3/3	3/3	3/3
						1/3			
7/10	5/10	7/10	6/10	1/10	2/10	2/3	2/3	0/3	0/3
3/10	5/10	3/10	4/10	1/10	8/10	1/3	1/3	1/3	3/3
1/10				9/10	2/10			2/3	
8/10	10/10			10/10	9/10	3/3	3/3	3/3	2/3
					1/10				1/3
2/10									
6/8	9/9			10/10	9/9	1/2	3/3	3/3	3/3
2/8						1/2			
10/10	10/10			10/10	10/10	3/3	3/3	3/3	3/3
10/10	9/10			10/10	10/10	3/3	3/3	2/2	2/2
	1/10								
9/9	10/10			10/10	9/9	3/3	3/3	3/3	3/3
9/9	10/10			9/9	6/6	3/3	3/3	3/3	3/3
10/10	10/10			10/10	10/10	3/3	3/3	3/3	3/3


 Ninety-Day Feeding Study in the Rat.

TABLE 30. Histopathologic Observations. Thirty-Day Interim Sacrifice.

Animal Number	Sex	Tissue	Comment
<u>Control:</u>			
14162	M	liver	Mild portal lymphocytic infiltrate.
14165	M		No lesion.
14168	M	urinary bladder	Seminal plug.
14142	F		No lesion..
14145	F	lung	Small focus of pneumonic consolidation.
14146	F		No lesion.
<u>2500 ppm.:</u>			
14282	M	liver	Slight hypertrophy of centrolobular hepatocytes.
14285	M	liver	Slight hypertrophy of centrolobular hepatocytes with loss of coarse cytoplasmic granularity.
14288	M	liver	Slight hypertrophy of centrolobular hepatocytes with loss of usual coarse granularity.
		spleen	Moderate hematopoietic activity.
14262	F	liver	Mild portal lymphocytic infiltrate.
		kidney	Mild focal interstitial lymphocytic infiltrate.
14265	F	lung	Moderate peribronchial lymphoid hyperplasia.
14268	F		No lesion.

( [REDACTED] ) Ninety-Day Feeding Study in the Rat.

TABLE 30. Continued. Histopathologic Observations. Sixty-Day Interim Sacrifice.

Animal Number	Sex	Tissue	Comment
<u>Control:</u>			
14167	M		No lesion.
14170	M		No lesion.
14178	M	liver	Slight portal lymphocytic infiltrate.
14143	F		No lesion.
14149	F	liver lung	Slight portal lymphocytic infiltrate. Few small scattered foci of pneumonic consolidation.
14152	F		No lesion.
<u>5000 ppm.:</u>			
14289	M	large intestine testes  liver	Nematodes. One testis was edematous and had reduced spermatogenic activity. Hypertrophy of hepatocytes which was more pronounced in the centrolobular area; hepatocytes appeared to have higher glycogen content than controls.
14293	M	liver	Moderate hypertrophy of hepatocytes, predominately centrolobular with loss of coarse granularity. Moderate portal lymphocytic infiltrate with scattered small nodules of proliferated reticuloendothelial cells in liver parenchyma.
14295	M	liver	Hypertrophy of hepatocytes, primarily centrolobular, hepatocytes appeared to contain more glycogen than control.
14263	F	liver	Slight hypertrophy of hepatocytes, predominately centrolobular.
14270	F	liver	Slight portal lymphocytic infiltrate.
14275	F	liver	Slight hypertrophy of centrolobular hepatocytes.



**[REDACTED]** Ninety-Day Feeding Study in the Rat.

TABLE 39. Continued. Histopathologic Observations. Ninety-Day Terminal Sacrifice.

Animal Number	Sex	Tissue	Comment
<u>Control:</u>			
14163	M	lung	Moderate perivascular lymphocytic cuffing.
14164	M	urinary bladder	Seminal plug.
14166	M		No lesion.
14169	M	kidney	Small numbers of hyaline droplets in epithelium of convoluted tubules.
		liver	Slight portal lymphocytic infiltrate and bile duct proliferation.
14171	M	lung	Moderate perivascular lymphocytic cuffing with localized pneumonitis.
14172	M	urinary bladder lung	Seminal plug. Slight perivascular lymphocytic cuffing, peribronchial lymphoid hyperplasia.
14173	M	lung kidney	Slight peribronchial lymphoid hyperplasia. Moderate numbers of hyaline droplets in epithelium of convoluted tubules.
14174	M		No lesion.
14175	M	liver	Slight portal lymphocytic infiltrate, centrolobular hepatocytes less coarsely granular.
		lung	Slight perivascular lymphocytic cuffing.
14176	M	liver heart	Slight portal lymphocytic infiltrate. Moderate mural necrosis and perivascular inflammatory infiltrate of a coronary vessel.
14144	F		No lesion.
14147	F		No lesion.
14148	F	liver	Slight portal lymphocytic infiltrate and bile duct proliferation.

[REDACTED] Ninety-Day Feeding Study in the Rat.

TABLE 30. Continued. Histopathologic Observations. Ninety-Day Terminal Sacrifice.

Animal Number	Sex	Tissue	Comment
<u>Control (cont'd):</u>			
14150	F		No lesion.
14151	F	liver	Slight portal lymphocytic infiltrate.
14153	F	lung liver	Small area of chronic pneumonitis. Slight portal lymphocytic infiltrate.
14154	F	uterus liver	Hydometra. Slight portal lymphocytic infiltrate.
14155	F	liver	Slight portal lymphocytic infiltrate.
14156	F		No lesion.
14157	F		No lesion.

[REDACTED] Ninety-Day Feeding Study in the Rat.

TABLE 30. Continued. Histopathologic Observations. Ninety-Day Terminal Sacrifice.

Animal Number	Sex	Tissue	Comment
<u>1000 ppm., Liver Only:</u>			
14243	M		No lesion.
14244	M		No lesion.
14247	M		Slight portal lymphocytic infiltrate.
14249	M		No lesion.
14250	M		No lesion.
14252	M		No lesion.
14254	M		No lesion.
14255	M		No lesion.
14256	M		Slight portal lymphocytic infiltrate.
14257	M		Slight portal lymphocytic infiltrate.
14224	F		No lesion.
14226	F		No lesion.
14227	F		No lesion.
14229	F		Slight portal lymphocytic infiltrate.
14231	F		No lesion.
14232	F		Slight portal lymphocytic infiltrate.
14233	F		Slight portal lymphocytic infiltrate.
14235	F		Slight portal lymphocytic infiltrate.
14236	F		No lesion.
14237	F		No lesion.

[REDACTED] Ninety-Day Feeding Study in the Rat.

TABLE 30. Continued. Histopathologic Observations. Ninety-Day Terminal Sacrifice.

Animal Number	Sex	Tissue	Comment
<u>5000 ppm.:</u>			
14283	M	liver	Centrolobular hepatocytes slightly hypertrophied cytoplasm less coarsely granular than in hepatocytes at periphery of lobules.
14284	M	liver	Centrolobular hepatocytes less coarsely granular than those at periphery.
		lung	Slight peribronchial lymphoid hyperplasia.
14286	M	brain	Glial nodules in medulla, structure resembling <u>Sarcosporidia</u> also present.
		lung	Slight perivascular lymphocytic cuffing, area of pneumonic consolidation.
		liver	Centrolobular hepatocytes less granular than those at periphery of lobule, slight portal lymphocytic infiltrate.
14287	M	adrenal...	Area of osteoid and bone in cortex of one adrenal.
		liver	Cytoplasm of centrolobular hepatocytes less coarsely granular than cytoplasm of hepatocytes at periphery, slight portal lymphocytic infiltrate.
14290	M		No lesion.
14291	M	liver	Cytoplasm of centrolobular hepatocytes less coarsely granular than cytoplasm of hepatocytes at periphery of lobules.
14292	M	liver	Centrolobular hepatocytes less coarsely granular than those at periphery of lobules.
		lung	Slight peribronchial lymphoid hyperplasia.
14294	M	liver	Centrolobular hepatocytes less coarsely granular than those at periphery of lobule.
14296	M	liver	Marked portal lymphocytic infiltrate, centrolobular hepatocytes slightly less granular than those at periphery of lobule.

**[REDACTED]** Ninety-Day Feeding Study in the Rat.

TABLE 30. Continued. Histopathologic Observations. Ninety-Day Terminal Sacrifice.

Animal Number	Sex	Tissue	Comment
<u>5000 ppm. (cont'd):</u>			
14297	M	liver	Centrolobular hepatocytes less coarsely granular than those at periphery of lobule.
14264	F	liver	Moderate portal lymphocytic infiltrate.
14266	F	liver	Centrolobular hepatocytes less coarsely granular than those at periphery, mild portal lymphocytic infiltrate.
		lung	Chronic murine pneumonia of moderate severity.
14267	F	liver	Mild portal lymphocytic infiltrate.
14269	F	liver	Slight portal lymphocytic infiltrate.
14271	F	kidney	Moderate interstitial lymphocytic infiltrate.
		liver	Mild portal lymphocytic infiltrate.
14272	F	liver	Mild portal lymphocytic infiltrate.
14273	F	liver	Mild portal lymphocytic infiltrate.
		large intestine	Nematodes.
14274	F	liver	Mild portal lymphocytic infiltrate, centrolobular hepatocytes less coarsely granular than those at periphery.
14276	F	lung	Moderate perivascular lymphocytic cuffing.
14277	F	lung	Moderate perivascular lymphocytic cuffing.

**[REDACTED]** Ninety-Day Feeding Study in the Rat.

TABLE 30. Continued. Histopathologic Observations. 21-Day Compound Withdrawal.

Animal Number	Sex	Tissue	Comment
<u>Control:</u>			
14177	M		No lesion.
14179	M	pancreas	Small area of necrosis and chronic inflammation.
14180	M	lung liver urinary bladder	Moderate peribronchial lymphoid hyperplasia. Slight portal lymphocytic infiltrate. Seminal plug.
14158	F		No lesion.
14159	F	lung	Moderate peribronchial lymphoid hyperplasia.
14161	F	liver lung	Slight portal lymphocytic infiltrate. Slight perivascular lymphocytic cuffing.
<u>5000 ppm.:</u>			
14298	M	liver lung	Slight portal lymphocytic infiltrate. Mild peribronchial lymphoid hyperplasia.
14299	M	urinary bladder liver	Seminal plug. Centrolobular hepatocytes appeared slightly hypertrophied.
14300	M	liver	Centrolobular hepatocytes appeared slightly hypertrophied.
14278	F	kidney  liver  lung	Slight interstitial lymphocytic infiltrate, few calcified tubules. Moderate lymphocytic inflammatory infiltrate, primarily in portal areas. Moderate peribronchial lymphoid hyperplasia.
14279	F	liver	Mild portal lymphocytic infiltrate, few scattered vacuolated hepatocytes.
14280	F	lung liver	Slight peribronchial lymphoid hyperplasia. Slight portal lymphocytic infiltrate.



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

*Mason Hayek*  
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M. Hayek, Orchem Tech.  
S. E. Krahler, Orchem-Jackson Lab.  
J. E. Smith, Orchem Tech.  
J. W. Clayton/H. Sherman, Haskell Lab.  
J. A. Nelson, Orchem-Jackson Lab.

LEGAL DEPARTMENT

Code 3411 715

March 23, 1966

H. A. LIPS  
ORGANIC CHEMICALS DEPARTMENT

FOOD ADDITIVE PETITION NO. 5B1747  
"ZONYL" RP PAPER FLUORIDIZER

S. E. Krahler, H. Sherman and the undersigned met with Messrs. Blumenthal, McLaughlin, Orr and Detwiler of FDA on March 22, 1966, to determine whether there is a basis upon which the above Food Additive Petition may be approved.

Initially, we inquired of the FDA officials as to the reasons for not accepting the Petition for filing. They indicated that with respect to compounds with which they are not familiar, two-year feeding studies are the usual standard requirement. In the event ninety-day studies are utilized, they now look for a no-effect level of 1,000. The migration data which we had submitted indicated that approximately 1 ppm might migrate into the food and, therefore, FDA would require no effect at the 1,000 ppm level. The toxicity data at the 1,000 ppm level did show some enlargement of livers, although unaccompanied by histological changes. Such an enlargement is considered an "effect" by FDA since, with only the ninety-day studies, they are unwilling to speculate as to whether the effect would increase or decrease after feeding was continued for two years. They indicated that often at the end of ninety days the results look their worst, and that, if continued for two years, the apparently adverse results at ninety days might well appear without significance at the end of two years.

There thus was no basis upon which we could persuade FDA to accept 1 ppm in food on the basis of our ninety-day studies. Since additional toxicity studies would appear to be out of the question for reasons of time and money, we approached the problem on the basis

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that undoubtedly actual migration would be significantly less than 1 ppm. We presented the revised calculations based on ZONYL RP treatment of 0.25% (OWP) and compared the average ppm extraction of ZONYL RP solids against such extractions based upon 0.50% (OWP) treatment. We basically told them we could live with a regulation limited to .5 ppm extraction. FDA indicated that this would be unsatisfactory.

However, FDA did indicate that .1 ppm probably would be acceptable based upon the toxicity data already submitted. They did not indicate exactly what number would be acceptable but at least we clearly know the limits: .5 ppm is not acceptable and .1 ppm would be acceptable.

Dr. McLaughlin advanced an interesting idea as to what actually is causing the toxic reaction in the compound. He recalled that diethylamine salt has been known to cause increased liver weight without histological change. Dr. Blumenthal confirmed this by reference to toxicity data developed by the Mellon Institute for Union Carbide. Thus it may be that the diethylamine salt rather than the perfluoroalkyl phosphate is the bad actor. If so, FDA would be more inclined to approve our petition since they have some familiarity with diethylamine salts and are reluctant to approve a petition involving perfluoroalkyl phosphates with which they are totally unfamiliar. Also, there is always the possibility that we could eliminate the diethylamine salt, thereby eliminating the toxicity problem. However, we did not indicate that this was a practical alternative since, even if the diethylamine salt were eliminated, we would still have to submit data on the perfluoroalkyl phosphate or else absolutely prove that the diethylamine salt was causing the toxicity.

Our task is now threefold. First, Technical Lab will have to determine the maximum level at which we can expect customers to apply ZONYL to the surface of paperboard. Obviously, this figure will have to be rather precise since we cannot afford to have a level of application any higher than is absolutely necessary for practical commercial use. Secondly, Jackson Lab will then



H. A. LIPS

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have to conduct extraction tests based upon the level of application determined by Technical Lab. Probably two or three sets of extraction tests should be run on levels of application close to the figure determined by Technical Lab. These extraction tests need only be run on water and Wesson Oil. Third, we will need a write-up by Haskell as to any ideas they might have on the toxicity of the diethylamine salt.

The above information will then be prepared as a supplement to the original petition and we will no doubt take it down to FDA and review it with them. It is extremely difficult to speculate on the ppm figure which FDA will accept but probably it will not accept anything more than .3. This must be borne in mind in determining levels of application.

  
RICHARD H. REA

RHR:df

FOR ENCLOSURE

DATE 6/2/71

TO: *J. R. Martin*  
*Jackson Lab. - Room 4124*

FROM: MASON HAYEK  
D. & C. Technical Laboratory  
Room 215, Ext. 2975

Please Discuss With	For Approval	For Attention	For Information	Note and Forward To File	Note and Return To Sender	Forwarded Per Your Request
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The attached table gives the range of extraction conditions. We are working on Condition D and hope to go to B and C. The letter of 9/20/66 is also attached.

- A. High temperature (a.s. over 200° F.)
- B. Boiling water
- C. Hot filled or pasteurized at 150° F.

- D. Hot filled or pasteurized at 150° F.

- E. Room temperature stored (no thermal treatment in the container).

- F. Refrigerated storage (no thermal treatment in the container).

- G. Frozen storage (no thermal treatment in the container).

- H. Frozen or refrigerated storage. Ready-prepared foods intended to be reheated in container at time of use:

- 1. Aqueous or oil-in-water emulsion of high or low fat.
- 2. Aqueous, high- or low-fat oil or fat.

I. N. F. Y. 7  
II. F. A. 7

**TABLE 1—TYPES OF RAW AND PROCESSED FOODS**

- I.** Nonacid, aqueous products; may contain salt or sugar or both (pH above 5.0).
- II.** Acid, aqueous products; may contain salt or sugar or both, and including oil-in-water emulsions of low- or high-fat content.
- III.** Aqueous, acid or nonacid products containing free oil or fat; may contain salt, and including water-in-oil emulsions of low- or high-fat content.
- IV.** Dairy products and modifications:
  - A.** Water-in-oil emulsions, high- or low-fat.
  - B.** Oil-in-water emulsions, high- or low-fat.
- V.** Low-moisture fats and oils.
- VI.** Beverages:
  - A.** Containing up to 8 percent of alcohol.
  - B.** Nonalcoholic.
  - C.** Containing more than 8 percent of alcohol.
- VII.** Bakery products other than those included under types VIII or IX of this table:
  - A.** Moist bakery products with surface containing free fat or oil.
  - B.** Moist bakery products with surface containing no free fat or oil.
- VIII.** Dry solids with the surface containing no free fat or oil (no end test required).
- IX.** Dry solids with the surface containing free fat or oil.

**TABLE 2—TEST PROCEDURES WITH TIME TEMPERATURE CONDITIONS FOR DETERMINING AMOUNT OF EXTRACTIVES FROM THE FOOD-CONTACT SURFACE OF UNCOATED OR COATED PAPER AND PAPERBOARD, USING SOLVENTS SIMULATING TYPES OF FOODS AND BEVERAGES**

Condition of use	Types of food (see table 1)	Food-simulating solvents			
		Water	Heptane †	8 percent alcohol	50 percent alcohol
<b>A.</b> High temperature heat-sterilized (a.e., over 212° F.).	I, IV-B, VII-B...	Time and temperature 250° F., 2 hr.	Time and temperature 150° F., 2 hr.	Time and temperature	Time and temperature
<b>B.</b> Boiling water sterilized	III, IV-A, VII-A... II, VII-B... III, VII-A...	250° F., 2 hr. 212° F., 30 min. 212° F., 30 min.	150° F., 2 hr. 120° F., 30 min.		
<b>C.</b> Hot filled or pasteurized above 150° F.	II, IV-B... III, IV-A...	Fill boiling, cool to 100° F. Fill boiling, cool to 100° F.	120° F., 15 min.		
<b>D.</b> Hot filled or pasteurized below 150° F.	V... II, IV-B, VI-B... III, IV-A... V... VI-A... VI-C...	150° F., 2 hr. 150° F., 2 hr.	120° F., 15 min. 100° F., 30 min. 100° F., 30 min.	150° F., 2 hr.	150° F., 2 hr.
<b>E.</b> Room temperature filled and stored (no thermal treatment in the container).	I, II, IV-B, VI-B, VII-B... III, IV-A, VII-A... V, IX... VI-A... VI-C...	120° F., 24 hr.	70° F., 30 min. 70° F., 30 min.	120° F., 24 hr.	120° F., 24 hr.
<b>F.</b> Refrigerated storage (no thermal treatment in the container).	III, IV-A, VII-A... I, II, IV-B, VI-B, VII-B... VI-A... VI-C...	70° F., 48 hr. 70° F., 48 hr.	70° F., 30 min.	70° F., 48 hr.	70° F., 48 hr.
<b>G.</b> Frozen storage (no thermal treatment in the container).	I, II, IV-B, VII-B... III, VII-A...	70° F., 24 hr. 70° F., 24 hr.	70° F., 30 min.		
<b>H.</b> Frozen or refrigerated storage: Ready-prepared foods intended to be reheated in container at time of use: <ul style="list-style-type: none"> <li><b>1.</b> Aqueous or oil-in-water emulsion of high- or low-fat.</li> <li><b>2.</b> Aqueous, high- or low-free oil or fat.</li> </ul>	I, II, IV-B, VII-B... III, IV-A, VII-A...	212° F., 30 min. 212° F., 30 min.	120° F., 30 min.		

*Industrial* **BIO-TEST** *Laboratories, Inc.*

1810 FRONTAGE ROAD

NORTHBROOK, ILLINOIS 60062

Telephone CRestwood 2-3030

REPORT TO

MINNESOTA MINING AND MANUFACTURING COMPANY

ACUTE ORAL TOXICITY STUDIES ON  
TWO MATERIALS

IBT NO. A4414

**Exhibit**

**1053**

State of Minnesota v. 3M Co.,  
Court File No. 27-CV-10-28862

REPORT TO  
MINNESOTA MINING AND MANUFACTURING COMPANY  
ACUTE ORAL TOXICITY STUDIES ON  
TWO MATERIALS

IBT NO. A4414

I. Introduction

Samples of two materials identified as L-1931 (20470-23P) and L-1932 (20430-39D) respectively, were received from Minnesota Mining and Manufacturing Company for the purpose of conducting acute oral toxicity studies employing albino rats as test animals.

II. Procedure

The procedure followed in the evaluation of each test material was the same and is described below.

Healthy, young albino rats of the Sprague-Dawley strain with a body weight range from 150 to 200 grams were used as test animals. The rats were divided into groups of four animals each (two male and two female) for dosing purposes.

All animals used were kept under observation for five days prior to experimental use, during which period they were checked for general physical health and suitability as test animals. The animals were housed

in stock cages and permitted a standard laboratory rat diet\* plus water ad libitum until 16 hours immediately prior to oral intubation.

On the morning of the first test day, after a 16-hour fast (water permitted), the selected dose groups of four rats each (two male and two female) were intubated with previously calculated doses of the test material in the form of an aqueous suspension. All doses were administered directly into the stomachs of the rats using a hypodermic syringe equipped with a ball-pointed intubating needle.

Following oral administration of the test material, the rats were housed individually in observation cages ( 10" x 8" x 8") and observed for the succeeding 30 days. All mortalities and/or reactions displayed were recorded.

Arrangements were made to autopsy any animal which might succumb during the study as well as all surviving animals at the end of the 30 days.

At the end of the observation period, all data were collected and arrangements were made to calculate the acute oral mean lethal dose (LD50) of the test material using the techniques of Weil\*\*, Thompson\*\*\*, and Thompson and Weil\*\*\*\*.

\* Wayne Lab Blox, Allied Mills, Chicago, Illinois.

\*\* Weil, Carrol S. : Tables for Convenient Calculation of Median-Effective Dose (LD50 or ED50) and Instructions in Their Use. Biometrics, Sept. 1952.

\*\*\* Thompson, William R. : Use of Moving Averages and Interpolation to Estimate Median-Effective Dose. Bact. Rev., Nov. 1947.

\*\*\*\* Thompson, William R. and Weil, Carrol S. : On the Construction of Tables for Moving Average Interpolation. Biometrics, March 1952.

III. ResultsA. Mortality

The mortality data are presented in Tables I and II.

TABLE I

TEST MATERIAL: L-1931

Acute Oral Toxicity - Albino Rats

Mortality Data

Dose* (g/kg)	Number Dead	Number Tested	Per Cent Dead
1.4	1	4	25
2.0	1	4	25
3.0	2	4	50
4.6	4	4	100

Acute Oral  $LD_{50} = 2.6$  g/kg  
Standard Deviation of  $LD_{50} = \pm 0.6$  g/kg

\* The test material was administered as a 25 per cent (w/v) aqueous suspension.

TABLE II

TEST MATERIAL: L-1932

Acute Oral Toxicity - Albino Rats

Mortality Data

Dose* (g/kg)	Number Dead	Number Tested	Per Cent Dead
0.6	0	4	0
0.9	2	4	50
1.4	4	4	100
2.0	4	4	100

Acute Oral  $LD_{50} = 0.9$  g/kg  
Standard Deviation of  $LD_{50} = \pm 0.1$  g/kg

\* The test material was administered as a 10 per cent (w/v) aqueous suspension.



B. Reactions

Summaries of the untoward behavioral reactions and time of death following oral administration of the respective test materials are presented in Tables III and IV.

Necropsy of animals which died during the study as well as those sacrificed at the end of the 30-day observation period revealed no significant gross pathologic alterations in the tissues and organs examined.

TABLE III

TEST MATERIAL: L-1931

Acute Oral Toxicity - Albino Rats

Summary of Reactions

Dose (g/kg)	Reaction	Time of Onset Following Dose Administration (minutes)	Duration of Reaction (days)	Time of Death Following Dose Administration (days)
1.4 and 2.0	Hypoactivity Ruffed fur Emaciation	90 6-22 hours 2-3 days	5 4-5 or until death	5
3.0 and 4.6	Hypoactivity Muscular weakness Ruffed fur Emaciation	30 30 6-22 hours 6-22 hours	5 or until death 4-5 or until death	2-3

TABLE IV

TEST MATERIAL: L-1932

Acute Oral Toxicity - Albino Rats

Summary of Reactions

Dose (g/kg)	Reaction	Time of Onset Following Dose Administration (hours)	Duration of Reaction (hours)	Time of Death Following Dose Administration (days)
0.6	Hypoactivity	1/2	1	-
	Ptosis	1/2	1	
	Muscular weakness	1/2	1	
0.9	Hypoactivity	1/2	5 days or until death	4-5
	Ptosis	1/2		
	Muscular weakness	1/2		
	Sneezing	1/2	5 minutes	
	Ruffed fur	6-22 hours	5 days or until death	
1.4 and 2.0	Ruffed fur	4 minutes	Until death	2-6
	Sneezing	8 minutes	2 minutes	
	Hypoactivity	20 minutes	Until death	
	Ptosis	20 minutes	6-22	
	Alopecia*	1-2 days	Until death	
	Emaciation	1-2 days	Until death	
	Bloody nasal discharge	1-2 days	Until death	

\* This reaction was noted only in one animal in the 1.4 g/kg dose group.

IV. Summary

Acute oral toxicity studies employing albino rats as test animals were conducted on two materials identified as L-1931 (20470-23P) and L-1932 (20430-39D) respectively. The test materials were administered in the form of aqueous suspensions. The acute oral mean lethal dose values (LD<sub>50</sub>), expressed in terms of undiluted test material, were  $2.6 \pm 0.6$  g/kg for L-1931 and  $0.9 \pm 0.1$  g/kg for L-1932 respectively.

Respectfully submitted,

INDUSTRIAL BIO-TEST LABORATORIES, INC.

Report prepared by:

*Gerald Schoenig*  
Gerald Schoenig, B. S.  
Departmental Director  
Acute Toxicity Department

Report approved by:

*Otis E. Fancher*  
Otis E. Fancher, Ph. D.  
Director

*J. C. Calandra*  
J. C. Calandra, Ph. D., M. D.  
President

September 21, 1966  
nk

**CHEMICAL CONCENTRATES**

*Corporation*

A SUBSIDIARY OF BAKER INDUSTRIES, INC.

FORT WASHINGTON, PA. 19034

PHONE (215) MI 6-9400

15 June 1970

The Editor  
Fire Journal  
National Fire Protection Association  
60 Batterymarch Street  
Boston, Massachusetts 02110

Sir:

At the recent NFPA Meeting in Toronto information about the toxicity of "Light Water" was asked of me frequently. We had made a limited study on the effects of "Light Water" on marine life in preparation for substantial and controlled field tests. These effects were highly derogatory to marine life and the entire test program had to be abandoned to avoid severe local stream pollution. I am asked by concerned people to report our data on the "Light Water" studied and do herewith comply.

The only commercially available product was FC-194 and this was checked over a range which allowed for 48-fold to 16,000-fold dilution. These results are reported. Other "Light Water" formulations not commercially available were also checked and the results were similar.

A series of five ten-gallon tanks were used and these were stocked and restocked with a recommended group of hardy fish. Tank temperatures were maintained at  $72^{\circ}\text{F} \pm 2^{\circ}\text{F}$ , uniform aeration maintained by Tiger pumps and filter.

Each tank, fitted with stainless lids, housed a) 3 goldfish (average length 2-1/4 inches, average weight 1-1/2 grams), b) 2 Blackmoors (average length 2-1/2 inches, average weight 3 grams) and c) 2 Calicos (average length 2 inches, average weight 1-1/2 grams). There were fed standard fish food at a rate of 0.025 grams per tank per day. The tanks contained nine gallons of tapwater and foam concentrate as shown in the following summary chart.

**Exhibit  
1083**

State of Minnesota v. 3M Co.,  
Court File No. 27-CV-10-28862

3M\_MN02267863

The Editor  
Fire Journal

16 June 1970  
Page 2

<u>Foam Liquid</u> Type	<u>Conc. :%</u>	<u>Fluorochemical</u> :ppm	<u>Surface Tension</u> :dynes/cm	<u>Survival</u> Time
FC-194	2.0	1,250	14.8	3-10 min.
"	0.2	125	16.3	5-60 min.
"	0.02	12.5	36.7	4-8 hrs.
"	0.006	4	39.7	2-7 days
"	0.002	1	52.5	Over 7 days
Blank	-	-	67.5	Over 10 weeks

*1/20 FC*

We regard the 4 parts per million as the threshold concentration with lower concentrations probably safe. However, at all listed concentrations (including the 1 part per million) erratic motion, loss of stability and other visibly odd effects were present.

There appeared to be two principal possible causes of death for all the fish. The erratic motion, rapid rotation and general inability to remain upright led to the apparent drowning of the fish. The same characteristic, by which fluorochemical greatly lowers the interfacial tension allowing for film-formation, also permits the intrusion of water as the oil film on which protection of the fish's stabilizing mechanism depends is destroyed by the fluorochemical. The fish appears to drown as a result. There also appears to be an attack on his nervous system as evidenced by high speed swimming and crashing headlong into the sides and bottom of the tank.

Faithfully yours,

CHEMICAL CONCENTRATES CORPORATION

*S. I. Kalkstein*  
S. I. Kalkstein  
President

SIK/k

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

CC: J. W. Amacher  
T. L. Miller  
File: 11-0-4

PLASTICS DEPARTMENT

February 18, 1970

COMPANY CONFIDENTIAL

TO: J. MITCHELL, JR.   
PLASTICS DEPARTMENT  
EXPERIMENTAL STATION

FROM: W. E. HILTON  
FLUOROCARBONS DIVISION  
WASHINGTON WORKS

(8-181-7239)

REQUEST FOR TOXICOLOGICAL INFORMATION  
"TEFLON"® DIVISION CHEMICALS

Past studies made at Haskell Laboratory have indicated that ammonium perfluorooctonate (C-8 APFC), which is used in the preparation of "Teflon"® dispersions, is highly toxic when inhaled and moderately toxic when injected. However, data are not available on the chronic local or chronic systemic effect of the compound in the solid state or dissolved in "Teflon" dispersions.

A review of the medical history of employees in the dispersion's area revealed that with the exception of two cases of dermatitis, which were probably due more to temperature and moisture than specific chemicals, there have not been any indications of toxicological effects of chemicals. The plant Medical group is aware of the potential effects of the area chemicals and monitor area employees, both individually and statistically.

We are interested in determining the systemic effect for repetitious skin contacts of short duration with C-8 APFC powder and with the aqueous dispersion of polytetrafluoroethylene containing C-8 (or chlorendic acid). We are also interested in determining the chronic effect of inhalation of minute quantities of C-8 APFC. In addition to knowing these effects, we would like guidance on the personnel equipment necessary for adequate protection against these effects. Would you determine the cost of each separate study, the potential for beneficial data

*3/3/70 RSW handling cost estimates & proposals*

000080

EID123138

*W. E. Hilton  
2/19/70*

DJP001005

J. MITCHELL, JR.

- 2 -

February 18, 1970

beyond our ability to extrapolate existing qualitative data, and if the following data is sufficient for Haskell Lab's purposes.

C-8 APFC Charging

**REDACTED**

**000081**



J. MITCHELL, JR.

- 3 -

February 18, 1970

REDACTED

000082

Haskell Number \_\_\_\_\_

HASKELL LABORATORY FOR TOXICOLOGY AND INDUSTRIAL MEDICINE

SAMPLE SUBMITTED FOR TOXICITY EVALUATION

Department Plastics Division Fluorocarbons Location Washington Works

Systematic Name of Chemical:

Ammonium Perfluorooctanoate

Synonym, Product Name and/or Designations:

3M's Wetting Agent FC-143, C-8 APFC

Formula:

$\text{CF}_3 (\text{CF}_2)_6 \text{COONH}_4$

Sample: Code No. \_\_\_\_\_ Lot No. \_\_\_\_\_ Amount \_\_\_\_\_

Grade \_\_\_\_\_ Color White Form \_\_\_\_\_ Mol. Wt. \_\_\_\_\_

Special Handling Requirements \_\_\_\_\_

Is it explosive in air? No in oxygen? ? if so, at what concentrations? \_\_\_\_\_

Active Ingredient C-8 APFC 95 %

Composition, if a mixture (are percentages by weight or volume?)

C-8 95% Minimum

C-6 5% Maximum

Impurities (Identity and amounts; are percentages by weight or volume?)

C-6 5% Maximum

Properties: Decomposes: 150°C @ 25°C Negligible (Attach MP curve if available)  
MP \_\_\_\_\_ BP \_\_\_\_\_ VP \_\_\_\_\_ @ Max. Process Temp. 100°C  
2% Aqueous Solution

Sp.Gr. \_\_\_\_\_ pH<sup>4</sup> Min Vapor Density (air = 1) \_\_\_\_\_

Flash Point (Open cup) \_\_\_\_\_ (Closed cup) \_\_\_\_\_

Solubility (Quantitative, if possible) in water Yes Acetone \_\_\_\_\_

Ethanol \_\_\_\_\_ Vegetable oil \_\_\_\_\_ Other Solvents \_\_\_\_\_

Proposed Use:

Ingredient in Polymerization Recipe

Present Stage of Development: Research Service Mfg. Sales

(OVER)

000083

EID123141

DUP001008

Employee Exposure

1. Concentration of material in process 1200 PPM
2. Solvent and other chemicals present Water, Disuccinic Acid Peroxide
3. Is exposure by inhalation X skin absorption X other \_\_\_\_\_
4. Maximum temperature of material in process 100°C
5. Is the compound present in the atmosphere in the form of vapor No  
mist No dust Slight
6. Maximum concentration likely to be present in atmosphere Unknown
7. What type of ventilation is in use? High Volume
8. What type of protective clothing is worn? Gloves, Goggles, Dust Mask
9. Exposure is for \_\_\_\_\_ hours \_\_\_\_\_ minutes per day See Attached Letter

Consumer Exposure

By Employee

What is possibility of:

- |                 |                |  |
|-----------------|----------------|--|
| 1. Ingestion    | Very Slight    | NOTE: See Attached Letter for Write-Up |
| 2. Inhalation   | Minor Quantity |  |
| 3. Skin contact | Fair           |  |
| 4. Eye contact  | Slight         |  |

Experience to Date

Have any clinical signs of toxicity such as headaches, difficulty in breathing, dizziness, nausea, skin or eye irritation, etc., been reported by persons who have been in contact with the chemical? If so, please list them and describe circumstances under which they occurred.

No

NOTE: Haskell Laboratory will retain the unused portion of stable, nonflammable, nonvolatile, low toxicity samples for 5 years unless requested to return to sender immediately after testing. After 5 years, the samples will be discarded or returned to the appropriate department. It is suggested that the sender also retain a suitably identified portion of the sample sent for toxicity evaluation for his own future reference.

Signature W. E. Hilton Date 2/13/70

000084

EID123142

DUP001009

TABLE I

Ammonium Perfluorooctanoate (C-8 APFC)

Formula:  $\text{CF}_3 (\text{CF}_2)_6 \text{COONH}_4$

Manufacturer: 3M (FC-143)

Previous Tests:

Inhalation, MR No. 1198, Haskell Laboratory Report  
No. 160-69

Oral, MR No. 604, Haskell Laboratory Report No. 55-61.

Chlorendic Acid

Formula:  $\text{C}_9\text{H}_4\text{O}_4\text{Cl}_6$

Manufacturer: Hooker Electrochemical Company

Previous Tests:

Oral, MR No. 604, Haskell Laboratory Report No. 1-63.

EID123143

000085

DUP001010

C. H. M. Jr.

Report No. 68-73

February 23, 1973

NINETY-DAY FEEDING STUDY IN  
RATS AND DOGS WITH ZONYL® RP

Medical Research Project No. 1491



HASKELL LABORATORY FOR TOXICOLOGY  
AND INDUSTRIAL MEDICINE

NINETY-DAY FEEDING STUDY IN  
RATS AND DOGS WITH ZONYL® RP

Haskell Laboratory Report No. 68-73

Medical Research Project No. 1491

- 1 C. F. Reinhardt/J. A. Zapp, Jr.
- 2 P. E. Smith, Jr./H. J. Trochimowicz/B. W. Karrh
- 3 J. R. Barnes/J. G. Aftosmis/R. S. Waritz
- 4 J. F. Morgan
- 5 D. B. Hood
- 6 H. Sherman
- 7 C. S. Hornberger
- 8 F. D. Griffith
- 9 Haskell Laboratory File
- 10-15 C. W. Maynard, Jr., Organic Chemicals Department  
Jackson Laboratory

NINETY-DAY FEEDING STUDY IN  
RATS AND DOGS WITH ZONYL® RP

Haskell Laboratory Report No. 68-73

Medical Research Project No. 1491

Report by: Henry Sherman  
Henry Sherman  
Chief, Oral Toxicology Section

Approved for Biochemistry: John R. Barnes  
John R. Barnes  
Chief, Biochemistry Section

Approved for Pathology: James G. Aftosmis  
James G. Aftosmis  
Chief, Pathology Section

Approved by: Charles F. Reinhardt  
Charles F. Reinhardt  
Assistant Director

NB 958, pp. 120-167

E0579

HS:dhg

Date: February 23, 1973

NINETY-DAY FEEDING STUDY IN  
RATS AND DOGS WITH ZONYL® RP

Haskell Laboratory Report No. 68-73

Medical Research Project No. 1491

This feeding study was performed by Mrs. Kathleen Carroll under the direction of Dr. Henry Sherman. The clinical laboratory and biochemistry tests were performed by Mrs. Louise L. Adams, Mr. Norman W. Henry, III, Mr. John R. Pennington, Jr., and Miss Adele M. Pochomis under the direction of Dr. John R. Barnes. Gross pathology and preparation of slides were carried out by Dr. Rudolf Culik, Dr. K. P. Lee, Mr. August H. Stenholm, Mr. William I. Swan, Mr. Francis Ulmer, Mr. Anthony T. DiLorenzo, Mrs. Jean A. Houck and Mrs. Joan A. Dimeler under the direction of Dr. James G. Aftosmis. Histopathologic evaluation of the tissues was conducted by Dr. Edwin F. Stula under Dr. Aftosmis' direction.



NINETY-DAY FEEDING STUDY IN  
RATS AND DOGS WITH ZONYL® RP

Haskell Laboratory Report No. 68-73

Medical Research Project No. 1491

SUMMARY

Zonyl® RP has been fed to rats and dogs for ninety days at dietary levels of 0, 500, 750, and 2,500 ppm. No nutritional or clinical signs of toxicity were observed in rats or dogs on any of the test levels.

Rats receiving the intermediate and highest dietary levels of Zonyl® RP showed lower erythrocyte counts, hemoglobin concentrations and hematocrits than those of the controls; those receiving the highest dietary level also showed a slightly higher incidence of hematuria and proteinuria. Male rats receiving the two higher dietary levels of Zonyl® RP showed slightly larger liver and kidney weights and higher organ/body weight ratios than did the controls or other test group; all female test rats had higher kidney weights and kidney/body weight ratios whereas the female rats on the highest dietary level also had greater liver weights and liver/body weight ratios. Of all the organs examined histologically, only the livers, and only those from the male animals receiving 2,500 ppm Zonyl® RP, showed changes, and these were considered to be reversible.

Dogs receiving the highest dietary level of Zonyl® RP had lower erythrocyte counts, hemoglobin concentrations and hematocrit values than did the controls. Alkaline phosphatase activities were elevated in the dogs in all test groups, whereas cholesterol values were higher in dogs receiving 750 and 2,500 ppm Zonyl® RP. The livers of the dogs from all three test groups were heavier than those from the control dogs. The liver was the only organ of those examined that exhibited histologic changes and this was confined to those coming from the highest level group.

Thus, the results of this ninety-day feeding study and those of the previous one support only 100 ppm as a "no-effect" level for Zonyl® RP.

A. RATS

PROCEDURE

Fifty male and 50 female weanling albino rats (ChR-CD) were housed in pairs, sexes separate, in suspended stainless steel wire cages and fed ground Purina Laboratory Chow (GPLC) with 1% corn oil (CO) added. During a pre-test period of five days, the animals were observed with respect to food consumption, eating habits, and weight gain. They were divided into four equal average-weight groups of 10 male and 10 female rats each at the end of this period on the basis of weight gain and freedom from gross respiratory disorders or other clinical signs of disease. The groups were then assigned at random to receive the following diets:

<u>Group</u>	<u>Computer Groups</u>	<u>Diet</u>
I (Control)	(1 and 2)	GPLC + 1% CO
II	(3 and 4)	GPLC + 1% CO + 500 ppm Zonyl® RP
III	(5 and 6)	GPLC + 1% CO + 750 ppm Zonyl® RP
IV	(7 and 8)	GPLC + 1% CO + 2,500 ppm Zonyl® RP

The highest dietary level (2,500 ppm) had been suggested by the FDA to act as a bridge between the present study and the one conducted earlier under MRO-840. The test material was added as active ingredient, Zonyl® RP, present in the slurry at a 35% concentration. An amount of water was added to the control diet equivalent to that contributed by the 35% aqueous slurry to the diet in Group IV. Diets were prepared fresh each week and stored at refrigerator temperature until used.

The animals were weighed once a week during the entire study. Food consumption data were obtained on a group and sex basis at the times the animals were weighed.

During the test, the animals were examined routinely for any abnormal behavior and any clinical manifestations of toxicity.

Hematological, urine and biochemical analyses were conducted on ten male and ten female rats from each group after they had been on their respective diets for one, two, and three months. Hematological evaluations included an erythrocyte count, a measure of hemoglobin concentration, a measure of the hematocrit, and a total and differential white blood cell count. Urine analysis consisted of a measure of the 24-hour urine volume, concentration in milliosmoles and creatinine, a test for sugar, blood, protein, and urobilinogen, and an observation of the color, appearance, and pH. Specimens with a negative test for blood were combined to form two pools of urine for each group and the sediment from these pools examined microscopically. All specimens with a positive test for blood were examined separately. In the biochemical tests, to measure liver function, alkaline phosphatase and glutamic-pyruvic transaminase activities and bilirubin concentration were measured in blood taken from the tails of 10 males and 10 females in each group.

## A. RATS (Continued)

### PROCEDURE (Continued)

After 91-98 days of continuous feeding, all the animals in each group were sacrificed by  $\text{CHCl}_3$  administration. The following organs were weighed: brain, heart, lungs, liver, spleen, kidney, testis, stomach, adrenal, and pituitary. Organs or tissues, preserved in formalin and stained with hematoxylin-eosin, included, in addition to those listed above, the following: eye, exorbital lacrimal gland, sciatic nerve, skin, mammary gland, bone marrow, lymph node, skeletal muscle, trachea, aorta, salivary gland, esophagus, colon, cecum, duodenum, urinary bladder, prostate and seminal vesicles, uterus, Fallopian tubes, ovary, thyroid, parathyroid, and thymus. The above tissues from the control and highest level group (2,500 ppm) were evaluated histopathologically; only the livers from the animals in the other two test groups were examined histopathologically.

### RESULTS

#### 1. Weight Gain

Average body weight curves for control and test groups of animals are plotted in Figure 1; average body weights and average weight gains are summarized in Tables I, II, V and VI.

The presence of 2,500 ppm Zonyl<sup>®</sup> RP in the diet of male and female rats did not adversely affect their rate of weight gain.

#### 2. Food Consumption

A summary of the average daily food consumption data, computed as grams ingested per rat for each group, is presented in Tables III, IV and V.

There were no meaningful differences among the control and test groups with respect to the amount of food they consumed over the entire test.

#### 3. Food Efficiency

Food efficiency data, calculated as gram weight gain per gram of food consumed, are presented in Tables III-V.

There were no meaningful differences among control and test groups with respect to food efficiency.

## A. RATS (Continued)

### RESULTS (Continued)

#### 4. Dose

The average daily ingestion of test material during each week was calculated in milligrams of Zonyl<sup>®</sup> RP per kilogram of body weight; these data are presented in Table VI.

The decline in the average dose of test material received by animals observed in this study is normal and typical of that observed in most feeding studies where rapidly growing animals are used initially and the concentration of test material is kept constant throughout the test.

#### 5. Clinical Observations

None of the test animals in any of the three test groups exhibited any clinical signs of toxicity during the entire test that could be attributed to the test material.

#### 6. Mortality

None of the animals in the control and test groups died during the entire feeding study.

#### 7. Hematology

The results of the periodic hematological examinations conducted on rats fed the various levels of Zonyl<sup>®</sup> RP and their controls are summarized in Table VII.

The erythrocyte count, hemoglobin concentration and hematocrit of the rats fed Zonyl<sup>®</sup> RP were lower than those of the controls at 750 ppm and above. A one-way analysis of variance showed that the treatment with Zonyl<sup>®</sup> RP had a significant effect on these measurements. The values for these measurements, observed in the males but not the females fed 750 ppm Zonyl<sup>®</sup> RP, were significantly ( $p < 0.05$ ) lower than those for the controls. Both sexes were affected by a level of 2,500 ppm in the diet. The calculated hematologic indices, mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH) indicated that the erythrocytes of the rats fed 2,500 ppm Zonyl<sup>®</sup> RP were macrocytic and hypochromic. There were no effects on the number or distribution of leucocytes.

A. RATS (Continued)

RESULTS (Continued)

8. Urinalysis

The results of the periodic urine analyses conducted on rats fed various levels of Zonyl® RP are summarized in Table VIII.

A slightly higher incidence of hematuria and proteinuria occurred in the rats fed 2,500 ppm Zonyl® RP than in the other groups. All other measurements and observations made on the urine of the treated rats did not differ from those of the controls.

9. Biochemistry

The results of the periodic biochemical measurements conducted on the blood of rats fed various levels of Zonyl® RP are summarized in Table IX.

No effect on any of the biochemical measurements was found that could be related to the presence of the Zonyl® RP in the diet.

10. Pathology

A summary of the average weights of select organs taken from the control and test groups is presented in Table X; organ/body weight ratios are summarized in Table XI.

Among the test groups, male animals receiving the intermediate and high levels of Zonyl® RP showed higher average liver and kidney weights; both liver/body weight and kidney/body weight ratios were also slightly greater in these groups. Among the female test groups, all showed higher average kidney weights and kidney/body weight ratios than did the controls; average liver weights and liver/body weight ratios were slightly greater in the animals receiving 2,500 ppm Zonyl® RP than in the other test groups or the control.

A summary of the histopathologic findings is presented in Table XII, where 0 = no abnormalities detected, + = slight degree of lesion present, and X = organ not on slide.

Test chemical-related histopathologic effects were found only in the livers of male rats fed the highest (2,500 ppm) dietary level of Zonyl® RP. Examination of the livers from rats that received the two lower levels of the test chemical did not reveal any compound-related effects.

A. RATS (Continued)

RESULTS (Continued)

10. Pathology (Continued)

The liver changes observed in the males in the highest dietary level group consisted of fatty change, cytomegaly, and cytoplasmic hyaline droplets. This type of liver change is considered to be reversible.

B. DOGS

PROCEDURE

During a pre-test period of approximately one month, 16 male and 16 female beagle dogs, 9 to 14 months old, were given Wayne Dog Krums<sup>®</sup> and water ad libitum between 3:00 p.m. and 7:00 a.m. Animals were examined daily for any abnormal behavior and any clinical manifestations of toxicity. During this period, specimens of blood and urine were collected from each dog twice for clinical laboratory examinations. The tests included in these examinations are listed below:

Hematology: Erythrocyte count, hemoglobin concentration, hematocrit, total and differential leucocyte count.

Urinalysis: An observation of color, appearance and pH; a measure of the 24-hour urine volume, creatinine, and osmolality; a test for sugar, blood, protein, acetone, urobilinogen, and bilirubin; a microscopic examination of the sediment.

Biochemistry: Glucose, urea-nitrogen, creatinine, cholesterol, alkaline phosphatase, glutamic-pyruvic transaminase, bilirubin, total protein, albumin, and albumin/globulin ratio

Four males and four females were allocated to each of the four groups on the basis of normal clinical and nutritional evaluations and assigned at random to receive the following diets:

<u>Group</u>	<u>Diet</u>
I (Control)	Wayne Dog Food (Krums <sup>®</sup> )
II	Krums <sup>®</sup> + 500 ppm Zonyl <sup>®</sup> RP
III	Krums <sup>®</sup> + 750 ppm Zonyl <sup>®</sup> RP
IV	Krums <sup>®</sup> + 2,500 ppm Zonyl <sup>®</sup> RP

Diets were prepared fresh each week. Diet was offered ad libitum to the dogs between 3:00 p.m. and 7:00 a.m.; water was available at all times.

## B. DOGS (Continued)

### PROCEDURE (Continued)

Diet consumption and body weight data were obtained each week and calculations were made to determine the approximate daily dose each week of Zonyl<sup>®</sup> RP per kilogram of body weight. Animals were examined daily for any clinical signs of toxicity.

The same clinical laboratory examinations made during the pre-test period were conducted on each dog after one, two, and three months of feeding.

After 98-105 days of continuous feeding, all dogs were sacrificed by electrocution and submitted to gross and histopathologic evaluation. Organ weights were obtained for the following: brain, heart, lungs, liver, spleen, pancreas, kidney, testis, prostate, stomach, thymus, adrenals, pituitary, and thyroid. Organs and tissues were preserved in formalin and stained with hematoxylin-eosin; these included, in addition to those mentioned above, the following: epididymis, Fallopian tubes, aorta, esophagus, uterus, ovary, duodenum, cecum, mammary gland, urinary bladder, spinal cord, trachea, salivary gland, bone marrow, lymph node, colon, sciatic nerve, skeletal muscle, eye, and skin. All tissues taken from the dogs on the control diet and highest test level diet were evaluated histologically; only the livers from the animals receiving the low and intermediate levels of Zonyl<sup>®</sup> RP were examined histologically. The livers from the control and highest level dogs were stained with Oil Red O.

### RESULTS

#### 1. Body Weight

The weekly body weight data of the individual dogs are presented in Tables XIII and XIV.

All dogs, control and test, showed normal body weight gains during the study.

#### 2. Diet Consumption

Values calculated for average daily diet consumption for each dog are presented in Tables XV and XVI.

The amount of diet consumed by each dog varied from week to week. However, there was no adverse effect by the test material upon diet intake.

#### 3. Dose

The average daily intake of Zonyl<sup>®</sup> RP in milligrams per kilogram of body weight was calculated for each dog; these results are presented in Tables XVII and XVIII.

## RESULTS (Continued)

### 3. Dose (Continued)

Since the body weights of most of the dogs remained relatively constant throughout the test, the dose of Zonyl<sup>®</sup> RP received by each treated dog each week fluctuated with diet intake.

### 4. Clinical Signs

Regular examination disclosed no clinical changes in any of the test dogs that could be attributed to the feeding of Zonyl<sup>®</sup> RP.

All dogs survived the 90-day feeding period without incident.

### 5. Hematology

The results of the hematological measurements conducted throughout the feeding study are summarized in Table XIX; the numbers represent average values obtained during pretreatment and treatment periods. Individual values are presented in Appendix I.

The erythrocyte count, hemoglobin concentration, and hematocrit of the dogs fed 2,500 ppm Zonyl<sup>®</sup> RP were generally lower than those of the other dogs, control and test, during treatment. A one-way analysis of variance indicated that the treatment with Zonyl<sup>®</sup> RP significantly affected the hemoglobin concentration and hematocrit, whether expressed as grams of hemoglobin per 100 ml and percent of packed cells, or as the change from the pre-test observation for these measurements.

### 6. Urinalysis

The results of the urine analysis measurements conducted throughout the feeding study are summarized in Table XX; the numbers represent average values obtained during the pretreatment and treatment periods. Individual values are presented in Appendix I.

No effect attributable to the addition of Zonyl<sup>®</sup> RP to the dogs' diet was found in any of the measurements or observations made on the urine.

### 7. Biochemistry

The results of the biochemical measurements conducted throughout the feeding study are summarized in Table XXI; the figures recorded represent average values obtained during the



## RESULTS (Continued)

### 7. Biochemistry (Continued)

pretreatment and treatment periods. Individual values are presented in Appendix I.

There was no effect on the glucose, urea-nitrogen, creatinine, transaminase, bilirubin, or plasma protein values. A one-way analysis of variance, however, showed that the total cholesterol and alkaline phosphatase activity of the dogs fed Zonyl<sup>®</sup> RP were significantly affected by the treatment. The alkaline phosphatase activity of the dogs fed the lowest dose, 500 ppm Zonyl<sup>®</sup> RP, was elevated significantly ( $p < 0.05$ ). Both alkaline phosphatase and cholesterol values were elevated in the dogs fed 750 ppm and 2,500 ppm Zonyl<sup>®</sup> RP. This effect occurred after one month, was maximum at two months, and then stabilized, or decreased slightly, at three months. The effect was significant for the relative change from the pre-exposure, expressed as a percent, as well as for the increase in Bessey units (alkaline phosphatase) or mg % (cholesterol) for the Zonyl<sup>®</sup> RP treated dogs.

### 8. Pathology

The individual organ weights of the dogs sacrificed after three months' feeding of Zonyl<sup>®</sup> RP are presented in Tables XXII and XXIII.

The small group sizes and large variation do not permit a complete statistical analysis of the liver weights. However, a variance analysis of liver/body weight ratios indicates that there was a difference between control and test groups with respect to this ratio, a heavier liver being the effect of treatment. This is summarized in Table XXIV, where mean values are recorded. It would appear that the livers of all three groups, i.e., low, intermediate, and high dietary levels of Zonyl<sup>®</sup> RP, were affected by the presence of Zonyl<sup>®</sup> RP in the diet.

A summary of the histopathologic findings is presented in Tables XXV-XXVIII.

The livers from the highest level of feeding were pale and had rounded edges with a tight capsule. Histologically, the liver from the dogs in the highest level of feeding (2,500 ppm Zonyl<sup>®</sup> RP) was the only organ affected. The liver changes were difficult to detect. They consisted of a slight enlargement of hepatocytes (hypertrophy) together with an uneven distribution of cytoplasmic particles (degeneration). Examination of the livers from control and high level dogs stained with Oil Red O did not reveal an increase of lipid.

## RESULTS (Continued)

### 8. Pathology (Continued)

No compound-related abnormalities were detected in the livers of the dogs receiving 500 and 750 ppm Zonyl<sup>®</sup> RP.

## DISCUSSION

In the previously-reported ninety-day feeding study in rats and dogs (MRO-840), the lowest level fed, 100 ppm, was considered the "no-effect" level. The other two levels, 500-1,000 ppm and 2,500-5,000 ppm, did produce changes in both rats and dogs. In rats, this consisted of hematological changes at 2,500-5,000 ppm, increased liver and kidney weights at 500-1,000 ppm and 2,500-5,000 ppm levels, and pale yellowish livers in some male rats at the mid and highest levels; histologic changes were observed in only the livers from the highest level, 2,500-5,000 ppm. In dogs, plasma cholesterol and alkaline phosphatase were elevated in those fed 2,500-5,000 ppm Zonyl<sup>®</sup> RP, suggesting liver damage. Liver weights were increased at the middle and highest dietary levels; histologic changes were observed in only the livers of dogs receiving the highest dietary level.

The present study confirms the results obtained earlier. There does not appear to be a "no-effect" level in this present study, since increased liver and kidney weights were observed at the lowest dietary level in rats and since increased alkaline phosphatase activity and increased liver weights were observed in the lowest level in the dogs.

Thus, the results of this ninety-day feeding study and those of the previous one support only 100 ppm as a "no-effect" level for Zonyl<sup>®</sup> RP.

## SUMMARY

Zonyl<sup>®</sup> RP has been fed to rats and dogs for ninety days at dietary levels of 0, 500, 750, and 2,500 ppm. No nutritional or clinical signs of toxicity were observed in rats or dogs on any of the test levels.

Rats receiving the intermediate and highest dietary levels of Zonyl<sup>®</sup> RP showed lower erythrocyte counts, hemoglobin concentrations and hematocrits than those of the controls; those receiving the highest dietary level also showed a slightly higher incidence of hematuria and proteinuria. Male rats receiving the two higher dietary levels of Zonyl<sup>®</sup> RP showed slightly larger liver and kidney weights and higher organ/body weight ratios than did the controls or other test group; all female test rats had higher kidney weights and kidney/body weight ratios whereas the female rats on the highest dietary level also had greater liver weights and liver/body weight ratios. Of all the organs examined histologically, only the livers, and only those from the male animals receiving 2,500 ppm Zonyl<sup>®</sup> RP, showed changes, and these were considered to be reversible.

Dogs receiving the highest dietary level of Zonyl<sup>®</sup> RP had lower erythrocyte counts, lower hemoglobin concentrations, and hematocrit values

SUMMARY (Continued)

than did the controls. Alkaline phosphatase activities were elevated in the dogs in all test groups, whereas cholesterol values were higher in dogs receiving 750 and 2,500 ppm Zonyl<sup>®</sup> RP. The livers of the dogs from all three test groups were heavier than those from the control dogs. The liver was the only organ of those examined that exhibited histologic changes and this was confined to those coming from the highest level group.

Thus, the results of this ninety-day feeding study and those of the previous one support only 100 ppm as a "no-effect" level for Zonyl<sup>®</sup> RP.

BODY WEIGHTS OF MALE & FEMALE RATS FED  
VARIOUS LEVELS OF ZONYL® RP

MR 1491

FIG. 1

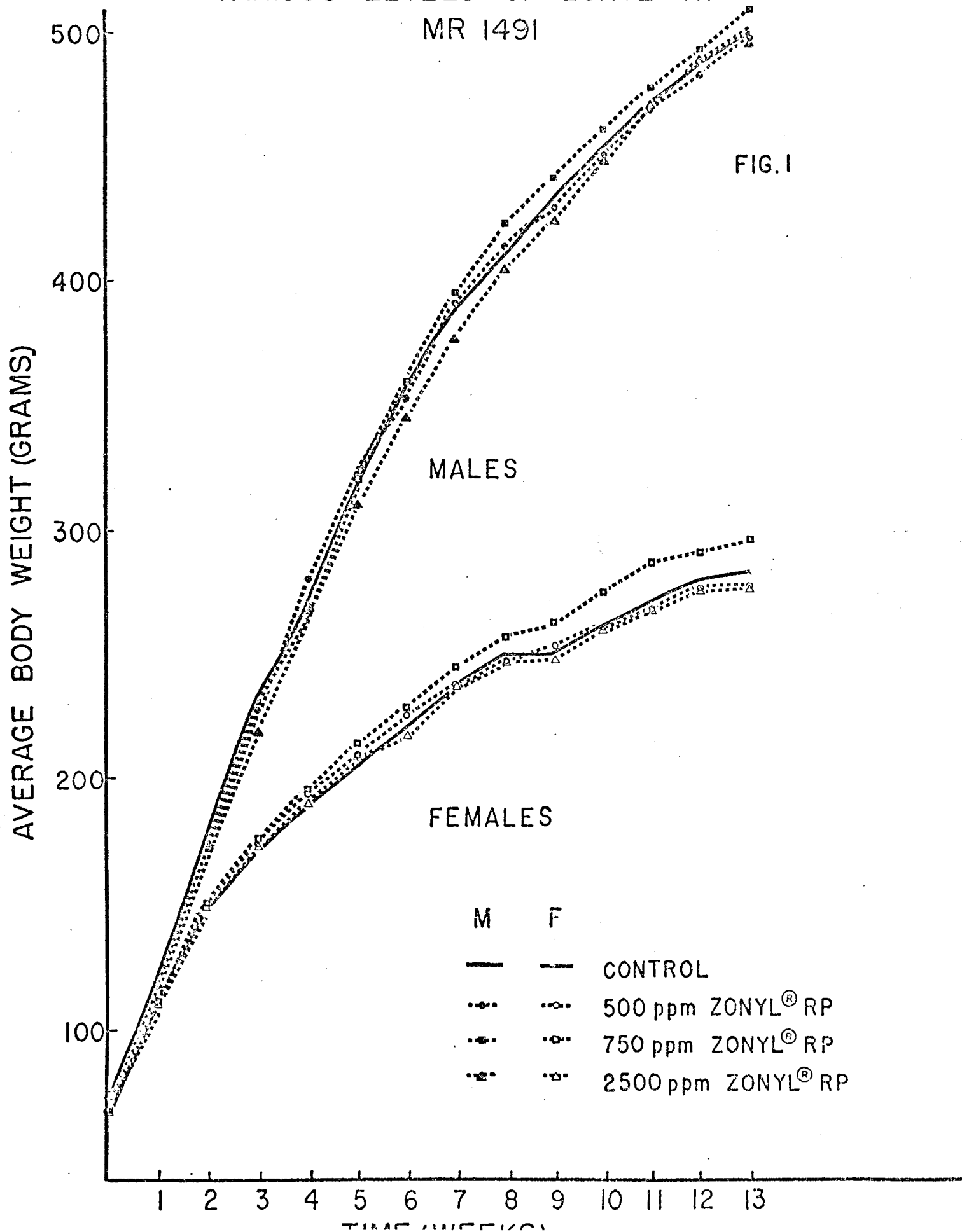


TABLE I

AVERAGE BODY WEIGHTS AND AVERAGE WEIGHT GAINS OF  
MALE RATS FED VARIOUS LEVELS OF ZONYL® RP

<u>Days on Test</u>	<u>Group I Control</u>	<u>Zonyl® RP</u>		
		<u>Group II 500 ppm</u>	<u>Group III 750 ppm</u>	<u>Group IV 2,500 ppm</u>
<u>Average Body Weight (gm)</u>				
0	70	69	69	68
7	123	120	119	117
14	179	174	172	168
21	233	227	229	221
28	266	280	267	267
35	321	323	320	310
42	358	358	358	345
49	388	390	396	377
56	411	413	423	404
63	432	431	441	423
70	452	451	461	448
77	472	470	478	471
84	487	482	492	489
91	500	497	510	495
<u>Average Weight Gain (gm)</u>				
0- 7	53	51	50	49
7-14	56	54	53	51
14-21	54	53	57	53
21-28	33	53	38	46
28-35	55	43	53	33
35-42	37	35	38	35
42-49	30	32	38	32
49-56	23	23	27	27
56-63	21	18	18	19
63-70	20	20	20	25
70-77	20	19	17	23
77-84	15	12	14	18
84-91	13	15	18	6

TABLE II

AVERAGE BODY WEIGHTS AND AVERAGE WEIGHT GAINS OF  
FEMALE RATS FED VARIOUS LEVELS OF ZONYL® RP

<u>Days on Test</u>	<u>Group I Control</u>	<u>Zonyl® RP</u>		
		<u>Group II 500 ppm</u>	<u>Group III 750 ppm</u>	<u>Group IV 2,500 ppm</u>
<u>Average Body Weight (gm)</u>				
0	69	70	70	70
7	113	114	110	112
14	147	148	149	150
21	170	173	175	176
28	189	194	196	195
35	205	208	214	208
42	220	225	228	217
49	238	236	244	236
56	250	247	257	246
63	250	253	262	247
70	259	260	273	261
77	270	268	286	267
84	279	276	290	275
91	282	277	295	276
<u>Average Weight Gain (gm)</u>				
0- 7	44	44	40	42
7-14	35	34	39	38
14-21	22	25	26	26
21-28	19	21	21	19
28-35	16	14	18	13
35-42	15	17	14	9
42-49	18	11	16	19
49-56	12	11	13	10
56-63	0	6	5	1
63-70	9	7	11	14
70-77	11	8	13	6
77-84	9	8	4	8
84-91	3	1	5	1

TABLE III

AVERAGE DAILY FOOD CONSUMPTION AND FOOD EFFICIENCY OF  
MALE RATS FED VARIOUS LEVELS OF ZONYL® RP

<u>Days on Test</u>	<u>Group I Control</u>	<u>Zonyl® RP</u>		
		<u>Group II 500 ppm</u>	<u>Group III 750 ppm</u>	<u>Group IV 2,500 ppm</u>
<u>Average Daily Food Consumption (gm)</u>				
0- 7	14.9	14.8	14.0	14.1
7-14	20.1	18.4	18.5	18.2
14-21	19.4	20.7	21.1	20.1
21-28	23.3	24.3	23.3	23.1
28-35	25.0	22.1	25.5	24.8
35-42	25.4	25.8	25.8	25.4
42-49	24.4	25.2	26.1	25.6
49-56	25.6	25.7	25.8	26.1
56-63	25.6	25.5	25.8	24.2
63-70	24.4	24.9	25.9	26.1
70-77	24.5	24.5	25.9	27.9
77-84	25.0	24.7	26.3	27.4
84-91	23.8	23.1	25.4	25.4
<u>Gram Weight Gain/Gram Food Consumed</u>				
0- 7	0.51	0.50	0.51	0.49
7-14	0.40	0.42	0.41	0.40
14-21	0.40	0.37	0.38	0.38
21-28	0.20	0.31	0.23	0.28
28-35	0.31	0.28	0.30	0.25
35-42	0.21	0.19	0.21	0.19
42-49	0.18	0.18	0.20	0.18
49-56	0.13	0.13	0.15	0.15
56-63	0.11	0.10	0.10	0.11
63-70	0.12	0.11	0.11	0.14
70-77	0.11	0.11	0.09	0.12
77-84	0.09	0.07	0.08	0.09
84-91	0.08	0.09	0.10	0.03

TABLE IV

AVERAGE DAILY FOOD CONSUMPTION AND FOOD EFFICIENCY OF  
FEMALE RATS FED VARIOUS LEVELS OF ZONYL<sup>®</sup> RP

<u>Days on Test</u>	<u>Group I Control</u>	<u>Zonyl<sup>®</sup> RP</u>		
		<u>Group II 500 ppm</u>	<u>Group III 750 ppm</u>	<u>Group IV 2,500 ppm</u>
<u>Average Daily Food Consumption (gm)</u>				
0- 7	13.5	14.0	13.0	13.1
7-14	16.1	16.9	16.0	15.9
14-21	12.9	16.8	15.6	14.7
21-28	17.0	18.2	17.3	17.4
28-35	17.6	16.9	17.2	16.9
35-42	17.8	19.1	18.1	17.3
42-49	18.2	17.7	18.8	18.1
49-56	18.8	20.4	19.1	18.9
56-63	21.2	20.6	18.3	18.2
63-70	16.2	17.6	17.3	17.3
70-77	18.2	19.2	20.2	18.9
77-84	18.5	19.0	18.6	18.3
84-91	17.0	16.9	17.9	16.5
<u>Gram Weight Gain/Gram Food Consumed</u>				
0- 7	0.46	0.45	0.44	0.46
7-14	0.31	0.29	0.35	0.34
14-21	0.24	0.21	0.24	0.25
21-28	0.16	0.16	0.17	0.16
28-35	0.13	0.12	0.15	0.11
35-42	0.12	0.13	0.11	0.08
42-49	0.14	0.09	0.12	0.15
49-56	0.09	0.08	0.10	0.08
56-63	-	0.04	0.04	0.01
63-70	0.08	0.06	0.09	0.11
70-77	0.08	0.06	0.09	0.05
77-84	0.07	0.06	0.03	0.06
84-91	0.02	0.01	0.04	0.01



TABLE V

AVERAGE WEIGHT GAIN, FOOD CONSUMPTION, AND FOOD EFFICIENCY DATA,  
CALCULATED AT APPROXIMATELY MONTHLY INTERVALS, OF MALE AND  
FEMALE RATS FED VARIOUS LEVELS OF ZONYL® RP

Group	Days on Test	MALES			FEMALES		
		Weight Gain (gm)	Food Consumption (gm)	Food Efficiency	Weight Gain (gm)	Food Consumption (gm)	Food Efficiency
I (Control)	0-28	196	544	0.36	120	417	0.29
	28-56	145	703	0.21	61	507	0.12
	56-91	89	863	0.10	32	637	0.05
	Total	430	2110	0.20	213	1561	0.14
II (500 ppm Zonyl® RP)	0-28	211	548	0.38	124	461	0.27
	28-56	133	692	0.19	53	519	0.10
	56-91	84	859	0.10	30	654	0.05
	Total	428	2099	0.20	207	1634	0.13
III (750 ppm Zonyl® RP)	0-28	198	539	0.37	126	434	0.29
	28-56	156	723	0.22	61	512	0.12
	56-91	87	905	0.10	38	647	0.06
	Total	441	2167	0.20	225	1593	0.14
IV (2500 ppm Zonyl® RP)	0-28	199	529	0.38	125	428	0.29
	28-56	137	713	0.19	51	499	0.10
	56-91	91	917	0.10	30	625	0.05
	Total	427	2159	0.20	206	1552	0.13

TABLE VI

AVERAGE DAILY INTAKE OF ZONYL® RP

Average Dose in Milligrams/Kilogram/Day

<u>Days on Test</u>	<u>Group I Control</u>	<u>Zonyl® RP</u>		
		<u>Group II 500 ppm</u>	<u>Group III 750 ppm</u>	<u>Group IV 2,500 ppm</u>
<u>MALES</u>				
0- 7	-	78	112	382
7-14	-	63	95	319
14-21	-	52	79	259
21-28	-	48	70	237
28-35	-	37	65	215
35-42	-	38	57	194
42-49	-	34	52	177
49-56	-	32	47	167
56-63	-	30	45	144
63-70	-	28	43	149
70-77	-	27	41	152
77-84	-	26	41	142
84-91	-	24	38	129
<u>FEMALES</u>				
0- 7	-	76	108	361
7-14	-	65	92	303
14-21	-	52	72	226
21-28	-	50	70	234
28-35	-	42	63	209
35-42	-	44	61	204
42-49	-	38	60	200
49-56	-	42	57	196
56-63	-	41	53	185
63-70	-	34	48	170
70-77	-	36	53	179
77-84	-	35	49	169
84-91	-	31	46	150

TABLE VII

SUMMARY OF HEMATOLOGIC MEASUREMENTS ON RATS FED ZONYL® RP FOR THREE MONTHS

	ppm in Diet	MALES			FEMALES		
		Months on Test			Months on Test		
		1	2	3	1	2	3
Erythrocytes x 10 <sup>6</sup> /mm <sup>3</sup>	0	5.98	6.14	6.38	5.87	4.65	6.04
	500	-	5.65	5.70	-	5.38	5.66
	750	5.64	5.39	6.12	5.86	4.33	5.93
	2,500	5.58	4.92	5.27	5.94	4.18	5.17
Hemoglobin gm/100 ml	0	14.9	15.6	16.7	15.4	13.5	16.6
	500	-	15.6	15.2	-	15.1	15.9
	750	13.9	14.5	15.6	15.2	12.1	16.2
	2,500	13.3	13.7	15.5	14.9	11.8	15.3
Hematocrit %	0	43	45	49	44	37	46
	500	-	43	43	-	43	44
	750	39	42	45	41	33	45
	2,500	38	39	44	41	32	42
Leucocytes x 10 <sup>3</sup> /mm <sup>3</sup>	0	13.4	13.2	12.6	10.8	10.5	7.1
	500	-	12.4	10.7	-	9.3	7.3
	750	12.9	14.1	12.3	10.2	10.6	8.9
	2,500	13.7	15.9	14.5	14.4	14.2	10.1
Neutrophils %	0	22	19	18	20	22	20
	500	-	22	23	-	17	20
	750	26	21	22	21	21	19
	2,500	25	19	21	19	19	21
Lymphocytes %	0	75	77	79	77	74	76
	500	-	75	75	-	80	77
	750	72	75	75	76	75	78
	2,500	73	79	76	78	77	75

TABLE VII (Continued)

SUMMARY OF HEMATOLOGIC MEASUREMENTS ON RATS FED ZONYL® RP FOR THREE MONTHS

	ppm in Diet	MALES			FEMALES		
		Months on Test			Months on Test		
		1	2	3	1	2	3
Monocytes %	0	1.0	0.9	0.1	0.3	1.0	0.5
	500	-	0.6	0.6	-	0.1	0.7
	750	1.5	0.4	0.1	1.0	0.9	0.5
	2,500	1.4	0.5	0.5	0.9	1.2	0.8
Eosinophils %	0	2.0	2.4	2.5	2.5	4.7	3.5
	500	-	2.3	2.1	-	2.7	2.7
	750	1.1	3.2	2.8	2.2	3.2	2.4
	2,500	1.4	2.2	2.9	2.1	3.2	3.4
Basophils %	0	0.1	0	0	0	0	0.1
	500	-	0.1	0.1	-	0	0
	750	0.1	0.1	0	0.1	0.1	0
	2,500	0	0	0.1	0	0.1	0.1
Atypical Cells %	0	0	0	0	0	0	0
	500	-	0	0	-	0	0
	750	0	0	0	0	0	0
	2,500	0	0	0	0	0	0
Nucleated RBC's per 100 WBC's	0	0	0.1	0	0	0	0
	500	-	0	0	-	0	0
	750	0	0.1	0	0	0	0
	2,500	0	0	0	0	0	0

TABLE VIII

SUMMARY OF URINALYSIS DATA ON RATS FED ZONYL® RP FOR THREE MONTHS

	ppm in Diet	MALES			FEMALES		
		Months on Test			Months on Test		
		1	2	3	1	2	3
Volume ml/24 hrs.	0	18	19	17	13	19	14
	500	-	22	16	-	15	12
	750	15	18	18	12	15	15
	2,500	15	20	21	13	18	13
Osmolality mOs/L	0	1349	1696	1857	1590	1473	1619
	500	-	1818	2012	-	1763	1926
	750	1578	1845	1860	1849	1792	1852
	2,500	1760	1757	1711	1783	1644	1842
Creatinine mg/24 hrs.	0	9.2	17.4	15.2	7.1	11.8	9.0
	500	-	16.7	15.9	-	10.0	8.2
	750	9.5	17.8	17.0	7.1	10.8	9.5
	2,500	9.3	16.7	16.7	7.4	10.3	8.6
Blood Number Positive	0	0	1	0	0	1	0
	500	-	0	0	-	1	0
	750	0	0	0	0	0	0
	2,500	1	2	1	1	3	1
Sugar Number Abnormal <sup>1)</sup>	0	0	0	0	0	0	0
	500	-	0	0	-	0	0
	750	0	0	0	0	0	0
	2,500	0	0	0	0	0	0
Bilirubin Number Positive	0	0	0	0	0	0	0
	500	-	0	0	-	0	0
	750	0	0	0	0	0	0
	2,500	0	0	0	0	0	0

1) Number ++ or greater by Clinitest®

TABLE VIII (Continued)

SUMMARY OF URINALYSIS DATA ON RATS FED ZONYL® RP FOR THREE MONTHS

	ppm in Diet	MALES			FEMALES		
		Months on Test			Months on Test		
		1	2	3	1	2	3
Protein Number Abnormal <sup>2)</sup>	0 500 750 2,500	0 0 0 1	0 0 0 1	0 0 0 0	0 0 0 0	1 2 1 3	
Urobilinogen	0 500 750 2,500	0.4 - 0.6 0.5	1.0 1.0 1.0 1.0	0.6 0.8 0.7 0.7	0.7 - 0.7 0.6	0.8 0.9 0.8 0.8	
Erythrocytes per hpf	0 500 750 2,500	0 - 0 0	0a) 0 0 0	0 0 0 0	0 - 0 0	0 0 0 1	
Leucocytes per hpf	0 500 750 2,500	0-1 - 0-1 0-1	0-1 0-1 0-1 0-1	0-1 0-1 0-1 0-1	0-1 - 0-1 0-3	0-1 0-3 0-1 0-1	
Epithelial Cells per hpf	0 500 750 2,500	0 - 0 0	0 0 0 0-1	0-1 0-1 0-1 0	0 - 0 0	0-1 0-1 0-1 0-1	
Casts per lpf	0 500 750 2,500	0 - 0 0	0 0 0 0	0 0 0 0	0 - 0 0	0 0 0 0	

2) Number ++ or greater by Albustix®

a) 1/10 hematuria

b) 1/10 with positive test for blood examined separately

c) 2/10 with positive test for blood examined separately

TABLE IX

SUMMARY OF BIOCHEMICAL MEASUREMENTS ON RATS FED ZONYL® RP FOR THREE MONTHS

	ppm in Diet	MALES			FEMALES		
		Months on Test			Months on Test		
		1	2	3	1	2	3
Alkaline Phosphatase Bessey Units	0	44	38	32	35	22	19
	500	-	-	-	-	-	-
	750	47	38	33	33	23	17
	2,500	41	33	24	26	20	14
Transaminase Reitman-Frankel Units	0	18	12	8	14	19	14
	500	-	-	-	-	-	-
	750	18	10	9	15	18	12
	2,500	18	12	18	17	21	15
Bilirubin mg %	0	1.1	1.7	0.6	1.0	0.3	0.5
	500	-	-	-	-	-	-
	750	0.6	1.5	0.3	0.9	0.4	0.5
	2,500	0.5	1.4	0.4	1.2	0.4	0.4

TABLE X

AVERAGE ORGAN WEIGHTS IN GRAMS OF MALE AND FEMALE RATS FED VARIOUS LEVELS OF ZONYL® RP

Group	Final Body Weight	Brain	Heart	Lungs	Liver	Spleen	Kidney	Testis	Stomach	Adrenal	Pituitary
<u>MALES</u>											
I (Control)	500	2.19	1.44	2.39	17.08	0.77	3.46	3.36	2.04	0.058	0.012
II (500 ppm Zonyl® RP)	505	2.24	1.50	2.40	17.95	0.71	3.78	3.27	1.90	0.053	0.014
III (750 ppm Zonyl® RP)	524	2.25	1.52	2.47	21.46	0.70	4.30	3.22	2.06	0.058	0.016
IV (2,500 ppm Zonyl® RP)	495	2.19	1.45	2.42	22.00	0.69	4.45	3.31	2.03	0.059	0.014
<u>FEMALES</u>											
I (Control)	286	2.06	0.91	1.84	10.72	0.57	2.13	-	1.43	0.074	0.017
II (500 ppm Zonyl® RP)	288	2.02	0.96	1.76	11.39	0.49	2.66	-	1.53	0.076	0.019
III (750 ppm Zonyl® RP)	306	2.08	0.97	1.86	11.76	0.53	2.87	-	1.65	0.078	0.016
IV (2,500 ppm Zonyl® RP)	276	2.06	0.97	1.85	13.24	0.53	3.04	-	1.46	0.089	0.019



TABLE XI

SUMMARY OF ORGAN/BODY WEIGHT RATIOS OF MALE AND FEMALE RATS FED VARIOUS LEVELS OF ZONYL® RP

Group	% of Body Weight									
	Brain	Heart	Lungs	Liver	Spleen	Kidney	Testis	Stomach	Adrenal	Pituitary
<u>MALES</u>										
I (Control)	0.44	0.29	0.48	3.41	0.15	0.69	0.67	0.41	0.012	0.002
II (500 ppm Zonyl® RP)	0.44	0.30	0.48	3.55	0.14	0.75	0.65	0.38	0.010	0.002
III (750 ppm Zonyl® RP)	0.43	0.29	0.47	4.10	0.13	0.82	0.61	0.39	0.011	0.003
IV (2,500 ppm Zonyl® RP)	0.44	0.29	0.49	4.44	0.14	0.90	0.67	0.41	0.011	0.002
<u>FEMALES</u>										
I (Control)	0.72	0.32	0.64	3.75	0.20	0.74	-	0.50	0.025	0.005
II (500 ppm Zonyl® RP)	0.70	0.33	0.61	3.95	0.17	0.92	-	0.53	0.026	0.006
III (750 ppm Zonyl® RP)	0.68	0.32	0.61	3.84	0.17	0.94	-	0.54	0.025	0.005
IV (2,500 ppm Zonyl® RP)	0.75	0.35	0.67	4.80	0.19	1.10	-	0.53	0.032	0.006













TABLE XII-C

HISTOPATHOLOGY IO-DATE SUMMARY - RAT

HASKELL # - 7247 TEST # - 33 HR. # - 1491001  
 COMPOUND - "ZONYL" HP (35% SLURRY OF "ZONYL" RP)

JULY 10, 1972

ANIMAL #	7576	7530	7631	7586	7599	7625	7627	7628	7604	7698	7626	7593	7577	7545	7551	7540
GROUP #	03	03	04	04	04	04	04	04	04	04	04	04	05	05	05	05
TREATMENT DAYS	96	96	97	97	97	97	97	97	97	97	97	97	97	97	97	97
POST-TREATMENT DAYS																
DIET/DOSE VALUE	500	500	500	500	500	500	500	500	500	500	500	500	750	750	750	750
INITIAL WEIGHT	96	96	56	61	63	68	70	72	75	77	78	82	63	63	63	66
LOW WEIGHT																
FINAL WEIGHT	482	542	245	242	259	268	301	293	281	333	346	309	507	549	552	525
SEX	M	M	F	F	F	F	F	F	F	F	F	F	M	M	M	M
DIED/SACRIFICED	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S

- EYE NORMAL MORPHOLOGIC STRUCTURES
- EXTRINSICAL LACRIMAL GLAND NORMAL MORPHOLOGIC STRUCTURES
- BRAIN NORMAL MORPHOLOGIC STRUCTURES
- MESENTERIC LYMPHATIC NORMAL MORPHOLOGIC STRUCTURES
- SCIATIC NERVE NORMAL MORPHOLOGIC STRUCTURES
- SKIN NORMAL MORPHOLOGIC STRUCTURES
- SKIN ORGAN MISSING
- MAMMARY GLAND NORMAL MORPHOLOGIC STRUCTURES
- MAMMARY GLAND ORGAN MISSING
- BONE MARROW NORMAL MORPHOLOGIC STRUCTURES
- SPLEEN NORMAL MORPHOLOGIC STRUCTURE
- LYMPH NODE NORMAL MORPHOLOGIC STRUCTURES
- LYMPH NODE ORGAN MISSING
- SKELTAL MUSCLE NORMAL MORPHOLOGIC STRUCTURES
- TRACHEA NORMAL MORPHOLOGIC STRUCTURES
- LUNG NORMAL MORPHOLOGIC STRUCTURES
- LUNG INTERSTITIAL PNEUMONIA
- HEART NORMAL MORPHOLOGIC STRUCTURES
- AORTA NORMAL MORPHOLOGIC STRUCTURES
- SALIVARY GLAND NORMAL MORPHOLOGIC STRUCTURES
- LIVER NORMAL MORPHOLOGIC STRUCTURES
- LIVER GRANULOMA
- LIVER CLOUDY SWELLING
- LIVER HYALINE DROPLET DEGENERATION
- LIVER CYTOPLASMIC LIPID DROPLET ALT

0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0



TABLE XII-C (Continued)

HISTOPATHOLOGY TO-DATE SUMMARY - RAT

PAGE - 4-A

HASKELL # - 7247 TEST # - 33 MR. # - 1491001  
 COMPOUND - "ZONYL" MP (35% SLURRY OF "ZONYL" RP)

JULY 10, 1972

ANIMAL # 7576 7538 7631 7586 7599 7625 7627 7628 7604 7608 7626 7593 7577 7545 7551 7540

LIVER HYPERTROPHY  
 PANCREAS NORMAL MORPH  
 OLOGIC STRUCTURES  
 ESOPHAGUS NORMAL MORPHOLOGIC STRUCT  
 URES  
 STOMACH NORMAL MORPHOLOGIC STRUCTUR  
 ES  
 DUODENUM NORMAL MORPHOLOGIC STRUCTU  
 RES  
 COLON NORMAL MORPHOLOGIC STRUCTURES  
 CECUM NORMAL MORPHOLOGIC STRUCTURES  
 KIDNEY NORMAL MORPHOLOGIC STRUCTURE  
 S  
 URINARY BLADDER NORMAL MORPHOLOGIC  
 STRUCTURES  
 PROSTATE AND SEMINAL VESICLE NORMAL  
 MORPHOLOGIC STRUCTURES  
 TESTIS NORMAL MORPHOLOGIC STRUCTURE  
 S  
 EPIDIDYMS NORMAL MORPHOLOGIC STRUC  
 TURES  
 UTERUS NORMAL MORPHOLOGIC STRU  
 CTURES  
 FALLOPIAN TUBE NORMAL MORPHOLOGIC S  
 TRUCTURES  
 OVARY NORMAL MORPHOLOGIC STRUCTURES  
 PITUITARY GLAND NORMAL MORPHOLOGIC  
 STRUCTURES  
 ADRENAL GLAND NORMAL MORPHOLOGIC ST  
 RUCTURES  
 THYROID GLAND NORMAL MORPHOLOGIC ST  
 RUCTURES  
 THYROID GLAND ORGAN MISSING  
 PARATHYROID GLAND NORMAL MORPHOLOGI  
 C STRUCTURES  
 PARATHYROID GLAND ORGAN MISSING  
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 S

TABLE XII-D

HISTOPATHOLOGY TO-DATE SUMMARY - RAT

JULY 10, 1972

HASKELL # - 7247 TEST # - 33 MR. # - 1491001  
 COMPOUND - "ZONYL" MP (35% SLURRY OF "ZONYL" NO.)

ANIMAL #	7552	7579	7567	7554	7562	7556	7583	7630	7615	7620	7622	7616	7601	7635	7607	7597
GROUP #	05	05	05	05	05	05	06	06	06	06	06	06	06	06	06	06
TREATMENT DAYS	97	97	97	97	97	97	98	98	98	98	98	98	98	98	98	98
POST-TREATMENT DAYS																
DIET/DOSE VALUE	750	750	750	750	750	750	750	750	750	750	750	750	750	750	750	750
INITIAL WEIGHT	68	68	70	73	76	78	60	62	63	63	67	71	74	77	77	82
LEW WEIGHT																
FINAL WEIGHT	482	488	540	492	520	575	345	304	303	323	291	271	316	272	313	312
SFX	M	M	M	H	M	M	F	F	F	F	F	F	F	F	F	F
DIED/SACRIFICED	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S

- EYE NORMAL MORPHOLOGIC STRUCTURES
- EXTRINSIC LACRIMAL GLAND NORMAL MORPHOLOGIC STRUCTURES
- BRAIN NORMAL MORPHOLOGIC STRUCTURES
- MEGALLA OBESGATA NORMAL MORPHOLOGIC STRUCTURES
- SCIATIC NERVE NORMAL MORPHOLOGIC STRUCTURES
- SKIN NORMAL MORPHOLOGIC STRUCTURES
- SKIN ORGAN MISSING
- MAMMARY GLAND NORMAL MORPHOLOGIC STRUCTURES
- MAMMARY GLAND ORGAN MISSING
- BONE MARROW NORMAL MORPHOLOGIC STRUCTURES
- SPLEEN NORMAL MORPHOLOGIC STRUCTURE S
- LYMPH NODE NORMAL MORPHOLOGIC STRUCTURES
- LYMPH NODE ORGAN MISSING
- SKELETAL MUSCLE NORMAL MORPHOLOGIC STRUCTURES
- TRACHEA NORMAL MORPHOLOGIC STRUCTURES
- LUNG NORMAL MORPHOLOGIC STRUCTURES
- LUNG INTERSTITIAL PNEUMONIA
- HEART NORMAL MORPHOLOGIC STRUCTURES
- ARTERIA NORMAL MORPHOLOGIC STRUCTURES
- SALIVARY GLAND NORMAL MORPHOLOGIC STRUCTURES
- LIVER NORMAL MORPHOLOGIC STRUCTURES
- LIVER GRANULOMA
- LIVER CLOUDY SWELLING
- LIVER HYALINE DROPLET DEGENERATION
- LIVER CYTOPLASMIC LIPID DROPLET ALT

TABLE XII-D (Continued)

HISTOPATHOLOGY TO-DATE SUMMARY - RAT

PAGE - 5-A  
JULY 10, 1972

YASKELL # - 7247	TEST # - 33	MR. # - 1491001	ANIMAL #	7552	7579	7567	7554	7562	7556	7538	7630	7615	7620	7622	7616	7601	7635	7607	7597
COMPOUND - "ZONYL" MP (35% SLURRY OF "ZONYL" RP)																			
LIVER	HYPERTROPHY																		
PANCREAS																			
OLOGIC STRUCTURES																			
ESOPHAGUS		NORMAL MORPHOLOGIC STRUCTURES																	
STOMACH		NORMAL MORPHOLOGIC STRUCTURES																	
DUODENUM		NORMAL MORPHOLOGIC STRUCTURES																	
COLON		NORMAL MORPHOLOGIC STRUCTURES																	
CECUM		NORMAL MORPHOLOGIC STRUCTURES																	
KIDNEY		NORMAL MORPHOLOGIC STRUCTURES																	
URINARY BLADDER																			
STRUCTURES																			
PROSTATE AND SEMINAL VESICLE																			
MORPHOLOGIC STRUCTURES																			
TESTIS																			
NORMAL MORPHOLOGIC STRUCTURES																			
EPIDIDYMIS																			
NORMAL MORPHOLOGIC STRUCTURES																			
UTERUS																			
NORMAL MORPHOLOGIC STRUCTURES																			
FALLOPIAN TUBE																			
NORMAL MORPHOLOGIC STRUCTURES																			
OVARY																			
NORMAL MORPHOLOGIC STRUCTURES																			
PIUTARY GLAND																			
NORMAL MORPHOLOGIC STRUCTURES																			
ADRENAL GLAND																			
NORMAL MORPHOLOGIC STRUCTURES																			
THYROID GLAND																			
NORMAL MORPHOLOGIC STRUCTURES																			
THYROID GLAND ORGAN																			
MISSING																			
PARATHYROID GLAND																			
NORMAL MORPHOLOGIC STRUCTURES																			
PARATHYROID GLAND ORGAN																			
MISSING																			
THYRUS																			
NORMAL MORPHOLOGIC STRUCTURES																			

TABLE XIII

BODY WEIGHTS OF INDIVIDUAL MALE DOGS FED ZONYL® RP

Group	Dog No.	Weekly Weight in Kilograms														Avg.
		0	1	2	3	4	5	6	7	8	9	10	11	12	13	
I (Control)	1150	8.1	8.2	8.6	8.1	7.9	8.0	8.1	8.2	8.2	8.3	8.2	8.4	8.4	8.4	8.2
	1154	11.2	10.9	11.0	11.1	11.0	11.0	10.8	11.2	11.1	11.3	11.4	11.2	11.4	11.5	11.2
	1158	11.4	11.4	11.4	11.6	11.1	11.5	11.5	11.8	11.8	12.0	11.8	11.7	12.0	11.9	11.6
	1162	9.5	9.2	9.2	9.4	9.3	9.5	9.6	9.7	9.5	9.6	9.7	9.6	9.7	9.9	9.5
II (500 ppm Zonyl® RP)	1153	10.8	11.3	11.1	11.4	11.1	11.2	11.3	11.5	11.4	11.6	11.2	11.5	11.7	11.6	11.3
	1155	9.3	9.2	9.4	9.5	9.4	9.4	9.5	9.6	9.8	9.8	9.8	10.0	10.0	9.8	9.6
	1159	14.0	14.9	14.0	14.3	14.2	14.2	14.3	14.6	14.4	14.8	14.8	14.9	14.7	15.0	14.5
III (750 ppm Zonyl® RP)	1165	9.0	9.5	9.5	9.8	9.5	9.6	9.4	9.8	9.6	9.8	9.9	9.8	9.7	9.9	9.6
	1152	11.2	11.0	11.1	11.3	11.4	11.2	11.3	11.7	11.3	11.4	11.7	11.5	11.6	11.8	11.4
	1156	8.3	8.2	8.3	8.4	8.1	8.1	8.2	8.3	8.4	8.2	8.6	8.3	8.4	8.3	8.3
IV (2,500 ppm Zonyl® RP)	1160	9.1	9.1	9.0	9.1	9.1	9.3	9.3	9.5	9.4	9.4	9.4	9.4	9.4	9.4	9.3
	1164	7.8	7.5	7.6	8.0	7.8	7.9	7.8	8.2	8.1	8.0	8.0	8.0	7.9	8.0	7.9
	1151	10.8	10.6	10.6	10.7	10.4	10.8	10.8	10.8	10.8	11.0	11.0	10.9	10.7	10.5	10.7
	1157	10.5	10.8	10.7	11.0	10.8	10.6	10.8	10.9	10.8	11.2	11.1	11.0	11.1	10.9	10.9
	1161	9.0	8.7	8.8	8.8	8.8	8.8	8.5	8.9	8.8	8.9	8.8	8.8	9.0	8.9	8.8
	1163	9.8	10.0	10.1	10.2	10.0	10.2	10.3	10.5	10.5	10.4	10.6	10.8	10.5	10.5	10.3

TABLE XIV

## BODY WEIGHTS OF INDIVIDUAL FEMALE DOGS FED ZONYL® RP

Group	Dog No.	Weekly Weight in Kilograms														Avg.
		0	1	2	3	4	5	6	7	8	9	10	11	12	13	
I (Control)	1137	10.5	10.6	10.7	10.9	10.7	10.8	10.9	11.0	11.0	10.8	10.9	11.0	11.0	10.9	10.8
	1141	11.8	12.0	12.0	12.0	12.2	12.0	11.7	11.6	11.5	11.5	11.4	11.5	11.4	11.5	11.7
	1145	8.0	7.8	8.0	8.3	8.2	8.2	8.4	8.5	8.5	8.4	8.8	9.0	9.2	9.1	8.4
	1147	8.9	8.7	8.6	8.8	8.6	9.0	9.1	9.2	9.2	9.4	9.3	9.4	9.5	9.4	9.1
II (500 ppm Zonyl® RP)	1135	8.2	8.1	8.1	8.3	8.4	8.5	8.7	8.5	8.5	8.5	8.2	8.3	8.2	8.4	8.4
	1140	7.0	7.0	7.0	7.2	7.0	7.1	7.2	7.3	7.3	7.2	7.3	7.3	7.3	7.2	7.2
	1143	8.1	8.8	8.0	8.2	8.0	8.4	8.8	8.6	8.6	8.4	8.5	8.6	8.8	8.6	8.4
	1149	6.3	6.5	6.6	6.8	6.8	6.8	6.8	6.9	6.9	7.0	7.1	7.0	6.9	7.0	6.8
III (750 ppm Zonyl® RP)	1136	13.0	13.3	13.2	13.3	13.4	13.7	13.8	14.1	14.4	14.9	15.2	15.3	15.3	15.1	14.1
	1139	8.0	7.8	7.8	7.9	7.9	8.0	8.0	8.4	8.5	8.4	8.4	8.7	8.7	8.5	8.2
	1144	9.2	8.5	8.3	8.8	8.6	8.8	8.7	8.9	8.8	8.8	8.8	8.9	9.0	9.0	8.8
	1148	9.6	9.8	9.4	9.6	9.4	9.8	9.8	10.0	10.0	10.2	10.2	10.2	10.7	10.6	10.0
IV (2,500 ppm Zonyl® RP)	1134	6.0	6.1	6.1	6.2	6.1	6.2	6.4	6.4	6.5	6.6	6.6	6.7	6.7	6.7	6.4
	1138	8.8	8.4	8.9	9.4	9.3	9.5	10.0	9.8	10.2	9.8	9.8	9.5	9.4	9.2	9.4
	1142	9.6	9.2	9.0	9.2	8.8	8.4	8.4	8.4	8.5	8.6	8.5	8.4	8.4	8.3	8.7
	1146	7.0	7.0	7.1	7.0	6.9	6.8	6.9	6.8	7.2	7.0	6.8	6.6	6.4	6.5	6.8

TABLE XV

DIET CONSUMPTION OF INDIVIDUAL MALE DOGS FED ZONYL<sup>®</sup> RP

Group	Dog No.	Average Daily Diet Consumption in Grams During Week													
		1	2	3	4	5	6	7	8	9	10	11	12	13	Avg.
I (Control)	1150	290	350	289	295	313	336	300	361	317	291	360	346	331	321
	1154	328	365	340	363	310	326	358	350	325	374	324	391	364	348
	1158	410	400	390	370	432	381	415	393	384	417	375	394	467	402
	1162	318	316	361	336	345	335	336	351	258	318	321	339	382	332
II (500 ppm Zonyl <sup>®</sup> RP)	1153	345	389	380	427	439	384	415	381	408	373	419	383	385	394
	1155	326	403	339	392	417	387	414	404	348	421	394	416	345	385
	1159	429	555	500	489	541	517	567	502	525	544	569	461	526	517
	1165	330	352	339	346	338	320	386	327	381	361	375	352	418	356
III (750 ppm Zonyl <sup>®</sup> RP)	1152	320	441	435	429	329	382	414	429	390	381	333	412	408	392
	1156	241	279	269	254	267	263	270	301	256	322	343	356	262	283
	1160	314	356	375	381	349	326	327	359	293	322	389	352	369	347
	1164	308	333	306	350	286	259	315	328	262	293	281	221	263	293
IV (2,500 ppm Zonyl <sup>®</sup> RP)	1151	320	416	396	421	438	370	409	429	430	380	448	371	330	397
	1157	379	417	418	404	432	387	426	426	361	382	420	382	421	404
	1161	260	306	370	347	305	314	371	384	314	304	355	355	362	334
	1163	296	333	352	318	370	341	330	356	293	314	323	300	341	328

TABLE XVI

## DIET CONSUMPTION OF INDIVIDUAL FEMALE DOGS FED ZONYL® RP

Group	Dog No.	Average Daily Diet Consumption in Grams During Week													Avg.
		1	2	3	4	5	6	7	8	9	10	11	12	13	
I (Control)	1137	382	396	406	364	338	378	386	391	312	316	338	358	374	364
	1141	428	373	372	453	287	286	280	395	356	401	470	452	500	389
	1145	263	287	303	284	281	331	290	259	226	311	312	285	274	285
	1147	200	198	280	321	334	262	327	321	359	295	318	306	330	296
II (500 ppm Zonyl® RP)	1135	251	323	329	332	320	329	319	356	355	305	244	202	331	307
	1140	265	267	283	277	254	273	329	301	232	231	200	261	285	266
	1143	258	297	375	380	392	386	343	338	362	326	247	349	338	338
	1149	349	362	369	362	412	359	369	366	373	337	367	381	409	370
III (750 ppm Zonyl® RP)	1136	427	409	368	426	388	429	450	492	538	449	455	403	420	435
	1139	304	317	317	357	309	283	319	331	293	257	332	310	240	305
	1144	197	290	304	305	298	313	291	271	259	296	308	317	307	289
	1148	346	372	361	364	382	353	335	318	360	244	272	402	380	345
IV (2,500 ppm Zonyl® RP)	1134	279	270	280	284	320	293	311	261	253	251	276	260	280	278
	1138	300	448	422	414	350	399	377	370	398	352	233	254	319	357
	1142	275	277	309	277	183	261	344	328	342	256	352	421	429	320
	1146	244	267	261	252	284	256	255	282	217	198	276	104	248	242

TABLE XVII

## AVERAGE DOSES OF INDIVIDUAL MALE DOGS FED ZONYL® RP

Group	Dog No.	Average Daily Dose in Milligrams/Kilograms during Week													Avg.
		1	2	3	4	5	6	7	8	9	10	11	12	13	
I Control	1150	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	1154	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	1158	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	1162	-	-	-	-	-	-	-	-	-	-	-	-	-	-
II 500 ppm Zonyl® RP	1153	45	50	17	19	20	17	18	17	18	16	18	16	17	22
	1155	51	62	18	21	22	21	22	21	18	22	20	21	17	26
	1159	42	55	18	17	19	18	20	17	18	18	19	16	18	23
	1165	51	53	18	18	18	17	20	17	20	18	19	18	21	24
III 750 ppm Zonyl® RP	1152	62	86	29	28	22	26	27	28	26	25	22	27	26	33
	1156	63	73	24	23	25	24	25	27	23	29	31	32	23	32
	1160	74	85	31	31	28	26	26	29	23	26	31	28	29	36
	1164	87	94	29	33	28	25	30	30	25	28	26	21	25	37
IV 2,500 ppm Zonyl® RP	1151	192	252	94	99	103	86	95	99	99	86	102	86	78	113
	1157	230	253	97	93	101	90	98	99	82	85	96	87	96	116
	1161	190	223	105	99	87	91	106	109	89	86	101	100	101	114
	1163	192	214	86	79	92	84	79	85	70	75	75	71	81	99



TABLE XVIII

## AVERAGE DOSES BY INDIVIDUAL FEMALE DOGS FED ZONYL® RP

Group	Dog No.	Average Daily Dose in Milligrams/Kilogram during Week													Avg.
		1	2	3	4	5	6	7	8	9	10	11	12	13	
I Control	1137	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	1141	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	1145	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	1147	-	-	-	-	-	-	-	-	-	-	-	-	-	-
II 500 ppm Zonyl® RP	1135	44	57	20	20	19	19	19	21	21	18	15	12	20	24
	1140	54	54	20	20	18	19	23	21	16	16	14	18	20	24
	1143	44	50	23	24	24	22	20	20	21	19	14	20	19	25
	1149	78	78	28	27	30	26	27	26	27	24	26	27	29	35
III 750 ppm Zonyl® RP	1136	69	66	21	24	21	23	24	26	28	22	22	20	21	30
	1139	82	87	30	34	29	26	29	30	26	23	29	27	21	36
	1144	50	74	26	26	26	27	25	23	22	25	26	26	26	31
	1148	76	83	28	29	30	27	25	24	27	18	20	29	27	34
IV 2,500 ppm Zonyl® RP	1134	300	284	115	114	129	116	121	102	96	95	105	97	105	137
	1138	224	334	115	110	93	108	95	92	99	90	61	67	86	121
	1142	188	195	85	77	53	78	102	98	99	104	105	125	128	110
	1146	224	245	93	90	104	94	94	101	76	72	103	40	97	110





TABLE XX

## SUMMARY OF URINALYSIS DATA ON DOGS FED ZONYL® RP FOR THREE MONTHS

	ppm in Diet	MALES				FEMALES			
		Pre-Test*	Months on Test			Pre-Test	Months on Test		
			1	2	3		1	2	3
Volume ml/24 hrs.	0	178	135	165	150	159	126	134	150
	500	228	209	180	176	169	140	149	150
	750	178	134	114	116	153	150	97	140
	2,500	266	163	159	199	203	209	178	151
Osmolality mOs/L	0	1549	1944	1775	2004	1648	1900	1794	1684
	500	1091	1482	1790	1638	1366	1639	1725	1861
	750	1612	1875	1951	1906	1635	1627	1348	1807
	2,500	1513	1500	1651	1516	1527	1456	1594	1859
Creatinine mg/100 ml	0	142	179	173	207	177	175	184	170
	500	96	114	153	158	130	113	154	160
	750	145	137	179	192	151	112	126	194
	2,500	116	98	158	145	119	106	125	170
Blood Number Positive	0	0	0	0	0	0	0	0	0
	500	0	0	0	0	1	0	0	0
	750	0	0	0	0	0	0	2	0
	2,500	1	0	0	0	0	0	0	0
Sugar Number Abnormal	0	0	0	0	0	0	0	0	0
	500	0	0	0	0	0	0	0	0
	750	0	0	0	0	0	0	0	0
	2,500	0	0	0	0	0	0	0	0
Acetone Number Positive	0	0	0	0	0	0	0	0	0
	500	0	0	0	0	0	0	0	0
	750	0	0	0	0	0	0	0	0
	2,500	0	0	0	0	0	0	0	0
Bilirubin Number Positive	0	8	4	3	4	2	3	4	3
	500	5	3	4	4	0	1	2	4
	750	6	4	4	4	3	3	4	4
	2,500	4	2	3	4	0	0	2	2

\* Average of the measurement per dog for volume, osmolality, creatinine; number positive or abnormal in eight specimens for blood, sugar, acetone, bilirubin, protein.



TABLE XXI

SUMMARY OF BIOCHEMICAL MEASUREMENTS ON DOGS FED ZONYL® RP FOR THREE MONTHS

	ppm in Diet	MALES				FEMALES			
		Pre-test	Months on Test			Pre-test	Months on Test		
			1	2	3		1	2	3
Glucose mg %	0	123	137	125	116	112	108	123	106
	500	128	140	126	112	119	112	108	106
	750	111	111	116	109	107	95	113	105
	2,500	114	101	117	107	107	96	114	112
Urea Nitrogen mg %	0	14	14	15	14	14	21	22	27
	500	16	22	19	20	17	21	25	25
	750	14	21	17	16	18	23	19	21
	2,500	17	23	21	19	16	21	21	18
Cholesterol mg %	0	137	133	150	138	158	164	168	195
	500	146	140	174	163	156	180	205	230
	750	161	184	206	189	157	209	258	293
	2,500	148	181	206	188	159	277	314	256
Alkaline Phosphatase Bessey Units	0	4.2	3.2	3.3	3.0	4.3	3.9	3.6	5.2
	500	6.4	6.9	8.1	8.2	6.4	8.4	7.4	6.1
	750	4.4	6.6	6.9	5.3	4.4	6.2	7.2	7.3
	2,500	4.4	10.7	11.3	11.7	5.2	10.5	12.3	11.6
Transaminase Reitman-Frankel Units	0	8	17	15	13	6	9	14	12
	500	7	14	15	11	6	12	10	10
	750	8	15	18	17	5	10	11	9
	2,500	7	11	15	13	5	13	18	15
Total Protein g/100 ml	0	6.3	6.1	6.2	6.4	6.2	6.0	6.0	6.3
	500	6.2	6.0	6.2	6.2	6.3	5.8	6.2	6.5
	750	6.1	5.9	6.1	6.2	6.1	6.1	6.5	6.8
	2,500	6.0	6.2	6.3	6.3	6.3	6.4	6.6	6.5

TABLE XXI (Continued)

SUMMARY OF BIOCHEMICAL MEASUREMENTS ON DOGS FED ZONYL® RP FOR THREE MONTHS

	ppm in Diet	MALES				FEMALES			
		Pre-test	Months on Test			Pre-test	Months on Test		
			1	2	3		1	2	3
Albumin/Globulin	0	1.12	1.27	0.97	1.00	1.11	1.29	1.09	1.29
	500	1.15	1.16	1.15	1.03	1.14	1.05	0.97	1.14
	750	1.11	1.25	1.18	1.02	1.19	1.16	1.07	0.91
	2,500	1.07	1.05	0.98	1.13	1.16	0.98	0.93	1.12
Albumin g/100 ml	0	3.3	3.4	3.0	3.2	3.2	3.3	3.2	3.5
	500	3.3	3.2	3.3	3.1	3.3	3.0	3.1	3.4
	750	3.2	3.3	3.3	3.1	3.3	3.2	3.4	3.2
	2,500	3.1	3.1	3.1	3.3	3.3	3.2	3.1	3.4
Creatinine mg %	0	0.6	0.6	0.8	0.8	0.7	0.7	0.9	1.1
	500	0.7	0.6	0.8	0.9	0.7	0.6	0.7	0.8
	750	0.6	0.5	0.7	0.9	0.7	0.7	0.8	0.7
	2,500	0.7	0.6	0.7	0.8	0.7	0.6	0.8	0.9
Bilirubin mg %	0	0.2	0.4	0.3	0.2	0.1	0.2	0.2	0.2
	500	0.1	0.4	0.2	0.2	0.1	0.3	0.4	0.6
	750	0.2	0.3	0.2	0.2	0.1	0.4	0.3	0.5
	2,500	0.2	0.3	0.2	0.2	0.2	0.3	0.3	0.3

TABLE XXII

ORGAN WEIGHTS IN GRAMS OF INDIVIDUAL MALE DOGS SACRIFICED AFTER THREE MONTHS' FEEDING OF ZONYL® RP

Group	Dog No.	Body Wt. (kg)	Brain	Heart	Lungs	Liver	Spleen	Pancreas	Kidney	Testis	Prostate	Bladder	Stomach	Thymus	Adrenals	Pituitary	Thyroid
I Control	1158	12.0	90	106	127	397	30	40	64	25	11.8	5.2	110	9.5	1.3	0.068	0.85
	1154	11.5	73	102	175	332	27	32	70	19	7.0	6.9	95	8.3	1.7	0.059	0.92
	1162	11.0	74	111	132	301	25	29	69	24	5.0	6.3	92	5.8	1.2	0.070	0.76
	1150	8.0	77	76	95	270	24	25	47	14	4.6	4.6	86	9.2	1.0	0.056	0.74
II 500 ppm Zonyl® RP	1153	11.5	73	108	174	408	36	25	73	24	5.3	5.6	93	36.3	1.5	0.077	0.45
	1165	9.9	71	90	144	400	26	29	59	21	4.9	5.0	93	13.7	1.2	0.068	0.25
	1159	14.9	85	143	191	593	22	32	77	32	8.0	8.8	128	13.7	1.8	0.084	1.02
	1155	10.0	75	74	153	370	20	25	70	14	8.9	4.9	86	4.3	1.1	0.066	0.91
III 750 ppm Zonyl® RP	1164	8.2	69	68	120	320	15	32	57	17	6.4	6.2	74	8.8	0.8	0.059	0.25
	1160	9.8	82	95	121	357	24	29	55	17	5.9	4.8	80	13.2	1.4	0.044	0.65
	1152	11.8	91	88	157	454	29	26	59	17	11.2	4.9	108	9.6	1.6	0.057	0.96
	1156	8.6	85	60	121	309	23	18	46	17	7.3	3.2	78	8.7	0.9	0.064	0.57
IV 2,500 ppm Zonyl® RP	1163	10.3	84	84	113	486	24	25	68	25	6.2	5.6	99	11.0	1.5	0.084	1.21
	1161	9.0	81	80	113	493	20	18	62	19	3.4	4.4	87	9.8	1.1	0.064	0.65
	1151	11.0	75	87	149	541	22	22	60	20	3.3	5.6	108	16.6	1.0	0.050	1.10
	1157	11.0	90	101	197	516	29	32	63	16	4.3	4.0	99	6.7	1.2	0.073	0.86



TABLE XXIII

ORGAN WEIGHTS IN GRAMS OF INDIVIDUAL FEMALE DOGS SACRIFICED AFTER THREE MONTHS' FEEDING OF ZONYL® RP

Group	Dog No.	Body Wt. (kg)	Brain	Heart	Lungs	Liver	Spleen	Pancreas	Kidney	Stomach	Thymus	Adrenal	Pituitary	Thyroid
I Control	1137	11.4	78	107	172	349	25	24	57	103	6.2	1.1	0.072	1.19
	1141	11.7	76	107	161	372	23	20	66	111	14.5	1.8	0.058	1.03
	1145	9.2	75	65	140	347	19	26	46	83	7.6	1.7	0.059	0.81
	1147	9.6	65	94	152	308	28	31	62	92	12.3	2.0	0.078	0.65
II 500 ppm Zonyl® RP	1135	8.3	68	81	114	356	22	22	54	87	5.3	1.0	0.076	0.37
	1140	7.5	65	60	98	274	22	21	43	76	10.2	1.0	0.072	0.70
	1143	8.8	81	70	121	397	19	30	46	88	17.6	1.4	0.074	0.78
	1141	7.6	75	78	134	357	15	22	42	80	4.4	1.6	0.054	1.06
III 750 ppm Zonyl® RP	1148	10.8	74	74	126	352	16	21	44	77	11.4	1.5	0.062	1.01
	1139	8.6	78	73	138	416	23	30	60	83	11.7	1.8	0.071	0.82
	1144	9.4	60	67	118	350	17	21	47	75	6.9	1.3	0.060	0.88
	1136	15.3	84	99	189	533	30	30	69	117	13.9	1.5	0.074	0.86
IV 2,500 ppm Zonyl® RP	1146	6.8	72	55	90	342	11	21	39	76	6.3	1.2	0.061	0.57
	1138	9.2	82	80	109	520	17	24	54	80	11.7	1.8	0.049	0.37
	1142	8.6	76	79	104	430	21	24	53	76	9.0	1.2	0.056	0.74
	1134	6.8	73	67	80	351	12	22	42	70	10.2	1.7	0.035	0.22

TABLE XXIV

AVERAGE LIVER WEIGHTS AND LIVER/BODY WEIGHT RATIOS OF DOGS FED ZONYL<sup>®</sup> RP

<u>Group</u>	<u>ppm Zonyl<sup>®</sup> RP</u>	<u>MALES</u>			<u>FEMALES</u>		
		<u>Final Body Weight (kg)</u>	<u>Liver Weight (g)</u>	<u>Liver Weight Body Weight X 100</u>	<u>Final Body Weight (kg)</u>	<u>Liver Weight (g)</u>	<u>Liver Weight Body Weight X 100</u>
I	0	10.4	325	3.15	10.5	344	3.29
II	500	11.6	442	3.82	8.0	346	4.28
III	750	9.6	360	3.75	11.0	413	3.83
IV	2,500	10.3	509	4.95	7.8	411	5.22

TABLE XXV

Histopathology - Group I (Control) - H-7247 - MR-1491

Dog No.	Sex	RESPIRATORY AND CARDIOVASCULAR					DIGESTIVE							GENITOURINARY				
		Lung	Upper trachea	Heart	Aorta	Stomach	Duodenum	Cecum	Colon	Salivary gland	Pancreas	Liver	Esophagus	Testis/ovary	Epididymis/ Fallopian tube	Uterus/ Prostate	Bladder	Kidney
1150	♂	A+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1154	♂	-	-	-	-	-	-	-	-	-	-	-	-	B+	-	-	-	-
1158	♂	C+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1162	♂	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1137	♀	C+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1141	♀	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1145	♀	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1147	♀	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Code: A = Focal pleural fibrosis.  
 B = Focal germinal cell atrophy or hypoplasia.  
 C = Focal suppurative pneumonitis.  
 - = No abnormalities detected.  
 + = Slight degree of lesion.

TABLE XXV (Continued)

Histopathology - Group I (Control) - H-7247 - MR-1491

Dog No.	Days On Test	Sex	ENDOCRINE				NERVOUS AND MUSCULOSKELETAL				SKIN AND APPENDAGES			HEMIC AND LYMPHATIC		
			Pituitary	Thyroid	Parathyroid	Adrenal	Skeletal Muscle	Sciatic Nerve	Brain	Spinal Cord	Eye	Mammary Gland	Skin	Bone Marrow	Spleen	Thymus
1150	104	♂	-	-	0	-	-	-	-	-	-	-	-	-	-	-
1154	98	♂	-	-	0	-	-	-	-	-	-	-	-	-	-	-
1158	98	♂	-	-	0	-	-	-	-	-	-	-	-	-	-	-
1162	98	♂	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1137	99	♀	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1141	99	♀	-	-	0	-	-	-	-	-	-	-	-	-	-	-
1145	99	♀	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1147	99	♀	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Code: - = No abnormalities detected.

0 = No tissue available on slide.

TABLE XXVI

Histopathology - Group IV - H-7247 - MR-1491

Dog No.	Sex	RESPIRATORY AND CARDIOVASCULAR					DIGESTIVE							GENITOURINARY				
		Lung	Upper Trachea	Heart	Aorta	Stomach	Duodenum	Cecum	Colon	Salivary Gland	Pancreas	Liver	Esophagus	Testis/ Ovary	Epididymis/ Fallopian Tube	Uterus/ Prostate	Bladder	Kidney
1157	♂	C+	-	-	-	-	-	-	-	-	-	D+	-	B+	-	-	-	-
1151	♂	-	-	-	-	-	-	-	-	-	-	D+	-	B+	-	-	-	-
1161	♂	-	-	-	-	-	-	-	-	-	-	D+	-	-	-	-	-	-
1163	♂	C+	-	-	-	-	-	-	-	-	-	D+	-	-	-	-	-	-
1134	♀	-	-	-	-	-	-	-	-	-	-	D+	-	-	-	-	-	-
1142	♀	C++	-	-	-	-	-	-	-	-	-	D+	-	-	-	-	-	-
1146	♀	-	-	-	-	-	-	-	-	-	-	D+	-	-	-	-	-	-
1138	♀	-	-	-	-	-	-	-	-	-	-	D+	-	-	-	-	-	-

Code: C = Focal suppurative pneumonitis.  
 B = Focal germinal cell atrophy or hypoplasia.  
 D = Hepatocyte hypertrophy and degeneration.  
 - = No abnormalities detected.  
 + = Slight degree of lesion.  
 ++ = Moderate degree of lesion.

TABLE XXVI (Continued)  
Histopathology - Group IV - H-7247 - MR-1491

Dog No.	Sex	ENDOCRINE				NERVOUS AND MUSCULOSKELETAL				SKIN AND APPENDAGES		HEMIC AND LYMPHATIC				
		Pituitary	Thyroid	Parathyroid	Adrenal	Skeletal Muscle	Sciatic Nerve	Brain	Spinal Cord	Eye	Mammary Gland	Skin	Bone Marrow	Spleen	Thymus	Lymph Node
1157	♂	-	-	-	-	-	-	-	-	-	0	-	-	-	-	-
1151	♂	-	-	-	-	-	-	-	-	-	0	-	-	-	-	-
1161	♂	-	-	-	-	-	-	-	-	-	0	-	-	-	-	-
1163	♂	-	-	-	-	-	-	-	-	-	0	-	-	-	-	-
1134	♀	-	-	-	-	-	-	-	-	-	0	-	-	-	-	-
1142	♀	-	-	-	-	-	-	-	-	-	0	-	-	-	-	E+
1146	♀	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1138	♀	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Code: E = Subcapsular eosinophil infiltration.  
 - = No abnormalities detected.  
 0 = No tissue available on slide.  
 + - Slight degree of lesion.

TABLE XXVII

Histopathology - Group II - H-7247 - MR-1491

<u>Dog No.</u>	<u>Sex</u>	<u>Liver</u>
1155	♂	-
1159	♂	-
1165	♂	-
1153	♂	-
1149	♀	-
1143	♀	-
1140	♀	-
1135	♀	-

---

Code: - = No abnormalities detected.

TABLE XXVIII

Histopathology - Group III - H-7247 - MR-1491

<u>Dog No.</u>	<u>Sex</u>	<u>Liver</u>
1156	♂	-
1152	♂	-
1160	♂	-
1164	♂	-
1136	♀	-
1144	♀	-
1139	♀	-
1148	♀	-

---

Code: - ■ No abnormalities detected.



APPENDIX I

APPENDIX I (1)  
 CLINICAL LABORATORY DATA ON INDIVIDUAL DOGS  
 FED ZONYL<sup>®</sup> RP FOR THREE MONTHS

Dog No.: 1150  
 Sex: Male  
 Group: Control

	Control		Months on Test		
			1	2	3
<u>Hematology</u>					
Erythrocytes	6.56	6.84	7.84	7.24	7.28
Hemoglobin	16.1	17.4	18.6	18.3	19.2
Hematocrit	40	45	50	49	49
Leucocytes	8.1	8.5	9.3	11.2	11.5
<u>DIFFERENTIAL</u>					
Neutrophils	63	52	74	50	57
Seg.	62	52	73	50	57
Juv.	1	0	1	0	0
Myel.	0	0	0	0	0
Lymphocytes	24	44	21	28	28
Eosinophils	10	4	4	22	13
Monocytes	2	0	1	0	2
Basophils	1	0	0	0	0
Atypicals	0	0	0	0	0
Nucleated RBC	0	0	0	0	0
<u>Biochemistry</u>					
Glucose	118	119	120	132	134
Urea Nitrogen	22	17	17	19	13
Cholesterol	110	130	120	130	120
Alk. Phos.	2.9	3.0	2.6	2.5	2.3
GPT	6	5	16	16	14
Total Protein	6.4	6.3	6.2	6.2	6.1
A/G	1.10	1.28	1.44	1.02	0.97
Creatinine	0.6	0.8	0.5	0.8	0.7
Bilirubin	0.3	0.4	0.4	0.5	0.2
Albumin	3.7	3.5	3.7	3.1	3.0
<u>Urinalysis</u>					
Volume	185	160	155	125	110
Appearance	D, Y, Cl, P	D, Y, Cl, P	A, Cl	D, Y, Cl, P	D, Y, Cl
Osmolality	1950	1373	2146	2159	2081
Occult Blood	N	N	N	N	N
Sugar	N	N	Tr	N	N
Acetone	N	N	N	N	N
pH	6.8	7.0	6.8	6.8	7.0
Urobilinogen	1.0	1.0	1.0	1.0	1.0
Bilirubin	P	P	P	P	P
Protein	Tr	Tr	1+	1+	1+
Creatinine	132	108	150	166	212
<u>MICROSCOPIC</u>					
RBC's	0	0	0	0	0
WBC's	0-2	1-4	0-3	0-1	0
Epithelial	0	0	0	0	0-1
Bacteria	2+	2+	2+	2+	2+
Cast	0	0	0	0	0
Sperm	0	1-5	0	2-6	5-10

APPENDIX I (2)  
 CLINICAL LABORATORY DATA ON INDIVIDUAL DOGS  
 FED ZONYL® RP FOR THREE MONTHS

Dog No.: 1154  
 Sex: Male  
 Group: Control

	Control		Months on Test		
			1	2	3
<u>Hematology</u>					
Erythrocytes	7.28	7.24	8.04	7.88	7.64
Hemoglobin	18.7	18.1	19.4	19.0	19.2
Hematocrit	51	48	54	49	51
Leucocytes	15.0	11.5	12.5	12.1	11.7
<u>DIFFERENTIAL</u>					
Neutrophils	74	70	67	63	68
Seg.	71	70	67	63	66
Juv.	3	0	0	0	2
Myel.	0	0	0	0	0
Lymphocytes	22	24	25	32	23
Eosinophils	4	6	7	5	9
Monocytes	0	0	1	0	0
Basophils	0	0	0	0	0
Atypicals	0	0	0	0	0
Nucleated RBC	0	0	0	0	0
<u>Biochemistry</u>					
Glucose	150	124	180	126	124
Urea Nitrogen	13	10	14	10	12
Cholesterol	145	175	145	160	150
Alk. Phos.	4.7	4.9	3.4	3.8	3.3
GPT	12	5	20	13	12
Total Protein	6.4	6.6	6.2	6.3	6.7
A/G	1.04	1.07	1.17	1.14	1.24
Creatinine	0.5	0.8	0.6	0.8	0.8
Bilirubin	0.1	0.1	0.4	0.2	0.2
Albumin	3.3	3.4	3.3	3.4	3.7
<u>Urinalysis</u>					
Volume	245	275	205	270	185
Appearance	Y, Cl, P	Y, Cl, P	A, Cl	Y, Cl, P	D, Y, Cl, P
Osmolality	1357	1194	1740	1142	1845
Occult Blood	N	N	N	N	N
Sugar	N	N	Tr	N	N
Acetone	N	N	N	N	N
pH	7.0	7.2	7.0	7.2	7.2
Urobilinogen	1.0	0.1	1.0	0.1	1.0
Bilirubin	P	P	P	N	P
Protein	1+	N	Tr	Tr	Tr
Creatinine	130	114	138	114	180
<u>MICROSCOPIC</u>					
RBC's	0	0	0	0	0
WBC's	0	0	0	0	0
Epithelial	0-1	0	0-1	0	0-1
Bacteria	2+	1+	2+	1+	1+
Cast	0	0	0	0	0
Sperm	0-1	0	1-4	2-4	0

APPENDIX I (3)  
 CLINICAL LABORATORY DATA ON INDIVIDUAL DOGS  
 FED ZONYL<sup>®</sup> RP FOR THREE MONTHS

Dog No.: 1158  
 Sex: Male  
 Group: Control

	Control		Months on Test		
			1	2	3
<u>Hematology</u>					
Erythrocytes	6.56	6.32	7.00	7.00	6.48
Hemoglobin	16.1	16.4	16.8	17.4	17.4
Hematocrit	42	44	46	47	47
Leucocytes	9.9	9.8	9.4	16.9	10.9
<u>DIFFERENTIAL</u>					
Neutrophils	67	72	62	68	73
Seg.	67	72	62	67	73
Juv.	0	0	0	1	0
Myel.	0	0	0	0	0
Lymphocytes	27	24	32	26	21
Eosinophils	4	3	2	3	6
Monocytes	2	0	4	3	0
Basophils	0	1	0	0	0
Atypicals	0	0	0	0	0
Nucleated RBC	0	0	0	0	0
<u>Biochemistry</u>					
Glucose	116	122	134	123	101
Urea Nitrogen	14	15	16	19	18
Cholesterol	135	145	135	165	150
Alk. Phos.	3.6	5.3	2.9	3.3	2.9
GPT	8	-	15	15	14
Total Protein	5.7	6.2	6.0	6.2	6.2
A/G	1.19	1.09	1.09	0.73	0.75
Creatinine	0.4	1.0	0.6	0.8	0.8
Bilirubin	0.1	0.1	0.3	0.3	0.3
Albumin	3.1	3.2	3.1	2.6	2.7
<u>Urinalysis</u>					
Volume	95	75	50	90	95
Appearance	A, Cl, P	L, A, Cl	A, Cl	D, Y, Cl	D, Y, Cl
Osmolality	1929	1520	1919	1908	1955
Occult Blood	N	N	N	N	N
Sugar	1+	Tr	1+	Tr	N
Acetone	N	N	N	N	N
pH	7.0	6.4	6.2	6.4	6.8
Urobilinogen	1.0	1.0	1.0	1.0	1.0
Bilirubin	P	P	P	P	P
Protein	1+	Tr	1+	1+	Tr
Creatinine	132	260	242	222	262
<u>MICROSCOPIC</u>					
RBC's	0	0	0	0	0
WBC's	0-1	0	0-2	0	0-1
Epithelial	0	0	0	0	0-1
Bacteria	1+	1+	1+	2+	2+
Cast	0	0	0	0	0
Sperm	0	0	1-5	0	4-5

APPENDIX I (4)  
 CLINICAL LABORATORY DATA ON INDIVIDUAL DOGS  
 FED ZONYL<sup>®</sup> RP FOR THREE MONTHS

Dog No.: 1162  
 Sex: Male  
 Group: Control

	Control		Months on Test		
			1	2	3
<u>Hematology</u>					
Erythrocytes	6.08	6.08	7.16	7.20	6.80
Hemoglobin	15.3	15.6	17.1	17.2	17.1
Hematocrit	40	40	47	46	46
Leucocytes	14.6	16.8	15.0	16.9	11.3
<u>DIFFERENTIAL</u>					
Neutrophils	67	67	65	70	54
Seg.	67	65	65	70	54
Juv.	0	2	0	0	0
Myel.	0	0	0	0	0
Lymphocytes	27	23	22	25	30
Eosinophils	2	8	13	4	15
Monocytes	4	2	0	1	1
Basophils	0	0	0	0	0
Atypicals	0	0	0	0	0
Nucleated RBC	0	0	0	0	0
<u>Biochemistry</u>					
Glucose	118	114	112	118	106
Urea Nitrogen	9	8	10	11	14
Cholesterol	115	140	130	145	130
Alk. Phos.	5.0	4.5	3.9	3.6	3.6
GPT	9	8	15	14	11
Total Protein	6.2	6.9	6.1	6.0	6.4
A/G	1.04	1.06	1.38	1.00	1.03
Creatinine	0.5	0.7	0.5	0.6	0.7
Bilirubin	0.1	0.1	0.3	0.2	0.2
Albumin	3.2	3.6	3.5	3.0	3.2
<u>Urinalysis</u>					
Volume	160	225	130	175	210
Appearance	D, Y, Cl, P	L, A, Cl, P	A, Cl, P	Y, Cl, P	L, A, Cl, P
Osmolality	1556	1514	1971	1892	2133
Occult Blood	N	N	N	N	N
Sugar	N	N	N	N	N
Acetone	N	N	N	N	N
pH	8.0	7.2	7.0	7.2	7.0
Urobilinogen	1.0	1.0	1.0	1.0	0.1
Bilirubin	P	P	P	P	P
Protein	1+	Tr	1+	1+	1+
Creatinine	128	132	184	190	172
<u>MICROSCOPIC</u>					
RBC's	0	0	0	0	0
WBC's	0-1	0	1-3	0	0
Epithelial	0	0-1	1-3	0	0-1
Bacteria	1+	2+	1+	2+	1+
Cast	0	0	0	0	0
Sperm	0	0-1	0-2	1-3	3-6

APPENDIX I (5)  
 CLINICAL LABORATORY DATA ON INDIVIDUAL DOGS  
 FED ZONYL® RP FOR THREE MONTHS

Dog No.: 1153  
 Sex: Male  
 Group: 500 ppm

	Months on Test				
	Control	1	2	3	
<u>Hematology</u>					
Erythrocytes	7.00	7.68	7.84	8.08	7.60
Hemoglobin	17.3	18.8	18.7	19.7	18.7
Hematocrit	48	50	52	53	50
Leucocytes	13.3	11.1	11.0	15.4	9.9
<u>DIFFERENTIAL</u>					
Neutrophils	66	60	62	62	63
Seg.	66	60	61	61	62
Juv.	0	0	1	1	1
Myel.	0	0	0	0	0
Lymphocytes	30	34	33	30	28
Eosinophils	4	2	2	8	7
Monocytes	0	4	3	0	2
Basophils	0	0	0	0	0
Atypicals	0	0	0	0	0
Nucleated RBC	0	0	0	0	0
<u>Biochemistry</u>					
Glucose	116	158	140	142	104
Urea Nitrogen	13	14	21	18	23
Cholesterol	140	160	100	170	160
Alk. Phos.	6.4	6.1	7.8	8.2	8.6
GPT	9	7	20	18	10
Total Protein	6.2	6.5	6.3	6.3	6.6
A/G	1.39	0.89	1.21	1.18	0.98
Creatinine	0.4	0.8	-	0.9	0.9
Bilirubin	0.1	0.1	0.4	0.3	0.4
Albumin	3.6	3.1	3.4	3.4	3.3
<u>Urinalysis</u>					
Volume	245	185	215	110	210
Appearance	Y, Cl, P	Y, Cl, P	A, Cl, P	Y, Cl, P	D, Y, Cl, P
Osmolality	605	1268	1147	1567	1331
Occult Blood	N	N	N	N	N
Sugar	N	N	N	N	N
Acetone	N	N	N	N	N
pH	8.0	7.0	7.0	7.6	6.8
Urobilinogen	0.1	1.0	0.1	0.1	1.0
Bilirubin	N	P	P	P	P
Protein	N	Tr	Tr	1+	Tr
Creatinine	66	118	88	130	138
<u>MICROSCOPIC</u>					
RBC's	0	0	0	0	0
WBC's	0	0-1	0-1	0	0
Epithelial	0	0	0	0	0-1
Bacteria	3+	2+	3+	2+	2+
Cast	0	0	0	0	0
Sperm	0	0	1-5	2-5	3-10

APPENDIX I (6)  
 CLINICAL LABORATORY DATA ON INDIVIDUAL DOGS  
 FED ZONYL® RP FOR THREE MONTHS

Dog No.: 1155  
 Sex: Male  
 Group: 500 ppm

	Months on Test				
	Control	1	2	3	
<u>Hematology</u>					
Erythrocytes	5.92	5.68	6.56	6.76	6.64
Hemoglobin	14.9	14.4	15.6	16.1	15.9
Hematocrit	39	37	42	41	41
Leucocytes	13.0	13.9	13.2	12.5	9.2
DIFFERENTIAL					
Neutrophils	65	62	66	68	69
Seg.	65	62	66	67	69
Juv.	0	0	0	1	0
Myel.	0	0	0	0	0
Lymphocytes	28	26	28	28	28
Eosinophils	6	9	6	4	3
Monocytes	1	3	0	0	0
Basophils	0	0	0	0	0
Atypicals	0	0	0	0	0
Nucleated NBC	0	0	0	0	0
<u>Biochemistry</u>					
Glucose	112	114	120	126	120
Urea Nitrogen	20	17	26	21	11
Cholesterol	115	135	135	160	145
Alk. Phos.	7.6	7.1	8.1	10.6	12.0
GPT	6	4	15	13	11
Total Protein	5.8	6.1	6.0	6.5	6.1
A/G	1.00	1.08	1.00	1.06	1.30
Creatinine	0.6	0.8	0.6	0.8	0.7
Bilirubin	0.1	0.1	0.5	0.2	0.1
Albumin	2.9	3.2	3.0	3.3	3.4
<u>Urinalysis</u>					
Volume	195	245	125	185	155
Appearance	Y, Cl, P	Y, Cl, P	A, Cl, P	D, Y, Cl, P	L, A, Cl, P
Osmolality	1478	1273	1819	1966	2181
Occult Blood	N	N	N	N	N
Sugar	N	N	Tr	N	N
Acetone	N	N	N	N	N
pH	7.0	7.0	7.0	7.0	6.8
Urobilinogen	0.1	1.0	1.0	1.0	1.0
Bilirutin	N	P	N	P	P
Protein	Tr	N	1+	1+	1+
Creatinine	104	92	124	158	208
MICROSCOPIC					
RBC's	0	0	0	0	0
WBC's	0	0-2	0	0	0
Epithelial	0-1	0	0	0	0
Bacteria	2+	2+	1+	2+	3+
Cast	0	0	0	0	0
Sperm	0	0	0-1	0	0-1

APPENDIX I (7)  
 CLINICAL LABORATORY DATA ON INDIVIDUAL DOGS  
 FED ZONYL® RP FOR THREE MONTHS

Dog No.: 1159  
 Sex: Male  
 Group: 500 ppm

	Control		Months on Test		
			1	2	2
<u>Hematology</u>					
Erythrocytes	5.64	5.60	5.84	5.48	5.60
Hemoglobin	15.2	15.3	14.9	14.3	14.3
Hematocrit	40	39	39	36	36
Leucocytes	11.0	12.3	12.6	13.7	12.9
<u>DIFFERENTIAL</u>					
Neutrophils	72	60	60	75	71
Seg.	71	60	58	75	70
Juv.	1	0	2	0	1
Myel.	0	0	0	0	0
Lymphocytes	27	32	31	17	23
Eosinophils	1	5	5	6	6
Monocytes	0	3	4	2	0
Basophils	0	0	0	0	0
Atypicals	0	0	0	0	0
Nucleated RBC	0	0	0	0	0
<u>Biochemistry</u>					
Glucose	116	124	118	118	109
Urea Nitrogen	18	17	17	17	19
Cholesterol	165	175	175	195	185
Alk. Phos.	6.6	6.4	7.5	8.7	8.3
GPT	7	6	9	13	12
Total Protein	6.2	6.3	6.1	6.0	6.1
A/G	0.92	1.48	1.40	1.13	0.93
Creatinine	0.5	0.9	0.5	0.8	0.9
Bilirubin	0.1	0.1	0.2	0.1	0.2
Albumin	3.0	3.8	3.6	3.2	2.9
<u>Urinalysis</u>					
Volume	350	165	185	235	275
Appearance	L, Y, C1, P	Y, C1, P	D, A, C1, P	A, C1, P	L, A, C1, P
Osmolality	560	1121	1945	1929	1656
Occult Blood	N	N	N	N	N
Sugar	N	N	Tr	Tr	N
Acetone	N	N	N	N	N
pH	6.2	7.2	7.4	7.0	7.0
Urobilinogen	0.1	1.0	1.0	0.1	1.0
Bilirubin	N	P	P	P	P
Protein	N	Tr	1+	Tr	Tr
Creatinine	52	130	168	182	170
<u>MICROSCOPIC</u>					
RBC's	0	0	0	0	0
WBC's	0	0	0	0	5-10
Epithelial	0	0	0-1	0	0-1
Bacteria	2+	3+	2+	2+	2+
Cast	0	0	0	0	0
Sperm	0	0	0-1	0-1	0-2



APPENDIX I (8)  
 CLINICAL LABORATORY DATA ON INDIVIDUAL DOGS  
 FED ZONYL<sup>®</sup> RP FOR THREE MONTHS

Dog No.: 1165  
 Sex: Male  
 Group: 500 ppm

	Control		Months on Test		
			1	2	3
<u>Hematology</u>					
Erythrocytes	4.96	5.92	7.24	6.48	6.32
Hemoglobin	13.2	15.7	17.6	16.6	16.3
Hematocrit	33	41	49	44	44
Leucocytes	10.4	16.2	15.0	13.2	12.2
DIFFERENTIAL					
Neutrophils	60	64	62	69	66
Seg.	60	64	62	69	66
Juv.	0	0	0	0	0
Myel.	0	0	0	0	0
Lymphocytes	38	35	35	30	27
Eosinophils	1	1	3	0	7
Monocytes	0	0	0	1	0
Basophils	1	0	0	0	0
Atypicals	0	0	0	0	0
Nucleated RBC	0	0	0	0	0
<u>Biochemistry</u>					
Glucose	134	150	182	116	116
Urea Nitrogen	12	16	23	18	25
Cholesterol	-	135	150	170	160
Alk. Phos.	6.5	4.8	4.2	4.8	4.0
GPT	12	7	13	14	12
Total Protein	6.2	6.2	5.5	6.1	6.1
A/G	1.11	1.35	1.03	1.22	0.91
Creatinine	-	0.9	0.6	0.8	0.9
Bilirubin	0.2	0.1	0.3	0.2	0.2
Albumin	3.3	3.6	2.8	3.4	2.9
<u>Urinalysis</u>					
Volume	200	240	310	190	65
Appearance	L, A, Cl, P	D, Y, Cl, P	Y, Cl	A, Cl, P	Y, Cl
Osmolality	1283	1136	1016	1698	1383
Occult Blood	N	N	N	N	N
Sugar	N	N	N	N	N
Acetone	N	N	N	N	N
pH	7.2	6.8	6.8	7.0	7.0
Urobilinogen	0.1	0.1	0.1	1.0	0.1
Bilirubin	P	P	P	P	P
Protein	Tr	Tr	N	1+	Tr
Creatinine	114	92	76	142	116
MICROSCOPIC					
RBC's	0	0	0	0	0
WBC's	0-1	0	0	0-1	0-3
Epithelial	0-1	0	0	0	0
Bacteria	3+	1+	3+	2+	2+
Cast	0	0	0	0	0
Sperm	0	0	0	0-3	0-1

APPENDIX I (9)  
 CLINICAL LABORATORY DATA ON INDIVIDUAL DOGS  
 FED ZONYL® RP FOR THREE MONTHS

Dog No.: 1152  
 Sex: Male  
 Group: 750 ppm

	Control		Months on Test		
			1	2	3
<u>Hematology</u>					
Erythrocytes	6.56	6.80	7.04	7.36	7.08
Hemoglobin	16.9	17.0	17.1	17.9	17.8
Hematocrit	45	45	48	49	49
Leucocytes	8.9	8.2	10.9	13.6	11.8
<u>DIFFERENTIAL</u>					
Neutrophils	69	66	78	74	68
Seg.	68	65	78	74	68
Juv.	1	1	0	0	0
Myel.	0	0	0	0	0
Lymphocytes	26	31	22	25	28
Eosinophils	2	0	0	1	1
Monocytes	3	3	0	0	3
Basophils	0	0	0	0	0
Atypicals	0	0	0	0	0
Nucleated RBC	0	0	0	0	0
<u>Biochemistry</u>					
Glucose	112	120	116	120	111
Urea Nitrogen	16	24	28	23	19
Cholesterol	150	165	185	215	195
Alk. Phos.	4.2	4.4	8.1	8.3	6.5
GPT	7	6	15	21	19
Total Protein	6.4	6.4	6.1	6.3	6.2
A/G	1.23	1.05	1.55	1.28	0.80
Creatinine	0.5	1.1	0.6	0.9	1.0
Bilirubin	0.1	0.1	0.2	0.3	0.2
Albumin	3.5	3.3	3.7	3.5	2.8
<u>Urinalysis</u>					
Volume	105	150	110	135	180
Appearance	A, C1, P	D, Y, C1, P	A, C1	D, Y, C1, P	D, Y, C1, P
Osmolality	1850	1987	2120	2420	1861
Occult Blood	N	N	N	N	N
Sugar	N	N	Tr	Tr	Tr
Acetone	N	N	N	N	N
pH	7.2	7.0	6.4	6.8	7.0
Urobilinogen	0.1	1.0	1.0	1.0	1.0
Bilirubin	P	P	P	P	P
Protein	1+	Tr	Tr	1+	1+
Creatinine	236	180	180	182	180
<u>MICROSCOPIC</u>					
RBC's	0	0	0	0	0
WBC's	0	0	0	0-4	0-1
Epithelial	0	0	0-1	0	0-2
Bacteria	2+	2+	2+	2+	2+
Cast	0	0	0	0	0
Sperm	0	0-1	0	0-1	0

APPENDIX I (10)  
 CLINICAL LABORATORY DATA ON INDIVIDUAL DOGS  
 FED ZONYL® RP FOR THREE MONTHS

Dog No.: 1156  
 Sex: Male  
 Group: 750 ppm

	Control		Months on Test		
			1	2	3
<u>Hematology</u>					
Erythrocytes	5.68	6.12	6.56	6.52	6.40
Hemoglobin	14.1	15.9	16.1	15.7	16.0
Hematocrit	36	42	43	42	43
Leucocytes	11.1	10.5	12.9	11.4	11.7
<u>DIFFERENTIAL</u>					
Neutrophils	66	64	61	57	65
Seg.	65	64	61	57	65
Juv.	1	0	0	0	0
Myel.	0	0	0	0	0
Lymphocytes	32	34	32	29	26
Eosinophils	2	1	3	12	9
Monocytes	0	1	4	2	0
Basophils	0	0	0	0	0
Atypicals	0	0	0	0	0
Nucleated RBC	0	0	0	0	0
<u>Biochemistry</u>					
Glucose	106	104	110	104	109
Urea Nitrogen	17	15	24	19	15
Cholesterol	160	180	180	190	180
Alk. Phos.	3.7	3.5	3.7	4.2	3.3
GPT	7	4	16	17	18
Total Protein	5.9	6.0	5.6	5.7	5.9
A/G	0.93	0.79	1.09	1.09	1.27
Creatinine	0.6	0.9	0.5	0.7	0.9
Bilirutin	0.1	0.1	0.5	0.1	0.1
Albumin	2.8	2.6	2.9	3.0	3.3
<u>Urinalysis</u>					
Volume	140	255	125	90	90
Appearance	Y, Cl, P	L, A, Cl, F	A, Cl, P	A, Cl, P	D, Y, Cl
Osmolality	1924	1777	2045	1971	2207
Occult Blood	N	N	N	N	N
Sugar	Tr	N	Tr	Tr	Tr
Acetone	N	N	N	N	N
pH	6.8	7.6	6.8	7.0	6.8
Urobilinogen	1.0	0.1	1.0	1.0	1.0
Bilirubin	N	P	P	P	P
Protein	Tr	Tr	1+	1+	1+
Creatinine	138	116	130	196	224
<u>MICROSCOPIC</u>					
RBC's	0	0	0	0	0
WBC's	2-6	0	0-2	0-2	2-5
Epithelial	0	0	0-1	0	0-1
Bacteria	2+	2+	1+	2+	2+
Cast	0	0	0	0	0
Sperm	1-2	0-1	0	4-8	1-6

APPENDIX I (11)  
 CLINICAL LABORATORY DATA ON INDIVIDUAL DOGS  
 FED ZONYL® RP FOR THREE MONTHS

Dog No.: 1160  
 Sex: Male  
 Group: 750 ppm

	Control		Months on Test		
			1	2	3
<u>Hematology</u>					
Erythrocytes	5.92	5.68	6.20	6.44	6.40
Hemoglobin	15.5	15.7	16.3	16.9	17.2
Hematocrit	39	42	43	42	46
Leucocytes	8.1	8.5	9.6	8.9	14.4
DIFFERENTIAL					
Neutrophils	69	67	74	70	80
Seg.	69	67	73	70	80
Juv.	0	0	1	0	0
Myel.	0	0	0	0	0
Lymphocytes	29	30	25	29	18
Eosinophils	1	2	0	1	2
Monocytes	1	1	1	0	0
Basophils	0	0	0	0	0
Atypicals	0	0	0	0	0
Nucleated RBC	0	0	0	0	0
<u>Biochemistry</u>					
Glucose	108	112	104	112	104
Urea Nitrogen	11	6	19	15	20
Cholesterol	160	180	200	230	200
Alk. Phos.	5.2	5.3	7.2	8.0	6.1
GPT	8	6	13	16	16
Total Protein	5.8	6.4	5.8	6.2	6.3
A/G	1.23	1.62	1.06	1.22	0.88
Creatinine	0.4	0.7	0.4	0.7	0.8
Bilirubin	0.1	0.1	0.2	0.2	0.2
Albumin	3.2	4.0	3.0	3.4	2.9
<u>Urinalysis</u>					
Volume	205	170	115	70	130
Appearance	Y, Cl, P	Y, Cl, P	A, Cl	D, Y, Cl, P	L, A, Cl, P
Osmolality	1215	1289	1992	1877	2151
Occult Blood	N	N	N	N	N
Sugar	N	N	Tr	Tr	N
Acetone	N	N	N	N	N
pH	7.0	7.0	6.8	7.2	6.8
Urobilinogen	0.1	1.0	0.1	0.1	1.0
Bilirubin	P	P	P	P	P
Protein	Tr	Tr	Tr	Tr	1+
Creatinine	112	130	140	196	180
MICROSCOPIC					
RBC's	0	0	0	0	0
WBC's	0	0	0-1	0	0
Epithelial	0	0	0	0	0-1
Bacteria	1+	2+	2+	2+	2+
Cast	0	0	0	0	0
Sperm	0-1	0	0-2	0	0-1

APPENDIX I (12)  
 CLINICAL LABORATORY DATA ON INDIVIDUAL DOGS  
 FED ZONYL® RP FOR THREE MONTHS

Dog No.: 1164  
 Sex: Male  
 Group: 750 ppm .

	Months on Test				
	Control	1	2	3	
<u>Hematology</u>					
Erythrocytes	6.24	5.76	6.40	6.28	6.56
Hemoglobin	16.8	15.1	15.7	16.0	16.7
Hematocrit	44	40	42	42	44
Leucocytes	17.1	16.3	18.3	16.2	14.6
<u>DIFFERENTIAL</u>					
Neutrophils	59	62	67	66	64
Seg.	59	62	67	64	64
Juv.	0	0	0	2	0
Myel.	0	0	0	0	0
Lymphocytes	32	29	27	20	25
Eosinophils	5	9	6	14	11
Monocytes	4	0	0	0	0
Basophils	0	0	0	0	0
Atypicals	0	0	0	0	0
Nucleated RBC	0	0	0	0	0
<u>Biochemistry</u>					
Glucose	114	114	114	127	110
Urea Nitrogen	11	9	12	12	11
Cholesterol	140	155	170	190	180
Alk. Phos.	4.2	4.4	7.4	7.2	5.3
GPT	8	15	14	17	15
Total Protein	5.8	6.3	6.1	6.1	6.2
A/G	1.04	1.00	1.29	1.12	1.13
Creatinine	0.4	0.8	0.5	0.6	0.7
Bilirubin	0.1	0.1	0.2	0.1	0.1
Albumin	3.0	3.2	3.4	3.2	3.3
<u>Urinalysis</u>					
Volume	225	170	185	160	65
Appearance	D, Y, Cl, P	Y, Cl, P	Y, Cl, P	D, Y, Cl, P	Y, Cl
Osmolality	1373	1478	1341	1535	1404
Occult Blood	N	N	N	N	N
Sugar	N	N	Tr	N	N
Acetone	N	N	N	N	N
pH	7.0	7.4	7.0	7.2	6.8
Urobilinogen	0.1	1.0	1.0	0.1	1.0
Bilirutin	P	N	P	P	P
Protein	Tr	N	1+	Tr	1+
Creatinine	132	118	96	142	182
<u>MICROSCOPIC</u>					
RBC's	0	0	0	0	0
WBC's	0	0	0-1	0	0-1
Epithelial	0	0	0	0	0-1
Bacteria	1+	2+	3+	2+	4+
Cast	0	0	0	0	0
Sperm	0	0-1	0-1	0	0

APPENDIX I (13)  
 CLINICAL LABORATORY DATA ON INDIVIDUAL DOGS  
 FED ZONYL® RP FOR THREE MONTHS

Dog No.: 1151  
 Sex: Male  
 Group: 2500 ppm

	Control		Months on Test		
			1	2	3
<u>Hematology</u>					
Erythrocytes	6.64	6.48	7.00	6.68	6.20
Hemoglobin	17.2	17.3	16.5	16.4	16.3
Hematocrit	46	45	45	43	43
Leucocytes	13.0	12.1	16.7	16.5	21.9
<u>DIFFERENTIAL</u>					
Neutrophils	72	62	67	67	80
Seg.	71	62	64	67	77
Juv.	1	0	3	0	3
Myel.	0	0	0	0	0
Lymphocytes	23	37	26	27	14
Eosinophils	5	1	7	5	5
Monocytes	0	0	0	1	1
Basophils	0	0	0	0	0
Atypicals	0	0	0	0	0
Nucleated RBC	0	0	0	0	0
<u>Biochemistry</u>					
Glucose	92	114	94	108	102
Urea Nitrogen	18	14	21	24	10
Cholesterol	150	155	185	230	200
Alk. Phos.	5.5	5.4	10.8	13.6	12.2
GPT	5	4	10	12	9
Total Protein	6.6	6.1	5.9	6.3	6.2
A/G	1.07	1.04	1.05	0.78	1.21
Creatinine	0.4	0.8	0.6	0.7	0.7
Bilirubin	0.2	0.1	0.2	0.1	0.1
Albumin	3.4	3.1	3.0	2.8	3.4
<u>Urinalysis</u>					
Volume	260	130	105	125	100
Appearance	L,A,C1,P	D,Y,C1,P	Y,C1	A,C1,P	D,Y,C1,P
Osmolality	1651	1745	1730	1908	1824
Occult Blood	N	N	N	N	N
Sugar	N	N	N	N	N
Acetone	N	N	N	N	N
pH	7.0	7.6	7.0	7.0	6.8
Urobilinogen	0.1	1.0	1.0	1.0	1.0
Bilirubin	P	P	P	P	P
Protein	Tr	Tr	Tr	Tr	Tr
Creatinine	128	154	108	168	220
<u>MICROSCOPIC</u>					
RBC's	0	0	0	0	0
WBC's	0	0-1	0-1	0	0-3
Epithelial	0	0	0	0	0-1
Bacteria	2+	2+	3+	2+	4+
Cast	0	0	0	0	0
Sperm	0	0	0-1	0-1	0

APPENDIX I (14)  
 CLINICAL LABORATORY DATA ON INDIVIDUAL DOGS  
 FED ZONYL® RP FOR THREE MONTHS

Dog No.: 1157  
 Sex: Male  
 Group: 2500 ppm

	Control		Months on Test		
			1	2	3
<u>Hematology</u>					
Erythrocytes	6.68	6.48	6.92	6.56	5.76
Hemoglobin	16.9	16.5	16.8	16.0	15.1
Hematocrit	44	44	45	42	40
Leucocytes	12.8	10.8	11.8	13.1	13.8
DIFFERENTIAL					
Neutrophils	78	73	67	73	68
Seg.	78	72	67	73	68
Juv.	0	1	0	0	0
Myel.	0	0	0	0	0
Lymphocytes	22	25	26	20	23
Eosinophils	0	2	7	4	9
Monocytes	0	0	0	3	0
Basophils	0	0	0	0	0
Atypicals	0	0	0	0	0
Nucleated RBC	0	0	0	0	0
<u>Biochemistry</u>					
Glucose	116	114	94	122	103
Urea Nitrogen	25	18	27	17	22
Cholesterol	145	145	170	185	170
Alk. Phos.	4.1	4.2	12.6	10.1	11.7
GPT	10	4	11	14	12
Total Protein	5.8	5.9	6.3	6.0	6.2
A/G	1.03	1.02	1.03	1.11	1.08
Creatinine	0.6	0.9	0.6	0.7	0.7
Bilirubin	0.1	0.1	0.3	0.1	0.1
Albumin	2.9	3.0	3.2	3.2	3.2
<u>Urinalysis</u>					
Volume	240	150	175	90	180
Appearance	A, Cl, P	Y, Cl, P	A, Cl, P	A, Cl, P	D, Y, Cl, P
Osmolality	2138	1619	1961	1814	1567
Occult Blood	N	N	N	N	N
Sugar	N	N	N	N	N
Acetone	N	N	N	N	N
pH	7.2	6.8	7.0	7.2	7.2
Urobilinogen	0.1	0.1	0.1	1.0	1.0
Bilirubin	P	P	N	P	P
Protein	Tr	N	Tr	Tr	Tr
Creatinine	136	122	130	226	144
MICROSCOPIC					
RBC's	0	0	0	0	0
WBC's	0	0	0-1	0-1	0-2
Epithelial	0	0-1	0	0	0-1
Bacteria	2+	1+	2+	3+	3+
Cast	0	0	0	0	0
Sperm	0	0	1-3	0-1	0-4

APPENDIX I (15)  
 CLINICAL LABORATORY DATA ON INDIVIDUAL DOGS  
 FED ZONYL® RP FOR THREE MONTHS

Dog No.: 1161  
 Sex: Male  
 Group: 2500 ppm

	Control		Months on Test		
			1	2	3
<u>Hematology</u>					
Erythrocytes	6.64	6.60	6.68	6.12	6.52
Hemoglobin	17.0	16.8	15.8	15.1	15.7
Hematocrit	44	44	42	39	42
Leucocytes	8.3	8.9	12.3	10.8	11.6
<u>DIFFERENTIAL</u>					
Neutrophils	61	56	66	65	67
Seg.	61	56	64	65	67
Juv.	0	0	2	0	0
Myel.	0	0	0	0	0
Lymphocytes	36	35	22	32	27
Eosinophils	3	8	11	3	5
Monocytes	0	1	1	0	1
Basophils	0	0	0	0	0
Atypicals	0	0	0	0	0
Nucleated RBC	0	0	0	0	0
<u>Biochemistry</u>					
Glucose	119	120	105	112	100
Urea Nitrogen	20	12	30	22	27
Cholesterol	130	140	160	190	180
Alk. Phos.	3.7	3.6	8.6	8.7	10.1
GPT	9	8	12	17	14
Total Protein	6.3	6.1	6.2	6.4	6.4
A/G	1.08	1.18	1.08	1.09	1.28
Creatinine	0.5	0.7	0.6	0.7	0.8
Bilirubin	0.1	0.1	0.3	0.2	0.2
Albumin	3.3	3.3	3.2	3.3	3.6
<u>Urinalysis</u>					
Volume	140	120	180	150	170
Appearance	D, Y, Cl, P	L, A, Cl, P	A, Cl	D, Y, Cl, P	D, Y, Cl
Osmolality	1961	2272	1955	2164	2103
Occult Blood	P	N	N	N	N
Sugar	N	N	N	N	N
Acetone	N	N	N	N	N
pH	6.8	7.0	6.8	7.0	6.8
Urobilinogen	1.0	1.0	1.0	1.0	1.0
Bilirubin	N	N	P	P	P
Protein	Tr	Tr	Tr	1+	Tr
Creatinine	156	174	120	152	150
<u>MICROSCOPIC</u>					
RBC's	0	0	0	0	0
WBC's	0-1	2-6	2-10	1-5	0-3
Epithelial	0	0	0-1	0	0-2
Bacteria	2+	1+	2+	2+	2+
Cast	0	0	0	0	0
Sperm	0-1	0	0-2	0	5-10



APPENDIX I (16)  
 CLINICAL LABORATORY DATA ON INDIVIDUAL DOGS  
 FED ZONYL® RP FOR THREE MONTHS

Dog No.: 1163  
 Sex: Male  
 Group: 2500 ppm

	Control		Months on Test		
			1	2	3
<u>Hematology</u>					
Erythrocytes	5.12	5.24	5.76	6.08	5.88
Hemoglobin	13.1	13.7	13.8	14.6	14.2
Hematocrit	33	35	36	38	37
Leucocytes	12.4	12.8	11.8	13.4	14.1
<u>DIFFERENTIAL</u>					
Neutrophils	76	67	63	68	73
Seg.	75	66	62	68	70
Juv.	1	1	1	0	3
Myel.	0	0	0	0	0
Lymphocytes	23	27	31	24	21
Eosinophils	1	6	6	6	6
Monocytes	0	0	0	2	0
Basophils	0	0	0	0	0
Atypicals	0	0	0	0	0
Nucleated RBC	0	0	0	0	0
<u>Biochemistry</u>					
Glucose	112	124	110	127	122
Urea Nitrogen	13	12	14	20	18
Cholesterol	150	165	210	220	200
Alk. Phos.	5.0	3.8	10.8	12.7	12.8
GPT	9	9	11	16	15
Total Protein	5.6	5.9	6.2	6.4	6.3
A/G	0.98	1.18	1.02	0.94	0.95
Creatinine	-	0.7	0.6	0.8	0.8
Bilirubin	0.1	0.1	0.2	0.5	0.5
Albumin	2.8	3.2	3.1	3.1	3.1
<u>Urinalysis</u>					
Volume	630	460	190	270	345
Appearance	L, Y, Cl, P	L, Y, Cl, P	L, Y, Cl	Y, Cl, P	Y, Cl, P
Osmolality	248	471	352	718	570
Occult Blood	N	N	N	N	N
Sugar	N	N	N	N	N
Acetone	N	N	N	N	N
pH	7.0	7.0	7.6	7.2	7.0
Urobilinogen	0.1	0.1	0.1	0.1	0.1
Bilirubin	N	N	N	N	P
Protein	N	N	N	Tr	N
Creatinine	18	38	34	84	66
<u>MICROSCOPIC</u>					
RBC's	0	0	0	0	0
WBC's	0-5	0-2	0-1	0-2	0
Epithelial	0	0	0-1	0	0-1
Bacteria	4+	3+	4+	3+	4+
Cast	0	0	0	0	0
Sperm	0	0	0	0	0

APPENDIX I (17)  
 CLINICAL LABORATORY DATA ON INDIVIDUAL DOGS  
 FED ZONYL® RP FOR THREE MONTHS

Dog No.: 1137  
 Sex: Female  
 Group: Control

	Control		Months on Test		
			1	2	3
<u>Hematology</u>					
Erythrocytes	6.72	6.68	7.40	7.28	7.76
Hemoglobin	17.8	18.3	18.5	18.9	19.7
Hematocrit	47	48	51	51	54
Leucocytes	12.4	11.4	13.1	10.6	9.1
<u>DIFFERENTIAL</u>					
Neutrophils	60	62	76	71	66
Seg.	58	62	76	71	66
Juv.	2	0	0	0	0
Myel.	0	0	0	0	0
Lymphocytes	28	25	20	24	25
Eosinophils	12	10	4	5	9
Monocytes	0	3	0	0	0
Basophils	0	0	0	0	0
Atypicals	0	0	0	0	0
Nucleated RBC	0	0	0	0	0
<u>Biochemistry</u>					
Glucose	114	109	111	110	101
Urea Nitrogen	10	12	16	19	26
Cholesterol	160	150	160	185	165
Alk. Phos.	4.5	4.7	4.1	3.9	10.1
GPT	7	3	8	9	15
Total Protein	6.4	5.5	6.3	6.4	6.7
A/G	1.20	1.15	1.50	1.21	1.06
Creatinine	0.5	0.5	0.5	0.7	0.8
Bilirubin	0.1	0.2	0.2	0.2	0.2
Albumin	3.5	2.9	3.8	3.5	3.4
<u>Urinalysis</u>					
Volume	125	235	165	190	140
Appearance	D, Y, Cl, P	D, Y, Cl, P	A, Cl, P	A, Cl, P	A, Cl, P
Osmolality	1359	1651	2142	2013	1646
Occult Blood	N	N	N	N	N
Sugar	N	N	Tr	N	N
Acetone	N	N	N	N	N
pH	8.5	6.8	6.8	7.0	6.8
Urobilinogen	1.0	1.0	1.0	1.0	1.0
Bilirubin	P	N	P	P	P
Protein	Tr	Tr	Tr	Tr	1+
Creatinine	146	114	164	168	156
<u>MICROSCOPIC</u>					
RBC 's	0	0	0	0	0
WBC 's	0	0-1	2-8	0	0
Epithelial	0-1	0	0	0	0
Bacteria	3+	1+	2+	2+	2+
Cast	0	0	0	0	0

APPENDIX I (18)  
 CLINICAL LABORATORY DATA ON INDIVIDUAL DOGS  
 FED ZONYL<sup>®</sup> RP FOR THREE MONTHS

Dog No.: 1141  
 Sex: Female  
 Group: Control

	Control		Months on Test		
			1	2	3
<u>Hematology</u>					
Erythrocytes	6.52	6.60	6.00	6.64	6.28
Hemoglobin	17.5	17.6	14.9	17.2	16.2
Hematocrit	45	46	39	45	43
Leucocytes	10.4	11.1	9.0	10.8	7.8
<u>DIFFERENTIAL</u>					
Neutrophils	72	74	73	77	65
Seg.	72	71	73	75	65
Juv.	0	3	0	2	0
Myel.	0	0	0	0	0
Lymphocytes	21	15	26	20	27
Eosinophils	6	8	1	2	6
Monocytes	1	3	0	1	2
Basophils	0	0	0	0	0
Atypicals	0	0	0	0	0
Nucleated RBC	0	0	0	0	0
<u>Biochemistry</u>					
Glucose	114	112	112	147	112
Urea Nitrogen	10	15	17	11	16
Cholesterol	145	140	190	160	145
Alk. Phos.	5.0	5.3	4.1	3.6	4.3
GPT	10	3	12	19	13
Total Protein	6.3	6.5	6.3	6.3	6.2
A/G	0.92	0.95	1.10	1.23	1.50
Creatinine	0.5	0.7	0.6	0.9	0.8
Bilirubin	0.1	0.3	0.2	0.1	0.1
Albumin	3.0	3.2	3.3	3.5	3.7
<u>Urinalysis</u>					
Volume	250	190	150	85	175
Appearance	D, Y, Cl, P	L, A, Cl, P	D, Y, Cl, P	D, Y, Cl, P	A, Cl, P
Osmolality	1546	2018	1625	1924	1856
Occult Blood	N	N	N	N	N
Sugar	N	N	Tr	N	N
Acetone	N	N	N	N	N
pH	7.0	6.6	7.0	8.0	7.0
Urobilinogen	1.0	1.0	1.0	1.0	1.0
Bilirubin	N	N	N	P	N
Protein	Tr	Tr	Tr	Tr	Tr
Creatinine	144	152	142	230	174
<u>MICROSCOPIC</u>					
RBC's	0	0	0	0	0
WBC's	0-1	0	0	0-1	0-2
Epithelial	0-2	0-1	0-3	0-1	1-5
Bacteria	2+	1+	3+	3+	2+
Cast	0	0	0	0	0

APPENDIX I (19)  
 CLINICAL LABORATORY DATA ON INDIVIDUAL DOGS  
 FED ZONYL<sup>®</sup> RP FOR THREE MONTHS

Dog No.: 1145  
 Sex: Female  
 Group: Control

	Months on Test				
	Control		1	2	3
<u>Hematology</u>					
Erythrocytes	7.08	6.72	6.88	7.20	6.76
Hemoglobin	16.9	16.0	16.2	17.8	16.8
Hematocrit	43	41	43	47	42
Leucocytes	9.2	11.2	9.5	12.6	8.3
<u>DIFFERENTIAL</u>					
Neutrophils	74	64	78	74	66
Seg.	74	63	78	74	65
Juv.	0	1	0	0	1
Myel.	0	0	0	0	0
Lymphocytes	24	30	20	26	33
Eosinophils	2	3	2	0	1
Monocytes	0	3	0	0	0
Basophils	0	0	0	0	0
Atypicals	0	0	0	0	0
Nucleated RBC	0	0	0	0	0
<u>Biochemistry</u>					
Glucose	106	118	110	118	102
Urea Nitrogen	13	11	14	15	21
Cholesterol	155	175	155	160	295
Alk. Phos.	3.5	3.4	3.5	3.2	3.1
GPT	7	6	10	17	13
Total Protein	5.9	5.9	5.4	5.7	6.5
A/G	1.16	1.47	1.58	0.99	1.32
Creatinine	0.7	0.8	0.6	0.8	1.0
Bilirutin	0.6	0.2	0.2	0.2	0.2
Albumin	3.2	3.5	3.3	2.8	3.7
<u>Urinalysis</u>					
Volume	190	80	110	120	175
Appearance	Y, Cl, P	A, Cl, P	A, Cl, P	D, Y, Cl, P	L, A, Cl, P
Osmolality	1289	2303	1824	1871	1735
Occult Blood	N	N	N	N	N
Sugar	N	N	Tr	N	Tr
Acetone	N	N	N	N	N
pH	7.6	8.0	7.2	7.0	7.0
Urobilinogen	1.0	1.0	1.0	1.0	1.0
Bilirubin	N	N	P	P	P
Protein	N	Tr	Tr	1+	1+
Creatinine	128	236	186	196	180
<u>MICROSCOPIC</u>					
RBC's	0	0	0	0	0
WBC's	0	0-1	0	0	0
Epithelial	0-1	0	0-1	0-1	0
Bacteria	2+	1+	2+	2+	1+
Cast	0	0	0	0	0

APPENDIX I (20)  
 CLINICAL LABORATORY DATA ON INDIVIDUAL DOGS  
 FED ZONYL® RP FOR THREE MONTHS

Dog No.: 1147  
 Sex: Female  
 Control

	Control		Months on Test		
			1	2	3
<u>Hematology</u>					
Erythrocytes	6.32	6.52	6.16	6.60	5.84
Hemoglobin	16.1	17.1	15.5	17.1	17.4
Hematocrit	43	44	43	45	46
Leucocytes	11.8	11.5	17.2	15.2	12.8
<u>DIFFERENTIAL</u>					
Neutrophils	61	60	60	66	60
Seg.	61	60	60	66	59
Juv.	0	0	0	0	1
Myel.	0	0	0	0	0
Lymphocytes	27	30	34	28	24
Eosinophils	11	6	5	5	16
Monocytes	1	4	1	1	0
Basophils	0	0	0	0	0
Atypicals	0	0	0	0	0
Nucleated RBC	0	0	0	0	0
<u>Biochemistry</u>					
Glucose	114	110	100	116	109
Urea Nitrogen	20	22	36	41	46
Cholesterol	170	170	150	165	175
Alk. Phos.	4.4	3.9	4.0	3.6	3.1
GPT	8	5	6	10	3
Total Protein	6.8	6.4	5.8	5.7	5.8
A/G	1.04	1.02	0.98	0.94	1.27
Creatinine	0.8	1.1	1.0	1.2	1.8
Bilirubin	0.1	0.1	0.2	0.1	0.1
Albumin	3.5	3.2	2.9	2.8	3.2
<u>Urinalysis</u>					
Volume	150	50	80	140	110
Appearance	Y, Cl, P	D, Y, Cl	D, Y, Cl	D, Y, Cl	D, Y, Cl
Osmolality	1073	1945	2003	1367	1499
Occult Blood	N	N	N	N	N
Sugar	N	N	Tr	N	N
Acetone	N	N	N	N	N
pH	6.8	7.0	6.4	7.2	7.0
Urobilinogen	0.1	1.0	1.0	0.1	1.0
Bilirubin	P	N	P	P	P
Protein	Tr	Tr	Tr	Tr	Tr
Creatinine	152	340	208	138	170
<u>MICROSCOPIC</u>					
RBC's	0	0	0	0	0
WBC's	0-2	0	0	0	0
Epithelial	0-1	0-2	0	0	0
Bacteria	2+	2+	1+	2+	2+
Cast	0	0	0	0	0

APPENDIX I (21)  
 CLINICAL LABORATORY DATA ON INDIVIDUAL DOGS  
 FED ZONYL<sup>®</sup> RP FOR THREE MONTHS

Dog No.: 1135  
 Sex: Female  
 Group: 500 ppm

	Control		Months on Test		
			1	2	3
<u>Hematology</u>					
Erythrocytes	7.04	5.68	7.08	7.24	6.64
Hemoglobin	18.8	14.6	17.8	18.0	16.5
Hematocrit	49	47	48	48	43
Leucocytes	10.9	11.2	11.4	12.4	11.2
DIFFERENTIAL					
Neutrophils	66	72	71	74	60
Seg.	66	71	69	72	58
Juv.	0	1	2	2	2
Myel.	0	0	0	0	0
Lymphocytes	32	25	24	24	22
Eosinophils	1	0	5	2	16
Monocytes	1	3	0	0	2
Basophils	0	0	0	0	0
Atypicals	0	0	0	0	0
Nucleated RBC	0	0	0	0	0
<u>Biochemistry</u>					
Glucose	102	110	118	104	110
Urea Nitrogen	13	8	11	23	22
Cholesterol	130	150	205	235	285
Alk. Phos.	7.0	6.6	8.0	6.5	4.2
GPT	6	2	9	10	9
Total Protein	6.7	5.8	5.8	6.6	6.8
A/G	1.40	1.09	1.05	0.90	0.80
Creatinine	0.5	0.5	0.6	0.7	0.8
Bilirubin	0.2	0.2	0.3	0.4	0.3
Albumin	3.9	3.0	3.0	3.1	3.0
<u>Urinalysis</u>					
Volume	60	165	160	160	145
Appearance	D, Y, Cl, P	Y, Cl, P	A, Cl, P	D, Y, Cl, P	A, Cl
Osmolality	1871	1136	1467	1488	2098
Occult Blood	N	N	N	N	N
Sugar	Tr	N	1+	Tr	N
Acetone	N	N	N	N	N
pH	6.6	7.0	8.0	7.0	6.8
Urobilinogen	0.1	0.1	1.0	1.0	1.0
Bilirubin	N	N	P	N	P
Protein	Tr	Tr	1+	Tr	Tr
Creatinine	202	88	118	154	182
MICROSCOPIC					
RBC 's	0	0	0	0	0
WBC 's	0-1	0-2	0-1	0	0
Epithelial	0-2	0	0-5	0-1	0
Bacteria	1+	1+	2+	2+	1+
Cast	0	0	0	0	0

APPENDIX I (22)  
 CLINICAL LABORATORY DATA ON INDIVIDUAL DOGS  
 FED ZONYL® RP FOR THREE MONTHS

Dog No.: 1140  
 Sex: Female  
 Group: 500 ppm

	Control		Months on Test		
			1	2	3
<u>Hematology</u>					
Erythrocytes	6.20	6.40	6.44	6.68	6.16
Hemoglobin	15.5	16.5	16.4	17.1	15.7
Hematocrit	41	44	45	45	41
Leucocytes	8.2	7.3	10.5	11.1	7.7
DIFFERENTIAL					
Neutrophils	62	72	61	69	68
Seg.	61	71	61	69	68
Juv.	1	1	0	0	0
Myel.	0	0	0	0	0
Lymphocytes	34	26	33	26	25
Eosinophils	3	2	4	5	7
Monocytes	0	0	2	0	0
Basophils	1	0	0	0	0
Atypicals	0	0	0	0	0
Nucleated RBC	0	0	0	0	0
<u>Biochemistry</u>					
Glucose	119	122	120	110	117
Urea Nitrogen	10	9	17	22	17
Cholesterol	170	170	165	170	200
Alk. Phos.	10.7	10.9	8.3	6.9	4.7
GPT	7	3	10	9	6
Total Protein	6.1	6.0	5.9	6.1	6.1
A/G	1.25	1.30	1.15	1.12	1.54
Creatinine	0.8	0.6	0.5	0.6	0.7
Bilirubin	0.1	0.1	0.3	0.3	0.3
Albumin	3.4	3.4	3.2	3.2	3.7
<u>Urinalysis</u>					
Volume	485	165	100	90	85
Appearance	L, Y, Cl, P	Y, Cl, P	D, Y, Cl	A, Cl	D, Y, Cl, P
Osmolality	209	951	1310	2151	1814
Occult Blood	N	N	N	N	N
Sugar	N	N	Tr	Tr	N
Acetone	N	N	N	N	N
pH	7.2	8.0	7.2	7.2	7.2
Urobilinogen	0.1	0.1	1.0	1.0	1.0
Bilirubin	N	N	N	P	P
Protein	N	N	Tr	1+	1+
Creatinine	14	74	102	204	196
MICROSCOPIC					
RBC's	0	0	0	0	0
WBC's	0-2	0	0-2	0-1	0
Epithelial	0-2	0-1	0	0	0-1
Bacteria	3+	2+	1+	1+	2+
Cast	0	0	0	0	0

APPENDIX I (23)  
 CLINICAL LABORATORY DATA ON INDIVIDUAL DOGS  
 FED ZONYL® RP FOR THREE MONTHS

Dog No.: 1143  
 Sex: Female  
 Group: 500 ppm

	Control		Months on Test		
			1	2	3
<u>Hematology</u>					
Erythrocytes	5.84	6.28	5.72	6.40	5.92
Hemoglobin	14.3	15.8	13.8	15.9	15.7
Hematocrit	36	41	37	40	41
Leucocytes	16.1	14.8	11.3	15.5	11.3
<u>DIFFERENTIAL</u>					
Neutrophils	70	74	68	60	67
Seg.	68	73	68	60	67
Juv.	2	1	0	0	0
Myel.	0	0	0	0	0
Lymphocytes	24	17	30	31	29
Eosinophils	1	5	2	9	4
Monocytes	5	4	0	0	0
Basophils	0	0	0	0	0
Atypicals	0	0	0	0	0
Nucleated RBC	0	0	0	0	0
<u>Biochemistry</u>					
Glucose	116	112	93	106	109
Urea Nitrogen	11	12	32	32	33
Cholesterol	145	160	170	200	220
Alk. Phos.	3.7	4.7	6.5	5.4	4.1
GPT	7	4	11	9	11
Total Protein	6.1	6.4	5.7	6.6	6.6
A/G	1.12	1.05	0.97	0.99	1.34
Creatinine	0.5	0.8	0.6	0.7	0.9
Bilirubin	0.1	0.1	0.3	0.8	0.9
Albumin	3.2	3.3	2.8	3.3	3.8
<u>Urinalysis</u>					
Volume	150	140	170	150	205
Appearance	Y, Cl, P	Y, Cl, P	D, Y, Cl	Y, Cl, P	D, Y, Cl, P
Osmolality	1205	1079	1320	1714	1252
Occult Blood	N	N	N	N	N
Sugar	N	N	N	N	N
Acetone	N	N	N	N	N
pH	7.0	6.8	6.8	6.8	7.0
Urobilinogen	1.0	1.0	1.0	1.0	0.1
Bilirubin	N	N	N	P	P
Protein	Tr	Tr	Tr	Tr	Tr
Creatinine	144	146	102	146	114
<u>MICROSCOPIC</u>					
RBC's	0	0	0	0	0
WBC's	0	0	0-1	0	0
Epithelial	0	0-1	0	0	0
Bacteria	2+	1+	3+	4+	1+
Cast	0	0	0	0	0



APPENDIX I (24)  
 CLINICAL LABORATORY DATA ON INDIVIDUAL DOGS  
 FED ZONYL® RP FOR THREE MONTHS

Dog No.: 1149  
 Sex: Female  
 Group: 500 ppm

	Control		Months on Test		
			1	2	3
<u>Hematology</u>					
Erythrocytes	6.64	6.40	4.20	6.76	6.52
Hemoglobin	15.9	15.6	14.8	16.6	16.0
Hematocrit	43	41	40	43	43
Leucocytes	11.5	16.0	9.2	12.6	11.0
<u>DIFFERENTIAL</u>					
Neutrophils	78	75	65	70	77
Seg.	78	74	65	70	75
Juv.	0	1	0	0	2
Myel.	0	0	0	0	0
Lymphocytes	22	23	33	28	22
Eosinophils	0	0	1	1	1
Monocytes	0	2	1	1	0
Basophils	0	0	0	0	0
Atypicals	0	0	0	0	0
Nucleated RBC	0	0	0	0	0
<u>Biochemistry</u>					
Glucose	104	170	116	112	89
Urea Nitrogen	39	37	23	23	26
Cholesterol	170	150	180	215	215
Alk. Phos.	2.0	5.2	10.7	10.7	11.2
GPT	9	9	19	11	13
Total Protein	6.7	6.2	5.6	5.6	6.4
A/G	0.76	1.11	1.03	0.87	0.89
Creatinine	-	1.1	0.6	0.7	0.8
Bilirubin	0.1	0.1	0.2	0.2	0.7
Albumin	2.9	3.3	2.8	2.6	3.0
<u>Urinalysis</u>					
Volume	100	90	130	195	165
Appearance	L, A, C1	A, C1	D, A, C1, P	D, Y, C1, P	L, A, C1, P
Osmolality	1950	2525	2460	1546	2281
Occult Blood	N	P	N	N	N
Sugar	N	N	1+	N	Tr
Acetone	N	N	N	N	N
pH	6.4	6.4	6.6	7.0	6.4
Urobilinogen	0.1	0.1	1.0	1.0	1.0
Bilirubin	N	N	N	N	P
Protein	N	N	Tr	Tr	Tr
Creatinine	184	184	130	112	148
<u>MICROSCOPIC</u>					
RBC's	0	0	0	0	0
WBC's	0	0	0-1	0-1	0-1
Epithelial	0-1	0-1	0-1	0	0-4
Bacteria	2+	3+	1+	2+	2+
Cast	0	0	0	0	0

APPENDIX I (25)  
 CLINICAL LABORATORY DATA ON INDIVIDUAL DOGS  
 FED ZONYL® RP FOR THREE MONTHS

Dog No.: 1136  
 Sex: Female  
 Group: 750 ppm

	Control		Months on Test		
			1	2	3
<u>Hematology</u>					
Erythrocytes	6.40	6.64	7.04	6.48	6.88
Hemoglobin	17.4	17.7	17.4	16.2	17.3
Hematocrit	46	46	48	42	46
Leucocytes	17.3	14.0	16.9	14.8	10.5
<u>DIFFERENTIAL</u>					
Neutrophils	68	64	71	63	49
Seg.	68	61	68	62	49
Juv.	0	3	3	1	0
Myel.	0	0	0	0	0
Lymphocytes	27	24	21	26	33
Eosinophils	5	10	8	11	18
Monocytes	0	2	0	0	0
Basophils	0	0	0	0	0
Atypicals	0	0	0	0	0
Nucleated RBC	0	0	0	0	0
<u>Biochemistry</u>					
Glucose	102	90	94	106	109
Urea Nitrogen	12	12	21	12	12
Cholesterol	120	140	210	275	235
Alk. Phos.	4.8	4.7	6.3	7.6	7.9
GPT	5	2	8	9	7
Total Protein	6.4	5.9	6.3	6.6	6.9
A/G	1.24	1.50	0.78	0.98	0.63
Creatinine	0.5	0.7	0.6	0.6	0.6
Bilirubin	0.1	0.2	0.4	0.1	0.2
Albumin	3.5	3.5	2.8	3.3	2.7
<u>Urinalysis</u>					
Volume	275	185	195	135	150
Appearance	D, Y, Cl, P	D, Y, Cl, P	A, Cl, P	D, Y, Cl, P	D, Y, Cl, P
Osmolality	1677	1388	1772	1609	1735
Occult Blood	N	N	N	N	N
Sugar	N	N	Tr	Tr	Tr
Acetone	N	N	N	N	N
pH	7.0	8.2	7.0	8.2	7.2
Urobilinogen	0.1	1.0	1.0	1.0	1.0
Bilirubin	N	N	N	P	P
Protein	Tr	Tr	Tr	Tr	Tr
Creatinine	130	110	114	132	242
<u>MICROSCOPIC</u>					
RBC's	0	0	0	0	0
WBC's	0	0	0-1	0	1-5
Epithelial	0-1	0-1	0-1	0-2	0-3
Bacteria	1+	2+	1+	1+	3+
Cast	0	0	0	0	0

APPENDIX I (26)  
 CLINICAL LABORATORY DATA ON INDIVIDUAL DOGS  
 FED ZONYL® RP FOR THREE MONTHS

Dog No.: 1139  
 Sex: Female  
 Group: 750 ppm

	Months on Test				
	Control		1	2	3
<u>Hematology</u>					
Erythrocytes	6.08	6.24	4.40	7.28	6.64
Hmoglobin	16.7	17.4	16.5	18.5	17.1
Hematocrit	43	45	44	48	45
Leucocytes	12.7	11.8	11.5	16.6	10.8
<u>DIFFERENTIAL</u>					
Neutrophils	66	70	71	79	62
Seg.	66	68	71	78	62
Juv.	0	2	0	1	0
Myel.	0	0	0	0	0
Lymphocytes	29	27	24	21	30
Eosinophils	2	1	5	0	5
Monocytes	3	2	0	0	3
Basophils	0	0	0	0	0
Atypicals	0	0	0	0	0
Nucleated RBC	0	0	0	0	0
<u>Biochemistry</u>					
Glucose	104	110	98	120	99
Urea Nitrogen	19	13	21	14	15
Cholesterol	170	170	190	250	340
Alk. Phos.	6.1	5.9	9.6	10.1	9.3
GPT	7	3	12	13	9
Total Protein	6.2	6.2	5.8	6.4	6.8
A/G	0.97	1.00	1.35	1.16	0.92
Creatinine	0.5	0.6	0.6	0.7	0.6
Bilirubin	0.1	0.1	0.3	0.1	0.1
Albumin	3.1	3.1	3.3	3.4	3.3
<u>Urinalysis</u>					
Volume	160	80	90	75	90
Appearance	Y,C1,P	Y,C1,P	Y,C1	Y,C1,P	Y,C1
Osmolality	1399	1210	1535	1493	1562
Occult Blood	N	N	N	P	N
Sugar	N	N	Tr	N	N
Acetone	N	N	N	N	N
pH	7.0	7.0	7.0	7.0	7.2
Urobilinogen	1.0	0.1	1.0	0.1	1.0
Bilirubin	N	N	P	P	P
Protein	Tr	Tr	Tr	Tr	Tr
Creatinine	112	128	94	172	158
<u>MICROSCOPIC</u>					
RBC's	0	0	0	1-6	0
WBC's	0-2	0-2	0-1	0-2	0-2
Epithelial	0-1	0	0-1	0-1	0-4
Bacteria	2+	2+	1+	2+	2+
Cast	0	0	0	0	0

APPENDIX I (27)  
 CLINICAL LABORATORY DATA ON INDIVIDUAL DOGS  
 FED ZONYL<sup>®</sup> RP FOR THREE MONTHS

Dog No.: 1144  
 Sex: Female  
 Group: 750 ppm

	Control		Months on Test		
			1	2	3
<u>Hematology</u>					
Erythrocytes	6.40	6.80	7.32	7.76	7.12
Hemoglobin	15.0	17.3	18.2	19.2	18.1
Hematocrit	38	45	50	50	48
Leucocytes	12.4	11.0	11.4	13.4	12.5
<u>DIFFERENTIAL</u>					
Neutrophils	80	72	79	77	70
Seg.	77	71	79	75	67
Juv.	3	1	0	2	3
Myel.	0	0	0	0	0
Lymphocytes	17	25	20	22	29
Eosinophils	2	3	1	1	1
Monocytes	1	0	0	0	0
Basophils	0	0	0	0	0
Atypicals	0	0	0	0	0
Nucleated RBC	0	0	0	0	0
<u>Biochemistry</u>					
Glucose	112	115	80	104	100
Urea Nitrogen	18	19	25	27	26
Cholesterol	160	185	200	245	285
Alk. Phos.	4.0	3.5	4.4	6.4	8.6
GPT	7	4	8	9	9
Total Protein	5.9	6.1	6.1	6.6	6.5
A/G	1.31	1.32	1.25	1.10	1.13
Creatinine	0.6	0.8	0.7	0.9	0.8
Bilirubin	0.2	0.2	0.6	0.7	1.2
Albumin	3.3	3.5	3.4	3.5	3.4
<u>Urinalysis</u>					
Volume	150	150	135	145	195
Appearance	D, Y, Cl, P	A, Cl, P	A, Cl	A, Cl	A, Cl, P
Osmolality	1898	1877	1766	1898	2194
Occult Blood	N	N	N	N	N
Sugar	N	Tr	1+	N	N
Acetone	N	N	N	N	N
pH	7.0	7.0	6.6	6.6	6.8
Urobilinogen	1.0	1.0	1.0	1.0	1.0
Bilirubin	P	N	P	P	P
Protein	Tr	Tr	Tr	N	Tr
Creatinine	184	188	126	148	166
<u>MICROSCOPIC</u>					
RBC's	0	0	0	0	0
WBC's	0	0	0	0	0-1
Epithelial	0	0	0-1	0	0-3
Bacteria	2+	2+	3+	1+	2+
Cast	0	0	0	0	0

APPENDIX I (28)  
 CLINICAL LABORATORY DATA ON INDIVIDUAL DOGS  
 FED ZONYL® RP FOR THREE MONTHS

Dog No.: 1148  
 Sex: Female  
 Group: 750 ppm

	Control		Months on Test		
			1	2	3
<u>Hematology</u>					
Erythrocytes	6.28	6.88	7.08	7.28	7.16
Hemoglobin	15.8	16.5	17.0	17.7	17.3
Hematocrit	42	43	47	46	46
Leucocytes	9.2	11.8	12.9	14.4	11.2
<u>DIFFERENTIAL</u>					
Neutrophils	59	71	56	51	67
Seg.	58	70	56	50	66
Juv.	1	1	0	1	1
Myel.	0	0	0	0	0
Lymphocytes	41	29	34	38	28
Eosinophils	0	0	8	9	5
Monocytes	0	0	2	2	0
Basophils	0	0	0	0	0
Atypicals	0	0	0	0	0
Nucleated RBC	0	0	0	0	0
<u>Biochemistry</u>					
Glucose	106	116	108	120	112
Urea Nitrogen	26	25	26	22	31
Cholesterol	150	160	235	260	310
Alk. Phos.	3.1	2.9	4.4	4.6	3.4
GPT	8	5	12	13	11
Total Protein	5.8	5.9	6.2	6.3	7.0
A/G	1.11	1.10	1.24	1.03	0.94
Creatinine	-	0.8	0.7	0.8	0.8
Bilirubin	0.2	0.4	0.3	0.3	0.4
Albumin	3.1	3.1	3.4	3.2	3.4
<u>Urinalysis</u>					
Volume	115	110	180	33	125
Appearance	D, Y, C1, P	D, Y, C1	D, Y, C1	Y, C1	D, Y, C1
Osmolality	1777	1856	1436	392	2034
Occult Blood	N	N	N	P	N
Sugar	N	N	N	N	Tr
Acetone	N	N	N	N	N
pH	6.8	6.8	7.0	7.0	7.0
Urobilinogen	1.0	1.0	1.0	0.1	1.0
Bilirubin	P	P	P	P	P
Protein	Tr	N	Tr	Tr	Tr
Creatinine	174	184	114	50	208
<u>MICROSCOPIC</u>					
RBC's	0	0	0	0	0
WBC's	0-1	0	0	0-1	0-1
Epithelial	0-1	0	0	0-2	0-2
Bacteria	2+	1+	3+	1+	2+
Cast	0	0	0	0	0

APPENDIX I (29)  
 CLINICAL LABORATORY DATA ON INDIVIDUAL DOGS  
 FED ZONYL® RP FOR THREE MONTHS

Dog No.: 1134  
 Sex: Female  
 Group: 2500 ppm

	Control		Months on Test		
			1	2	3
<u>Hematology</u>					
Erythrocytes	5.68	6.64	6.40	6.60	6.60
Hemoglobin	17.6	18.1	16.8	17.1	16.8
Hematocrit	44	46	45	44	44
Leucocytes	10.5	9.0	9.0	13.2	9.1
<u>DIFFERENTIAL</u>					
Neutrophils	57	65	67	75	68
Seg.	57	65	66	73	68
Juv.	0	0	1	2	0
Myel.	0	0	0	0	0
Lymphocytes	38	30	27	19	29
Eosinophils	3	3	3	4	3
Monocytes	2	2	3	2	0
Basophils	0	0	0	0	0
Atypicals	0	0	0	0	0
Nucleated RBC	0	0	0	0	0
<u>Biochemistry</u>					
Glucose	112	100	91	110	124
Urea Nitrogen	11	9	13	16	17
Cholesterol	140	155	235	290	285
Alk. Phos.	4.4	6.8	15.4	17.7	15.2
GPT	8	3	14	24	17
Total Protein	6.3	6.2	6.5	6.7	6.8
A/G	1.37	1.15	0.94	0.97	1.05
Creatinine	0.5	0.6	0.6	0.8	0.8
Bilirubin	0.1	0.2	0.3	0.2	0.5
Albumin	3.6	3.3	3.1	3.3	3.5
<u>Urinalysis</u>					
Volume	120	130	165	175	170
Appearance	D, Y, Cl, P	D, Y, Cl, P	A, Cl, P	D, Y, Cl, P	A, Cl, P
Osmolality	2116	1798	1724	1436	1903
Occult Blood	N	N	N	N	N
Sugar	Tr	Tr	Tr	N	N
Acetone	N	N	N	N	N
pH	7.0	7.0	6.8	7.0	7.2
Urobilinogen	1.0	1.0	1.0	1.0	1.0
Bilirubin	N	N	N	P	P
Protein	Tr	Tr	N	N	1+
Creatinine	156	124	116	100	144
<u>MICROSCOPIC</u>					
RBC's	0	0	0	0	0
WBC's	0-1	0	0-1	0	0
Epithelial	0	0-1	0	0	0-1
Bacteria	2+	1+	3+	3+	3+
Cast	0	0	0	0	0

APPENDIX I (30)  
 CLINICAL LABORATORY DATA ON INDIVIDUAL DOGS  
 FED ZONYL<sup>®</sup> RP FOR THREE MONTHS

Dog No.: 1138  
 Sex: Female  
 Group: 2500 ppm

	Control		Months on Test		
			1	2	3
<u>Hematology</u>					
Erythrocytes	6.52	6.68	5.84	5.16	6.16
Hemoglobin	17.1	17.4	14.9	12.8	15.3
Hematocrit	44	45	39	31	41
Leucocytes	8.5	7.7	10.7	9.7	7.4
<u>DIFFERENTIAL</u>					
Neutrophils	73	69	70	77	63
Seg.	72	69	70	75	60
Juv.	1	0	0	2	3
Myel.	0	0	0	0	0
Lymphocytes	22	20	28	23	34
Eosinophils	3	8	2	0	1
Monocytes	2	3	0	0	2
Basophils	0	0	0	0	0
Atypicals	0	0	0	0	0
Nucleated RBC	0	0	0	0	0
<u>Biochemistry</u>					
Glucose	104	102	90	120	108
Urea Nitrogen	14	11	21	17	16
Cholesterol	175	180	348	436	275
Alk. Phos.	8.0	7.6	12.8	14.1	13.5
GPT	6	3	11	16	17
Total Protein	6.5	6.3	6.6	6.7	6.3
A/G	1.09	0.97	0.88	0.78	1.28
Creatinine	0.4	0.6	0.6	0.7	0.8
Bilirubin	0.1	0.2	0.3	0.2	0.2
Albumin	3.4	3.1	3.1	2.9	3.5
<u>Urinalysis</u>					
Volume	270	380	415	290	175
Appearance	Y, Cl, P	Y, Cl, P	Y, Cl	Y, Cl, P	A, Cl, P
Osmolality	1399	931	684	862	1420
Occult Blood	N	N	N	N	N
Sugar	N	N	N	N	N
Acetone	N	N	N	N	N
pH	7.0	7.0	6.8	7.0	7.2
Urobilinogen	0.1	0.1	0.1	1.0	1.0
Bilirubin	N	N	N	P	N
Protein	Tr	N	N	N	Tr
Creatinine	104	72	48	82	158
<u>MICROSCOPIC</u>					
RBC's	0	0	0	0	0
WBC's	0-1	0-1	0	0-1	0
Epithelial	0-1	0-1	0-1	0	0-1
Bacteria	2+	4+	3+	4+	2+
Cast	0	0	0	0	0

APPENDIX I (31)  
 CLINICAL LABORATORY DATA ON INDIVIDUAL DOGS  
 FED ZONYL<sup>®</sup> RP FOR THREE MONTHS

Dog No.: 1142  
 Sex: Female  
 Group: 2500 ppm

	Control		Months on Test		
			1	2	3
<u>Hematology</u>					
Erythrocytes	5.76	6.00	5.28	5.96	5.72
Hemoglobin	16.1	16.3	13.6	15.9	14.9
Hematocrit	41	41	36	40	39
Leucocytes	16.1	12.3	13.3	14.7	8.5
DIFFERENTIAL					
Neutrophils	41	65	54	66	61
Seg.	40	65	52	66	61
Juv.	1	0	2	0	0
Myel.	0	0	0	0	0
Lymphocytes	23	28	30	27	25
Eosinophils	36	7	16	7	13
Monocytes	0	0	0	0	1
Basophils	0	0	0	0	0
Atypicals	0	0	0	0	0
Nucleated RBC	0	0	0	0	0
<u>Biochemistry</u>					
Glucose	110	120	92	108	102
Urea Nitrogen	29	20	23	28	21
Cholesterol	140	155	265	260	235
Alk. Phos.	3.5	3.8	6.1	9.2	8.6
GPT	8	3	16	16	15
Total Protein	6.4	6.6	6.1	6.6	6.3
A/G	1.11	1.02	1.02	0.89	0.86
Creatinine	0.6	0.8	0.7	0.8	1.0
Bilirubin	0.6	0.2	0.2	0.5	0.2
Albumin	3.4	3.3	3.1	3.1	2.9
<u>Urinalysis</u>					
Volume	240	175	115	165	165
Appearance	D, Y, Cl, P	L, A, Cl, P	A, Cl, P	A, Cl, P	A, Cl, P
Osmolality	1766	2272	2168	2355	2168
Occult Blood	N	N	N	N	N
Sugar	N	Tr	Tr	N	N
Acetone	N	N	N	N	N
pH	6.8	6.8	7.0	6.6	7.0
Urobilinogen	0.1	1.0	1.0	1.0	1.0
Bilirubin	N	N	N	N	N
Protein	N	Tr	Tr	Tr	Tr
Creatinine	108	144	164	158	178
MICROSCOPIC					
RBC's	0	0	0	Contaminated with Bacteria	0
WBC's	0	0	0-1		0
Epithelial	0	0-3	0-1		0
Bacteria	1+	1+	2+		2+
Cast	0	0	0		0



APPENDIX I (32)  
 CLINICAL LABORATORY DATA ON INDIVIDUAL DOGS  
 FED ZONYL® RP FOR THREE MONTHS

Dog No.: 1146  
 Sex: Female  
 Group: 2500 ppm

	Control		Months on Test		
			1	2	3
<u>Hematology</u>					
Erythrocytes	6.48	6.72	5.88	6.00	5.88
Hemoglobin	15.4	16.7	14.3	14.2	15.0
Hematocrit	40	44	38	36	39
Leucocytes	11.1	8.6	11.4	12.3	12.6
<u>DIFFERENTIAL</u>					
Neutrophils	67	64	61	65	62
Seg.	66	64	61	65	62
Juv.	1	0	0	0	0
Myel.	0	0	0	0	0
Lymphocytes	31	35	31	34	30
Eosinophils	0	1	5	0	6
Monocytes	2	0	3	1	2
Basophils	0	0	0	0	0
Atypicals	0	0	0	0	0
Nucleated RBC	0	0	0	0	0
<u>Biochemistry</u>					
Glucose	97	108	110	116	112
Urea Nitrogen	18	15	26	23	19
Cholesterol	155	175	260	270	230
Alk. Phos.	3.8	4.0	7.8	8.3	8.9
GPT	6	3	11	17	12
Total Protein	5.8	6.0	6.3	6.2	6.6
A/G	1.17	1.41	1.07	1.06	1.28
Creatinine	0.6	0.7	0.6	0.8	0.9
Bilirubin	0.1	0.1	0.2	0.1	0.1
Albumin	3.1	3.5	3.3	3.2	3.7
<u>Urinalysis</u>					
Volume	200	110	140	80	95
Appearance	Y, Cl, P	Y, Cl	Y, Cl, P	D, Y, Cl	D, A, Cl, P
Osmolality	1189	743	1247	1724	1942
Occult Blood	N	N	N	N	N
Sugar	N	N	N	N	Tr
Acetone	N	N	N	N	N
pH	7.2	7.0	7.0	7.2	8.0
Urobilinogen	0.1	0.1	0.1	1.0	1.0
Bilirubin	N	N	N	N	P
Protein	Tr	Tr	Tr	Tr	1+
Creatinine	108	136	96	158	198
<u>MICROSCOPIC</u>					
RBC'S	0	0	0	0	0
WBC's	0	0-2	0-1	0	0
Epithelial	0-1	0-1	0-1	0-2	0-1
Bacteria	2+	3+	4+	2+	2+
Cast	0	0	0	0	0

August 20, 1975

Subject: Fluorocarbons in Human  
Blood Plasma

CONFIDENTIAL

(Ju) TO: L. C. KROGH - COMMERCIAL CHEMICAL DIVISION - 223-6SE  
J. D. LAZERTE - COMMERCIAL CHEMICAL DIVISION - 236-1  
R. A. NEWMARK - CENTRAL RESEARCH - 201-2W  
J. A. PENDERGRASS - MEDICAL DEPARTMENT - 220-2E  
FROM: G. H. CRAWFORD - PHOTOGRAPHIC PRODUCTS - 209-1S

Record of a Telephone Conversation - August 14, 1975

Person calling - Dr. William Guy  
College of Medicine  
University of Florida  
Gainesville, Florida

Dr. Guy called again, following up on the subject (vide my earlier memo) to see if we had any further ideas as to possible sources of the fluorocarbon carboxylic acids found in human blood samples from Texas and New York. I got John Pendergrass on the line and Guy brought in a Dr. Tays (who apparently was involved in the original observation).

The original sampling involved plasma specimens from Albany, New York, Rochester, New York (low natural fluoride in the water) Hillsborough, Texas, Andrews, Texas, and Corpus Christi, Texas (high natural fluoride). There was no measurable difference by region ( $10^{-6}$  molar  $F^-$ ).  $F^{19}$  NMR studies run by Prof. Wallace Brey (Dept. of Chem., U. of F.) indicate that the fluorine is organic and the suspected species is fluorocarbon carboxylic acid with a  $C_6$  or  $C_7$  fluoroalkyl group. Dr. Brey suspects a branched end on the chain, e.g. perfluoro t-butyl.

The discussion involved Dr. Guy's speculative questions as to where such a "universal" presence of such compounds in human blood could come from. (The compounds are not present in laboratory animals.) These included:

1. Biosynthesis from inorganic  $F^-$ .
2. Biosynthesis from aerosol freons (but they don't find chlorine).

**Exhibit  
1118**

State of Minnesota v. 3M Co.,  
Court File No. 27-CV-10-28862

3. Teflon cookware.

4. "Scotchgard" fabrics.

Somewhere he got the information that 3M's fluorocarbon carboxylic acids are used as surfactants and wanted to know if they were present in "Scotchgard" or other items in general use by the public. We plead ignorance but advised him that "Scotchgard" was a polymeric material not a F.C. acid.

Apparently an earlier ('59-'60) study turned up similar quantities of F<sup>-</sup> in human plasma (not necessarily FC derived); this would presumably antedate the increased use of either "Scotchgard" or "Teflon" cookware.

They have done experiments involving water boiled in Teflon cookware with negative results.

We suggested obtaining plasma specimens from uncivilized areas, e.g. New Guinea where they don't use too much "Teflon" cookware or "Scotchgard".

Of all the unlikely explanations above, the least unlikely is residual FC 143 (or whatever) we sell to DuPont to polymerize TFE in Teflon cookware. This is still pretty far-fetched. This was not (I hasten to say) suggested to Dr. Guy.

We adopted a position of scientific curiosity and desire to assist in any way possible and suggested that our own analytical people might be able to clarify Dr. Brey's NMR findings (I know Wallace Brey from way back. He is highly respected, conservative and not given to frivolous speculations).

After we hung up I called CRL Analytical, talked to John McBrady and Richard Newmark. It turns out that Newmark is acquainted with Brey and has, in fact, published in a NMR journal edited by Brey.

My recommendation (with J.P.'s concurrence) is to get Richard in touch with Brey, obtain spectra for his own interpretation perhaps samples to run on our equipment, etc. in other words, keep scientists talking to scientists in the spirit of cooperative scientific inquiry.

On the positive side - if it is confirmed to our satisfaction that everybody is going around with fluorocarbon surfactants in their bloodstreams with no apparent ill-effects, are there some medical possibilities that would bear looking into? We

Telephone Conversation - Dr. William Guy  
August 20, 1975  
Page -3-

know that fluorocarbons are good oxygen carriers (but this is straight FC-75, not dissolved FC 143). Can fluorocarbon surfactants improve the hemodynamics, wetting and capillary permeation of blood in cases of atherosclerosis, kidney blockage, senility and the like? Can hemolysis, platelet destruction and other blood damage during hemodialysis and cardiovascular surgical procedures be reduced by fluorocarbon surfactants? This is speculation (but not completely wild). I would like to suggest that we consider some animal experiments to see just how much of these materials can, in fact, be tolerated in the bloodstream - both from a defensive point of view and for the above (to me) intriguing reasons. What do you think, John?



GHC/lr

MINNESOTA MINING & MFG. CO.

FIELD LETTER OF R. J. SEFFL - 236-2A

CITY Wilmington, Delaware

DATE 12/5/75

TO J. D. LAZERTE - 236-1

cc: L.S. Cove  
223-6SE  
L.J. Hals  
W. Caldwell  
L.C. Krogh  
223-6SE  
J.E. Long  
220-2E  
W.H. Petersen  
223-6SE  
W.F. Scown  
223-6SE

COMPANY: E. I. duPont  
PERSONNEL: duPont  
Henry Moncure, Jr., Research Manager,  
Fluorocarbon Div.  
Peter Phimmer, Research Supervisor, Fluorocarbon  
Homopolymers  
Henry Gilman, Toxicologist, Haskell Labs.  
Mike Kaplan, Toxicologist, Haskell Labs.  
Jim Terrill, Toxicologist, Haskell Labs.

3M  
L. J. Hals  
L. S. Cove  
J. E. Long  
R. J. Seffl

DuPont is concerned about the possible toxic effects of FC-143 in the Teflon K used in Harshaw "Dustless Process" because of its possible use in, or in contact with, food products.

Henry Moncure emphasized that duPont is not advocating the use of Teflon K in food applications and actually recommends against such use. They do not plan to file for an FDA clearance for Teflon K. However, they do recognize that they can not control all applications and they would like to have defensive information available to support their position in the event that a problem does arise.

Henry Gibson reported on toxicity studies carried out with FC-143 and Teflon K (containing 0.25% FC-143) some 10-15 years ago.

Acute oral toxicity: FC-143--~~AD~~ 670 mg/kg.  
Teflon K--"Innocuous"  
Sub acute toxicity: 10 day study on rats fed a diet of 25% Teflon K (25-30 g/kg).

Observations: Enlarged livers, up to three times the weight of the control depending upon which (of several tested) fluoro surfactant was used. This appeared to be a function of the surface activity of the surfactant. Others were worse than FC-143. With FC-143 the liver to body weight ratio was 4.8 vs the control of 3.5. In all cases the enlargement effect was reversible but did require "much time". The other sub acute effect

THIS MARGIN TO BE USED FOR OFFICE NOTES, ETC.

Form FL-10

**Exhibit**  
**1124**  
State of Minnesota v. 3M Co.,  
Court File No. 27-CV-10-28862

3MA10024380


1124.0001

Field Letter of R.J. Seffl  
E.I. DuPont - 12/5/75  
Page 2

noted was the reduction in sleeping times of rats treated with pentathol thus indicating possible rapid de-toxification of the drug by the test group. Further details of this study will be discussed on a one-to-one basis between H. Gilman and J. Long.

DuPont claims that unsintered Teflon dispersion which does not contain FC-143 does not produce the above <sup>stated</sup> sub acute effects. Therefore they desire defensive information and want to know to what extent 3M will be willing to provide them with information on FC-143 relative to possible inhalation toxicity--sub acute, and data with which to establish an oral "no effect level".

It was agreed that Jim Long and Henry <sup>Gilman</sup> would get back together regarding the details of their tests to date. Subsequently Jim will determine what tests he deems advisable and make his recommendations to the Commercial Chemicals Division who in turn will communicate with duPont as to what our program, if any, will be.

  
RJS:df

78 in Blood (Illum)

C. Rick Davis  
10/24/77  
T.O.

Interoffice Correspondence **3M**

Subject:

October 19, 1977

CONFIDENTIAL

RECEIVED

OCT 20 1977

I. J. SCHEUERMAN

TO: J. D. LAZERTE  
T. J. SCHEUERMAN  
F. A. UBEL

FROM: L. C. KROGH

In order to help you with your preparations for the presentation on November 7 to the Corporate Responsibility Committee, I am enclosing copies of the transparencies used in the last report.

I was in error yesterday, we last reported to the Corporate Responsibility Committee on November 8, 1977, not in February.

*LCK*  
LCK:jmb  
encl.



3MA10067215

Exhibit  
1145

State of Minnesota v. 3M Co.,  
Court File No. 27-CV-10-28862

Trial Exhibit 1202

3M\_MN00000479

1145.0001

## ORGANIC FLUORINE COMPOUNDS IN BLOOD

### CHRONOLOGY

- AUGUST 22, 1975 - DR. J. D. LA ZERTE RECEIVES CALL FROM W. S. GUY OF UNIVERSITY OF ROCHESTER.
- AUGUST 25, 1975 - W. S. GUY, D. R. TAVES, AND W. S. BREY, JR. PRESENT A PAPER AT CHICAGO ACS MEETING ENTITLED "CHARACTERISTICS AND CONCENTRATIONS OF ORGANIC FLUORO COMPOUNDS FOUND IN HUMAN TISSUES."
- SEPTEMBER 17-21, 1975 - CENTRAL RESEARCH ANALYTICAL TO COMPARE  $C_7F_{15}COOH$  AND  $C_8F_{17}SO_3H$  NMR SPECTRA WITH THAT REPORTED BY GUY ET AL.
- SEPTEMBER 22, 1975 - TAVES CALLS J. D. LA ZERTE TO DETERMINE IF 3M WILL FURTHER ANALYZE THEIR SAMPLE OF FLUROCHEMICAL. ALSO ASKS 3M TO OPEN CONTENTS OF FDA PETITION ON "SCOTCHBAN."
- OCTOBER, 1975 - CENTRAL RESEARCH ANALYTICAL AGREES TO DETERMINE QUANTITY AND CHARACTER OF ORGANIC FLUORINE COMPOUNDS IN HUMAN BLOOD.
- NOVEMBER 6, 1975 - CENTRAL RESEARCH REPORTS THAT  $C_8F_{17}SO_3H$  SPECTRA MATCHES THAT PRESENTED BY GUY, ET AL.
- DECEMBER 16, 1975 - LA ZERTE, FREIER, AND LONG OF 3M VISIT GUY AND TAVES AT THE UNIVERSITY OF ROCHESTER. 3M PROPOSES, AND GUY AND TAVES AGREE THAT 3M WILL ATTEMPT TO ISOLATE AND IDENTIFY ORGANIC FLUORINE COMPOUNDS IN HUMAN BLOOD.

3MA10067216

3M\_MN00000480



CHRONOLOGY - PAGE 2

- FEBRUARY 17, 1976 - CENTRAL RESEARCH ANALYTICAL DEVELOPS AN ACCURATE ANALYTICAL METHOD FOR DETERMINING PARTS PER BILLION QUANTITIES OF ORGANIC FLUORINE COMPOUNDS IN HUMAN BLOOD. METHOD TESTED ON BLOOD FROM AMERICAN RED CROSS AND VALUE AGREES WITH THOSE IN LITERATURE.
- APRIL 14, 1976 - FOUR LABORATORY PERSONNEL HAVE BLOOD SAMPLES ANALYZED. CONCENTRATION OF ORGANIC FLUORINE COMPOUNDS IN SOME PERSONNEL 100 TIMES NORMAL.
- JUNE 29, 1976 - SOME CHEMOLITE PERSONNEL SHOW ORGANIC FLUORINE COMPOUNDS AT 1,000 TIMES NORMAL.
- AUGUST 23, 1976 - CORDOVA PERSONNEL EXPOSED TO FLUORO-CHEMICALS HAVE UP TO 50 TIMES NORMAL VALUES.
- AUGUST 26, 1976 - CENTRAL RESEARCH ISOLATES AND IDENTIFIES ORGANIC FLUORINE COMPOUNDS FROM BLOOD OF CHEMOLITE PERSON AS  $C_{715}F_{15}COOH$ .
- SEPTEMBER 9, 1976 - MICE FED "SCOTCHBAN." HAD 4,000 TIMES NORMAL ORGANIC FLUORINE COMPOUNDS.
- SEPTEMBER 17, 1976 -  $C_{8173}F_{173}SO_3H$  IDENTIFIED AS ORGANIC FLUORINE COMPOUND IN MICE FED "SCOTCHBAN."
- OCTOBER 8, 1976 - DECATUR PLANT PERSONNEL FOUND TO HAVE UP TO 300 TIMES NORMAL LEVELS.
- INDIVIDUALS EXPOSED TO FLUORO-CHEMICALS OVER 20 YEARS AGO HAVE NORMAL ORGANIC FLUORINE COMPOUND LEVELS.

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3M\_MN00000481

CHRONOLOGY - PAGE 3

OCTOBER 28, 1976

- DR. LEON SINGER OF THE BIOCHEMISTRY DEPARTMENT, UNIVERSITY OF MINNESOTA, CALLS TO OBTAIN SAMPLES OF  $C_7F_{15}COOH$ .
- DR. SINGER REPORTS THAT HE HAS HAD CONVERSATIONS WITH TAVES. ALSO REPORTS THAT ANIMALS FED INORGANIC FLUORIDE SHOW INCREASES IN ORGANIC FLUORINE BLOOD LEVEL.

3MA10067218

3M\_MN00000482

1145.0004

ORGANIC FLUORINE COMPOUNDS IN BLOOD

STATUS AT 3M

1. NO EVIDENCE NOW OF RELATED HEALTH PROBLEMS.
2. 3M MEDICAL DEPARTMENT INITIATING PROGRAM TO STUDY BLOOD CHEMISTRY OF EXPOSED PERSONNEL.
3. NO EVIDENCE THAT THE PROBLEM EXISTS WITH 3M'S CUSTOMERS.
4. FUNDS ARE BUDGETED TO CONTINUE THE PROGRAM STUDY UNTIL WE ARE SATISFIED:
  - (A.) THAT THERE IS NO HEALTH HAZARD INCURRED BY 3M EMPLOYEES; AND,
  - (B.) THAT THE CONTINUED SALE OF OUR FLUORO-CHEMICALS FOR VARIOUS PURPOSES DOES NOT ENDANGER THE PUBLIC'S HEALTH.

3MA10067219

3M\_MN00000483

FLUORINE CONTENT OF BLOOD SERUM

	<u>ORGANIC FLUORINE P.P.B.</u>	<u>INORGANIC FLUORINE P.P.B.</u>
1. LITERATURE VALUES	2 - 130 AVE. 30	3 - 170 AVE. 20
2. 3M CONTROLS	10 - 80	40 - 60
3. LABORATORY BUILDING 236	430 - 3,100	30 - 360
4. CORDOVA	160 - 630	50 - 870
5. CHEMOLITE	510 - 38,800	50 - 90
6. DECATUR	130 - 9,840	40 - 210

3MA10067220

3M\_MN00000484

SOURCES OF FLUORINE

INORGANIC

$\text{Sn F}_2$  TOOTHPASTE, DENTAL CARE.

$\text{F}^-$  FLUORIDIZED WATER

ORGANIC

3M  $\text{CF}_3 \text{CF}_2 \text{CF}_2 \text{CF}_2 \text{CF}_2 \text{CF}_2 \text{CF}_2 \text{COOH}$   
 $\text{CF}_3 \text{CF}_2 \text{CF}_2 \text{CF}_2 \text{CF}_2 \text{CF}_2 \text{CF}_2 \text{CF}_2 \text{SO}_3 \text{H}$

OTHERS

STEROIDS  
TRANQUILIZERS  
ANESTHETICS  
HERBICIDES  
TEFLON  
AEROSOL PROPELLANTS  
REFRIGERANTS

3MA10067221

3M\_MN00000485

FC-95, FC-143 and FM-3422 - 90 Day Subacute Toxicity Studies

Conducted at IRDC - Review of Final Reports and Summary

OVERALL SUMMARY AND RECOMMENDATIONS

FC-95 was the most toxic of the three compounds studied and certainly more toxic than anticipated. It produced mortalities in rats at a dietary dose of 100 ppm ( $\approx$ 10 mg/kg/day) and in monkeys at an oral dose of 4.5 mg/kg/day. The primary target organs in rats were the liver, hematopoietic tissues and upper gastrointestinal tract and in monkeys, the gastrointestinal tract although no pathological lesions were reported. FC-143 appeared to be the least toxic of the three compounds studied and produced no mortalities in rats at dietary doses as high as 1000 ppm ( $\approx$  100 mg/kg/day). However, definite evidence of liver toxicity was seen at the high dose. In monkeys, FC-143 caused deaths at oral doses of 100 (4/4) and 30 (3/4) mg/kg/day and evidence of effects on hematopoietic tissue at these lethal doses. Like FC-95 and FM-3422, FC-143 also produced clinical evidence of gastrointestinal toxicity but no associated pathological lesions. FM-3422 caused deaths in rats at dietary doses of 1000, 3000 and 10,000 ppm ( $\approx$  100, 300 and 1000 mg/kg/day respectively) and in monkeys (1/4) at an oral dose of 30 mg/kg/day. The primary target organ in rats appeared to be the liver although there was some gross evidence of kidney and upper gastrointestinal tract involvement as well. In monkeys, the gastrointestinal tract was affected clinically, but there were no pathological lesions reported at necropsy.

The goals of conducting these 90 day subacute toxicity studies of 1) defining doses for chronic experiments and 2) obtaining general toxicological information on the three compounds appear to have been met. However, several questions surfaced that deserve further clarification. The apparent effect of FC-95 on the liver and hematopoietic system of rats should be studied for reversibility. The question of clinical gastrointestinal signs in monkeys with all three compounds without any gross or microscopic pathology is certainly perplexing, but may not be worth further pursuit since the oral route is not a likely one for man. If another study with FC-143 is conducted to help define the gastrointestinal and hematopoietic effects, the dog should be considered. Since the most likely route of exposure in plant workers is by inhalation, an inhalation study, probably with FM-3422, could be useful in evaluating any effects via pulmonary exposure. Marv Case and Bill McCormick are preparing protocols for follow-up to the toxicity questions mentioned.

Because of the apparent persistence of these fluorochemicals in the body, the most important question remains possible long term effects. Although lifetime rodent studies have limitations in predicting chronic effects (carcinogenesis) for man, they are still considered the most reliable indicators available. Unless there are adequate data through human epidemiological evaluations that can reasonably assure relative safety of these compounds following long term exposure, lifetime rodent studies should be undertaken as soon as possible. It may be possible to limit the number of compounds evaluated in lifetime rodent studies to one or two if metabolic data can be used to establish a common linkage between compounds.

**Exhibit  
1199**

State of Minnesota v. 3M Co.,  
Court File No. 27-CV-10-28862

INDIVIDUAL SUMMARIES

FC-95

Study No. 137-085 - 90 Day Subacute Rat Toxicity Study

Dietary levels of FC-95 were administered to five male and five female rats/level at 30, 100, 300, 1000 and 3000 ppm which approximates 3, 10, 30, 100 and 300 mg/kg/day respectively. All rats at the three highest doses and 5/10 at 100 ppm died during the study. Predominant signs observed included emaciation, convulsions, altered posture, ocular, oral and anal discharges, hyperreactivity and reduced motor activity. Mortalities occurred in a sequence related to dose, with earlier deaths seen at the highest level. There was compound and dose related evidence of reduced body weight gain and food consumption with actual weight loss at higher lethal doses. At 30 ppm only slight body weight effects were present. The most notable clinical pathology effects were observed at 100 ppm (values not obtained at higher levels) and consisted of enzyme level increases suggestive of possible liver toxicity and decreased erythrocytic values (principally hemoglobin and hematocrit with slight lowering of red cell counts) indicating an anemia. Pathologically, the most consistent and apparent compound related effect involved liver, hematopoietic tissues (thymus, bone marrow, spleen, mesenteric lymph nodes), gastrointestinal tract, muscle and skin.

In summary, FC-95 was relatively toxic to rats causing mortalities at dietary doses as low as 100 ppm ( $\approx$  10 mg/kg/day). Primary target organs appeared to be liver, hematopoietic tissues, stomach and small intestine with some indication of a compound related effect in muscle and skin.

Study No. 137-087 - 90 Day Subacute Rhesus Monkey Toxicity Study

FC-95 was administered by gastric intubation as an aqueous suspension to two male and two female rhesus monkeys/level at doses of 10, 30, 100 and 300 mg/kg/day for up to 20 days. Because of unexpected early mortalities in all monkeys at all levels (days 2-4 at 300, 3-5 at 100, 7-10 at 30 and 11-20 at 10 mg/kg/day), the study was inconclusive. Prominent signs observed consisted of anorexia, decreased activity, emesis with some diarrhea, body stiffening, general body trembling and twitching, weakness, convulsions and prostration. No clinical pathology work was done because of the short study duration. The only pathological lesions reported consisted of gross yellowish-brown liver coloration at 100 and 300 mg/kg/day but no histopathologic basis for this finding was observed.

In summary, FC-95 proved to be considerably more toxic to monkeys than anticipated resulting in early deaths preceded by gastrointestinal and central nervous system signs. Although far from definitive, this study suggested the gastrointestinal tract and possibly liver as target organs.

Study No. 137-092 - 90 Day Subacute Rhesus Monkey Toxicity Study (Second Study)

Since all monkeys died in the first FC-95 study (137-087), a second experiment was conducted using oral gavage doses of 0.5, 1.5 and 4.5 mg/kg/day administered to two male and two female monkeys/dose. The controls were the same monkeys used in the first FC-95 experiment. All 4.5 mg/kg monkeys exhibited signs of gastrointestinal tract toxicity (anorexia, emesis, black stools, dehydration) starting on day 1 or 2 of the study, and all died or were sacrificed in extremis between weeks 5-7. Prior to death, these monkeys showed marked or severe rigidity, convulsions, general body tremors, prostration and loss of body weight. The monkeys at lower doses all survived, but evidence of gastrointestinal toxicity was observed both at 1.5 and 0.5 mg/kg/day. The only consistent clinical pathology observation reported was decreased alkaline phosphatase values at all three doses. No gross pathological lesions considered compound related were observed and the only microscopic pathology of apparent compound relationship consisted of lipid depletion in the adrenals, atrophy of pancreatic exocrine cells and atrophy of the serous alveolar cells of the submandibular salivary glands in high dose monkeys. These latter effects may be due to general debilitation of the animals.

In summary, FC-95 was relatively toxic to rhesus monkeys producing deaths at doses as low as 4.5 mg/kg/day in 5-7 weeks. The apparent target organ was the upper gastrointestinal tract although no pathological lesions were reported even at the high dose.

FC-143

Study No. 137-089 - 90 Day Subacute Rat Toxicity Study

Dietary levels of FC-143 administered to five male and five female rats/level were 10, 30, 100, 300 and 1000 ppm which approximates 1, 3, 10, 30 and 100 mg/kg/day respectively. Clinically, the only effect observed was slightly decreased body weight gains at 300 and 1000 ppm. Clinical pathology abnormalities reported in high dose male rats only included slightly lowered erythrocyte counts, and elevated BUN and alkaline phosphatase values. There were several other variations from control groups in the clinical pathology parameters including fairly consistent lowering of calcium levels at all doses, but these were not considered abnormal based on the contract laboratory's comparison to background control data. Pathological abnormalities were confined to the liver and included gross enlargement and discoloration at 1000 ppm, increased organ weights at 1000 and 300 ppm and several microscopic changes at 1000 ppm.

In summary, FC-143 was well tolerated in rats at doses up to and including 300 ppm ( $\approx$  30 mg/kg/day). There was obvious liver toxicity at 1000 ppm ( $\approx$  100 mg/kg/day), but no mortalities occurred.



Study No. 137-090 - 90 Day Subacute Rhesus Monkey Toxicity Study

FC-143, suspended in 0.5% methocel, was administered by gastric intubation to two male and two female rhesus monkeys/dose at 3, 10, 30 and 100 mg/kg/day. All high dose monkeys died during weeks 2-5 and 3/4 30 mg/kg monkeys died during the last half of the study. All monkeys that died showed clinical evidence of gastrointestinal toxicity (anorexia, emesis, dark stools), but there were no associated pathological lesions found at necropsy. No mortalities occurred and only occasional signs of gastrointestinal effects were reported at the two lower doses except for one 10 mg/kg monkey that had signs of gastrointestinal toxicity for several days late in the study. There were a few abnormalities reported in clinical pathology parameters, but no consistent pattern was observed. Gross and microscopic pathological lesions were restricted to the two highest dose levels and consisted of lipid depletion in adrenals, hypocellularity of bone marrow and atrophy of lymphoid follicles of the spleen and lymph nodes.

In summary, FC-143 was less toxic than FC-95 in rhesus monkeys but, at lethal doses (100 and 30 mg/kg/day), evidence of effects on hematopoietic tissue was seen. Like FC-95, the gastrointestinal tract also appeared to be a target organ although this was not confirmed on histopathological examination.

FM-3422

Study No. 137-086 - 90 Day Subacute Rat Toxicity Study

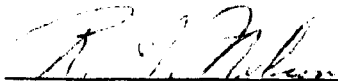
FM-3422 was administered in the diet to five male and five female rats/level at 30, 100, 300, 1000, 3000 and 10,000 ppm which corresponds to approximately 3, 10, 30, 100, 300 and 1000 mg/kg/day respectively. All rats at the 1000, 3000 and 10,000 ppm levels died between days 9 and 29. Prominent signs observed in these rats included emaciation, altered posture, convulsions, reduced motor activity and/or increased sensitivity. At 30 ppm, a slight decrease in body weight gain in females was the only clinical effect reported. There were also some slight abnormalities in serum enzyme levels, but no pronounced trends. Likewise, minimal effects were seen at 100 ppm. At 300 ppm there appeared to be increased compound related clinical signs, decreased body weight gain and food consumption, depressed hematological parameters and several alterations in clinical chemistry values. Pathologically, the liver was grossly enlarged with accentuated lobulation and discoloration with the 300 ppm group being more severely effected than the 1000 or 3000 ppm rats. This apparent reversed order of toxicity related to dose could be due to the early mortalities of the high dose rats and, therefore, a short dosing duration. The liver abnormalities seen grossly were associated with increased liver weights and microscopic lesions. Some kidney discoloration and evidence of stomach irritation were also observed grossly at 300 ppm.

In summary, FM-3422 was lethal at doses of 1000, 3000 and 10,000 ppm which is approximately 100, 300 and 1000 mg/kg/day respectively. The liver appeared to be the primary target organ, but there was gross pathological evidence of possible kidney and stomach involvement at the 300 ppm level also.

Study 137-088 - 90 Day Subacute Rhesus Monkey Toxicity Study

FM-3422, suspended in propylene glycol, was administered by gavage to two male and two female monkeys/level using doses of 1, 3, 10 or 30 mg/kg/day. The vehicle appeared to cause anorexia early in the study necessitating volume reduction from 5 to 2 ml/kg. The only mortality occurred in one high dose monkey the last week of dosing. Gastrointestinal signs consisting of emesis, diarrhea and black stools with mucus or bloody mucus were seen in most monkeys from most groups. There were no clinical pathology observations that appeared to be significant compound effects. Pathological lesions reported included lipid depletion of adrenals and atrophy of pancreatic exocrine glands at 30 mg/kg only.

In summary, FM-3422 caused mortality at 30 mg/kg in 1/4 monkeys and appeared to primarily effect the gastrointestinal tract although there was no supporting microscopic evidence.

  
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Date

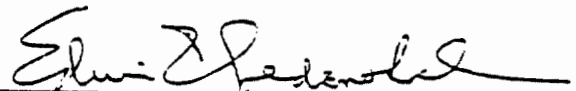
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*International Research and Development Corporation*

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SPONSOR: 3M Company  
COMPOUND: Fluorad® Fluorochemical FC-143  
SUBJECT: Ninety Day Subacute Rhesus Monkey Toxicity Study.



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I. SYNOPSIS

In a ninety day oral study in rhesus monkeys, Fluorad® Fluorochemical FC-143 was administered at dosage levels of 0 (control, treated only with 0.5% Methocel®), 3, 10, 30 and 100 mg/kg/day. Two male and two female monkeys were initiated at each dosage level and also in a control group. The monkeys were observed twice daily for general physical appearance and behavior and pharmacotoxic signs. Body weights were recorded weekly. Hematological, biochemical and urinalysis studies were conducted once in the control period, at the end of the first and third months of study.

The monkeys treated with the higher dose, (100 mg/kg/day) all died during weeks 2 through 5 of the study. At the 30 mg/kg/day dosage level, three monkeys died during weeks 7-12. They all showed signs of toxicity in the gastrointestinal tract (anorexia, emesis, sometimes brown in color, black stools), pale face and gums, swollen face and eyes, slight to severe decreased activity and prostration. The monkeys of the 30 and 100 mg/kg/day dosage level showed body weight losses from the first week of the study.

Because of the early deaths of the monkeys at the 100 mg/kg/day dosage level, the clinical laboratory tests were not conducted.

The monkeys at the 30 mg/kg/day dosage level showed, in the first month of the study, slight increase in prothrombin time and in activated partial thromboplastin time (A.P.T.T.) values, as well as decreased alkaline phosphatase activity in the serum (statistically significant). Only one monkey from this dosage level in this period showed a low albumin value. At the end of the study, the only remaining monkey from the 30 mg/kg/day dosage level showed apparent anemia, low blood glucose, alkaline phosphatase, total protein and albumin values.

There was no mortality at the 10 mg/kg/day dosage level. One monkey had black stool on several days in week 12 and occasionally

anorexia and one monkey exhibited pale face and gums. At this dosage level there was a very slight increase in the activated P.T.T. values in the female monkeys during the first month of the study (not statistically significant). There were no changes in the other indices and no changes in the body weight. In single monkeys from the 3 and 10 mg/kg/day dosage levels, there were trends toward decreased alkaline phosphatase in the serum.

In the control and the 3 mg/kg/day dosage level there was no mortality, no changes in the body weights and no signs of toxicity. Soft stool, diarrhea or emesis were observed occasionally.

The mortality and the above mentioned signs of toxicity in the 30 and 100 mg/kg/day dosage levels were compound-related. There was a trend toward the same signs of toxicity in single monkeys at the 10 mg/kg/day dosage level. The 3 mg/kg/day dosage level seems to be free of signs of toxicity. There is an evident relationship between the administered doses and the degree of the toxicity.

No gross or microscopic lesions which were considered compound-related were seen in tissues other than the adrenals, bone marrow, spleen and lymph nodes for male and female monkeys at the 30 and 100 mg/kg/day dosage levels. Microscopically, the adrenals from male and female monkeys at the 30 and 100 mg/kg/day dosage levels had compound-related marked diffuse lipid depletion; the bone marrow from male and female monkeys at the 30 and 100 mg/kg/day dosage levels had compound-related slight to moderate hypocellularity; the spleen and lymph nodes from male and female monkeys at the 30 and 100 mg/kg/day dosage levels had compound related moderate atrophy of lymphoid follicles.

Statistically significant variations in sex group mean weights of a few organs occurred between the control and experimental groups. These variations were of unknown biological significance and were not accompanied by morphologic alterations.

*International Research and Development Corporation*

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II. COMPOUND

The compound was received from 3M Company, Saint Paul, Minnesota on October 24, 1977 as shown below:

<u>Label</u>	<u>Description</u>
Fluorad® Fluorochemical FC-143 3M Stock No. 98-0211-0008-0 Lot 340	white powder

137-090

**G01727**

III. CLINICAL STUDIES

A. METHODS:

1. General Procedure:

Ten male rhesus monkeys (weighing from 2.60 to 3.90 kilograms) and 10 females (weighing from 2.95 to 3.80 kilograms) were initiated on this study. The monkeys were purchased from Primate Imports Corporation, Port Washington, N. Y. 11050. The monkeys were housed individually in hanging wire mesh, "squeeze type" cages and maintained in a temperature, humidity and light controlled environment. Purina® Monkey Chow® was fed twice each day and fresh apples were fed 3 times a week. Water was available ad libitum.

During the conditioning period, the monkeys were tattooed on the inner surface of the thigh and intrapalpebral tuberculin tests were conducted. Tuberculin tests were conducted at bimonthly intervals during the treatment period. Also a complete physical examination was conducted by the staff veterinarian prior to initiation of compound administration. Only monkeys in good health were selected for the study.

This study was initiated on January 11, 1978. Terminal sacrifices were conducted on April 12, 1978.

2. Compound Administration:

At the end of the conditioning period the monkeys were divided into five groups on a random basis, so that the initial average body weights were similar:

<u>Number of Monkeys</u>		<u>Dosage Level</u>
<u>Male</u>	<u>Female</u>	
2	2	Control
2	2	3 mg/kg/day
2	2	10 mg/kg/day
2	2	30 mg/kg/day
2	2	100 mg/kg/day



The test compound, suspended in 0.5% Methocel®, was administered by gavage, 7 days each week. All doses were given in a constant volume. Also the same volume of 0.5% Methocel® was given to the vehicle control group. Individual daily doses were based upon the body weights obtained weekly.

3. Observations:

The monkeys were observed twice daily for general physical appearance and behavior and pharmacotoxic signs. Individual body weights were recorded weekly. General physical examinations were conducted in the control period and monthly during the study.

4. Clinical Laboratory Tests:

Blood and urine samples were obtained for analysis from all monkeys once during the control period and at 1 and 3 months of study. The monkeys were fasted overnight prior to the collection of blood and urine samples.

a. Hematology:

Hematological studies included: hemoglobin<sup>1</sup>, hematocrit<sup>2</sup>, erythrocyte count<sup>3</sup>, total<sup>3</sup> and differential leucocyte counts, reticulocyte count<sup>4</sup>, platelet count<sup>5</sup>, prothrombin time<sup>6</sup>, activated partial thromboplastin time<sup>7</sup> (A.P.T.T.). Mean corpuscular hemoglobin, mean corpuscular volume and mean corpuscular hemoglobin concentration were calculated.

b. Biochemistry:

Biochemical studies included: fasting blood glucose<sup>8</sup>, blood urea nitrogen<sup>8</sup>, serum alkaline phosphatase<sup>8</sup>, serum glutamic oxalacetic and pyruvic transaminase activities<sup>8,9</sup>, cholesterol<sup>9</sup>, total protein<sup>9</sup>, albumin<sup>8</sup>, sodium<sup>10</sup>, potassium<sup>10</sup>, chloride<sup>9</sup>, inorganic phosphate<sup>9</sup>,  $\gamma$ -glutamyl transpeptidase<sup>11</sup> ( $\gamma$ -G.T.P.) and creatinine phosphokinase<sup>9</sup>.

c. Urinalysis:

Urinalysis included: measurement of volume, pH<sup>12</sup> and specific gravity; description of color and appearance; qualitative tests for protein<sup>12</sup>, glucose<sup>12</sup>, ketones<sup>12</sup>, occult blood<sup>12</sup> and microscopic examination of the sediment.

d. Statistical Analysis:

Analysis of body weights and clinical laboratory tests were performed. All statistical analyses compared the treatment groups with the control group, by sex. The tests were compared by analysis of variance (one-way classification) Bartlett's test for homogeneity and the appropriate t-test (for equal or unequal variances) as described by Steel and Torrie<sup>13</sup> using Dunnett's<sup>14</sup> multiple comparison tables to judge significance of differences.

B. RESULTS:

1. General Behavior, Appearance and Survival:

There was no mortality in monkeys at 0, 3 and 10 mg/kg/day dosage levels.

The monkeys from the control and 3 mg/kg/day dosage levels did not show any unusual behavior or signs of toxicity. Soft stool or moderate to marked diarrhea were noted occasionally. Frothy emesis was also noted occasionally.

At the 10 mg/kg/day dosage level the monkeys did not show any unusual signs of toxicity, except Monkey 7363. In week 7 its face appeared swollen and pale. It had been occasionally anorexic in week 4 and black stools appeared for several days in week 12 of the study.

At the 30 mg/kg/day dosage level, three monkeys died during weeks 7, 12 and 13 of the study. From week 4, the monkeys were anorexic. Slight to moderate and sometimes severe decreased activity was noted occasionally to frequently for the four monkeys. Emesis and ataxia were very rarely noted, for one monkey.

Swollen face, eyes and vulva, as well as pallor of the face and gums were noted. From week 6, for two monkeys, black stools were noted. Monkey 7387 showed slight to moderate dehydration and ptosis of the eyelids.

All monkeys from the 100 mg/kg/day dosage level died during weeks 2 through 5 of study. They showed the same symptoms of toxicity as the previous group, but they appeared sooner in the study (from week 1) and were more marked: anorexia, frothy emesis (sometimes brown in color) pale face and gums, swollen face and eyes, decreased activity from slight to severe, prostration and body trembling.

2. Body Weights (Tables 1-3):

Changes in body weight were similar for monkeys from the control and the 3 and 10 mg/kg/day dosage levels. Monkeys at the 30 and 100 mg/kg/day dosage levels lost body weight after the first week of study. There was statistically significant decreases in the body weight for the male monkeys at the 30 mg/kg/day dosage level in week 13 of the study. The female monkeys of the same dosage level and the monkeys from the 100 mg/kg/day dosage level were dead in this period.

3. Laboratory Test (Tables 4-15):

a. Hematology:

There were no noteworthy changes in monkeys from the 3 and 10 mg/kg/day dosage levels. In the first month of the study there was a slight increase (not statistically significant) of the A.P.T.T. values in the females at the 10 mg/kg/day dosage level and a statistically significant increase of the A.P.T.T. and prothrombin time values in monkeys at the 30 mg/kg/day dosage level. In the third month of the study there was a high increase in the above mentioned indices for the one surviving monkey from the 30 mg/kg/day dosage level. The same monkey (#7455) had pronounced anemia as well.

The statistically significant increase in the hematocrit in monkeys at the 10 mg/kg/day dosage level and in the platelet count in monkeys at the 3 mg/kg/day dosage level at 3 months of study, were within the normal physiological limits.

b. Biochemistry:

There were no noteworthy changes in monkeys from the control, 3 and 10 mg/kg/day dosage level. Only one monkey from the 3 mg/kg/day dosage level and one monkey from the 10 mg/kg/day dosage level showed trends toward decreases of alkaline phosphatase (432 and 474 units/l, respectively), without statistical significance.

In the first month of the study, decrease in serum alkaline phosphatase was noted in monkeys at the 30 mg/kg/day dosage level (statistically significant) and in one monkey in the same dosage level, the albumin in the serum was lower (3.22 g/100ml). The one surviving monkey (7455) from the 30 mg/kg/day dosage level showed decreasing of: blood sugar (66 mg/100ml), total protein (5.52 g/100ml) with albumin (2 g/100ml) and alkaline phosphatase (360 units/l) and slightly elevated cholesterol (240 mg/100ml).

c. Urinalysis:

No changes considered to be related to compound were seen in the urinalysis studies.

IV. PATHOLOGICAL STUDIES

A. METHODS:

1. Gross Pathology:

After completion of the compound administration period all surviving monkeys were anesthetized with Sernylan<sup>®</sup>\*, exsanguinated and necropsied. At necropsy, the heart, liver, adrenals, spleen, pituitary, kidneys, testes/ovaries and brain were weighed and representative tissues were collected in buffered neutral 10% formalin. Eyes were fixed in Russell's fixative. The thyroid/parathyroid was weighed after fixation.

Monkeys which died during the study were necropsied as above.

2. Histopathology:

Microscopic examination of formalin fixed hematoxylin and eosin stained paraffin sections was performed for all monkeys in the control and treatment groups. The following tissues were examined:

adrenals	kidneys	lumbar spinal cord
aorta	liver	pituitary
bone	lung	stomach
brain	skin	testes/ovaries
esophagus	mesenteric lymph node	thyroid
eyes	retropharyngeal lymph node	parathyroid
gallbladder	mammary gland	thymus
heart (with coronary vessels)	nerve (with muscle)	trachea
duodenum	spleen	tonsil
ileum	pancreas	tongue
jejunum	prostate/uterus	urinary bladder
cecum	rib junction (bone marrow)	vagina
colon	salivary gland	tattoo
rectum		

and any other tissue(s) with lesions

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\*Phencyclidine HCl - Bio-Ceutic Laboratories, Inc.,  
St. Joseph, Missouri.

B. RESULTS:

1. Gross Pathology (Table 16) and Organ Weights (Table 17):

No gross lesions considered compound related were seen in male and female rhesus monkeys which died on study or were sacrificed after 90 days of study.

Statistically significant variations in sex group mean weights of few organs occurred between the control and experimental groups. The following statistically significant organ weight variations occurred:

<u>Organ</u>	<u>Dosage Level</u> mg/kg/day	<u>S</u> e	<u>Weight</u>	<u>Change</u>	<u>P&lt;</u>
Heart	10	F	absolute,relative	decrease,decrease	0.05,0.01
Brain	10	F	absolute	decrease	0.01
Pituitary	3	M	relative	increase	0.05

The biological significance of these variations is unknown. These organ weight variations were not accompanied by morphologic changes which were considered compound related.

2. Histopathology (Table 18):

One male and two female rhesus monkeys at the 30 mg/kg/day dosage level and all male and female rhesus monkeys at the 100 mg/kg/day dosage level had marked diffuse lipid depletion in the adrenals. All male and female rhesus monkeys at the 30 and 100 mg/kg/day dosage levels had slight to moderate hypocellularity of the bone marrow. All male and female rhesus monkeys at the 30 and 100 mg/kg/day dosage levels had moderate atrophy of lymphoid follicles in the spleen. One female at the 30 mg/kg/day dosage level and all male and female rhesus monkeys at the 100 mg/kg/day dosage level had moderate atrophy of the lymphoid follicles in the lymph nodes.

No microscopic changes considered compound related were seen in the adrenals, bone marrow, spleen and lymph nodes of male and female rhesus monkeys at the 3 and 10 mg/kg/day dosage levels. No microscopic

lesions in tissues other than the adrenals, bone marrow, spleen and lymph nodes at the 30 and 100 mg/kg/day dosage levels were considered compound-related.

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Ninety Day Subacute Rhesus Monkey Toxicity Study.

TABLE 1.

Mean Body Weights of Monkeys Week 13 of Study.

<u>Sex</u>	<u>Group I</u> (Control)	<u>Group II</u> (3 mg/kg/day)	<u>Group III</u> (10 mg/kg/day)	<u>Group IV</u> (30 mg/kg/day)	<u>Group V</u> (100 mg/kg/day)
M	3.78	3.50	3.68	2.30*	dead
F	3.55	3.68	3.78	dead	dead

\*Statistical significance.

FC-143:

## Ninety Day Subacute Rhesus Monkey Toxicity Study.

TABLE 2.

## Individual Body Weights, Kilograms.

Group, Monkey Number	Sex	Control		Week of Study												
		1	2	1	2	3	4	5	6	7	8	9	10	11	12	13
<b>Control:</b>																
7362	M	3.15	3.30	3.15	3.30	3.35	3.10	3.20	3.20	3.00	3.15	3.20	3.05	3.20	3.40	3.50
7365	M	3.50	3.50	3.50	3.50	3.50	3.40	3.55	3.60	3.60	3.80	3.75	3.75	3.80	4.00	4.05
7336	F	3.05	3.20	3.25	3.25	3.35	3.15	3.00	3.15	3.20	3.30	3.45	3.30	3.35	3.35	3.60
7386	F	3.90	3.70	3.70	3.65	3.55	3.45	3.40	3.55	3.40	3.40	3.55	3.40	3.50	3.35	3.50
Mean		3.40	3.43	3.40	3.43	3.44	3.28	3.29	3.38	3.30	3.41	3.49	3.38	3.46	3.56	3.66
<b>3 mg/kg/day:</b>																
7364	M	3.70	3.90	3.85	3.95	3.85	3.85	3.80	3.80	3.85	4.10	4.10	4.05	4.05	4.20	4.30
7366	M	2.60	2.60	2.70	2.60	2.65	2.65	2.70	2.70	2.50	2.70	2.70	2.45	2.55	2.50	2.70
7384	F	3.55	3.60	3.70	3.80	3.80	3.80	3.70	3.70	3.60	3.55	3.80	3.55	3.70	3.90	3.75
7385	F	3.50	3.55	3.45	3.45	3.45	3.45	3.40	3.40	3.50	3.55	3.60	3.55	3.70	3.40	3.60
Mean		3.34	3.41	3.43	3.45	3.44	3.44	3.40	3.40	3.36	3.48	3.55	3.36	3.40	3.50	3.59
<b>10 mg/kg/day:</b>																
7363	M	3.55	3.70	3.70	3.65	3.65	3.65	3.65	3.60	3.60	3.70	3.65	3.75	3.85	3.90	3.90
7458	M	3.10	3.10	3.25	3.20	3.10	3.05	2.95	3.20	3.00	3.15	3.10	3.10	3.25	3.25	3.45
7328	F	3.30	3.30	3.45	3.40	3.40	3.30	3.20	3.30	3.25	3.45	3.60	3.50	3.40	3.60	3.75
7383	F	3.60	3.60	3.50	3.80	3.60	3.55	3.50	3.60	3.60	3.65	3.80	3.65	3.75	3.75	3.80
Mean		3.39	3.43	3.48	3.51	3.44	3.39	3.33	3.43	3.36	3.49	3.54	3.50	3.56	3.63	3.73

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TABLE 2. Cont.

Individual Body Weights, Kilograms.

Group, Monkey Number	Sex	Control		Week of Study												
		1	2	1	2	3	4	5	6	7	8	9	10	11	12	13
<u>30 mg/kg/day:</u>																
7367	M	3.40	3.40	3.25	3.25	3.10	2.95	2.65	2.30	2.10*	Died					
7455	M	3.50	3.30	3.20	3.05	2.85	2.65	2.45	2.50	2.55	2.60	2.70	2.70	2.65	2.50	2.30
7382	F	3.25	3.30	3.20	3.20	3.05	3.00	2.85	2.80	2.80	2.80	2.80	2.80	2.80	2.60	2.30
7387	F	3.70	3.75	3.50	3.55	3.50	3.45	3.10	2.95	2.85	2.80	2.80	2.80	2.80	2.60	2.25* Died
Mean		3.46	3.44	3.29	3.26	3.13	3.01	2.76	2.64	2.73	2.75	2.73	2.72	2.65	2.50	2.25* Died
<u>100 mg/kg/day:</u>																
7361	M	3.50	3.85	3.50	3.30	3.00	2.55	2.40*	Died							
7456	M	3.10	3.10	2.60	2.70*	Died										
7335	F	2.80	2.95	2.70	2.45	2.05*	Died									
7381	F	3.85	3.80	3.55	3.20	2.80	2.60*	Died								
Mean		3.31	3.43	3.09	2.98	2.90	2.55									

\*Terminal weight not included in mean.

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TABLE 3. T-Test Comparison of Body Weights.

Study Week	Sex	Control	3 mg/kg/day	10 mg/kg/day	30 mg/kg/day	100 mg/kg/day
13	M	3.78	3.50	3.68	2.30 <sup>a</sup>	-
	F	3.55	3.68	3.78	-	-

\*p<0.05

\*\*p<0.01

<sup>a</sup>Not included in statistical analysis due to only one surviving animal.

- Line indicates animals had died prior to week 13.

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## Ninety Day Subacute Rhesus Monkey Toxicity Study.

TABLE 4. Means and Significance of Hematological Values.

Hematology	Month of Study	Control	3 mg/kg/day	10 mg/kg/day	30 mg/kg/day
Erythrocytes, 10 <sup>6</sup> /cmm	1	4.46	4.26	4.71	4.53
	3	4.90	4.74	5.47	3.84 <sup>a</sup>
Hemoglobin, g/100 ml	1	11.7	11.4	12.1	11.7
	3	12.9	12.7	13.3	9.7 <sup>a</sup>
Hematocrit, %	1	38	37	39	36
	3	37	37	40**	30 <sup>a</sup>
Platelets, 10 <sup>3</sup> /cmm	1	253	233	210	219
	3	210	285*	216	261 <sup>a</sup>
Reticulocytes, %	1	0.2	0.5	0.5	0.2
	3	0.3	0.2	0.2	0.2 <sup>a</sup>
Prothrombin Time, sec	1	12	12	13	15**
	3	11	11	11	30 <sup>a</sup>
Activated P.T.T., sec	1	28	28	31	35**
	3	26	26	24	65 <sup>a</sup>
Leucocytes, 10 <sup>3</sup> /cmm	1	9.49	9.78	9.93	8.44
	3	9.40	9.83	11.96	10.14 <sup>a</sup>
Neutrophils, %	1	24	19	26	15
	3	16	19	25	36 <sup>a</sup>
Lymphocytes, %	1	75	76	72	85
	3	80	76	67	54 <sup>a</sup>
Eosinophils, %	1	1	5*	2	0
	3	3	3	6	3 <sup>a</sup>
Monocytes, %	1	0	0	0	0
	3	1	2	2	7 <sup>a</sup>
Basophils, %	1	0	0	0	0
	3	0	0	0	0 <sup>a</sup>
MCV, f <sup>3</sup>	1	86	86	82	80
	3	75	78	73	78 <sup>a</sup>
MCH, µg	1	27	27	26	26
	3	26	27	24	25 <sup>a</sup>
MCHC, g/100 ml	1	31	31	32	32*
	3	36	35	34	32 <sup>a</sup>

\*Significantly different from control group, p&lt;0.05.

\*\*Significantly different from control group, p&lt;0.01.

<sup>a</sup>Value not used in statistical analysis due to only one animal surviving.

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TABLE 5.

Individual Hematological Values - Control 1.

Group, Monkey Number	Sex	Erythrocytes 10 <sup>6</sup> /cmm	Hemoglobin g/100 ml	Hemato-crit %	Platelets 10 <sup>3</sup> /cmm	Reti-cu-locytes %	Prothrombin Time sec	Activated P.T.T. sec	Leuco-cytes 10 <sup>3</sup> /cmm	Neutrophils Seg. %	Non-Seg. %	Lympho-cytes <sup>a</sup> %	Eosino-philis <sup>a</sup> %	Mono-cytes <sup>a</sup> %	Baso-philis <sup>a</sup> %	MCV μ <sup>3</sup>	MCH OPG	MCHC g/100 ml
<b>Control:</b>																		
7362	M	5.08	13.0	40	207	0.1	13											
7365	M	4.72	11.9	38	219	0.3	13	29	10.96	36	1	62	1	0	0	79	26	33
7336	F	5.27	12.8	39	226	0.6	14	30	14.79	27	0	72	1	0	0	81	25	31
7386	F	4.20	11.1	34	227	0.5	14	29	7.86	38	0	59	3	0	0	74	24	33
Mean		4.82	12.2	38	245	0.4	14	21	12.09	59	0	39	1	1	0	81	26	33
<b>3 mg/kg/day:</b>																		
7364	M	4.50	11.5	37	155	0.4	13	27	11.43	40	0	58	2	0	0	79	25	31
7366	M	4.48	12.0	37	297	0.3	14	25	8.98	42	0	57	0	1	0	82	26	31
7384	F	4.55	11.7	38	160	0.2	13	29	7.39	41	0	59	0	0	0	83	27	32
7385	F	4.19	11.4	35	145	0.6	13	30	14.72	31	0	64	5	0	0	84	26	31
Mean		4.43	11.7	37	232	0.4	13	24	8.16	38	0	59	3	0	0	84	27	33
<b>10 mg/kg/day:</b>																		
7363	M	5.24	13.7	42	264	0.4	13	27	9.81	38	0	60	2	0	0	83	27	32
7458	M	5.29	12.2	36	263	0.2	13	31	12.97	46	0	49	5	0	0	80	26	33
7328	F	5.32	12.5	39	192	0.8	13	29	17.34	16*	0	78	6	0	0	68	23	34
7383	F	5.04	13.5	42	120	0.4	13	31	7.89	35	0	65	0	0	0	73	23	32
Mean		5.22	13.0	40	210	0.5	13	28	8.22	47	0	48	4	1	0	83	27	32
<b>30 mg/kg/day:</b>																		
7367	M	4.98	12.4	38	143	0.2	12	36	11.61	36	0	60	4	0	0	76	25	33
7455	M	5.16	13.6	40	133	0.5	12	28	10.84	41	0	57	2	0	0	76	25	33
7382	F	4.84	12.8	38	157	0.6	13	24	8.65	21	0	76	3	0	0	78	26	34
7387	F	4.67	12.2	35	113	0.6	14	26	5.83	26	0	73	1	0	0	79	26	34
Mean		4.91	12.8	38	137	0.5	13	27	5.10	29	0	68	1	2	0	75	26	35
<b>100 mg/kg/day:</b>																		
7361	M	4.75	12.4	36	282	0.3	12	26	7.61	29	0	68	2	1	0	77	26	34
7456	M	5.36	13.4	42	196	0.2	11	27	10.77	30	0	67	3	0	0	76	26	34
7335	F	5.46	12.8	40	185	0.2	14	28	5.84	38	0	60	0	1	1	78	25	32
7381	F	4.82	11.5	36	115	0.5	14	28	12.8	38	0	57	5	0	0	73	23	32
Mean		5.10	12.5	39	195	0.3	13	27	10.36	54	0	44	1	0	1	75	24	32
									9.58	40	0	57	2	0	1	76	25	31

\*Repeat determination

<sup>a</sup>The differential leucocyte means have been adjusted to equal 100%.

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TABLE 6.

Individual Hematological Values - 1 Month.

Group, Monkey Number	Sex	Erythrocytes 10 <sup>6</sup> /cmm	Hemoglobin g/100 ml	Hemato-crit %	Platelets 10 <sup>3</sup> /cmm	Reticu-locytes %	Prothrombin Time sec	Activated P.T.T. sec	Leuco-cytes 10 <sup>3</sup> /cmm	Neutrophils		Lympho-cytes <sup>a</sup> %	Eosino-philis <sup>a</sup> %	Mono-cytes <sup>a</sup> %	Baso-philis <sup>a</sup> %	MCV $\mu^3$	MCH $\mu\mu\text{g}$	MCHC g/100 ml
										Seg. %	Non-Seg. %							
Control:																		
7362	M	4.80	11.9	38	224	0.2	12	30	6.91	28	0	69	3	0	0	79	25	31
7365	M	4.71	11.9	39	349	0.2	12	28	14.58	15	0	84	1	0	0	83	25	31
7336	F	4.20	11.2	37	246	0.2	13	28	7.46	11	0	89	0	0	0	88	27	30
7386	F	4.13	11.9	38	191	0.3	12	27	8.99	42	0	58	0	0	0	92	29	31
Mean		4.46	11.7	38	253	0.2	12	28	9.49	24	0	75	1	0	0	86	27	31
2 mg/kg/day:																		
7364	M	4.35	11.6	37	264	0.5	11	27	6.81	17	0	80	3	0	0	85	27	31
7366	M	3.96	10.7	35	188	0.4	12	28	5.83	16	0	78	6	0	0	88	27	31
7384	F	4.46	11.9	39	234	0.2	13	28	17.07	22	1	73	3	1	0	87	27	31
7385	F	4.25	11.2	35	247	0.9	12	29	9.41	18	0	73	9	0	0	82	26	32
Mean		4.26	11.4	37	233	0.5	12	28	9.78	19	0	76	5	0	0	86	27	31
10 mg/kg/day:																		
7363	M	4.42	12.3	38	168	1.0	13	27	8.08	42	0	57	1	0	0	86	28	32
7458	M	4.81	11.3	37	281	0.3	13	31	17.98	11	0	87	1	0	1	77	23	31
7328	F	4.70	12.0	39	181	0.5	13	33	7.01	35	0	63	2	0	0	83	26	31
7383	F	4.92	12.8	40	209	0.1	12	33	6.64	18	0	79	3	0	0	81	26	32
Mean		4.71	12.1	39	210	0.5	13	31	9.93	26	0	72	2	0	0	82	26	32
30 mg/kg/day:																		
7367	M	4.59	11.2	36	135	0.1	13	34	7.92	12	0	88	0	0	0	78	24	31
7455	M	4.44	11.8	37	237	0.2	14	33	11.11	27	0	73	0	0	0	83	27	32
7382	F	4.51	11.9	35	268	0.3	15	35	6.19	9	0	90	1	0	0	78	26	34
7387	F	4.56	12.0	37	237	0.2	16	38	8.54	13	0	87	0	0	0	81	26	32
Mean		4.53	11.7	36	219	0.2	15	35	8.44	15	0	85	0	0	0	80	26	32
100 mg/kg/day:																		
7361	M	Died, week 5																
7456	M	Died, week 2																
7335	F	Died, week 3																
7381	F	Died, week 4																

<sup>a</sup>The differential leucocyte means have been adjusted to equal 100%.

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TABLE 7.

Individual Hematological Values - 3 Months.

Group, Monkey Number	Sex	Erythrocytes 10 <sup>6</sup> /cmm	Hemoglobin g/100 ml	Hematocrit %	Platelets 10 <sup>3</sup> /cmm	Reticulocytes %	Prothrombin Time sec	Activated P.T.T. sec	Leucocytes 10 <sup>3</sup> /cmm	Neutrophils		Lymphocytes <sup>c</sup> %	Eosinophils <sup>c</sup> %	Monocytes <sup>c</sup> %	Basophils <sup>c</sup> %	MCV μ <sup>3</sup>	MCH μg	MCHC g/100 ml
										Seg. %	Non-Seg. %							
<b>Control:</b>																		
7362	M	4.89	12.9	37	217	0.2	11	32	7.82	20	0	74	4	2	0	76	26	35
7365	M	5.29	13.1	37	218	0.3	10	25	12.84	10	0	85	4	1	0	70	25	35
7336	F	4.72	12.9	36	170	0.4	11	25	8.41	16	0	79	4	1	0	76	27	36
7386	F	4.69	12.8	36	234	0.3	11	20	8.51	18	1	80	0	1	0	77	27	36
Mean		4.90	12.9	37	210	0.3	11	26	9.40	16	0	80	3	1	0	75	26	36
<b>3 mg/kg/day:</b>																		
7364	M	4.86	12.9	37	299	0.1	11	24	7.33	24	0	71	4	1	0	76	27	35
7366	M	4.46	12.0	34	278	0.2	11	26	5.44	25	0	74	0	0	1	76	27	35
7384	F	4.92	13.0	39	313	0.2	11	28	18.21	16	0	76	5	3	0	79	26	33
7385	F	4.71	13.0	37	248	0.2	11	24	8.35	10	0	82	5	3	0	79	28	35
Mean		4.74	12.7	37	285	0.2	11	26	9.83	19	0	76	3	2	0	78	27	35
<b>10 mg/kg/day:</b>																		
7363	M	5.04	13.6	40	214	0.2	11	24	8.41	34	0	60	4	2	0	79	27	36
7458	M	5.70	12.6	40	218	0.3	11	23	20.18	4	0	94	2	0	0	70	22	32
7328	F	5.47	13.4	40	219	0.3	11	23	10.72	33	0	51	11	5	0	73	24	34
7383	F	5.65	13.5	39	212	0.1	11	27	8.52	30	0	64	5	1	0	69	24	35
Mean		5.47	13.3	40	216	0.2	11	24	11.96	25	0	67	6	2	0	73	24	34
<b>30 mg/kg/day:</b>																		
7367	M	Died, week 7																
7455	M	3.84 <sup>a,b</sup>	9.7	30	261	0.2	30	65	10.14	36	0	54	3	7	0	78	25	32
7382	F	Died, week 13																
7387	F	Died, week 12																
Mean		3.84	9.7	30	261	0.2	30	65	10.14	36	0	54	3	7	0	78	25	32
<b>100 mg/kg/day:</b>																		
7361	M	Died, week 5																
7456	M	Died, week 2																
7335	F	Died, week 3																
7381	F	Died, week 4																

<sup>a</sup>2+ Polkilocytosis

<sup>b</sup>2 Nucleated erythrocytes/100 leucocytes

<sup>c</sup>The differential leucocyte means have been adjusted to equal 100%.



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TABLE 8. Means and Significance of Biochemical Values.

Biochemistry	Month of Study	Control	3 mg/kg/day	10 mg/kg/day	30 mg/kg/day
Glucose, mg/100 ml	1	89	117*	104	122
	3	81	96	88	66 <sup>a</sup>
B.U.N., mg/100 ml	1	23.0	21.2	22.5	26.1
	3	27.6	20.2	22.0	22.6 <sup>a</sup>
Alk. Phos., int'l units/l	1	597	847	601	365*
	3	851	783	743	360 <sup>a</sup>
S.G.O.T., int'l units/l	1	29	35	34	59**
	3	45	41	35	88 <sup>a</sup>
S.G.P.T. int'l units/l	1 <sup>b</sup>	15	21	34*	44
	3 <sup>c</sup>	31	31	34	46 <sup>a</sup>
Cholesterol, mg/100 ml	1	165	154	158	174
	3	165	141	154	240 <sup>a</sup>
Total Protein, g/100 ml	1	7.94	8.23	8.66	8.36
	3	8.21	8.24	8.43	5.52 <sup>a</sup>
Albumin, g/100 ml	1	4.78	5.05	4.66	4.28
	3	4.82	5.12	5.17	2.00 <sup>a</sup>
Sodium, meq/liter	1	153	152	155	152
	3	151	154	159**	150 <sup>a</sup>
Potassium, meq/liter	1	5.1	5.1	5.2	5.7
	3	5.5	5.6	6.0	5.9 <sup>a</sup>
Chloride, meq/liter	1	112	110	113	112
	3	113	112	114	113 <sup>a</sup>
γ-G.T.P., Sigma units/ml	1	61	49	47	33
	3	44	38	51	49 <sup>a</sup>
C.P.K., Sigma units/ml	1	9	14	16	19*
	3	7	6	9	10 <sup>a</sup>
Inorganic Phosphate, mg/100 ml	1	7.9	7.2	7.0	6.7
	3	6.9	6.3	7.3	5.0 <sup>a</sup>

\*Significantly different from control group,  $p < 0.05$ .

\*\*Significantly different from control group,  $p < 0.01$ .

<sup>a</sup>Value not used in statistical analysis due to only one animal surviving.

<sup>b</sup>I.U./l

<sup>c</sup>Sigma units/ml

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TABLE 9.

## Individual Biochemical Values - Control 1.

Group, Monkey Number	Sex	Glucose mg/100 ml	B.U.N. mg/100 ml	Alk. Phos. int'l units/l	S.G.O.T. int'l units/l	S.G.P.T. int'l units/l	Cholesterol mg/100 ml	Total Protein g/100 ml	Albumin g/100 ml	Sodium meq/l	Potassium meq/l	Chloride meq/l	Inorganic Phosphate mg/100 ml	γ-G.T.P. Sigma u/ml	Creatinine Phosphokinase Sigma u/ml
<b>Control:</b>															
7362	M	94	41.0	780	40	99									
7365	M	82	16.7	659	61	88	219	8.68	5.40	160	5.0	111	6.5	67	15
7336	F	79	24.0	915	30	80	123	9.50	4.30	155	5.3	110	6.7	44	18
7386	F	85	21.0	960	39	86	185	9.52	5.30	156	4.3	110	6.5	41	85
Mean		85	25.7	829	43	88	190	8.52	5.12	162	5.0	111	6.5	37	16
<b>3 mg/kg/day:</b>															
7364	M	111	19.0	880	42	94									
7366	M	71	28.7	580	60	89	197	9.08	5.28	155	4.3	108	5.0	50	12
7384	F	96	22.0	570	38	106	172	9.12	5.80	157	4.9	108	7.1	30	26
7385	F	107	22.0	1320	60	76	133	10.12	5.19	162	6.0	113	6.1	32	16
Mean		96	22.9	838	50	91	154	8.72	4.80	158	5.2	116	5.4	41	29
<b>10 mg/kg/day:</b>															
7363	M	89	27.2	1167	46	118									
7458	M	180	24.2	806	63	136	237	9.84	5.10	167	6.2	117	6.7	78	16
7328	F	98	20.0	776	26	75	107	10.08	3.99	150	4.9	107	7.7	55	14
7383	F	98	27.3	581	31	91	189	8.48	5.14	157	4.4	109	6.3	51	34
Mean		116	24.7	833	42	105	168	8.32	5.25	159	5.1	112	6.0	59	64
<b>30 mg/kg/day:</b>															
7367	M	108	26.9	970	47	114									
7455	M	110	24.0	687	37	86	150	9.38	5.60	170	6.2	116	6.9	65	15
7382	F	132	27.9	641	40	79	205	9.50	5.31	163	5.3	111	6.6	59	9
7387	F	117	23.8	978	45	138	176	11.10	5.72	165	5.5	112	6.8	43	18
Mean		117	25.7	819	42	104	194	9.44	5.60	155	3.9	113	5.4	39	16
<b>100 mg/kg/day:</b>															
7361	M	93	29.0	598	43	80									
7456	M	100	23.0	799	40	104	155	8.60	5.00	159	5.9	116	6.9	64	17
7335	F	75	28.0	570	40	96	202	9.00	5.69	157	4.5	109	5.7	44	22
7381	F	119	22.1	1233	40	103	151	8.98	5.19	157	5.2	111	5.6	58	20
Mean		97	25.5	800	41	96	124	9.60	4.89	159	5.2	112	6.7	47	10
							158	9.05	5.19	158	5.2	112	6.2	53	17

## Ninety Day Subacute Rhesus Monkey Toxicity Study.

TABLE 10.

## Individual Biochemical Values - 1 Month.

Group, Monkey Number	Sex	Glucose mg/100 ml	B.U.N. mg/100 ml	Alk. Phos. Int'l units/l	S.G.O.T. int'l units/l	S.G.P.T. int'l units/l	Cholesterol mg/100 ml	Total Protein g/100 ml	Albumin g/100 ml	Sodium meq/l	Potassium meq/l	Chloride meq/l	Inorganic Phosphate mg/100 ml	Y-G.T.P. Sigma u/ml	Creatinine Phosphokinase Sigma u/ml
<b>Control:</b>															
7362	M	87	33.9	611	27	18	191	7.30	4.82	153	5.4	117	6.6	81	8
7365	M	84	14.2	626	33	17	121	8.40	4.11	153	5.4	111	8.4	50	11
7336	F	87	23.9	672	25	15	142	7.90	4.89	148	4.2	109	8.4	68	7
7386	F	96	14.9	480	31	10	206	8.15	5.30	158	5.4	112	8.1	44	11
Mean		89	23.0	597	29	15	165	7.94	4.78	153	5.1	112	7.9	61	9
<b>3 mg/kg/day:</b>															
7364	M	112	18.0	970	30	36	173	8.15	5.20	150	4.3	106	6.9	77	4
7366	M	131	23.3	693	39	19	148	8.05	5.42	154	4.9	110	6.6	26	7
7384	F	105	24.2	539	30	15	141	8.70	4.85	152	5.8	111	7.5	47	39
7385	F	120	19.1	1185	40	13	153	8.00	4.72	152	5.2	114	7.8	47	7
Mean		117	21.2	847	35	21	154	8.23	5.05	152	5.1	110	7.2	49	14
<b>10 mg/kg/day:</b>															
7363	M	98	24.9	552	40	35	219	9.40	4.62	161	6.3	118	6.9	65	7
7458	M	97	22.5	732	40	43	134	9.05	4.32	151	4.9	109	8.4	44	20
7328	F	98	22.7	640	23	19	145	8.20	4.50	152	4.3	111	5.4	37	24
7383	F	124	20.0	480	31	37	132	8.00	5.19	154	5.2	113	7.2	43	14
Mean		104	22.5	601	34	34	158	8.66	4.66	155	5.2	113	7.0	47	16
<b>30 mg/kg/day:</b>															
7367	M	112	35.2	376	48	30	180	8.20	4.70	157	6.0	110	6.6	40	25
7455	M	86	21.0	322	61	80	177	8.55	3.22	148	5.2	112	6.9	40	16
7382	F	104	25.2	400	83	43	161	8.15	4.21	149	5.9	111	6.0	28	17
7387	F	185	22.8	360	45	23	179	8.55	5.00	153	5.6	114	7.2	24	18
Mean		122	26.1	365	59	44	174	8.36	4.28	152	5.7	112	6.7	33	19
<b>100 mg/kg/day:</b>															
7361	M	Died, week 5													
7456	M	Died, week 2													
7335	F	Died, week 3													
7381	F	Died, week 4													

## Ninety Day Subacute Rhesus Monkey Toxicity Study.

TABLE 11.

## Individual Biochemical Values - 3 Months.

Group, Monkey Number	Sex	Glucose mg/100 ml	B.U.N. mg/100 ml	Alk. Phos. Int'l units/l	S.G.O.T. Int'l units/l	S.G.P.T. Sigma units/ml	Cholesterol mg/100 ml	Total Protein g/100 ml	Albumin g/100 ml	Sodium meq/l	Potassium meq/l	Chloride meq/l	Inorganic Phosphate mg/100 ml	γ-G.T.P. Sigma u/ml	Creatinine Phosphokinase Sigma u/ml
<b>Control:</b>															
7362	M	95	41.9	804	55	44	197	7.59	4.99	150	5.5	114	5.6	37	7
7365	M	77	17.4	744	47	30	135	9.18	4.40	151	6.1	113	8.0	53	8
7336	F	67	33.1	786	39	24	150	8.31	4.98	151	5.1	114	7.3	42	7
7386	F	86	18.1	1068	39	27	177	7.76	4.90	153	5.1	109	6.7	45	6
Mean		81	27.6	851	45	31	165	8.21	4.82	151	5.5	113	6.9	44	7
<b>3 mg/kg/day:</b>															
7364	M	106	17.1	1092	41	28	164	7.72	5.09	153	5.8	112	7.0	45	7
7366	M	111	18.1	594	39	33	126	8.09	5.52	153	5.5	109	5.3	51	6
7384	F	94	23.4	432	39	33	132	8.93	4.91	153	5.2	112	6.5	27	6
7385	F	74	22.0	1014	43	29	142	8.21	4.97	155	6.0	114	6.4	29	6
Mean		96	20.2	783	41	31	141	8.24	5.12	154	5.6	112	6.3	38	6
<b>10 mg/kg/day:</b>															
7363	M	87	24.9	936	42	42	194	8.44	5.61	164	7.0	119	8.0	43	7
7458	M	88	21.1	936	38	31	139	9.71	4.69	159	6.2	112	9.0	52	12
7328	F	75	21.8	624	30	25	155	7.93	5.27	156	4.8	110	5.6	60	7
7383	F	100	20.0	474	30	37	128	7.62	5.11	158	5.8	113	6.5	48	9
Mean		88	22.0	743	35	34	154	8.43	5.17	159	6.0	114	7.3	51	9
<b>30 mg/kg/day:</b>															
7367	M	Died, week 7													
7455	M	66	22.6	360	88	46	240	5.52	2.00	150	5.9	113	5.0	49	10
7382	F	Died, week 13													
7387	F	Died, week 12													
Mean		66	22.6	360	88	46	240	5.52	2.00	150	5.9	113	5.0	49	10
<b>100 mg/kg/day:</b>															
7361	M	Died, week 5													
7456	M	Died, week 2													
7335	F	Died, week 3													
7381	F	Died, week 4													

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## Ninety Day Subacute Rhesus Monkey Toxicity Study.

TABLE 12. Means and Significance of Urinalysis Values.

Urinalysis	Month of Study	Control	3 mg/kg/day	10 mg/kg/day	30 mg/kg/day
Volume, ml	1	35	33	51	41
	3	71	94	51	40 <sup>a</sup>
pH	1	8.5	8.5	8.1	8.1
	3	8.3	7.6	8.2	6.6 <sup>a</sup>
Specific Gravity	1	1.028	1.026	1.026	1.026 <sup>a</sup>
	3	1.018	1.015	1.024	1.031 <sup>a</sup>

<sup>a</sup>Value not used in statistical analysis due to only one animal surviving.

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Ninety Day Subacute Rhesus Monkey Toxicity Study.

TABLE 13.

Individual Urinalysis Values - Control 1.

Group, Monkey Number	Sex	Volume ml	Color and Appear.	pH	Spec. Grav.	Protein	Glucose	Occult Blood	Ketones	Leuco-cytes	Erythro-cytes	Epi. Cells	Urates	Triple Phos.	Cal. Oxal.	Uric Acid Crystals	Bacteria	Casts
<b>Control:</b>																		
7362	M	100	LS-cl	7.6	1.010	N	N	tr	N	-	-	-	-	-	-	-	-	-
7365	M	28	LS-cl	7.2	1.037	N	N	N	N	-	occ	occ	F	occ	-	-	M	-
7336	F	27	LS-C	7.0	1.036	N	N	N	N	-	1-3	occ	F	occ	-	-	N	-
7386	F	70	LS-cl	8.4	1.023	N	N	N	1+	-	-	-	occ	occ	occ	-	F	-
Mean		56		7.6	1.027			4+	N	-	-	occ	occ	occ	M	-	N	-
<b>3 mg/kg/day:</b>																		
7364	M	25	LS-cl	7.8	1.032	N	N	tr	N	-	-	-	-	-	-	-	-	-
7366	M	25	LS-cl	7.2	1.035	N	N	tr	N	-	-	occ	F	F	F	-	-	-
7384	F	215	LS-C	8.3	1.026	N	N	N	N	-	-	occ	F	occ	occ	-	M	-
7385	F	35	LS-cl	8.3	1.020	N	N	N	N	-	-	occ	occ	occ	-	-	N	-
Mean		75		7.9	1.028					-	-	occ	F	occ	-	-	N	-
<b>10 mg/kg/day:</b>																		
7363	M	20	LS-cl	7.7	1.020	N	N	tr	N	-	-	-	-	-	-	-	-	-
7458	M	50	LS-cl	7.5	1.036	N	N	tr	N	-	-	occ	F	F	-	-	-	-
7378	F	35	LS-cl	7.8	1.036	N	N	tr	N	-	-	occ	F	occ	F	-	M	-
7383	F	35	LS-cl	8.2	1.020	N	N	tr	N	-	-	1-3	F	occ	M	-	N	-
Mean		35		7.8	1.028			3+	N	-	-	occ	occ	occ	-	-	F	-
<b>30 mg/kg/day:</b>																		
7367	M	20	LS-cl	7.1	1.050	N	N	tr	N	-	-	-	-	-	-	-	-	-
7455	M	35	LS-cl	6.8	1.030	N	N	tr	N	-	1-3	1-3	occ	occ	occ	-	-	-
7382	F	20	LS-cl	7.0	1.055	N	N	tr	N	-	1-3	1-3	occ	F	-	-	M	-
7387	F	48	LS-cl	8.2	1.030	N	N	N	N	-	-	1-3	F	occ	-	-	M	-
Mean		31		7.3	1.041					-	-	occ	F	occ	occ	-	F	-
<b>100 mg/kg/day:</b>																		
7361	M	21	LS-cl	7.6	1.035	N	N	tr	N	-	-	-	-	-	-	-	-	-
7456	M	25	LS-cl	7.1	1.042	N	N	tr	N	-	occ	-	F	occ	-	-	-	-
7335	F	25	LS-cl	7.2	1.041	N	N	tr	3+	-	-	occ	F	occ	F	-	M	-
7381	F	40	LS-cl	8.1	1.042	N	N	tr	1+	-	1-3	-	occ	occ	F	-	M	-
Mean		28		7.5	1.040			1+	1+	-	-	1-3	occ	occ	M	-	F	-

Code: tr - Trace  
 1+ - Trace to slight  
 2+ - Slight to moderate  
 3+ - Moderate  
 4+ - Marked

S - Straw  
 LS - Light Straw  
 DS - Dark Straw  
 LAm - Light Amber  
 DAm - Dark Amber  
 cl - Cloudy  
 C - Clear

N - Negative  
 F - Few  
 L - Loaded  
 M - Many  
 R - Rare  
 occ - Occasional

QNS - Quantity not sufficient  
 norm - Normal  
 - None seen

Ninety Day Subacute Rhesus Monkey Toxicity Study.

TABLE 14.

Individual Urinalysis Values - 1 Month.

Group, Monkey Number	Sex	Volume ml	Color and Appear.	pH	Spec. Grav.	Protein	Glucose	Occult Blood	Ketones	Leuco-cytes	Erythro-cytes	Epi. Cells	Urates	Triple Phos.	Cal. Oxal.	Uric Acid Crystals	Bacteria	Casts
<u>Control:</u>																		
7362	M	55	LS-C	8.5	1.021	N												
7365	M	35	LS-C	8.5	1.028	N	N	N	N	-	occ	-	occ	occ	M	-	M	-
7336	F	20	LS-C	8.5	1.033	N	N						occ	F	occ	-	M	-
7386	F	30	LS-C	8.5	1.030	N	N	3+	N	-	-	1-3	F	F	F	-	M	-
Mean		35		8.5	1.028			tr	N	-	-	occ	M	F	M	-	M	-
<u>3 mg/kg/day:</u>																		
7364	M	20	LS-C	8.8	1.019	N	N	N	N	-	-	occ	F	M	occ	-	M	-
7366	M	20	LS-C	8.5	1.036	N	N	N	N	-	-	occ	F	F	F	-	M	-
7384	F	40	DS-cl	8.0	1.021	1+	N	4+	2+	-	8-12	-	F	occ	F	-	M	-
7385	F	50	LS-cl	8.5	1.027	N	N	N	N	-	-	occ	F	occ	F	-	M	-
Mean		33		8.5	1.026					-	-	occ	F	occ	M	-	M	-
<u>10 mg/kg/day:</u>																		
7363	M	65	LS-cl	7.5	1.023	N	N	N	N	-	occ	-	F	occ	M	-	M	-
7458	M	35	LS-C	8.0	1.028	N	N	N	N	-	-	-	occ	occ	M	-	M	-
7328	F	55	LS-cl	8.5	1.026	N	N	N	N	-	-	-	occ	occ	M	-	M	-
7383	F	50	LS-cl	8.5	1.028	N	N	N	N	-	-	1-3	occ	occ	M	-	M	-
Mean		51		8.1	1.026			tr	N	-	occ	occ	F	occ	M	-	M	-
<u>30 mg/kg/day:</u>																		
7367	M	30	LS-C	7.5	1.024	N	N	N	N	-	-	occ	occ	occ	-	-		-
7455	M	30	LS-cl	8.0	1.026	N	N	N	N	-	-	occ	occ	M	F	-		-
7382	F	60	LS-cl	8.3	1.022	N	N	N	N	-	occ	occ	M	F	-	-	L	-
7387	F	45	LS-cl	8.5	1.032	N	N	N	N	-	occ	-	F	F	-	-	M	-
Mean		41		8.1	1.026					-	-	occ	F	occ	occ	-	M	-
<u>100 mg/kg/day:</u>																		
7361	M	Died, week 5																
7456	M	Died, week 2																
7335	F	Died, week 3																
7381	F	Died, week 4																

Code:

tr - Trace  
 1+ - Trace to slight  
 2+ - Slight to moderate  
 3+ - Moderate  
 4+ - Marked

S - Straw  
 LS - Light Straw  
 DS - Dark Straw  
 LAm - Light Amber  
 DAm - Dark Amber  
 cl - Cloudy  
 C - Clear

N - Negative  
 F - Few  
 L - Loaded  
 M - Many  
 R - Rare  
 occ - Occasional

QNS - Quantity not sufficient  
 norm - Normal  
 - None seen

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Ninety Day Subacute Rheus Monkey Toxicity Study.

TABLE 15.

Individual Urinalysis Values - 3 Months.

Group, Monkey Number	Sex	Volume ml	Color and Appear.	pH	Spec. Grav.	Protein	Glucose	Occult Blood	Ketones	Leucocytes	Erythrocytes	Epi. Cells	Urates	Triple Phos.	Cal. Oxal.	Uric Acid Crystals	Bacteria	Casts
<b>Control:</b>																		
7362	M	110	LS-C	8.2	1.012	N	N	N	N	-	-	occ	F	occ	-	-	M	-
7365	M	40	LS-cl	8.1	1.029	N	N	N	1+	-	-	occ	F	occ	-	-	M	-
7336	F	85	LS-C	8.2	1.015	N	N	N	tr	-	-	occ	F	occ	F	-	M	-
7386	F	50	LS-C	8.8	1.016	N	N	N	tr	-	-	occ	F	occ	F	-	M	-
Mean		71		8.3	1.018			3+	N	occ	-	occ	F	F	F	-	M	-
<b>3 mg/kg/day:</b>																		
7364	M	50	LS-C	6.0	1.020	N	N	N	tr	-	-	-	F	occ	-	-	M	-
7366	M	150	LS-C	7.9	1.007	N	N	N	N	-	-	-	F	occ	-	-	M	-
7384	F	125	LS-C	8.1	1.010	N	N	N	N	-	-	occ	F	occ	-	-	M	-
7385	F	50	LS-C	8.5	1.021	N	N	N	N	-	-	occ	F	F	F	-	M	-
Mean		94		7.6	1.015			tr	N	-	occ	1-3	M	F	M	-	M	-
<b>10 mg/kg/day:</b>																		
7363	M	40	LS-C	8.0	1.027	N	N	N	N	-	-	occ	F	occ	occ	-	M	-
7458	M	35	LS-cl	8.7	1.022	N	N	N	N	-	-	-	F	occ	-	-	M	-
7328	F	50	LS-C	9.0	1.029	N	N	N	N	-	-	occ	F	occ	-	-	M	-
7383	F	80	LS-cl	7.0	1.019	N	N	N	N	-	occ	occ	F	occ	-	-	M	-
Mean		51		8.2	1.024			N	N	-	occ	occ	F	-	-	-	M	-
<b>30 mg/kg/day:</b>																		
7367	M	Died, week 7																
7455	M	40	S-C	6.6	1.031	N	N	1+	N	1-3	occ	-	F	M	occ	-	M	-
7382	F	Died, week 13																
7387	F	Died, week 12																
Mean		40		6.6	1.031													
<b>100 mg/kg/day:</b>																		
7361	M	Died, week 5																
7456	M	Died, week 2																
7335	F	Died, week 3																
7381	F	Died, week 4																

Code:

tr - Trace  
 1+ - Trace to slight  
 2+ - Slight to moderate  
 3+ - Moderate  
 4+ - Marked

S - Straw  
 LS - Light Straw  
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 cl - Cloudy  
 C - Cloud

N - Negative  
 F - Few  
 L - Loaded  
 M - Many  
 R - Rare  
 occ - Occasional

QNS - Quantity not sufficient  
 norm - Normal  
 - None seen



FC-143: Ninety Day Subacute Rhesus Monkey Toxicity Study.

TABLE 16. Summary of Gross Necropsy Observations, Terminal Sacrifice.

Site Lesion	0 mg/kg/day				3 mg/kg/day				10 mg/kg/day				30 mg/kg/day				100 mg/kg/day			
	M	M	F	F	M	M	F	F	M	M	F	F	M*	M	F*	F*	M*	M*	F*	F*
No Gross Lesions																				
<b>External</b>																				
swelling, eye area																				
alopecia																				
dehydrated																				
emaciated																				
red vaginal discharge																				
scab, facial area																				
<b>Lung</b>																				
mite lesion																				
adhesions	x	x																		
dark red foci/reddish purple area		x																		
yellow, white foci																				
nodules																				
<b>Heart</b>																				
hemorrhage, subendocardial																				
gelatinized fat, endocardial																				
atrophy																				
<b>Lymph Nodes</b>																				
enlarged																				
reddish black in color																				
<b>Thymus</b>																				
atrophy																				
<b>Abdominal Cavity</b>																				
depletion of fat																				
<b>Stomach</b>																				
dark red foci																				
erosion, mucosa, pyloric portion																				
mucosal hyperemia																				
yellowish gelatinous material, fundic portion																				
hemorrhage, fundic mucosa																				
ulcers																				
<b>Small Intestine</b>																				
greenish-gray mucoid material																				
dark red/brown mucoid material																				
liquid, blood tinged fluid																				
reddish brown in color																				
congestion, mucosa																				
hemorrhage, mucosa																				
<b>Large Intestine</b>																				
congestion, mucosa																				
hemorrhage, mucosa																				
dark reddish black foci																				
semi solid, blood stained contents																				

\*Died on Study

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Ninety Day Subacute Rhesus Monkey Toxicity Study.

TABLE 16. Cont.

Summary of Gross Necropsy Observations.

Site Lesion	Group, Monkey Number	0 mg/kg/day				3 mg/kg/day				10 mg/kg/day				30 mg/kg/day				100 mg/kg/day				
		M	M	F	F	M	M	F	F	M	M	F	F	M*	M*	F*	F*	M*	M*	F*	F*	
Pancreas		7362	7365	7336	7386	7364	7366	7384	7385	7363	7458	7328	7383	7167	7455	7382	7387	7361	7456	7335	7381	
accessory spleen									*													
Liver																						
cyst																						
brownish color											x											
accentuated lobulations														x								
granular surface														x						x		
yellowish mottling														x								
reddish yellow color															x							x
Kidneys																						
brownish discoloration														x								
Skin																						
subcutaneous edema, abdomen																						
subcutaneous hemorrhage, abdomen																x						x

\*Died on Study

Ninety Day Subacute Rhesus Monkey Toxicity Study.

TABLE 17.

Absolute (Grams) and Relative (% Body Weight) Organ Weights, Terminal Sacrifice and Deaths.

Group, Monkey Number	Sex	Body Wt. kg	Spleen		Liver		Adrenals		Kidneys		Testes/Ovaries	
			g	%	g	%	g	%x10	g	%	g	%x10 <sup>2</sup>
<u>Terminal Sacrifice:</u>												
<u>Control:</u>												
7362	M	3.25	2.35	0.07	70.73	2.18	0.65	0.20				
7365	M	3.85	7.87	0.20	79.15	2.06	0.71	0.18	11.82	0.36	0.85	0.03
Mean		3.55	5.11	0.14	74.94	2.12	0.68	0.19	17.06	0.44	1.23	0.08
7336	F	3.40	5.03	0.15	84.79	2.49	-	-	14.44	0.40	2.04	0.06
7386	F	3.50	3.87	0.11	77.77	2.22	-	-	13.80	0.41	0.28	0.82
Mean		3.45	4.45	0.13	81.28	2.36	0.62 <sup>a</sup>	0.18 <sup>a</sup>	19.58	0.56	0.27	0.77
<u>3 mg/kg/day:</u>												
7364	M	4.10	4.67	0.11	91.40	2.23	0.77	0.19				
7366	M	2.65	1.87	0.07	63.17	2.38	0.82	0.31	19.76	0.48	3.66	0.09
Mean		3.38	3.27	0.09	77.29	2.31	0.80	0.25	12.40	0.47	0.85	0.03
7384	F	3.70	6.82	0.18	102.64	2.77	0.78	0.21	16.08	0.47	2.26	0.06
7385	F	3.45	2.94	0.09	67.25	1.95	0.55	0.16	17.60	0.48	0.18	0.49
Mean		3.58	4.88	0.13	84.95	2.36	0.67	0.19	14.44	0.42	0.16	0.46
<u>10 mg/kg/day:</u>												
7363	M	3.80	2.39	0.06	87.25	2.30	0.74	0.19				
7458	M	3.25	4.91	0.15	82.30	2.53	0.67	0.21	16.84	0.44	1.75	0.05
Mean		3.53	3.65	0.11	84.78	2.41	0.71	0.20	16.54	0.51	1.99	0.06
7328	F	3.55	4.06	0.11	83.00	2.34	0.66	0.19	16.69	0.48	1.87	0.05
7383	F	3.70	3.99	0.11	85.35	2.31	0.86	0.23	15.32	0.43	0.29	0.82
Mean		3.63	4.03	0.11	84.18	2.32	0.76	0.21	13.56	0.37	0.39	1.05
<u>30 mg/kg/day<sup>a</sup>:</u>												
7455	M	2.40	3.50	0.15	70.76	2.95	0.84	0.35	14.44	0.40	0.34	0.94
<u>Deaths:</u>												
<u>30 mg/kg/day:</u>												
7367	M	2.10	1.45	0.07	75.33	3.59	1.63	0.78				
7382	F	2.25	3.01	0.13	112.87	5.02	1.74	0.77	16.34	0.78	1.94	0.09
7387	F	2.25	1.97	0.09	85.17	3.79	1.20	0.53	19.03	0.85	0.21	0.93
<u>100 mg/kg/day:</u>												
7361	M	2.40	1.65	0.07	79.02	3.29	1.59	0.66	15.96	0.71	0.32	1.42
7456	M	2.70	1.76	0.07	85.08	3.15	1.45	0.54				
7335	F	2.05	2.49	0.12	74.28	3.62	1.03	0.50	21.88	0.91	1.37	0.06
7381	F	2.60	3.05	0.12	82.58	3.18	1.16	0.45	14.77	0.55	0.71	0.03
									15.40	0.75	0.10	0.51
									18.28	0.70	0.13	0.50

Group mean relative organ weights shown in this table were calculated by averaging the individually calculated relative organ weights.  
<sup>a</sup>Significantly different from Control group mean, p<0.05.  
<sup>\*\*</sup>Significantly different from Control group mean, p<0.01.  
<sup>a</sup>Not included in analysis.  
<sup>a</sup>g not available

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Ninety Day Subacute Rhesus Monkey Toxicity Study.

TABLE 17. Cont.

Absolute (Grams) and Relative (% Body Weight) Organ Weights, Terminal Sacrifice and Deaths.

Group, Monkey Number	Sex	Body Wt. kg	Heart		Thyroid/Parathyroid		Brain		Pituitary	
			g	%	g	%x10	g	%	g	%x10 <sup>2</sup>
<u>Terminal Sacrifice:</u>										
<u>Control:</u>										
7362										
7365	M	3.25	11.69	0.36	1.050	0.32				
Mean		3.85	18.17	0.47	0.296	0.08	87.04	2.68	0.053	0.16
		3.55	14.93	0.42	0.673	0.20	90.39	2.35	0.063	0.16
7376	F	3.40	15.30	0.45	-	-	88.72	2.51	0.058	0.16
7386	F	3.50	14.75	0.42	0.839	0.24	82.64	2.43	0.050	0.15
Mean		3.45	15.03	0.44	0.839 <sup>a</sup>	0.24 <sup>a</sup>	81.55	2.33	0.073	0.21
<u>3 mg/kg/day:</u>										
7364										
7366	M	4.10	18.90	0.46	0.893	0.22	96.01	2.36	0.080	0.20
Mean		2.65	12.70	0.48	0.378	0.14	83.50	3.15	0.051	0.19
		3.38	15.80	0.47	0.636	0.18	89.76	2.75	0.066	0.19*
7384	F	3.70	16.87	0.46	0.694	0.19	78.66	2.13	0.086	0.23
7385	F	3.45	15.19	0.44	0.543	0.16	80.21	2.32	0.053	0.15
Mean		3.58	16.03	0.45	0.619	0.17	79.44	2.23	0.070	0.19
<u>10 mg/kg/day:</u>										
7363										
7458	M	3.80	15.10	0.40	1.211	0.32	77.73	2.05	0.063	0.17
Mean		3.25	14.14	0.44	0.488	0.15	83.38	2.57	0.047	0.14
		3.53	14.62	0.42	0.850	0.23	80.56	2.31	0.055	0.16
7328	F	3.55	11.85	0.33	0.461	0.13	77.19	2.17	-	-
7381	F	3.70	11.69	0.32	0.537	0.15	75.88	2.05	0.071	0.19
Mean		3.63	11.77*	0.32**	0.499	0.14	76.54**	2.11	0.071 <sup>a</sup>	0.19 <sup>a</sup>
<u>30 mg/kg/day<sup>a</sup>:</u>										
7455	M	2.40	10.50	0.44	0.292	0.12	75.01	3.13	0.049	0.20
<u>Deaths:</u>										
<u>30 mg/kg/day:</u>										
7367										
7382	M	2.10	10.39	0.49	0.532	0.25	82.27	3.92	0.068	0.32
7387	F	2.25	11.93	0.53	0.543	0.24	83.22	3.70	0.070	0.31
Mean		2.25	10.21	0.45	0.845	0.38	91.45	4.06	0.057	0.25
<u>100 mg/kg/day:</u>										
7361										
7456	M	2.40	14.54	0.61	0.791	0.33	92.43	3.85	0.072	0.30
7335	M	2.70	15.55	0.58	0.718	0.27	95.42	3.53	0.046	0.17
7381	F	2.05	11.44	0.56	0.479	0.23	74.28	3.62	0.056	0.27
	F	2.60	12.95	0.50	0.417	0.16	86.20	3.32	0.082	0.32

Group mean relative organ weights shown in this table were calculated by averaging the individually calculated relative organ weights.  
 \*Significantly different from Control group mean, p 0.05.  
 \*\*Significantly different from Control group mean, p<0.01.  
<sup>a</sup>Not included in analysis.  
 - = Not available

FC-143:

Ninety Day Subacute Rhesus Monkey Toxicity Study.

TABLE 18. Microscopic Observations.

Tissue Lesion	Group, S Monkey e Number x	Control				3 mg/kg/day				10 mg/kg/day				30 mg/kg/day				100 mg/kg/day			
		M	M	F	F	M	M	F	F	M	M	F	F	M	M	F	F	M	M	F	F
Brain focal perivascular lymphoid infiltrates		7362	7365	7336	7386	7364	7366	7384	7385	7363	7458	7328	7383	7455	7367*	7382*	7387*	7456*	7361*	7335*	7381*
		1	1	1	1	1	1	1	1	1		1	1	1	1	1	1	1	1	1	1
										3											
Spinal cord		1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Peripheral nerve		1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Eye Sarcocystis sp. in ocular muscle		1		1	1	1	1					1		1	1	1	1	1			1
focal lymphoid infiltrates in sclera			x						x												x
focal lymphoid infiltrates in lacrimal gland											3										
focal lymphoid infiltrate in palpebral conjunctiva								3					3								
cystic tarsal gland										3	3		3								
											3	3									3
Pituitary diffuse congestion		1	1	1	1	1	1	1	1	1		1	1	1		1				1	
small parenchymal cyst															3		3	3		3	3
											x										
Thyroid foci of interstitial lymphoid infiltrates		1	1	1		1		1	1	1	1	1	1	1						1	1
focal interstitial fibrosis					3		2									2					
diffuse congestion				3											3	2					3
Parathyroid diffuse congestion		1	1	1	1	1	1	-	-	-	-	-	-	-	1	-	-			-	-
																	3				1
Tongue foci of inflammatory cell infil- trates in lamina propria and mucosal epithelium		1								1		1			1	1	1	1			
foci of inflammatory cell infil- trates in muscle			3	3	4	2	3	2	3		3	3		2	2					2	2
Sarcocystis sp.			2					3			3	2		2						2	
								x				x									

Code: x - condition present      4 - moderate  
a - autolyzed                      5 - marked  
1 - not remarkable                6 - extreme  
2 - very slight                    - = not available  
3 - slight                            \*Died or sacrificed in extremis

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Ninety Day Subacute Rhesus Monkey Toxicity Study.

TABLE 18. Cont. Microscopic Observations.

Tissue Lesion	Group, S Monkey e Number x	Control				3 mg/kg/day				10 mg/kg/day				30 mg/kg/day				100 mg/kg/day			
		M	M	F	F	M	M	F	F	M	M	F	F	M	M	F	F	M	M	F	F
Tonsil																					
foci of inflammatory cell infiltrates in mucosal epithelium and tonsillar crypt					1											1					
Sarcocystis sp. in muscle		3	4	2	3		4	3	3	3	3	4	4		2		3				4
Gongylonema sp. in mucosal epithelium			x																		
atrophy of lymphoid follicles					x															4	4
Adrenal									1												
foci of dystrophic mineralization		3	3	2	2	3		2			3	2	2				2				
diffuse congestion															3	4	3			4	3
diffuse lipid depletion														5	5	5	5	5	5	5	5
foci of lymphoid infiltrates in sinusoids				3		2		2	3	3	3		2								
acidophilic degeneration of individual to small groups of cells														2			3				
Trachea			1												1		1	1	1		
foci of inflammatory cell infiltrates in lamina propria		3		3	3	3	2	2	3	3	3	3	2	2		3				3	3
Salivary gland				1		1				1									1	1	
focal interstitial lymphoid infiltrates		2	3		2		3	4	3		2	2	3	3		2	3				
diffuse congestion														3	3					3	
decreased cell size, loss of cytoplasmic granules															4		4				
Lung																					
acarian pigment (peribronchial, peribronchiolar, perivascular)		3	2	2	2	3	2	2	2	2	2	3	2	3	2	2	4	2		2	2
focal perivascular lymphoid infiltrates						3					3	3									
focal peribronchial/peribronchiolar lymphoid aggregates		4	4	3	4	3	3	4	3	3	4	4	3	3		2	2			3	3
lung mite in bronchiolar lumen		x			x																
interstitial pneumonia		3	4		4	3		3	4	3											
diffuse congestion												3	4		4			3			
foreign body pneumonia			5											3	3	3	4				
focal hemorrhage			3					5													
acute focal bronchopneumonia		4				3												3			
numerous aggregates of pigment laden alveolar macrophages											4										5

Code: x - condition present      4 - moderate  
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 1 - not remarkable                6 - extreme  
 2 - very slight                      - = not available  
 3 - slight                            \*Died or sacrificed in extremis

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## Ninety Day Subacute Rhesus Monkey Toxicity Study.

TABLE 18. Cont.

## Microscopic Observations.

Tissue Lesion	Group, S Monkey e Number x1	Control				3 mg/kg/day				10 mg/kg/day				30 mg/kg/day				100 mg/kg/day			
		M	M	F	F	M	M	F	F	M	M	F	F	M	M	F	F	M	M	F	F
Heart		7362	7365	7336	7386	7364	7366	7384	7385	7363	7458	7328	7383	7455	7367*	7382*	7387*	7456*	7361*	7335*	7381*
focal interstitial lymphoid infiltrates			1			1				1	1		1	1		1				1	
focus of lymphoid infiltrate in endocardium		3		3	3		2	3	3						3			2		2	
focal subendocardial hemorrhage												3						3		4	4
atrophy of epicardial fat																					4
Aorta		1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Spleen		1	1	1	1	1	1	1	1	1	1										
atrophy of lymphoid follicles																					
diffuse congestion														4	4	4	4	4	4	4	4
focal amyloidosis in lymphoid follicles											3	3		3	3	4	3	4	4	3	4
increased amount of hemosiderin pigment																				3	
Lymph node		1		1	1	1	1	1	1	1	1	1	1	1	1			4	4	4	4
atrophy of lymphoid follicles																					
increased amount of hemosiderin pigment			3																		
neutrophil infiltrate in sinuses																		3			
diffuse congestion															3			3	5		
lymphoid hyperplasia		3													3						3
Esophagus		1			1		1											1		1	1
foci of inflammatory cell infiltrates in lamina propria			3	2		2		3	2		3	2	2	3	2		2				
foci of interstitial lymphoid infiltrates in muscularis			2					2			2	2	2								
Congyloema sp. in mucosal epithelium																					x
Stomach																					
foci of inflammatory cell infiltrate in lamina propria		3	4	3	3	3	3	4	4	4	3	4	3	3			3	3	2	4	3
diffuse congestion																					
foci of inflammatory cell infiltrates in submucosa															3	3				3	
foci of inflammatory cell infiltrates in muscularis					4					4		4	3								
foci of inflammatory cell infiltrates in serosa								3			3										
parasitic granuloma in omentum											3										
focal mucosal hemorrhage											x										
focal coagulation necrosis in mucosa													2		2					2	

Code: x - condition present      4 - moderate  
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2 - very slight                    - = not available  
3 - slight                            \*Died or sacrificed in extremis

FC-143:

## Ninety Day Subacute Rhesus Monkey Toxicity Study.

TABLE 18. Cont.

## Microscopic Observations.

Tissue Lesion	Group, S Monkey e Number x	Control				3 mg/kg/day				10 mg/kg/day				30 mg/kg/day				100 mg/kg/day				
		M	M	F	F	M	M	F	F	M	M	F	F	M	M	F	F	M	M	F	F	
Small intestine																						
diffuse villous atrophy		1	1	1	1	1	1	1	1	1	1	1	1	1								
focal hemorrhage																			5	5		
diffuse congestion																		3		3	3	
focal aggregate of brown pigment-laden foamy macrophages in mesentery																		3	3	3		3
inflammatory cell infiltrates in serosa																						x
atrophy of lymph nodule																			4		4	
																		4		4		
Cecum																						
transmural inflammatory cell infiltrates		1	1	-	1	1	1	1	1		1	1	1						1			1
diffuse congestion																				4		
focal mucosal hemorrhage																		3	3	3		3
inflammatory cell infiltrates in serosa																		2		3	3	
parasitic granuloma in muscularis										2												
atrophy of lymph nodule																	x					
																			4		4	
Colon																						
diffuse congestion		1	1	1	1	1	1	1	1	1	1	1	1	1								1
parasitic granuloma in submucosa																		3	3	3		3
transmural inflammatory cell infiltrates																				x		
focal mucosal hemorrhage																				4		
atrophy of lymph nodule																		3				4
																				4		4
Rectum																						
diffuse congestion		1	1	1	1	1	1	1	1	1	1	1	1	1								
inflammatory cell infiltrates in muscularis																		3	3	3		3
atrophy of lymphoid nodule																						3
																				4		4
Pancreas																						
focal periductal lymphoid infiltrates		1	1				1			1		1	1							a	1	1
focal interstitial lymphoid infiltrates																		3	2	3		3
diffuse congestion																				3	2	
																				3	3	3
Thymus																						
		1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	-	-	-	-	-

Code: x - condition present      4 - moderate  
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2 - very slight                    - = not available  
3 - slight                          \*Died or sacrificed in extremis

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Ninety Day Subacute Rhesus Monkey Toxicity Study.

TABLE 18. Cont.

Microscopic Observations.

Tissue Lesion	Group, S Monkey e Number x	Control				3 mg/kg/day				10 mg/kg/day				30 mg/kg/day				100 mg/kg/day				
		M	M	F	F	M	M	F	F	M	M	F	F	M	M	F	F	M	M	F	F	
<b>Liver</b>																						
portal inflammatory cell infiltrates																					1	
parenchymal inflammatory cell infiltrates		3	3	3	3			3	2	3	3	2	2		2						2	
diffuse congestion		2	2	2	3	3	3	3	3	3	3	2										
acidophilic degeneration of individual to small groups of hepatocytes														4	3	3	3				3 2	
diffuse hepatocellular hypertrophy with cytoplasmic vacuolation								3								3					3 3	
neutrophil infiltrates in sinusoids														3		3						
														3								
<b>Gallbladder</b>																						
foci of inflammatory cell infiltrates in lamina propria		3	3	4	3	3	2	2	3	2	3	3	3	3	3	3	3	1	a	a	a	a
																					1 a	
<b>Kidney</b>																						
focal interstitial lymphoid infiltrates		2	2		2	3	3	4	2	2	3	2	3	2		2	2				2 2	
multinucleated lining epithelium in papillary ducts		x	x					x														
cyst in medulla		x										x										
chronic interstitial nephritis				3																		
diffuse congestion																						
microlith in renal tubules														4	3	3	3	3	3	3	3	
small foci of dystrophic mineralization					2															x	3 3	
														2		2					2 2	
<b>Urinary bladder</b>																						
foci of inflammatory cell infiltrates in lamina propria		3	2	3	2	2	3	2	3	3	3	3	1	1	1	1		1			1	
diffuse congestion																						
																					3 3	
<b>Testes</b>																						
prepuberal development		x	x																			
chronic focal vasculitis			4		x	x				x	x										x x	
focal perivascular lymphoid infiltrate														x	x						x x	
																					2	
<b>Ovaries</b>																						
small foci of dystrophic mineralization				1		1	1			1	1					1					1 1	
diffuse congestion			2																		2 3	

Code: x - condition present      4 - moderate  
a - autolyzed                      5 - marked  
1 - not remarkable                6 - extreme  
2 - very slight                    - = not available  
3 - slight                            \*Died or sacrificed in extremis

FC-143:

## Ninety Day Subacute Rhesus Monkey Toxicity Study.

TABLE 18. Cont.

## Microscopic Observations.

Tissue Lesion	Group, S Monkey e Number x	Control				3 mg/kg/day				10 mg/kg/day				30 mg/kg/day				100 mg/kg/day			
		M	M	F	F	M	M	F	F	M	M	F	F	M	M	F	F	M	M	F	F
Prostate		7362	7365	7336	7386	7364	7366	7384	7385	7363	7458	7328	7383	7455	7367*	7382*	7387*	7456*	7361*	7335*	7381*
focal interstitial lymphoid infiltrates														1			1				
focal lymphoid infiltrate in corpus cavernosum		3	3			2	3			2	3			2							
			3				2			2				3							
Uterus																					
diffuse congestion											1	1				1					
blood in uterine glands				2	2			2							3				3	3	
small foci of hemorrhage in endometrium				2	2			3							2				2		
brown pigment-laden macrophages in endometrium									3												
inflammatory cell infiltrates in endometrium									3												
proteinaceous fluid and inflammatory cells in uterine lumen			3	2				4	2											3	
Vagina																					
foci of lymphoid infiltrates in lamina propria and mucosal epithelium			3	4				3	3		4	4			2	3			2	5	
foci of lymphoid infiltrates in muscularis				2					2			3								3	
Sarcocystis sp.									x												
focal lymphoid infiltrate in tunica adventitia									3												
diffuse congestion																					
focal neutrophil infiltrate in mucosa															3						
Skeletal muscle		1		1	1	1	1			1	1							1			
Sarcocystis sp.			x					x	x											x	
focal interstitial inflammatory cell infiltrates			3					4	2		3	2									
interstitial fibrosis																					
focal/multifocal atrophy of muscle																				3	
increased sarcolemmal nuclei														4	4	4		4		4	
Skin																					
brown/black pigment in dermis		x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	
dermal inflammatory cell infiltrates			2					3	3												
diffuse acanthosis		3		3																	
diffuse congestion																					
hyperkeratosis						3	3			3	3						3				
few large areas of hemorrhage in subcutis														3	3	3			3	3	
							3													5	

Code: x - condition present      4 - moderate  
a - autolyzed                      5 - marked  
1 - not remarkable                6 - extreme  
2 - very slight  
3 - slight                            - = not available  
   \*Died or sacrificed in extremis

FC-143:

Ninety Day Subacute Rhesus Monkey Toxicity Study.

TABLE 18. Cont.

Microscopic Observations.

Tissue Lesion	Control				3 mg/kg/day				10 mg/kg/day				30 mg/kg/day				100 mg/kg/day			
	M	M	F	F	M	M	F	F	M	M	F	F	M	M	F	F	M	M	F	F
	7362	7365	7336	7386	7364	7366	7384	7385	7363	7458	7328	7383	7455	7367*	7382*	7387*	7456*	7361*	7335*	7381*
Mammary gland																				
brown pigment in dermis							1													
hyperkeratosis	x	x		x	x				x	x	x		x	x		x	x	x	x	x
dermal inflammatory cell infiltrates	3		3	3	3	3	3			3		3	3	3	3	x	x	x	x	x
inflammatory exudate in acinar lumen/ducts			3	3	2		3		3		3	3	2							
inflammatory cell infiltrates in intralobular connective tissue		2		2												2				
diffuse congestion		3							2											
intraepidermal microabscess																	3			
Femur	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	1	1	1	1	1
Bone marrow (Rib junction)																				
hypocellular marrow	1	1	1	1	1	1	1	1	1	1	1	1								
congestion													3	4	4	3	4	4	4	4
Miscellaneous														3	3	4	3	3	4	3
acute focal cheilitis, lip																				4

Code: x - condition present      4 - moderate  
 a - autolyzed                5 - marked  
 1 - not remarkable          6 - extreme  
 2 - very slight              - = not available  
 3 - slight                    \*Died or sacrificed in extremis

Interoffice Correspondence **3M**

CONFIDENTIAL

Subject: Meeting Minutes - Review  
of Animal Studies

cc: R.J. Davis - 220-12E  
J.D. LaZerte - 236-1  
L.J. Magill - 223-6SE  
A.L. Rosenthal - 230-3  
T.J. Scheuerman - 220-12E  
F.A. Ubel - 220-2E

May 17, 1978

THOSE PRESENT:

M. T. CASE	218-2
J. E. LONG	220-2E
R. A. NELSON	218-3
R. E. OBER	218-2
R. A. PROKOP	236-3B

Those present met on April 28, 1978 to discuss results of the 90 day animal studies carried out at I.R.D.C. The dosing phase of studies on rats using FC-95, FM-3422 and FC-143 have been completed. Dosing of the monkeys on FC-143 is complete while the FM-3422 and FC-95 monkey dosing will be completed in May. An up-to-date status summary of all studies was supplied by J. E. Long and is attached to these minutes. A complete report from I.R.D.C., including histopathological data is due in June or July for the rat studies and later in the fall for the monkey experiments.

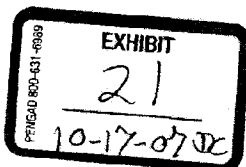
After a very brief discussion of the most recent results from the animal studies, M. T. Case, J. E. Long, R. A. Nelson and R. E. Ober agreed that FC-95, FM-3422 and FC-143 should be regarded as toxic although the degree of toxicity was left undefined.

R. E. Ober inquired as to the types and amounts of impurities present in FC-95, FM-3422 and FC-143. Some impurities, if sufficiently toxic, could cause erroneous conclusion from the animal studies. During the discussion, it was pointed out that FC-95 has been identified in the blood of rats which were fed FM-3422. The question arose as to whether FC-95 might be an impurity in FM-3422. The answer was not known. R. A. Prokop agreed to supply the committee with all available information on impurities present in FC-95, FM-3422 and FC-143.

RECEIVED

MAY 22 1978

R. J. DAVIS



3MA10067059

R. E. Ober raised the question as to which compounds related to FM-3422 and FC-95 would cause greatest worker exposure. Because of the many products related to these compounds, no definite answer could be given. However, because of the large volumes involved, it is believed that FM-3422 itself and FC-807 would contribute most to exposure. It was agreed that Industrial Hygiene should spend more time in identifying the intensity of employee exposure related to FM-3422 and FC-95.

A discussion then took place on analytical methods for determining FC-95 in serum and tissue. Due to the (high?) toxicity of FC-95, it would be advisable to sample the blood of workers exposed to FC-95 or related compounds in order to determine  $C, F, SO_2$  levels. The analytical method which has been developed for FC-95 is claimed to be sensitive down to a level of 0.5 ppm, however supporting data is lacking. Also, if the method is satisfactory down to a level of 0.5 ppm, it should be adequately sensitive for determining the amount of FC-95 in serum and liver from I.R.D.C. rat studies. However, supporting data are not available. It was concluded that R.E. Ober and R.A. Prokop should meet with Central Research Analytical personnel and analyze the data on determination of FC-95 in serum and tissue in order to assess the reliability of the method.

A discussion then took place on having the capability of analyzing for FC-95 in serum and liver of animals before starting the two year animal studies. R. A. Nelson and J. E. Long felt that FC-95 should be identified as being present before proceeding with the studies since it is possible that a metabolite of FC-95 might be responsible for toxic effects rather than FC-95 itself. R. E. Ober regarded such studies as supplemental. It was agreed that analytical work on FC-95 in the serum and liver of rats should be completed as rapidly as possible.

It was questioned why FC-95, FM-3422 and FC-143 were chosen for the animal studies. FC-143 and FC-95 have been found in the employees. FM-3422 is an intermediate which goes into a variety of products. R. E. Ober suggested that a two year study on FM-3422 would give information on the effects of FM-3422, and possible metabolites. It was agreed that this suggestion should be given further consideration.

R.A. Nelson stated that I.R.D.C. is now saving monkeys for the two year animal studies. If we are to use these animals, we must purchase them now at a cost of \$61,280 and pay \$7800 per month to maintain them. I.R.D.C. wants an answer by May 1, 1978. If we do not purchase and maintain these monkeys, none may be available later for the animal studies. However, since it has not yet been decided with certainty that monkeys will be used in the two year studies, those present recommended not purchasing the monkeys at this time.

3MA10067060

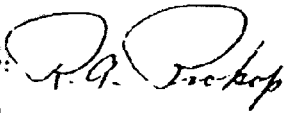
Those present again considered the available toxicity data on FC-95, FM-3422 and FC-143. It was pointed out that male rats fed FC-143 at the 1000 ppm level had about 50 ppm FC-143 in their blood and that one Chemolite worker had a level of 53 ppm in his blood. At the 1000 ppm feeding level, male rats had liver discoloration (females had none). It was concluded that the liver discoloration in rats associated with a blood level of 50 ppm suggests a possible human health problem for individuals who have this level (or above) in their blood for long periods of time. Those present also concluded the following:

As concluded previously by the full committee, available data in man indicates that no substantial risk exists under the Toxic Substances Control Act. However, those present urgently recommended that all reasonable steps be taken immediately to reduce exposure of employees to these compounds.

It was also agreed that:

1. R. E. Ober will make proposals on metabolic studies and make a presentation to the committee on such studies.
2. R. A. Prokop and J. E. Long will make certain that Riker has all previous analytical and toxicological data involving fluorochemicals in blood.
3. A protocol should be written for sampling of employees blood.
4. It will be necessary to have a method for analyzing FC-95, FM-3422 and FC-143 in the food used in animal studies.

Submitted by:



R. A. Prokop

RAP:df

attachment

3MA10067061



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

EMPLOYEE RELATIONS DEPARTMENT

CC: B. W. Karrh, M.D.  
V. A. Brewster, M.D.  
S. Pell  
W. A. Bower-PP&R-Parkersburg  
R. Dyer-PP&R-Parkersburg  
G. A. Ploeger-PP&R-Parker  
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.

*copy of Doughty*  
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

- 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
- 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.

*Report to Y. L. Power*

*copy original*

AJP001399

File to: Jim Hillman  
Pack 12/1/81

THIRD DRAFT  
FC-143 Decatur  
Standby Press Statement  
April 15, 1981  
Lowell Ludford (3-6154)

C O N F I D E N T I A L

APR 27 1981

HOLD FOR RELEASE

As a precautionary measure, approximately 25 women of childbearing potential have received job reassignments at the 3M Decatur plant this week so they will not be exposed to a type of fluorochemical that can cause birth defects in rats.

Preliminary results of a recent 3M toxicology study showed that three related fluorochemicals affected eye development in the fetuses of rats, according to Phil Rath, manager of the Chemical Resources Division plant.

The study currently is being repeated on rats and other species to clarify the initial finding, Rath explained. Until these results are known and evaluated, he said the 3M Medical Department felt it was prudent to recommend this action.

The women are being reassigned to jobs in the adjacent 3M Film and Allied Products Division plant. This is being done in an equitable way that will protect their present seniority status, benefits and pay, Rath pointed out.

-more-

**Exhibit  
1253**  
State of Minnesota v. 3M Co.,  
Court File No. 27-CV-10-28862



"While we are not aware of any adverse health effects on our men and women employees," Raths said, "we are transferring these women as a precautionary step pending further tests."

The three fluorochemicals are used in the manufacture of specialty chemicals by 3M and various other industrial firms.

For further information contact:

Lowell Ludford  
3M Public Relations Department  
3M Company, 3M Center  
St. Paul, Minnesota 55133  
Telephone: (612) 733-6154

Internal Correspondence

CONFIDENTIAL

F. D. GRIFFITH - MEDICAL - 220-2E-02  
L. F. LUDFORD - CORPORATE INFORMATION - 225-5N-04  
W. C. MC CORMICK - MEDICAL - 220-2E-02  
To: D. E. ROACH, M.D. - MEDICAL - 220-2E-02  
From: F. A. UBEL, M.D. - (3-5181) -MEDICAL - 220-2E-02  
Subject: Phone conversation from Dr. McKusick - DuPont - 12/14/81  
Date: December 14, 1981

**3M**


"This is what we are going to tell our employees and we are going to start telling them at 1:00 o'clock on Wednesday, December 16."

"On April 1 we advised you that 3M in a preliminary study had observed birth defects in the eyes of unborn rats when C-8, also known as FC-143 or ammonium perfluorooctanoate, was fed to pregnant female rats. Based upon those findings, we decided it was necessary to exclude female employees of childbearing capability from areas where there is potential for exposure to C-8. We indicated further studies by DuPont and 3M would be undertaken promptly to determine the significance, if any, of the findings as they might relate to employee exposure. We would like to share with you the results of these studies that we have today."

"Thus far, based on our review of the results of the further studies, it does not seem that the observed effects on the eyes of the unborn rats were due to C-8. Also, in the new studies, rat pups delivered by C-8 exposed females showed no eye defects. Rather, it is believed that in the original studies, 3M's technique for the very difficult job of preparing the fetal eye tissue for microscopic examination resulted in the alterations noted".

"3M has another toxicological test underway that will be completed in the first quarter of 1982. At that time we expect to have all the data available and will assess if it is necessary to continue excluding female employees of childbearing capability from areas of potential exposure. Until final determination is made, we continue to advise that employees defer giving blood until the blood level of C-8 returns to background levels. We also advise that females who have an organic flouride level above background should consult with their personal physician prior to contemplating pregnancy. We will provide all information we have on C-8 to employees' personal physicians".

FAU:mam



**Exhibit  
1266**

State of Minnesota v. 3M Co.,  
Court File No. 27-CV-10-28862

TO: R. L. BOHON - ENVIRONMENTAL LAB (EE&PC) - 21-2W-05  
M. T. CASE - RIKER SAFETY EVALUATION - 218-3S-03  
R. J. DAVIS - OFFICE OF GENERAL COUNSEL - 220-12E-02  
J. D. JOHNSON - RIKER DRUG METABOLISM - 270-3S-05  
W. C. MCCORMICK - TOXICOLOGY SERVICES - 220-2E-02  
A. M. NORBERG - I&CS R&D REGULATORY AFFAIRS - 223-5N-06  
W. H. PEARLSON - COMMERCIAL CHEMICALS DIV. - 223-6S-04  
P. F. RIEHLE - CHEMOLITE FACTORY ADMINISTRATION - 41-1  
D. E. ROACH - MEDICAL DEPARTMENT - 220-2E-02  
T. J. SCHEUERMAN - OFFICE OF GENERAL COUNSEL - 220-12E-02  
W. F. SCOWN - COMMERCIAL CHEMICALS DIV. MARKETING - 223-5S-04  
S. D. SORENSON - INDUSTRIAL HYGIENE - 220-2E-02  
J. K. SUGG - INDUSTRIAL HYGIENE ADM. - 220-2E-02  
A. C. WEST - COMMERCIAL CHEMICALS LAB - 236-2B-01

c: F. D. Griffith - Toxicology Services - 220-2E-02  
D. F. Hagen - Gen. Res. Analytical Services - 201-1W-29  
L. C. Krogh - Executive - 220-14W-03  
J. D. LaZerte - Commercial Chemicals Lab - 236-1B-21  
S. M. Leahy - Executive - 220-13E-33  
J. J. McKeown - I&CS R&D Administration - 220-4E-01  
R. E. Ober - Riker Drug Metabolism - 270-3S-05  
F. A. Ubel - Medical Department - 220-2E-02

*Fluorochemicals in Blood*

RECEIVED

APR 12 1983

R. J. DAVIS

Exhibit  
1279

State of Minnesota v. 3M Co.,  
Court File No. 27-CV-10-28862

3MA10067173

1279.0001

To: TO LIST  
 From: A. M. NORBERG - I&CS R&D REGULATORY AFFAIRS - 223-5N-06  
 W. H. PEARLSON - COMMERCIAL CHEMICALS DIV. - 223-6S-04  
 Subject: Minutes of Fluorochemical Study Committee Meeting, March 16, 1983  
 Date: April 8, 1983



The Fluorochemical Study Committee met on March 16, 1983 with the following members and invited participants in attendance:

- R. L. BOHON - ENVIRONMENTAL LAB (EE&PC) - 21-2W-05
- \* M. T. CASE - RIKER SAFETY EVALUATION - 218-3S-03
- R. J. DAVIS - OFFICE OF GENERAL COUNSEL - 220-12E-02
- J. D. JOHNSON - RIKER DRUG METABOLISM - 270-3S-05
- \* W. C. MCCORMICK - TOXICOLOGY SERVICES - 220-2E-02
- \* A. M. NORBERG - I&CS R&D REGULATORY AFFAIRS - 223-5N-06
- \* W. H. PEARLSON - COMMERCIAL CHEMICALS DIV. - 223-6S-04
- \* P. F. RIEHLE - CHEMOLITE FACTORY ADMINISTRATION - 41-1
- \* D. E. ROACH - MEDICAL DEPARTMENT - 220-2E-02
- T. J. SCHEUERMAN - OFFICE OF GENERAL COUNSEL - 220-12E-02
- W. F. SCOWN - COMMERCIAL CHEMICALS DIV. MARKETING - 223-5S-04
- \* S. D. SORENSON - INDUSTRIAL HYGIENE - 220-2E-02
- J. K. SUGG - INDUSTRIAL HYGIENE ADM. - 220-2E-02
- \* A. C. WEST - COMMERCIAL CHEMICALS LAB - 236-2B-01

- - - - -  
 \*Members of Fluorochemical Study Committee  
 - - - - -

The Committee met to review the current status of "fluorochemicals (FC)-in-blood" and discuss future FC worker monitoring and laboratory research directions.

■ Bill McCormick (Toxicology Services) reviewed toxicity studies completed and on-going. He summarized the FC toxicity standing today as:

- Building a stronger data base to describe the results of toxicity testing of FC.
- The results from teratogenicity studies are not cause for concern.
- Fluorochemicals are not mutagenic and to date not carcinogenic.

In follow-up discussions he suggested those areas for potential animal in vitro research with highest priority given to:

- First: platelet aggregation effects
- Second: immunosuppressive effects
- Third: male reproductive effects or Third: hemopoetic system.

- Jim Johnson (Riker Drug Metabolism) reviewed FC metabolism studies and stressed the significant increased experience 3M has gained with methods that will greatly facilitate future research activities.

Summarizing his research, Johnson stated radiometric data from small numbers of rats indicate:

- FC-807 absorption after oral administration is due mostly to absorption of the monoester.
- FC-95, FC-143, and N-ethyl FOSE are well absorbed after oral administration.
- The anions of FC-95 and FC-143 and metabolites of N-ethyl FOSE are slowly excreted.
- Cholestyramine treatment for several days enhances fecal elimination of radioactivity and decreases plasma and liver radioactivity after administration of labeled FC-95 or FC-143. In addition, probenecid appears to enhance urinary elimination of the anion of FC-143.

- Don Roach (Medical Department) presented a summary of the clinical work and future planned medical activities.

Clinical Work:

- Epidemiology mortality study results revealed no adverse deaths, slightly decreased incidence of coronary heart disease (CHD), and the healthy worker syndrome.
- Clinical Health Evaluation continues with 80-90% voluntary participation.
- Decatur Control Study results were presented.
- Blood fluorine testing using Dr. V's biphenyl system. Generally the worker's blood fluorine levels are falling. In some specific areas where opportunities for higher exposure exist, the workers' fluorine blood levels have dropped at a slower rate and in a few incidences leveled.

**Future Planning Includes:**

- Phase II Health Evaluations changed to every two year basis, from more frequent intervals.
- Epidemiology to be brought up-to-date using records accumulated during last five years. Leonard M. Schuman did original FC study. 3M would contract with him for update.
- Assessment in higher exposure areas of ways to reduce further exposure, for example through personal hygiene and engineering modifications.

Roach will continue to monitor the health of 3M workers through the clinical Health Evaluations (every two years) and the FC levels of workers with 5-10 ppm in blood (every six months).

He expressed concern that the FC in worker's blood is not falling to the extent anticipated, and he asked if further reduction in worker exposure could be accomplished.

GENERAL RECOMMENDATIONS EMERGING:

- Continue to monitor workers' health and FC blood levels by Health Evaluations--that is, physical examinations every two years.
- Measure FC in blood every six months in workers with FC levels greater than 5 ppm.
- Advise workers through meetings to continue reducing FC exposure by different handling procedures and through altered personal hygiene.
- Eliminate dried FC materials in plant environment, if possible.
- Schuman should update epidemiology study.
- Investigate engineering modifications in higher exposure areas.
- In workers with higher FC blood levels (e.g., 15 ppm and greater), specific organic fluorine compounds in blood should be identified.

RESEARCH QUESTION POSED:

- What are the specific organic fluorine compounds in blood of workers?  
This involves working with Don Hagen (CRL) to see if it is feasible to adapt Hagen's gas chromatographic/Helium Microwave plasma detector (GC/MPD) system to worker blood samples. It is crucial to initiate this soon since larger volumes of blood will need to be drawn during the current and on-going Health Evaluations to accommodate these further studies.

SPECIFIC RECOMMENDATIONS:

- Once we know the specific organic fluorine compounds in workers' blood, efforts to relate human metabolism of FC to animal metabolism of FC should continue.

Although a data base on metabolism and toxicity of FC in rats has been started, further toxicity studies might be suggested from identification of specific compounds in human blood and urine samples if these compounds have not been previously studied in toxicity tests. In addition, specific questions as to protein binding, body burden and the possibility of affecting the rate of elimination may be further addressed by experiments in the metabolism laboratory.

It was agreed that we need to continue the Health Evaluation monitoring program and laboratory research programs to try to elucidate the effects 3M's FC have on our workers and potentially on consumers using our products.

*A.M. Norberg*

A. M. Norberg  
Secretary  
Fluorochemical Study Committee

*W. H. Pearson*

W. H. Pearson  
Chair  
Fluorochemical Study  
Committee

Internal Correspondence

AR226-0483

cc: R. Ahlness - Spec. Chem. - 223-6S-04  
D. D. Dworak - Chem. Fluoro Mfg. Chemolite Center - 41-1  
S. D. Sorenson - Medical, Ind. Hygiene Serv. - 220-2E-02  
F. A. Ubel, M.D. - Medical - 220-2E-02

A. Riddlehoover  
F. Riehle  
D. Fong

It's put renewed emphasis on personal hygiene and engineering controls to reverse this trend.

RLA - 9/19/84

To: P. F. RIEHLE - SPECIALTY CHEMICAL DIVISION - CHEMOLITE CENTER 41-1

From: D. E. ROACH, M.D. - MEDICAL SERVICES - 220-2E-02

Subject: Organic Fluorine Levels

Date: August 31, 1984



Since our meeting on the latest results of Building 15 organic fluorine levels, test results have been distributed to Bldg. 15 employees. In addition, we have now re-sampled Bldg. 15 workers for updated organic fluorine measurements.

The tests results that were reviewed at our meeting seem to substantiate a trend that has been developing over the past 12-18 months - a tendency for these levels in a number of people to no longer show the previous pattern of decline, in fact, a fair number are now demonstrating an increase in blood fluorine levels. Handling procedures, proper use of protective equipment, showering, etc. may obviously be important factors in the changes being seen. Unfortunately, our sampling had not allowed sufficient time to elapse since you initiated a major program to review personnel handling of fluorochemicals. Therefore, our most recent testing will of great interest.

However, unless we see some evidence of improvement in this last batch of tests from Bldg. 15, we must view this present trend with serious concern. It is certainly possible that with steady and concentrated production of these surfactants in Bldg. 15, and despite our controls, exposure opportunities are providing a potential uptake of fluorochemicals that exceeds excretion capabilities of the body. If this is true, additional protective measures will be needed.

I am enclosing some copies of the slides that demonstrate the above situation. As soon as we receive the recent test sampling, we will be in contact with you.

*DER*  
DER/cr

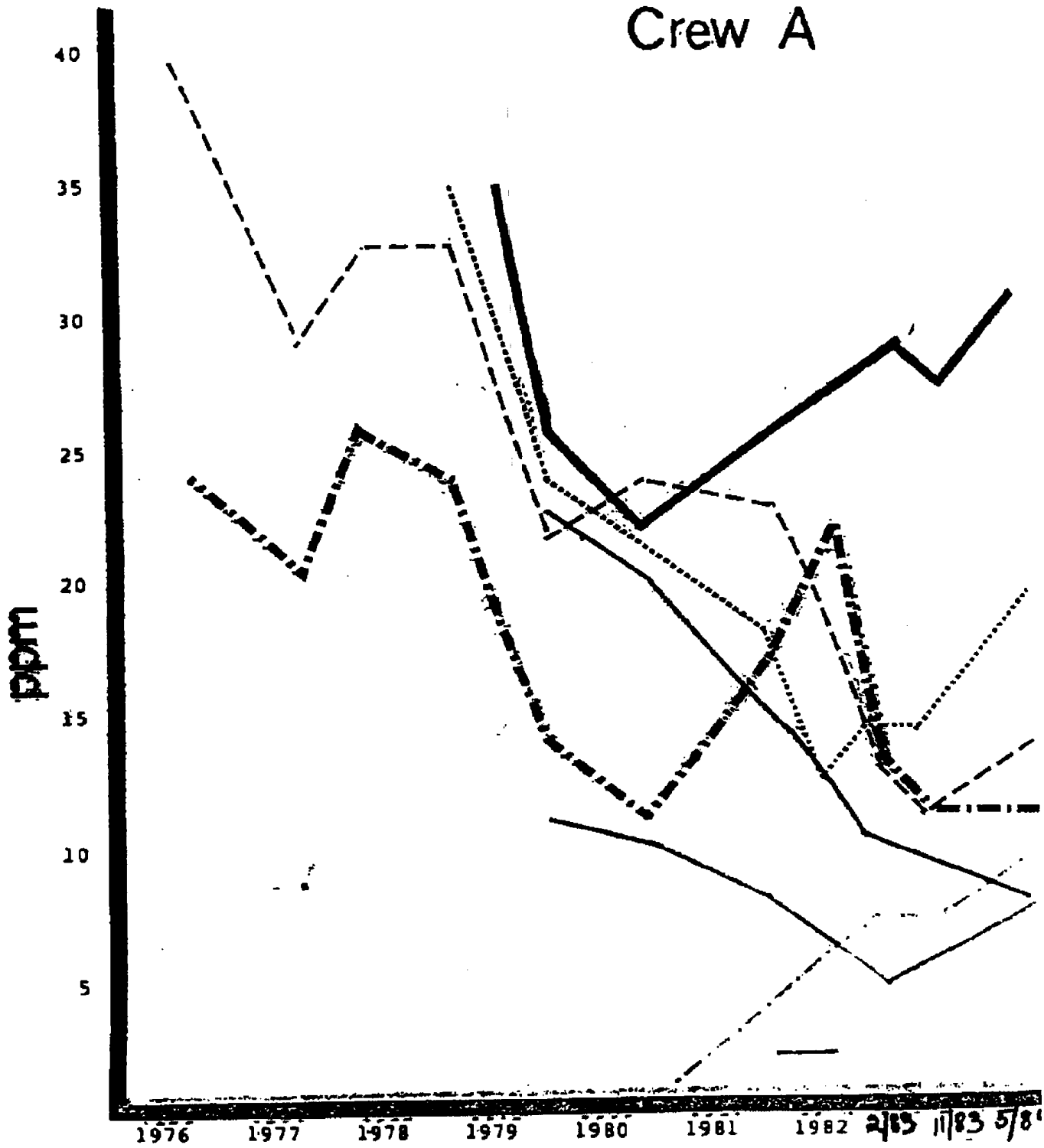
Attachments

Exhibit  
1313  
State of Minnesota v. 3M Co.,  
Court File No. 27-CV-10-28862

003586

3M\_MN03269963

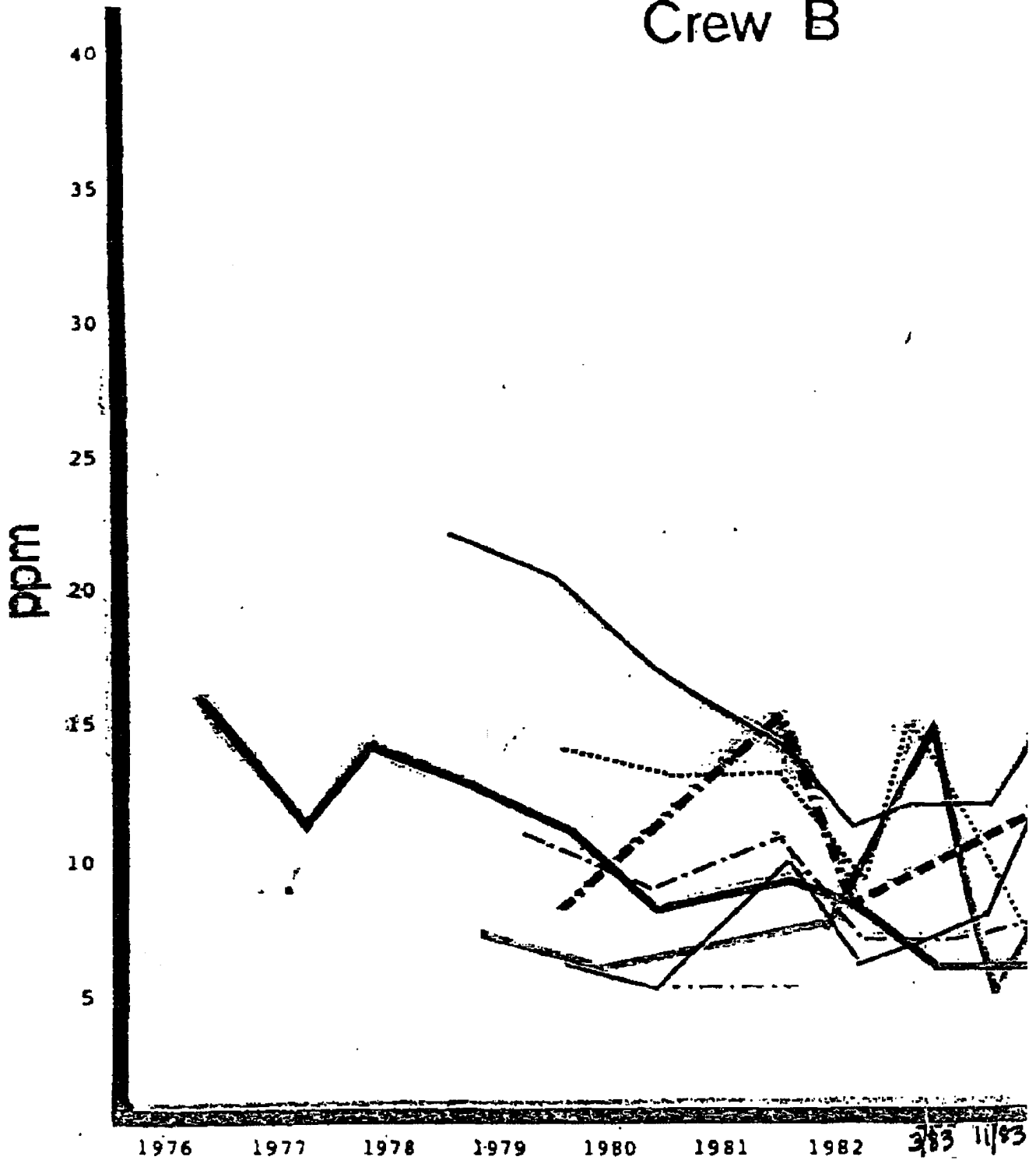
# Crew A



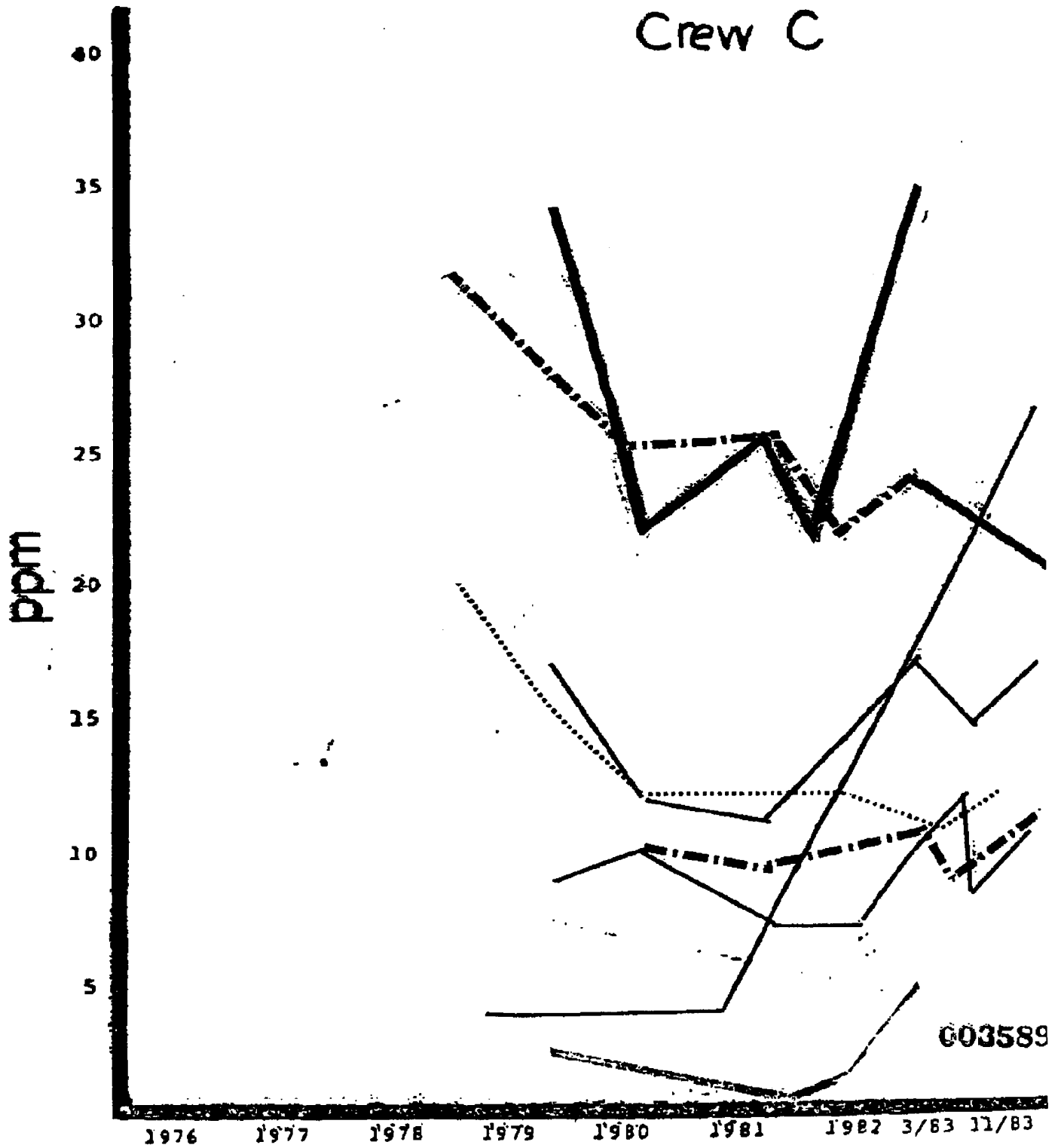
003587



# Crew B



003588



3M\_MN03269966

-Pcm

C-8 SAMPLING (MARCH - JUNE 1984)

<u>LOCATION</u>	<u>DISTANCE (MILES)</u>	<u>C-8 PPM(0.6 LIMIT)</u>
PKSBG-HOME TAP	7.5 UPSTREAM	<
WW-DRINK FTN	---	<
DIST. CTR-WELL	0.25 DOWN	<
WASHINGTON-STORE TAP	0.25 DOWN	1.2, 1.0
LUBECK-STORE TAP	0.25 DOWN	1.5
L. HOCKING-STORE TAP	3 DOWN	0.8, 0.6
BELLEVILLE-PRIVATE WELL	12 DOWN	<
REEDSVILLE-STORE TAP	14 DOWN	<
RAVENSWOOD-STORE TAP	29 DOWN	<
RACINE-STORE TAP	50 DOWN	<
POINT PLEASANT-STORE TAP	74 DOWN	<
GALLIPOLIS-STORE TAP(*)	79 DOWN	<

(\*) NEAREST COMMUNITY TO TAKE WATER DIRECTLY FROM OHIO RIVER.

AS1010163

EID103022

000875

Phone Conversation Report

**3M**

Form 6916 6-D  
COPIES TO

J. L. Allen 270-35-05  
A. R. Picher 236-1B-10  
C. W. Olson 236-GL-04

Person talked with: Robert Geil, D.V.M.

Title:

Firm:

cc: F.D. Griffith 220-2E-02  
F.A. Ubel, M.D. 220-2E-02

Address: 3030 South 9th St.

City, State, Zip Code: Kalamazoo, Michigan 49009

Telephone Number: (616) 375-9051

Date: Dec 9, 1987

ACN: T-3141 / 12951

Call Taken/Placed By: Roger G. Perkins

Time: 9:15 a.m.

SUBJECT: Review of FC-143 final report: Leydig cell tumor incidence.

COMMENTS: Dr. Geil states that he has reviewed the information sent to him and ~~felt~~ <sup>says</sup> that his opinion remains unchanged. He has been contacted by Greg Greg Sykes of Haskell Labs and discussed the report. Geil states: 1) Effect in the testis does seem to be test article related. 2) findings in testis are not within normal biological variation. 3) Effects may be secondary effects resulting from effects on the liver. 4) No urgent need to section more testicular tissues - tumors were grossly visible.

ACTION TO BE TAKEN: 5) He will meet with Sykes and Conrad King here in St. Paul to review the slides of testicular tissues already done. This meeting is to be arranged after Geil and King have met on Dec. 14, 1987.

6) He believes the Dunsafe meeting information is pertinent and that 3M should obtain copies of those presentations and/or any published references cited in the conference.

7) He will send us a letter summarizing his review and any additional suggestions that may arise from his meeting with Conrad King.

Action: J. Allen can you pursue to Dunsafe conf. reference through Ethel? Thanks

Signature: Roger G. Perkins

Exhibit 1342  
State of Minnesota v. 3M Co.,  
Court File No. 27-CV-10-28862



UNIVERSITY OF MINNESOTA  
TWIN CITIES

Division of Environmental  
and Occupational Health  
School of Public Health  
Box 197 Mayo  
420 Delaware Street S.E.  
Minneapolis, Minnesota 55455

April 6, 1989

Larry R. Zobel, M.D.  
Staff Physician  
3M Center  
Medical Department  
220-2E-02  
St. Paul, MN 55144-1000

Dear Larry;

Enclosed please find the tables containing the results of the comparison with the Minnesota population. As I mentioned on the telephone, these must be interpreted cautiously because of the uncertainty regarding the Minnesota rates prior to 1959. Deaths among the study cohort occurred in 41 states; therefore, the U.S. rates may be more appropriate.

As you will see from the tables, the results are similar to those presented previously which used the U.S. rates. The only consistent finding between the two comparisons is for prostatic cancer which we addressed in our initial report. For cancer of the digestive organs and peritoneum there was a statistically significant excess (SMR=176, 95% C.L.=1.09, 2.69) for the entire cohort. However, this was not found among the Clinical Division employees. Furthermore, no single site within the gastrointestinal tract was elevated suggesting that this was probably due to chance. Also worth noting is the fact that these are not sites typically associated with chemical exposures.

If you have any questions please feel free to call me at 626-4810.

Yours sincerely,

Jack S. Mandel, Ph.D.  
Associate Professor

HEALTH SCIENCES



TABLE 5. OBSERVED AND EXPECTED DEATHS BY CAUSE, STANDARDIZED MORTALITY RATIO (SMR), 95 PERCENT CONFIDENCE LIMITS AND CHI SQUARE VALUES, MALES, CHEMICAL DIVISION (COMPARED TO MINNESOTA DEATH RATES)

	OBSERVED	EXPECTED	OBS/EXP	LL	UL	CHISQ
0111 CAUSES OF DEATH	100	89.79	1.11	0.91	1.35	1.05
1111 ALL HALLIGANT NEOPLASMS	23	16.39	1.40	0.89	2.11	2.27
2111 ALL INFECTIVE AND PARASITIC DISEASE	0	0.68	0.00	0.00	5.41	0.05
9111 TUBERCULOSIS	0	0.20	0.00	0.00	18.48	0.46
1401 CANCER OF BUCCAL CAVITY AND PHARYNX	0	0.52	0.00	0.00	7.05	0.00
1491 CANCER OF DIGESTIVE ORGANS AND PERITONEUM (1925-APPROXIMATE)	6	4.23	1.42	0.52	3.09	0.38
1501 CANCER OF ESOPHAGUS (1925-APPROXIMATE)	1	0.40	2.49	0.03	13.83	0.02
1511 CANCER OF STOMACH	0	0.71	0.00	0.00	5.16	0.06
1531 CANCER OF LARGE INTESTINE (1925-APPROXIMATE)	3	1.40	2.14	0.43	6.25	0.86
1541 CANCER OF RECTUM (1925-APPROXIMATE)	0	0.45	0.00	0.00	8.23	0.01
1551 ALL CANCER OF LIVER (1925-APPROXIMATE) 1970 PLUS-PRIMARY ONLY	0	0.27	0.00	0.00	13.68	0.20
1571 CANCER OF PANCREAS (1925-APPROXIMATE)	2	0.88	2.28	0.26	8.23	0.44
1601 CANCER OF RESPIRATORY SYSTEM (1925-APPROXIMATE)	5	4.66	1.07	0.35	2.50	0.01
1611 CANCER OF LARYNX (1925-,1930- APPROXIMATE)	1	0.19	5.16	0.07	28.73	0.48
1621 ALL CANCER OF LUNG-PRIMARY AND SECONDARY (1925-,1930-APPROXIMATE)	4	4.41	0.91	0.24	2.32	0.00
1701 CANCER OF BOOE (1925-,1930,1945-APPROXIMATE)	0	0.12	0.00	0.00	29.60	1.14
1721 CANCER OF SKIN	1	0.40	2.49	0.03	13.85	0.02
1851 CANCER OF PROSTATE (1925-APPROXIMATE)	4	0.51	7.80	2.10	19.96	17.39
1861 CANCER OF TESTIS (OTHER GENITAL ORGANS-1925-49) (1925-,1930-APPROXIMATE)	1	0.38	2.61	0.03	14.55	0.04
1881 CANCER OF BLADDER (1925-APPROXIMATE)	0	0.26	0.00	0.00	14.08	0.22
1891 CANCER OF KIDNEY (1925-APPROXIMATE)	0	0.53	0.00	0.00	6.91	0.00
1901 CANCER OF EYE (1950-1969 ONLY)	0	0.02	0.00	0.00	181.46	11.39
1911 CANCER OF BRAIN AND OTHER CENTRAL NERVOUS SYSTEM (1925-APPROXIMATE)	1	0.87	1.15	0.02	6.42	0.15
1931 CANCER OF THYROID (1950-1969 ONLY)	0	0.04	0.00	0.00	93.44	5.41
2001 LYMPHOSARCOMA AND RETICULOSARCOMA (1950-1969 ONLY)	0	0.52	0.00	0.00	7.04	0.00
2011 HODGKIN'S DISEASE (1940-,1945-APPROXIMATE)	0	0.55	0.00	0.00	6.62	0.01

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3MA000632314

204LEUKEMIA AND ALEUKEMIA	0	2.63	0.00	0.00	1.40	1.72
208CANCER OF OTHER LYMPHATIC TISSUE (1950-1969 ONLY)	3	1.42	2.11	0.42	6.15	0.61
209ALL LYMPHOBLASTIC CANCER	6	6.69	0.90	0.33	1.95	0.01
210BENIGN HEMOPLASMS (PLUS UNSPECIFIED)	0	0.49	0.00	0.00	7.50	0.00
260ALLERGIC, ENDOCRINE, METABOLIC, NUTRITIONAL DISEASES (1950-1969 ONLY)	4	4.74	0.84	0.23	2.16	0.01
250DIABETES MELLITUS	3	3.71	0.81	0.16	2.36	0.01
280ALL DISEASES OF BLOOD AND BLOOD-FORMING ORGANS (1925-,1930-APPROXIMATE)	1	0.44	2.28	0.03	12.68	0.01
319NEURAL, PSYCHONEUROTIC, AND PERSONALITY DISORDERS (1950-1969 ONLY)	1	1.86	0.54	0.01	3.00	0.07
320ALL DISEASES OF NERVOUS SYSTEM AND SENSE ORGANS	3	3.21	0.94	0.19	2.73	0.03
390ALL DISEASES OF CIRCULATORY SYSTEM	104	96.73	1.08	0.88	1.30	0.47
393CHRONIC RHEUMATIC HEART DISEASE (1925-APPROXIMATE)	3	2.89	1.04	0.21	3.03	0.05
410ARTERIOSCLEROTIC HEART DISEASE, INCLUDING CHD (1925-APPROXIMATE)	64	72.88	1.15	0.92	1.43	1.55
430ALL VASCULAR LESIONS OF CNS	7	10.58	0.66	0.26	1.36	0.90
460ALL RESPIRATORY DISEASES (1925-,1930-APPROXIMATE)	7	8.68	0.81	0.32	1.66	0.16
480ALL PNEUMONIA (1925-,1930-APPROXIMATE)	3	3.09	0.97	0.20	2.04	0.05
492EMPHYSEMA (1950-,1955 APPROXIMATE)	1	1.95	0.51	0.01	2.86	0.10
493ASTHMA (1925-,1930-APPROXIMATE)	1	0.43	2.34	0.03	13.02	0.01
520ALL DISEASES OF DIGESTIVE SYSTEM	12	11.45	1.05	0.54	1.83	0.00
531ALL GASTRIC AND DUODENAL ULCER	2	1.12	1.78	0.20	6.44	0.13
571CIRRHOSIS OF LIVER	3	6.88	0.44	0.09	1.27	1.66
580ALL DISEASES OF GENITO-URINARY SYSTEM	0	2.16	0.00	0.00	1.70	1.27
582CHRONIC HEPATITIS	0	0.91	0.00	0.00	4.04	0.18
709ALL DISEASES OF THE SKIN AND CELLULAR TISSUE	0	0.14	0.00	0.00	26.14	0.92
739ALL DISEASES OF THE BONES AND ORGANS OF MOVEMENT	1	0.52	1.94	0.03	10.78	0.00
799SVRPTIONS,SENILITY,AND ILL DEFINED CONDITIONS	1	1.66	0.60	0.01	3.36	0.01
800ALL EXTERNAL CAUSES OF DEATH	46	52.17	0.88	0.65	1.18	0.62
801ALL ACCIDENTS	34	36.27	0.89	0.62	1.24	0.37
810MOTOR VEHICLE ACCIDENTS	27	21.42	1.26	0.83	1.83	1.20
959SUICIDE	9	10.86	0.63	0.38	1.57	0.17
TOTAL RESIDUAL	1	-65.08	-0.02			

CANCER RESIDUAL

13

3.00

4.33

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3MA00632316

1357.0004



AGENDA  
C-8 REVIEW - APRIL 26, 1990

- I. INTRODUCTION - HOWARD SMITH
- II. TOXICITY STATUS - GERRY KENNEDY
- o RESULTS OF HASKELL TESTICULAR EFFECTS STUDY
  - o STATUS OF 3M STUDY - NOEL FOR LIVER EFFECTS
- III. REVIEW OF ALL PERSONNEL AIR MONITORING AND BLOOD DATA - HOWARD SMITH
- IV. REVIEW OF AIR, WATER, LAND PROGRAMS - BILL CRAWLEY  
- CALVIN CHIEN (HYDROGEOLOGIST)
- V. PATH FORWARD - HOWARD SMITH

HAS/IS  
4/3/90

ALP001661

EID924242



INVESTIGATION OF HORMONAL MECHANISMS  
FOR C-8 INDUCED LEYDIG CELL ADENOMA  
MARK HURTT & JON COOK, HASKELL

- O IN THE TWO-YEAR 3M FEEDING STUDY, A DOSE-DEPENDENT INCREASE IN LEYDIG CELL ADENOMAS (TESTICULAR TUMORS) WAS OBSERVED. BECAUSE C-8 WAS NEGATIVE IN TWO SHORT-TERM TESTS, THE MECHANISM OF THE TUMOR INDUCTION BY C-8 WAS INVESTIGATED. SPECIFICALLY, THE EFFECT OF C-8 TREATMENT ON THE HORMONAL STATUS WAS EXAMINED.
  
- O THE RESULTS FROM THE STUDY SUGGEST THAT THE INDUCTION OF LEYDIG CELL ADENOMA BY C-8 IS HORMONALLY MEDIATED.
  
- O ALTHOUGH THE DATA FROM THE STUDY WAS FAR FROM FIRM OR COMMANDING, THE STUDY SCIENTISTS DID FEEL IT DID NOT PRECLUDE A HUMAN ROUTE FOR TESTICULAR TUMORS.
  
- O THIS STUDY IS SUPPORTIVE OF THE LOW LEVEL BENIGN TESTICULAR TUMORS IN THE 3M STUDY AND SUPPORTS OUR EXISTING LOW AEL.

HASMITH/IS  
4/12/90

AJP001662

EID924243



DU PONT HUMAN RESOURCES  
Wilmington, Delaware 19898

cc: A.J. Playtis  
G.A. Ploeger  
W.E. Fayerweather  
(letter only)

August 28, 1992

**CONFIDENTIAL**

**Y.L. POWER, M.D.  
WASHINGTON WORKS  
POLYMERS**

**WASHINGTON WORKS - SURVEILLANCE DATA  
MORTALITY AND CANCER INCIDENCE**

As requested, attached for Washington Works are results of cancer incidence surveillance for 1956-1989 and mortality surveillance for 1957-1991. These data are generated from Du Pont's Company-wide epidemiologic surveillance program.

To assist in your interpretation and evaluation of the findings, I've included a description of the methodology used. Accompanying the surveillance tables is a summary of the major findings. If these findings include a statistically significant excess(es), further follow-up may be recommended.

Please call me at 773-4552 after you have had a chance to review the surveillance results so we can discuss what additional follow-up, if any, is warranted.

**JUDY WALRATH  
EPIDEMIOLOGY SECTION  
N-11510  
773-4552**

Better Things for Better Living

HR-1 REV. 1:

AJP009997

AJP009997

**Exhibit  
1376**

State of Minnesota v. 3M Co.,  
Court File No. 27-CV-10-28862

3MA0074830

1376.0001

**FLUOROCARBONS AND HUMAN HEALTH:  
STUDIES IN AN OCCUPATIONAL COHORT**

**A THESIS  
SUBMITTED TO THE FACULTY OF THE GRADUATE SCHOOL  
OF THE UNIVERSITY OF MINNESOTA  
BY  
FRANK DAVIS GILLILAND**

**IN PARTIAL FULFILLMENT OF THE REQUIREMENTS  
FOR THE DEGREE OF  
DOCTOR OF PHILOSOPHY/ENVIRONMENTAL HEALTH**

**OCTOBER, 1992**

## **ACKNOWLEDGEMENTS**

**I am indebted to Dr. Jack Mandel whose competent research and career advice were invaluable. Not only did Dr Mandel guide me to this research project; he directed me to the NIOSH occupational medicine fellowship that has enriched my clinical medicine knowledge and supported my research efforts over the last three years. His generosity with his time and patience are deeply appreciated. Dr. Timothy Church, Dr. William Toscano, Dr. Ian Greaves, and Dr. Thomas Sellers served on my committee. They deserve a special thanks for their efforts.**

**I wish to thank the Division of Environmental and Occupational Health at the University of Minnesota and the Occupational Medicine Section at St. Paul Ramsey Medical Center for the superb training opportunities I have had over the past three years. Dr. William Lohman, Dr. Samuel Hall, and Paula Geiger at St. Paul Ramsey Medical Center provided much appreciated support during the arduous task of residency and doctoral training. Several members of the Division of Environmental and Occupational health staff were instrumental in the successful completion of this research effort. Sarah Wolgamot and Maralyn Zappia provided excellent administrative support. Gavin Watt, Mindy Geisser, Richard Hoffbeck, and other members of Colon Cancer Control Study provided outstanding computer and statistical support.**

**Dr. Larry Zobel and Dr. Jeffrey Mandel of the 3M Corporation's Medical Department provided advice and support. Their help was an essential element in the success of this project. Stan Sorenson, Dr. Roger Perkins, and other 3M Medical Department members shared their invaluable experience and knowledge. I would also like to acknowledge the support of the Dow Chemical Corporation over the last two years of my training.**

**Last, but not least, this work could not have been accomplished without the loving support of Susan, my wife. Her understanding and excellent editorial comments are greatly appreciated.**

## ABSTRACT

Perfluorooctanoic acid (PFOA) has been reported to be a nongenotoxic hepatocarcinogen and reproductive hormonal toxin in rats. Although PFOA is the major component of total fluorine in humans, little information is available concerning human toxicities. The health effects of PFOA were assessed in two studies conducted in occupationally exposed workers. The associations between PFOA and reproductive hormones, hepatic enzymes, lipoproteins, hematology parameters, and leukocyte counts were studied in 115 male employees. Serum PFOA was positively associated with estradiol and negatively associated with free testosterone (TF) but was not significantly associated with luteinizing hormone. The negative association between TF and PFOA was stronger in older men. Thyroid stimulating hormone and PFOA were positively associated. PFOA and prolactin were positively associated in moderate drinkers. The effect of adiposity on serum glutamyl oxaloacetic and glutamyl pyruvic transaminase decreased as PFOA increased. The induction of gamma glutamyl transferase by alcohol was decreased as PFOA increased. The effect of alcohol on HDL was reduced as PFOA increased. A positive association between hemoglobin, mean cellular volume, and leukocyte counts with PFOA was observed. These results suggest that PFOA affects male reproductive hormones and that the liver is not a significant site of toxicity in humans at the PFOA levels observed in this study. However, PFOA appears to modify hepatic and immune responses to xenobiotics. A retrospective cohort mortality study of 2788 male and 749 females workers employed between 1947-1984 at a PFOA production plant was conducted. Overall, there were no significantly increased cause specific SMRs. Among men, ten years of employment in PFOA production was associated with a significant three fold increase in prostate cancer mortality compared to no employment in production. Given the small number of prostate cancer deaths and the natural history of the disease, the association between production work and prostate cancer must be viewed as hypothesis generating and should not be over interpreted. If the prostate cancer mortality excess is related to PFOA, the results of the two studies suggest that PFOA may increase prostate cancer mortality through endocrine alterations.

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## 1. INTRODUCTION

Fluorine was first isolated as an element in 1880 by Moisser <sup>1</sup>. Five years later he synthesized the first fluorocarbons through uncontrolled reactions of carbon with elemental fluorine. It was not until the late 1930s that the controlled synthesis of fluorocarbons became possible. In the 1940s, Frigidaire and DuPont developed chlorofluorocarbons, the first commercially available fluorocarbons, for use in refrigeration <sup>1</sup>. During the same period perfluorocarbons, a subclass of perfluorinated organic fluorocarbons with unique properties, were first synthesized to meet the special needs of the Manhattan project <sup>2</sup>. The electrochemical fluorination method for perfluorocarbon production made commercial production of perfluorocarbons possible and opened the door to widespread use of perfluorocarbons <sup>3, 4</sup>.

Fluorocarbons are wide ranging in their structures and uses. Many commercial applications have been developed for chlorofluorocarbon compounds including refrigeration, degreasing, aerosol dispensing, polymerization, polymer foam blowing, drugs, and reactive intermediates or catalysts. Perfluorocarbons (PFCs) have extensive applications because of their unique physical and chemical properties. These applications include use as artificial blood substitutes, computer coolants, polymers such as teflon, surfactants, lubricants, foaming agents, ski waxes, and in an extensive specialty chemical industry which produces grease and oil repellent coatings for paper and cloth, polymers, insecticides, and a variety of consumer products. Perfluorocarbons are currently being tested as replacements for chlorofluorocarbons in industrial processes and products.

For many years fluorocarbons were generally thought to be nontoxic. Perfluorocarbons were considered to be particularly nontoxic because they were chemically and physically inert and showed low acute toxicity in animals <sup>4</sup>. Recent epidemiological and experimental studies have associated exposure to chlorofluorocarbons, a subclass of fluorocarbons previously classified as nontoxic, with direct and indirect adverse human health effects. Subsequently, researchers and regulators turned their attention to the study of other fluorocarbons. The discovery that one perfluorocarbon, perfluorooctanoic acid

(PFOA), was present in measurable quantities in residents of several U.S. cities<sup>5-7</sup>, the recognition that some perfluorocarbons including PFOA have long half lives in the humans<sup>8</sup> and the observations that PFOA produced toxic effects in animals, including hepatotoxicity, endocrine toxicity, immunotoxicity, and carcinogenesis<sup>9</sup>, has led to a re-evaluation of the toxic potential of perfluorocarbons, particularly PFOA, in humans.

Despite widespread exposure to perfluorocarbons, little is known about their effects on human health. It was apparent that additional studies designed to explore their physiologic effects and potential adverse health outcomes and conducted in an occupational cohort with high exposure to PFCs, were necessary. The 3M Chemolite Plant located in Cottage Grove, Minnesota is one of a few PFC production facilities in the world. Biological monitoring data from studies of the Chemolite workforce showed that employees have had high levels and long durations of exposure to PFOA<sup>8, 10</sup>. This occupational cohort provided the opportunity to study the effects of PFOA on humans. The specific goals and objectives of this study were:

**GOAL 1)** To quantify the human effects of perfluorooctanoic acid on the following physiologic parameters:

- a) **Hormones:** free and bound testosterone, estradiol, lutenizing hormone, thyroid stimulating hormone, prolactin, and follicle stimulating hormone.
- b) **Serum lipids and lipoproteins:** cholesterol, low density lipoprotein, high density lipoprotein, and triglycerides.
- c) **Hematologic parameters:** hemoglobin, mean corpuscular volume, white blood cell count, polymorphonuclear leukocyte count, band count, lymphocyte count, monocyte count, platelet count, eosinophil count, and basophil count.

d) Hepatic enzymes: serum glutamic oxaloacetic transaminase, serum glutamic pyruvic transaminase, gamma glutamyl transferase, and alkaline phosphatase.

**OBJECTIVE 1:** to conduct a cross-sectional study of production workers to estimate the relationships between total serum fluoride, a surrogate assay for prefluorooctanoic acid, and physiologic parameters.

**GOAL 2)**To quantify the mortality in an occupational cohort with long term exposure to perfluorooctanoic acid production.

**OBJECTIVE 2:** to conduct a retrospective cohort occupational study to assess the mortality experience of workers using expected mortality based on Minnesota mortality rates.

## 2. REVIEW OF THE LITERATURE

### 2.1 Introduction

The presence of small amounts of fluoride in human blood was recognized in 1856 <sup>11</sup>. More than 100 years later, Taves <sup>5, 6</sup> presented evidence that fluorine exists in two major forms in humans and animals; in a free ionic state and in a covalently bound organic state. Prior to this report, it was assumed that fluorine existed primarily as inorganic ionic fluoride in biological systems. Taves' observations have since been confirmed by several other investigators <sup>12-16</sup>. The discovery that organofluorine compounds constitute the majority of fluorine found in humans focused research on characterizing these undefined compounds. Guy identified a perfluorinated compound, perfluorooctanoic acid (PFOA), as a major constituent of the serum organic fluorine fraction <sup>7, 17</sup>. Perfluorooctanoic acid (PFOA) is the only organic fluorine compound to be identified in human serum <sup>18</sup>. The recognition of human and animal toxicities associated with perfluorochemicals <sup>9, 19</sup>, has renewed interest in understanding the human health effects of perfluorocarbons (PFC), particularly PFOA.

### 2 Organic Fluorochemicals

Organic fluorochemicals, otherwise referred to as fluorocarbons, are compounds composed of fluorine, carbon and other elements such as oxygen, nitrogen and sulfur. Perfluorocarbons have structures analogous to hydrocarbons, except the hydrogens are exhaustively replaced by fluorine <sup>20</sup>. A limited number of organic fluorochemicals occur in nature <sup>21-23</sup>, however no PFCs occur naturally <sup>24, 25</sup>.

The first report of the synthesis of a fluorocarbon was published in 1890 when Moissan claimed to have purified carbon tetrafluoride. It is likely he isolated fluorographite, however <sup>1</sup>. Pure carbon tetrafluoride was not obtained until 1930 <sup>26</sup>. Work by Ruff and the Belgian chemist, Swarts, in the late 19th and early 20th centuries laid the foundation of organic fluoride chemistry. Midegly and Henne extended Swarts' work and reported the synthesis of dichlorodifluoromethane,

$\text{Cl}_2\text{F}_2$ , in 1930<sup>27</sup>. This chlorofluorocarbon with the trade name Freon 12 is an inert, non-toxic refrigerant which was vastly superior to other refrigerants available in the 1930s. After commercial production of Freon 12 began in 1936, it rapidly became a major industrial chemical<sup>2, 26</sup>. A number of chlorofluoromethanes and chlorofluoroethanes have been produced on a commercial scale in many regions of the world. These chlorofluorocarbons have been used in large amounts as aerosol propellants and degreasers, in addition to their use as refrigerants. Currently, their production is being reduced as a result of their ozone depleting properties<sup>28, 29</sup>.

In 1937, Simons and Block developed a method to produce laboratory quantities of perfluorocarbons, such as  $\text{C}_3\text{F}_8$ ,  $\text{C}_4\text{F}_{10}$ , cyclo $\text{C}_5\text{F}_{10}$  and cyclo $\text{C}_6\text{F}_{12}$ <sup>2, 3</sup>. The analysis of these compounds led to the understanding that many of the structures of saturated hydrocarbons could be replicated in the form of perfluorocarbons. Research in the area of perfluorocarbons was stimulated by two developments. First, Plunkett discovered the polymer, polytetrafluoroethylene, or Teflon<sup>1</sup>. Second, the development of perfluorocarbon chemistry was stimulated by the U.S. effort to develop atomic weapons during World War II under the Manhattan Project. The  $^{235}\text{U}$  isotope of uranium was required for the development of atomic bombs. One method of uranium isotope separation was gaseous diffusion. The only volatile uranium compound available for use in this diffusion process was uranium hexafluoride,  $\text{UF}_6$ , an extremely reactive gas. Materials were needed for use as coolants, lubricants, sealers and buffer gases in equipment exposed to this highly reactive gas<sup>1, 2, 26</sup>. Perfluorocarbons prepared by Simons were found to be inert to  $\text{UF}_6$ . This discovery led to a research effort directed toward understanding the properties of a variety of perfluorocarbons and developing commercial methods for preparation of perfluorocarbons. The development by Simons of the electrochemical fluorination (ECF) was a major milestone in the fluorochemical industry. Since World War II there has been much interest and work in this new branch of organic chemistry based on perfluorocarbons.

The use of Simons' ECF method has allowed the production of a wide variety of perfluorocarbons including perfluorinated alkanes, alkenes, ethers, esters, amides, sulfonamides and compounds with cyclic and ring structures<sup>2</sup>. The 'inert' perfluorocarbons are compounds made up of only carbon and fluorine. This class

of compounds ranges from carbon tetrafluoride to complex multiple ring structures such as perfluorodecalin. Perfluorinated surfactants include carboxylic acids, sulfonic acids, and their derivatives. These compounds form the basis of an extensive fluorochemical industry. A variety of perfluorinated polymers and elastomers exist. The most widely used are polytetrafluoroethylene and Kel-F, a elastomer of vinylidene fluoride and hexafluoropropylene.

### 2.3 Physical Properties

Perfluorooctanoic acid is a straight chain eight carbon carboxylic acid with a molecular weight of 414.16. The melting point of POFA is 59-60°C. Its boiling point is 189°C at standard conditions <sup>30</sup>. Perfluorooctanoic acid is produced as a complex mixture of branched chain isomers. In practice, all eight carbon carboxylic acid isomers are referred to as PFOA. The ammonium salt of PFOA (APFOA) is the common industrially used form of PFOA. It is a white crystalline powder that easily becomes airborne and sublimates at 130°C.

Perfluorocarbons have unique chemical and physical properties <sup>20, 26, 31, 32</sup>. The importance of perfluorination in producing these properties cannot be overemphasized. Perfluorocarbons are not just another hydrocarbon-like molecule. Chemically, perfluorocarbons are remarkably inert. They are stable to boiling in strong acids and bases. Very few oxidizing or reducing agents react appreciably with perfluorocarbons. Perfluorocarbons that contain other organic molecules such as nitrogen, oxygen and sulfur will participate in reaction at the site of these molecules. For instance, perfluorooctanoyl sulfonic acid will react and form the sulfonamide derivative. The amide portion of this molecule can then be conjugated with many other organic compounds. The perfluorinated portion of these larger molecules remains non-reactive.

Perfluorocarbons are heat stable. They can be heated to greater than 250°C without breakdown. At high temperatures, greater than 400°C, some compounds will breakdown. For example, PTFE, breaks down to perfluoroisobutylene (PFIB), an extremely toxic gas <sup>1</sup>. Because most perfluorochemicals are heat stable they are used in high temperature applications.



The inert perfluorocarbons are excellent insulators. Polymers, such as PTFE, and inert PFCs, such as perfluorohexane, are used in electrical applications because of their superior dielectric properties. Their heat stability and insulation properties make perfluorocarbon materials the insulators of choice <sup>20</sup>.

Perfluorinated surfaces are the most non-wettable and non-adhesive surfaces known <sup>20, 26</sup>. Fluorochemical surfactants are some of the most potent surface active agents yet discovered <sup>31</sup>. Very low concentrations of fluorochemical surfactants effectively reduce the surface tension at interphase boundaries.

Most perfluorocarbons are poorly soluble in both aqueous and organic solutions. They form a group of fluorophilic compounds, however some perfluorocarbons with functional groups such as the salts of PFOA, are highly water soluble <sup>31, 32</sup>. Perfluorocarbon liquids dissolve oxygen avidly. This unique property is the basis for the use of perfluorocarbons as blood substitutes <sup>33</sup>.

Perfluorinated carboxylic and sulfonic acids are some of the strongest organic acids known <sup>31</sup>. The  $pK_a$  of PFOA is 2.5 <sup>34</sup>. Thus, when in physiologic solutions, they exist in primarily anionic forms. The anionic forms have a strong propensity to form complex ion pairs\* .

In the past, some investigators have assumed that the chemical and physical properties of many fluorocarbons is synonymous with lack of activity in biologic systems <sup>35, 36</sup>. However, abundant evidence exists that their chemical and physical inertness does not imply biologic inertness <sup>19, 30, 37, 38</sup>.

## 2.4 Synthesis

Synthesis of fluorocarbons has been accomplished using four major methods; electrochemical fluorination (ECF), direct fluorination, teleomerization, and catalytic methods using high valence heavy metals. The ECF was developed by Simons in 1941 <sup>3</sup>. The Simons process is the oldest commercial technique and remains a commercial method to obtain many perfluorocarbons. A solution of

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\* personal communication from James Johnson, 3M Corporation

organic substrate is electrolyzed in anhydrous HF at a low voltage, high current, nickel anode. The products of these electrolysis cell reactions are largely perfluorinated. The spectrum of material produced by the ECF process is defined by the starting material. Commercial products from this process include perfluoroalkanes, perfluoroalkyl ethers, perfluoroalkenes, perfluoroalkyl esters, perfluorotrialkyl amines, perfluorocarboxylic acids and perfluorosulfonic acids <sup>2</sup>. Products of ECF often include a significant proportion of complex isomers and fragmentation products. For example, ECF production of PFOA from straight chain octanoic acid produces 30% complex branch chain isomers <sup>39</sup>. The mixture of products from each ECF run is unpredictably variable. These isomeric mixes are difficult to separate and purify <sup>33</sup>. Workers producing PFCs using ECF may be exposed to a complex mixture that changes composition over time.

Direct fluorination is another method used to produce perfluorocarbons. It is not subjected to the impurity problems associated with the ECF process. Direct fluorination reacts fluorine gas with hydrocarbon substrate. Because fluorine gas is extremely reactive, direct fluorination is a technically difficult process and has only recently been pilot tested for commercial production of fluorocarbons.

World production of fluorocarbons is limited to a handful of commercial plants. The 3M Corporation operates PFC production plants in Minnesota, Illinois, Alabama and Antwerp, Belgium. A plant in Italy owned by a Japanese and Italian consortium produces limited amount of fluorocarbons. Perfluorocarbons are also produced in Germany and have been produced, in the past, in the former Soviet Union.

### 2.5 Sources Of Organic Fluoride Exposure

Guy <sup>17</sup> presented possible candidates for the organic fluorine constituents of human blood based on observation made during the isolation of PFOA from serum. The organic fluorine was not likely to be a macromolecule such as a protein or nucleic acid, because of its solubility in organic solvents such as ether or chloroform/methanol. It was not covalently bound to albumin since it was removed on charcoal at pH 3 at room temperature. The solubility characteristics suggested that multiple compounds existed with different polarities. The major

compound was a polar lipid like molecule that was identified as PFOA. Other less polar compounds appeared to be present. This data suggests that fluorocompounds other than PFOA were bound to albumin. These compounds were not esters of C<sub>13</sub> - 18 fatty acids and were less polar than PFOA. Perfluorooctanyl sulfonamide (PFOS) and its derivative compounds fit this description and may be constituents of the organic fluorine fraction. Although exposure is probably low, the properties of PFOS suggest that it may accumulate to measurable levels.

In contrast to ionic fluoride, little has been reported concerning the organic fluorine content of water and beverages. The fluorine content of ground water is essentially all in ionic form. Some fluorochemicals, such as the perfluorinated carboxylic acid surfactants and their salts, are soluble in water. Such water soluble compounds may locally contaminate surface and ground water near industrial plants that use these compounds. Other perfluorinated compounds such as the alkanes, alkenes, and ethers are fluorophilic and are insoluble in aqueous solutions. Although data on the oral organic fluorine intake is limited, it is unlikely that water and beverages are significant sources of organic fluorine in humans.

The diet as a source of the organic fluorine found in human serum has been the subject of speculation <sup>5, 6, 18, 40</sup>. Non-perfluorinated fluorocompounds have been found in biological systems. Marais showed that fluoroacetate was the compound responsible for toxicity from the poisonous plant *Dichapetalum cymosum* <sup>41</sup>. Other investigators have found plant species that synthesize fluoroacetate, fluorocitrate, and monofluorinated fatty acids. Peters reported that a few toxic plants produce fluoroacetate <sup>42</sup>. Fluoroacetate and fluorocitrate have been found in beans grown in high fluoride soil <sup>23</sup>. Peters <sup>21</sup> and Lovelace et al. <sup>22</sup> have reported the occurrence of fluorocitrate in a few plants and foods. In animals, the metabolic activation of fluoroacetate into (-)-erythro-fluorocitrate blocks the transport of citrate into the mitochondria and citrate breakdown by aconitase <sup>42, 43</sup>. Other omega-fatty acids with even numbers of carbon atoms are highly toxic as a result of oxidation that produces fluoroacetate. Fluorocitrate also undergoes rapid defluorination in rat liver in the presence of glutathione (GSH) <sup>44</sup>. Given the low environmental levels, the infrequent occurrence, the toxicity, and the rapid

metabolism of these compounds in mammalian species, it is unlikely that these monofluorinated compounds contribute substantially to the organic fluorine content in humans.

Taves measured the organic and inorganic fluorine in 93 food items <sup>45</sup>. No significant organic fluorine was found in the tested foods. Ophaug and Singer tested a market basket of food. They concluded that there was no significant organic fluorine content in food. Although food and beverages generally do not contain PFCs, it is possible that they may be contaminated by fluorochemical packaging materials. Water and grease repellent coatings in packaging material could leach into food items in small quantities. This could occur when materials that are not designed for microwave use are used in microwave ovens. Studies have not been reported that quantify human exposures from food packaging sources.

Perfluorocarbons are contained in many consumer products. Fluorocarbon surfactants such as PFOA, PFOS, and its derivatives are present in window cleaning products, floor waxes and polishes, fabric and leather coatings and carpet and upholstery treatments <sup>20</sup>. Additionally these compounds are used to coat food wraps and are incorporated into plastic food storage bags. Fluorocarbons are the basis for a new generation of cross country ski waxes. Teflon and Teflon related products are widely used as lubricants, electrical insulators, heat and chemical stable gaskets and linings and in non-stick cookware. Fluoroalkanes such as perfluorohexane are being evaluated as CFC replacements. If perfluorohexane or other fluorocarbons are used as replacements for CFC's, consumer exposure from aerosols and other products will increase dramatically. PFC's have several experimental medical uses including use as blood substitutes, x-ray and magnetic resonance imaging contrast agents <sup>46</sup>, vitreous replacement and in liquid ventilation therapeutic methods <sup>47</sup>. Recently, a potent fluorocarbon insecticide has been marketed to control fire ants <sup>48</sup>.

Perfluorocarbons have a variety of industrial uses. Teflon and other polymers are used where heat stable and chemically inert liners, gaskets and lubricants are necessary. In addition, they are used as electrical insulators both in solid and

liquid form and used as inert non-conductive liquid coolants in electrical devices such as Cray supercomputers. Perfluorinated surfactants are important fire suppression materials. Perfluorocarbons have been used to control the metal vapors in electroplating processes and to prevent the release of toxic gases from landfills<sup>20</sup>. Perfluorocarbons are being considered to replace CFC's in many processes such as refrigeration, polymer foam blowing and building insulation. New applications are being continually developed for these unique compounds, making increased exposure to workers probable.

## 2.6 Toxicokinetics of PFOA

Since Taves and Guy's observations, perfluorocarboxylic acids, perfluorosulfonic acids and their derivatives have been the subject of numerous toxicokinetic and toxicodynamic studies in animals. These studies have focused primarily on two compounds, PFOA, and perfluorodecanoic acid (PFDA).

Perfluorooctanoic acid or its salts are well absorbed by ingestion, inhalation or dermal exposure. Absorption has been studied primarily in rats, although a number of other species have been studied.

Five male and five female rats were exposed to airborne APFOA for one hour. In this experiment the nominal air concentration of ammonium perfluorooctanoate was 18.6 mg/l. No animals died during the inhalation exposure or the 14 day post exposure observation period. Pooled serum samples contained 42 ppm of organic fluorine for males and 2 ppm for females. Inorganic fluoride content was 0.02 ppm for males and 0.01 ppm for females<sup>9</sup>. Kennedy and Hall<sup>38</sup> studied the inhalation toxicity in male rats of ammonium perfluorooctanoate using both single dose and repeated dose schedules. They found a LC<sub>50</sub> of 980 mg/m<sup>3</sup> for a 4 hour exposure placing PFOA in the moderately toxic by inhalation category. Following ten repeated doses at levels of 1.0, 7.6, and 84 mg/m<sup>3</sup> blood ammonium PFOA levels were obtained. At the 1.0 mg/m<sup>3</sup> level PFOA levels were 13 ppm, at the 7.6 mg/m<sup>3</sup> level PFOA levels were 47 ppm and at 84 mg/m<sup>3</sup> level PFOA levels were 108 ppm. Therefore it appears that PFOA is well absorbed by inhalation. It should be noted that the exposures were to APFOA dust, the likely form for occupational exposure.

Ammonium perfluorooctanoate in food and PFOA administered by gavage in propylene glycol or corn oil vehicles are well absorbed in rats. In an acute oral LD50 study<sup>9</sup>, rats displayed a dose dependent spectrum of toxicities indicating that PFOA was absorbed after ingestion. PFOA levels were not measured in this study. In a subacute oral toxicity study, rats were fed PFOA for 90 days<sup>9</sup>. Serum concentration of organic fluorine showed a dose response relationship in both sexes. A marked gender difference in organic fluorine levels was observed. Males had organic fluorine 50 times higher than females at each dose level.

Studies have since demonstrated excellent oral absorption of PFOA in a variety of species including rats, mice, guinea pigs, dogs, hamsters and monkeys<sup>9, 19</sup>. Of most immediate relevance to humans have been studies in a small number of rhesus monkeys<sup>9</sup>. In a 90 day oral toxicity study, monkeys were given 3, 10, and 30 mg/kg/day doses of APFOA. In monkeys at the 3 mg/kg/day dose, mean serum PFOA was 50 ppm in males and 58 ppm in females. At the same dose, males had 3 ppm and females 7 ppm in liver samples. At 10 mg/kg/day doses, male monkeys had a mean serum PFOA of 63 ppm and females 75 ppm. Liver levels were 9 and 10 ppm for males and females, respectively. Because all but 1 monkey died at the 30 and 100 mg/kg/day dose levels, only 1 serum sample from a male monkey in the 30 mg/kg/day dose group was available. In this monkey the serum level of PFOA was 145 ppm. In the 30 and 100 mg/kg/day dose group mean liver levels were greater than 100 ppm. Thus, the oral route of absorption may be a significant contributor to the body burden of PFOA in exposed workers.

Dermal absorption of PFOA has been studied in rats and rabbits. Ammonium perfluorooctanoate is a fine white powder that may come into contact with skin and be absorbed. In rats dermally exposed to ammonium perfluorooctanoate at 4 dose levels, PFOA was absorbed in a dose dependent fashion<sup>37</sup>. In single dose dermal exposure experiments using rabbits, PFOA appeared to be absorbed. Levels of fluorine were not measured, but dose dependent toxic changes were noted<sup>9</sup>. In a multi-dose experiment, ten male and ten female rabbits were injected dermally with a 100 mg/kg dose of PFOA on a five day a week schedule for two weeks. Total serum fluorine levels were increased in a dose-dependent fashion. Dose-dependent changes in weight were noted<sup>49</sup>. From these studies,

it appears that dermal exposure to the salts of PFOA are absorbed in animals. In the past, Chemolite workers have been exposed to large dermal doses of ammonium perfluorooctonate. It appears that dermal exposure may have played a significant role in the absorption of PFOA in these workers. Upon recognition that PFOA could be absorbed dermally, work practices were changed and engineering controls were adopted that reduced dermal exposures. The role that dermal exposures currently play in PFOA absorption at Chemolite has not been well studied.

Once absorbed, PFOA enters the plasma probably by diffusing as a neutral ion pair. In plasma, PFOA is strongly bound to proteins in the serum with more than 97.5 percent in bound form<sup>50</sup>. It is likely that albumin is the major site for high affinity binding<sup>5-7, 50-54</sup>. There does not appear to be a sex difference in protein binding<sup>50, 54</sup>. Hanhijarvi et al. have suggested that protein binding is saturable in rats<sup>55</sup>. Using human serum, Ophaug and Singer<sup>39</sup> found that PFOA was 99% protein bound at PFOA levels up to 16 ppm total fluorine, however. Guy suggested that perfluorocarboxylic acids bind to albumin in a similar fashion to fatty acids<sup>24</sup>. This hypothesis is consistent with the results of several studies. Taves observed that the organic fraction of serum co-migrated with albumin during electrophoresis<sup>6</sup>. Dialysis and ultrafiltration studies observed the retention of organic fluorine during dialysis and ultrafiltration<sup>7, 17, 56</sup>. Belisle and Hagen reported that PFOA appeared to be strongly protein bound in human serum<sup>51</sup>. Extraction of PFOA from acidified water is quantitatively complete using hexane. When PFOA is extracted from plasma, recovery is only 35 percent. Plasma appeared to complex PFOA and PFDA. The partitioning of the bound into organic phase during extraction was more difficult and necessitated the use of more polar solvents. Klevens<sup>53</sup> suggested that CF<sub>2</sub> and CF<sub>3</sub> groups complex with polar groups that are present in the amino acids in proteins such as albumin. In protein precipitation studies using bovine serum albumin, PFOA bound to albumin at an estimated 28 binding sites per molecule<sup>52</sup>. Nordby and Luck studied the precipitation of human albumin by PFOA. Under acidic pH conditions, PFOA produced reversible precipitation of albumin<sup>57</sup> by binding to high affinity sites. These studies do not rule out significant binding to other plasma proteins or erythrocyte components. In studies using serum protein electrophoresis, the protein bound organic fluorine was distributed in a diffuse

pattern <sup>6, 17</sup> suggesting that PFOA protein binding may be nonspecific. The large amount bound to albumin may reflect the abundance of albumin in plasma and serum.

In rats, PFOA is distributed to all tissues studied except adipose tissue. The highest concentrations of PFOA are in the serum, liver, and kidneys. Ylinen et al. <sup>34</sup> studied the disposition of PFOA in male and female rats after single and 28 day oral dosing. After a single dose of 50 mg/kg, PFOA was concentrated in the serum. Twelve hours after dosing 40% of the PFOA dose was found in the serum of males and 10% in females. Males retained 3.5% of the dose in serum after 14 days. PFOA was retained in the liver for much longer than in serum. In females, the half-life of PFOA in liver was 60 hours compared to 24 hours in serum. In males the half-life was 210 hours in liver and 105 hours in serum. It is noteworthy that PFOA was not found in adipose tissue in detectable quantities. After 28 days of PFOA treatment, PFOA was distributed to the following sites in descending amounts: serum, liver, lung, spleen, brain, and testis. Again, no PFOA was found in adipose tissue. The distribution of PFO from serum to the tissues occurred in a dose dependent manner for females. In male rats, the concentrations of PFOA in testis and spleen followed a dose dependent trend. The levels in male rat serum and liver was the same for the 10 mg/kg and 30 mg/kg dose group. Johnson and Gibson <sup>58, 59</sup> studied the distribution of <sup>14</sup>C labeled ammonium perfluorooctanoate after a single iv dose in rats. Their findings were similar to those of Ylinen et al. The primary sites of distribution were the liver, kidneys, and plasma. Other sites, including adipose tissue, had less than 1% of the administered dose. The level of PFOA in the testis of male rats was not reported. As discussed previously, the 90 day oral toxicity study in rhesus monkeys showed that the relative amounts of PFOA in serum and liver was different in monkeys compared to rats. In the low dose group of monkeys (3 and 10 mg/kg/day) serum had 5 to 10 times the PFOA levels found in liver. However, at higher dose levels, the PFOA levels were equally distributed. Additionally, no sex differences were noted in the monkeys liver and serum PFOA levels.

There is no evidence that perfluorinated compounds including PFOA are biotransformed by living organisms. Several studies have examined whether



PFOA is conjugated or incorporated into tissue constituents such as triglycerides or lipids. Ylinen et al. found no evidence in Wistar rats for metabolism or incorporation of PFOA into lipids<sup>34</sup>. Although the lipid content in PFOA treated rats was different than that in untreated rats, Pastoor et al. did not find evidence for PFOA incorporation into lipids or of metabolism<sup>60</sup>. Vander Heuvel et al. showed that PFOA was not incorporated into triacylglycerols, phospholipids, or cholesterol esters in the liver, kidney, heart, fat pad, or testis of male or female rats<sup>61</sup>. No evidence has been found that PFOA is conjugated in phase II metabolism<sup>61</sup>. Kuslikis et al. studied the formation of activated coenzyme A (CoA) derivatives of PFOA using rat liver microsomes. They found no evidence for the formation of a CoA derivative.

Sex related differences in the toxicokinetics of PFOA have been reported for rats. The mechanism of PFOA excretion appears to be species-dependent since these gender differences are not seen in mice, monkeys, rabbits, or dogs<sup>9, 62</sup>. The half-life of PFOA in female rats has been estimated to be less than one day<sup>39</sup>, whereas the half-life of PFOA in males is five to seven days<sup>34, 38</sup>. It is of note that PFDA does not exhibit this gender difference<sup>63</sup>. It is hypothesized that the sex differences in sensitivity to the toxicities of PFOA are as a result of the slower excretion of PFOA in male rats compared to female rats. Investigators have reported that rats have an estrogen-dependent active renal excretion mechanism for PFOA which can be inhibited by probenecid<sup>50, 54</sup>. As noted previously, females have a much shorter half-life than male rats. The half-life in males can be reduced by castration or estrogen administration. It can be reduced to the female half-life by a combination of castration and estrogen treatment. Estrogen administration alone is almost as effective as the combination of castration and estradiol treatment in reducing the PFOA half-life. This treatment increased the renal excretion of PFOA in male rats to those observed in female rats. Other investigators have reported that the gender difference in half-life depends on a testosterone mediated increase in PFOA tissue binding<sup>64</sup>. This hypothesis is consistent with the gender difference in tissue half-life discussed previously<sup>34</sup>. Johnson has suggested that the primary method of excretion in intact males is via the hepatobiliary route<sup>58, 59</sup>. He reported that cholestyramine enhanced the fecal elimination of carbon 14 labeled PFOA in male rats. These data suggest there was biliary excretion with enterohepatic circulation of PFOA, particularly in

male rats. However, in a male worker with high serum PFOA levels who was treated with cholestyramine, little if any change in excretion of PFOA was noted. In this study PFOA was excreted slowly in the urine.

In humans, the half-life of PFOA appears to be extremely long and is not sex dependent. Ubel and Griffith <sup>8</sup> reported kinetic data for one highly exposed worker. At the time he was removed from exposure his serum organic fluorine was 66 ppm, 80 percent of which was PFOA. Over the next 18 months his organic fluorine level decreased to 39 ppm. Urinary excretion of PFOA fell from 387 micrograms/24 hours to 80 micrograms/24 hours. The decline in organic fluorine levels was consistent with two compartment kinetics, with a calculated half-life of 2 to 5 years. Additional unpublished biological monitoring data from three Chemolite workers is consistent with the 2 to 5 year half-life. In the Chemolite workforce, male and female workers employed in jobs with similar PFOA exposure have increased PFOA levels. Since men and women with similar exposures have similar levels, a large gender difference in PFOA toxicokinetics is unlikely. Therefore, the relevance of the rat data in assessing the effects of PFOA in humans is questionable.

## 2.7 Toxicodynamics of PFOA

### 2.7.1 Male Reproductive Toxicities

Both PFOA and PFDA have been found to produce significant toxicities in the reproductive systems of male rodents <sup>19, 63, 65</sup>. The testis has been reported as the target organ of toxicity for both PFOA and PFDA <sup>19, 66</sup>. Additional evidence exists suggesting that these compounds affect the function of the hypothalamic-pituitary-gonad axis (HPG) <sup>19, 65</sup>.

Perfluorodecanolic acid, but not PFOA, has been shown to produce degenerative changes in rat seminiferous tubules that could progress to tubular necrosis. Van Rafeighem et al. reported that a single ip dose of 50 mg/kg of PFDA, produced degenerative changes in rat seminiferous tubules 8 days after injection <sup>66</sup>. Similar but lesser changes were noted in the seminiferous tubules of hamsters and guinea pigs treated in the same manner. They reported no such change in

treated mice. Bookstaff and Moore <sup>65</sup> did not observe similar changes in rats treated with 20-80 mg/kg of PFDA. They used a different strain of rats in their experiments which is less susceptible to testicular toxicants than those used by Van Rafeighem et al. Thus, the effects of perfluorocarboxylic acids on seminiferous tubules may be limited to a specific compound, PFDA, in a specific strain of rats. The effects observed by Van Rafeighem et al. in other species were not consistent and did not demonstrate a dose-response relationship. In monkeys treated orally with PFOA, no compound related histopathologic changes in the seminiferous tubules were noted <sup>6</sup>.

In a two year rat feeding study, PFOA treated animals were observed to have increased numbers of Leydig cell tumors\*. Male and female rats were fed PFOA containing diets resulting in a mean intake of 1.5 and 15 mg/kg/day. A statistically significant increase in Leydig cell adenomas of 0%, 7%, and 14% in the control, low dose, and high dose groups, respectively, was observed at the end of the two year study. The result was statistically significant as a result of the unexpectedly low number of adenomas in control animals. Historically, CD rats experience a lifetime mean Leydig cell incidence of 6.3 percent with a range of 2 to 12 percent. The high dose group incidence is outside the expected range and may represent a compound related effect. Although the evidence was not definitive, it suggested that PFOA may alter the histology as well as the function of Leydig cells in rats. Perfluorooctanoic acid was not mutagenic in the standard tests including the Ames assay using five species of *Salmonella typhimurium* and in *Saccharomyces cerevisiae* <sup>9</sup>. Mammalian cell transformation assays using C3H 10T 1/2 cells were also negative <sup>67</sup>. These data suggest that PFOA is not a genotoxic xenobiotic. The increase in Leydig cell tumors may be the result of an epigenetic mechanism.

The observation that rats fed PFOA for 2 years had an increased incidence of Leydig cell adenomas prompted researchers to examine the hormonal effects of PFOA in male rats <sup>19</sup>. Adult male CD rats were treated orally with PFOA in doses of 1 to 50 mg/kg. Serum estradiol levels were elevated in the rats treated with more than 10 mg/kg of PFOA. In the highest dose group estradiol was 2.7 times

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\* Report: 3M Riker Laboratories. Two Year Oral Toxicity/Carcinogenicity Study of FC143 in Rats #281CR0012, 1983

greater than the estradiol levels in pair fed control group rats. Serum testosterone levels were significantly decreased in a dose dependent manner when compared with *ad libitum* feed control animals. No significant differences were observed between the high dose rats and their pair fed controls, however. No significant differences were noted in serum luteinizing hormone (LH) levels. Additionally, the accessory sex organ relative weights of the highest group were significantly less than those of their pair-fed controls.

In order to clarify the site of PFOA action, Cook <sup>19</sup> conducted a set of challenge experiments in PFOA treated rats. The results of these experiments demonstrate that the altered testosterone levels were PFOA related. Human chorionic gonadotropin (hCG) challenge can be used to identify abnormalities in the steroidogenic pathway. Human chorionic gonadotropin binds to the LH receptors on Leydig cells and stimulates sex steroid hormone synthesis <sup>68</sup>. Abnormalities in Leydig cell function can be detected by challenging Leydig cells with hCG and measuring steroid hormone production. Similarly, abnormalities in pituitary secretion of gonadotropins can be identified using a gonadotropin releasing hormone (GnRH) challenge that stimulates LH release <sup>69</sup>. Hypothalamic dysfunction can be identified using a naloxone challenge to stimulate GnRH release <sup>70</sup>. In rats treated with PFOA for 14 days at the same dose level as the initial experiment, the Leydig cell production of testosterone was significantly blunted after hCG challenge in the highest dose group compared to *ad libitum* fed controls. A small, non-significant blunting of the testosterone production in response to GnRH and naloxone was observed. Following GnRH and naloxone stimulation, LH levels were not significantly different in the treatment and control animals. The hCG challenge showed that the decrease in testosterone in PFOA treated rats resulted from altered steroidogenesis in the Leydig cell. The results from the GnRH and naloxone stimulation were not definitive. The results were compatible with an effect at the pituitary level as well as at the Leydig cell level. Cook et al. examined the site at which testosterone steroidogenesis was affected by PFOA. Progesterone, 17 alpha-hydroxyprogesterone and androstenedione were measured after hCG challenge. Progesterone and 17 alpha-hydroxyprogesterone were unaffected. Androstenedione levels were significantly decreased in PFOA treated rats compared to controls. Given that the conversion of 17 alpha-hydroxyprogesterone to androstenedione by C17/20 lyase is

necessary for testosterone synthesis, these results suggest that decreased testosterone is the result of a block in this conversion step. In hCG stimulated rat Leydig cells, the 17 alpha hydroxylase/C-17/20 lyase is inhibited by estradiol. Taken together, these data are consistent with the hypothesis that the elevated estradiol levels associated with PFOA treatment inhibit the C-17/20 lyase enzyme and thereby depress testosterone levels. Cook et al. suggested that the blunted response of LH to low testosterone may be mediated, in part, by elevated estradiol levels. A subtle hypothalamic or pituitary effect may also be present, however. The mechanism for the estradiol elevation was not studied.

Perfluorodecanoic acid alters reproductive hormones in male rats in a fashion similar to PFOA. In male rats treated with doses of PFDA ranging from 20 to 80 mg/kg, given as a single ip dose, PFDA decreased plasma androgen levels in a dose dependent fashion <sup>65</sup>. Both plasma testosterone and 5-alpha dihydrotestosterone were significantly reduced. Compared to *ad libitum* fed control rat values, mean plasma testosterone was decreased by 88 percent in PFDA treated animals and DHT was decreased by 82 percent. These changes were reflected in accessory sex organ weight and histology. The changes in accessory sex organs after PFDA administration were found to be reversed by testosterone replacement. The PFDA decrease in androgens was the result of decreased responsiveness of Leydig cells to LH. There was no evidence for altered metabolism of testosterone. Additionally, plasma LH concentrations did not increase appropriately in the face of low plasma testosterone concentrations. This suggests that PFDA may alter the normal feedback mechanisms of the HPG axis.

It is of interest to note that 2,3,7,8 tetrachlorodibenzo-p-dioxin (TCDD), which, like PFOA, is a nongenotoxic rat carcinogen, a peroxisome proliferators, and an inducer of P-450 system, has been shown to produce hormonal effects in male rats similar to those observed for PFOA and PFDA. Moore et al. <sup>71</sup> studied the effect of TCDD on steroidogenesis in rat Leydig cells. Exposure of cell to TCDD resulted in depression testosterone and 5-alpha-DHT concentrations without altering LH concentration or testosterone metabolism. Moore concluded that TCDD treatment inhibits the early phase of the synthetic pathway and the mobilization of cholesterol to cytochrome P450<sub>scc</sub>. However, Moore et al.

observed decreased estradiol. TCDD has been shown to increase the estrogen mediated feedback inhibition of LH secretion <sup>72</sup> Additionally, in studies using MCF-7 breast tumor cells, the antiestrogenic effect of TCDD was mediated by alterations in the cytochrome P450 metabolism of estradiol <sup>73</sup>. The decreased testosterone in rats could be mediated by the effect of TCDD on Leydig cells directly, by alterations in testosterone metabolism, or through increased negative feedback at the pituitary or hypothalamic level. Recently, reports from occupational studies of TCDD exposed workers have associated TCDD exposure with hormonal alterations in human males. Egeland et al. <sup>74</sup> reported that men with high TCDD levels had significantly depressed serum testosterone levels. The changes in testosterone were not associated with altered LH values. Estradiol values were not reported. They concluded that dioxin has a similar effects in men and male rodents. The observations that PFOA, PFDA, and TCDD have overlapping spectrums of rodent toxicities suggests that peroxisome proliferators, inducers of the P-450 system and non-genotoxic carcinogens may also alter the hypothalamic -pituitary-gonad function in male animals.

### 2.7.2 Female Reproductive Toxicities

In the two year rat feeding study, female rats treated with PFOA were observed to have an increased number of mammary fibroadenomas compared to control animals. All mammary carcinomas occurred in control animals. Hyperplasia of the ovarian stroma was observed, but specific histopathological studies were not reported <sup>\*</sup>. No information is available concerning the effect of PFOA and PFDA on HPG axis in women or female animals.

### 2.7.3 Thyroid Toxicities

Altered thyroid hormone dynamics have been observed in rats exposed to PFDA <sup>75-78</sup>. A single ip dose of PFDA in rats results in a rapid and persistent decrease in thyroxin (T4) and T3 <sup>78</sup>. Gutshall reported that the decrease in thyroid hormones occurred as early as eight hours after treatment and persistent for at least 90 days <sup>79</sup>. These changes were associated with a hypothyroid-like state in

<sup>\*</sup> Report: 3M Riker Laboratories. Two Year Oral Toxicity/Carcinogenicity Study of FC143 in Rats #281CR0012, 1983.

the treated rats. The alterations in serum thyroid levels occurred at dose levels that did not produce a hypothyroid syndrome <sup>78</sup>. Animals with depressed T4 levels were found to be metabolically euthyroid <sup>77</sup>. Replacement of T4 resulted in normal food intake, but did not reverse the hypothyroid-like syndrome of hypothermia and bradycardia <sup>78</sup>. This suggests that PFDA has a marked effect on cellular metabolism that is independent of its effect on thyroid homeostasis. The low T4 was thought to be a result of two mechanisms. First, PFDA readily displaces T4 from albumin which results in increased metabolic turn over of the hormone. Second, the response of the hypothalamic-pituitary-thyroid (HPT) axis appeared to be depressed as assessed by thyrotropin releasing hormone simulation testing <sup>75</sup>. In these studies, the animals had increased levels of thyroid responsive hepatic enzyme activities suggesting that the PFDA treated rats were not functionally hypothyroid. The histological appearance of the thyroid glands were unremarkable, although treated rats had significantly lower thyroid weights. TSH levels were not studied. No similar studies are available for PFOA. PFOA has been noted to produce a transient weight loss in treated rats <sup>30</sup>. The hypothyroid-like syndrome observed in PFDA treated rats has not been studied in PFOA treated rats, however. Since the thyroid hormone effects of PFDA do not cause the hypothyroid-like state in rats, PFOA may alter the HPT axis without producing this syndrome.

#### 2.7.4 Hepatic Toxicities

The primary site of PFOA toxicity in rodents is the liver. Peroxisome proliferation (PP), induction of enzymes involved in  $\beta$ -oxidation of fatty acids, and induction of cytochrome P450 occur after a single PFOA dose. Marked hepatomegaly has been noted coincident to the PP and enzyme induction. Increased liver size was the result of a combination of both hypertrophy and hyperplasia. Cell hypertrophy predominated after an initial burst of cell proliferation. The initial hyperplasia is evidenced by large hepatocytes and markers of DNA synthesis <sup>80</sup>. Areas of increased necrosis in the periportal regions have been observed <sup>81</sup>.

The relationship between hepatic enlargement, peroxisome proliferation, and increased  $\beta$ -oxidation is unclear. Xenobiotic induced changes in one specific peroxisomal enzyme are not necessarily linked to changes in other peroxisomal

enzymes or hepatic enlargement <sup>82</sup>. Studies have suggested that xenobiotic induced hepatomegaly and PP may be related to the endocrine status of experimental animals or to oxidative stress <sup>80, 83-86</sup>. Adrenal and thyroid hormones may play a role in peroxisomal proliferation. <sup>80, 85</sup>. Studies of clofibrate, a PP, have shown that endocrine manipulation can modify its hepatic effects. In adrenalectomized and thyroidectomized rats, clofibrate-induced hepatomegaly was reduced compared to the effect in control rats <sup>83, 84</sup>. Conversely, in thyroidectomized or hypophysectomized rats, clofibrate induced peroxisomal  $\beta$ -oxidation enzymes were increased compared to normal rats <sup>83</sup>. Thottassery et al. compared the PFOA-induced hepatomegaly in normal rats, adrenalectomized rats and adrenalectomized rats with cortisol replacement <sup>80</sup>. They found that hepatomegaly was cortisol dependent and was primarily a result of hepatocyte hypertrophy. Hyperplastic responses were also cortisol dependent and were noted in periportal regions of the liver. Peroxisomal proliferation did not depend on cortisol and was observed in centrilobular regions. They concluded that PFOA-induced hepatomegaly and peroxisome proliferation were separate processes.

In oral feeding studies, PFOA and other PP were reported to cause increased hepatomegaly in males compared to females. This difference could be reduced by exogenous estradiol administration or castration and eliminated by castration and estradiol administration <sup>54</sup>. These observations may be explained by an estrogen dependent renal excretion mechanism or a testosterone mediated increase in tissue binding <sup>85, 87, 88</sup>.

Issemann and Green have cloned a mouse PP activated receptor, mPPAR, a member of the nuclear hormone receptor superfamily of ligand-activated transcription factors that is activated by peroxisome proliferators <sup>89</sup>. This receptor directly mediates the effects of peroxisome proliferators (PPs). Tugwood has shown that PPs activated PPAR recognizes a specific response unit on the Acyl-CoA oxidase gene promoter in a manner similar to the steroid hormone receptor <sup>90</sup>. The action of PFOA and other PPs may be mediated by a family of cytosolic receptors that regulate gene transcription in a manner similar to other nuclear hormone receptors.



### 2.7.5 Nongenotoxic Carcinogenesis

In initiation, selection, and promotion experiments in rats, PFOA produced an increased number of hepatocellular carcinomas <sup>91, 92</sup> Several mechanisms for PFOA associated nongenotoxic carcinogenesis have been suggested.

Perfluorooctanoic acid is an archetypal member of a unique sub class of PPs that are not metabolized. Reddy has argued that the structurally diverse peroxisome proliferators (PP) are a distinct class of nongenotoxic carcinogens <sup>86</sup>. Reddy proposed that PPs induce oxidative stress which results in increased tumor formation. According to this theory, the observed increase in hydrogen peroxide formation associated with increased  $\beta$ -oxidation is not associated with an increase of similar magnitude in detoxifying catalase activity <sup>86</sup>. Oxidative attack by hydrogen peroxide and other reactive oxygen species on cell constituents and membranes leads to DNA damage and increased cell proliferation. Increased proliferation in concert with DNA damage produces increased cell transformation and malignancies.

Studies testing the theory that PFOA induces HCC by increasing oxidative stress have lead to conflicting results. Takagi et al. observed an increase in 8-hydroxydeoxyguanosine in liver DNA from rats exposed to PFOA. They concluded that rat hepatocytes were under increased oxidative stress <sup>83</sup>. Handler et al. found no increase in hydrogen peroxide production in intact livers exposed to PFOA <sup>84</sup>. Lake et al. failed to find an association between hepatic tumor formation and peroxisome proliferation <sup>95</sup>. Thottassery et al. observed that the PFOA induction of  $\beta$ -oxidation was independent of adrenal hormone status. A PFOA associated increase in catalase activity depended on cortisol <sup>80</sup>. Therefore, the hormonal status in animals used in experiments could confound studies of oxidative stress and account for the conflicting results.

### 2.7.6 Immunotoxicity

In the 90 day monkey feeding study, bone marrow and lymphoid tissue were a site of histopathology <sup>9</sup>. Treated monkeys in the highest two dose groups were observed to have moderate hypocellularity of the bone marrow. Specific

histopathological findings were not reported. Atrophy of lymphoid follicles in lymph nodes and the spleen were noted in the same treatment groups. No follow-up studies of these observations have been reported. Studies in PFOA treated rats have not shown histological changes in the immune system <sup>9</sup>.

### 2.7.7 Mechanisms of Action

The mechanism of toxicity of perfluorinated surfactants may be mediated by their effect on cell membranes. Olson and Andersen <sup>30</sup> suggested that PFOA may alter membrane function through changes in fatty acid composition and oxidation status. Levitt and Liss hypothesized that the effect of perfluorinated surfactants is mediated by their alteration of membrane organization or fluidity <sup>96, 97</sup>.

Shindo <sup>32</sup> reported that miscibility of fluorocarbon and hydrocarbon surfactants depends strongly on carbon chain length. A carbon chain length greater than eight carbons is necessary for immiscibility. Perfluorocarbon surfactants with eight or fewer carbon atoms are miscible with hydrocarbon surfactants with carbon chain lengths up to nine. These observations could have important implications for biological systems that contain fluorocarbon surfactants. Cellular membranes are a phase boundary, usually between a lipid phase and an aqueous phase. Surfactants will segregate to this phase boundary. Two immiscible surfactants may form two coexistent monolayers on the inside and outside of the membrane whereas miscible surfactants will form only one such monolayer. The presence of two monolayers will maximally reduce the surface tension at the boundary, whereas a single monolayer will affect surface tension to a lesser degree. Changes in surface tension may alter membrane fluidity and affect its function in such processes as signal recognition and transduction. It is interesting to note that the change in miscibility in Shindo's experimental system occurred for fluorocarbon surfactants with carbon chain lengths greater than eight. This change in miscibility depended on hydrocarbon surfactant chain length as well.

The effects of PFOA and PFDA on experimental membrane systems and cellular membranes have been investigated. Inoue studied the differential effects of octanoic acid and perfluorooctanoic acid on experimental cell membrane

properties <sup>98</sup>. The phase transition temperature of dipalmitoylphosphatidylcholine vesicles decreased linearly as PFOA increased in concentration up to one mM and then reach a plateau. This suggested that PFOA may form aggregates in the membrane above a critical concentration. Such a phase separation is observed to occur in micelles <sup>32</sup>. The partition coefficient between water and the membranes for PFOA,  $K = 8910$ , was larger than the coefficient for ionized octanoic acid,  $K = 135$ , possibly because of the difference in hydrophobicity between hydrocarbon and fluorocarbon chains in aqueous solution. The differences between the toxicokinetics and toxicodynamics of PFOA and PFDA may be the result of their differing miscibilities with cell membrane surfactants.

Levitt and Liss investigated the effect of PFOA and PFDA on the plasma membranes of cells from F4 human B-lymphoblastoid cell line using the dye merocyanine 540 (MC540) <sup>97</sup>. The dye binds to phospholipids that are loosely packed on the outer cell membrane, but does not bind to highly organized lipids and does not penetrate the membrane of healthy cells <sup>99</sup>. A large decrease in MC540 cell surface binding was observed after treatment with sub-lethal concentrations of PFOA and PFDA but not other non-perfluorinated fatty acids. Albumin or serum reduced the change in MC540 binding. This effect may be a result of the strong protein binding of PFOA and PFDA by albumin <sup>50</sup>. These observations suggest that PFOA and PFDA either interact directly with MC540 lipid binding sites or alter the structure of the lipids in the membranes.

In experiments examining functional changes in the lymphoblastoid cell lines, Levitt and Liss observed that PFOA and PFDA could cause direct damage to cells resulting in the release of membrane bound cell proteins and immunoglobulins in soluble form <sup>96</sup>. PFDA was significantly more potent than PFOA in solubilizing proteins and killing cells. This may be the result of different miscibilities in the cell membrane of these compounds. However, neither PFOA nor PFDA reduces the ability of surface immunoglobulins to migrate and undergo capping after antigen recognition <sup>97</sup>. In the PFOA concentration ranges that decreased MC540 binding, PFOA did not affect immunoglobulin migration and capping. Capping involves the cytoskeletal mediated polar migration of immunoglobulins within the plane of the membrane <sup>100</sup>. Apparently, the PFOA

and PFDA associated membrane changes do not affect membrane characteristics that are important for receptor migration.

The membrane effects of PFDA have been studied in greater detail. Plicher et al. reported that a single injection of PFDA in rats significantly reduced the apparent number of  $\beta$  adrenergic receptors in cardiac cells <sup>101</sup>. This change in number of receptors was reflected in the diminished response of adenylyl cyclase (AC) to epinephrine in PFDA treated rat cardiac cells. The intrinsic properties of AC were not altered. The action of PFOA was on the epinephrine receptor. The fatty acid composition of the treated rat cardiac cell membranes was significantly altered <sup>101</sup>. Palmitic (16:0) acid was elevated 13 percent, eicosatrienoic (20:3 w6) was elevated 71 percent, and docosahexaenoic acid (22:6 w3) was elevated 18 percent. Arachidonic acid (20:4) was reduced by 18 percent. Several other investigators have reported changes in membrane function following PFDA exposure. Wigler and Shaw <sup>102</sup> demonstrated that PFDA inactivated a membrane transport channel for 2-aminopurine in L 5178 Y mouse lymphoma cells. *In vitro* experiments reported by Olson et al. <sup>103</sup> showed that erythrocytes exposed to PFDA exhibited decreased osmotic fragility and increased fluidity. Taken together, these studies indicate that perfluorinated surfactants exert their effects on cell membranes. The effects appear to be limited to the outer portion of the membranes as the result of differential partitioning within the membrane or binding to specific membrane constituents. Although PFOA and PFDA can be cytotoxic as a result of their detergent action on membranes, their membrane effects at lower doses are not related to their detergent action. From available data, it appears that functional membrane changes may be limited to specific receptor mediated functions.

### 2.8 Occupational Fluorine Exposures At Chemolite

In workers employed in fluorochemical production plants, blood organic fluorine has far outweighed ionic fluoride <sup>8, 12, 14, 51, 56</sup>. More than 98 percent of the total fluorine in these groups has been reported to be organic fluorine. Therefore, the use of total fluoride levels, which consist predominantly of organic fluorine compounds, is a valid surrogate for organic fluorine in occupationally exposed groups. In workers at the Chemolite plant, PFOA has been identified in the serum

of these workers and was estimated to account for 90 percent of organic fluorine found in the serum samples <sup>8</sup>. In this cohort of workers, total fluorine is a good surrogate measure for PFOA.

Industrial hygiene measurement of fluorochemicals have been conducted at the Chemolite plant since the 1970s <sup>8</sup>. These measurements include area samples, personal breathing samples and surface wipe samples. In 1977, a comprehensive effort at evaluating fluorochemical exposures was conducted at the Chemolite plant. During certain operations breathing zone PFOA concentrations were as high as 165 ppm. After extensive engineering control alterations, the plant was serially re-surveyed. In general, airborne exposures were below the recommended limit of 0.1 mg/m<sup>3</sup>. However, there was evidence of surface contamination in production buildings <sup>8</sup>. In 1986, airborne PFOA, as well as breathing zone samples were less than 0.1 mg/m<sup>3</sup> based on 8 hour time weighted averages. Levels as high as 1.5 mg/m<sup>3</sup> were measured in breathing zone samples during certain clean-up and maintenance zone samples. Perfluorobutyric acid was also found, but in much lower concentrations. Spray dryer operators had consistently higher exposures, even following extensive equipment improvements. \*

It appears that airborne exposure to PFOA was low for most workers. Spray dry operators and workers involved in clean up and maintenance activities have higher intermittent exposures. Although personal protection devices are required in high exposure jobs, worker compliance has not been evaluated. The role that surface contamination plays in worker exposure has not been defined\*. The route of PFOA exposure in worker has not been clearly identified.

### 2.9 Epidemiological Studies

A retrospective cohort mortality study of employees at the Chemolite Plant in the period of 1948-1978 was conducted by Mandel and Schuman <sup>8</sup>. Of the 3,688 male employees who were employed for at least 6 months, 159 deaths were identified. There was no excess mortality in the employees as compared to all

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\* personal communication from Stan Sorenson, 3M Corporate Medical Department  
\* personal communication from Stan Sorenson, 3M Corporate Medical Department

cause or cause specific mortality in the U.S. white male population. The subcohort of all chemical division workers did not show any all cause or cause-specific excess in mortality.

Starting in 1976 medical surveillance examinations were offered to Chemolite employees in the Chemical division<sup>8</sup>. Approximately 90 percent of the workers participated in the program. No health problems related to the exposure to fluorocarbons were encountered in participants. Serially conducted surveillance examinations have failed to reveal any relationship between blood levels of organic fluorine and clinical pathology<sup>\*</sup>.

### 2.10 Summary

Animal studies have suggested that there are five areas of toxicity associated with PFOA exposure. These include hepatotoxicity, immune system alterations, reproductive hormone alterations, Leydig cell adenomas, and non-genotoxic hepatocarcinogenicity. Toxicity studies have primarily used rodents. There is considerable variability between strains of rats for some of the toxic endpoints such as Leydig cell adenomas. Additionally, some of the effects seen in rats have not been seen in other rodent species such as mice, hamsters or guinea pigs. The limited data available on PFOA exposed rhesus monkeys and occupationally exposed workers suggests that any extrapolation of the results from rodent experiments to humans requires more information about the mechanism of PFOA toxicity. From this data it does not appear that the liver is a major site for PFOA toxicity in humans. Of greater human health concern are the potential effects on the immune system and the reproductive hormones.

In the past, workers have been found to have significant blood levels of PFOA. Many workers have levels above one ppm. These blood levels are 50-1000 times background levels in the general population. These levels may be high enough to produce toxicities in occupationally exposed humans. A confident estimate of risk cannot be made until further information on the adverse health effects of PFOA exposure in humans is obtained.

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\* personal communication from Larry Zobel; 3M Corporation Medical Department

### 3. METHODS

#### 3.1 Introduction

The effects of perfluorooctanoic acid (PFOA) exposure on human health were studied in employees of the 3M Chemolite plant (hereafter referred to as Chemolite) located in Cottage Grove, Minnesota. Two studies were conducted to investigate of the human health effects associated with PFOA exposure. First, mortality associated with occupational PFOA exposure was studied using a retrospective cohort design. Second, a cross sectional study design was used to estimate the relationships between PFOA exposure and selected physiologic parameters.

A retrospective cohort study was designed to examine mortality among workers. All workers ever employed at the Chemolite plant for greater than six months were included in the cohort. All causes and cause-specific mortality were compared to expected mortality. Expected mortality was calculated by applying sex and race specific quinquennial age, calendar period, and cause-specific mortality rates for the United States and Minnesota populations to the distribution of observed person-time<sup>104, 105</sup>. Age adjusted standardized rate ratios were calculated<sup>106</sup>. A relative risk (RR) for PFOA exposed workers compared to unexposed workers was calculated using proportional hazard regression models<sup>107</sup>. The RR were stratified by gender and adjusted for age at first employment, duration of employment and calendar period of first employment. Any significant differences between observed and expected cause-specific mortality were to be explored using nested case control studies. Case studies were completed for causes of death with 5 or more deaths and standardized mortality rates greater than 1.5. Each deceased individual's record was examined for commonalties in job history information including age at first employment, calendar period of employment, years in the Chemical Division, and duration of employment.

Selected physiologic effects of PFOA exposure were studied using a cross sectional study design. The relationships between total serum fluorine and biochemical parameters including reproductive hormones, hepatic biochemical parameters, lipid and lipoprotein parameters, and hematologic parameters, were

explored. A sample of the work force employed on November 1, 1990 was invited to participate. All employees in high exposure jobs were asked to participate. A sample of workers employed in low exposure jobs was frequency matched to the age and sex distribution of the high exposure group. Each participant completed a questionnaire which included medical history and information concerning alcohol, tobacco, and medication use. The questionnaire is provided in Appendix 3-1. Blood was drawn for determination of hematologic and biochemical parameters. Total serum fluorine, free (FT) and bound testosterone (BT), estradiol (E), thyroid stimulating hormone (TSH), follicle stimulating hormone (FSH), prolactin (P) and luteinizing hormone (LH) were assayed. The PFOA-hormone dose-response relationship for each hormone was estimated using linear regression techniques to adjust for the effects of age, sex, body mass, alcohol consumption, tobacco use, and other potential confounders. The PFOA-hormone dose-response relationship was further explored by fitting linear multivariate models to hormone ratios. All unique ratios between the seven hormones were defined. Twenty-one hormone ratios were calculated for each participant. The prevalence of hormone values outside the laboratory reference range for men was compared to the expected prevalence assuming a normal distribution for assay values.

### 3.2 Retrospective Cohort Mortality Study

#### 3.2.1 Definition Of The Cohort

The Chemolite facility opened in 1947. Individuals who were employed at the Chemolite plant between January 1, 1947 and December 31, 1983 were identified from company records. Workers with fewer than six months employment were excluded. In October 1951 large scale commercial PFOA production facilities became operational (Abe 1982). Because large scale PFOA production did not begin until 1951, a second cohort with potentially significant PFOA exposure was defined as those workers employed between October 1, 1951 and December 31, 1983. Subjects with greater than six months employment were included in this second PFOA cohort.



The cohort was initially assembled in 1979. Subsequently, the cohort was updated to include new employees through 1983. Personnel records for employees working prior to 1979 were coded for demographic items and work history by trained abstractors. Computerized corporate personnel databases were utilized to provide information for workers employed in the 1979 to 1983 period. Abstracted work history included year of first employment, year of last employment, years employed at Chemolite, and months worked in the chemical division. Individual job histories were not abstracted because job titles were defined by wage grades and did not correspond to specific jobs or locations within the plant.

### 3.2.2 Study Databases And Files

A Chemolite cohort database was created on a VAX computer using Ingres software. Data stored on magnetic tape were transferred to the VAX. Duplicate records were identified and removed. Missing data were identified. The Ingress update function was used for data editing. Final analytic files for the Monson program, SAS programs, and custom programs were constructed using the Ingress report writer.

### 3.2.3 Data Editing

The Ingres relational database allowed extensive internal consistency checks to be made. All dates were checked for plausibility. Those records with implausible, inconsistent, or improperly formatted dates were edited and corrected if information was available. Records of workers with fewer than six months employment were flagged and excluded from the analytic data set. A random check of 50 of the 364 workers with fewer than six month employment found no errors in classification of employment length. Extensive attempts were made to obtain all missing data items. Sources of information included plant personnel records, corporate personnel databases, benefit records, archived corporate records, plant medical records, and death certificates. No individual employees or next-of-kin were contacted. Four employees were excluded from the cohort as a result of missing demographic data items.

### 3.2.4 Validation Of The Historical Cohort Information

#### 3.2.4.1 Assessment Of Completeness Of Ascertainment

The cohort was initially defined from personnel records stored at the Chemolite plant. Complete records were maintained on all workers ever employed at the plant. Hourly and salaried workers were included in these files, as were all transferred, terminated and retired former employees. Records for workers first employed in the 1947-1978 period were abstracted from documents, coded and computerized. A corporate computerized database was used to update the cohort through December 1, 1983. Since insufficient induction time had lapsed between 1983 and 1989, no new employees or work history information was added to the cohort database for the post 1983 period for this study.

Verifying the ascertainment of all eligible cohort members was problematic. The assumption that the personnel records represented a complete roster was difficult to check because of a lack of independent information. Several sources were used to exclude major errors in the enumeration of the cohort. The historical plant hiring pattern based on seniority dates was compared with the distribution of dates of first employment. Qualitatively, dates of major plant expansion corresponded to peaks in the distribution of dates of first employment and to seniority dates. Large increases in hiring due to new plant openings were reflected in peaks in the distribution of starting dates in the cohort. A sample of 25 annuity beneficiaries retired from the Chemolite plant were obtained from the corporate personnel office. All 25 were found to be included in the enumerated cohort.

Several plant personnel record systems were randomly sampled. Separate files were maintained for active workers, retirees, transferred and terminated workers, and workers whose employment at Chemolite ended prior to 1960. A sample of records for current employees with start dates prior to December 31, 1983 was compared to the cohort. All 12 records from the 1945-1960 period for start dates were found in the cohort database. Of 30 records sampled from the 1961-1969, 28 (93%) were included in the cohort. Fifty two records had starting dates in the 1970-1978 period. Of these 52 records, forty seven (90%) were found in the

database. In the 1979-1980 period 18 of 44 (41%) records were in the database. Lastly, in the 1981 through 1983 period, 36 of 37 records were in the database (97%). The low ascertainment for workers first employed in the 1979-1980 period was further examined. Of the 34 workers not in the cohort database, 16 (47%) were first employed in the 7/79-1/80 period. These omissions occurred in the transition period between document abstracting and electronic updating of the cohort. Using seniority lists, 44 workers currently employed were hired between 1979 and 1980. They represent approximately 1% of the total number of individuals in the workforce and less than 0.5% of the total person time at risk for the cohort. Records for retired workers were sampled from files containing all workers retired from Chemolite. Forty seven of the 48 (98%) sampled records were present in the database. A sample of the files containing the personnel records of employees completing employment before 1960 was randomly drawn. Of the 67 selected records, 65 (97%) were in the database. Finally, files containing records of all transferred, terminated, or disabled employees were randomly sampled. Of the 120 sampled records, 116 (97%) were present in the cohort database.

#### 3.2.4.2 Validation Of Cohort Information

Information in the edited database was compared to information in the personnel records. A random sample of 25 records was drawn from the personnel files. Database names, social security numbers (SSN), dates of birth (DOB), and dates of employment were verified against record information. The sole error occurred in coding the last digit of one SSN. All other information was correctly entered into the database.

The reliability of ICD8 coding of death certificates for underlying cause of death was evaluated by resubmitting a sample of death certificates for coding by the same nosologist. The sample consisted of 25 death certificates from 1970 -1989. No change in the major categories of cause of death was noted. All cancer deaths were coded concordantly. Within cardiovascular causes of death, two certificates were discordant.

#### 3.2.5 Vital Status Ascertainment

The vital status was ascertained from the Social Security Administration (SSA) and the National Death Index (NDI). All individuals with unknown vital status were traced successfully and vital status determined. Vital status determination in the 1979-1989 period was obtained through the NDI. Death certificates were requested from the appropriate state health departments for those individuals identified as, or presumed to be, deceased. A professional nosiologist coded the death certificates for underlying cause of death according to International Classification of Diseases, 8th revision (ICD8). Information concerning the date and cause of two deaths which occurred outside the United States was obtained from family members or other available sources. Date of death and the ICD8 code for the underlying cause of death were entered into the database.

### 3.2.6 Validation of Vital Status Ascertainment

The vital status determination procedures for the cohort was evaluated. Corporate benefit records were utilized as an independent source for vital status among the retirees. Vital status from the database was compared to vital status in corporate records. A list of all retirees in the 1947-1984 cohort was sent to 3M benefits department. These individuals were matched to retirees who had received 3M death benefits. 3M records were not complete for periods prior to 1975. In the pre-1983 period, 4 deaths in retirees were identified by 3M records. Vital status was correctly ascertained by the SSA matching procedure for only one of these retirees. In the 1983-1989 period, 34 deaths in retirees were identified in 3M records. The NDI matching procedure ascertained all 34 of these deaths. The NDI was not available for 1990. 3M records indicate that 8 retirees died during 1990. The incomplete SSA ascertainment in the period 1975 to 1983 resulted in extending the NDI search to include 1979 to 1983. All 3M identified deaths were also identified in the subsequent NDI search covering the 1979 to 1983 period.

### 3.2.7 Analysis

Analytic methods employed in this study were appropriate for cohort studies. The relative risk was estimated by calculating an adjusted standardized mortality ratio

(SMR) <sup>105</sup>. This study used both national and Minnesota mortality rates for comparisons. Mortality for men in the Chemolite cohort was compared to expected national and Minnesota mortality, adjusted for age, calendar period, sex and race. The use of mortality rates in the rural counties surrounding the plant were not considered to be stable for many causes of death and were not used. Since less than one percent of plant employees are non-white, white male and female rates were used for comparison. For women, only U.S. rates were used because cause- and calendar period-specific Minnesota rates were not available. SMRs were calculated for all cause, all cancer, and cause-specific mortality. The effects of disease latency, duration of employment, duration of follow-up, and work in the Chemical Division were examined using stratified SMR analyses.

Three additional methods of analysis were used to assess the validity of the SMR contrasts. The three methods were: standardized rate ratios (SRR) <sup>106</sup>, Mantel Haenszel adjusted relative rates (RRMH) <sup>108</sup>, and proportional hazard regression adjusted RR <sup>107</sup>.

Limited exposure data were available from plant records. Exposed workers were defined as all workers who worked for 1 month or more in the chemical division. Exposed and unexposed workers' all cause, all cancer, and cause-specific mortality was compared using stratified SMRs, SRRs <sup>106</sup>, and stratified Mantel Haenszel analysis <sup>108, 109</sup>. Additionally, the same summary measures were calculated contrasting the rates for workers with at least ten years duration of employment and those with less than ten years employment.

The relative risk (RR) and 95% CI for the RR for deaths from all causes, cancer, cardiovascular diseases, and selected specific causes were estimated using a proportional hazard model (PH) <sup>107, 109</sup>. The time to event or censoring was defined as time from first employment to event or December 31, 1989. In PH models for specific causes of death, deaths from other causes were censored at the time of death. Exposure was quantified by months of chemical division employment. Covariates included in the models were age at first employment, year of first employment, and duration of employment. The analyses were stratified by gender. The appropriateness of the proportional hazard assumptions were tested using stratified models with graphical analysis of log (-log(survival))

versus follow-up time relationships and models that tested the significance of a product term between exposure and log(follow-up time) <sup>109, 110</sup>.

### 3.3 Cross Sectional Study Of PFOA Exposed Workers

#### 3.3.1 Population Definition And Recruitment

Medical screening of workers employed at the Chemolite plant occurs every two years. The general medical screening program included a medical questionnaire (Appendix 3-1), measurement of height, weight and vital signs, pulmonary function evaluation, urinalysis, serum assays, and hematology indices. This screening program offered an opportunity to assess the physiologic effects of PFOA exposure in workers engaged in commercial production of a limited spectrum of PFCs. Of particular interest were the effects of PFOA, the primary fluorochemical found in the serum of Chemolite workers. (Griffith and Ubel, 1980).

Participation in the Physiologic Effects Study required the subjects' willingness to undergo hormonal and biochemical testing and to have an additional 15 ml of blood drawn for total fluorine assay. In the cross-sectional study, exposure classification was based on the potential for PFOA exposure in a workers job and plant location. All workers engaged in any facet of PFOA production in the previous five years were considered to have potentially high PFOA exposure. The jobs considered to have high exposure potential included all jobs in the production buildings (bldg 6 and 15), all maintenance workers who were assigned to the PFOA production areas, and all management jobs requiring physical presence in the production building. Plant records and job history information was used to assign exposure status to individual workers. A random sample of workers in jobs with low exposure potential was frequency matched to the age and sex distribution of the high exposure workers. Workers with low exposure potential were defined as those assigned to jobs not involved in the production of PFCs for at least five years. A roster of workers meeting the low exposure potential was defined from plant records and knowledge of plant personnel about the location of high exposure jobs. A gender stratified sample from the group of workers in low exposure jobs with an age (5 year strata) distribution similar to the exposed group was identified and invited to participate. If a worker in a job with

low exposure declined to participate, another worker in the same age and sex stratum was randomly selected and invited to participate. In all cases informed consent was obtained. Participation in this study was voluntary.

### 3.3.2 Data Collection

#### 3.3.2.1 Study Logs And Files

A roster of participants was maintained by the plant occupational health nurses. A log for biological sample information was completed by the laboratory technician. The date and time of sample collection was recorded. Quality assurance samples were recorded on a separate log. Results reported on paper records were maintained as medical records. Results for other tests were transmitted electronically to a computerized database and coded as SAS datasets. All records were stored with employee medical records or in the corporate medical offices for confidentiality purposes. Printed laboratory results and questionnaire data were entered into a SAS dataset.

#### 3.3.2.2 Questionnaire

Each participant completed a medical questionnaire prior to reporting to the plant medical office. (Appendix 3-1) Items included demographic information, symptoms, illness history and diagnoses, and medication usage. Detailed questions concerning tobacco use and alcohol use were included. Workers were not re-contacted to obtain missing information or to correct inconsistencies. Responses were not validated. Two plant occupational health nurses collected the questionnaires and returned them to the corporate medical department. In the corporate medical office, data were coded and entered into a SAS data base.

#### 3.3.2.3 Laboratory Procedures

##### 3.3.2.3.1 Height and Weight

Upon reporting to the plant medical office, participants had their height and weight determined by an occupational health nurse. Height and weight were measured once on the same calibrated scale.

#### 3.3.2.3.2 Blood

##### 3.3.2.3.2.1 Drawing And Handling

Four vacutainers of blood were drawn from a single venipuncture by a laboratory technologist. Two 15 ml red top vacutainers of blood were drawn and allowed to clot. One 10 ml purple top vacutainer was drawn for hematology studies. A specially prepared fluorine free 15 ml vacutainer was used to collect blood for total serum fluorine determination. Venipunctures were scheduled to occur at the same time of day and on the same shift for each worker. All blood was drawn between 6:30 and 8:00 a.m. Workers in the Chemical Division of the Chemolite plant rotate shifts on a weekly basis. Blood was drawn after a worker was assigned to the day shift for at least 3 days.

All specimens were refrigerated at the plant prior to transport to the appropriate laboratory. Clotted red top vacutainer specimens were centrifuged for 12 minutes to separate serum from cells before transport to the contract laboratory. In order to render the total serum fluorine specimens non-infectious, serum for total fluorine assays was either extracted in the corporate medical department prior to sending the samples to the 3M Chemical Division analytic laboratories.

##### 3.3.2.3.2.2 Assays

Serum samples were analyzed for total serum fluorine, hepatic biochemical parameters, cholesterol, lipoproteins, and seven hormones. Assayed biochemical parameters included serum glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT), gamma glutamyl transferase (GGT), and alkaline phosphatase (AKPH). The following hormones were assayed: bound testosterone, free testosterone, estradiol, prolactin, luteinizing hormone (LH), follicle stimulating hormone (FSH), and thyroid stimulating hormone (TSH). EDTA preserved whole blood samples underwent routine hematologic analysis including



complete blood count with erythrocyte indices and leukocyte differential cell count (CBC). Analyses were done without knowledge of the subject status or purpose of the study.

Total serum fluorine was determined in 3M's Chemical Division analytic laboratory using the sodium biphenyl extraction method (Venkateswarlu, 1982). The accurate determination of total fluorine in the parts per million (ppm) range required specialized equipment, procedures, and personnel. Assays were completed in a dedicated laboratory following tested protocols.

Upon receipt of extracted serum samples divided aliquots were frozen at -70 degrees centigrade. After all samples had been received, batches of 15 samples were assayed on successive working days. Each batch included high and low quality control samples. Each sample was assayed twice. If the difference in assayed values was greater than 1 ppm, the sample was re-assayed. The total serum fluorine value was reported as a mean value and a rounded integer value.

Serum glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT), gamma glutamyl transferase (GGT), and alkaline phosphatase (AKPH) were assayed by the United Health Services Laboratory in Apple Valley, Minnesota using clinical colorimetric assays. CBCs were determined using automated Coulter counters. Light microscopy was utilized for differential counts.

Estradiol, prolactin, thyroid stimulating hormone (TSH), luteinizing hormone (LH), and follicle stimulating hormone (FSH) were assayed by the United Health Services laboratory using radioimmunoassay (RIA) and enzyme linked immunosorbent assay (ELISA). FSH, LH, and prolactin were assayed using Abbott laboratories IMX microparticle enzyme linked immunoassays. TSH was assayed using London Diagnostics chemiluminescence immunometric assay. Estradiol was determined using Diagnostic Products Corporation's Coat-a-count assay.

Testosterone was assayed by the Mayo Clinic clinical laboratories. Total testosterone was determined by RIA using proprietary immunoglobulins. Free and bound testosterone was determined using equilibrium dialysis.<sup>111</sup>.

#### 3.3.2.3.2.3 Quality Assurance

Two methods were used to assess the accuracy and reliability of the laboratory assays. The laboratories routinely followed quality assurance programs. Three standards were run with each batch. If the control values were outside two standard deviations of the intra assay mean value for each standard, the assay was repeated. If 10 controls were outside 1 standard deviation of the mean, the assay was flagged for review. The reliability of each of these assays was assessed. For each assay, five specimens were randomly selected and split into two aliquots. The aliquots were labeled with different identifiers ensuring that the assays were carried out in a blinded fashion. Both aliquots were submitted on the same day to the laboratory. The coefficient of variation was calculated for each hormone.

#### 3.3.3 Analysis

There were two analytic strategies. First, assay results were treated as continuous parameters and modeled using regression methods. Models were fit to assess the relationship between assay results and total fluorine, body mass index, alcohol consumption, and smoking. Second, hormonal assay results were dichotomized into those within the reference range and those outside the reference range. The hormone assay categories were based on published sex specific normal reference values for each assay. The purpose of this dichotomization was to evaluate the possibility that highly susceptible individuals may be affected at lower levels of exposure and not follow the adjusted dose-response curve.

The relationships between total serum fluorine and the assayed parameters were estimated by fitting linear multivariate regression models to the data. The clinical parameters and ratios of selected parameters were first modeled as functions of nominally categorized exposure and covariates. Dependent variables that were

not normally distributed were appropriately transformed. Total serum fluorine was categorized into mutually distinct categories. Cutoff values for the categories were chosen to assure adequate numbers in each category while maintaining the fullest range of exposure values possible. Accordingly, total serum fluorine level categories were defined as the following: less than 1 ppm, greater than 1 ppm to less than 4 ppm, 4 ppm to 10 ppm, greater than 10 ppm to 15 ppm, and greater than 15 ppm. If insufficient numbers of events occurred within individual categories, the number of categories was reduced by combining adjacent categories. Additionally, models were fitted with total serum fluorine entered as a continuous variable using linear, square, square root transformations.

Age, body mass index (BMI), alcohol use and tobacco use were included in the model as potential confounders. Age was included in the models as both a categorical variable and a continuous variable. Age was grouped into four ten year age categories. Age was treated as a continuous variable using linear, square, square root, and log transformations. BMI was entered in the models as a categorical variable and as a continuous variable. BMI categories were less than 25 kg/m<sup>2</sup>, 25-30 kg/m<sup>2</sup>, and greater than 30 kg/m<sup>2</sup>. Additionally, BMI was dichotomized into obese, greater than 28 kg/m<sup>2</sup>, and non-obese, less than or equal to 28 kg/m<sup>2</sup>. The continuous variable was entered as linear, square, log, and square transformations. Alcohol use was categorized into 3 categories: less than 1 drink per day, greater than one to 3 drinks per day, and non response to the questionnaire item. Smoking was categorized as current nonsmokers and current smokers. A nonresponse category was not included since only two individuals were in this category. These two individuals were excluded from analyses that required smoking history. Smoking was quantified as cigarettes smoked per day. Linear, square and square root transformations of cigarettes per day were used in regression models.

The choice of the final model was somewhat subjective. For each dependent variable, other covariates were included in the final model if they were potential confounders. Other potential confounding hormones and biochemical parameters were included in the models if they produced significant changes in effect estimates.

Total serum fluorine and confounding covariates were entered into models as continuous variables. Significant nonlinear dose-response relationships were evaluated by comparing model fit and parameter estimates using categorical variables and continuous variables. Square, square root, exponential, and logarithmic transformations were used if the transformed variables produced models of superior predictive power as assessed by model fit. All two way interactions between total serum fluorine and the included covariates were evaluated. Interaction terms were included in the final model if the parameter estimate for the interaction term was significant at the  $\alpha = .10$  level.

The potential for susceptibility to confound the relationship between PFOA exposure and the assayed parameters was examined by comparing the observed prevalence of assay results outside of the reference range with the expected prevalence. The prevalence of abnormal assays was based on published reference values for the adult male US population. Reference ranges for test parameters were defined as being within 2 standard deviations above or below the mean value for the parameter. The laboratory maintains laboratory and assay specific reference range for each assay. Given that the distribution of values is approximately normal, about 2.5% of individual values are expected to fall above the upper limit and 2.5% below the lower limit. It follows that the prevalence for a high test is .025. The prevalence for a low value is .025. Using these prevalences, an expected number of tests outside of the reference range can be defined. A priori hypotheses based upon animal and in vitro studies defined the expected direction of the effect. The calculation of an observed to expected ratio allowed the estimation of the relative prevalence for a test outside of the normal range in the study subjects as compared to the general population. The 95% CI for the ratio was calculated assuming that the expected number is a constant and the observed number is a random variable with a Poisson distribution.

## 4. RESULTS

### 4.1 Cross Sectional Perfluorocarbon Physiologic Effects Study

In October 1990, at the time of the cross sectional study, the workforce at Chemolite consisted of 880 salaried and hourly employees. There were 50 men and 2 women in high exposure potential jobs. Since there were only 2 women in this group, the study was restricted to males. Forty-eight (96%) of the 50 male workers in high exposure potential jobs agreed to participate. The exact number of low exposure workers invited to participate in the study was not recorded. However, few individuals in this group refused to participate. Thus, it is estimated that over 80% of low exposure workers participated.

#### 4.1.1 Participant Characteristics

Since frequency matching for age was used to select study participants, the overall age distribution reflected the age distribution of workers in high exposure potential jobs (Table 4.1.1). Ages ranged from 24 to 59 years, with a median age of 37 years and a mean age of 39.2 years.

Table 4.1.2 presents the alcohol and tobacco use profile of the study participants. The light drinkers category included 22 participants who reported no alcohol use. Consumption of one to three ounces of ethanol per day was reported by 20 (18.7%) participants. No participants reported drinking greater than three ounces of ethanol per day. Eight workers (7.0%) did not complete this item of the questionnaire. There were 28 (24.8%) smokers who smoked an average of 21.7 cigarettes per day. Smoking status was not available for two workers (1.8%). The association between smoking and alcohol consumption is presented in Table 4.1.3. Thirteen (15.3%) of 85 nonsmokers and seven (25.0%) of 28 smokers reported moderate drinking ( $p=.24$ ). Table 4.1.4 displays the age distribution for alcohol and tobacco use categories. There were no significant differences in mean ages among smoking or drinking categories.

Total fluorine was not significantly correlated with age, BMI, alcohol, or tobacco use (Table 4.1.5). BMI and age were correlated ( $r=.26$ ,  $p=.005$ ). Alcohol use and tobacco use were not significantly correlated ( $r=.08$ ;  $p>.7$ ).

BMI ranged from 18.8 to 40.5 kg/m<sup>2</sup> with a median value of 26.3 kg/m<sup>2</sup> and a mean of 26.9 kg/m<sup>2</sup> (Table 4.1.6). Half of all workers had BMIs between 25 and 30 kg/m<sup>2</sup>. The mean BMI in smokers was not significantly different from that of nonsmokers (Table 4.1.7). The mean BMI for moderate drinkers was not significantly different from the BMI of light drinkers. Smoking status and BMI were not significantly associated (Table 4.1.8). There was a significant linear relationship between BMI and age ( $\beta=.10$  SE( $\beta$ )=.035). This relationship was not substantially altered after adjusting for smoking status, alcohol use, and total serum fluorine level.

#### 4.1.2 Total Serum Fluorine

The total serum fluorine values ranged from zero to 26 with a median value of two ppm, a mean of 3.27 ppm and a standard deviation of 4.68 ppm (Table 4.1.9). The inter-assay coefficient of variation was 66% calculated from repeated assays on different days.

Twenty-three (20.0%) of 115 workers had total serum fluorine values less than one ppm. This group included eight workers values reported as zero ppm (below the limits of detection). Eighty-eight workers (76.5%) had levels less than or equal to three ppm. Six (5.2%) of 115 workers had values between 10 and 15 ppm and five (4.4%) had values greater than 15 ppm. All workers with levels greater than ten had worked in Building 15, the primary PFC production area at the Chemolite Plant.

There were no significant differences in total serum fluoride mean values among the BMI, age, alcohol use and tobacco use categories (Table 4.1.10). No statistically significant differences in mean age between total fluorine categories were observed (Table 4.1.11).

Participants with less than one ppm total fluorine smoked the least (16.3) number of cigarettes per day (Table 4.1.12). Those with one ppm to three ppm total fluorine smoked the greatest number of cigarettes per day (24.5). This difference was statistically significant ( $p < .005$ ). As estimated in a regression model, the linear relationship between total fluorine and smoking status, adjusted for age and BMI, was small in magnitude ( $\beta = 0.10$ ,  $SE(\beta) = 0.062$ ,  $p = .09$ ). Smokers average total serum fluorine was estimated to be 0.1 ppm higher than nonsmokers. The number of cigarettes smoked per day was weakly correlated with total serum fluorine (Table 4.1.5).

Drinking status was not associated with total fluorine (Table 4.1.13). Overall, eight (7.0%) participants did not respond to this question. Four had less than one ppm total serum fluorine.

Table 4.1.14 presents the distribution of BMI in the total fluorine categories defined previously. BMI mean values were not significant differences among the total serum fluorine categories. The linear relationship between BMI and total fluorine, adjusted for age, smoking, and alcohol use, was weak and not significant ( $\beta = -.016$ ,  $SE(\beta) = .069$ ,  $p > .5$ ).

#### 4.1.3 Hormone Assays

The intra-assay coefficient of variation (CV) for the bound and free testosterone, estradiol, TSH, LH, prolactin, and FSH assays are provided in Table 4.1.15. The estradiol assay had the highest CV, 18.3%. The prolactin assay had the lowest CV of 3.1%.

Table 4.1.16 presents the observed and expected number of hormone assays out of the assay reference range, the observed to expected (O/E) ratio, and the 95% confidence limits. The O/E ratio was significantly greater than one for estradiol, free testosterone, bound testosterone and prolactin. The O/E ratios for LH, FSH, and TSH were not significantly different from one.

The Pearson correlation coefficients among the seven hormones assayed in study participants are presented in Table 4.1.17. As expected, estradiol was

correlated with free testosterone ( $r=.40$ ,  $p=.0001$ ) and bound testosterone ( $r=.32$ ,  $p=.0006$ ). Bound testosterone was correlated with free testosterone ( $r=.74$ ,  $p=.0001$ ), LH ( $r=.28$ ,  $p=.003$ ) and FSH ( $r=.16$ ,  $p=.04$ ). LH and FSH were significantly correlated ( $r=.63$ ,  $p=.0001$ ). FSH and TSH were significantly correlated ( $r=.23$ ,  $p=.01$ ).

As shown in Table 4.1.18, total fluorine was significantly correlated with prolactin ( $r=.19$ ,  $p=.045$ ) and TSH ( $r=.26$ ,  $p=.005$ ). Age was negatively correlated with estradiol ( $r=-.25$ ,  $p=.01$ ), free testosterone ( $r=-.45$ ,  $p=.0001$ ), bound testosterone ( $r=-.24$ ,  $p=.01$ ), and prolactin ( $r=-.19$ ,  $p=.01$ ). Age was positively correlated with FSH ( $r=.33$ ,  $p=.0003$ ). As expected, BMI was negatively correlated with free and bound testosterone ( $r=-.26$ ,  $p=.005$  and  $r=-.36$ ,  $p=.0001$  respectively). BMI was correlated positively with LH ( $r=.20$ ,  $p=.03$ ). Alcohol consumption was significantly correlated with FSH ( $r=-.24$ ,  $p=.01$ ).

Bound testosterone ranged from 141 to 1192 ng/dl with a mean of 572 ng/dl and a median of 561 ng/dl (Table 4.1.19). The standard deviations were large. The mean bound testosterone values were not significantly different among the total serum fluorine groups. As expected, the mean bound testosterone decreased significantly as BMI increased. The mean bound testosterone values were significantly different among the age categories ( $p=.016$ ).

There was a significant nonlinear relationship between total serum fluorine and bound testosterone (BT) in the final regression model (Table 4.1.20). Bound testosterone, which was positively associated with both LH and estradiol, decreased as both age and BMI increased. Alcohol and cigarette use were weakly associated with BT. There was a significant interaction between age and total serum fluorine. There was a negative association between bound testosterone and total serum fluorine in young workers than in older workers. In workers greater than 45 years of age, total serum fluorine was associated with a slight increase in BT. The relationship between bound testosterone and total serum fluorine is presented for four different sets of covariate value (Figure 2). Dose-response curves for bound testosterone were plotted for young, lean individuals aged 30 with BMIs of 25, young obese individuals aged 30 with BMIs of 35, middle aged lean individuals aged 50 with BMIs of 25, and middle aged



obese individuals aged 50 with BMIs of 35. Each of the relationships is for nonsmoking, light drinking men with the sample mean LH value (5.4 mU/l) and mean estradiol value (33.4 pg/ml). In 30 year old workers, bound testosterone decreased as total serum fluorine increased in both BMI groups. The dose-response relationship for 40 year old workers was approximately flat (not shown). In workers greater than 50 year of age, BT increased as total serum fluorine increased.

Total serum fluoride was not significantly associated with free testosterone (FT) (Table 4.1.21). Within BMI categories, free testosterone was highest in the less than 25 kg/m<sup>2</sup> group and lowest in the greater than 30 kg/m<sup>2</sup> category. The difference in mean FT among BMI categories was statistically significant (p=.03).

There was a significant nonlinear dose-response relationship between total serum fluorine and FT in the final regression model (Table 4.1.22). As total serum fluorine increased, free testosterone decreased. There was a significant interaction between age and total serum fluorine. Figure 4.2 illustrates the modifying effect of age on the total serum fluorine free testosterone relationship. The covariate vectors (nonsmoker, light drinker, mean LH and estradiol, age=30 and BMI=25 or 25, age=50 and BMI=25 or 35) were the same as used Figure 1. Lean or obese 50 year old men had low free testosterone (less than 9 ng/dl) for all values of total serum fluorine. In 30 year olds, free testosterone decreased asymptotically toward the 50 year old values. In this model, a 50 year old, obese, moderate drinker with any total serum fluorine level (the lower limit of assay sensitivity was approximately 1 ppm total serum fluorine) had free testosterone below nine ng/dl.

As shown in Table 4.1.23, the estradiol means in the three BMI groups were not significantly different (p=.88). As the age of participants increased, mean estradiol levels decreased. In the greater than 30 to 40 year age group, mean estradiol was 36.8 pg/ml compared to 25.9 pg/ml in the greater than 50 to 60 year age group. The age group means were significantly different (p=.018). There was a nonsignificant positive association between mean estradiol and total serum fluorine.

As shown in Table 4.1.24, estradiol and total serum fluorine were positively associated in the final regression model. Total serum fluorine followed a nonlinear relationship with estradiol. No interaction terms were statistically significant. As expected, free testosterone and estradiol were positively associated ( $\beta=.85$   $p=.0007$ ). The relationship between total serum fluorine and estradiol is illustrated in Figure 3. The plotted curves depict the relationship for lean ( $25 \text{ kg/m}^2$ ) and obese ( $35 \text{ kg/m}^2$ ) male workers who were 30 years old with sample mean free testosterone and who were nonsmokers and light drinkers. As total serum fluorine increased over the observed range, estradiol increased quadratically. In obese men ( $\text{BMI}=35 \text{ kg/m}^2$ ) aged 30, estradiol exceeded 44 pg/ml when total serum fluorine was between 15 and 20 ppm. The highest estradiol levels were in young, obese smokers who consumed 1 to 3 ounces of ethanol per day.

LH was not significantly associated with serum fluorine, but was negatively associated with BMI ( $p=.003$ ) and positively associated with smoking ( $p=.025$ ), age, and BT. There was no association between total serum fluorine and FT. (Table 4.1.25, Table 4.1.26, and Figure 4).

FSH was not significantly related to total serum fluorine levels but was positively associated with age ( $p=.014$ ) (Table 4.1.27, Table 4.1.28). The final regression model for FSH is illustrated in Figure 5. The relationship was essentially flat over the total fluorine range.

**TSH was positively associated with total serum fluorine in both univariate and multivariate analyses (Table 4.1.29, Table 4.1.30 and Figure 7). TSH was not significantly related to age, BMI, alcohol use, smoking, and other hormones.**

Prolactin was positively associated with total serum fluorine and smoking (Table 4.1.31, Table 4.1.32). Moderate drinkers had a different prolactin-total serum fluorine relationship compared to light drinkers and nonrespondents. Figure 6 illustrates the relationship of prolactin with total serum fluorine and the modifying effect of alcohol use. Total serum fluorine was weakly associated with prolactin in light and moderate drinkers. However, in moderate drinkers (1-3 oz/day), there was a positive association between prolactin and total serum fluorine.

#### 4.1.4 Hormone Ratios

The univariate distributions of the 21 ratios are provided in Appendices 4.1 and 4.2. A table is presented for each of the 21 ratios showing the number of participants, mean ratio value with the standard deviation, median ratio value, and the range of ratio values in each of the previously defined categories of BMI, age, alcohol use, tobacco use, and total serum fluorine

Correlations between total serum fluorine (ppm), age (years), BMI ( $\text{kg}/\text{m}^2$ ), alcohol use (oz/day), and cigarette consumption (cigarettes/day) and all possible ratios between E, free testosterone TF, TB, and LH are displayed in Table 4.1.33. The estradiol to bound testosterone ratio (E/TB) and estradiol to free testosterone ratio (E/TF) were significantly correlated with BMI ( $r=.32$ ,  $p=.001$  and  $r=.27$ ,  $p=.004$  respectively). The estradiol to luteinizing hormone ratio (E/LH) was negatively correlated with age ( $r=-.26$ ,  $p=.005$ ), and positively correlated with BMI ( $r=.18$ ,  $p=.06$ ). The bound testosterone to luteinizing hormone ratio (TB/LH) followed a different pattern as compared to E/LH. The correlation coefficient between TB/LH and age was  $-.32$  ( $p=.001$ ) while the coefficient between TB/LH and BMI was  $-.14$ , ( $p=.13$ ). The free testosterone to luteinizing hormone ratio (TF/LH) had the strongest correlation with age ( $r=-.40$ ,  $p=.0001$ ) but was not significantly correlated with BMI. The bound testosterone to free testosterone ratio (TB/TF) followed a unique pattern. TB/TF was positively correlated with age ( $r=.24$ ,  $p=.01$ ), and negatively correlated with BMI ( $r=-.16$ ,  $p=.08$ ).

Prolactin ratios with bound testosterone (TB/P), free testosterone (TF/P), estradiol (E/P), follicle stimulating hormone (FSH/P), luteinizing hormone (P/LH), and thyroid stimulating hormone (P/TSH) are presented in Table 4.1.34. None of the prolactin-hormone ratios were significantly correlated with total serum fluorine or BMI. All except P/TSH were significantly correlated with cigarette consumption.

Table 4.1.35 presents the Pearson correlation coefficients for the bound testosterone to thyroid stimulating hormone (TB/TSH) ratio, the free testosterone to thyroid stimulating hormone (TF/TSH), and the estradiol to thyroid stimulating

hormone (E/TSH). Total serum fluorine and TF/TSH were negatively correlated ( $r=-.18, p=.05$ ). All three ratios were significantly and negatively correlated with age. TB/TSH and TF/TSH were negatively correlated with BMI, ( $r=-.24, p=.01$  and  $r=-.23, p=.01$  respectively).

The Pearson correlation coefficients for the bound testosterone to follicle stimulating hormone (TB/FSH) ratio, the free testosterone to follicle stimulating hormone (TF/FSH), and the estradiol to follicle stimulating hormone (E/FSH) are provided in Table 4.1.36. Age was the only covariate that was significantly correlated with the three ratios.

The correlation coefficients for selected ratios between pituitary glycoprotein hormones, TSH, LH, and LH, are presented in Table 4.1.37. The thyroid stimulating hormone to follicle stimulating hormone (TSH/FSH), the thyroid stimulating hormone to luteinizing hormone (TSH/LH), and the follicle stimulating hormone to luteinizing hormone (FSH/LH) are provided. Age was significantly correlated with the FSH/LH ratio and the TSH/FSH ratio. Alcohol consumption was correlated with both TSH/FSH and TSH/LH.

As shown in the final regression models, the TB/TF ratio increased as total serum fluorine increased (Tables 4.38 and 4.39). Alcohol consumption, cigarette consumption, estradiol, prolactin, and TSH were not significantly related to the TB/TF ratio in either model. These covariates do not substantially alter the estimated relationship between total serum fluorine and TB/TF ratio when included in the regression model. The quadratic increase of the TB/TF ratio over the observed range of total serum fluorine is illustrated in Figure 4.8. The covariates used were: nonsmoker, less than one ounce of alcohol consumed per day, 30 years of age, and a BMI of  $30 \text{ kg/m}^2$ .

Table 4.1.40 presents the full regression model for the estradiol to bound testosterone ratio (E/TB). Total serum fluorine was not significantly associated with the E/TB ratio. BMI was a determinant of the E/TB ratio. Free testosterone was negatively related to the E/TB ratio.

The full regression model for estradiol to free testosterone ratio (E/TF) is displayed in Table 4.1.41. There was a significant positive dose-response relationship between the E/TF ratio and total serum fluorine. Although the dose-response relationship for free testosterone was modified by age, the dose-response relationship for the ratio was not modified by age.

As shown in Tables 4.1.42, 4.1.43 and 4.1.44, total serum fluorine was not significantly associated with E/LH and TB/LH, but was positively associated with the TF/LH ratio ( $\beta = -.05$ ,  $p = .09$ ). Bound testosterone and FSH were associated with the TF/LH ratio ( $\beta = .003$ ,  $p = .0001$ ) and ( $\beta = -.33$ ,  $p = .0001$ ).

Cigarette consumption and free testosterone were strongly and significantly related to the TB/P ratio ( $\beta = 1.49$ ,  $p = .02$  and  $\beta = 3.93$ ,  $p = .008$  respectively) (Table 4.1.45). Cigarette consumption and bound testosterone were significantly related to the TF/P ratio ( $\beta = .04$ ,  $p = .03$  and  $\beta = .002$ ,  $p = .03$  respectively) (Table 4.1.46). Only cigarette consumption was significantly related to the E/P ratio ( $\beta = .10$ ,  $p = .005$ ) (Table 4.1.47).

Tables 4.48 through 4.50 present full regression models for the ratios of prolactin to FSH (P/FSH), prolactin to LH (P/LH), and prolactin to TSH (P/TSH). In each of the three regression models total serum fluorine was positively and significantly associated with the prolactin-hormone ratio. Moderate drinkers had a significantly different ratio total serum fluorine dose-response relationship compared to the relationships in light drinker and nonrespondents.

The full regression models for the glycoprotein hormone ratios are presented in Table 4.1.51 to 4.1.59. As shown in table 4.1.52, total serum fluorine was significantly related to TF/TSH ( $\beta = -.28$ ,  $p = .03$ ) and bound testosterone and FSH were significantly related to the TF/TSH ratio ( $\beta = .01$ ,  $p = .006$  and  $\beta = .68$ ,  $p = .04$  respectively). Total serum fluorine was not significantly associated with the other glycoprotein hormone ratios.

#### 4.1.5 Cholesterol, Low Density Lipoprotein, High Density Lipoprotein, And Triglycerides

Table 4.1.60 provides the correlation coefficients for serum lipids, specifically cholesterol, low density lipoprotein (LDL), and high density lipoprotein (HDL), with total serum fluorine, age, BMI, alcohol consumption, and cigarette consumption. Total serum fluorine was not significantly correlated with cholesterol, LDL, HDL, or triglycerides. Cholesterol and triglycerides were correlated with age ( $r=.25$ ,  $p=.008$  and  $r=.19$ ,  $p=.04$ , respectively), and BMI ( $r=.19$ ,  $p=.04$  and  $r=.27$ ,  $p=.004$ , respectively). Cigarette smoking was positively and significantly correlated with cholesterol ( $r=.35$ ,  $p=.0001$ ), LDL ( $r=.28$ ,  $p=.002$ ), and triglycerides ( $r=.19$ ,  $p=.04$ ). HDL was not significantly correlated with any variable, although the correlation with alcohol consumption was suggestive ( $r=.18$ ,  $p=.06$ ).

Total fluorine was not significantly associated with cholesterol, LDL or triglycerides (Tables 4.1.61, Table 4.1.62, Table 4.1.64). Smoking, age, and GGT were positively and significantly associated with cholesterol. Smoking and prolactin were positively and significantly associated with LDL. Smoking and free testosterone were positively associated and bound testosterone was negatively associated with triglycerides.

The final regression model for HDL, displayed in Table 4.1.63, presents a different picture. HDL decreased as total fluorine increased in moderate drinkers. In light drinkers, there was a negligible change in HDL as total fluorine increased. Self-reported moderate alcohol consumption was positively associated with HDL. Additionally, bound testosterone was positively associated with HDL, while free testosterone was negatively associated.

#### 4.1.6 Hepatic Parameters: Serum Glutamic Oxaloacetic Transaminase (SGOT), Serum Glutamic Pyruvic Transaminase (SGPT), Alkaline Phosphatase (AKPH), Gamma Glutamyl Transferase (GGT).

Table 4.1.65 presents the correlation coefficients between the hepatic parameters, SGOT, SGPT, GGT, AKPH, and total serum fluorine, age, BMI, alcohol consumption, and cigarette consumption. The hepatic parameters were not significantly correlated with total serum fluorine. SGOT was not significantly correlated with any of the participant characteristics. SGPT and GGT were correlated significantly only with BMI ( $r=.20$ ,  $p=.02$  and  $r=.27$ ,  $p=.004$

respectively). AKPH was significantly correlated with age, BMI, alcohol consumption, and cigarette consumption.

The correlation coefficients between the hepatic parameters and cholesterol, LDL, HDL, triglycerides, estradiol, TF, TB, and prolactin are displayed in Table 4.1.66. SGOT and AKPH were significantly correlated with prolactin. SGPT was correlated with cholesterol and triglycerides. GGT was correlated with cholesterol, triglycerides, and free testosterone. As expected, SGOT, SGPT, and GGT were highly correlated (Table 4.1.67). AKPH was only correlated with GGT.

The SGOT, SGPT, GGT, and AKPH mean values were not significantly different among the five total serum fluorine categories (Table 4.1.68). SGOT and SGPT mean values were not significantly different for BMI, age, alcohol use, and smoking (Tables 4.1.69 to 4.1.72). Mean GGT was significantly higher in the greater than thirty BMI group ( $p=.03$ ). As shown in Table 4.1.72, mean and median AKPH values were significantly higher in smokers compared to nonsmokers ( $p=.012$ ).

Tables 4.1.73 A, B, and C present three linear multiple regression models for SGOT. In non-obese workers (BMI=25), SGOT decreased as total fluorine increased. In obese workers (BMI= 35), the association between total serum fluorine and SGOT was in the opposite direction. Model 2 included GGT as a covariate (Table 4.1.73 B). The association between total fluorine and SGOT, as well as the effect modification by BMI, were present after adjusting for GGT. When SGPT was included in the regression model (Table 4.1.73 C), the association between total fluorine and SGOT was weak and nonsignificant. The effect modification by BMI was no longer present. AKPH had little effect on the regression estimates when included in the model.

Three linear multiple regression models for SGPT are provided in Tables 4.1.74 A, B, and C. In non-obese workers (BMI=25), SGPT decreased as total fluorine increased. However, in obese workers (BMI= 35), the association between total serum fluorine and SGPT was in the opposite direction. Little change occurred in the estimates after adjusting for GGT. As seen in Table 4.1.74 C, the association was significant, although weaker in strength, after adjusting for SGOT. The effect

modification by BMI was present. When AKPH was included in the model, effect estimates did not change significantly.

The final regression models for GGT, provided in Tables 4.75 A, B, and C, present a different picture. GGT decreased as total fluorine increased in moderate drinkers. In light drinkers, GGT decreased less steeply as total fluorine increased. Controlling for SGOT and SGPT (model 2 and 3) did not significantly alter the relationship between total fluorine and GGT. Moderate alcohol consumption was positively associated with GGT.

Table 4.1.76 presents the final regression model for AKPH. In nonsmokers, total serum fluorine was negatively associated with AKPH. As the number of cigarettes smoked per day increased to more than five per day, AKPH increased as total serum fluorine increased.

4.1.7 Hematology Parameters: Hemoglobin, White Blood Count, Polymorphonuclear Leukocyte Count, Band Count, Eosinophil Count, Lymphocyte Count, Monocyte Count, Platelet Count, And Basophil Count.

Table 4.1.77 presents the correlation coefficients between the nine hematology parameters and total serum fluorine, age, BMI, alcohol use, and cigarette consumption. The only parameter that was significantly correlated with total serum fluorine was lymphocyte count ( $r=.19$ ,  $p=.04$ ). Monocyte count was correlated with BMI ( $r=-.22$ ,  $p=.04$ ) and alcohol consumption, ( $r=-.21$ ,  $p=.03$ ). All the parameters, except the basophil and band counts, were strongly associated with cigarette consumption. Alcohol consumption was correlated with hemoglobin, ( $r=-.20$ ,  $p=.04$ ), and band count ( $r=.26$ ,  $p=.005$ ).

The final regression models for hemoglobin and the erythrocyte indices, mean corpuscular hemoglobin (MCH) and mean corpuscular volume (MCV), are presented in Tables 4.1.78, 4.1.79, and 4.1.80 respectively. Total serum fluorine was significantly associated with hemoglobin.. The association hemoglobin and MCV were modified by smoking. In smokers who smoked seven or more cigarettes per day, hemaglobin and MCV increased significantly as total fluorine increased. In nonsmokers, hemaglobin and MCVdecreased as total fluorine



increased . The association of total fluorine with MCH was modified by smoking and by alcohol use. The increase in MCH as total fluorine increased was enhanced with increased smoking. In light drinkers, total serum fluorine had a weak association with MCH. In moderate drinkers, MCH increased as total fluorine increased. There was a positive association of both MCH and MCV with alcohol consumption. None of the estimated associations are of clinically significant magnitude over the range of total fluorine values.

The white blood cell count (WBC) increased significantly in nonrespondents as total fluorine increased above 2ppm, increased less in moderate drinkers, and increased the least in light drinkers (Table 4.1.81). As expected, cigarette smoking intensity was positively associated with WBC. PMN increased significantly in alcohol use nonrespondents as total fluorine increased and increased less steeply in moderate drinkers (Table 4.1.82). In light drinkers, total serum fluoride above 10 ppm was associated with a decreased in PMN. Cigarette smoking was positively associated with PMN. As shown in Table 4.1.83, the final regression models for band count provides little evidence that total fluorine was associated with band count. Moderate alcohol use was estimated to reduce the band count. Smoking was positively associated with band count.

The negative association between total fluorine and lymphocyte count was modified by adiposity, alcohol consumption, and cigarette smoking (Table 4.1.84). The decrease in lymphocyte count was smaller as BMI increased. The decrease in lymphocyte count associated with total fluorine above 3 ppm was greater in moderate drinkers compared to nonrespondents. As cigarette consumption increased, the decrease in lymphocyte count increased.

The positive association between total fluorine and monocyte count (MONO) was modified by adiposity (Table 4.1.85). As BMI increased, the association with MONO was weaker. Cigarette smoking and LH were positively associated with MONO. Alcohol consumption was negatively associated with MONO. The association between total fluorine and eosinophil count (EOS) was negative for nonsmokers, but was positive as more than ten cigarettes per day were smoked

(Table 4.1.86). As smoking increased, the PFOA associated decrease in BASO was smaller (Table 4.1.88).

The association between total fluorine and platelet count (PLAT) was modified by adiposity and cigarette smoking intensity (Table 4.1.87.). In lean participants (BMI=25), PLAT increased as total fluorine increased. In obese participants (BMI=40), the PLAT decreased as total fluorine increased. As smoking increased, the rate of increase in PLAT associated with total fluorine above 10 ppm decreased.

#### 4.1.8 Summary Of Results

The serum fluorine levels in Chemolite workers were 20-100 times higher than expected in workers not directly involved in PFOA production. All workers with levels above 10 ppm fluorine work in PFOA production areas. Smoking was associated with a small increase in serum fluorine. Age was not associated with serum fluorine levels. The two women employed in the PFOA production areas had total serum fluorine levels similar to men.

Alcohol use, smoking, age, BMI, and hormones had the expected associations with peripheral leukocyte counts, hematology parameters, cholesterol, HDL, LDL, and hepatic enzymes.

The main hormone results are:

1. The number of male workers with hormone values outside of the laboratory reference range was greater than expected for estradiol, free testosterone, bound testosterone, and prolactin.
2. Total serum fluorine was negatively associated with free testosterone and positively associated with estradiol. No association was noted between total serum fluorine and LH.
3. E/TF and TB/TF, but not E/TB, were positively associated with total serum fluorine.
4. E/LH and TB/LH were not associated with total serum fluorine. However, the relationship between total serum fluorine and TF/LH was suggestive.

5. TSH was positively associated with total serum fluorine. TF/TSH was negatively associated with total serum fluorine; TB/TSH and E/TSH were not.
6. Prolactin and total serum fluorine were positively associated in moderate drinkers, but not in light drinkers.
7. P/FSH, P/LH, P/TSH were positively associated with total serum fluorine. TB/P, TF/P, and E/P were not associated with total serum fluorine

The main hepatic parameter results are:

1. The increase in SGOT and SGPT levels associated with adiposity was enhanced by total serum fluorine.
2. The induction of GGT by alcohol was decreased as total serum fluorine increased.
3. The induction of AKPH by smoking was increased by increasing levels of total serum fluorine.

The main cholesterol and lipoprotein results are:

1. Cholesterol and triglyceride levels were not associated with total serum fluorine.
2. LDL was not associated with total serum fluorine.
3. The positive association between moderate alcohol use and HDL levels was reduced as total serum fluorine increased.

The main hematology parameter and peripheral leukocyte count results are:

1. The effect of smoking on hemoglobin and MCV was enhanced by total serum fluorine.
2. Total serum fluorine was negatively associated with all peripheral leukocyte counts except PMNs and MONOs, which were positively associated.
3. The associations between cell counts and total serum fluorine were modified by smoking, drinking, and adiposity.

#### 4.2 The 1990 Chemolite Retrospective Cohort Mortality Study

A total of 3,537 individuals who were employed at the Chemolite plant between January 1, 1947 and December 31, 1983 were identified from company records. The cohort consisted of 2,788 (79%) male and 749 (21%) females employees (Tables 4.2.1 and 4.2.2). The majority of women (67.3%) never worked in the Chemical Division. Of the 19,309 person years (PY) observed for women, 68.8% occurred in those who were never employed in the Chemical Division. The mean follow-up for women was 25.8 years in the overall cohort, 24.6 years in the Chemical Division (CD) cohort, and 26.4 years in the non-CD cohort. The distribution of follow-up periods was similar in the women's CD and non-CD cohorts.

The women's mean age at first employment was 27.6 years. Sixty-eight percent were less than 30 years old at employment; 9.7% were older than 40 at first employment at Chemolite. The CD cohort was slightly older than the non-CD cohort. The CD and non-CD distributions of latency times were not statistically different ( $p=.66$ ). The mean duration of employment for women was 8.7 years and ranged from six months to 41.4 years. The distribution of years of employment was significantly different for CD and non-CD women ( $p<.0001$ ). Of non-CD women, 11.9% were employed for more than twenty years. Of 245 women in the CD cohort, 51(21.1%) were employed for more than twenty years.

As shown in Table 4.2.2, the 2,788 men who were ever employed for more than six months at Chemolite contributed a total of 71,117.7 PY which was about equally divided between the CD and non-CD cohorts. The mean follow-up for the overall male cohort was 25.5 years. The distribution of follow-up periods and distribution of year of first employment was similar in the male CD and non-CD cohorts. The average age at death was higher in the male non-CD group, 58.1 years, compared to the CD group, 54.2 years. The duration of employment for men (mean 13.6 years, median 9.8 years) was longer than for women. The distribution of years of employment was significantly different for CD and non-CD men ( $p<.0001$ ). Of non-CD men, 25.5% were employed for longer than twenty

years. Of men in the CD cohort, 38.0% were employed for longer than twenty years.

Vital status was obtained for 100% of the women's cohort (Table 4.2.3). Among the 749 women there were 50 deaths; 11 in the CD cohort and 39 in the non-CD cohort. Vital status was obtained for 100% of the men's cohort. Among the 2788 men there were 348 deaths; 148 deaths in the CD group and 200 in the non-CD group. Six individuals who had employment records that were missing information were excluded from the cohort and their vital status was not ascertained. Death certificates were obtained for 99.5% of deaths. Two deaths occurred outside the U.S. and causes of death were ascertained by other means.

#### 4.2.1 Standardized Mortality Ratios (SMRs)

##### 4.2.1.1 SMRs For Women

The numbers of deaths, the SMRs and 95% confidence intervals (CI) among women in the 1947-1989 follow-up period are shown in Table 4.2.5. The SMRs for all causes of death (SMR=.75, 95% CI .56-.99), and cancer (SMR=.71, 95% CI .42-1.14) were significantly lower than expected in comparison to national rates. No association was found with duration of employment or latency for deaths from all causes, cancer, and cardiovascular diseases (Tables 4.2.6 and 4.2.7). SMRs for CD women and non-CD women are displayed in Table 4.2.8. The estimated SMR for the CD cohort of women were less than expected. In CD women, the all causes SMR was .46 (95% CI .23,.86) and the cancer SMR was .31 (95% CI .07,1.05). The SMRs for the non-CD women were closer to unity.

##### 4.2.1.2 SMRs For Men

The number of male deaths, the expected number of male deaths based on U.S. national white male rates, and age and calendar period adjusted SMRs with associated 95% CIs are presented in Table 4.2.9. The SMR for all causes (.73, 95% CI .66,.81), for cardiovascular diseases (SMR=.71, 95% CI .60,.86), for all gastrointestinal (GI) diseases (.50,95% CI .26,.87) and for all respiratory diseases (.50,95% CI .27,.86) were significantly less than one. None of the

cause-specific SMRs were large nor were the estimates significantly different from one. As shown in Table 4.2.10, the results were similar when the expected numbers of male deaths was based on Minnesota white male rates.

Table 4.2.11, Table 4.2.12, and Table 4.2.13 present adjusted SMRs and 95% CI for males based on Minnesota mortality rates for three latency intervals 10, 15, and 20 years respectively. The three latency intervals the all causes SMR ranged from .75 to .77. For all cancers, SMRs ranged from 1.06 to 1.12 and were nonsignificant. Among men there was no association between any cause of death and duration of employment (Table 4.2.14, Table 4.2.15, and Table 4.2.16).

Table 4.2.17 and 4.2.18 display the SMRs and 95% CI for CD and non-CD male workers. The all causes SMRs were .69 (.59,.79) for the non CD group and .86 (.72,1.01) for the CD group. The SMRs for prostate cancer, based on a comparison with Minnesota population rates, were 2.03 (95% CI .55,4.59) in the CD group and .58 (95% CI .07,2.09) in the non-CD cohort. There were 4 observed deaths from prostate cancer compared to 2 expected in the CD group. The latency analysis for non-CD and CD men are presented in Tables 4.2.19 and 4.2.20. There was no associations between any cause of death and latency in either group.

As shown in Table 4.2.21 and 4.2.22, male CD cohort members with more than 10 or more than 20 years of employment had SMRs that were less than one for all causes of death, all malignancy, cardiovascular diseases and all respiratory diseases. Among male non-CD cohort members with more than ten years of employment or more 20 years of employment, the SMRs for all causes, cardiovascular disease and all respiratory diseases were significantly less than expected (Table 4.2.23 and 4.2.24). There was no association of any cause of death with duration of employment at Chemolite in either CD or non-CD groups.

#### 4.2.2 Standardized Rate Ratios (SRRs)

Age adjusted standardized rate ratios (SRRs) were calculated for all causes, all cancer, and cardiovascular diseases mortality comparing men employed at the

plant for ten years or more to men employed for less than ten years. The SRRs are presented in Table 4.2.25. The 95% CIs for all causes, all cancer, and all cardiovascular diseases were wide and include one. Confounding variables such as year of first employment and length of follow-up were not controlled in this analysis due to small numbers and unstable rates within the large number of strata.

Table 4.2.26 presents the age adjusted SRRs for all causes, all cancers, lung cancer, GI cancer, and all cardiovascular diseases mortality comparing men ever employed in the CD with men never employed in the CD. All SRRs were slightly greater than one, however, none was statistically significant.

#### 4.2.3 Mantel- Relative Risks (RRMH)

Age stratified RRMH, contrasting the rates in men ever employed in the CD compared to the rates in men never employed in the CD, were calculated for all causes, all cancer, and all cardiovascular diseases mortality and are displayed in Table 4.2.27. The estimated RR for CD employment versus non-CD employment did not follow a monotonic pattern and the 95% CIs include one for each of the three endpoints.

Table 4.2.28 presents the RRMH for men employed for less than ten years to those employed for more than ten years. The all causes RRMH (2.16, 95% CI 1.52, 2.70) in the 30 to 39 year age at first employment strata was reflected in both the RRMH for all cancers (1.75, 95% CI 1.19, 2.61) and cardiovascular diseases (3.53, 95% CI 1.68, 6.21). The RRMH were not adjusted for important time covariates such as the year of first employment.

#### 4.2.4 Proportional Hazard Regression Model Relative Risk Estimates

##### 4.2.4.1 Proportional Hazard Models For Male Workers

Table 4.2.29 to 4.2.36 show the final proportional hazard (PH) model for death from all causes, cardiovascular diseases, all cancers, lung cancer, GI cancer, prostate cancer, pancreatic cancer, and diabetes among the 2788 male workers

ever employed at Chemolite for greater than six months. There was no evidence for violation of the PH assumptions or for significant nonlinear associations between the independent variables and mortality. As expected, age at first employment was positively associated with all causes of death. The RR for a one year increase in age at first employment was 1.082 (95% CI 1.069,1.094). Year of first employment and duration of employment were negatively associated with all causes mortality. The risk of death associated with months in the Chemical Division was small and nonsignificant.

For cardiovascular diseases mortality, the RR for a one year increase in age at first employment was 1.126 (95% CI 1.069,1.094). Year of first employment was negatively associated with cardiovascular diseases mortality. Time in the CD was not associated with death from cardiovascular diseases.

Age at first employment was positively associated with cancer mortality. The RR for a one year increase in age of employment was 1.08 (95% CI 1.06,1.10). Duration of employment was negatively associated with cancer. The RR was .972 (95% CI .96,.99) for a one year increase in employment. There was no association of cancer mortality with employment time in the CD.

The final prostate cancer mortality proportional hazard model for male cohort members is shown in Table 4.2.34. Time in the Chemical Division was positively and significantly associated with prostate cancer mortality. The relative risk for a one year increase in CD employment time was 1.13 (95% CI 1.01,1.43). Age at first employment was positively associated with prostate cancer mortality risk. A one year increase in age at first employment was associated with a RR of 1.09 (95% CI .99,1.19). The RR for lung cancer mortality was 1.07 (95% CI .03,1.12) for a one year increase in age of employment. Months in the chemical division was not significantly associated with lung cancer mortality. Table 4.2.33 shows the final proportional hazard (PH) model for all GI cancer mortality. The estimated RR for a one year increase in age at first employment was 1.14 (95% CI 1.09,1.19). Year of first employment, duration of employment and time employed in the CD were not associated with GI cancer risk. Age at first employment was positively associated with pancreatic cancer mortality. The other covariates were weakly associated with pancreatic cancer risk and were



not significantly different from one. A one year increase in age at first employment was positively associated with diabetes mortality (RR = 1.10, 95% CI 1.01,1.19).

#### 4.2.4.2 Proportional Hazard Models For Female Workers

Table 4.2.37, 4.2.38 and 4.2.39 show the final PH model for death from all causes, cardiovascular diseases and all cancers among the 749 female cohort members. Age at first employment was positively associated with all causes mortality. The RR for all causes of death among women employed for two to ten years (3.72) and among women employed for greater than ten years (2.33) were significantly greater than the all causes mortality in women employed for less than two years. Time in the CD was not related to mortality. The RR for death from cardiovascular diseases associated with a one year increase in age at first employment was 1.13 (1.07,1.18). The year at first employment, duration of employment, and time in the CD were not significantly associated with female cardiovascular diseases mortality. The RR for death from cancer was associated with age at first employment. A one year increase in age at first employment increase the RR for death from cancer (1.09 (1.04,1.14). The year at first employment, duration of employment, and time in the chemical division were weakly and non-significantly associated with female cancer mortality.

4.3 Physiologic Effects Tables

**TABLE 4.1.1 AGE DISTRIBUTION IN FIVE YEAR AGE GROUPS  
3M CHEMOLITE PLANT, COTTAGE GROVE, MINNESOTA**

<b>AGE</b>	<b>NUMBER</b>	<b>PERCENT</b>
<b>21-25</b>	<b>3</b>	<b>2.6</b>
<b>26-30</b>	<b>18</b>	<b>15.7</b>
<b>31-35</b>	<b>26</b>	<b>22.6</b>
<b>36-40</b>	<b>22</b>	<b>19.1</b>
<b>41-45</b>	<b>18</b>	<b>15.7</b>
<b>46-50</b>	<b>9</b>	<b>7.8</b>
<b>51-55</b>	<b>13</b>	<b>11.3</b>
<b>56-60</b>	<b>6</b>	<b>5.2</b>
<b>TOTAL</b>	<b>115</b>	<b>100.0</b>
<b>MEAN</b>	<b>39.2</b>	
<b>SD</b>	<b>8.91</b>	
<b>MEDIAN</b>	<b>37</b>	
<b>RANGE</b>	<b>24-59</b>	

**TABLE 4.1.2 DISTRIBUTION OF ALCOHOL AND TOBACCO USE  
3M CHEMOLITE PLANT, COTTAGE GROVE, MINNESOTA**

<b>USE STATUS</b>	<b>NUMBER</b>	<b>PERCENT</b>
<b>TOBACCO USE</b>		
CURRENT SMOKER	28	24.3
NONSMOKER	85	73.9
MISSING VALUES	2	1.8
<b>TOTAL</b>	<b>115</b>	<b>100.0</b>
<b>ALCOHOL USE</b>		
<1oz ETHANOL/DAY*	87	75.6
1-3oz ETHANOL/DAY	20	17.4
MISSING VALUES	8	7.0
<b>TOTAL</b>	<b>115</b>	<b>100.0</b>

\*Includes 22 nondrinkers

**TABLE 4.1.3 THE JOINT DISTRIBUTION OF TOBACCO AND ALCOHOL USE  
3M CHEMOLITE PLANT, COTTAGE GROVE, MINNESOTA**

	TOBACCO USE			TOTAL
	SMOKER	NONSMOKER	MISSING	
<b>ALCOHOL USE</b>				
<1oz/day	19 (67.9%)	67 (78.8%)	1 (50.0%)	87 (75.6%)
1-3oz/day	7 (25.0%)	13 (15.3%)	0 (0%)	20 (17.4%)
missing	2 (7.1%)	5 (5.9%)	1 (50.0%)	8 (7.0%)
<b>TOTAL</b>	<b>28 (100%)</b>	<b>85 (100%)</b>	<b>2 (100%)</b>	<b>115 (100%)</b>

TABLE 4.1.4 DISTRIBUTION OF AGE BY SMOKING AND DRINKING STATUS.  
3M CHEMOLITE PLANT, COTTAGE GROVE, MINNESOTA

	N	AGE(years)			RANGE	TEST#
		MEAN	SD	MEDIAN		
<b>Alcohol</b>						
<1oz/d	87	39.9	9.31	37	24-59	
1-3oz/d	20	37.5	6.95	37	27-51	*p=.29
missing	8	36.6	8.70	35	27-54	*p=.17
<b>Tobacco</b>						
smoker	28	40.4	7.59	39	28-54	
nonsmoker	85	39.0	9.35	37	24-59	*p=.47
missing	2	32.5	3.53	32	30-35	
<b>TOTAL</b>	<b>115</b>	<b>39.2</b>	<b>8.91</b>	<b>37</b>	<b>24-59</b>	

\*Student t test, Prob>T, reference groups <1oz/day, smoker

TABLE 4.1.5 PEARSON CORRELATION COEFFICIENTS BETWEEN TOTAL SERUM FLUORINE, AGE, BODY MASS INDEX (BMI), DAILY ALCOHOL USE, AND DAILY TOBACCO CONSUMPTION. 3M CHEMOLITE PLANT, COTTAGE GROVE, MINNESOTA

	TOTAL FLUORINE (ppm)	AGE (years)	BMI (kg/m <sup>2</sup> )	ALCOHOL (oz/day)	TOBACCO (cigs/day)
TOTAL FLUORINE	1	.004	.0002	-.007	.006
AGE	-	1	.26 p=.005	-.14	.15
BMI	-	-	1	.08	-.04
ALCOHOL	-	-	-	1	.08
TOBACCO	-	-	-	-	1

**TABLE 4.1.6 BODY MASS INDEX DISTRIBUTION  
3M CHEMOLITE PLANT, COTTAGE GROVE, MINNESOTA**

<b>BMI (kg/m<sup>2</sup>)</b>	<b>NUMBER</b>	<b>PERCENT</b>
>15-20	1	0.9
>20-25	40	34.8
>25-30	57	49.5
>30-35	15	13.0
>35-45	2	1.8
<b>TOTAL</b>	<b>115</b>	<b>100.0</b>
<b>MEAN BMI</b>	<b>26.9</b>	
<b>SD</b>	<b>3.4</b>	
<b>MEDIAN BMI</b>	<b>26.3</b>	
<b>RANGE</b>	<b>18.8-40.5</b>	

**TABLE 4.1.7 BODY MASS INDEX BY SMOKING AND DRINKING STATUS.  
3M CHEMOLITE PLANT, COTTAGE GROVE, MINNESOTA**

	N	BMI(kg/m <sup>2</sup> )		MEDIAN	RANGE	TEST#
		MEAN	SD			
<b>Alcohol</b>						
<1oz/d	87	26.9	3.54	26.1	18.8-40.5	*p=.71
1-3oz/d	20	27.2	3.10	27.0	22.8-33.7	
missing	8	25.9	3.64	26.1	21.3-30.4	
<b>Tobacco</b>						
smoker	28	26.6	3.63	26.3	18.8-28.2	*p=.57
nonsmoker	85	27.0	2.99	26.6	21.4-33.7	
missing	2	26.2	2.87	26.2	24.1-28.2	
<b>Total</b>	<b>115</b>	<b>26.9</b>	<b>3.45</b>	<b>26.3</b>	<b>18.8-40.5</b>	

\*Student t test, t test p-value, reference groups <1oz/day, smoker



TABLE 4.1.8 THE DISTRIBUTION OF AGE, ALCOHOL AND TOBACCO USE BY  
 BODY MASS INDEX  
 3M CHEMOLITE PLANT, COTTAGE GROVE, MINNESOTA

	BMI mg/kg <sup>2</sup>		
	<25	25-30	>30
<b>TOBACCO USE</b>			
SMOKER	11 (26.8%)	15 (26.3%)	2 (11.8%)
NONSMOKER	29 (70.7%)	41 (71.9%)	15 (88.2%)
MISSING	1 (2.5%)	1 (1.8%)	0 (0%)
TOTAL	41 (100%)	57 (100%)	17 (100%)
<b>ALCOHOL USE</b>			
<1 oz/day	31 (75.6%)	43 (75.4%)	13 (76.4%)
1-3 oz/day	6 (14.6%)	11 (19.3%)	3 (17.7%)
MISSING	4 (9.8%)	3 (5.3%)	1 (5.9%)
TOTAL	41 (100%)	57 (100%)	17 (100%)
<b>AGE</b>			
<40 years	31 (75.6%)	28 (49.1%)	6 (35.3%)*
>40 years	10 (24.4%)	29 (50.9%)	11 (64.7%)
TOTAL	41 (100%)	57 (100%)	17 (100%)

\*t test p=.005

**TABLE 4.1.9 TOTAL SERUM FLUORIDE DISTRIBUTION  
3M CHEMOLITE PLANT, COTTAGE GROVE, MINNESOTA**

<b>TOTAL FLUORINE (PPM)</b>	<b>NUMBER</b>	<b>PERCENT</b>
<1	23	20.0
1-3	65	56.5
>3-10	16	13.9
>10-15	6	5.2
>15-26	5	4.4
<b>TOTAL</b>	<b>115</b>	<b>100.0</b>
<b>MEAN TF</b>	<b>3.3</b>	
<b>SD</b>	<b>4.7</b>	
<b>MEDIAN TF</b>	<b>2</b>	
<b>RANGE</b>	<b>0-26</b>	

TABLE 4.1.10 TOTAL SERUM FLUORIDE BY BODY MASS INDEX, AGE,  
SMOKING AND DRINKING STATUS.  
3M CHEMOLITE PLANT, COTTAGE GROVE, MINNESOTA

	N(%)	MEAN	FLUORINE (ppm)		RANGE	TEST#
			SD	MEDIAN		
<b>BMI</b>						
<25	41(35.7)	2.8	3.74	2	0-19	F=1.47#
25-30	57(49.6)	4.0	5.47	2	0-26	P=.24
>30	17(14.8)	2.1	3.51	1	0-14	
<b>AGE</b>						
<31	21(18.3)	3.7	4.95	2	0-20	F=.10#
31-40	48(41.7)	3.2	4.08	2	0-14	P=.96
41-50	27(23.5)	3.3	4.26	2	0-19	
51-60	19(16.5)	3.0	6.42	1	0-26	
<b>Alcohol</b>						
<1oz/d	87(75.6)	3.4	5.15	2	0-26	p=.83*
1-3oz/d	20(17.4)	3.2	2.87	2	0-12	
missing	8(7.0)	2.1	2.53	1	0-6	
<b>Tobacco</b>						
smoker	28(24.8)	3.6	4.36	2	0-20	p=.66*
nonsmoker	85(75.2)	3.2	4.13	2	0-26	
missing	2(1.7)	3.0	4.24	3	0-6	
<b>TOTAL</b>	115	3.3	4.67	2	0-26	

#univariate Anova

\*Student t test, Prob>T

**TABLE 4.1.11 AGE DISTRIBUTION BY TOTAL SERUM FLUORINE  
CATEGORY.  
3M CHEMOLITE PLANT, COTTAGE GROVE, MINNESOTA**

	<b>TOTAL SERUM FLUORINE (ppm)</b>				
	<b>&lt;1</b>	<b>1-3</b>	<b>&gt;3-10</b>	<b>&gt;10-15</b>	<b>&gt;15-26</b>
<b>AGE</b>	<b>NUMBER (PERCENT)</b>				
<b>20-25</b>	1 (4.4)	1 (1.5)	0 (0)	1 (16.7)	0 (0)
<b>26-30</b>	3 (13.0)	10 (15.4)	4 (25.0)	0 (0)	1 (20.0)
<b>31-35</b>	6 (26.1)	13 (20.0)	4 (25.0)	2 (33.2)	1 (20.0)
<b>36-40</b>	4 (17.4)	12 (18.5)	5 (31.2)	0 (0)	1 (20.0)
<b>41-45</b>	2 (8.7)	13 (20.0)	2 (12.5)	1 (16.7)	0 (0)
<b>46-50</b>	0 (0)	7 (10.7)	0 (0)	1 (16.7)	1 (20.0)
<b>51-55</b>	6 (26.1)	6 (9.3)	0 (0)	1 (16.7)	0 (0)
<b>56-60</b>	1 (4.3)	3 (4.6)	1 (6.3)	0 (0)	1 (20.0)
<b>TOTAL</b>	23 (100)	65 (100)	16 (100)	6 (100)	5 (100)
<b>MEAN AGE SD</b>	39.9	39.6	36.0	39.3	41.6
	10.2	8.5	7.5	11.1	10.5
<b>MEDIAN AGE</b>	37	38	35.5	37.5	40
<b>AGE RANGE</b>	25-59	24-56	27-57	25-54	30-57

TABLE 4.1.12 DISTRIBUTION OF TOBACCO USE BY TOTAL SERUM FLUORIDE CATEGORY.  
3M CHEMOLITE PLANT, COTTAGE GROVE, MINNESOTA

TOTAL SERUM FLUORINE (ppm)						
	<1	1-3	>3-10	>10-15	>15-26	TOTAL
<b>Tobacco use</b>	NUMBER(%)					
Smoker	3 (13.0)	16 (24.6)	6 (37.5)	2 (33.3)	1 (20.0)	28 (24.3)
Nonsmoker	19 (82.7)	49 (75.4)	9 (56.2)	4 (66.7)	4 (80.0)	85 (73.9)
Missing	1 (4.3)	0 (0)	1 (6.3)	0 (0)	0 (0)	2 (1.7)
<b>Total</b>	<b>23 (100)</b>	<b>65 (100)</b>	<b>16 (100)</b>	<b>6 (100)</b>	<b>5 (100)</b>	<b>115 (100)</b>
<b>Cigarettes/day (among smokers)</b>						
MEAN	16.3	24.5*	18.0	20	20	21.5
SD	14.0	8.8	9.9	0	-	10.1
MEDIAN	17	20	20	20	20	20
RANGE	2-30	7-40	3-30	20	20	2-40

\*significantly different from <1 ppm mean (p<.005)

TABLE 4.1.13 DISTRIBUTION OF ALCOHOL USE BY TOTAL SERUM FLUORIDE CATEGORY.  
3M CHEMOLITE PLANT, COTTAGE GROVE, MINNESOTA

	TOTAL SERUM FLUORINE (ppm)				
	<1	1-3	>3-10	>10-15	>15-26
ALCOHOL USE	NUMBER (PERCENT)				
<1 oz/day	17 (73.9)	51 (78.5)	9 (56.3)	5 (83.3)	5 (100)
1-3 oz/day	2 (8.7)	13 (20.0)	4 (25.0)	1 (16.7)	0 (0)
MISSING	4 (17.4)	1 (1.5)	3 (18.7)	0 (0)	0 (0)
TOTAL	23 (100)	65 (100)	16 (100)	6 (100)	5 (100)

TABLE 4.1.14 BODY MASS INDEX DISTRIBUTION BY TOTAL SERUM FLUORINE CATEGORY.  
3M CHEMOLITE PLANT, COTTAGE GROVE, MINNESOTA

BMI(kg/m <sup>2</sup> )	TOTAL SERUM FLUORINE (ppm)				
	<1	1-3	>3-10	>10-15	>15-26
	NUMBER (PERCENT)				
>15-20	1 (4.4)	0 (0)	0 (0)	0 (0)	0 (0)
>20-25	9 (39.1)	21 (32.3)	8 (50.0)	1 (16.7)	1 (20.0)
>25-30	5 (21.7)	39 (60.0)	5 (31.2)	4 (66.6)	4 (80.0)
>30-35	7 (30.4)	5 (7.7)	3 (18.8)	0 (0)	0 (0)
>35-40	0 (0)	0 (0)	0 (0)	1 (16.7)	0 (0)
>40-45	1 (4.4)	0 (0)	0 (0)	0 (0)	0 (0)
TOTAL	23 (100)	65 (100)	16 (100)	6 (100)	5 (100)
MEAN BMI	27.6	26.6	26.3	29.4	26.0
SD	5.3	2.6	3.3	3.7	1.4
MEDIAN	27	26.8	25.7	29.8	25.6
BMI RANGE	18.8-40.5	22.5-33.7	21.4-32.5	24.5-35.5	24.1-27.6

TABLE 4.1.15 COEFFICIENT OF VARIATION FOR SEVEN HORMONE  
ASSAYS.  
3M CHEMOLITE PLANT, COTTAGE GROVE, MINNESOTA

HORMONE	CV
BOUND TESTOSTERONE	10.6%
FREE TESTOSTERONE	12.1%
ESTRADIOL	18.3%
TSH	10.0%
LH	8.6%
PROLACTIN	3.1%
FSH	5.6%



**TABLE 4.1.16 THE OBSERVED VERSUS EXPECTED NUMBER OF WORKERS WITH HORMONE ASSAYS OUTSIDE THE ASSAY REFERENCE RANGE. 3M CHEMOLITE PLANT, COTTAGE GROVE, MINNESOTA**

	<b>OBSERVED</b>	<b>EXPECTED</b>	<b>O/E*</b>	<b>95% CI**</b>
Estradiol >=44 pg/ml	17	2.8	6.0	(3.6,9.8)
Testosterone bound <=300 ng/dl	13	2.8	4.5	(2.6,8.1)
Testosterone free <=9 ng/dl	11	2.8	3.9	(2.0,7.1)
Prolactin >=15 ng/ml	10	2.8	3.5	(1.8,6.7)
LH 2-12 mU/ml	3	2.8	1.1	(0.3,3.3)
FSH 1-12 mU/ml	1	2.8	.4	(0.1,2.0)
TSH >=4.6 mU/ml	1	2.8	.4	(0.1,2.0)

\*O/E - OBSERVED TO EXPECTED RATIO  
 \*\*CI -95% CONFIDENCE INTERVAL

TABLE 4.1.17 PEARSON CORRELATION COEFFICIENTS BETWEEN SERUM HORMONES.  
3M CHEMOLITE PLANT, COTTAGE GROVE, MINNESOTA.

	ESTRADIOL	FREE TEST.	BOUND TEST.	PROLACTIN	LnLH++	FSH+	LnTSH#
ESTRADIOL@	1	.40 p=-.0001	.32 p=-.0006	.16 p=-.08	.06	-.14 p=-.15	.05
FREE TESTOSTERONE*	-	1	.74 p=-.0001	.13	.10	-.05	.07
BOUND TESTOSTERONE*	-	-	1	.21 p=-.03	.28 p=-.003	.16 p=-.04	-.02
PROLACTIN#	-	-	-	1	.15	.004	.11
LnLH++	-	-	-	-	1	.63 p=-.0001	-.15 p=-.11
FSH+	-	-	-	-	-	-	-.23 p=-.01

@pg/ml

\*ng/dl

#ng/ml

++LOG LUTENIZING HORMONE (mU/ml)

+ FOLLICLE STIMULATING HORMONE (mU/ml)

#LOG THYROID STIMULATING HORMONE (mU/ml)

**TABLE 4.1.18 PEARSON CORRELATION COEFFICIENTS BETWEEN TOTAL SERUM FLUORIDE, AGE, BODY MASS INDEX (BMI), DAILY ALCOHOL USE, DAILY TOBACCO CONSUMPTION, AND SERUM HORMONES. 3M CHEMOLITE PLANT, COTTAGE GROVE, MINNESOTA**

	TOTAL FLUORINE (ppm)	AGE (years)	BMI (kg/m <sup>2</sup> )	ALCOHOL (oz/day)	TOBACCO (cigs/day)
ESTRADIOL@	.13 p=.16	-.25 p=.01	-.01	.05	.12 p=.2
FREE TESTOSTERONE*	.03	-.45 p=.0001	-.26 p=.005	-.08	.05
BOUND TESTOSTERONE*	.08	-.24 p=.01	-.38 p=.0001	-.16 p=.11	.11
PROLACTIN#	.19 p=.045	-.19 p=.01	-.06	.03	-.16 p=.09
LnLH++	.04	.11	.20 p=.03	-.14	.18 p=.06
FSH+	-.03	.33 p=.0003	-.08	-.24 p=.01	.17 p=.06
LnTSH#	.26 p=.005	.09	.04	.15 p=.15	-.03

@pg/ml

\*ng/dl

#ng/ml

++LOG LUTENIZING HORMONE (mU/ml)

+ FOLLICLE STIMULATING HORMONE (mU/ml)

#LOG THYROID STIOMULATING HORMONE (mU/ml)

TABLE 4.1.19 BOUND TESTOSTERONE (TB) BY BODY MASS INDEX, AGE, SMOKING, DRINKING STATUS AND TOTAL SERUM FLUORIDE 3M CHEMOLITE PLANT, COTTAGE GROVE, MINNESOTA.

	N(%)	MEAN	TB(ng/dl) SD	MEDIAN	RANGE	TEST#
<b>BMI (kg/m<sup>2</sup>)</b>						
<25	40(35.4)	641	242.9	592	275-1192	F=5.64
25-30	58(49.8)	565	196.8	560	141-854	p=.005
>30	17(15.0)	436	172.7	438	210-803	
<b>Age</b>						
<31	20(17.7)	598	232.8	673	278-1192	F=3.60
31-40	48(42.5)	634	214.1	605	275-1189	p=.016
41-50	26(23.0)	512	185.8	498	141-947	
51-60	19(16.8)	470	226.1	409	210-954	
<b>Alcohol</b>						
<1oz/d	86(76.1)	581	212.5	574	210-1192	F=1.23
1-3oz/d	19(16.8)	484	215.1	417	141-1039	p=.27
missing	8(7.1)	690	272.8	602	409-1101	
<b>Tobacco</b>						
smoker	27(23.9)	622	177.7	617	379-1039	F=1.69
nonsmoker	84(74.3)	559	233.0	556	141-1192	p=.20
missing	2(1.8)	432	97.6	432	363-501	
<b>Total Fluorine</b>						
<1 ppm	23(20.4)	584	295.4	438	275-1192	F=0.39
1-3	64(56.6)	567	202.9	572	141-1039	p=.82
>3-10	15(13.3)	530	189.3	574	210-819	
>10-15	6(5.3)	600	234.8	563	244-947	
>15-26	5(4.4)	662	149.8	659	517-880	
<b>Total</b>	113(100)	572	220.7	561	141-1192	

#univariate Anova

**TABLE 4.1.20 LINEAR MULTIVARIATE REGRESSION MODEL OF FACTORS  
PREDICTING THE BOUND TESTOSTERONE (ng/dl) AMONG 112 MALE  
WORKERS.  
3M CHEMOLITE PLANT, COTTAGE GROVE, MINNESOTA**

<b>Variable</b>	<b>B</b>	<b>SE(B)</b>	<b>p-value</b>
Intercept	1027	190.7	.0001
Total Fluorine (ppm)*	-148	67.2	.05
Age (years)	-9	3.3	.009
Age X Total Fluoride*	3	1.6	.04
BMI (kg/m <sup>2</sup> )	-16	5.4	.003
Smoker**	74	45.0	.28
Alcohol (<1oz/day)#	89	47.5	.11
Estradiol (pg/ml)	2	1.0	.02
LH (mU/ml)	116	6.1	.004
Prolactin (ng/ml)	8	4.1	.04

R<sup>2</sup> = .39

\*Square root transformation of total serum fluoride measured in ppm.

\*\*Reference category is nonsmokers.

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

TABLE 4.1.21 FREE TESTOSTERONE (TF) BY BODY MASS INDEX, AGE, SMOKING AND DRINKING STATUS AND TOTAL SERUM FLUORIDE  
3M CHEMOLITE PLANT, COTTAGE GROVE, MINNESOTA

	N(%)	MEAN	TF(ng/dl) SD	MEDIAN	RANGE	TEST#
<b>BMI kg/m<sup>2</sup></b>						
<25	40(35.4)	17.4	6.22	16.7	7.4-45.3	F=3.58
25-30	56(49.6)	15.1	4.13	15.8	3.2-23.8	p=.03
>30	17(15.0)	13.7	6.08	13.5	5.6-30.5	
<b>Age years</b>						
<30	20(17.7)	18.7	7.64	16.7	9.3-45.3	F=9.14
31-40	48(42.5)	17.0	3.75	17.1	7.4-29.37	p=.0001
41-50	26(23.0)	14.1	4.73	14.3	3.2-23.8	
51-60	19(16.8)	11.5	3.78	11.5	5.6-19.0	
<b>Alcohol</b>						
<1 oz/d	86(76.1)	15.8	5.36	15.8	5.6-45.3	F=1.45
1-3 oz/d	19(16.8)	14.2	4.79	15.3	3.2-23.9	p=.23
missing	8(7.1)	18.1	6.40	17.2	11.0-29.7	
<b>Tobacco</b>						
smoker	27(23.9)	16.6	3.71	17.1	8.4-24.3	F=.95
nonsmoker	84(74.3)	15.4	5.84	15.3	3.2-45.3	p=.33
missing	2(1.8)	15.9	4.45	15.9	12.7-19.0	
<b>Total Fluorine</b>						
<1 ppm	23(20.4)	16.4	8.4	13.9	6.4-45.3	F=0.13
1-3	64(56.6)	15.6	4.5	15.8	3.2-30.5	p=.97
>3-10	15(13.3)	15.2	3.8	15.3	7.1-19.7	
>10-15	6(5.3)	15.9	5.2	17.6	5.6-19.9	
>15-26	5(4.4)	15.3	2.2	14.1	13.3-18.2	
<b>Total</b>	<b>113(100)</b>	<b>15.7</b>	<b>5.4</b>	<b>16</b>	<b>3.2-45.3</b>	

#univariate Anova

TABLE 4.1.22 LINEAR MULTIVARIATE REGRESSION MODEL OF FACTORS  
 PREDICTING THE FREE TESTOSTERONE VALUE (ng/dl)  
 AMONG 111 MALE WORKERS.  
 3M CHEMOLITE PLANT, COTTAGE GROVE, MINNESOTA

Variable	B	SE(B)	p-value
Intercept	29.72	4.57	.0001
Total Fluorine (ppm)*	-3.56	1.62	.03
Age (years)	-.34	.08	.0001
Age X Total Fluoride*	.07	.04	.05
BMI (kg/m <sup>2</sup> )	-.21	.13	.11
Smoker**	1.46	1.03	.16
Alcohol (<1 oz/day)#	1.65	1.14	.15
Estradiol (pg/ml)	.10	.03	.003
LH (mU/ml)	.18	.15	.20

R<sup>2</sup>= .39

\*Square root transformation of total serum fluoride measured in ppm.

\*\*Reference category is nonsmokers.

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

TABLE 4.1.23 PARTICIPANT ESTRADIOL BY BODY MASS INDEX, AGE, SMOKING DRINKING STATUS AND TOTAL SERUM FLUORIDE. 3M CHEMOLITE PLANT, COTTAGE GROVE, MINNESOTA

	N(%)	MEAN	ESTRADIOL (pg/ml)			TEST#
			SD	MEDIAN	RANGE	
<b>BMI (kg/m<sup>2</sup>)</b>						
<25	40(35.4)	34.1	12.91	40	8-89	F=.13
25-30	56(49.6)	33.2	13.89	33	8-83	p=.88
>30	17(15.0)	32.2	12.36	27	18-57	
<b>AGE</b>						
<30	20(17.7)	34.4	10.15	34	19-56	F=3.50
31-40	48(42.5)	36.8	11.54	36	12-69	p=.018
41-50	26(23.0)	31.6	18.48	28	8-83	
51-60	19(16.8)	25.9	7.93	24	15-47	
<b>Alcohol</b>						
<1 oz/d	86(76.1)	33.0	11.78	33	8-66	F=.14
1-3 oz/d	19(16.8)	31.8	16.61	30	8-69	p=.71
missing	8(7.1)	41.1	18.20	40	23-83	
<b>Tobacco</b>						
smoker	27(23.9)	36.3	17.40	34	14-83	F=.13
nonsmoker	84(74.3)	32.5	11.63	32	8-66	p=.88
missing	2(1.8)	30.5	13.44	30	21-40	
<b>Total Fluorine</b>						
<1 ppm	23(20.4)	36.2	13.1	34	14-60	F=1.27
>=1-3	64(56.6)	31.4	13.6	30	8-83	p=.29
>3-10	15(13.3)	32.8	10.6	34	10-58	
>10-15	6(5.3)	38.2	15.2	35.5	22-66	
>15-26	5(4.4)	41.2	11.4	42	26-56	
<b>Total</b>	<b>113(100)</b>	<b>33.4</b>	<b>13.2</b>	<b>33</b>	<b>8-83</b>	

#univariate Anova



**TABLE 4.1.24 LINEAR MULTIVARIATE REGRESSION MODEL OF FACTORS  
PREDICTING THE ESTRADIOL VALUE (pg/dl) AMONG 113 MALE WORKERS.  
3M CHEMOLITE PLANT, COTTAGE GROVE, MINNESOTA**

<b>Variable</b>	<b>B</b>	<b>SE(B)</b>	<b>p-value</b>
Intercept	12.89	12.13	.29
Total Fluorine (ppm)*	.03	.01	.03
Age (years)	-.22	.15	.14
BMI (kg/m <sup>2</sup> )	.51	.34	.14
Cigarettes/day	.16	.11	.15
Alcohol (<1oz/day)#	.09	.11	.98
Free Testosterone (ng/dl)	.85	.24	.0007

R<sup>2</sup> = .24

\*Square transformation of total serum fluoride measured in ppm.

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

TABLE 4.25 LUTENIZING HORMONE (LH) BY BODY MASS INDEX, AGE, SMOKING AND DRINKING STATUS, AND TOTAL SERUM FLUORINE  
3M CHEMOLITE PLANT, COTTAGE GROVE, MINNESOTA

	N(%)	MEAN	LH (mU/ml)		RANGE	TEST#
			SD	MEDIAN		
<b>BMI mg/kg<sup>2</sup></b>						
<25	40(35.4)	5.49	3.06	4.60	2.6-21.7	F=6.19
25-30	56(49.6)	5.84	3.25	5.15	1.7-23.0	p=.003
>30	17(15.0)	3.72	1.21	3.60	2.0-7.2	
<b>Age years</b>						
<30	20(17.7)	4.81	2.26	4.45	1.7-10.1	F=.69
31-40	48(42.5)	5.49	3.14	4.75	2.4-21.7	p=.58
41-50	28(23.0)	5.33	1.64	5.15	2.5-9.6	
51-60	19(16.8)	5.90	4.73	4.10	2.0-23.0	
<b>Alcohol</b>						
<1oz/d	86(76.1)	5.60	3.34	4.70	1.7-23.0	F=1.24
1-3oz/d	19(16.8)	4.69	1.80	4.21	2.3-10.1	p=.27
missing	8(7.1)	4.86	1.00	4.05	3.4-6.2	
<b>Tobacco</b>						
smoker	27(23.9)	6.30	3.78	5.30	2.6-21.7	F=5.16
nonsmoker	84(74.3)	5.05	2.71	4.52	1.7-23.0	p=.025
missing	2(1.8)	7.45	2.47	7.45	5.7-9.2	
<b>Total Fluorine</b>						
<1 ppm	23(20.4)	5.0	2.1	4.4	2.5-9.3	F=0.16
>=1-3	64(56.6)	5.6	3.6	4.8	1.7-23.0	p=.98
>3-10	15(13.3)	5.1	2.7	4.9	2.0-13.9	
>10-15	6(5.3)	5.4	0.3	4.9	3.7-7.5	
>15-26	5(4.4)	5.3	1.3	5.5	3.7-7.2	
<b>Total</b>	<b>113(100)</b>	<b>5.4</b>	<b>3.0</b>	<b>4.7</b>	<b>1.7-23.0</b>	

#univariate Anova

**TABLE 4.1.26 LINEAR MULTIVARIATE REGRESSION MODEL #1 OF  
FACTORS PREDICTING THE LUTENIZING HORMONE\* VALUE (mU/ml)  
AMONG 113 MALE WORKERS.  
3M CHEMOLITE PLANT, COTTAGE GROVE, MINNESOTA**

<b>Variable</b>	<b><math>\beta</math></b>	<b>SE(<math>\beta</math>)</b>	<b>p-value</b>
Intercept	1.26	.40	.002
Total Fluorine (ppm)*	.001	.008	.93
Age (years)	.01	.005	.03
BMI (kg/m <sup>2</sup> )	-.02	.01	.15
Smokers**	.24	.23	.29
Alcohol (<1oz/day)#	.06	.10	.60
Bound Testosterone (ng/dl)	.001	.0002	.008

R<sup>2</sup>= .28

\*logarithmic transformation of lutenizing hormone (LH).

\*\* Reference category is nonsmokers.

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

TABLE 4.1.27 FOLLICLE STIMULATING HORMONE (FSH) BY BODY MASS INDEX, AGE, SMOKING AND DRINKING STATUS, AND TOTAL SERUM FLUORINE  
3M CHEMOLITE PLANT, COTTAGE GROVE, MINNESOTA

	FSH(mU/ml)					TEST#
	N(%)	MEAN	SD	MEDIAN	RANGE	
<b>BMI mg/kg<sup>2</sup></b>						
<25	40(35.4)	5.02	2.39	4.6	1.8-10.3	F=1.27
25-30	56(49.6)	5.39	2.71	4.5	1.4-14.8	p=.29
>30	17(15.0)	4.31	1.75	3.9	1.6-8.3	
<b>Age years</b>						
<30	20(17.7)	3.88	1.86	3.6	1.4-9.8	F=3.72
31-40	48(42.5)	4.86	2.24	4.6	1.6-10.3	p=.014
41-50	26(23.0)	5.65	2.55	4.6	2.1-14.8	
51-60	19(16.8)	6.22	3.01	5.0	2.7-14.8	
<b>Alcohol</b>						
<1oz/d	86(76.1)	5.37	2.62	4.6	1.4-14.8	F=3.47
1-3oz/d	19(16.8)	4.18	1.92	3.9	2.0-9.8	p=.065
missing	8(7.1)	4.38	1.49	4.8	2.6-6.4	
<b>Tobacco</b>						
smoker	27(23.9)	5.77	2.46	4.9	2.6-11.9	F=2.80
nonsmoker	84(74.3)	4.85	4.49	4.2	1.4-14.8	p=.09
missing	2(1.8)	6.10	0.42	6.1	5.8-6.4	
<b>Total Fluorine</b>						
<1 ppm	23(20.4)	4.4	1.95	4.4	1.6-10.3	F=0.75
1-3	64(56.6)	5.4	2.75	4.6	1.4-14.8	p=.56
>3-10	15(13.3)	4.8	2.23	4.9	2.1-9.7	
>10-15	6(5.3)	5.4	2.14	4.4	3.5-8.9	
>15-26	5(4.4)	4.9	2.36	3.7	2.6-7.7	
<b>Total</b>	<b>113(100)</b>	<b>5.1</b>	<b>2.49</b>	<b>4.5</b>	<b>1.4-14.8</b>	

#univariate Anova

TABLE 4.1.28 LINEAR MULTIVARIATE REGRESSION MODEL OF FACTORS  
 PREDICTING THE FOLLICLE STIMULATING HORMONE VALUE (mU/ml)  
 AMONG 113 MALE WORKERS.  
 13M CHEMOLITE PLANT, COTTAGE GROVE, MINNESOTA

Variable	$\beta$	SE( $\beta$ )	p-value
Intercept	1.20	1.62	.46
Total Fluorine(ppm)*	.004	.04	.91
Age (years)	.08	.02	.0006
BMI (kg/m <sup>2</sup> )	-.04	.05	.41
Cigarettes/day	.02	.02	.29
Alcohol(<1oz/day)#	.45	.48	.34
TSH (mU/ml)@	-.43	.22	.05
LH (mU/ml)##	.44	.06	.0001

R<sup>2</sup> = .48

\*logarithmic transformation of follicle stimulating hormone (FSH).

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

@Thyroid Stimulating Hormone

##Lutienizing Hormone

TABLE 4.1.29 THYROID STIMULATING HORMONE (TSH) BY BODY MASS INDEX, AGE, SMOKING AND DRINKING STATUS, AND TOTAL SERUM FLUORINE.  
3M CHEMOLITE PLANT, COTTAGE GROVE, MINNESOTA

	N(%)	TSH(mU/ml)		MEDIAN	RANGE	TEST#
		MEAN	SD			
<b>BMI mg/kg<sup>2</sup></b>						
<25	40(35.4)	1.55	0.66	1.04	0.37-3.14	F=.35
25-30	56(49.6)	1.64	1.01	1.038	0.45-6.80	p=.70
>30	17(15.0)	1.72	0.71	1.55	0.62-3.23	
<b>Age years</b>						
<30	20(17.7)	1.43	0.56	1.42	0.38-2.33	F=.47
31-40	48(42.5)	1.66	1.04	1.46	0.37-6.80	p=.70
41-50	26(23.0)	1.64	0.75	1.34	0.75-3.56	
51-60	19(16.8)	1.70	0.74	1.53	0.62-3.09	
<b>Alcohol</b>						
<1oz/d	86(76.1)	1.57	0.70	1.40	0.38-3.56	F=1.23
1-3oz/d	19(16.8)	1.93	1.39	1.55	0.60-6.80	p=.27
missing	8(7.1)	1.49	0.63	1.61	0.37-2.22	
<b>Tobacco</b>						
smoker	27(23.9)	1.53	0.61	1.37	0.61-3.03	F=.09
nonsmoker	84(74.3)	1.66	0.92	1.49	0.37-6.80	p=.76
missing	2(1.8)	1.28	0.42	1.28	0.98-1.57	
<b>Total fluorine</b>						
<1 ppm	23(20.4)	1.5	0.64	1.5	0.3-3.3	F=2.30
>=1-3	64(56.6)	1.6	0.94	1.3	0.4-6.8	p=.08
>3-10	15(13.3)	1.6	0.67	1.4	0.6-3.0	
>10-15	6(5.3)	2.4	0.87	2.5	0.83-3.5	
>15-26	5(4.4)	2.2	1.66	2.1	1.7-3.5	
<b>Total</b>	<b>113(100)</b>	<b>1.6</b>	<b>0.85</b>	<b>1.4</b>	<b>0.3-6.8</b>	

#univariate Anova

TABLE 4.1.30 LINEAR MULTIVARIATE REGRESSION MODEL OF FACTORS  
 PREDICTING THE THYROID STIMULATING HORMONE\* VALUE (mU/ml)  
 AMONG 113 MALE WORKERS.  
 3M CHEMOLITE PLANT, COTTAGE GROVE, MINNESOTA

Variable	B	SE(B)	p-value
Intercept	-.190	.465	.68
Total Fluorine (ppm)*	.027	.009	.004
Age (years)	.006	.005	.29
BMI (kg/m <sup>2</sup> )	-.002	.013	.89
Cigarettes/day	-.001	.004	.74
Alcohol (<3oz/day)#	-.140	.194	.26
Free Testosterone**	.020	.009	.04
FSH##	.060	.019	.003

R<sup>2</sup> = .30

\*logarithmic transformation of thyroid stimulating hormone (TSH).

#Reference category is moderate drinkers who consume 3 oz ethanol/day.

\*\* ng/dl

##Follicle stimulating hormone mU/ml

TABLE 4.1.31 PROLACTIN BY BODY MASS INDEX, AGE, SMOKING,  
DRINKING STATUS, AND TOTAL SERUM FLUORINE  
3M CHEMOLITE PLANT, COTTAGE GROVE, MINNESOTA

	N(%)	MEAN	PROLACTIN		RANGE	TEST#
			SD	(ng/ml) MEDIAN		
<b>BMI (kg/m<sup>2</sup>)</b>						
<25	40(35.4)	9.10	5.13	8.4	2.7-24.3	F=.69
25-30	56(49.8)	8.71	5.18	7.8	1.2-33.7	p=.51
>30	17(15.0)	7.45	3.08	7.2	2.5-13.6	
<b>Age</b>						
<30	20(17.7)	9.63	4.20	8.5	3.9-18.3	F=.96
31-40	48(42.5)	9.01	5.30	8.7	12-33.7	p=.51
41-50	26(23.0)	8.38	5.52	6.2	2.9-23.5	
51-60	19(16.8)	7.16	3.37	6.8	2.5-15.1	
<b>Alcohol</b>						
<1oz/d	86(76.1)	8.61	4.57	7.8	1.2-24.3	F=.44
1-3oz/d	19(16.8)	9.46	6.87	8.7	2.9-33.7	p=.50
missing	8(7.1)	7.25	2.33	6.9	43.-10.5	
<b>Tobacco</b>						
smoker	27(23.9)	6.97	3.14	6.6	1.2-12.8	F=4.18
nonsmoker	84(74.3)	9.13	5.18	8.5	2.5-33.7	p=.043
missing	2(1.8)	11.65	9.40	11.7	5.0-18.3	
<b>Total Fluorine</b>						
<1 ppm	23(20.4)	7.9	3.19	7.5	2.5-18.3	F=3.02
>=1-3	64(56.6)	8.5	4.34	8.1	1.2-24.3	p=.02
>3-10	15(13.3)	4.9	1.15	6.6	1.4-18.1	
>10-15	6(5.3)	15.1	11.01	9.4	6.8-33.7	
>15-26	5(4.4)	8.3	4.16	7.7	3.8-15.1	
<b>Total</b>	<b>113(100)</b>	<b>8.7</b>	<b>4.90</b>	<b>7.7</b>	<b>1.2-33.7</b>	

#univariate Anova



**TABLE 4.1.32 LINEAR MULTIVARIATE REGRESSION MODEL OF FACTORS  
PREDICTING THE PROLACTIN VALUE (ng/ml) AMONG 113 MALE WORKERS.  
3M CHEMOLITE PLANT, COTTAGE GROVE, MINNESOTA**

<b>Variable</b>	<b>B</b>	<b>SE(B)</b>	<b>p-value</b>
Intercept	7.41	4.14	.07
Total Fluorine (ppm)	1.43	.36	.0002
Age (years)	-.04	.05	.41
BMI (kg/m <sup>2</sup> )	-.08	.13	.53
Cigarettes/day#	-.08	.04	.08
Estradiol (pg/ml)	.06	.03	.07
<b>Alcohol Use##</b>			
Light (<1 oz/day)	3.21	1.65	.05
Nonresponse (NR)	2.14	2.69	.43
Light X total fluoride	-1.67	.77	.03
NR X total fluoride	-1.34	.37	.0006

R<sup>2</sup>= .22

##Reference category is moderate drinkers who consume 1-3 oz ethanol/day.  
Nonrespondants (NR) failed to complete the alcohol use questionnaire items.  
Light X total fluoride and NR X total fluoride are interaction terms for alcohol  
categories and total serum fluoride.

TABLE 4.1.33 PEARSON CORRELATION COEFFICIENTS BETWEEN HORMONE RATIOS AND TOTAL FLUORIDE, AGE, BODY MASS INDEX, ALCOHOL AND TOBACCO CONSUMPTION  
3M CHEMOLITE PLANT, COTTAGE GROVE, MINNESOTA

	TOTAL FLUORINE (ppm)	AGE (years)	BMI (kg/m <sup>2</sup> )	ALCOHOL (oz/day)	TOBACCO (cigs/day)
E/TB@	-.01	.004	.32 p=.001	.05	.05
E/TF*	.11	.15 p=.15	.27 p=.004	.01	.01
E/LH**	.001	-.26 p=.005	.18 p=.08	.05	.04
TB/LH+	.002	-.32 p=.001	-.14 p=.13	-.01	-.01
TF/LH++	-.09	-.40 p=.0001	-.02	.03	.03
TB/TF"	.16 p=.09	.24 p=.01	-.16 p=.08	-.12	.09

@ESTRADIOL TO BOUND TESTOSTERONE RATIO

\*ESTRADIOL TO FREE TESTOSTERONE

\*\*ESTRADIOL TO LUTENIZING HORMONE RATIO

+BOUND TESTOSTERONE TO LUTENIZING HORMONE RATIO

++FREE TESTOSTERONE TO LUTENIZING HORMONE RATIO

"BOUND TESTOSTERONE TO FREE TESTOSTERONE RATIO

**TABLE 4.1.34 PEARSON CORRELATION COEFFICIENTS BETWEEN  
 PROLACTIN HORMONE RATIOS AND TOTAL FLUORIDE, AGE, BODY MASS  
 INDEX, ALCOHOL AND TOBACCO CONSUMPTION  
 3M CHEMOLITE PLANT, COTTAGE GROVE, MINNESOTA**

	TOTAL FLUORINE (ppm)	AGE (years)	BMI (kg/m <sup>2</sup> )	ALCOHOL (oz/day)	TOBACCO (cigs/day)
<b>TB/P@</b>	-.05	-.04	-.13	-.03	.24 p=.01
<b>TF/P*</b>	-.08	-.11	-.08	.06	.22 p=.02
<b>E/P+</b>	-.03	-.05	.03	.007	.25 p=.008
<b>FSH/P**</b>	-.09	.37 p=.0001	.004	-.13 p=.16	.21 p=.02
<b>P/LH**</b>	.11	-.24 p=.003	.09	.15 p=.11	-.22 p=.02
<b>P/TSH**</b>	.07	.17 p=.07	.07	.17 p=.07	.09

- @Free testosterone to prolactin ratio
- \*Free testosterone to prolactin ratio
- +Estradiol to prolactin ratio
- \*\*Follicle stimulating hormone to prolactin ratio
- \*\*Prolactin to lutenizing hormone ratio
- ++Prolactin to thyroid stimulating hormone ratio

TABLE 4.1.35 PEARSON CORRELATION COEFFICIENTS BETWEEN THYROID STIMULATING HORMONE RATIOS AND TOTAL FLUORIDE, AGE, BODY MASS INDEX, ALCOHOL AND TOBACCO CONSUMPTION 3M CHEMOLITE PLANT, COTTAGE GROVE, MINNESOTA

	TOTAL FLUORINE (ppm)	AGE (years)	BMI (kg/m <sup>2</sup> )	ALCOHOL (oz/day)	TOBACCO (cigs/day)
TB/TSH#	-.13	-.23 p=.01	-.24 p=.01	-.16 p=.09	.03
TF/TSH*	-.18 p=.05	-.34 p=.0002	-.23 p=.01	-.13	.01
E/TSH**	-.13	-.24 p=.01	-.05	-.05	.04

#Bound testosterone to thyroid stimulating hormone ratio

\*Free testosterone to thyroid stimulating hormone ratio

+Estradiol to thyroid stimulating hormone ratio

TABLE 4.1.36 PEARSON CORRELATION COEFFICIENTS BETWEEN FOLLICLE STIMULATING HORMONE RATIOS AND TOTAL FLUORIDE, AGE, BODY MASS INDEX, ALCOHOL AND TOBACCO CONSUMPTION 3M CHEMOLITE PLANT, COTTAGE GROVE, MINNESOTA

	TOTAL FLUORINE (ppm)	AGE (years)	BMI (kg/m <sup>2</sup> )	ALCOHOL (oz/day)	TOBACCO (cigs/day)
TB/FSH#	.07	-.43 p=.0001	-.16 p=.08	.06	-.06
TF/FSH*	-.01	-.47 p=.0001	.04	.08	-.12
E/FSH**	.04	-.36 p=.0001	.07	.04	-.02

#Bound testosterone to follicle stimulating hormone ratio

\*Free testosterone to follicle stimulating hormone ratio

+Estradiol to follicle stimulating hormone ratio

**TABLE 4.1.37 PEARSON CORRELATION COEFFICIENTS BETWEEN  
PITUITARY GLYCOPROTEIN HORMONE RATIOS AND TOTAL FLUORIDE,  
AGE, BODY MASS INDEX, ALCOHOL AND TOBACCO CONSUMPTION  
3M CHEMOLITE PLANT, COTTAGE GROVE, MINNESOTA**

	TOTAL FLUORINE (ppm)	AGE (years)	BMI (kg/m <sup>2</sup> )	ALCOHOL (oz/day)	TOBACCO (cigs/day)
TSH/FSH@	.12	-.16 p=.08	.04	.24 p=.01	-.14
TSH/LH*	.09	-.02	.15	.21 p=.03	-.14
F/LH*	-.05	.28 p=.003	.13	-.14	.05

@Thyroid stimulating hormone to follicle stimulating hormone ratio

\*Thyroid stimulating hormone to lutenizing hormone ratio

+Follicle stimulating hormone to lutenizing hormone ratio

**TABLE 4.1.38 LINEAR MULTIVARIATE REGRESSION MODEL1 OF FACTORS  
PREDICTING THE BOUND-FREE TESTOSTERONE RATIO AMONG 112 MALE  
WORKERS.  
3M CHEMOLITE PLANT, COTTAGE GROVE, MINNESOTA**

<b>Variable</b>	<b>B</b>	<b>SE(B)</b>	<b>p-value</b>
Intercept	36.60	6.87	.0001
Total Fluorine (ppm)*	.02	.008	.02
Age (years)	.19	.101	.07
BMI (kg/m <sup>2</sup> )	-.48	.244	.05
LH+	.12	.337	.73
FSH@	.92	.440	.04

R<sup>2</sup> = .21

\*square transformation of total serum fluoride

+lutienizing hormone mU/ml

@ follicle stimulating hormone mU/ml

TABLE 4.1.39 LINEAR MULTIVARIATE REGRESSION MODEL2 OF FACTORS  
 PREDICTING THE BOUND-FREE TESTOSTERONE RATIO AMONG 112 MALE  
 WORKERS.  
 3M CHEMOLITE PLANT, COTTAGE GROVE, MINNESOTA

Variable	$\beta$	SE( $\beta$ )	p-value
Intercept	37.3	6.97	.0001
Total Fluorine (ppm)*	.02	.009	.03
Age (years)	.25	.097	.009
BMI (kg/m <sup>2</sup> )	-.52	.250	.03
LH+	.55	.271	.05

R<sup>2</sup> = .17

\*square transformation of total serum fluoride

+luteinizing hormone mU/ml

@ follicle stimulating hormone mU/ml

TABLE 4.1.40 LINEAR MULTIVARIATE REGRESSION MODEL OF FACTORS  
 PREDICTING THE ESTRADIOL-BOUND TESTOSTERONE RATIO AMONG 112  
 MALE WORKERS.  
 3M CHEMOLITE PLANT, COTTAGE GROVE, MINNESOTA

Variable	B	SE(B)	p-value
Intercept	.05	.027	.05
Total Fluorine (ppm)	.00001	.00001	.74
Age (years)	-.0004	.0004	.29
BMI (kg/m <sup>2</sup> )	.002	.0007	.006
Cigarettes/day	-.00001	.00002	.96
Alcohol (<1oz/day)#	.003	.007	.63
Free Testosterone*	-.001	.0006	.008
LH+	.0001	.0006	.94
FSH@	-.002	.001	.12
TSH++	-.003	.003	.30
Prolactin**	.0001	.0005	.78

R<sup>2</sup> = .21

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

\* ng/dl

+luteinizing hormone mU/ml

@ follicle stimulating hormone mU/ml

++ Thyroid stimulating hormone (mU/ml)

\*\* prolactin ng/ml



**TABLE 4.1.41 LINEAR MULTIVARIATE REGRESSION MODEL OF FACTORS  
PREDICTING THE ESTRADIOL-FREE TESTOSTERONE RATIO AMONG 112  
MALE WORKERS.**

**3M CHEMOLITE PLANT, COTTAGE GROVE, MINNESOTA**

<b>Variable</b>	<b>B</b>	<b>SE(B)</b>	<b>p-value</b>
Intercept	1.31	.880	.15
Total Fluorine (ppm)	.002	.001	.03
Age (years)	.012	.011	.34
BMI (kg/m <sup>2</sup> )	.048	.026	.07
Cigarettes/day	.005	.008	.51
Alcohol (<1oz/day)#	.090	.730	.70
Bound Testosterone*	-.001	.0004	.01
LH+	.012	.035	.73
FSH@	-.059	.046	.21
TSH++	-.204	.110	.05
Prolactin**	.027	.018	.15

**R<sup>2</sup> = .22**

**#Reference category is moderate drinkers who consume 1-3 oz ethanol/day.**

**\* ng/dl**

**+lutenizing hormone mU/ml**

**@ follicle stimulating hormone mU/ml**

**++ Thyroid stimulating hormone (mU/ml)**

**\*\* prolactin ng/ml**

TABLE 4.1.42 LINEAR MULTIVARIATE REGRESSION MODEL OF FACTORS  
 PREDICTING THE ESTRADIOL-LH+ RATIO AMONG 112 MALE WORKERS.  
 3M CHEMOLITE PLANT, COTTAGE GROVE, MINNESOTA

Variable	B	SE(B)	p-value
Intercept	3.07	3.58	.39
Total Fluorine (ppm)	.02	.07	.80
Age (years)	-.03	.05	.39
BMI (kg/m <sup>2</sup> )	.27	.10	.008
Cigarettes/day	.009	.03	.77
Alcohol (<1 oz/day)#	.37	.90	.68
Free Testosterone*	.13	.10	.21
Bound Testosterone*	.001	.002	.71
FSH@	-.75	.15	.0001
TSH++	-.39	.42	.35
Prolactin**	-.03	.07	.72

R<sup>2</sup>= .34

+estradiol to lutenizing hormone (mU/ml) ratio

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

\* ng/dl

@follicle stimulating hormone mU/ml

++ Thyroid stimulating hormone (mU/ml)

\*\* prolactin ng/ml

TABLE 4.1.43 LINEAR MULTIVARIATE REGRESSION MODEL OF FACTORS  
PREDICTING THE BOUND TESTOSTERONE-LH+ RATIO AMONG 112 MALE  
WORKERS.

3M CHEMOLITE PLANT, COTTAGE GROVE, MINNESOTA

Variable	B	SE(B)	p-value
Intercept	74.46	48.53	.13
Total Fluorine (ppm)	.27	.98	.79
Age (years)	.29	.62	.64
BMI (kg/m <sup>2</sup> )	-.43	1.35	.75
Cigarettes/day	-.15	.44	.73
Alcohol (<1 oz/day)#	7.55	12.1	.54
Free Testosterone*	5.96	1.01	.0001
estradiol@	-.28	.38	.45
FSH@@	-8.65	2.03	.0001
TSH++	-.23	5.69	.97
Prolactin**	.11	.95	.90

R<sup>2</sup>= .43

+bound testosterone to lutenizing hormone (mU/ml) ratio

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

\* ng/dl

@ pg/ml

@@follicle stimulating hormone mU/ml

++ Thyroid stimulating hormone (mU/ml)

\*\* prolactin ng/ml

TABLE 4.1.44 LINEAR MULTIVARIATE REGRESSION MODEL OF FACTORS  
 PREDICTING THE FREE TESTOSTERONE-LH+ RATIO AMONG 112 MALE  
 WORKERS.  
 3M CHEMOLITE PLANT, COTTAGE GROVE, MINNESOTA

Variable	$\beta$	SE( $\beta$ )	p-value
Intercept	3.00	1.38	.03
Total Fluorine (ppm)	-.05	.03	.09
Age (years)	.001	.01	.91
BMI (kg/m <sup>2</sup> )	.07	.04	.08
Cigarettes/day	-.007	.01	.58
Alcohol (<1 oz/day)#	.30	.36	.41
Bound Testosterone*	.003	.0007	.0001
Estradiol@	.001	.01	.91
FSH@@	-.33	.06	.0001
TSH++	.18	.17	.30
Prolactin**	-.05	.03	.08

R<sup>2</sup> = .46

+free testosterone to lutenizing hormone (mU/ml) ratio

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

\* ng/dl

@ pg/ml

@@follicle stimulating hormone mU/ml

++ Thyroid stimulating hormone (mU/ml)

\*\* prolactin ng/ml

TABLE 4.1.45 LINEAR MULTIVARIATE REGRESSION MODEL OF FACTORS  
 PREDICTING THE BOUND TESTOSTERONE-PROLACTIN RATIO AMONG 111  
 MALE WORKERS.  
 13M CHEMOLITE PLANT, COTTAGE GROVE, MINNESOTA

Variable	B	SE(B)	p-value
Intercept	60.05	68.04	.38
Total Fluorine (ppm)	-.15	1.38	.91
Age (years)	.84	.88	.34
BMI (kg/m <sup>2</sup> )	-1.54	1.92	.42
Cigarettes/day	1.49	.62	.02
Alcohol (<1oz/day)#	13.9	17.2	.42
Estradiol++	-.22	.53	.68
Free Testosterone *	3.93	1.45	.008
LH**	-2.23	2.63	.40
FSH@	-2.55	3.50	.47
TSH+	-.95	.80	.24

R<sup>2</sup> = .17

++pg/ml

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

\* ng/dl

\*\* lutenizing hormone mU/ml

@ follicle stimulating hormone mU/ml

+ Thyroid stimulating hormone mU/ml

TABLE 4.1.46 LINEAR MULTIVARIATE REGRESSION MODEL OF FACTORS  
 PREDICTING THE FREE TESTOSTERONE-PROLACTIN RATIO AMONG 111  
 MALE WORKERS.  
 3M CHEMOLITE PLANT, COTTAGE GROVE, MINNESOTA

Variable	B	SE(B)	p-value
Intercept	2.41	1.76	.17
Total Fluorine (ppm)	-.03	.04	.35
Age (years)	-.004	.02	.95
BMI (kg/m <sup>2</sup> )	-.004	.05	.93
Cigarettes/day	.04	.02	.03
Alcohol (<1 oz/day)#	-.03	.76	.97
Estradiol++	-.0001	.01	.99
Bound Testosterone *	.002	.0001	.03
LH**	-.08	.07	.24
FSH@	-.12	.09	.21
TSH+	-.18	.21	.40

R<sup>2</sup> = .15

++pg/ml

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

\* ng/dl

\*\* lutenizing hormone mU/ml

@ follicle stimulating hormone mU/ml

+ Thyroid stimulating hormone mU/ml

TABLE 4.1.47 LINEAR MULTIVARIATE REGRESSION MODEL OF FACTORS  
 PREDICTING THE ESTRADIOL-PROLACTIN RATIO AMONG 111 MALE  
 WORKERS.  
 3M CHEMOLITE PLANT, COTTAGE GROVE, MINNESOTA

Variable	B	SE(B)	p-value
Intercept	2.65	4.01	.51
Total Fluorine (ppm)	.005	.081	.95
Age (years)	.01	.05	.80
BMI (kg/m <sup>2</sup> )	.07	.116	.53
Cigarettes/day	.10	.036	.005
Alcohol (<1oz/day)#	.86	1.01	.40
Bound Testosterone*	-.001	.003	.95
Free Testosterone *	.12	.12	.31
LH**	-.13	.15	.39
FSH@	-.29	.21	.17
TSH+	-.67	.47	.16

R<sup>2</sup> = .16

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

\* ng/dl

\*\* lutenizing hormone mU/ml

@ follicle stimulating hormone mU/ml

+ Thyroid stimulating hormone mU/ml

TABLE 4.1.48 LINEAR MULTIVARIATE REGRESSION MODEL OF FACTORS  
 PREDICTING THE PROLACTIN-FSH@ RATIO AMONG 111 MALE WORKERS.  
 3M CHEMOLITE PLANT, COTTAGE GROVE, MINNESOTA

Variable	B	SE(B)	p-value
Intercept	2.56	1.52	.09
Total Fluorine (ppm)	.31	.11	.008
Alcohol #			
low (<1 oz/day)	.81	.52	.13
nonresponse (NR)	.19	.85	.82
low X Fluoride	-.31	.12	.01
NR X Fluoride	-.08	.29	.78
Age (years)	-.05	.02	.01
BMI (kg/m <sup>2</sup> )	.01	.04	.86
Cigarettes/day	-.03	.01	.03
Estradiol <sup>++</sup>	.02	.01	.06
Bound Testosterone*	-.001	.001	.92
Free Testosterone *	.01	.04	.75
LH <sup>**</sup>	-.07	.05	.15
TSH <sup>+</sup>	.31	.17	.07

R<sup>2</sup> = .31

<sup>++</sup> pg/ml

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

\* ng/dl

\*\* lutenizing hormone mU/ml

@ follicle stimulating hormone mU/ml

+ Thyroid stimulating hormone mU/ml



TABLE 4.1.49 LINEAR MULTIVARIATE REGRESSION MODEL OF FACTORS  
 PREDICTING THE PROLACTIN-LH\*\* RATIO AMONG 111 MALE WORKERS.  
 3M CHEMOLITE PLANT, COTTAGE GROVE, MINNESOTA

Variable	B	SE(B)	p-value
Intercept	1.07	1.27	.38
Total Fluorine (ppm)	.34	.09	.0003
Alcohol #			
low (<1oz/day)	.68	.43	.11
nonresponse (NR)	.41	.69	.55
low X Fluoride	-.35	.09	.0004
NR X Fluoride	-.39	.20	.05
Age (years)	-.02	.01	.12
BMI (kg/m <sup>2</sup> )	.05	.04	.17
Cigarettes/day	-.02	.01	.09
Estradiol <sup>++</sup>	.003	.009	.76
Bound Testosterone*	.001	.0008	.17
Free Testosterone *	-.04	.03	.30
FSH@	-.11	.05	.03
TSH+	.15	.14	.29

R<sup>2</sup> = .31

\*\*lutenizing hormone

++ pg/ml

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

\* ng/dl

@ follicle stimulating hormone mU/ml

+ Thyroid stimulating hormone mU/ml

TABLE 4.1.50 LINEAR MULTIVARIATE REGRESSION MODEL OF FACTORS  
 PREDICTING THE PROLACTIN-TSH+ RATIO AMONG 111 MALE WORKERS.  
 3M CHEMOLITE PLANT, COTTAGE GROVE, MINNESOTA

Variable	B	SE(B)	p-value
Intercept	5.06	4.83	.30
Total Fluorine (ppm)	1.61	.37	.0001
Alcohol #			
low (<1oz/day)	4.16	1.67	.01
nonresponse (NR)	3.85	2.72	.16
low X Fluoride	-1.76	.38	.0001
NR X Fluoride	-2.11	.77	.008
Age (years)	-.19	.06	.003
BMI (kg/m <sup>2</sup> )	.11	.14	.43
Cigarettes/day	-.06	.04	.14
Estradiol++	.03	.04	.46
Bound Testosterone*	.008	.003	.02
Free Testosterone *	-.35	.14	.01
FSH@	.51	.20	.01

R<sup>2</sup> = .34

++ pg/ml

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

\* ng/dl

@ follicle stimulating hormone mU/ml

+ Thyroid stimulating hormone mU/ml

TABLE 4.1.51 LINEAR MULTIVARIATE REGRESSION MODEL OF FACTORS  
 PREDICTING THE BOUND TESTOSTERONE-TSH+ RATIO AMONG 112 MALE  
 WORKERS.  
 3M CHEMOLITE PLANT, COTTAGE GROVE, MINNESOTA

Variable	$\beta$	SE( $\beta$ )	p-value
Intercept	559.5	360.7	.12
Total Fluorine (ppm)	37.7	109.8	.73
Age (years)	-1.1	6.2	.85
BMI (kg/m <sup>2</sup> )	-9.8	8.9	.27
Cigarettes/day	-.66	2.9	.82
Alcohol (<1oz/day)#	74.9	78.3	.34
Free Testosterone*	12.5	6.6	.06
Estradiol@	-1.6	2.5	.51
FSH@@	47.1	15.9	.004
LH++	5.7	12.2	.64
Prolactin**	-1.1	6.2	.85

R<sup>2</sup> = .29

+bound testosterone to thyroid stimulating hormone (mU/ml) ratio

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

\* ng/dl

@ pg/ml

@@follicle stimulating hormone mU/ml

++ Thyroid stimulating hormone (mU/ml)

\*\* prolactin ng/ml

TABLE 4.1.52 LINEAR MULTIVARIATE REGRESSION MODEL OF FACTORS  
 PREDICTING THE FREE TESTOSTERONE-TSH+ RATIO AMONG 112 MALE  
 WORKERS.  
 3M CHEMOLITE PLANT, COTTAGE GROVE, MINNESOTA

Variable	$\beta$	SE( $\beta$ )	p-value
Intercept	15.65	6.34	.02
Total Fluorine (ppm)	-.28	.13	.03
Age (years)	-.29	.08	.003
BMI (kg/m <sup>2</sup> )	-.01	.19	.94
Cigarettes/day	-.03	.06	.65
Alcohol (<1oz/day)#	1.50	1.64	.36
Bound Testosterone*	.01	.003	.006
Estradiol@	-.01	.05	.80
FSH@@	.68	.33	.04
LH++	-.001	.25	.99
Prolactin**	-.18	.13	.17

R<sup>2</sup>= .37

+free testosterone to thyroid stimulating hormone (mU/ml) ratio

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

\* ng/dl

@ pg/ml

@@follicle stimulating hormone mU/m++ Thyroid stimulating hormone (mU/ml)

\*\* prolactin ng/ml

TABLE 4.1.53 LINEAR MULTIVARIATE REGRESSION MODEL OF FACTORS  
 PREDICTING THE ESTRADIOL-TSH+ RATIO AMONG 112 MALE WORKERS.  
 3M CHEMOLITE PLANT, COTTAGE GROVE, MINNESOTA

Variable	B	SE(B)	p-value
Intercept	30.80	16.10	.06
Total Fluorine (ppm)	-.425	.31	.18
Age (years)	-.53	.20	.01
BMI (kg/m <sup>2</sup> )	.32	.46	.50
Cigarettes/day	.06	.14	.70
Alcohol (<1oz/day)#	2.36	4.00	.55
Free Testosterone*	-.28	.46	.55
Bound Testosterone*	.009	.01	.42
FSH@	.81	.81	.31
LH++	.20	.62	.75
Prolactin**	-.07	.32	.83

R<sup>2</sup> = .15

+estradiol to thyroid stimulating hormone (mU/ml) ratio

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

\* ng/dl

@follicle stimulating hormone mU/ml

++ thyroid stimulating hormone (mU/ml)

\*\* prolactin ng/ml

**TABLE 4.1.54 LINEAR MULTIVARIATE REGRESSION MODEL OF FACTORS  
PREDICTING THE BOUND TESTOSTERONE-FSH+ RATIO AMONG 112 MALE  
WORKERS.  
3M CHEMOLITE PLANT, COTTAGE GROVE, MINNESOTA**

<b>Variable</b>	<b>B</b>	<b>SE(B)</b>	<b>p-value</b>
Intercept	101.89	61.25	.10
Total Fluorine (ppm)	.66	1.24	.60
Age (years)	-.13	.75	.14
BMI (kg/m <sup>2</sup> )	-1.08	1.70	.53
Cigarettes/day	-.37	.55	.50
Alcohol (<1 oz/day)#	.29	15.30	.98
Free Testosterone*	6.87	1.28	.0001
LH@@	-7.61	1.93	.0002
Estradiol@	.77	.47	.11
TSH++	8.90	7.03	.21
Prolactin**	-.03	1.20	.97

R<sup>2</sup> = .50

+bound testosterone to follicle stimulating hormone (mU/ml) ratio

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

\* ng/dl

@luteinizing hormone mU/ml

@@estradiol pg/ml

++ Thyroid stimulating hormone (mU/ml)

\*\* prolactin ng/ml

TABLE 4.1.55 LINEAR MULTIVARIATE REGRESSION MODEL OF FACTORS  
 PREDICTING THE FREE TESTOSTERONE-FSH+ RATIO AMONG 112 MALE  
 WORKERS.  
 3M CHEMOLITE PLANT, COTTAGE GROVE, MINNESOTA

Variable	B	SE(B)	p-value
Intercept	4.31	2.01	.03
Total Fluorine (ppm)	-.04	.04	.27
Age (years)	-.10	.02	.0001
BMI (kg/m <sup>2</sup> )	.06	.06	.28
Cigarettes/day	-.02	.02	.31
Alcohol (<1 oz/day)#	.18	.52	.74
Bound Testosterone*	.003	.001	.02
LH@@	-.25	.07	.0003
Estradiol@	.03	.04	.27
TSH++	.49	.24	.04
Prolactin**	-.05	.04	.23

R<sup>2</sup> = .43

+free testosterone to follicle stimulating hormone (mU/ml) ratio

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

\* ng/dl

@luteinizing hormone mU/ml

@@estradiol pg/ml

++ Thyroid stimulating hormone (mU/ml)

\*\* prolactin ng/ml

**TABLE 4.1.56 LINEAR MULTIVARIATE REGRESSION MODEL OF FACTORS  
PREDICTING THE ESTRADIOL-FSH+ RATIO AMONG 112 MALE WORKERS.  
3M CHEMOLITE PLANT, COTTAGE GROVE, MINNESOTA**

<b>Variable</b>	<b>B</b>	<b>SE(B)</b>	<b>p-value</b>
Intercept	6.91	5.69	.23
Total Fluorine (ppm)	.006	.006	.34
Age (years)	-.19	.07	.008
BMI (kg/m <sup>2</sup> )	.27	.16	.10
Cigarettes/day	.03	.05	.57
Alcohol (<1 oz/day)#	.52	1.42	.71
Free Testosterone*	.26	.16	.11
Bound Testosterone*	-.002	.004	.62
LH@	-.49	.18	.009
TSH++	.08	.65	.90
Prolactin**	.04	.11	.70

R<sup>2</sup> = .26

+estradiol to follicle stimulating hormone (mU/ml) ratio

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

\* ng/dl

@luteinizing hormone mU/ml

++ Thyroid stimulating hormone (mU/ml)

\*\* prolactin ng/ml



**TABLE 4.1.57 LINEAR MULTIVARIATE REGRESSION MODEL OF FACTORS  
PREDICTING THE BOUND TSH-FSH+ RATIO AMONG 112 MALE WORKERS.  
3M CHEMOLITE PLANT, COTTAGE GROVE, MINNESOTA**

<b>Variable</b>	<b>B</b>	<b>SE(B)</b>	<b>p-value</b>
Intercept	.72	.33	.03
Total Fluorine (ppm)	.01	.006	.14
Age (years)	.002	.004	.59
BMI (kg/m <sup>2</sup> )	.00007	.01	.94
Cigarettes/day	-.003	.003	.37
Alcohol (<1 oz/day)#	-.16	.08	.05
Estradiol++	-.001	.003	.56
Bound Testosterone*	-.0002	.0002	.28
Free Testosterone*	-.01	.009	.15
Prolactin**	.002	.007	.73
LH@	-.03	.01	.005

R<sup>2</sup> = .26

++pg/ml

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

\* ng/dl

\*\* prolactin ng/ml

@ lutenizing hormone mU/ml

+ thyroid stimulating hormone (mU/ml) to follicle stimulating hormone (mU/ml) ratio

TABLE 4.1.58 LINEAR MULTIVARIATE REGRESSION MODEL OF FACTORS  
 PREDICTING THE TSH-LH+ RATIO AMONG 112 MALE WORKERS.  
 3M CHEMOLITE PLANT, COTTAGE GROVE, MINNESOTA

Variable	$\beta$	SE( $\beta$ )	p-value
Intercept	.32	.25	.21
Total Fluorine (ppm)	.006	.005	.21
Age (years)	.004	.003	.26
BMI (kg/m <sup>2</sup> )	.008	.007	.27
Cigarettes/day	-.001	.002	.53
Alcohol (<1oz/day)#	-.07	.06	.26
Estradiol++	-.004	.002	.07
Bound Testosterone*	-.0001	.001	.84
Free Testosterone*	.007	.007	.32
Prolactin**	.001	.005	.91
FSH@	-.05	.01	.0001

R<sup>2</sup> = .26

++pg/ml

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

\* ng/dl

\*\* prolactin ng/ml

@ follicle stimulating hormone mU/ml

+ Thyroid stimulating hormone (mU/ml) to lutenizing hormone (mU/ml) ratio

TABLE 4.1.59 LINEAR MULTIVARIATE REGRESSION MODEL OF FACTORS  
 PREDICTING THE BOUND LH-FSH+ RATIO AMONG 112 MALE WORKERS.  
 3M CHEMOLITE PLANT, COTTAGE GROVE, MINNESOTA

Variable	B	SE(B)	p-value
Intercept	.60	.43	.17
Total Fluorine (ppm)	-.0001	.009	.98
Age (years)	.009	.005	.09
BMI (kg/m <sup>2</sup> )	.01	.01	.40
Cigarettes/day	.0001	.004	.82
Alcohol (<1oz/day)#	.04	.11	.71
Estradiol++	.004	.003	.18
Bound Testosterone*	.0001	.0002	.18
Free Testosterone*	-.004	.01	.78
Prolactin**	-.005	.009	.57
TSH@	-.05	.05	.29

R<sup>2</sup>= .12

++pg/ml

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

\* ng/dl

\*\* prolactin ng/ml

@ thyroid stimulating hormone mU/ml

+ lutenizing hormone (mU/ml) to follicle stimulating hormone  
 (mU/ml) ratio

TABLE 4.1.60 PEARSON CORRELATION COEFFICIENTS BETWEEN TOTAL SERUM FLUORIDE, AGE, BODY MASS INDEX (BMI), DAILY ALCOHOL USE, DAILY TOBACCO CONSUMPTION, AND LIPOPROTEINS 3M CHEMOLITE PLANT, COTTAGE GROVE, MINNESOTA

	TOTAL FLUORIDE (ppm)	AGE (years)	BMI (kg/m <sup>2</sup> )	ALCOHOL (oz/day)	TOBACCO (cigs/day)
CHOLESTEROL*	.07	.25 p=.008	.19 p=.05	.09	.35 p=.0001
LDL**	.02	.13	.06	-.008	.28 p=.002
HDL#	-.01	.03	-.13	.18 P=.06	-.09
TRIGLYCERIDES*	.09	.19 p=.04	.27 p=.004	.07	.19 p=.04

\*mg/dl

\*\*low density lipoprotein

#high density lipoprotein

TABLE 4.1.61 LINEAR MULTIVARIATE REGRESSION MODEL OF FACTORS  
 PREDICTING THE CHOLESTEROL AMONG 111 MALE WORKERS.  
 3M CHEMOLITE PLANT, COTTAGE GROVE, MINNESOTA

Variable	$\beta$	SE( $\beta$ )	p-value
Intercept	107.30	33.00	.002
Total Fluoride (ppm)	.52	.67	.44
Cigarettes/day	1.12	.31	.0005
BMI (kg/m <sup>2</sup> )	1.44	1.01	.16
Age (years)	.77	.38	.05
Alcohol #			
low (<1 oz/day)	-5.50	8.71	.53
nonresponse (NR)	-13.53	14.75	.35
GGT (IU/dl)*	.41	.12	.001
Bound Testosterone**	.03	.02	.07

R<sup>2</sup> = .29

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

\*gamma glutamyl transferase

\*\*ng/dl

TABLE 4.1.62 LINEAR MULTIVARIATE REGRESSION MODEL OF FACTORS  
 PREDICTING THE LOW DENSITY LIPOPROTEIN AMONG 111 MALE WORKERS.  
 3M CHEMOLITE PLANT, COTTAGE GROVE, MINNESOTA

Variable	B	SE(B)	p-value
Intercept	73.93	32.00	.03
Total Fluoride (ppm)	.22	.65	.73
Cigarettes/day	.69	.30	.02
BMI (kg/m <sup>2</sup> )	1.26	.95	.19
Age (years)	.37	.37	.32
Alcohol #			
low (<1oz/day)	-3.02	8.33	.71
nonresponse (NR)	-10.85	13.93	.43
Prolactin (ng/ml)	-1.59	.66	.02
Bound Testosterone(ng/dl)	.04	.02	.0071

R<sup>2</sup> = .19

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

TABLE 4.1.63 LINEAR MULTIVARIATE REGRESSION MODEL OF FACTORS  
 PREDICTING THE HIGH DENSITY LIPOPROTEIN (HDL) AMONG 111 MALE  
 WORKERS.  
 3M CHEMOLITE PLANT, COTTAGE GROVE, MINNESOTA

Variable	B	SE(B)	p-value
Intercept	65.00	10.07	.0001
Total Fluoride (ppm)	-1.61	.77	.04
Alcohol #			
low (<1oz/day)	-9.92	3.51	.006
nonresponse (NR)	-6.77	5.73	.24
low X Fluoride	1.62	.80	.04
NR X Fluoride*	2.05	1.63	.21
Age (years)	-.004	.12	.97
BMI (kg/m <sup>2</sup> )	-.31	.29	.28
Cigarettes/day	-.12	.09	.18
Bound Testosterone**	.018	.007	.009
Free Testosterone**	-.77	.28	.008

R<sup>2</sup> = .17

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

\*interaction terms between total fluoride and alcohol category

\*\* ng/dl

TABLE 4.1.64 LINEAR MULTIVARIATE REGRESSION MODEL OF FACTORS  
 PREDICTING THE TRIGLYCERIDES AMONG 111 MALE WORKERS.  
 3M CHEMOLITE PLANT, COTTAGE GROVE, MINNESOTA

Variable	B	SE(B)	p-value
Intercept	-114.50	117.20	.33
Total Fluoride (ppm)	2.38	2.31	.15
Cigarettes/day	2.28	1.05	.03
BMI (kg/m <sup>2</sup> )	6.07	3.39	.08
Age (years)	2.32	1.44	.11
Alcohol #			
low (<1oz/day)	-11.48	29.4	.70
nonresponse (NR)	-19.94	49.03	.69
Free Testosterone*	7.34	3.37	.03
Bound Testosterone*	-.21	.08	.009

R<sup>2</sup> = .19

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

\*ng/dl



TABLE 4.1.65 PEARSON CORRELATION COEFFICIENTS BETWEEN TOTAL SERUM FLUORIDE, AGE, BODY MASS INDEX (BMI), DAILY ALCOHOL USE, DAILY TOBACCO CONSUMPTION, AND HEPATIC PARAMETERS  
3M CHEMOLITE PLANT, COTTAGE GROVE, MINNESOTA

	TOTAL FLUORINE (ppm)	AGE (years)	BMI (kg/m <sup>2</sup> )	ALCOHOL (oz/day)	TOBACCO (cigs/day)
SGOT*	.01	-.10	.09	.12	-.11
SGPT**	.01	.01	.20 p=.02	.03	-.11
GGT#	-.04	.12	.27 p=.004	.15	.03
AKPH##	-.03	.27 p=.004	.19 p=.04	-.19 p=.05	.26 p=.006

\*SERUM GLUTAMIC OXALOACETIC TRANSAMINASE IU/dl

\*\*SERUM GLUTAMIC PYRUVIC TRANSAMINASE IU/dl

#GAMMA GLUTAMYL TRANSFERASE IU/dl

##ALKALINE PHOSPHATASE IU/dl

TABLE 4.1.66 PEARSON CORRELATION COEFFICIENTS BETWEEN HEPATIC ENZYMES, SERUM HORMONES, AND LIPOPROTEINS  
3M CHEMOLITE PLANT, COTTAGE GROVE, MINNESOTA

	SGOT	SGPT	GGT	AKPH
CHOLESTEROL*	.07	.25 p=.008	.19 p=.05	.09
LDL**	.02	.13	.06	-.008
HDL#	-.01	.03	-.13	.18 p=.06
TRIGLYCERIDES*	.09	.19 p=.04	.27 p=.004	.07
ESTRADIOL+	-.16 p=.09	-.04	.03	-.003
FREE TESTOSTERONE*	-.12	-.14	-.23 p=.01	-.03
BOUND TESTOSTERONE*	-.16 p=.09	-.10	-.12	-.12
PROLACTIN*	-.20 p=.03	-.15	-.16 p=.09	-.20 p=.03

\*mg/dl

\*\*low density lipoprotein

#high density lipoprotein

+pg/ml

\*ng/dl

**TABLE 4.1.67 PEARSON CORRELATION COEFFICIENTS BETWEEN  
HEPATIC PARAMETERS  
3M CHEMOLITE PLANT, COTTAGE GROVE, MINNESOTA**

	<b>SGOT</b>	<b>SGPT</b>	<b>GGT</b>	<b>AKPH</b>
<b>SGOT*</b>	1	.68 P=,0001	.43 P=,0001	.04
<b>SGPT**</b>	-	1	.60 P=,0001	.09
<b>GGT#</b>	-	-	1	.21 P=,02
<b>AKPH##</b>	-	-	-	1

\*SERUM GLUTAMIC OXALOACETIC TRANSAMINASE IU/dl  
 \*\*SERUM GLUTAMIC PYRUVIC TRANSAMINASE IU/dl  
 #GAMMA GLUTAMYL TRANSFERASE IU/dl  
 ##ALKALINE PHOSPHATASE IU/dl

TABLE 4.1.68 SERUM GLUTAMIC OXALOACETIC TRANSAMINASE (SGOT),  
 GLUTAMIC PYRUVIC TRANSAMINASE (SGPT), GAMMA GLUTAMYL  
 TRANSFERASE (GGT), AND ALKALINE PHOSPHATASE (AKPH) BY TOTAL  
 SERUM FLUORINE  
 3M CHEMOLITE PLANT, COTTAGE GROVE, MINNESOTA

	N	MEAN	SD	MEDIAN	RANGE	TEST#
<b>TOTAL FLUORINE</b>						
			<b>SGOT</b>			
			(IU/dl)			
<1 ppm	23	22.5	4.1	22	13-29	F=0.41
≥1-3	65	24.1	8.6	23	10-74	p=.80
>3-10	16	25.8	14.5	22.5	17-77	
>10-15	6	25.7	11.3	22.5	17-47	
>15-26	5	22.2	5.1	22	14-27	
<b>TOTAL</b>	<b>115</b>	<b>24.0</b>	<b>8.9</b>	<b>23</b>	<b>10-77</b>	
<b>SGPT</b>						
			(IU/dl)			
<1	23	47.7	10.7	46	30-69	F=1.19
≥1-3	65	51.3	30.2	45	4-263	p=.32
>3-10	16	53.0	14.0	50.5	29-40	
>10-15	6	73.2	53.2	52.5	38-177	
>15-26	5	44.6	8.6	42	34-54	
<b>TOTAL</b>	<b>115</b>	<b>51.7</b>	<b>26.8</b>	<b>47</b>	<b>4-263</b>	
<b>Alkaline Phosphatase</b>						
			(IU/dl)			
<1 ppm	23	86.1	25.6	85	43-153	F=0.43
≥1-3	65	85.9	19.9	80	38-137	p=.78
>3-10	16	77.9	20.3	71.5	54-123	
>10-15	6	87.2	34.0	75.5	61-153	
>15-26	5	89.0	42.1	84	41-153	
<b>TOTAL</b>	<b>115</b>	<b>83.3</b>	<b>22.9</b>	<b>80</b>	<b>38-153</b>	
<b>GGT</b>						
			(IU/dl)			
<1 ppm	23	37.2	29.4	27	6-117	F=0.39
≥1-3	65	32.4	26.7	25	5-174	p=.81
>3-10	16	35.4	35.4	26	10-158	
>10-15	6	38.3	16.7	36.5	19-60	
>15-26	5	22.2	11.5	20	11-37	
<b>TOTAL</b>	<b>115</b>	<b>33.7</b>	<b>27.6</b>	<b>26</b>	<b>5-174</b>	

#univariate Anova

TABLE 4.1.69 SERUM GLUTAMIC OXALOACETIC TRANSAMINASE (SGOT)  
 BY BODY MASS INDEX, AGE, SMOKING AND DRINKING STATUS  
 3M CHEMOLITE PLANT, COTTAGE GROVE, MINNESOTA

	SGOT (IU/dl)					TEST#
	N(%)	MEAN	SD	MEDIAN	RANGE	
<b>BMI</b>						
<25	41(35.7)	24	12.4	22	13-77	F=.92
25-30	57(49.6)	23	5.8	23	10-42	p=.40
>30	17(14.8)	27	8.1	26	17-47	
<b>AGE</b>						
<30	21(18.3)	25	12.7	23	17-77	F=.78
31-40	48(41.7)	24	9.1	23	10-74	p=.51
41-50	27(23.5)	22	5.4	23	13-40	
51-60	19(16.5)	26	7.8	23	14-47	
<b>Alcohol</b>						
<1oz/d	87(81.3)	26	13.5	22	16-77	F=.61
1-3oz/d	20(18.7)	24	8.0	23	10-74	p=.44
missing	8	23	4.3	21	19-31	
<b>Tobacco</b>						
smoker	28(24.8)	24	8.4	23	13-77	F=.02
nonsmoker	85(75.2)	24	11.0	22	10-42	p=.89
missing	2	20	3.5	20	17-47	
<b>TOTAL</b>	115					

#univariate Anova

TABLE 4.1.70 SERUM GLUTAMIC PYRUVIC TRANSAMINASE (SGPT) BY  
 BODY MASS INDEX, AGE, SMOKING AND DRINKING STATUS  
 3M CHEMOLITE PLANT, COTTAGE GROVE, MINNESOTA

	N(%)	MEAN	SGPT(U/dl)		RANGE	TEST
			SD	MEDIAN		
<b>BMI</b>						
<25	41(35.7)	49	35.4	41	29-263	F=.21
25-30	57(49.6)	50	14.2	49	4-95	p=.12
>30	17(14.8)	64	32.8	55	38-177	
<b>AGE</b>						
<30	21(18.3)	49	11.5	45	31-80	F=.61
31-40	48(41.7)	53	33.6	47	29-263	p=.61
41-50	27(23.5)	47	15.2	46	4-99	
51-60	19(16.5)	57	32.0	50	34-177	
<b>Alcohol</b>						
<1oz/d	87(81.3)	53	29.35	47	29-263	F=.68
1-3oz/d	20(18.7)	47	16.9	46	4-99	p=.41
missing	8	51	10.9	52	35-67	
<b>Tobacco</b>						
smoker	28(24.8)	48	15.2	47	4-90	F=.76
nonsmoker	85(75.2)	53	29.6	48	30-263	p=.39
missing	2	49	25.5	49	31-67	
<b>TOTAL</b>	115					

#univariate Anova

TABLE 4.1.71 GAMMA GLUTAMYL TRANSFERASE (GGT) BY BODY MASS INDEX, AGE, SMOKING AND DRINKING STATUS  
3M CHEMOLITE PLANT, COTTAGE GROVE, MINNESOTA

	N(%)	GGT (IU/dl)			RANGE	TEST#
		MEAN	SD	MEDIAN		
<b>BMI</b>						
<25	41(35.7)	28	31.1	17	5-174	F=3.54
25-30	57(49.6)	34	23.1	19	6-158	p=.03
>30	17(14.8)	48	28.6	44	19-117	
<b>AGE</b>						
<30	21(18.3)	32	23.4	25	11-111	F=1.58
31-40	48(41.7)	31	32.7	22	5-174	p=.36
41-50	27(23.5)	33	17.2	29	8-72	
51-60	19(16.5)	44	29.3	35	11-117	
<b>Alcohol</b>						
<1oz/d	87(81.3)	40	25.5	35	8-89	F=1.64
1-3oz/d	20(18.7)	32	25.3	26	6-174	p=.36
missing	8	41	50.4	23	12-158	
<b>Tobacco</b>						
smoker	28(24.8)	36	21.3	33	5-89	F=.55
nonsmoker	85(75.2)	32	26.3	25	6-174	p=.46
missing	2	85	103.2	85	12-158	
<b>TOTAL</b>	115					

#univariate Anova

TABLE 4.1.72 ALKALINE PHOSPHATASE (AKPH) BY BODY MASS INDEX,  
AGE, SMOKING AND DRINKING STATUS  
3M CHEMOLITE PLANT, COTTAGE GROVE, MINNESOTA

	AKPH (IU/dl)					TEST#
	N(%)	MEAN	SD	MEDIAN	RANGE	
<b>BMI</b>						
<25	41(35.7)	79	22.1	75	38-153	F=1.53
25-30	57(49.6)	84	21.9	81	41-153	p=.22
>30	17(14.8)	90	27.1	90	43-153	
<b>AGE</b>						
<30	21(18.3)	78	22.2	76	38-153	F=2.78
31-40	48(41.7)	80	20.3	76	50-15.	p=.45
41-50	27(23.5)	86	24.1	83	43-153	
51-60	19(16.5)	95	24.1	94	41-130	
<b>Alcohol</b>						
<1oz/d	87(81.3)	85	24.0	2	38-153	F=2.05
1-3oz/d	20(18.7)	77	16.9	75	51-124	p=.16
missing	8	82	22.5	70	60-115	
<b>Tobacco</b>						
smoker	28(24.8)	85	23.8	85	61-153	F=6.48
nonsmoker	85(75.2)	77	22.0	77	38-153	p=.012
missing	2	86	24.8	86	68-103	
<b>TOTAL</b>	115					

#univariate Anova



TABLE 4.1.73A LINEAR MULTIVARIATE REGRESSION MODEL 1 OF FACTORS PREDICTING THE SERUM GLUTAMIC OXALOACETIC TRANSAMINASE (SGOT) AMONG 111 MALE WORKERS. 3M CHEMOLITE PLANT, COTTAGE GROVE, MINNESOTA

Variable	B	SE(β)	p-value
Intercept	26.71	7.1	.0003
Total Fluorine (ppm)	-3.23	1.31	.02
BMI (kg/m <sup>2</sup> )	-.0004	.23	.99
BMI X T. Fluorine*	.12	.05	.015
Age (years)	-.003	.08	.97
Alcohol #			
low (<1oz/day)	.70	1.85	.71
nonresponse (NR)	-1.10	3.10	.72
Cigarettes/day	-.09	.07	.16
Prolactin (ng/ml)	-.37	.15	.01

R<sup>2</sup> = .17

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

\* interaction term between total serum fluoride and BMI.

TABLE 4.1.73B LINEAR MULTIVARIATE REGRESSION MODEL 2 OF  
FACTORS PREDICTING THE SERUM GLUTAMIC OXALOACETIC  
TRANSAMINASE (SGOT) AMONG 111 MALE WORKERS.  
3M CHEMOLITE PLANT, COTTAGE GROVE, MINNESOTA

Variable	B	SE(B)	p-value
Intercept	27.71	6.22	.0001
Total Fluorine (ppm)	-2.70	1.23	.02
BMI (kg/m <sup>2</sup> )	-.09	.06	.11
BMI X T. Fluorine*	.10	.04	.02
Age (years)	-.02	.07	.74
Cigarettes/day	-.11	.06	.11
Alcohol #			
low (<1oz/day)	1.84	1.61	.28
nonresponse (NR)	-1.3	2.7	.64
Prolactin (ng/ml)	-.27	.13	.04
GGT (IU/dl)**	.13	.02	.0001

R<sup>2</sup> = .35

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

\* Interaction term between total serum fluoride and BMI

\*\* Gamma glutamyl transferase

TABLE 4.1.73C LINEAR MULTIVARIATE REGRESSION MODEL 3 OF FACTORS PREDICTING THE SERUM GLUTAMIC OXALOACETIC TRANSAMINASE (SGOT) AMONG 111 MALE WORKERS. 3M CHEMOLITE PLANT, COTTAGE GROVE, MINNESOTA

Variable	$\beta$	SE(B)	p-value
Intercept	120.60	4.0	.002
Total Fluorine (ppm)	.63	.78	.42
BMI (kg/m <sup>2</sup> )	-.07	.13	.58
BMI X T. Fluorine*	-.03	.03	.34
Age (years)	.06	.05	.23
Cigarettes/day	-.02	.04	.45
Alcohol #			
low (<1oz/day)	-.65	1.03	.53
nonresponse (NR)	-1.40	1.72	.42
Prolactin (ng/ml)	-.09	.08	.29
SGPT (IU/dl)**	.24	.01	.0001

R<sup>2</sup> = .74

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

\* Interaction term between total serum fluoride and BMI

\*\* Serum glutamic pyruvic transaminase

TABLE 4.1.74A LINEAR MULTIVARIATE REGRESSION MODEL 1 OF  
FACTORS PREDICTING THE SERUM GLUTAMIC PYRUVIC TRANSAMINASE  
(SGPT) AMONG 111 MALE WORKERS.  
3M CHEMOLITE PLANT, COTTAGE GROVE, MINNESOTA

Variable	$\beta$	SE( $\beta$ )	p-value
Intercept	58.13	24.26	.02
Total Fluorine (ppm)	-15.80	4.58	.0008
BMI (kg/m <sup>2</sup> )	.30	.82	.72
BMI X T. Fluorine*	.62	.17	.0004
Age (years)	-.24	.28	.39
Alcohol #			
low (<1oz/day)	5.54	6.36	.39
nonresponse (NR)	1.31	10.63	.90
Cigarettes/day	-.27	.23	.24
Prolactin (ng/ml)	-1.18	.51	.02

R<sup>2</sup> = .21

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

\* interaction term between total serum fluoride and BMI.

**TABLE 4.1.74B LINEAR MULTIVARIATE REGRESSION MODEL 2 OF  
FACTORS PREDICTING THE SERUM GLUTAMIC PYRUVIC TRANSAMINASE  
(SGPT) AMONG 111 MALE WORKERS.  
3M CHEMOLITE PLANT, COTTAGE GROVE, MINNESOTA**

<b>Variable</b>	<b>B</b>	<b>SE(B)</b>	<b>p-value</b>
Intercept	62.09	19.63	.002
Total Fluoride (ppm)	-13.70	3.64	.0003
BMI (kg/m <sup>2</sup> )	-.70	.66	.30
BMI X T. Fluorine*	.54	.14	.0001
Age (years)	-.33	.22	.14
Cigarettes/day	-.027	.18	.14
Alcohol #			
low (<1oz/day)	10.02	5.09	.05
nonresponse (NR)	.48	8.44	.95
Prolactin (ng/ml)	-.74	.41	.07
GGT (IU/dl)**	.56	.07	.0001

R<sup>2</sup> = .51

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

\* Interaction term between total serum fluoride and BMI

\*\* Gamma glutamyl transferase

TABLE 4.1.74C LINEAR MULTIVARIATE REGRESSION MODEL 3 OF  
FACTORS PREDICTING THE SERUM GLUTAMIC PYRUVIC TRANSAMINASE  
(SGPT) AMONG 111 MALE WORKERS.  
3M CHEMOLITE PLANT, COTTAGE GROVE, MINNESOTA

Variable	B	SE(B)	p-value
Intercept	-18.25	14.36	.21
Total Fluorine (ppm)	-6.65	2.61	.01
BMI (kg/m <sup>2</sup> )	.30	.45	.51
BMI X T. Fluorine*	.27	.10	.007
Age (years)	-.23	.16	.14
Cigarettes/day	-.001	.13	.99
Alcohol #			
low (<1 oz/day)	3.55	3.53	.32
nonresponse (NR)	4.39	5.91	.46
Prolactin (ng/ml)	-.11	.29	.72
SGOT (IU/dl)**	2.85	.19	.0001

R<sup>2</sup> = .76

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

\* Interaction term between total serum fluoride and BMI

\*\* serum glutamic oxaloacetic transaminase

**TABLE 4.1.75A LINEAR MULTIVARIATE REGRESSION MODEL 1 OF  
FACTORS PREDICTING THE GAMMA GLUTAMYL TRANSFERASE (GGT)  
AMONG 111 MALE WORKERS.  
3M CHEMOLITE PLANT, COTTAGE GROVE, MINNESOTA**

<b>Variable</b>	<b>B</b>	<b>SE(B)</b>	<b>p-value</b>
Intercept	-12.59	22.62	.58
Total Fluorine (ppm)	-1.93	2.11	.36
Alcohol #			
low (<1oz/day)	-12.37	9.50	.20
nonresponse (NR)	-28.13	15.46	.07
low X Fluorine*	1.59	2.18	.47
NR X Fluorine*	13.90	4.48	.003
Age (years)	.29	.30	.33
BMI (kg/m <sup>2</sup> )	1.71	.76	.03
Cigarettes/day	.09	.24	.72

R<sup>2</sup> = .18

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

\*interaction terms between total fluoride and alcohol category

TABLE 4.1.75B LINEAR MULTIVARIATE REGRESSION MODEL 2 OF  
FACTORS PREDICTING THE GAMMA GLUTAMYL TRANSFERASE (GGT)  
AMONG 111 MALE WORKERS.  
3M CHEMOLITE PLANT, COTTAGE GROVE, MINNESOTA

Variable	B	SE(B)	p-value
Intercept	-58.78	21.55	.008
n			
Total Fluoride (ppm)	-1.79	1.83	.33
Alcohol #			
low (<1oz/day)	-9.04	8.25	.28
nonresponse (NR)	-20.08	13.49	.14
low X Fluorine*	1.39	1.90	.47
NR X Fluorine*	12.18	3.91	.002
Age (years)	.15	.26	.57
BMI (kg/m <sup>2</sup> )	1.30	.66	.05
Cigarettes/day	.01	.23	.96
Cholesterol (mg/dl)	.15	.06	.02
SGOT (IU/dl)	1.18	.24	.0001

R<sup>2</sup> = .38

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

\*interaction terms between total fluoride and alcohol category



TABLE 4.1.75C LINEAR MULTIVARIATE REGRESSION MODEL 3 OF  
FACTORS PREDICTING THE GAMMA GLUTAMYL TRANSFERASE (GGT)  
AMONG 111 MALE WORKERS.  
3M CHEMOLITE PLANT, COTTAGE GROVE, MINNESOTA

Variable	B	SE(B)	p-value
Intercept	-32.39	18.47	.08
Total Fluorine (ppm)	-1.63	1.60	.31
Alcohol #			
low (<1oz/day)	-13.58	7.17	.06
nonresponse (NR)	-26.68	11.75	.025
low X Fluorine*	.92	1.66	.58
NR X Fluorine*	12.04	3.41	.0006
Age (years)	.25	.23	.27
BMI (kg/m <sup>2</sup> )	.51	.59	.38
Cigarettes/day	.09	.20	.65
Cholesterol (mg/dl)	.12	.06	.04
SGPT (IU/dl)**	.59	.07	.0001

R<sup>2</sup> = .53

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

\*interaction terms between total fluoride and alcohol category

\*\* serum glutamic pyruvic transaminase

TABLE 4.1.76 LINEAR MULTIVARIATE REGRESSION MODEL 1 OF FACTORS  
 PREDICTING THE ALKALINE PHOSPHATASE (AKPH) AMONG 111 MALE  
 WORKERS.  
 3M CHEMOLITE PLANT, COTTAGE GROVE, MINNESOTA

Variable	B	SE(B)	p-value
Intercept	24.50	15.69	.09
Total Fluorine (ppm)	-1.03	.43	.02
Cigarettes/day	-.06	.22	.79
Cigarettes/day X Fluorine*	.22	.05	.0001
BMI (kg/m <sup>2</sup> )	1.10	.55	.05
Age (years)	.54	.22	.02
Alcohol #			
low (<1oz/day)	5.78	4.90	.24
nonresponse (NR)	8.12	8.13	.32

R<sup>2</sup> = .31

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

\* interaction term between total serum fluoride and cigarettes/day.

TABLE 4.1.77 PEARSON CORRELATION COEFFICIENTS BETWEEN TOTAL SERUM FLUORIDE, AGE, BODY MASS INDEX (BMI), DAILY ALCOHOL USE, DAILY TOBACCO CONSUMPTION, AND HEMATOLOGY PARAMETERS 3M CHEMOLITE PLANT, COTTAGE GROVE, MINNESOTA

	TOTAL FLUORINE (ppm)	AGE (years)	BMI (kg/m <sup>2</sup> )	ALCOHOL (oz/day)	TOBACCO (cigs/day)
HEMAGLOBIN*	-.07	-.03	.04	-.20 p=.04	.20 p=.008
WBC**	.10	.07	.07	-.07	.70 p=.0001
PMN COUNT+	.05	.08	.09	-.10	.64 p=.0001
EOSINOPHILS	-.10	.13	.05	.02	.23 p=.003
LYMPHOCYTES	.19 p=.04	-.05	.04	.15	.28 p=.002
MONOCYTES	.05	.04	-.22 p=.02	-.21 p=.03	.32 p=.0004
PLATLETS	.10	-.13	-.11	.05	.29 p=.002
BASOPHILS	.04	-.08	-.02	-.14	-.05
BANDS				.26 p=.005	-.14

\* g/dl

\*\*white blood cell count

+ polymorphonuclear leukocyte count

TABLE 4.1.78 LINEAR MULTIVARIATE REGRESSION MODEL OF FACTORS  
 PREDICTING THE HEMAGLOBIN AMONG 111 MALE WORKERS.  
 3M CHEMOLITE PLANT, COTTAGE GROVE, MINNESOTA

Variable	B	SE(B)	p-value
Intercept	14.51	.67	.0001
Total Fluorine (ppm)*	-.002	.0009	.02
Alcohol #			
low (<1 oz/day)	.22	.20	.27
nonresponse (NR)	.56	.33	.09
Age (years)	.001	.009	.88
BMI (kg/m <sup>2</sup> )	.01	.02	.65
Cigarettes/day	.01	.007	.20
Cigs/day X Fluorine <sup>2</sup> **	.0003	.0001	.0005
Estradiol (pg/ml)	.01	.006	.07

R<sup>2</sup> = .23

\*square transformation of total fluoride

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

\*\* interaction term between cigarettes per day and square transformation of total fluoride

TABLE 4.1.79 LINEAR MULTIVARIATE REGRESSION MODEL OF FACTORS  
 PREDICTING THE MEAN CORPUSCULAR HEMOBLOBIN (MCH) AMONG 111  
 MALE WORKERS.  
 3M CHEMOLITE PLANT, COTTAGE GROVE, MINNESOTA

Variable	B	SE(B)	p-value
Intercept	31.65	.95	.0001
Total Fluorine (ppm)	.15	.09	.10
Alcohol #			
low (<1 oz/day)	-.29	.65	.65
nonresponse (NR)	.03	.01	.02
low X Fluorine	-.16	.09	.08
NR X Fluorine	-.04	.19	.80
Age (years)	.03	.01	.02
BMI (kg/m <sup>2</sup> )	-.07	.03	.01
Cigarettes/day	.02	.01	.13
Cigs/day X Fluorine*	.006	.003	.03

R<sup>2</sup> = .24

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

\* interaction terms; alcohol category by total fluoride, cigarettes per day by total fluoride

TABLE 4.1.1.80 LINEAR MULTIVARIATE REGRESSION MODEL OF FACTORS  
 PREDICTING THE MEAN CORPUSCULAR VOLUME (MCV) AMONG 111 MALE  
 WORKERS.  
 3M CHEMOLITE PLANT, COTTAGE GROVE, MINNESOTA

Variable	B	SE(B)	p-value
Intercept	8.74	2.50	.0001
Total Fluorine (ppm)	-.04	.07	.52
Alcohol #			
low (<1oz/day)	-.61	.78	.43
nonresponse (NR)	-.95	1.27	.46
Age (years)	.11	.03	.002
BMI (kg/m <sup>2</sup> )	-.06	.08	.05
Cigarettes/day	.04	.03	.21
Cigs/day X Fluorine*	.02	.007	.004
TSH (mU/ml)	.38	.35	.29

R<sup>2</sup> = .28

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

\* interaction term; cigarettes per day by total fluoride

TABLE 4.1.81 LINEAR MULTIVARIATE REGRESSION MODEL OF FACTORS  
 PREDICTING THE WHITE BLOOD CELL COUNT (WBC)\* AMONG 111 MALE  
 WORKERS.  
 3M CHEMOLITE PLANT, COTTAGE GROVE, MINNESOTA

Variable	B	SE(B)	p-value
Intercept	2.87	1.32	.03
Total Fluorine (ppm)	.07	.10	.49
Alcohol #			
low (<1 oz/day)	.44	.46	.33
nonresponse (NR)	-1.08	.74	.15
low X Fluorine	-.04	.10	.68
NR X Fluorine	.59	.21	.006
Age (years)	-.007	.02	.64
BMI (kg/m <sup>2</sup> )	.07	.04	.05
Cigarettes/day	.13	.01	..0001
Free Testosterone(ng/dl)	.04	.03	.13
LH@	.10	.04	.02

R<sup>2</sup> = .67

\*WBC/1000

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

@ lutenizing hormone mU/ml

TABLE 4.1.82 LINEAR MULTIVARIATE REGRESSION MODEL OF FACTORS  
 PREDICTING THE POLYMORPHONUCLEAR LEUKOCYTE COUNT (POLY)  
 AMONG 111 MALE WORKERS.  
 3M CHEMOLITE PLANT, COTTAGE GROVE, MINNESOTA

Variable	B	SE(B)	p-value
Intercept	368	1151	.75
Total Fluorine (ppm)	165	88	.06
Alcohol #			
low (<1oz/day)	746	399	.06
nonresponse (NR)	-49	651	.94
low X Fluorine	-161	90	.08
NR X Fluorine	370	185	.05
Age (years)	6	14	.66
BMI (kg/m <sup>2</sup> )	45	33	.17
Cigarettes/day	95	10	.0001
LH (mU/ml) <sup>++</sup>	79	36	.03
Bound Testosterone*	-1.62	.8	.04
Free Testosterone *	84	32	.01

R<sup>2</sup> = .55

<sup>++</sup> Lutenizing hormone

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

\* ng/dl



**TABLE 4.1.83 LINEAR MULTIVARIATE REGRESSION MODEL OF FACTORS  
PREDICTING THE BAND COUNT (BAND) AMONG 111 MALE WORKERS.  
3M CHEMOLITE PLANT, COTTAGE GROVE, MINNESOTA**

<b>Variable</b>	<b>B</b>	<b>SE(B)</b>	<b>p-value</b>
Intercept	-11.4	129.6	.93
Total Fluorine (ppm)	-3.4	3.2	.30
Alcohol #			
low (<1 oz/day)	78.2	40.3	.05
nonresponse (NR)	14.9	67.8	.83
Age (years)	1.0	1.8	.56
BMI (kg/m <sup>2</sup> )	2.2	4.6	.63
Cigarettes/day	4.2	1.5	.005

R<sup>2</sup> = .12

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

**TABLE 4.1.84 LINEAR MULTIVARIATE REGRESSION MODEL OF FACTORS  
PREDICTING THE LYMPHOCYTE COUNT (LYMPH) AMONG 111 MALE  
WORKERS.  
3M CHEMOLITE PLANT, COTTAGE GROVE, MINNESOTA**

<b>Variable</b>	<b>B</b>	<b>SE(B)</b>	<b>p-value</b>
Intercept	2205.6	611.1	.0005
Total Fluorine (ppm)	-342.7	125.3	.007
Alcohol #			
low (<1 oz/day)	-526.6	222.7	.02
nonresponse (NR)	-977.1	355.7	.007
low X Fluorine	189.0	52.3	.0005
NR X Fluorine	247.9	103.9	.02
Cigarettes/day	34.0	6.9	.0001
Cigs/day X Fluorine*	-3.3	1.45	.02
BMI (kg/m <sup>2</sup> )	1.58	19.6	.94
BMI X Fluorine*	7.15	4.1	.08
Age (years)	-16.1	8.6	.06
Prolactin (ng/ml)	38.5	14.2	.008
TSH (mU/ml)+	170.4	77.2	.03

R<sup>2</sup> = .35

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

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

+ thyroid stimulating hormone mU/ml

TABLE 4.1.85 LINEAR MULTIVARIATE REGRESSION MODEL OF FACTORS  
 PREDICTING THE MONOCYTE COUNT (MONO) AMONG 111 MALE  
 WORKERS.  
 3M CHEMOLITE PLANT, COTTAGE GROVE, MINNESOTA

Variable	B	SE(B)	p-value
Intercept	397.4	198.9	.05
Total Fluorine (ppm)	110.4	38.6	.005
Alcohol #			
low (<1 oz/day)	132.1	53.8	.02
nonresponse (NR)	40.1	89.1	.66
Age (years)	-.37	2.4	.88
BMI (kg/m <sup>2</sup> )	-2.66	7.0	.70
BMI X Fluorine*	-4.0	1.42	.006
Cigarettes/day	7.0	1.9	.0004
LH@	13.9	6.8	.04

R<sup>2</sup> = .30

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

\*interaction term, BMI by total fluoride.

@ lutenizing hormone mU/ml

TABLE 4.1.86 LINEAR MULTIVARIATE REGRESSION MODEL OF FACTORS  
 PREDICTING THE EOSINOPHIL COUNT (EOS)  
 AMONG 111 MALE WORKERS.  
 3M CHEMOLITE PLANT, COTTAGE GROVE, MINNESOTA

Variable	B	SE(B)	p-value
Intercept	50.45	122.30	.68
Total Fluorine (ppm)	-7.31	3.35	.03
Alcohol #			
low (<1oz/day)	-12.10	37.91	.75
nonresponse (NR)	21.79	62.25	.73
Age (years)	1.56	1.67	.35
BMI (kg/m <sup>2</sup> )	2.10	4.13	.61
Cigarettes/day	3.04	1.69	.08
Cigs/day X Fluorine*	.62	.35	.08
TSH@	30.1	17.1	.08

R<sup>2</sup> = .18

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

\* interaction term, cigarettes per day by total fluoride

@ Thyroid stimulating hormone mU/ml

TABLE 4.1.87 LINEAR MULTIVARIATE REGRESSION MODEL OF FACTORS  
 PREDICTING THE PLATELET COUNT (PLATE) AMONG 111 MALE  
 WORKERS.  
 3M CHEMOLITE PLANT, COTTAGE GROVE, MINNESOTA

Variable	B	SE(B)	p-value
Intercept	264.7	54.8	.0001
Total Fluorine (ppm)	29.8	9.5	.002
Alcohol #			
low (<1oz/day)	8.2	13.3	.54
nonresponse (NR)	.9	22.5	.97
Age (years)	-1.3	.6	.04
BMI (kg/m <sup>2</sup> )	1.1	1.7	.53
BMI X Fluorine*	-1.0	.4	.004
Cigarettes/day	2.7	.6	.0001
Cigs/day X Fluorine*	-.3	.1	.04
Prolactin (ng/ml)	2.6	.03	.09
Bound Testosterone**	-.04	.03	.10

R<sup>2</sup>=.28

#Reference category is moderate drinkers who consume 1-3 oz ethanol/day.  
 \*interaction terms, BMI by total fluoride, cigarettes per day by total fluoride.  
 \*\* ng/dl

TABLE 4.1.88 LINEAR MULTIVARIATE REGRESSION MODEL OF FACTORS  
 PREDICTING THE BASOPHIL COUNT (BASO) AMONG 111 MALE WORKERS.  
 3M CHEMOLITE PLANT, COTTAGE GROVE, MINNESOTA

Variable	B	SE(B)	p-value
Intercept	44.35	54.19	.42
Total Fluorine (ppm)*	-.03	.06	.61
Alcohol #			
low (<1 oz/day)	-1.73	13.61	.90
nonresponse (NR)	-.56	22.54	.81
Age (years)	-.07	.68	.92
BMI (kg/m <sup>2</sup> )	-.61	1.58	.70
Cigarettes/day	-.80	.52	.12
Cigs/day X Fluorine <sup>2**</sup>	.02	.007	.007
Bound Testosterone##	-.07	.04	.06
Free Testosterone##	2.5	1.6	.11
LH@	5.5	1.8	.002

R<sup>2</sup> = .17

\*square transformation of total serum fluoride

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

\*\*interaction term, cigarettes per day by total fluoride.

##ng/dl

@ lutenizing hormone mU/ml

#### 4.4 Mortality Tables

**TABLE 4.2.1 CHARACTERISTICS OF 749 FEMALE EMPLOYEES, 1947-1989.**

	<b>Chemical Division</b>	<b>Non chemical Division</b>	<b>Total</b>
<b>number of workers</b>	<b>245</b>	<b>504</b>	<b>749</b>
<b>person years of observation</b>	<b>6029.0</b>	<b>13280.4</b>	<b>19309.4</b>
<b>mean follow-up (years)</b>	<b>24.6</b>	<b>26.4</b>	<b>25.8</b>
<b>mean age at employment (years)</b>	<b>28.8</b>	<b>26.9</b>	<b>27.6</b>
<b>mean year of employment (years)</b>	<b>1965.0</b>	<b>1962.8</b>	<b>1963.5</b>
<b>mean year of death (years)</b>	<b>1981.3</b>	<b>1979.2</b>	<b>1979.6</b>
<b>mean age at death (years)</b>	<b>58.7</b>	<b>54.4</b>	<b>55.4</b>

**TABLE 4.2.2 CHARACTERISTICS OF 2788 MALE EMPLOYEES, 1947-1990.**

	<b>Chemical Division</b>	<b>Non chemical Division</b>	<b>Total</b>
<b>number of workers</b>	<b>1339</b>	<b>1449</b>	<b>2788</b>
<b>person years of observation</b>	<b>33385.3</b>	<b>37732.4</b>	<b>71117.7</b>
<b>mean follow-up (years)</b>	<b>24.8</b>	<b>26.0</b>	<b>25.5</b>
<b>mean age at employment (years)</b>	<b>25.6</b>	<b>28.9</b>	<b>27.3</b>
<b>mean year of employment (years)</b>	<b>1963.8</b>	<b>1962.3</b>	<b>1963.0</b>
<b>mean year of death (years)</b>	<b>1978.3</b>	<b>1978.1</b>	<b>1978.2</b>
<b>mean age at death (years)</b>	<b>54.2</b>	<b>58.1</b>	<b>56.4</b>



**TABLE 4.2.3 VITAL STATUS AND CAUSE OF DEATH ASCERTAINMENT  
AMONG 749 FEMALE EMPLOYEES, 1947-1990.**

Vital status	Chemical Division		Non chemical Division		Total	
	No.	%	No.	%	No.	%
Alive	234	95.3	465	91.6	699	93.3
Dead*	11	4.7	39	8.4	50	6.7
Total	245	100.0	504	100.0	749	100

\*two deaths occurred outside the U.S. with cause of death ascertained from sources other than death certificates.

**TABLE 4.2.4 VITAL STATUS AND CAUSE OF DEATH ASCERTAINMENT  
AMONG 2788 MALE EMPLOYEES, 1947-1989.**

Vital status	Chemical Division		Non chemical Division		Total	
	No.	%	No.	%	No.	%
Alive	1191	88.9	1249	86.2	2440	87.5
Dead*	148	11.1	200	13.8	348	12.5
Total	1339	100.0	1449	100.0	2788	100.0

\*two deaths occurred outside the U.S. with cause of death ascertained from sources other than death certificates.

**TABLE 4.2.5 NUMBERS OF DEATHS AND STANDARDIZED MORTALITY RATIOS (SMRs) AMONG 749 FEMALE EMPLOYEES, 1947-1989.**

Cause of Death	Obs	Exp	SMR	95% CI
All causes	50	66.74	.75	.56-.99
Cancer	17	23.04	.71	.42-1.14
Gastrointestinal	2	4.54	.44	.05-1.59
Respiratory	4	4.72	.95	.26-2.43
Breast	3	5.87	.51	.10-1.49
Genital	2	3.37	.59	.07-2.14
Lymphopoietic	3	2.04	1.47	.30-4.29
Heart disease	10	12.39	.81	.49-1.29
Cerebrovascular	3	3.51	.86	.01-4.80
Gastrointestinal	3	3.41	.88	.18-2.57
Injuries	4	6.23	.64	.17-1.64
Suicide	1	1.78	.56	.01-3.13

**TABLE 4.2.6 NUMBERS OF DEATHS AND STANDARDIZED MORTALITY RATIOS (SMRs) BY DURATION OF EMPLOYMENT AMONG FEMALE EMPLOYEES, 1947-1989.**

<b>Cause of Death</b>	<b>Obs</b>	<b>Exp</b>	<b>SMR</b>	<b>95% CI</b>
<b>Duration ≤10 years</b>				
All causes	50	66.74	.75	.56-.99
Cancer	17	23.04	.71	.42-1.14
Cardiovascular	18	22.00	.82	.48-1.29
<b>Duration &gt;10 years</b>				
All causes	20	26.62	.75	.46-1.16
Cancer	6	9.42	.64	.23-1.39
Cardiovascular	8	10.27	.78	.34-1.54

Abbreviations used are: Obs, observed; Exp, expected; CI, confidence interval.

**TABLE 4.2.7 NUMBERS OF DEATHS AND STANDARDIZED MORTALITY RATIOS (SMRs) BY LATENCY AMONG FEMALE EMPLOYEES, 1947-1989.**

Cause of Death	Obs	Exp	SMR	95% CI
<b>Latency&gt;10 years</b>				
All causes	41	56.94	.72	.52-.98
Cancer	16	20.93	.76	.44-1.24
Cardiovascular	13	19.86	.65	.35-1.12
<b>Latency&gt;15 years</b>				
All causes	37	49.37	.75	.53-1.03
Cancer	14	18.25	.77	.42-1.29
Cardiovascular	13	17.79	.73	.39-1.25
<b>Latency&gt;20 years</b>				
All causes	29	39.20	.74	.49-1.08
Cancer	11	14.47	.76	.38-1.36
Cardiovascular	10	14.67	.68	.33-1.25

Abbreviations used are: Obs, observed; Exp, expected; CI, confidence interval.

TABLE 4.2.8 NUMBERS OF DEATHS AND STANDARDIZED MORTALITY RATIOS (SMRs) BY ANY EMPLOYMENT IN THE CHEMICAL DIVISION AMONG FEMALE EMPLOYEES, 1947-1989.

Cause of Death	Obs	Exp	SMR	95% CI
Not employed in CD				
All causes	39	43.05	.91	.64-1.24
Cancer	14	15.46	.91	.49-1.52
Cardiovascular	13	13.82	.94	.50-1.61
Heart disease	8	7.69	1.04	.45-2.05
All GI	2	2.23	.90	.10-3.23
All respiratory	2	2.23	.90	.10-3.23
Injuries	3	1.48	2.02	.41-5.90
employed in CD				
All causes	11	23.69	.46	.23-.83
Cancer	3	8.38	.36	.07-1.05
Cardiovascular	5	8.19	.61	.20-1.43
Heart disease	2	4.69	.43	.05-1.54
All GI	1	1.18	.85	.01-4.73
All respiratory	1	1.28	.78	.01-4.81
Injuries	1	1.98	.51	.51-2.81

Abbreviations used are: Obs, observed; Exp, expected; CI, confidence interval; CD, Chemical Division.

TABLE 4.2.9 NUMBERS OF DEATHS AND STANDARDIZED MORTALITY RATIOS (SMRs), BASED ON U.S. WHITE MALE RATES, AMONG 2788 MALE EMPLOYEES, 1947-1989.

Cause of Death	Obs	Exp	SMR	95% CI
All causes	347	473.56	.73	.66-.81
Cancer	103	107.80	.95	.77-1.15
Gastrointestinal	24	25.94	.93	.59-1.38
Colon	9	9.11	.99	.45-1.88
Pancreas	8	5.33	1.50	.65-2.96
Respiratory	31	40.53	.76	.52-1.09
Lung	29	38.72	.75	.50-1.08
Prostate	6	5.10	1.18	.43-2.56
Testis	1	.82	1.22	.02-6.80
Bladder	3	2.20	1.36	.27-3.98
Lymphopietic	13	11.42	1.14	.54-1.84
Cardiovascular	145	203.31	.71	.60-.84
CHD	110	147.04	.75	.61-.90
Cerebrovascular	10	19.92	.50	.24-.92
All Gastrointestinal	12	23.99	.50	.26-.87
All respiratory	13	25.89	.50	.27-.86
Diabetes	8	6.53	1.23	.53-2.42
Injuries	38	46.56	.82	.58-1.12
Suicide	12	17.10	.70	.32-1.23

Abbreviations used are: Obs, observed; Exp, expected; CI, confidence interval; CHD, coronary and atherosclerotic heart disease.

TABLE 4.2.10 NUMBERS OF DEATHS AND STANDARDIZED MORTALITY RATIOS (SMRs), BASED ON MINNESOTA WHITE MALE RATES, AMONG 2788 MALE EMPLOYEES, 1947-1989.

Cause of Death	Obs	Exp	SMR	95% CI
All causes	347	450.79	.77	.69-.86
Cancer	103	97.29	1.05	.86-1.27
Gastrointestinal	24	26.78	.90	.57-1.33
Colon	9	9.42	.96	.44-1.81
Pancreas	8	5.58	1.43	.62-2.83
Respiratory	31	30.42	1.02	.69-1.45
Lung	29	28.94	1.00	.67-1.44
Prostate	6	6.07	.99	.36-2.15
Testis	1	.92	1.09	.01-6.05
Bladder	3	2.18	1.37	.28-4.01
Lymphopoietic	13	12.07	1.09	.57-1.84
Cardiovascular	145	212.19	.68	.58-.80
CHD	110	159.09	.69	.57-.83
Cerebrovascular	10	24.66	.60	.32-1.02
All Gastrointestinal	12	21.13	.57	.29-.99
All respiratory	13	21.75	.60	.32-1.06
Diabetes	8	6.52	1.23	.53-2.42
Injuries	38	47.74	.80	.56-1.08
Suicide	12	15.09	.79	.41-1.39

Abbreviations used are: Obs, observed; Exp, expected; CI, confidence interval; CHD, coronary and atherosclerotic heart disease.

TABLE 4.2.11 NUMBERS OF DEATHS AND STANDARDIZED MORTALITY RATIOS (SMRs) BY LATENCY, BASED ON MINNESOTA WHITE MALE RATES, AMONG MALE EMPLOYEES, 1947-1989.

LATENCY ≥ 10 YEARS				
Cause of Death	Obs	Exp	SMR	95% CI
All causes	299	398.27	.77	.68-.86
Cancer	98	88.71	1.10	.90-1.35
Gastrointestinal	24	24.78	.97	.62-1.44
Pancreas	8	5.20	1.54	.66-3.03
Respiratory	29	28.81	1.01	.67-1.45
Lung	27	27.44	.98	.65-1.43
Skin	3	1.53	1.96	.39-5.73
Prostate	6	5.94	1.01	.37-2.20
Bladder	3	1.75	1.72	.34-5.01
Lymphopoietic	11	10.03	1.10	.55-1.96
Cardiovascular	130	195.91	.66	.55-.79
All Gastrointestinal	8	18.58	.43	.19-.86
All respiratory	11	20.16	.55	.27-.98
Diabetes	8	5.37	1.49	.64-2.94
Injuries	21	27.61	.76	.47-1.16
Suicide	11	10.19	1.08	.54-1.93

Abbreviations used are: Obs, observed; Exp, expected; CI, confidence interval; CHD, coronary and atherosclerotic heart disease.



TABLE 4.2.12 NUMBERS OF DEATHS AND STANDARDIZED MORTALITY RATIOS (SMRs) BY LATENCY, BASED ON MINNESOTA WHITE MALE RATES, AMONG MALE EMPLOYEES, 1947-1989.

LATENCY ≥ 15 YEARS				
Cause of Death	Obs	Exp	SMR	95% CI
All causes	266	344	.77	.68-87
Cancer	90	80.64	1.12	.90-1.37
Gastrointestinal	24	22.63	1.06	.68-1.51
Pancreas	8	4.72	1.69	.73-3.32
Respiratory	27	26.71	1.01	.67-1.47
Lung	25	25.45	.98	.64-1.45
Skin	3	1.29	2.33	.47-6.80
Prostate	5	5.73	.87	.28-2.04
Bladder	3	1.96	1.53	.37-4.47
Lymphopoietic	9	8.68	1.04	.47-1.97
Cardiovascular	119	178.25	.67	.55-.80
All Gastrointestinal	8	16.17	.49	.21-.97
All respiratory	9	18.60	.48	.22-.92
Diabetes	7	4.54	1.54	.62-3.18
Injuries	23	29.21	.79	.50-1.16
Suicide	9	7.47	1.21	.55-2.29

Abbreviations used are: Obs, observed; Exp, expected; CI, confidence interval; CHD, coronary and atherosclerotic heart disease.

TABLE 4.2.13 NUMBERS OF DEATHS AND STANDARDIZED MORTALITY RATIOS (SMRs) BY LATENCY, BASED ON MINNESOTA WHITE MALE RATES, AMONG MALE EMPLOYEES, 1947-1989.

LATENCY ≥ 20 YEARS				
Cause of Death	Obs	Exp	SMR	95% CI
All causes	216	286.9	.75	.66-.86
Cancer	73	68.74	1.06	.83-1.34
Gastrointestinal	15	19.33	.77	.43-1.28
Pancreas	4	4.06	.99	.27-2.52
Respiratory	25	23.06	1.08	.70-1.60
Lung	23	21.06	1.05	.66-1.57
Skin	2	.98	2.02	.23-7.34
Prostate	5	5.29	.95	.30-2.21
Bladder	3	1.75	1.72	.34-5.01
Lymphopoietic	7	7.01	.99	.39-2.03
Cardiovascular	99	151.80	1.06	.83-1.34
All Gastrointestinal	8	12.90	.62	.27-1.21
All respiratory	9	16.3	.55	.25-1.05
Diabetes	7	3.65	1.92	.77-3.95
Injuries	13	19.47	.67	.36-1.14
Suicide	7	5.01	1.40	.56-2.80

Abbreviations used are: Obs, observed; Exp, expected; CI, confidence interval; CHD, coronary and atherosclerotic heart disease.

TABLE 4.2.14 NUMBERS OF DEATHS AND STANDARDIZED MORTALITY RATIOS (SMRs) BY DURATION OF EMPLOYMENT, BASED ON MINNESOTA WHITE MALE RATES, AMONG MALE EMPLOYEES, 1947-1989.

DURATION ≥ 5 YEARS				
Cause of Death	Obs	Exp	SMR	95% CI
All causes	256	321.20	.80	.70-.90
Cancer	80	72.21	1.11	.88-1.38
Gastrointestinal	22	20.21	1.09	.68-1.65
Colon	8	7.10	1.13	.49-2.22
Pancreas	7	4.22	1.66	.66-3.42
Respiratory	25	23.72	1.08	.70-1.59
Lung	23	22.10	1.04	.66-1.56
Prostate	4	4.47	.84	.23-2.15
Bladder	2	1.68	1.19	.13-4.29
Brain	3	2.51	1.20	.24-1.50
Lymphopoietic	6	8.41	.71	.26-1.55
Cardiovascular	114	159.50	.71	.59-.86
CHD	90	120.20	.75	.60-.92
Cerebrovascular	6	18.44	.33	.12-.71
All Gastrointestinal	7	15.20	.46	.18-.95
All respiratory	9	16.30	.55	.25-1.05
Diabetes	8	4.53	1.77	.76-3.48
Injuries	29	36.60	.79	.53-1.14
Suicide	9	8.81	1.02	.47-1.94

Abbreviations used are: Obs, observed; Exp, expected; CI, confidence interval; CHD, coronary and atherosclerotic heart disease.

TABLE 4.2.15 NUMBERS OF DEATHS AND STANDARDIZED MORTALITY RATIOS (SMRs) BY DURATION OF EMPLOYMENT, BASED ON MINNESOTA WHITE MALE RATES, AMONG MALE EMPLOYEES, 1947-1989.

DURATION ≥ 10 YEARS				
Cause of Death	Obs	Exp	SMR	95% CI
All causes	203	257.30	.79	.68-.91
Cancer	67	59.36	1.13	.87-1.43
Gastrointestinal	20	16.75	1.19	.73-1.84
Colon	7	5.92	1.18	.47-2.44
Pancreas	6	3.50	1.71	.63-3.71
Respiratory	22	19.38	1.13	.71-1.72
Lung	20	18.47	1.08	.66-1.67
Prostate	4	4.20	.95	.26-2.44
Bladder	1	1.44	.69	.01-3.85
Brain	3	1.89	1.59	.32-4.64
Lymphopoietic	5	6.58	.76	.24-1.77
Cardiovascular	92	132.13	.70	.56-.85
CHD	75	99.75	.73	.57-.92
Cerebrovascular	5	15.49	.32	.10-.75
All Gastrointestinal	4	11.96	.33	.09-.86
All respiratory	7	13.80	.51	.20-1.05
Diabetes	8	3.49	2.29	.99-4.51
Injuries	19	23.46	.68	.34-1.22
Suicide	8	5.88	1.36	.59-2.68

Abbreviations used are: Obs, observed; Exp, expected; CI, confidence interval; CHD, coronary and atherosclerotic heart disease.

**TABLE 4.2.16 NUMBERS OF DEATHS AND STANDARDIZED MORTALITY RATIOS (SMRs) BY DURATION OF EMPLOYMENT, BASED ON MINNESOTA WHITE MALE RATES, AMONG MALE EMPLOYEES, 1947-1989.**

DURATION ≥ 20 YEARS				
Cause of Death	Obs	Exp	SMR	95% CI
All causes	104	152.36	.68	.56-.83
Cancer	35	37.31	.94	.65-1.30
Gastrointestinal	10	10.52	.95	.46-1.75
Colon	5	3.77	1.33	.43-3.09
Pancreas	1	2.21	.45	.01-2.52
Respiratory	11	12.69	.87	.43-1.55
Lung	10	12.10	.83	.40-1.52
Prostate	2	2.83	.71	.08-2.55
Bladder	1	.94	1.06	.01-5.91
Brain	1	1.03	.97	.01-5.40
Lymphopoietic	4	3.82	1.05	.28-6.02
Cardiovascular	48	80.6	.58	.19-1.36
CHD	39	61.25	.64	.45-.87
Cerebrovascular	1	9.13	.11	.00-.61
All Gastrointestinal	2	6.87	.29	.03-1.05
All respiratory	5	8.61	.58	.19-1.36
Diabetes	5	1.94	2.58	.83-6.02
Injuries	2	6.61	.30	.03-1.09
Suicide	3	2.54	1.18	.24-3.45

Abbreviations used are: Obs, observed; Exp, expected; CI, confidence interval; CHD, coronary and atherosclerotic heart disease.

TABLE 4.2.17 NUMBERS OF DEATHS AND STANDARDIZED MORTALITY RATIOS (SMRs), BASED ON MINNESOTA WHITE MALE RATES, AMONG 1339 MALE EMPLOYEES EVER EMPLOYED IN THE CHEMICAL DIVISION, 1947-1989.

Cause of Death	Obs	Exp	SMR	95% CI
All causes	148	172.96	.86	.72-1.01
Cancer	40	36.31	1.10	.79-1.50
Gastrointestinal	9	9.77	.92	.42-1.75
Colon	4	3.46	1.15	.31-4.01
Pancreas	4	2.04	1.96	.53-5.01
Respiratory	12	11.26	1.07	.55-1.86
Lung	11	10.70	1.03	.51-1.84
Prostate	4	1.97	2.03	.55-4.59
Testis	1	.44	2.28	.03-12.66
Bladder	1	.75	1.33	.02-7.40
Lymphopietic	5	4.76	1.05	.34-2.45
Cardiovascular	54	76.65	.70	.53-.92
CHD	43	57.74	.74	.54-1.00
Cerebrovascular	4	8.53	.47	.13-1.20
All Gastrointestinal	8	8.27	.97	.42-1.91
All respiratory	7	7.770	.91	.36-1.87
Diabetes	3	2.55	1.18	.24-3.44
Injuries	31	31.72	.98	.66-1.39
Suicide	10	6.99	1.43	.68-2.63

Abbreviations used are: Obs, observed; Exp, expected; CI, confidence interval; CHD, coronary and atherosclerotic heart disease.

TABLE 4.2.18 NUMBERS OF DEATHS AND STANDARDIZED MORTALITY RATIOS (SMRs), BASED ON MINNESOTA WHITE MALE RATES, AMONG 1449 MALE EMPLOYEES NEVER EMPLOYED IN THE CHEMICAL DIVISION, 1947-1989.

Cause of Death	Obs	Exp	SMR	95% CI
All causes	200	291.25	.69	.59-.79
Cancer	63	67.56	.93	.72-1.19
Gastrointestinal	15	16.46	.91	.51-1.50
Colon	5	5.79	.89	.28-2.01
Pancreas	4	3.37	1.19	.32-3.04
Respiratory	19	25.58	.74	.45-1.16
Lung	18	24.44	.74	.44-1.16
Prostate	2	3.45	.58	.07-2.09
Testis	0	.43	.00	.00-8.45
Bladder	2	1.45	1.38	.16-4.99
Lymphopoietic	8	6.89	1.16	.50-2.29
Cardiovascular	91	129.77	.70	.56-.86
CHD	67	93.84	.71	.55-.91
Cerebrovascular	6	12.93	.46	.17-1.01
All Gastrointestinal	4	14.56	.27	.07-.70
All respiratory	6	16.77	.36	.13-.78
Diabetes	5	4.05	1.24	.40-2.88
Injuries	23	38.28	.60	.38-.98
Suicide	2	9.26	.60	.02-.78

Abbreviations used are: Obs, observed; Exp, expected; CI, confidence interval; CHD, coronary and atherosclerotic heart disease.

TABLE 4.2.19 NUMBERS OF DEATHS AND STANDARDIZED MORTALITY RATIOS (SMRs) BY LATENCY, BASED ON MINNESOTA WHITE MALE RATES, AMONG MALE EMPLOYEES NEVER EMPLOYED IN THE CHEMICAL DIVISION, 1947-1989.

LATENCY ≥ 15 YEARS				
Cause of Death	Obs	Exp	SMR	95% CI
All causes	161	216.10	.75	.63-.87
Cancer	56	50.70	1.10	.83-1.43
Gastrointestinal	15	14.37	1.05	.59-1.73
Colon	5	5.13	.98	.31-2.28
Pancreas	4	2.99	1.34	.36-3.43
Respiratory	17	16.73	1.02	.59-1.67
Lung	16	15.94	1.00	.57-1.63
Prostate	2	3.86	.52	.06-1.87
Lymphopoietic	5	5.40	.93	.30-2.16
Cardiovascular	75	113.60	.66	.52-.83
All Gastrointestinal	4	9.80	.41	.11-1.05
All respiratory	4	12.14	.33	.09-.84
Diabetes	5	2.82	1.77	.57-4.14
Injuries	7	11.17	.63	.25-1.29

Abbreviations used are: Obs, observed; Exp, expected; CI, confidence interval; CHD, coronary and atherosclerotic heart disease; CD, Chemical Division.



**TABLE 4.2.20 NUMBERS OF DEATHS AND STANDARDIZED MORTALITY RATIOS (SMRs) BY LATENCY, BASED ON MINNESOTA WHITE MALE RATES, AMONG MALE EMPLOYEES EVER EMPLOYED IN THE CHEMICAL DIVISION, 1947-1989.**

LATENCY ≥ 15 YEARS				
Cause of Death	Obs	Exp	SMR	95% CI
All causes	105	128.4	.82	.67-.99
Cancer	34	29.95	1.14	.79-1.59
Gastrointestinal	9	8.30	1.08	.49-2.06
Colon	7	3.00	1.33	.36-3.42
Pancreas	4	1.75	2.28	.61-5.85
Respiratory	10	9.98	1.00	.48-1.94
Lung	9	9.50	.95	.43-1.80
Prostate	3	1.87	1.61	.32-4.70
Lymphopoietic	4	3.28	1.72	.33-3.12
Cardiovascular	44	64.67	.68	.49-.91
All Gastrointestinal	4	6.37	.63	.17-1.61
All respiratory	5	6.49	.77	.25-1.80
Diabetes	2	1.72	1.17	.43-4.21
Injuries	6	8.54	.70	.26-1.53

Abbreviations used are: Obs, observed; Exp, expected; CI, confidence interval; CHD, coronary and atherosclerotic heart disease; CD, Chemical Division.

TABLE 4.2.21 NUMBERS OF DEATHS AND STANDARDIZED MORTALITY RATIOS (SMRs) BY DURATION OF EMPLOYMENT, BASED ON MINNESOTA WHITE MALE RATES, AMONG MALE EMPLOYEES EVER EMPLOYED IN THE CHEMICAL DIVISION, 1947-1989.

DURATION ≥ 10 YEARS				
Cause of Death	Obs	Exp	SMR	95% CI
All causes	90	108.7	.83	.67-1.02
Cancer	27	24.4	1.08	.71-1.58
Gastrointestinal	6	6.92	.87	.22-1.89
Colon	3	2.47	1.22	.24-3.55
Pancreas	2	1.46	1.37	.75-4.86
Respiratory	8	8.16	.98	.42-1.93
Lung	7	7.78	.90	.36-1.86
Prostate	3	1.55	1.94	.39-5.66
Lymphopoietic	4	2.84	1.41	.38-3.61
Cardiovascular	38	54.60	.70	.50-.97
All respiratory	3	5.42	.55	.11-1.62
Diabetes	3	1.51	1.99	.40-5.80
Injuries	7	8.11	.86	.35-1.78

Abbreviations used are: Obs, observed; Exp, expected; CI, confidence interval; CHD, coronary and atherosclerotic heart disease; CD, Chemical Division.

TABLE 4.2.22 NUMBERS OF DEATHS AND STANDARDIZED MORTALITY RATIOS (SMRs) BY DURATION OF EMPLOYMENT, BASED ON MINNESOTA WHITE MALE RATES, AMONG MALE EMPLOYEES EVER EMPLOYED IN THE CHEMICAL DIVISION, 1947-1989.

DURATION ≥ 20YEARS				
Cause of Death	Obs	Exp	SMR	95% CI
All causes	45	66.29	.68	.50-.91
Cancer	16	16.21	.99	.56-1.20
Gastrointestinal	3	4.53	.66	.13-1.94
Colon	3	1.61	1.84	.37-5.38
Pancreas	0	.96	.00	0-3.84
Respiratory	5	5.57	.90	.29-2.09
Lung	4	5.31	.75	.20-1.93
Prostate	2	1.10	1.82	.20-6.58
Lymphopoietic	3	1.67	1.79	.36-5.24
Cardiovascular	18	34.48	.52	.31-.83
All respiratory	2	3.52	.57	.06-2.52
Diabetes	2	.84	2.37	.27-8.56
Injuries	2	3.27	.61	.07-2.21

Abbreviations used are: Obs, observed; Exp, expected; CI, confidence interval; CHD, coronary and atherosclerotic heart disease; CD, Chemical Division.

**TABLE 4.2.23 NUMBERS OF DEATHS AND STANDARDIZED MORTALITY RATIOS (SMRs) BY DURATION OF EMPLOYMENT, BASED ON MINNESOTA WHITE MALE RATES, AMONG MALE EMPLOYEES NEVER EMPLOYED IN THE CHEMICAL DIVISION, 1947-1989.**

DURATION ≥ 10 YEARS				
Cause of Death	Obs	Exp	SMR	95% CI
All causes	113	148.60	.76	.63-.91
Cancer	40	34.43	1.16	.83-1.58
Gastrointestinal	14	9.82	1.43	.78-2.39
Colon	4	3.45	1.16	.31-2.97
Pancreas	4	2.04	1.96	.53-5.01
Respiratory	14	11.22	1.25	.68-2.09
Lung	13	10.69	1.22	.65-2.08
Prostate	1	2.65	.38	.01-2.10
Lymphopoletic	1	3.47	.27	.01-1.49
Cardiovascular	54	78.31	.69	.52-.90
All respiratory	4	8.35	.48	.13-1.27
Diabetes	5	1.98	2.52	.81-3.87
Injuries	4	7.96	.50	0.14-1.29

Abbreviations used are: Obs, observed; Exp, expected; CI, confidence interval; CHD, coronary and atherosclerotic heart disease; CD, Chemical Division.

**TABLE 4.2.24 NUMBERS OF DEATHS AND STANDARDIZED MORTALITY RATIOS (SMRs) BY DURATION OF EMPLOYMENT, BASED ON MINNESOTA WHITE MALE RATES, AMONG MALE EMPLOYEES NEVER EMPLOYED IN THE CHEMICAL DIVISION, 1947-1989.**

Cause of Death	DURATION ≥ 20YEARS			
	Obs	Exp	SMR	95% CI
All causes	59	86.1	.69	.52-.88
Cancer	19	21.09	.90	.54-1.41
Gastrointestinal	7	5.99	1.17	.47-2.41
Colon	2	2.15	.93	.10-3.32
Pancreas	1	1.25	.80	.01-4.45
Respiratory	6	7.12	.84	.31-1.83
Lung	6	6.79	.88	.32-1.92
Prostate	0	1.73	.00	.0-2.12
Lymphopoietic	1	2.15	.46	.01-2.58
Cardiovascular	30	46.14	.65	.44-.93
All respiratory	3	5.43	.59	.12-1.72
Diabetes	3	1.10	2.74	.55-8.00
Injuries	0	3.34	.00	.00-1.14

Abbreviations used are: Obs, observed; Exp, expected; CI, confidence interval; CHD, coronary and atherosclerotic heart disease; CD, Chemical Division.

**TABLE 4.2.25 AGE ADJUSTED STANDARDIZED RATE RATIOS (SRRs) FOR ALL CAUSE, CANCER, AND CARDIOVASCULAR MORTALITY BY DURATION OF EMPLOYMENT, AMONG MALE EMPLOYEES, 1947-1989.**

Cause of death	SRR*	95%CI
all causes	.81	.63-1.03
all cancers	1.04	.67-1.61
all cardiovascular	.91	.62-1.34

Abbreviations used are: SRR, standardized rate ratio ; CI, confidence interval.  
 \* less than 10 years of employment as referent category

**TABLE 4.2.26 AGE ADJUSTED STANDARDIZED RATE RATIOS (SRRs) FOR ALL CAUSE, CANCER, LUNG CANCER, GI CANCER, AND CARDIOVASCULAR MORTALITY BY EVER/NEVER EMPLOYED IN THE CHEMICAL DIVISION, AMONG MALE EMPLOYEES, 1947-1989.**

Cause of death	SRR*	95%CI
all causes	1.18	(.95,1.47)
all cancers	1.10	(.74,1.65)
lung cancer	1.09	(.67,2.31)
GI cancer	1.16	(.50,2.69)
all cardiovascular	1.05	(.76,1.48)

Abbreviations used are: SRR, standardized rate ratio ; CI, confidence interval; GI, gastrointestinal.

\* Never employed in the Chemical Division as referent category

**TABLE 4.2.27 AGE STRATIFIED, YEARS OF FOLLOW-UP ADJUSTED RATE RATIOS (RR<sub>MH</sub>) FOR ALL CAUSE, CANCER, AND CARDIOVASCULAR MORTALITY BY EVER/NEVER EMPLOYED IN THE CHEMICAL DIVISION, AMONG MALE EMPLOYEES, 1947-1989.**

<b>Age at employment</b>	<b>RR<sub>MH</sub><sup>*</sup></b>	<b>95%CI</b>
<b>All causes</b>		
15-19 years	1.22	(.62, 2.40)
20-29 years	.95	(.68, 1.32)
30-39 years	.95	(.61, 1.50)
40-65 years	1.02	(.72-1.44)
<b>All cancers</b>		
15-19 years	.95	(.21, 4.34)
20-29 years	.72	(.38, 1.35)
30-39 years	1.10	(.62, 1.90)
40-65 years	.66	(.27, 1.60)
<b>All cardiovascular</b>		
15-19 years	1.40	(.39, 5.03)
20-29 years	.86	(.44, 1.67)
30-39 years	.78	(.44, 1.29)
40-65 years	1.11	(.73, 1.82)

Abbreviations used are: RR<sub>MH</sub>, Mantel-Haenszel age adjusted rate ratio ; CI, confidence interval.

\* Adjusted for years of follow-up and stratified by four age categories. Never employed in the Chemical Division as referent category



**TABLE 4.2.28 AGE STRATIFIED, YEARS OF FOLLOW-UP ADJUSTED RATE RATIOS (RR<sub>MH</sub>) FOR ALL CAUSE, CANCER, AND CARDIOVASCULAR MORTALITY BY DURATION OF EMPLOYMENT IN THE CHEMICAL DIVISION, AMONG MALE EMPLOYEES, 1947-1989.**

<b>Age at employment</b>	<b>RR<sub>MH</sub><sup>*</sup></b>	<b>95%CI</b>
<b>All causes</b>		
15-19 years	1.30	(.58, 3.28)
20-29 years	1.16	(.81, 1.65)
30-39 years	2.16	(1.52, 2.70)
40-65 years	1.69	(1.07, 2.60)
<b>All cancers</b>		
15-19 years	2.17	(.40, 11.61)
20-29 years	.84	(.44, 1.51)
30-39 years	1.75	(.95, 3.21)
40-65 years	2.67	(.995, 7.14)
<b>All cardiovascular</b>		
15-19 years	.88	(.25, 3.33)
20-29 years	1.38	(.73, 2.60)
30-39 years	3.53	(1.68, 6.21)
40-65 years	1.50	(.81, 2.79)

Abbreviations used are: RR<sub>MH</sub>, Mantel-Haenszel age adjusted rate ratio ; CI, confidence interval.

\* Adjusted for years of follow-up and stratified by four age categories. less than 10 years employment as referent category

**TABLE 4.2.29 PROPORTIONAL HAZARD REGRESSION MODEL OF FACTORS PREDICTING THE ALL CAUSE MORTALITY AMONG 2788 MALE WORKERS.**

<b>Variable</b>	<b><math>\beta</math></b>	<b>SE(<math>\beta</math>)</b>	<b>p-value</b>	<b>RR<sup>#</sup></b>
Year of first employment	-0.55	.009	.0001	.946
Age at first employment*	.079	.006	.0001	1.082
Duration of employment*	-.34	.001	.0001	.967
Months in chemical division	.001	.001	.24	1.001

Abbreviations used are:  $\beta$ , regression parameter; SE( $\beta$ ), standard error of the slope parameter; RR, relative risk.

# relative risk for one unit change in independent variable

\* years

**TABLE 4.2.30 PROPORTIONAL HAZARD REGRESSION MODEL OF FACTORS PREDICTING THE CARDIOVASCULAR MORTALITY AMONG 2788 MALE WORKERS.**

<b>Variable</b>	<b><math>\beta</math></b>	<b>SE(<math>\beta</math>)</b>	<b>p-value</b>	<b>RR<sup>#</sup></b>
Year of first employment	-0.075	.016	.001	.928
Age at first employment*	.119	.009	.0001	1.126
Duration of employment*	.230	.294	.45	.852
Months in chemical division	.0002	.001	.85	1.00

Abbreviations used are:  $\beta$ , regression parameter; SE( $\beta$ ), standard error of the slope parameter; RR, relative risk.

# relative risk for one unit change in independent variable

\* years

TABLE 4.2.31 PROPORTIONAL HAZARD REGRESSION MODEL OF FACTORS PREDICTING THE CANCER MORTALITY AMONG 2788 MALE WORKERS.

Variable	B	SE(B)	p-value	RR#
Year of first employment	-.031	.019	.11	.969
Age at first employment*	.078	.011	.0001	1.081
Duration of employment*	-.028	.009	.002	.972
Months in chemical division	.002	.001	.20	1.002

Abbreviations used are:  $\beta$ , regression parameter; SE( $\beta$ ), standard error of the slope parameter; RR, relative risk.

# relative risk for one unit change in independent variable

\* years

TABLE 4.2.32 PROPORTIONAL HAZARD REGRESSION MODEL OF FACTORS PREDICTING THE LUNG CANCER MORTALITY AMONG 2788 MALE WORKERS.

Variable	B	SE(B)	p-value	RR#
Year of first employment	-.019	.042	.65	.981
Age at first employment*	.070	.021	.001	1.072
Duration of employment*	-.062	.133	.64	.940
Months in chemical division	-.026	.016	.11	.975

Abbreviations used are:  $\beta$ , regression parameter; SE( $\beta$ ), standard error of the slope parameter; RR, relative risk.

# relative risk for one unit change in independent variable

\* years

**TABLE 4.2.33 PROPORTIONAL HAZARD REGRESSION MODEL OF FACTORS PREDICTING THE GI CANCER MORTALITY AMONG 2788 MALE WORKERS.**

Variable	B	SE(B)	p-value	RR#
Year of first employment	.015	.038	.71	1.015
Age at first employment*	.130	.021	.001	1.139
Duration of employment*	.005	.020	.82	1.005
Months in chemical division	.001	.002	.56	1.001

Abbreviations used are: GI, Gastrointestinal; B, regression parameter; SE(B), standard error of the slope parameter; RR, relative risk.  
 # relative risk for one unit change in independent variable  
 \* years

**TABLE 4.2.34 PROPORTIONAL HAZARD REGRESSION MODEL OF FACTORS PREDICTING THE PROSTATE CANCER MORTALITY AMONG 2788 MALE WORKERS.**

Variable	B	SE(B)	p-value	RR#
Year of first employment	.010	.081	.90	1.011
Age at first employment*	.082	.045	.06	1.085
Duration of employment*	-.070	.052	.18	.932
Months in chemical division	.010	.005	.03	1.010

Abbreviations used are: B, regression parameter; SE(B), standard error of the slope parameter; RR, relative risk.  
 # relative risk for one unit change in independent variable  
 \* years

**TABLE 4.2.35 PROPORTIONAL HAZARD REGRESSION MODEL OF FACTORS PREDICTING THE PANCREATIC CANCER MORTALITY AMONG 2788 MALE WORKERS.**

<b>Variable</b>	<b>B</b>	<b>SE(B)</b>	<b>p-value</b>	<b>RR<sup>#</sup></b>
Year of first employment	.046	.066	.48	1.047
Age at first employment*	.136	.034	.0001	1.146
Duration of employment*	-.012	.035	.73	.988
Months in chemical division	-.002	.006	.73	.998

Abbreviations used are: B, regression parameter; SE(B), standard error of the slope parameter; RR, relative risk.

# relative risk for one unit change in independent variable

\* years

**TABLE 4.2.36 PROPORTIONAL HAZARD REGRESSION MODEL OF FACTORS PREDICTING THE DIABETES MELLITUS MORTALITY AMONG 2788 MALE WORKERS.**

<b>Variable</b>	<b>B</b>	<b>SE(B)</b>	<b>p-value</b>	<b>RR<sup>#</sup></b>
Year of first employment	-.405	.221	.06	.667
Age at first employment*	.092	.044	.04	1.096
Duration of employment*	.009	.030	.75	1.009
Months in chemical division	-.001	.004	.76	.999

Abbreviations used are: B, regression parameter; SE(B), standard error of the slope parameter; RR, relative risk.

# relative risk for one unit change in independent variable

\* years

**TABLE 4.2.37 PROPORTIONAL HAZARD REGRESSION MODEL OF FACTORS PREDICTING THE ALL CAUSE MORTALITY AMONG 749 FEMALE WORKERS.**

<b>Variable</b>	<b>B</b>	<b>SE(B)</b>	<b>p-value</b>	<b>RR#</b>
Year of first employment	-0.02	.03	.41	.977
Age at first employment*	.08	.02	.0001	1.08
Duration of employment*				
2-10 years	1.31	.54	.01	3.72
>10 years	.85	.57	.14	2.33
Months in chemical division	-.003	.004	.48	.997

Abbreviations used are: B, regression parameter; SE(B), standard error of the slope parameter; RR, relative risk.

# relative risk for one unit change in independent variable

\* years

**TABLE 4.2.38 PROPORTIONAL HAZARD REGRESSION MODEL OF FACTORS PREDICTING THE CARDIOVASCULAR MORTALITY AMONG 749 FEMALE WORKERS.**

<b>Variable</b>	<b>B</b>	<b>SE(B)</b>	<b>p-value</b>	<b>RR#</b>
Year of first employment	-.034	.048	.48	.966
Age at first employment*	.119	.024	.0001	1.126
Duration of employment*	-.011	.025	.67	.986
Months in chemical division	-.015	.017	.37	.985

Abbreviations used are: B, regression parameter; SE(B), standard error of the slope parameter; RR, relative risk.

# relative risk for one unit change in independent variable

\* years

TABLE 4.2.39 PROPORTIONAL HAZARD REGRESSION MODEL OF FACTORS PREDICTING THE CANCER MORTALITY AMONG 749 FEMALE WORKERS.

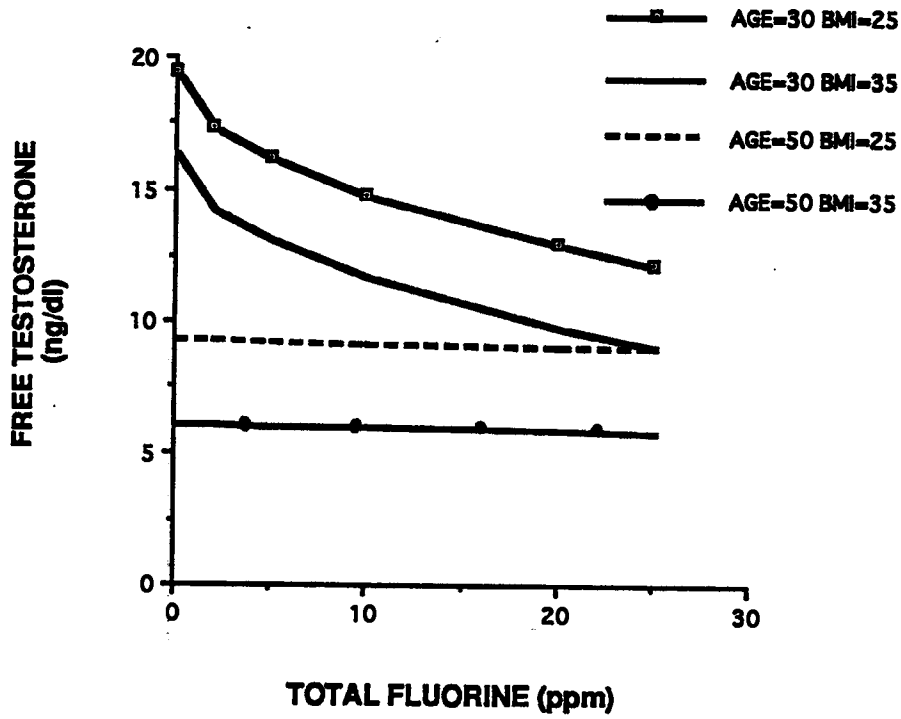
Variable	B	SE( $\beta$ )	p-value	RR <sup>#</sup>
Year of first employment	-.043	.053	.42	.958
Age at first employment*	.085	.025	.001	1.089
Duration of employment*	-.021	.025	.65	.980
Months in chemical division	.001	.005	.87	1.001

Abbreviations used are:  $\beta$ , regression parameter; SE( $\beta$ ), standard error of the slope parameter; RR, relative risk.

# relative risk for one unit change in independent variable

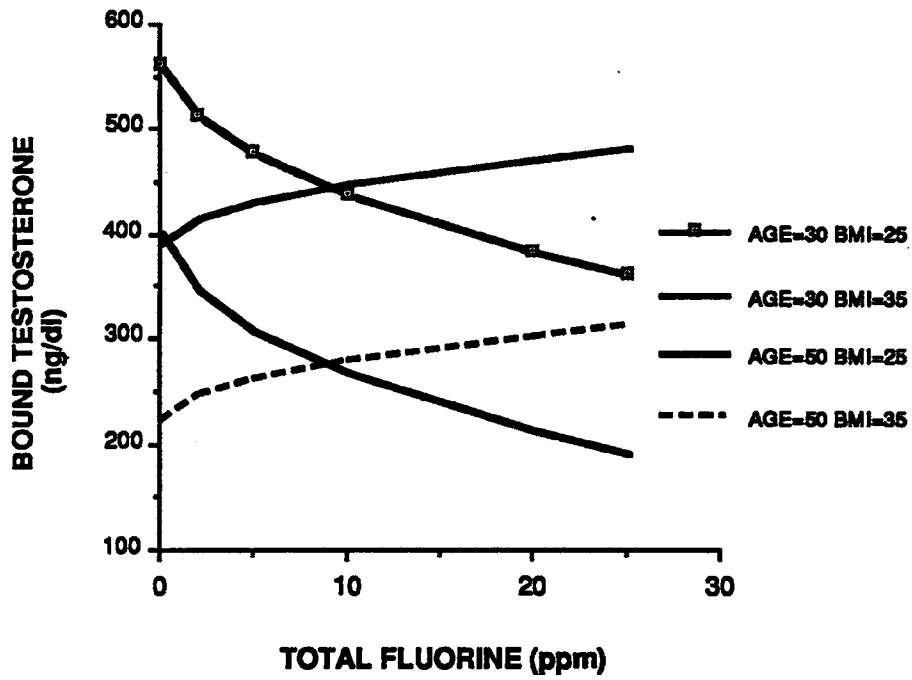
\* years

**FIGURE 1. Free testosterone and total serum fluorine  
1990 3M Chemolite study**

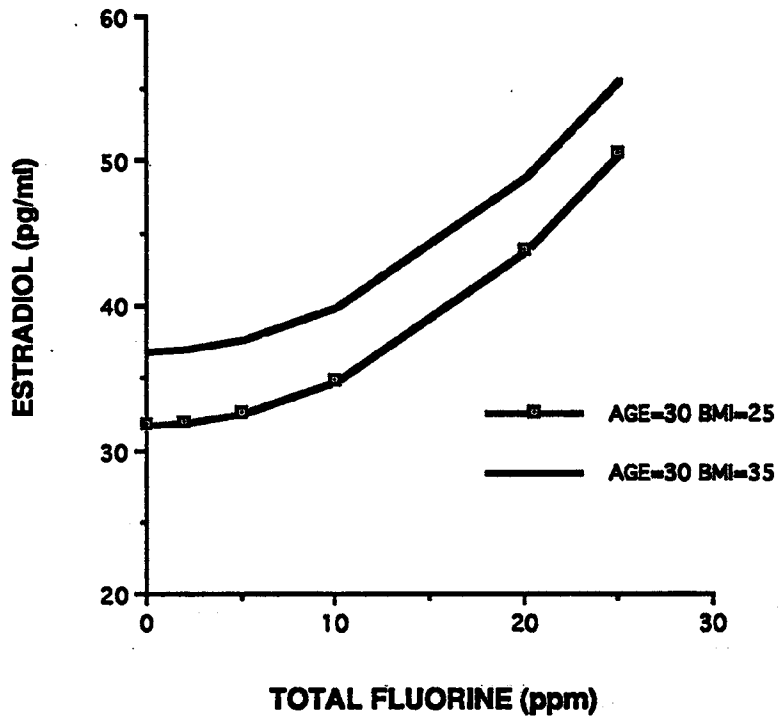




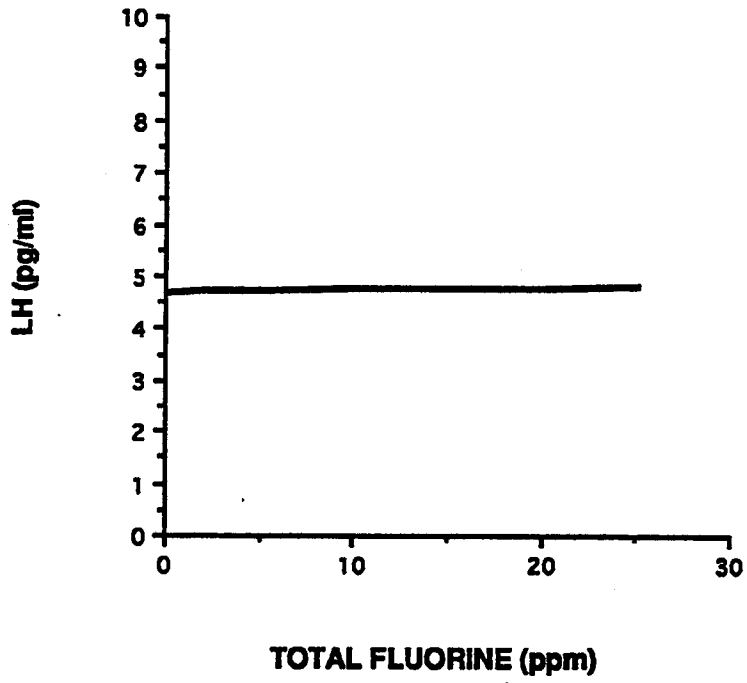
**Figure 2. Bound testosterone and total serum fluoride  
1990 3M Chemolite study**



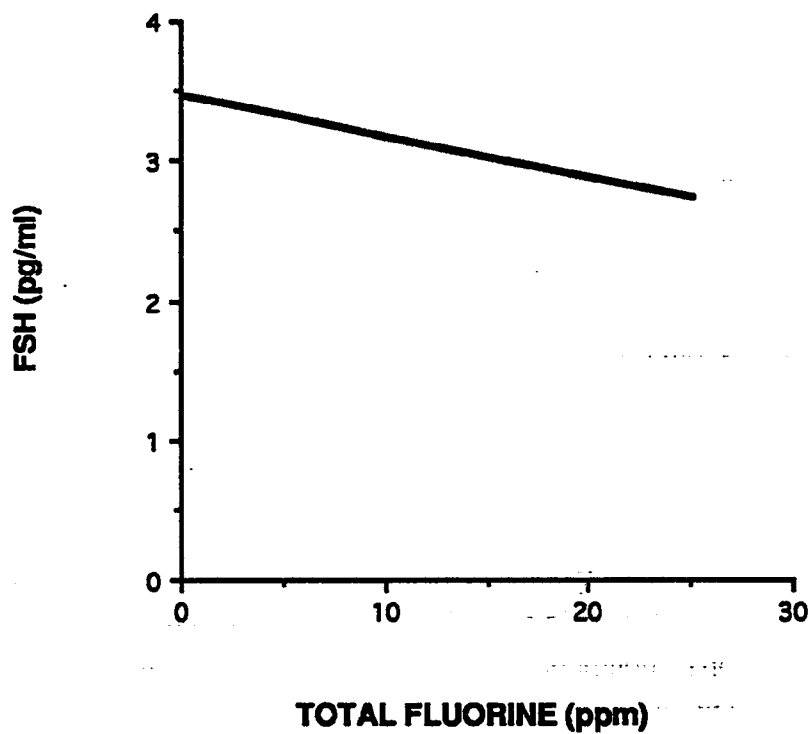
**Figure 3. Estradiol and total serum fluorine  
1990 3M Chemolite study**



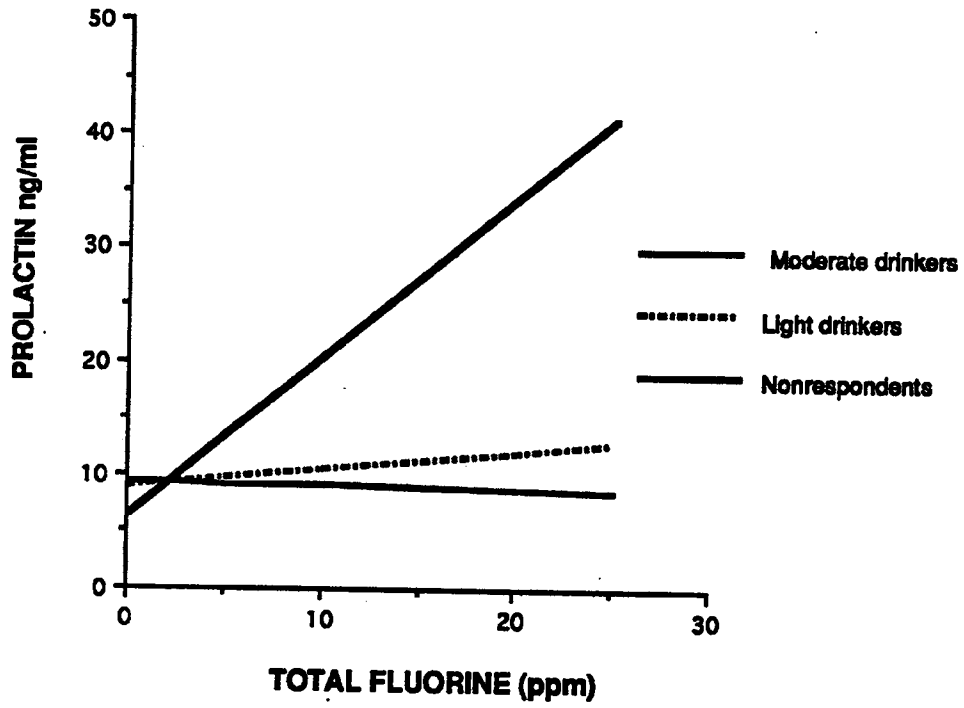
**Figure 4. Lutenizing hormone and total serum fluorine  
1990 3M Chemolite study**



**Figure 5. Follicle stimulating hormone and total serum fluorine  
1990 3M Chemolite study**



**Figure 6. Prolactin and total serum fluorine  
1990 3M Chemolite study**



**Figure 7. Thyroid stimulating hormone and total serum fluorine  
1990 3M Chemolite study**

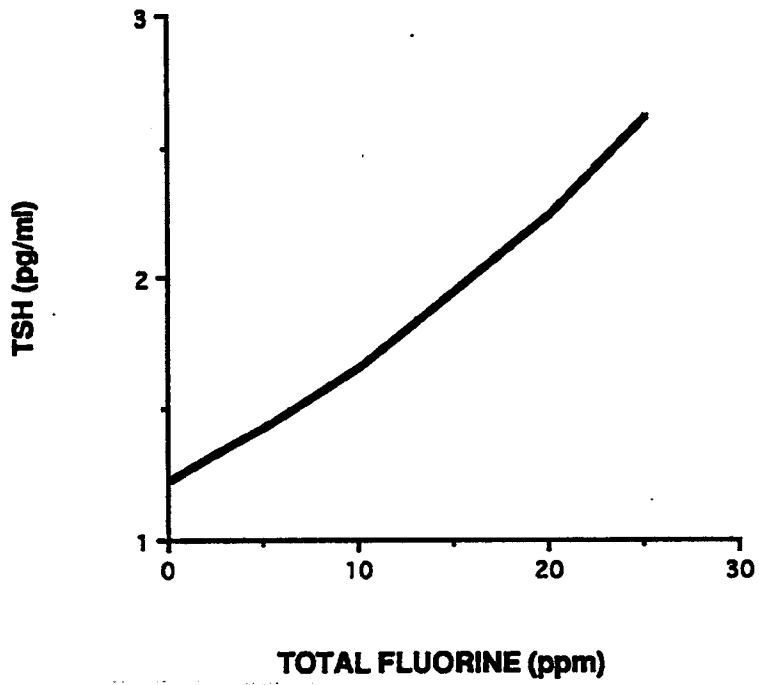
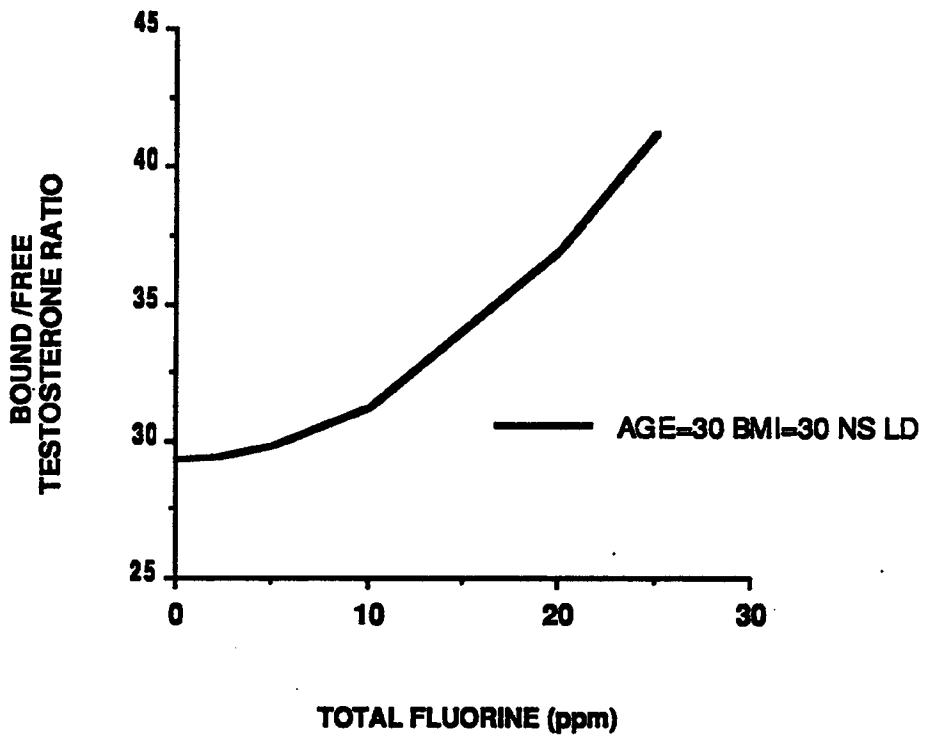


Figure 8. Bound to free testosterone ratio and total serum fluorine  
1990 3M Chemolite study



## 5. DISCUSSION

### 5.1 Physiologic Effects Study

#### 5.1.1 Introduction

This was a cross-sectional study of the relationship between selected physiologic parameters and PFOA exposure which was assessed using total serum fluorine. Participants were recruited from workers employed during November, 1990 in the Chemical Division of the 3M Chemolite Plant in Cottage Grove, Minnesota. All current workers who had worked in high exposure jobs at any time in the five previous years were invited to participate. A sample of workers employed in low exposure jobs was frequency matched to the age distribution of workers in high exposure jobs.

Participants completed a corporate medical history questionnaire and had vital parameters measured by an occupational health nurse. Blood was drawn for assays of total serum fluorine, seven hormones involved in the hypothalamic-pituitary-gonadal axis, serum lipids, lipoproteins, hepatic function parameters, and hematology indices. Blood was drawn in the morning after workers were assigned to the day shift for at least three days.

In 93% of participants serum fluorine levels were at least 10 times the background levels in the general population and in 3M workers not employed at Chemolite. Many workers who lacked PFOA exposure by job history had elevated PFOA levels. The sources of the unexpected PFOA exposure are unknown.

#### 5.1.2 Hormones

The findings from this study are consistent with the hypothesis that perfluorooctanoic acid (PFOA) affects the human hypothalamic-pituitary-gonadal axis. This study showed that relatively low levels of serum PFOA (20µM) depressed free testosterone and elevated estradiol but did not affect LH or FSH levels. The association between free testosterone and PFOA was different in



older men than in younger men. In older men, free testosterone (FT) was depressed below 10 ng/ml at serum fluoride levels below one part per million (estimated PFOA levels below 1  $\mu$ M). In younger men, FT decreased toward 10 ng/ml at serum fluoride levels above 15 ppm (estimated PFOA levels below 15  $\mu$ M). **Increasing age may increase men's susceptibility to the testosterone lowering effects of PFOA.** The associations between PFOA and the hormone levels may reflect a true causal relationship, or may be a result of chance, bias, or uncontrolled confounding. There are no human studies of PFOA associated reproductive toxicity available for comparison. **Studies of the effects of PFOA in rodents have demonstrated a similar decrease in testosterone, increase in estradiol, and little change in LH** <sup>19</sup>.

The association between PFOA and free testosterone may have been mediated by elevated estradiol and prolactin. Elevated estradiol decreases testosterone and other steroid hormone synthesis in Leydig cells. LH response to low testosterone is attenuated by estradiol through negative feedback mechanisms at the pituitary and hypothalamic levels <sup>112, 113</sup>. Elevated prolactin sensitizes the hypothalamus and pituitary to estrogens feedback. The combined effect of elevated estradiol and prolactin could have reduced the secretion of LH and the subsequent Leydig cell response. Estradiol has direct effects on Leydig cell testosterone synthesis. In rats, PFOA decreased androstenedione and testosterone, but not 17 alpha-hydroxyprogesterone <sup>19</sup>. The metabolism of 17 alpha-hydroxyprogesterone to androstenedione was inhibited at the step of the C-17,20 lyase. The activity of the rate limiting C-17,20 lyase has been reported to be under estradiol regulation in rat Leydig cells <sup>114, 115</sup>. Thus, in PFOA treated rats, elevated levels of estradiol may inhibit the C-17,20 lyase and thereby reduce testosterone synthesis. The increase in the estradiol-testosterone ratio observed in workers is compatible with this mechanism for decreased free testosterone.

The primary source of estradiol in males is the P450 (P450 19) mediated aromatization of testosterone <sup>116, 117</sup>. Additional estradiol is secreted directly from Leydig cells. The observed increase in estradiol may be the result of increased production from one of these two sources or may be the result of inhibition of P450 mediated estradiol metabolism <sup>118</sup>. Perfluorooctanoic acid, a

prototype peroxisome proliferator, may regulate steroidogenesis by binding to a member of a new family of cytosolic receptors (PPAR) belonging to the nuclear hormone receptor superfamily and transactivating the transcription of genes involved in steroid synthesis <sup>119-121</sup>.

PFOA was positively associated with the TB/TF and E/TF ratios. PFOA binding to sex hormone binding globulin (SHBG) may have produced changes in the bound to free testosterone ratio. However, this would result in a change in the TB/TF that is in the opposite direction to the observed association between PFOA and TB/TF. The associations of PFOA with these ratios are consistent with a mechanism that involves decreased production of testosterone and increased production of estradiol.

The HPG axis of older men appeared to be more susceptible to PFOA compared to that of younger men. No animal data has been reported concerning age related sensitivity to the effects of PFOA. However, the onset of Leydig cell tumors has been reported to occur late in two year rat feeding studies <sup>122</sup>. This finding may represent increased susceptibility for hormonal alterations in aged rats. Further animal research is needed to define any age related susceptibility factors.

Prolactin levels were positively associated with total serum fluoride in participants who reported moderate drinking (1-3 drinks/day). Since the function of prolactin in men is uncertain, the clinical significance of such an association is unclear. Alcohol ingestion is a stimulus for prolactin secretion. The mechanism of this effect appears to be mediated by alterations in calcium mediated signal transduction pathways <sup>123</sup>. This suggests that the elevation of prolactin associated with PFOA and alcohol may be mediated by alterations in calcium mediated events such as transmembrane signal transduction pathways.

Thyroid stimulating hormone was positively associated with total serum fluoride. Animal studies have shown that perfluorodecanoic acid depressed peripheral thyroid hormone levels without producing a hypothyroid response <sup>75, 79, 101</sup>. In the present study, peripheral thyroid hormone levels were not assayed. Therefore, it is not possible to assess whether the observed association between

PFOA and TSH could be a direct hypothalamic effect, a pituitary regulatory effect, or an effect mediated by changes in peripheral thyroid hormone levels.

In summary, this is the first report of hormonal changes associated with PFOA in humans. The present findings in humans are consistent with those previously reported in animal studies <sup>19</sup>. The consistent findings include low free testosterone, increased estradiol, and unchanged LH. Rodent and human reproductive endocrine systems differ greatly, yet the suggested effects of PFOA are similar. In light of the observed similarities in effect, it is tempting to speculate that PFOA may effect the humans and rodents reproductive endocrine system through the same mechanism. A hypothesis that PFOA alters a calcium mediated cellular signal transduction pathway, such as the cAMP or Inositol triphosphate mediated second messenger response, may provide a unified mechanism for the multiple loci of putative effects.

No adverse health effects have been observed in exposed <sup>8</sup>. The present study did not examine adverse health effects, although several adverse outcomes associated with hormonal alterations are possible. The etiology of a number of cancers including adenocarcinomas of the prostate, endometrium, colon, rectum, pancreas and breast, have been linked to changes in endogenous hormones <sup>124</sup>. Cancers in this etiologic category include.

Perfluorooctanoic acid is not a genotoxic carcinogen in standard assays <sup>9</sup>. However, PFOA is a nongenotoxic rodent carcinogen. In rats exposed to PFOA over a two year period, there was associated increase in Leydig cell tumors <sup>125</sup>. Leydig cell tumors have been observed in association with other peroxisome proliferators in rats <sup>122</sup>. It has been hypothesized that chronically elevated LH produced testicular neoplasms <sup>19, 122</sup>. However, in PFOA treated rats, LH was not elevated. This may be due to estrogens feedback inhibition as discussed previously, or due to insufficient experimental induction time <sup>19</sup>. Alternatively, another mechanism may have been operative in producing Leydig cell tumors. Exogenous estradiol produces Leydig cell tumors in mice <sup>126</sup>. High estradiol levels are associated with Leydig cell tumors in both rats and humans <sup>127, 128</sup>. The tissue surrounding the Leydig cell adenomas also produces increased estrogens <sup>127</sup>. High estradiol may be a stimulus for Leydig cell

proliferation and tumor formation. This hypothesis is supported by the observation that estradiol stimulates TGF- $\alpha$  secretion in Leydig cells. TGF- $\alpha$  binds to EGF receptors expressed on Leydig cells<sup>129</sup> and stimulates cell proliferation. The hormonal changes associated with PFOA may be a mechanism for nongenotoxic carcinogenesis. The role of PFOA in human nongenotoxic carcinogenesis needs to be clarified.

Adequate androgen levels are necessary for maintenance of potency, spermatogenesis, libido and male reproductive organs. Low testosterone and high estrogens may decrease libido, and fertility in males<sup>130</sup>. Decreased male fertility may be one potential adverse outcome of PFOA. The reproductive toxicity of PFOA has not been extensively studied. No studies have been conducted in humans. PFOA was not teratogenic in rats<sup>9, 131, 132</sup>. No adverse effects on fertility were noted for female rats in a teratogenesis study<sup>9</sup>. Male rats were not studied. No other reproductive studies in animals have been reported. Studies of human reproductive function are needed since human reproductive processes are thought to be more sensitive to xenobiotic insults compared to other animal species<sup>133</sup>.

#### 5.1.3 Cholesterol, Triglycerides, and Lipoproteins

Cholesterol, triglycerides, and LDL were not significantly associated with PFOA. The lack of association of PFOA with cholesterol or triglycerides is consistent with observations in experimental animal models. No animal studies of PFOA's effect on LDL are available for comparison. There are no studies in humans concerning the relationship of PFOA with LDL, cholesterol, or triglycerides.

In light drinkers, PFOA had little effect on HDL levels. In moderate drinkers, increasing PFOA reduced HDL. The putative effects of PFOA and alcohol may be mediated by alteration of a common HDL regulatory process. The findings are limited by the small number of exposed workers, the limited range of total fluoride values, and the limitations of the study design. The conclusion and suggested mechanism must be considered preliminary.

The mechanism by which PFOA modifies the alcohol-HDL relationship could be mediated by alterations in fatty acid metabolism or fatty acid binding. Alcohol intake induces specific P450 metabolic enzymes including 2E1 and alters lipid metabolism <sup>134</sup>. PFOA induces a specific P450 A1 family of metabolic enzymes and alters lipid metabolism in rodents. The joint effect of alcohol and PFOA on P450 mediated lipid metabolism could alter HDL dynamics. The primary structure of PFOA suggests that PFOA could affect the ligand binding of fatty acid in hepatocytes and HDLs. The competition for NEFA binding sites could reduce the effect of alcohol on HDL levels. Studies of the joint effect of PFOA and alcohol on HDL may clarify the regulatory mechanisms for HDL.

The decrease in HDL associated with increasing PFOA levels may be clinically significant. In a meta-analysis of 12 prospective studies of the relationship between HDL levels and coronary heart disease (CHD), Gordon estimated that the change in CHD risk associated with a one mg/dl change in HDL level is approximately the same as the change in risk associated with a 2-4 mg/dl change in LDL level <sup>135</sup>. The predicted drop in HDL for a moderate drinking participant with a total fluoride of 20 ppm is 30 mg/dl. A change of this order of magnitude may have a measurable impact on the occurrence of cardiovascular disease. In the retrospective mortality study, there was no increase in mortality from cardiovascular disease. However, there are a limited number of workers with total serum fluoride levels of 20 ppm or more. Any increase in risk for cardiovascular diseases among a small group of highly exposed workers may not be readily apparent in a study of all Chemolite or CD employees. Further research is needed to confirm and clarify the association between PFOA and HDL level. Future studies could test the hypothesis that PFOA and alcohol jointly alter NEFA metabolism resulting in a decrease in HDL and an increase in cardiovascular morbidity and mortality risks for exposed workers who drink alcohol.

#### 5.1.4 Hepatic Parameters

Changes in SGOT (AST) and SGPT (ALT) appear to be associated with total serum fluoride through an interaction with adiposity. In obese participants, both SGOT and SGPT increased with increasing PFOA. However, there did not

appear to be an independent effect of PFOA on SGOT after adjusting for SGPT. The findings are limited by the small number of exposed workers, the limited range of total fluoride values, and the previously discussed limitations of the study design. The conclusion and suggested mechanisms must be considered preliminary.

Compared to SGOT, SGPT is a relatively specific marker for hepatocyte disruption <sup>136</sup>. The lack of association of SGOT with PFOA after adjusting for SGPT suggests that the liver is the primary source for the small PFOA associated changes in transaminases. Since SGPT is an enzyme associated with the ER membrane, the increase in SGPT may have been the result of PFOA associated ER proliferation. It may indicate a disruption in the integrity of hepatocyte membranes which allows increased release of cytosolic hepatic enzymes. The tissue specific effect suggested for hepatocyte membranes could be due to a higher hepatic concentration of PFOA.

Liver injury is generally considered to be a multifactorial process. There is evidence that interactions between endogenous and exogenous factors play a role in hepatotoxicity observed in workers <sup>137</sup>. The modification of the adiposity-SGPT association by PFOA suggests that the mechanisms of transaminase elevation may be linked. Obesity has been associated with elevation of transaminases as well as clinically important hepatitis <sup>138, 139</sup>. The observation that some obese individuals evidence little adiposity effect while other obese individuals develop hepatic fibrosis has not been explained. It has been hypothesized that metabolic polymorphisms or other hepatotoxin exposure may play a role <sup>140</sup>. Animal studies and limited human data suggest that xenobiotics, such as certain solvents and alcohol, may potentiate the effects of other hepatotoxins <sup>141, 142</sup>. Following this model, PFOA may directly or indirectly potentiate the hepatotoxic effect of obesity.

A mitochondrial site of PFOA action may occur. The mitochondria plays an essential role in fat metabolism. Disruption of mitochondrial function can produce impairment of mitochondrial oxidation of long chain and medium chain fatty acids. Studies of fatty acid metabolism in PFOA exposed humans have not been carried out. Valproic acid, an eight carbon branched chain fatty acid (2 propyl-pentanoic

acid) that impairs mitochondrial function and fatty acid metabolism, is an example of a hepatotoxic xenobiotic of similar carbon structure to PFOA <sup>143</sup>. Commercial grade PFOA contains isomers with carbon backbones identical to valproic acids structure <sup>39</sup>. The valproate-like isomers of PFOA could produce toxicity similar to that of valproate. The modification of the association between PFOA and the transaminases by adiposity could be mediated by disturbances of mitochondrial fatty acid metabolism in humans.

GGT increased as alcohol use increased. The increase in GGT was smaller as PFOA increased. This association was independent of changes in SGOT, SGPT, and AKPH. Perfluorooctanoic acid may inhibit the hepatotoxic effects of alcohol. The GGT-alcohol dose response relationship is thought to be secondary to the induction and increased release of GGT. Increased serum GGT levels indicate proliferation of the endoplasmic reticulum and induction of cytochrome P450 system, leakage from hepatocytes, or injury to other tissues <sup>144-147</sup>. Perfluorooctanoic acid may decrease serum GGT by altering cell membrane permeability, by reducing the alcohol mediated induction of GGT, or by changing alcohol oxidation pathways and reducing the production of toxic intermediates such as acetaldehyde.

Perfluorooctanoic acid was negatively associated with AKPH in non-smokers. In workers who smoke greater than five cigarettes per day, PFOA was positively associated with AKPH. The association of AKPH with PFOA was independent of GGT, transaminases, and hormones. Smoking has been reported to elevate AKPH <sup>148</sup>. The mechanism of this effect is thought to be the result of AKPH induction by compounds in cigarette smoke. The joint effect of smoking and PFOA could increase the induction of AKPH.

In summary, the associations between PFOA and hepatic enzymes are weak and are not clinically significant. In the retrospective mortality study, there was no increased mortality associated with liver disease. Future studies of the effects of PFOA may elucidate possible mechanisms of action of nongenotoxic hepatic carcinogens. The hepatic enzyme results are illustrative of the problem of extrapolating findings observed in rodent animal models to other species, including humans <sup>149</sup>. In humans, PFOA does not cause the dramatic hepatic

effects observed in rodents. Instead, the observed associations may result from PFOA modification of the hepatic effects of obesity, alcohol consumption, and smoking. Each of these factors are independently associated with hepatotoxicity. Further studies of the joint effects of PFOA and BMI, alcohol, and smoking on hepatic enzymes are needed.

#### 5.1.5 Hematology Counts and Parameters

PFOA was weakly, but significantly associated with hemoglobin levels, MCV, and MCH. The associations between PFOA and erythrocyte indices appeared to be mediated through interactions with smoking, and perhaps alcohol consumption. The findings in animal studies<sup>9, 150</sup> are consistent with a decrease in red cell volume and a larger decrease in red cell number. Together, these changes produce an increase in cellular hemoglobin concentration. The estimated changes in erythrocyte indices are not of clinical significance over the range of total serum fluoride. However, these findings suggest that further studies of the effect of PFOA on red cell regulation and function are needed. The findings are limited by the small number of exposed workers, the limited range of total fluoride values, and the previously discussed limitations of the study design.

Pharmacological doses of androgens increase erythrocyte number and mass but produce little change in MCV or MCH<sup>151, 152</sup>. The mechanisms by which androgens increase hemoglobin appear to be mediated by modulating the erythropoietin responsiveness of multi-potential stem cells and by stimulating erythropoietin production<sup>151, 153-155</sup>. In physiologic doses, the effect of testosterone on erythrocyte indices is controversial. Palacios et al. and Cunningham et al. reported that testosterone is associated with a small increase in hemoglobin, but no change in MCV or MCH<sup>156, 157</sup>. Mauss et al. reported no change in red cell indices for physiologic levels of testosterone<sup>158</sup>. In the present study, the testosterone level was not strongly or significantly related to the red cell indices. Estradiol was weakly associated with HGB but not MCV or MCH. The effect of physiologic estradiol levels on the male hematological system is poorly understood. Tell et al. reported that the effect of smoking on red cell indices was different in male than in female adolescents<sup>159</sup>. This suggests that estrogen levels may play a role in the effect of xenobiotics on red cell



indices. Taken together, the evidence suggests that the association between PFOA and erythrocyte indices was not mediated by the PFOA associated changes in testosterone, but may have been mediated in part by changes in estradiol.

Thyroid hormone was associated with changes in HGB and MCV. A decreased availability of thyroxin (T<sub>4</sub>) to myxedema levels produces a mild macrocytic anemia in humans. The increased cell volume is due to alterations in lipid deposition in erythrocyte membranes that occurs during ineffective erythropoiesis<sup>160</sup>. TSH confounded the association between PFOA and MCV. Decrease in T<sub>4</sub> could explain some of the increase in MCV and TSH. However, PFOA appeared to have an independent and opposite effect on MCV. Therefore, the association between PFOA and changes in red cell indices was probably not related to changes in thyroid function.

The immune system effects associated with PFOA present a complex picture. As expected, smoking had a strong effect on leukocyte counts. Smoking modified the association between cell count and PFOA for total lymphocytes, eosinophils, platelets and basophils. However, smoking did not modify the estimated PFOA effect on WBC, PMN, band count, or monocyte count. Alcohol modified the association between PFOA and cell count for WBC, PMN, and lymphocyte count. Adiposity modified the association between PFOA and lymphocyte count, monocyte count, and platelet count. Taken together, this preliminary data suggests that PFOA is associated with changes in peripheral leukocyte counts. The negative association with lymphocyte count is consistent with the lymphocytes effects observed in primate studies. PFOA could modulate cell counts by altering the effects of smoking, alcohol consumption, and adiposity on peripheral leukocyte counts.

The magnitude of the WBC and PMN associations were not clinically significant from an infectious disease perspective. Increased WBC is positively associated with mortality from all causes, cardiovascular diseases, cancer and myocardial infarction<sup>161-168</sup>. It is unclear if the alteration in WBC is a consequence of, or the cause of, ongoing pathological processes. Judgment as to the clinical relevance of the PFOA associated changes in WBC must await further study.

Adiposity modified the association between cell count and PFOA for monocytes. Alcohol and cigarette consumption were independent determinants in the present study. Monocyte counts have been reported to be low in massively obese individuals <sup>169</sup>. The biological basis for these effects are not clear. The univariate and joint effects of adiposity and PFOA on monocyte count may be a fruitful area for future research.

In the present study, the complex relationships between lymphocyte count, PFOA, alcohol use, cigarette use, and body mass may have been the result of the differential effect on T cell subsets. In order to clarify these associations, specific subsets need to be measured. The association of lymphocyte subsets with disease endpoints have yet to be clarified. The interpretation of the observed association requires further research.

Smoking was negatively associated with basophil count. As PFOA level increased, the smoking effect was diminished. Taylor et al. reported an increase in blood basophils in smokers compared to nonsmokers <sup>170</sup>. Walter et al. studied smokers and nonsmokers and found that acute smoking causes degranulation and loss of basophils. However, chronic smoking is associated with an elevated basophil count. <sup>171-174</sup>. No attempt was made to prohibit subjects from smoking prior to the time of blood sampling. The negative association observed in this study may reflect recent smoking by participants prior to blood drawing. The apparent reduction in the degranulating effect of smoking suggests that PFOA may interact with the basophil degranulation process.

Exposure to PFOA may be associated with changes in immune function beyond simple changes in cell number. The avid oxygen binding by PFCs may alter the effectiveness of peroxidatic killing by PMNs. Cytokine signaling is important in immune function and could be altered by PFOA exposure <sup>175</sup>. The response to antigen binding depends upon rearrangement of membrane proteins. Changes in the membrane physical characteristics produced by the potent surfactant action of PFOA could alter immune responses. More research is needed in the area of PFOA immunotoxicity. The findings of the present study need to be confirmed. Lymphocyte could be immunophenotyped using well established flow

cytometry methods <sup>176, 177</sup>. The standard immunotoxicologic assessment defined by the National Toxicology Program <sup>178</sup> should be carried out for PFOA.

Smoking has been observed to increase platelet number, survival, adhesiveness, activation, and aggregation when exposed to ADP <sup>179-184</sup>. Adhesiveness may change as a result of the effects of smoking on nonesterified fatty acids (NEFA). Smoking increases NEFA which may compete with PFOA for platelet membrane binding sites. Such competition could alter the smoking associated increase in platelet count. This hypothesized mechanism could be tested by in vitro modeling of platelet function in the presence of NEFA and PFOA. The relationship between obesity and platelet count has not been well studied. BMI has been reported to be negatively associated with platelet count <sup>185</sup>. The mechanism for this effect is not clear, but may be related to changes in NEFA associated with obesity. Thus, the effect modification of the PFOA effect by smoking and obesity may have resulted from a common effect on NEFA. Changes in platelet count have been associated with risk for cardiovascular disease <sup>186, 187</sup>. Direct and indirect mechanisms have been hypothesized for the observed increase in disease occurrence. Thus, PFOA associated changes in platelet count may be a marker for increased cardiovascular disease risk. Further study of potential effects of PFOA on platelet count and function are needed.

#### 5.1.6 Total Fluorine

Smoking and total serum fluorine were weakly associated in participants. The adjusted estimate for the difference in mean fluorine between smokers and nonsmokers was small (0.1 ppm) and probably not of biological significance. Smoking intensity was not significantly correlated with total serum fluorine levels. It is unlikely that smoking affects the pharmacokinetics of serum fluorine or PFOA. It is unlikely that smoking was a primary route for absorption of PFOA.

Exposure reduction does not need to await the results of future studies. In rodents, removal from exposure results in the reversal of the marked hepatic responses to PFOA <sup>188</sup>. Intervention to reduce the PFOA body burdens of employees would prevent any potential adverse effects in the future. The reduction of exposure is especially important since PFOA has an unusually long

biological half-life. A significant reduction in body burden will require years of reduced exposure.

#### 5.1.7 Methodological Considerations

##### 5.1.7.1 Selection Bias

Given the occupational study setting, the voluntary participation, and the requirements for blood sample collection, the overall participation was unexpectedly high. Past medical screening programs at Chemolite had participation rates of 60% to 70%. The present study's participation rate exceeded 80%. Given the high participation, non-response bias is likely to be small.

Selection bias is an important validity issue for cross sectional studies <sup>189</sup>. Only active Chemical Division workers were included in this study. Workers not included may have had a different response pattern than those who were included. If continued employment depended on response to exposure and the exposure was associated with the endpoint of interest, then selection bias may have occurred. A finding of the present study was that PFOA was associated with decreased free testosterone and increased estradiol. If workers who had high susceptibility to the effect of PFOA changed jobs, then the overall slope of the dose response curve could be underestimated. Conversely, if workers with low testosterone associated with PFOA changed jobs less often, then the overall dose response curve may be overestimated. Migration out of the high exposure jobs is unlikely to be the result of subclinical changes in hormone levels. All current Chemical Division employees who worked in high exposure jobs over the last five years were included in the sample. Many workers who had been employed in the high exposure jobs, but who changed jobs were included as participants. The vast majority of workers who had significant exposure over the previous five years would be included in the study sample as the turn-over rate in Chemolite employees was low (three percent per year) and the study included all current employees with appropriate job histories. Selection bias is not a likely explanation for the findings in this study.

### **5.1.7.2 Information Bias**

No worker was unexposed. The lowest potential exposure group had significantly elevated levels of total serum fluorine. In view of this, the observed effects may represent an underestimate of the true effect.

Total serum fluorine was used as a surrogate variable for PFOA exposure. The use of total serum fluorine has been validated in past biological monitoring in the Chemolite Plant and other plants using PFOA<sup>8</sup>. Direct measurement of PFOA using gas chromatographic techniques have been highly correlated with total serum fluorine in Chemolite workers. Approximately 90% of total serum fluorine in Chemolite workers was reported to be in the form of PFOA<sup>8, 12</sup>. The validity of using this surrogate measure was not directly assessed in the current study due to cost. Small amounts of PFCs other than PFOA may have been present in serum. The half-life of PFC compounds is directly related to molecular weight. Compounds with six or less carbon backbones are likely to be rapidly excreted by exhalation<sup>190</sup>. Short chain PFCs are unlikely to contribute appreciably to total serum fluorine. Longer chain PFC, such as perfluorodecanoic acid (PFDA), are not produced at Chemolite. The high toxicity of PFDA excludes it from commercial applications<sup>63, 77, 79, 101, 102</sup>. Longer chain PFC are unlikely to be a significant component of total serum fluorine. Other organic fluorine containing compounds exist in biological systems and the environment. However, the small amounts absorbed from the environment in the form of drugs or plant products are rapidly metabolized and excreted<sup>191</sup>. Inorganic fluorine was not a large constituent of the total fluorine levels. Serum ionic fluorine levels in the 1-5 ppm range are associated with death in unintentional occupational exposures<sup>192</sup>. Total serum fluorine is a good surrogate measure for PFOA in this cohort.

The coefficient of variation for total serum fluorine was 66%. The repeatability of the assay was better at total serum fluorine levels above five ppm. At the low end of the spectrum (< 1 ppm), where the assay is limited by sensitivity, the total serum fluorine values may overestimate the true value. These measurement errors are likely to lead to an underestimate of the effect of PFOA on the physiologic endpoints.

Commercial PFOA is a complex mixture of isomers and related compounds <sup>39</sup>. It is clear that structurally related compounds, such as valproic acid, exhibit toxicity for certain isomeric forms, but not others <sup>143, 193</sup>. It is widely recognized that different drug enantiomers have different pharmacokinetic and pharmacodynamic properties <sup>191</sup>. Thus, different isomers of PFOA may have different toxicities. If one isomer of PFOA is associated with toxicity, then the use of total serum fluorine or total PFOA levels could have produced an underestimate of the true strength of association. However, in animal studies using straight chain PFOA, the spectrum of toxicities is similar to those observed in studies using mixed isomer of PFOA <sup>37, 38, 88, 194</sup>. Further research is needed to clarify the role of PFOA isomers.

The toxicokinetics of PFOA in humans are different from those observed in rodents. Extrapolating the tissue distribution of PFOA from animals to humans may not be valid. No data exist on the relationship between serum and tissue PFOA distribution or body burden in humans. The use of serum levels to extrapolate to the concentrations at the site of PFOA action may have been inappropriate. Obtaining pharmacokinetic data in humans or appropriate animal models is an important area for future research efforts.

The temporal variability of physiologic parameters is recognized. The ultradian, circadian, and circannual variability of the study endpoints was not assessed directly. Instead, blood samples were drawn at the same time of day, on the same shift for all participants. One sample was drawn to estimate mean parameter values. Considerable measurement error is inherent in this procedure for hormones with short pulsatile intervals such as LH, FSH, and testosterone. However, studies have shown that one sample is as good as three samples in estimating mean values <sup>195</sup>. The use of a single sample to estimate mean hormone level produced random measurement error and would be expected to attenuate the observed relationships. Mean serum values of the assayed hormones may not represent the biologically important quantities at the site of action.

Validation studies of self-reported smoking status, using biochemical markers such as exhaled carbon monoxide, serum and urine thiocyanate, and serum

thiocyanate, have shown that smokers underreport their smoking <sup>196</sup>. Smoking is associated with changes in physiologic parameters such as hematological counts <sup>197-199</sup>, cholesterol <sup>200</sup>, lipoproteins <sup>201, 202</sup>, and hepatic enzymes <sup>148</sup>. The strength and direction of the association between self-reported smoking information and these parameters can be used to indirectly assess the validity of the smoking information <sup>203</sup>.

In this study, smoking status and intensity was strongly and significantly associated with leukocyte count, band count, eosinophil count, platelet count, and monocyte count. As expected, smoking intensity was negatively associated with basophil count.

No participant reported drinking more than 3 ounces of alcohol per day. This may reflect the company's success in discouraging heavy alcohol consumption in employees, a reporting bias, or the fact that heavy drinkers may not be able to continue employment due to the demands of Chemical Division jobs.

Alcohol consumption is associated with changes in physiologic parameters such as hepatic enzymes <sup>204</sup>, erythrocyte mean corpuscular volume, triglycerides, and high density lipoprotein <sup>144</sup>. As with smoking, the strength and direction of the association between self-reported alcohol consumption information and these parameters can be used to indirectly assess the validity of the alcohol information. Increased serum HDL is associated with moderate alcohol intake <sup>144, 205, 206</sup>. The expected relationship between alcohol intake and HDL was observed in this study in individuals with low PFOA levels. As expected, there was a positive association between alcohol intake and triglycerides. Alcohol has a direct toxic effect upon erythrocyte size, maturation, and number <sup>207, 208</sup>. The specificity of MCV is 90% in identifying alcoholics from social drinkers with a positive predictive value of 96% <sup>209</sup>. In the present study alcohol consumption of 1 to 3 drinks per day was associated with an increase in MCV of the same order as reported previously <sup>208</sup>. Alcohol induces GGT. The sensitivity of GGT in detecting alcohol use varies from 52% to 94%. GGT is highly non-specific for alcohol consumption or for hepatic abnormalities <sup>146, 210</sup>. Heavy drinking of two to five drinks per day over one week or more are necessary to induce GGT <sup>145, 208</sup>. GGT may be the only commonly assayed hepatic enzyme to increase with

heavy drinking. A significant positive association between GGT and self-reported alcohol use was observed. The presence of these associations indicates that the misclassification of alcohol use was unlikely to produce a bias large enough to explain the observed associations.

Alcohol use was weakly associated with hepatic transaminases. SGOT and SGPT are less sensitive indicators of alcohol use than GGT. In alcohol induced liver disease, SGOT may be slightly elevated and SGPT little changed. SGPT has been shown to decrease in some cases of alcohol induced liver disease <sup>211</sup>. Considering the relationship known to exist between alcohol use, SGOT and SGPT, little alcohol associated change in transaminases would be expected. The observed weak association is not an unexpected finding and therefore probably does not reflect misclassification of alcohol use.

Nonrespondents to the alcohol item were different than respondents. They were treated as a separate group in the analysis since the difference could not be explained by measured covariates. Although the power of the study is diminished by treating alcohol information as a nominal categorical variable, the potential for bias was reduced.

#### 5.1.7.3 Confounding Bias

Information on the duration of employment in exposed jobs was not collected. Plant records did not contain sufficient information to reconstruct exposures more than five years in the past. The duration of exposure may be an important determinant of PFOA effect. Duration of employment may be related to PFOA level since PFOA has the potential for bioaccumulation. The duration of exposure may have been a confounder for peptide hormonal endpoints in this study. In rodents, steroid hormonal and hepatic enzyme effects of PFOA exposure occur after two weeks of exposure, whereas peptide hormonal effects may require longer exposures <sup>19</sup>. Leydig cell tumors may require a considerable length of exposure or latency to develop <sup>122</sup>.

There are many compounds in complex androgen-estrogen system. The present study measured only a few of them. Other biologically important steroid



hormones include cortisol, androstenedione, dihydroepiandrosterone sulfate (DHEAS), estrone, estriol, estrogenic catechols, and dihydrotestosterone (DHT). A total estrogen index or estrogen to testosterone ratio (E/T) may be more important than assays of individual compounds <sup>212</sup>. Sex hormone binding globulin (SHBG), a major determinant of the estrogen to testosterone balance at the tissue level <sup>213</sup>, was not assayed. More research is needed to clarify the potential role of these hormones as confounders of the observed associations.

The relationship for bound testosterone may have been confounded by steroid hormone binding globulin (SHBG). Sex hormone binding globulin is an important determinant of testosterone and estradiol levels in different tissues as well as their metabolism <sup>213</sup>. Plasma SHBG levels are positively associated with estrogens and negatively associated with androgens <sup>214</sup>. Thyroid hormone levels affect SHBG <sup>215</sup>. The ratios of estradiol to testosterone and testosterone to DHT may be regulated by SHBG levels <sup>213</sup>. The association between PFOA and bound testosterone may have been, in part, related to estradiol and thyroid hormone changes in SHBG levels. Adult rats do not express SHBG <sup>216</sup>. The decline in total testosterone observed in rats is not the result of changes in the amount or binding characteristic of SHBG. The observed depression of free testosterone in men is analogous to changes in total testosterone in rats and is probably not significantly related to changes in SHBG binding.

Major stresses, such as surgical procedures, have been shown to markedly affect hormones in men <sup>217</sup>. It is unlikely that major physical stresses were associated with PFOA. Therefore, stress was not a significant confounder in the present study.

Shiftwork has been shown to affect a variety of physiologic endpoints including biochemical parameters, hematologic indices, and hormones <sup>218</sup>. Study participants rotated weekly through three shifts. All samples were collected on the day shift at least three days post shift change. Given the rotating shifts and standard day shift sampling, it is unlikely that shiftwork and PFOA were associated. Shiftwork did not appear to be a significant confounder of the estimated dose response relationships.

Several dietary factors are determinants of the endpoints considered in this study. The effects of dietary fat and cholesterol on serum lipoproteins and lipids is widely appreciated <sup>200</sup>. Dietary calories, fat, and carbohydrates affect steroid hormones <sup>219, 220</sup>. Diet can also affect the metabolism of steroid hormones <sup>221, 222</sup>. Since it is unlikely that diet is associated with PFOA, it is probably not a confounding covariate in this study.

Physical activity affects many physiologic parameters including hormones <sup>223</sup>, enzymes <sup>224</sup>, lipoproteins <sup>200</sup>, and hematologic indices. For hormones, only maximal exercise produced an effect. No effect was noted for submaximal physical activity. It is unlikely that many participants engaged in maximal physical activity. Therefore, in this group, it is unlikely that physical activity is a determinant of the hormonal endpoints under study. Physical activity may effect HDL levels, but it is unlikely that physical activity was associated with PFOA. Therefore, physical activity was unlikely to be a significant confounder in this study.

Medication usage and diseases such as diabetes mellitus are important determinants for some of the physiologic parameters measured in this study <sup>193, 195</sup>. Questionnaire items concerning medication use and medical history were incomplete and were not validated. PFOA exposure has not been associated with any medical conditions <sup>8</sup>. If the use of medication or the diagnosis of a medical condition that affects one of the physiologic endpoints is associated with PFOA exposure, then confounding may occur. However, no such relationships have been described.

Inflammatory processes, which are major determinants of WBC, were not assessed in this study. There is no evidence that inflammatory processes are related to total serum fluorine or serum PFOA. Therefore, these determinants of leukocyte count are unlikely to confound the estimated relationships.

#### 5.1.7.4 Analytic Model Specification Bias

The analytic multivariate approach used in this study assumed that a linear model with additive effects was an adequate model with which to summarize the

data. A normal error term was used. Similar models of physiologic variables have been extensively used in the past and their assumptions tested <sup>225</sup>. The model form was partially defined a priori based on a biological hypothesis. The choice of a final model was based on biological knowledge plus best predictive power. The variable transformations used were not based on a specific biological mechanism, but instead reflect the basic form of dose response relationships observed in nature.

## 5.2 1990 Chemolite Mortality Study

### 5.2.1 Introduction

This was a retrospective cohort study of mortality in workers employed in a PFOA production plant for greater than six months during the period from January 1, 1947 to December 31, 1989. Completeness of the cohort was assessed from independent sources. Demographic and work history data were collected from plant records and verified from independent sources where possible. Cohort members were not individually contacted for additional information on confounding variables such as smoking. Vital status was confirmed for 100% of the cohort. Cause of death was obtained from death certificates for 99.6% of deaths and other sources for 0.4% of deaths. Cause of death was coded by ICD-8 categories by a nosologist. Reliability of death certificate coding was assessed by random resubmission of death certificates for recoding. The concordance was 100% for three digit ICD-8 codes.

### 5.2.2 Participant Characteristics

The 749 women were observed for 19,309 person-years, had a mean age at first employment of 27 years and mean follow-up of 26 years. The number of expected events given the age and size of the cohort was small. The study had limited power to detect moderate increases in cause-specific mortality.

The 2788 men were observed for over 70,000 person years. The mean age at first employment was 27 years, the mean length of follow-up was 25 years and a the mean age of death was 56 years. Non-CD men were older on average than

CD men and had more person-years in the older age groups where mortality was the highest. Internal comparisons were confounded by age as well as other time correlated factors such as length of follow-up.

### 5.2.3 Mortality Results

In females, 6.7% were deceased compared to 12.5% in the males. Given that the mean age at first employment and mean length of follow-up was similar for males and females, this reflects the expected survival advantage of women. For both males and females the proportion of deaths was smaller in the CD cohort. Employment in the Chemical Division did not produce a large survival disadvantage.

The all causes, all cancer, and all cardiovascular mortality among women was less than expected in the overall cohort. The SMRs were remarkably stable when stratified on ten year exposure groups, and ten, fifteen, and twenty year latency periods. The all causes SMR was .75 in the total cohort, .75 in those employed for at least ten years or for those employed longer than ten years, and .75 in all three latency periods. Cardiovascular diseases and cancer mortality followed a similar pattern.

In males, the all causes, cardiovascular diseases, all gastrointestinal, and all respiratory diseases SMRs were significantly less than one. The all causes SMR was .77 using Minnesota mortality rates and .73 using national rates. The low SMRs are most likely a result of the healthy worker effect (HWE). As expected, the cancer SMR is less affected by the HWE. The all causes SMRs were .75 for all three latency groups. Latency did not have a strong relationship with the HWE. The all causes SMR was .80 in the greater than five year employment duration group and .68 in the greater than 20 year employment group. The low all causes SMR in the greater than 20 year duration group suggests that working for 20 or more years was associated with continued selection based on good health. The all causes SMR decreased with duration of employment in one meta-analysis of retrospective cohort studies<sup>226</sup> but increased in the meta-analysis by Fox and Collier<sup>227</sup>.

The SRRs for all causes, all cancer, and all cardiovascular diseases for less than ten years employment to more than ten years employment were not significantly different from one. Because the rates were based on small numbers of events, the 95% CI were wide. Due to the small number of events in the females, SRRs were not calculated. The SRRs are similar to the SMRs for the less than ten year employment and greater than ten year employment groups.

The SRRs for CD versus non-CD male workers for all causes, all cancer, and all cardiovascular diseases were not significant and were similar to the SMRs. Working in the CD did not substantially alter the rates of death. The small number of events observed for rare causes of death or specific causes of death make it unlikely that moderate increases in rates could be detected in this cohort for the follow-up period through 1989. More follow-up time will be needed to allow sufficient power to detect moderate increases in rates for specific causes of death.

The results from the adjusted RRMH contrasting the mortality rates for all causes, all cancer, and all cardiovascular diseases between CD and non-CD male workers were similar to those for the SRRs and SMRs. None of RRMH point estimates were statistically different from one. The contrast of rates between less than ten years of employment and greater than ten years of employment presented a different picture. All cause RRMH were significantly elevated in the oldest two age groups, while the RRMH for cardiovascular diseases was significantly elevated in the 30 to less than 40 year age group. The all cancer RRMH displayed a trend toward a statistically significant elevation in the oldest two groups. The RRMH were not adjusted for year of first employment. They may have been substantially confounded by changes of exposure over time since year of first employment. As seen in several PH regression models, year of first employment was significantly associated with the mortality. After age and length of follow-up, calendar time is the strongest time factor associated with mortality<sup>189</sup>. Hence, it is likely that the elevated RRs for composite categories of cause of death in the oldest groups were a result of uncontrolled confounding by calendar period. Given the small number of events in strata, it was not feasible to further stratify the data on year of first employment.

In the PH regression analysis, prostate cancer mortality was positively and significantly related to time in the Chemical Division. Ten years of employment in the CD was associated with a 3 fold increase in prostate cancer mortality compared to men never employed in the CD. This trend was evident in the SMR analysis stratified by CD and non-CD employment. This association was independent of duration of employment and year of first employment. As expected, age at first employment was positively related to prostate cancer mortality rate. The interpretation of this estimated relative rate is tempered by a number of factors. The estimates were based on six prostate cancer deaths, four in the CD cohort and two in the non-CD cohort. A change of one case could significantly alter the estimates. Ascertainment of all prostate cancer deaths may have been incomplete. Diagnosis may have been more complete in the CD cohort. Given that death certificate cause of death information is known to be imperfect, misclassification of one or more deaths could occur. The use of mortality as the event of interest for etiologic studies of prostate cancer is not the best study endpoint because of the long natural history and low mortality of prostate cancer. The majority of incident prostate cancers do not progress and cause death <sup>228, 229</sup>. For localized disease, an 80% ten year survival in untreated patients have been reported <sup>230</sup>. Studies of prostate cancer incidence in this workforce are needed to clarify the suggested increase in prostate cancer risk. The findings of hormonal alterations in PFOA exposed men suggests a possible biologic mechanism for the increase in prostate cancer mortality <sup>231</sup>. Incidence studies of other diseases that are hormonally mediated may be indicated if the PFOA associated hormonal changes are confirmed.

#### 5.2.4 Methodological Considerations

##### 5.2.4.1 Information Bias

The use of death certificates to categorize cause of death imperfect <sup>232-235</sup>. The size of the potential bias depends on the cause of death. In one study cancer as a cause of death was under-reported by 13% <sup>232</sup>. Leukemias and lymphomas were underreported in 19% of autopsy confirmed cases. Colorectal cancers were underreported in 12% of cases. Therefore, it appears that cancer deaths were not severely misclassified. All cardiovascular diseases as a group may

have been inaccurate. Individual disease with the whole may be severely misclassified and may produce large biases. For example, specific causes of death in the cardiovascular group, such as cerebrovascular disease, are inaccurately designated on death certificates.

Three measures of PFOA exposure based on job history were used in this study. First, the cohort was dichotomized into those who ever worked in the CD and those who never worked in the CD. Second, the number of months worked in the CD until 1985 was used as a continuous parameter for PFOA exposure. Third, the total duration of Chemolite employment was used as a continuous parameter for the effect of work in a plant producing PFOA among a large number of products. Each of these surrogate variables may produce a different spectrum of misclassification. Categorization of workers into ever versus never employed in the CD may not reflect the biological effective dose of PFOA. Many CD jobs do not entail PFOA exposure. A number of workers were employed in the CD for short periods in the distant past. Their exposure may not have been significant. This categorization may misclassify unexposed workers as exposed. Conversely, PFOA exposure was widespread among Chemical Division (CD) employees working in jobs with no exposure to PFOA. No exposure measurements have been done in non-CD employees. It is possible that non-CD employees had significant body burdens of PFOA. If this was the case, exposed workers would have been classified as unexposed. Such misclassification would be expected to bias the effect estimates toward the null. The months of employment in the CD was the best available estimate of PFOA dose. Not all CD jobs have PFOA exposure. The misclassification produced by classifying unexposed workers as exposed could have biased the estimate toward the null. The use of duration of employment at Chemolite as a continuous exposure parameter is less specific for PFOA than time in the CD. If another xenobiotic exposure in the plant has modulated disease occurrence rates, the use of duration may produce less misclassification than use of duration in the CD.

#### 5.2.4.2 Confounding and Selection Bias

The healthy worker effect strongly affects the validity of many occupational studies<sup>189, 236</sup>. It is a complex bias that results, in part, from the selection of

individuals for employment who are healthier than those in the comparison population. The HWE is usually stronger for cardiovascular diseases and respiratory diseases. Because cardiovascular diseases mortality accounts for a significant portion of all causes mortality, the HWE usually reduces the all causes SMR. The age at first employment, age at risk, length of follow-up, and duration of employment are four time factors that are associated with changes in the HWE<sup>189</sup>. Generally, the HWE diminishes with age and time.

Collection of confounder information for individuals is difficult in retrospective cohort mortality studies. The present study included workers followed for more than 40 years. It was not feasible to collect individual information on such covariates as smoking, health status, medical history, or dietary habits. The proportion of workers at Chemolite who smoke has been lower than in other facilities owned by the same corporation. In recent health maintenance studies, the self-reported smoking prevalence (25%) is lower than the statewide smoking prevalence. The observation that all respiratory diseases and lung cancer rates are lower than expected may be the result of historically low smoking prevalence. The low smoking prevalence may depress the all causes SMR, all cancer SMR, and all respiratory disease SMR. The use of internal comparison groups may reduce this smoking related bias<sup>237</sup>.

Time factors such as age at risk, age at first employment, year of first employment, and duration of employment are associated with the occurrence of many diseases<sup>189</sup>. The use of an internal comparison group may reduce certain selection effects, but may not control confounding if the exposure defined internal comparison groups have different distributions of these time factors. Although the mean age at first employment and mean year of first employment are similar in the CD and non-CD cohorts of men and women, the comparisons of the rates of disease are confounded by differences in the distribution of age at risk. These time factors are strongly correlated, with some being exact linear combinations of others. The relationship between measures of exposure and disease occurrence may be complex functions of these inter-related time factors. Adjustment for time factors may reduce the effects of confounding, but may not control confounding<sup>238</sup>. If the disease occurrence relationship is defined in terms of cumulative



exposure, the true effect of exposure may be biased toward the null by uncontrolled confounding due to the complex time factors <sup>189</sup>.

Some workers were exposed to many other potentially disease causing xenobiotics, such as benzene and asbestos, during their employment at Chemolite. Adjustment for their effects was not possible in this study. Even if information was available, exposures are often highly correlated making the separation of individual effects impossible.

#### 5.2.4.4 Analytic Model Specification Bias

Comparison of SMRs and RRMH between exposure groups may not be strictly valid. However, if the distribution of the person time in the comparison groups is not strongly discordant, then such a comparison may be useful. In the current study, the person-time distributions are different in the exposed groups. However, the differences appear to be of a magnitude that makes useful comparisons of SMRs possible.

Although the proportional hazard (PH) model has been used frequently for cohort studies and clinical trials, it has not been widely used in occupational studies. In the past, it has been suggested that Poisson regression was the analytic strategy of choice because computational costs were less and the conceptualization of the model straight forward <sup>189</sup>. However, PH models are now easily run with standard computer packages <sup>109</sup>. Their wide application in clinical trials and cohort studies has fostered the understanding of the PH models. Poisson models appear less frequently in the literature and may not be as well understood. Poisson regression and PH models have theoretical links and have been shown to give similar results when used to analyze the same data set <sup>189</sup>. Cox PH regression was chosen as the multivariate model to employ in this study. The validity of the proportional hazards assumptions was examined using the two standard techniques. The assumptions did not appear to be grossly violated. However, in analyses involving a small number of events, the assessment of the validity of assumptions may be limited. The use of the factors as continuous variables was based on lack of statistical evidence for a significant nonlinear

effect. Although this strategy has been widely used for control of confounding, it has not been extensively validated in simulation studies.

## **6. SUMMARY, CONCLUSIONS AND RECOMMENDATIONS**

### **6.1 Cross-Sectional Study of the Physiologic Effects of PFOA**

This was a cross-sectional study of selected physiologic effects of PFOA, as quantified by total serum fluorine. Participants were recruited from workers employed during November 1990 in the Chemical Division of the 3M Chemolite Plant in Cottage Grove, Minnesota. All current workers who were employed in high exposure jobs at any time during the previous five years and an age matched sample of workers employed in low exposure jobs were invited to participate.

Participants completed a corporate medical history questionnaire and had vital parameters measured by an occupational health nurse. Blood was drawn for assays of total serum fluorine, seven hormones involved in the hypothalamic-pituitary-gonadal axis, serum lipids, lipoproteins, hepatic function parameters, and hematology indices. Blood was drawn in the morning after workers were assigned to the day shift for at least three days.

In past studies, the majority of total serum fluorine found in Chemolite workers was in the form of PFOA. Thus, total serum fluorine is a valid surrogate measure of PFOA in Chemolite employees. For 93% of workers, total serum fluorine levels were 20 times greater than community and corporate background levels. Findings in the current study are consistent with other data suggesting that PFOA has a long biological half-life in both men and women. The long half-life of PFOA may result in significant bioaccumulation from small frequent doses or large, infrequent doses.

The hormonal findings from this study are consistent with the hypothesis that PFOA depresses the human hypothalamic-pituitary-gonadal axis. The results show that low levels of serum PFOA (20 $\mu$ M) depressed free testosterone and elevated estradiol with little observed change in LH levels. In older men, free testosterone was depressed below 9 ng/dl at serum fluorine levels below one ppm (estimated PFOA levels below 1  $\mu$ M).

Mean prolactin levels were positively associated with PFOA in moderate drinkers, but not in light drinkers. Since the function of prolactin in men is uncertain, the clinical significance of this finding is unclear.

Mean thyroid stimulating hormone was positively associated with PFOA. Since peripheral thyroid hormone levels were not assayed, it was not possible to assess whether the observed association between PFOA and TSH was the result of a direct effect on the hypothalamus, pituitary, thyroid gland, or peripheral thyroid hormone metabolism.

Cholesterol, triglycerides, and LDL were not significantly associated with PFOA. PFOA was negatively associated with HDL in moderate drinkers.

PFOA was not associated with the marked hepatic changes in humans that have been observed in rodents. PFOA appeared to alter the hepatic response to endogenous factors and xenobiotics.

PFOA was significantly associated with hemoglobin levels, MCV, and MCH. The estimated changes in erythrocytes are not of clinical significance over the range of observed total serum fluorine.

The changes in leukocyte counts associated with PFOA exposure presented a complex picture. For example, the negative association between PFOA and lymphocytes was increased by smoking more than 10 cigarettes per day and decreased by alcohol use and adiposity. The magnitude of these associations are not clinically significant from an infectious disease perspective. However, elevated WBC has been associated with increased all causes, cardiovascular diseases, and cancer mortality as well as increased incidence of myocardial infarction.

#### 6.2 Retrospective Cohort Mortality Study Of The Chemolite Workforce, 1947-1990

This was a retrospective cohort study of mortality in workers employed in a PFOA production plant. All causes mortality in both male and female Chemolite employees were significantly less than expected based on comparisons to the

mortality experience of the Minnesota and United States population. The SMRs for several other causes of death including all respiratory diseases were less than expected. Since the healthy worker effect was apparently strong in the Chemolite cohort, internal comparisons of SMRs were made between Chemical Division (CD) and non-Chemical Division (non-CD) employees. These comparisons did not suggest any significant excesses in mortality in CD or non-CD employees.

Generally, the findings from this study provide no evidence that employment at Chemolite results in elevated mortality rates from any cause. However, prostate cancer mortality may be associated with length of employment in the Chemical Division. Ten years of employment in the CD was associated with a significant three fold increase in prostate cancer mortality. There was no association between prostate cancer mortality and employment (ever/never) in the Chemical Division. Given the small number of deaths from prostate cancer in this study and the natural history of the disease, the association between employment in the CD and prostate cancer must be viewed as hypothesis generating and should not be over interpreted. However, the biological plausibility for any association between CD employment and prostate cancer is increased by animal and human toxicological data suggesting an association between PFOA and steroid sex hormone changes.

### 6.3 Conclusion

Perfluorooctanoic acid was associated with reproductive hormonal changes in exposed workers. The clinical significance of these findings are unknown. The associations of PFOA with hormones, HDL, hematology parameters, prostate cancer mortality in men indicates the need for further research.

### 6.4 Recommendations

Research is needed in five areas.

1. An assessment of the hormonal effect of PFOA in women is needed. A cross-sectional study should be conducted using specific assays for PFOA and accounting for temporal hormonal variations.

2. The clinical significance of the associations of PFOA with the physiologic parameters need clarification. Since morbidity from diseases such as prostate cancer is reflected in mortality, an update of the retrospective mortality study is needed in five years. Morbidity studies should be conducted of endpoints that may be produced by hormonal changes. Since exposed workers are relatively young and are limited in number, the feasible endpoints for a short term morbidity study are limited. Pooling of workers from a number of plants could increase the number of exposed workers and allow endpoints with lower incidence to be studied. The morbidity study should be a long term which would allow the study of endpoints that occur at higher frequency in older age groups. In men, endpoints should include the incidence of benign prostatic hypertrophy and prostate cancer. The feasibility of including inflammatory bowel disease and colorectal cancer as endpoints should also be evaluated. In women, endpoints should include the age of menopause, the incidence of osteoporosis and related fractures, uterine fibroids, and cholelithiasis. If there are a sufficient number of events, endometrial cancer and inflammatory bowel disease should be evaluated. If the cross-sectional hormonal study in women finds no association between PFOA and hormones, then the morbidity study can be limited to men.

3. Studies of reproductive outcomes in both men and women are needed. Libido, potency, and fertility are directly associated with steroid hormones levels. The feasibility of a retrospective study of reproductive endpoints or a prospective study of time-to-pregnancy needs to be explored.

4. The mechanisms of action of PFOA need to be studied concurrently with morbidity. Mechanistic studies are needed to define the relevance of animal studies for humans and provide a firm biological basis for the findings of the mortality, morbidity, and reproduction studies.

*In vitro* and cell line studies could clarify the mechanisms of action of PFOA on the pituitary secretion of LH, FSH, TSH, and prolactin. Pituitary cultures may be

helpful in evaluating the direct effect of PFOA pituitary function. The effect of PFOA on other autocrine or paracrine factors such as TGF- $\alpha$ , TGF- $\beta$ , FGF, and TNF could also be evaluated. Human adipocyte cultures could be used to study the effect of PFOA on aromatase activity. Additionally, studies are needed to clarify the relationship between PFOA and the temporal variability of reproductive hormones.

5. Studies are needed to better define the PFOA exposure profile of all workers employed at Chemolite, to ascertain the source of their PFOA exposure and route of continued absorption and to clarify the toxicokinetics and toxicodynamics of PFOA in humans.

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**APPENDIX 1**

**PHYSIOLOGIC EFFECTS STUDY QUESTIONNAIRE**

## Medical History Questionnaire

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# Medical History Questionnaire

Form 22004 - 1/90

Name		Employee Number		Date	
Address		Business		Social Security Number	
Sex <input type="checkbox"/> Male <input type="checkbox"/> Female		Height		Blood Pressure	

Medical Symptoms	Yes	No	Medical Diagnoses	Yes	No
<p><i>(The presence of these symptoms may be medically significant. Consultation with your personal physician is recommended if any have occurred.)</i></p> <p>Check all that you have had in the last two years.</p>			<p><i>(Check yes for all medical problems you have that have been diagnosed by a physician.)</i></p>		
1. Persistent, bothersome cough	<input type="checkbox"/>	<input type="checkbox"/>	21. Allergies that cause nose or eye symptoms	<input type="checkbox"/>	<input type="checkbox"/>
2. Recurrent wheezing	<input type="checkbox"/>	<input type="checkbox"/>	22. Chronic bronchitis	<input type="checkbox"/>	<input type="checkbox"/>
3. Becoming easily short of breath with exertion	<input type="checkbox"/>	<input type="checkbox"/>	23. Emphysema	<input type="checkbox"/>	<input type="checkbox"/>
4. Recurring chest pain	<input type="checkbox"/>	<input type="checkbox"/>	24. Asthma	<input type="checkbox"/>	<input type="checkbox"/>
5. Ongoing difficulty with swallowing	<input type="checkbox"/>	<input type="checkbox"/>	25. Other lung disease	<input type="checkbox"/>	<input type="checkbox"/>
6. Persisting abdominal pain	<input type="checkbox"/>	<input type="checkbox"/>	26. <input style="width: 100%;" type="text"/>		
7. Blood in stool or black stools	<input type="checkbox"/>	<input type="checkbox"/>	27. Coronary artery disease	<input type="checkbox"/>	<input type="checkbox"/>
8. Change in bowel habits	<input type="checkbox"/>	<input type="checkbox"/>	28. Heart attack	<input type="checkbox"/>	<input type="checkbox"/>
9. Change in mole or other skin lesion	<input type="checkbox"/>	<input type="checkbox"/>	29. Angina	<input type="checkbox"/>	<input type="checkbox"/>
10. Persistent swollen lymph nodes	<input type="checkbox"/>	<input type="checkbox"/>	30. Arrhythmia (heart rhythm disturbance)	<input type="checkbox"/>	<input type="checkbox"/>
11. Blood in urine	<input type="checkbox"/>	<input type="checkbox"/>	31. Heart failure	<input type="checkbox"/>	<input type="checkbox"/>
12. Burning with urination	<input type="checkbox"/>	<input type="checkbox"/>	32. Hepatitis	<input type="checkbox"/>	<input type="checkbox"/>
13. Difficulty with balance or coordination	<input type="checkbox"/>	<input type="checkbox"/>	33. Cirrhosis of the liver	<input type="checkbox"/>	<input type="checkbox"/>
14. Temporary loss of vision or other visual disturbance	<input type="checkbox"/>	<input type="checkbox"/>	34. Gall bladder disease	<input type="checkbox"/>	<input type="checkbox"/>
15. Persisting numbness in hands or feet	<input type="checkbox"/>	<input type="checkbox"/>	35. Other liver disease	<input type="checkbox"/>	<input type="checkbox"/>
16. Fainting	<input type="checkbox"/>	<input type="checkbox"/>	36. Stomach ulcer	<input type="checkbox"/>	<input type="checkbox"/>
17. Trouble speaking	<input type="checkbox"/>	<input type="checkbox"/>	37. Duodenal ulcer	<input type="checkbox"/>	<input type="checkbox"/>
18. Weakness in arm or leg	<input type="checkbox"/>	<input type="checkbox"/>	38. Colon polyps	<input type="checkbox"/>	<input type="checkbox"/>
<p><i>(The presence of these symptoms may be medically significant. Consultation with your personal physician is recommended if any have occurred.)</i></p>			39. Kidney failure or insufficiency	<input type="checkbox"/>	<input type="checkbox"/>
19. Do you take medications?	<input type="checkbox"/>	<input type="checkbox"/>	40. Bladder polyps or tumors	<input type="checkbox"/>	<input type="checkbox"/>
20. If, so please list (include non prescription medications.)	<div style="border: 1px solid black; height: 100px; width: 100%;"></div>		41. Diabetes	<input type="checkbox"/>	<input type="checkbox"/>
			42. Anemia (low blood)	<input type="checkbox"/>	<input type="checkbox"/>
			43. Low white blood cell count	<input type="checkbox"/>	<input type="checkbox"/>
			44. Blood clotting disorder	<input type="checkbox"/>	<input type="checkbox"/>
			45. Other blood disorder	<input type="checkbox"/>	<input type="checkbox"/>
			46. <input style="width: 100%;" type="text"/>		

	Yes	No		Yes	No
47. Neuropathy (nerve abnormality in arms or legs.)	<input type="checkbox"/>	<input type="checkbox"/>	Have you ever smoked a pipe regularly? (Yes means more than 12 oz. of tobacco in a lifetime.)	65. <input type="checkbox"/>	<input type="checkbox"/>
48. Seizure disorder	<input type="checkbox"/>	<input type="checkbox"/>	If yes:		
49. Multiple sclerosis	<input type="checkbox"/>	<input type="checkbox"/>	How old were you when you started to smoke a pipe regularly? Age >	66. <input type="text"/>	
50. Other nervous system disease	<input type="checkbox"/>	<input type="checkbox"/>	If you have stopped smoking a pipe completely, how old were you when you stopped? Age stopped >	67. <input type="text"/>	Still smoking <input type="checkbox"/>
51. <input type="text"/>			On the average over the entire time you smoked a pipe, how much pipe tobacco did you smoke per week? > (Standard pouch of tobacco contains 1 1/2 oz. per week.)	68. <input type="text"/>	Not smoking a pipe <input type="checkbox"/>
52. Carpal Tunnel syndrome	<input type="checkbox"/>	<input type="checkbox"/>	How much pipe tobacco are you smoking now? Oz. per week? >	69. <input type="text"/>	
53. Chronic low back pain	<input type="checkbox"/>	<input type="checkbox"/>	Do you or did you inhale the pipe smoke?		
54. Herniated or ruptured disc in the low back	<input type="checkbox"/>	<input type="checkbox"/>	70. <input type="checkbox"/> Never smoked <input type="checkbox"/> Slightly <input type="checkbox"/> Deeply		
55. Herniated or ruptured disc in the neck	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/> Not at all <input type="checkbox"/> Moderately		
56. Any cancer	<input type="checkbox"/>	<input type="checkbox"/>	Have you ever smoked cigars regularly? (Yes means more than 1 cigar a week for a year)	71. <input type="checkbox"/>	<input type="checkbox"/>
57. <input type="text"/>			If yes:		
<b>Tobacco Smoking</b>			How old were you when you first started smoking cigars regularly? Age in year >	72. <input type="text"/>	
Have you ever smoked cigarettes? (No means less than 20 packs of cigarettes or 12 oz. of tobacco in a lifetime or less than 1 cigarette a day for 1 year.)	58. <input type="checkbox"/>	<input type="checkbox"/>	If you have stopped smoking cigars completely, how old were you when you stopped? Age stopped >	73. <input type="text"/>	Still smoking <input type="checkbox"/>
If yes: (Complete the following questions)			On the average over the entire time you smoked cigars, how many cigars did you smoke a week? Cigars per week >	<input type="text"/>	Not smoking cigars <input type="checkbox"/>
Do you now smoke cigarettes (as of one month ago?)	59. <input type="checkbox"/>	<input type="checkbox"/>	How many cigars are you smoking per week now? Cigars per week >	75. <input type="text"/>	
How old were you when you first started regular cigarette smoking?	60. <input type="text"/>		Do or did you inhale the cigar smoke?		
If you have stopped smoking cigarettes completely, how old were you when you stopped? Age stopped >	61. <input type="text"/>		76. <input type="checkbox"/> Never smoked <input type="checkbox"/> Slightly <input type="checkbox"/> Deeply		
How many cigarettes do you smoke per day now? Per day >	62. <input type="text"/>		<input type="checkbox"/> Not at all <input type="checkbox"/> Moderately		
On the average of the entire time you smoked, how many cigarettes did you smoke per day? Per day >	63. <input type="text"/>		Check if you use snuff or chewing tobacco.	77. <input type="checkbox"/>	
Do or did you inhale the cigarette smoke?			If yes, how many years have you used it? Years used >	78. <input type="text"/>	
64. <input type="checkbox"/> Does not apply <input type="checkbox"/> Slightly <input type="checkbox"/> Deeply			What is the best description of the number of alcoholic beverages you consume (1 drink = 1 12oz. beer, 1 glass of wine or 1 oz. of hard liquor)		
<input type="checkbox"/> Not at all <input type="checkbox"/> Moderately			79. <input type="checkbox"/> None <input type="checkbox"/> Less than 1 drink per day		
			<input type="checkbox"/> 1 to 3 drinks a day <input type="checkbox"/> 4 or more drinks per day		

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**APPENDIX 2**

**TABLES OF HORMONE RATIOS BY BODY MASS INDEX, AGE, SMOKING  
STATUS, AND ALCOHOL CONSUMPTION**

**TABLE A4.1.1 BOUND TESTOSTERONE TO FREE TESTOSTERONE RATIO  
(TB/TF) BY BODY MASS INDEX, AGE,  
SMOKING AND DRINKING STATUS  
1990 PERFLUORO-CHEMICAL EFFECTS STUDY,  
3M CHEMOLITE PLANT, COTTAGE GROVE, MINNESOTA**

	<b>N</b>	<b>MEAN</b>	<b>TB/TF SD</b>	<b>MEDIAN</b>	<b>RANGE</b>	<b>TEST#</b>
<b>BMI</b>						
<25	40	37.2	9.06	37.1	22.3-62.5	F=1.47
25-30	56	37.6	9.31	37.1	19.3-62.4	p=.23
>30	17	33.3	9.18	31.2	19.7-52.4	
<b>AGE</b>						
<31	20	32.4	6.92	31.2	20.0-43.6	F=.239
31-40	48	37.3	9.37	37.1	19.3-62.6	p=.07
41-50	26	37.1	9.30	38.8	19.7-58.6	
51-60	19	39.9	9.90	39.9	22.3-62.4	
<b>Alcohol</b>						
<1oz/d	86	37.4	9.90	37.2	19.3-62.6	F=2.06
1-3oz/d	19	33.9	6.70	32.2	22.7-44.1	p=.15
missing	8	38.0	5.70	38.6	26.4-43.5	
<b>Tobacco</b>						
smoker	27	37.9	7.96	37.2	25.0-68.8	F=.32
nonsmoker	84	36.7	9.64	36.3	19.3-62.6	p=.57
missing	2	27.5	1.57	27.5	26.4-28.6	
<b>TOTAL</b>	113					

#univariate Anova

TABLE A4.1.2 ESTRADIOL TO FREE TESTOSTERONE RATIO (E/TF)  
 BY BODY MASS INDEX, AGE, SMOKING AND DRINKING STATUS  
 1990 PERFLUORO-CHEMICAL EFFECTS STUDY,  
 3M CHEMOLITE PLANT, COTTAGE GROVE, MINNESOTA

	N	MEAN	E/TF SD	MEDIAN	RANGE	TEST#
<b>BMI</b>						
<25	40	2.07	0.88	1.94	0.73-5.0	*
25-30	56	2.28	0.92	2.17	0.76-5.39	>30vs<=30
>30	17	2.56	0.98	2.42	1.44-5.31	T=2.35 p=.02
<b>AGE</b>						
<31	20	1.94	0.56	1.81	1.44-3.27	F=1.19
31-40	48	2.25	0.81	2.17	0.77-4.18	p=.32
41-50	26	2.31	1.20	2.04	0.77-5.39	
51-60	19	2.48	1.05	2.42	1.07-5.31	
<b>Alcohol</b>						
<1oz/d	86	2.23	0.92	2.10	0.74-5.31	F=.01
1-3oz/d	19	2.21	0.76	2.21	0.73-4.18	p=.92
missing	8	2.46	1.96	2.09	1.41-5.39	
<b>Tobacco</b>						
smoker	27	2.19	0.98	2.06	0.74-5.39	F=.15
nonsmoker	84	2.27	0.92	2.19	0.73-5.31	p=.70
missing	2	1.88	0.32	1.88	1.65-2.11	
<b>TOTAL</b>	113					

#univariate Anova

\*Student t test, Prob>T

TABLE A4.1.3 ESTRADIOL TO BOUND TESTOSTERONE RATIO (E/TB)  
 BY BODY MASS INDEX, AGE, SMOKING AND DRINKING STATUS  
 1990 PERFLUORO-CHEMICAL EFFECTS STUDY,  
 3M CHEMOLITE PLANT, COTTAGE GROVE, MINNESOTA

	N	MEAN	E/TB x100 SD	MEDIAN	RANGE	TEST#
<b>BMI</b>						
<25	40	6.8	2.65	5.7	11.8-13.5	F=3.70
25-30	58	6.3	2.73	5.5	2.2-13.4	p=.03
>30	17	8.0	2.91	7.6	3.7-14.4	
<b>AGE</b>						
<31	20	6.1	1.79	5.9	3.0-9.5	F=.07
31-40	48	6.4	2.78	5.6	11.8-13.5	p=.98
41-50	26	6.5	3.53	5.0	1.7-14.4	
51-60	19	6.4	2.76	6.0	2.3-11.6	
<b>Alcohol</b>						
<1oz/d	86	6.3	2.90	5.7	1.2-14.4	F=.08
1-3oz/d	19	6.5	1.98	6.8	3.0-10.5	p=.98
missing	8	6.6	3.45	5.4	3.9-13.5	
<b>Tobacco</b>						
smoker	27	5.9	2.73	5.1	11.8-13.5	F=1.01
nonsmoker	84	6.5	2.84	6.0	2.2-13.4	p=.32
missing	2	6.9	1.55	6.9	3.7-14.4	
<b>TOTAL</b>	113					

#univariate Anova



**TABLE A4.1.4 ESTRADIOL TO LUTENIZING HORMONE RATIO (E/LH)  
BY BODY MASS INDEX, AGE, SMOKING AND DRINKING STATUS  
1990 PERFLUORO-CHEMICAL EFFECTS STUDY,  
3M CHEMOLITE PLANT, COTTAGE GROVE, MINNESOTA**

	<b>N</b>	<b>MEAN</b>	<b>E/LH SD</b>	<b>MEDIAN</b>	<b>RANGE</b>	<b>TEST#</b>
<b>BMI</b>						
<25	40	7.0	3.11	7.3	2.0-16.4	F=2.59
25-30	56	7.0	4.29	6.5	1.0-20.6	p=.08
>30	17	9.3	3.92	8.8	3.3-18.4	
<b>AGE</b>						
<31	20	8.7	4.58	7.6	2.3-20.6	F=2.51
31-40	48	7.8	3.35	7.6	1.5-16.4	p=.06
41-50	26	6.4	4.58	5.1	1.6-18.8	
51-60	19	5.8	2.89	6.2	1.0-11.5	
<b>Alcohol</b>						
<1oz/d	86	7.2	3.95	7.0	1.0-20.6	F=.04
1-3oz/d	19	7.4	4.16	6.7	1.6-16.4	p=.86
missing	8	8.5	3.19	8.7	4.3-15.4	
<b>Tobacco</b>						
smoker	27	7.0	4.42	6.2	1.5-16.4	F=.34
nonsmoker	84	7.5	3.77	7.1	1.0-20.6	p=.56
missing	2	4.7	3.35	4.7	2.3-7.0	
<b>TOTAL</b>	113					

#univariate Anova

**TABLE A4.1.5 FREE TESTOSTERONE TO LUTENIZING HORMONE RATIO (TF/LH) BY BODY MASS INDEX, AGE, SMOKING AND DRINKING STATUS  
1990 PERFLUORO-CHEMICAL EFFECTS STUDY,  
3M CHEMOLITE PLANT, COTTAGE GROVE, MINNESOTA**

	N	MEAN	TF/LH SD	MEDIAN	RANGE	TEST#
<b>BMI</b>						
<25	40	3.6	1.74	3.3	1.1-11.3	F=1.47
25-30	56	3.2	1.74	2.9	0.7-9.1	p=.24
>30	17	3.9	1.75	3.4	1.4-7.1	
<b>AGE</b>						
<31	20	4.8	2.55	3.9	3.9	F=7.21
31-40	48	3.6	1.43	3.3	3.3	p=.0002
41-50	26	2.9	1.30	2.7	2.7	
51-60	19	2.5	1.14	2.5	2.5	
<b>Alcohol</b>						
<1oz/d	86	3.4	1.85	3.2	0.7-11.3	F=.06
1-3oz/d	19	3.3	1.44	3.2	0.7-6.4	p=.81
missing	6	3.8	1.43	3.6	2.1-6.2	
<b>Tobacco</b>						
smoker	27	3.2	1.34	3.2	1.1-6.9	F=1.20
nonsmoker	84	3.6	1.87	3.2	0.7-11.3	p=.28
missing	2	2.4	1.38	2.3	1.4-3.3	
<b>TOTAL</b>	113					

#univariate Anova

**TABLE A4.1.6 BOUND TESTOSTERONE TO LUTENIZING HORMONE RATIO  
(TB/LH)  
BY BODY MASS INDEX, AGE, SMOKING AND DRINKING STATUS  
1990 PERFLUORO-CHEMICAL EFFECTS STUDY,  
3M CHEMOLITE PLANT, COTTAGE GROVE, MINNESOTA**

	<b>N</b>	<b>MEAN</b>	<b>TB/LH SD</b>	<b>MEDIAN</b>	<b>RANGE</b>	<b>TEST#</b>
<b>BMI</b>						
<25	40	131	57.9	125	39-298	F=.79
25-30	56	116	60.6	107	24-288	p=.46
>30	17	122	43.4	121	55-199	
<b>AGE</b>						
<31	20	147	69.7	136	39-298	F=4.72
31-40	48	133	58.0	131	36-288	p=.004
41-50	26	101	36.7	93	29-163	
51-60	19	96	47.6	95	24-208	
<b>Alcohol</b>						
<1oz/d	86	122	57.1	121	24-298	F=.32
1-3oz/d	19	114	58.1	92	29-202	p=.57
missing	8	147	63.2	142	77-234	
<b>Tobacco</b>						
smoker	27	116	52.4	114	41-288	F=.54
nonsmoker	84	125	58.8	121	24-298	p=.46
missing	2	64	34.3	64	39-88	
<b>TOTAL</b>	<b>113</b>					

#univariate Anova

**TABLE A4.1.7 THYROID STIMULATING HORMONE TO  
LUTENIZING HORMONE RATIO (TSH/LH)  
BY BODY MASS INDEX, AGE, SMOKING AND DRINKING STATUS  
1990 PERFLUORO-CHEMICAL EFFECTS STUDY,  
3M CHEMOLITE PLANT, COTTAGE GROVE, MINNESOTA**

	N	TSH/LH x10			RANGE	TEST#
		MEAN	SD	MEDIAN		
<b>BMI</b>						
<25	40	3.2	1.78	3.2	0.6-8.3	F=3.40
25-30	56	3.5	2.80	3.0	0.4-17.0	p=.02
>30	17	5.3	3.34	4.4	1.7-13.5	
<b>AGE</b>						
<31	20	3.7	2.45	3.1	1.0-9.9	F=.14
31-40	48	3.8	3.16	3.2	0.4-17.0	p=.93
41-50	28	3.4	1.89	2.9	0.8-8.3	
51-60	19	3.8	2.48	3.5	0.4-11.0	
<b>Alcohol</b>						
<1oz/d	86	3.5	2.29	3.1	0.4-11.0	F=2.92
1-3oz/d	19	4.7	4.05	3.2	1.0-17.0	p=.09
missing	8	3.3	1.74	3.6	0.8-5.8	
<b>Tobacco</b>						
smoker	27	3.0	1.88	2.8	0.4-7.6	F=2.89
nonsmoker	84	4.0	2.89	3.3	0.4-17.0	p=.09
missing	2	1.7	0.01	1.7	1.7-1.7	
<b>TOTAL</b>	113					

#univariate Anova

**TABLE A4.1.8 FOLLICLE STIMULATING HORMONE TO  
LUTENIZING HORMONE RATIO (FSH/LH) BY BODY MASS INDEX, AGE,  
SMOKING AND DRINKING STATUS  
1990 PERFLUORO-CHEMICAL EFFECTS STUDY,  
3M CHEMOLITE PLANT, COTTAGE GROVE, MINNESOTA**

	<b>N</b>	<b>MEAN</b>	<b>FSH/LH SD</b>	<b>MEDIAN</b>	<b>RANGE</b>	<b>TEST#</b>
<b>BMI</b>						
<25	40	1.0	0.42	0.8	0.4-2.3	F=2.54
25-30	56	1.0	0.37	0.9	0.4-1.9	p=.08
>30	17	1.2	0.46	1.1	0.4-2.0	
<b>AGE</b>						
<31	20	0.9	0.4	0.8	0.5-1.9	F=3.06
31-40	48	0.9	0.46	0.9	0.4-1.9	p=.03
41-50	26	1.1	0.45	1.0	0.4-1.8	
51-60	19	1.2	0.49	1.1	0.5-2.3	
<b>Alcohol</b>						
<1oz/d	86	1.0	0.42	0.9	0.4-2.3	
1-3oz/d	19	0.9	0.41	0.9	0.4-1.7	F=.48
missing	8	0.9	0.24	0.9	0.5-1.3	p=.49
<b>Tobacco</b>						
smoker	27	1.0	0.40	0.9	.04-1.8	
nonsmoker	84	1.0	0.42	0.9	0.4-2.3	F=0.0
missing	2	0.9	0.43	0.9	0.7-1.0	p=.98
<b>TOTAL</b>	<b>113</b>					

#univariate Anova

**TABLE A4.1.9 PROLACTIN TO LUTENIZING HORMONE RATIO (P/LH)  
 BY BODY MASS INDEX, AGE, SMOKING AND DRINKING STATUS  
 1990 PERFLUORO-CHEMICAL EFFECTS STUDY,  
 3M CHEMOLITE PLANT, COTTAGE GROVE, MINNESOTA**

	<b>N</b>	<b>MEAN</b>	<b>P/LH SD</b>	<b>MEDIAN</b>	<b>RANGE</b>	<b>TEST#</b>
<b>BMI</b>						
<25	40	1.84	1.14	1.53	0.50-6.39	F=.73
25-30	58	1.78	1.40	1.46	0.39-9.11	p=.49
>30	17	2.21	1.20	1.83	1.18-4.91	
<b>AGE</b>						
<31	20	2.29	1.70	1.83	1.18-4.91	F=1.55
31-40	48	1.95	1.56	1.67	0.39-9.11	p=.21
41-50	26	1.61	0.93	1.16	0.56-3.70	
51-60	19	1.54	0.87	1.39	0.35-3.37	
<b>Alcohol</b>						
<1oz/d	86	1.78	1.04	1.61	0.35-6.39	F=3.19
1-3oz/d	19	2.37	2.14	1.70	0.56-9.11	p=.08
missing	8	1.57	0.71	1.47	0.88-3.00	
<b>Tobacco</b>						
smoker	27	1.27	0.73	1.12	0.39-2.74	F=8.25
nonsmoker	84	2.07	1.38	1.72	0.35-9.11	p=.005
missing	2	1.43	0.78	1.43	0.88-2.00	
<b>TOTAL</b>	113					

#univariate Anova

**TABLE A4.1.10 BOUND TESTOSTERONE TO THYROID STIMULATING  
HORMONE RATIO (TB/TSH)  
BY BODY MASS INDEX, AGE, SMOKING AND DRINKING STATUS  
1990 PERFLUORO-CHEMICAL EFFECTS STUDY,  
3M CHEMOLITE PLANT, COTTAGE GROVE, MINNESOTA**

	N	MEAN	TB/TSH SD	MEDIAN	RANGE	TEST#
<b>BMI</b>						
<25	40	500	331	413	170-1682	F=2.42
25-30	58	461	364	329	51-2102	p=.09
>30	17	296	152	297	87-589	
<b>AGE</b>						
<31	20	522	357	416	122-1682	F=2.64
31-40	48	521	388	421	51-2102	p=.05
41-50	26	359	203	345	131-1035	
51-60	19	328	231	286	87-1122	
<b>Alcohol</b>						
<1oz/d	86	468	352	353	87-2102	F=2.74
1-3oz/d	19	329	210	278	51-900	p=.10
missing	8	563	326	456	184-1154	
<b>Tobacco</b>						
smoker	27	468	232	403	184-1185	F=.07
nonsmoker	84	448	364	321	51-2102	p=.80
missing	2	371	198	371	231-511	
<b>TOTAL</b>	113					

#univariate Anova

TABLE A4.1.11 FREE TESTOSTERONE TO THYROID STIMULATING  
HORMONE RATIO (TF/TSH)  
BY BODY MASS INDEX, AGE, SMOKING AND DRINKING STATUS  
1990 PERFLUORO-CHEMICAL EFFECTS STUDY,  
3M CHEMOLITE PLANT, COTTAGE GROVE, MINNESOTA

	N	MEAN	TF/TSH SD	MEDIAN	RANGE	TEST#
<b>BMI</b>						
<25	40	13.8	8.62	10.8	3.8-43.7	F=2.43
25-30	56	11.7	7.01	9.7	1.7-35.8	p=.09
>30	17	9.3	5.05	8.0	2.0-19.7	
<b>AGE</b>						
<31	20	15.8	9.87	10.8	6.1-43.7	F=5.36
31-40	48	13.4	7.62	11.9	1.7-35.8	p=.002
41-50	26	9.7	4.58	8.7	3.7-22.0	
51-60	19	8.1	4.46	7.8	2.0-21.3	
<b>Alcohol</b>						
<1oz/d	86	12.4	1.65	10.0	2.0-43.7	F=2.48
1-3oz/d	19	9.5	5.10	8.7	1.7-20.7	p=.12
missing	8	15.3	9.35	12.3	5.0-33.5	
<b>Tobacco</b>						
smoker	27	12.5	5.53	11.3	5.0-27.2	F=.12
nonsmoker	84	11.9	8.06	9.8	1.7-43.7	p=.73
missing	2	13.7	7.99	13.7	8.1-19.4	
<b>TOTAL</b>	113					

#univariate Anova



**TABLE A4.1.12 ESTRADIOL TO THYROID STIMULATING HORMONE RATIO (E/TSH) BY BODY MASS INDEX, AGE, SMOKING AND DRINKING STATUS 1990 PERFLUORO-CHEMICAL EFFECTS STUDY, 3M CHEMOLITE PLANT, COTTAGE GROVE, MINNESOTA**

	<b>N</b>	<b>MEAN</b>	<b>E/TSH SD</b>	<b>MEDIAN</b>	<b>RANGE</b>	<b>TEST#</b>
<b>BMI</b>						
<25	40	26.7	17.73	23.1	3.3-108.1	F=.27
25-30	56	25.4	14.04	21.3	1.8-59.0	p=.76
>30	17	23.4	15.79	18.4	7.7-54.8	
<b>AGE</b>						
<31	20	27.8	12.00	24.8	9.7-50.0	F=3.21
31-40	48	29.5	18.40	22.3	1.8-108.1	p=.03
41-50	26	21.8	13.06	19.0	3.3-52.2	
51-60	19	18.2	10.80	16.8	7.8-54.8	
<b>Alcohol</b>						
<1oz/d	88	25.1	13.08	21.5	3.3-59.0	F=.57
1-3oz/d	19	22.4	15.30	18.0	1.8-54.2	p=.45
missing	8	37.8	31.81	28.5	10.4-108.1	
<b>Tobacco</b>						
smoker	27	26.7	15.13	23.4	10.3-59.0	F=.20
nonsmoker	84	25.1	15.85	20.7	1.8-108.1	p=.65
missing	2	27.1	19.40	27.1	13.4-40.8	
<b>TOTAL</b>	113					

#univariate Anova

TABLE A4.1.13 THYROID STIMULATING HORMONE TO  
 FOLLICLE STIMULATING HORMONE RATIO (TSH/FSH)  
 BY BODY MASS INDEX, AGE, SMOKING AND DRINKING STATUS  
 1990 PERFLUORO-CHEMICAL EFFECTS STUDY,  
 3M CHEMOLITE PLANT, COTTAGE GROVE, MINNESOTA

	N	MEAN	TSH/FSH SD	MEDIAN	RANGE	TEST#
<b>BMI</b>						
<25	40	0.39	0.25	0.34	0.08-1.09	F=.39
25-30	56	0.41	0.39	0.31	0.06-2.34	p=.68
>30	17	0.48	1.29	0.43	0.15-1.26	
<b>AGE</b>						
<31	20	0.46	0.31	0.44	0.12-1.26	F=.93
31-40	48	0.46	0.40	0.35	0.06-2.34	p=.43
41-50	26	0.37	0.28	0.30	0.09-1.21	
51-60	19	0.33	0.18	0.31	0.06-0.75	
<b>Alcohol</b>						
<1oz/d	86	0.38	0.26	0.32	.06-1.26	F=5.36
1-3oz/d	19	0.58	0.54	0.39	0.15-2.34	p=.02
missing	8	0.40	0.24	0.39	0.08-0.76	
<b>Tobacco</b>						
smoker	27	0.34	1.04	0.26	0.06-0.92	F=2.39
nonsmoker	84	0.45	2.28	0.40	0.06-2.34	p=.12
missing	2	0.21	0.05	0.21	0.17-0.25	
<b>TOTAL</b>	113					

#univariate Anova

**TABLE A4.1.14 FREE TESTOSTERONE TO FOLLICLE STIMULATING  
HORMONE RATIO (TF/FSH)  
BY BODY MASS INDEX, AGE, SMOKING AND DRINKING STATUS  
1990 PERFLUORO-CHEMICAL EFFECTS STUDY,  
3M CHEMOLITE PLANT, COTTAGE GROVE, MINNESOTA**

	N	MEAN	TF/FSH SD	MEDIAN	RANGE	TEST#
<b>BMI</b>						
<25	40	4.3	2.86	3.6	1.3-15.6	F=1.03
25-30	56	3.6	2.18	3.0	0.7-11.1	p=.36
>30	17	4.0	2.86	3.1	0.8-11.3	
<b>AGE</b>						
<31	20	5.8	3.34	5.0	1.7-15.6	F=10.35
31-40	48	4.2	2.18	3.7	1.7-11.3	p=.0001
41-50	26	3.0	1.62	2.5	0.7-6.6	
51-60	19	2.2	1.37	2.0	0.7-6.3	
<b>Alcohol</b>						
<1oz/d	86	2.8	2.60	3.1	0.7-15.6	F=.01
1-3oz/d	19	3.9	2.18	3.7	1.3-10.1	p=.91
missing	8	4.4	1.67	4.7	2.2-7.3	
<b>Tobacco</b>						
smoker	27	3.5	1.76	3.0	0.7-7.5	F=1.14
nonsmoker	84	4.1	2.67	3.5	0.7-15.6	p=.28
missing	2	2.6	0.91	2.6	2.2-7.3	
<b>TOTAL</b>	113					

#univariate Anova

**TABLE A4.1.15 BOUND TESTOSTERONE TO FOLLICLE STIMULATING  
HORMONE RATIO (TB/FSH)  
BY BODY MASS INDEX, AGE, SMOKING AND DRINKING STATUS  
1990 PERFLUORO-CHEMICAL EFFECTS STUDY,  
3M CHEMOLITE PLANT, COTTAGE GROVE, MINNESOTA**

	N	MEAN	TF/FSH SD	MEDIAN	RANGE	TEST
<b>BMI</b>						
<25	40	155	87.3	138	39-411	F=2.00
25-30	56	126	67.9	113	23-297	p=.14
>30	17	122	76.7	115	34-306	
<b>AGE</b>						
<31	20	182	90.3	184	39-411	F=8.75
31-40	48	154	74.5	141	45-348	p=.0001
41-50	26	101	52.9	91	39-227	
51-60	19	87	53.2	78	23-264	
<b>Alcohol</b>						
<1oz/d	86	133	76.5	120	23-411	F=0.0
1-3oz/d	19	133	78.6	112	39-325	p=.98
missing	8	173	80.6	190	82-303	
<b>Tobacco</b>						
smoker	27	130	74.4	106	39-325	F=.28
nonsmoker	84	139	78.5	131	23-411	p=.60
missing	2	72	70.9	72	57-86	
<b>TOTAL</b>	113					

#univariate Anova

TABLE A4.1.16 ESTRADIOL TO FOLLICLE STIMULATING HORMONE RATIO (E/FSH) BY BODY MASS INDEX, AGE, SMOKING AND DRINKING STATUS  
1990 PERFLUORO-CHEMICAL EFFECTS STUDY,  
3M CHEMOLITE PLANT, COTTAGE GROVE, MINNESOTA

	N	MEAN	E/FSH SD	MEDIAN	RANGE	TEST#
<b>BMI</b>						
<25	40	8.7	5.51	6.8	1.3-23.3	F=.50
25-30	56	7.8	5.76	6.6	1.4-29.6	p=.61
>30	17	9.3	7.04	7.6	2.65-33.1	
<b>AGE</b>						
<31	20	10.8	5.80	9.6	3.1-25.0	F=5.00
31-40	48	9.5	6.01	7.3	1.4-33.1	p=.003
41-50	26	6.9	6.13	4.8	1.3-29.6	
51-60	19	4.9	2.27	4.6	1.5-9.3	
<b>Alcohol</b>						
<1oz/d	86	8.1	5.83	6.6	1.3-33.1	F=.01
1-3oz/d	19	8.3	4.99	7.1	3.1-19.1	p=.91
missing	8	10.9	7.94	8.3	4.6-29.6	
<b>Tobacco</b>						
smoker	27	8.1	6.82	4.7	1.4-29.6	F=.13
nonsmoker	84	8.5	5.59	7.0	1.3-33.1	p=.72
missing	2	5.1	2.56	5.1	3.2-6.9	
<b>TOTAL</b>	113					

#univariate Anova

TABLE A4.1.17 THYROID STIMULATING HORMONE TO PROLACTIN RATIO (TSH/P) BY BODY MASS INDEX, AGE, SMOKING AND DRINKING STATUS  
1990 PERFLUORO-CHEMICAL EFFECTS STUDY,  
3M CHEMOLITE PLANT, COTTAGE GROVE, MINNESOTA

	N	MEAN	TSH/P SD	MEDIAN	RANGE	TEST#
<b>BMI</b>						
<25	40	0.22	0.17	0.18	0.06-0.83	F=.71
25-30	56	0.25	0.22	0.19	0.02-1.21	p=.49
>30	17	0.28	0.19	0.26	0.07-0.81	
<b>AGE</b>						
<31	20	0.17	0.09	0.15	0.05-0.39	F=1.38
31-40	48	0.25	0.23	0.17	0.02-1.21	p=.25
41-50	26	0.26	0.18	0.22	0.06-0.83	
51-60	19	0.29	0.20	0.20	0.07-0.81	
<b>Alcohol</b>						
<1oz/d	86	0.24	0.19	0.19	0.04-1.21	F=2.15
1-3oz/d	19	0.29	0.24	0.17	0.02-1.00	p=.15
missing	8	0.21	0.08	0.20	0.09-0.30	
<b>Tobacco</b>						
smoker	27	0.29	0.25	0.21	0.09-1.21	F=1.00
nonsmoker	84	0.23	0.18	0.18	0.02-1.00	p=.32
missing	2	0.14	0.08	0.14	0.09-0.30	
<b>TOTAL</b>	113					

#univariate Anova

TABLE A4.1.18 FREE TESTOSTERONE TO PROLACTIN RATIO (TF/P)  
 BY BODY MASS INDEX, AGE, SMOKING AND DRINKING STATUS  
 1990 PERFLUORO-CHEMICAL EFFECTS STUDY,  
 3M CHEMOLITE PLANT, COTTAGE GROVE, MINNESOTA

	N	MEAN	TF/P SD	MEDIAN	RANGE	TEST#
<b>BMI</b>						
<25	40	2.5	1.47	2.2	0.6-7.8	F=.19
25-30	56	2.3	2.13	1.9	0.5-15.0	p=.83
>30	17	2.1	1.12	2.0	0.8-4.6	
<b>AGE</b>						
<31	20	2.4	1.67	2.0	0.7-7.8	F=.72
31-40	48	2.6	2.27	2.1	0.5-15.0	p=.54
41-50	26	2.1	1.05	2.0	0.7-4.2	
51-60	19	2.0	1.19	1.6	0.8-5.6	
<b>Alcohol</b>						
<1oz/d	86	2.4	1.94	2.0	0.6-15.0	F=.19
1-3oz/d	19	2.0	1.20	1.5	0.5-5.1	p=.37
missing	8	2.6	0.75	2.7	1.5-3.8	
<b>Tobacco</b>						
smoker	27	3.2	2.81	2.4	1.1-15.0	F=9.58
nonsmoker	84	2.1	1.18	1.9	0.5-7.8	p=.003
missing	2	2.2	2.20	2.2	0.7-3.8	
<b>TOTAL</b>	113					

#univariate Anova

**TABLE A4.1.19 BOUND TESTOSTERONE TO PROLACTIN RATIO (TB/P)  
 BY BODY MASS INDEX, AGE, SMOKING AND DRINKING STATUS  
 1990 PERFLUORO-CHEMICAL EFFECTS STUDY,  
 3M CHEMOLITE PLANT, COTTAGE GROVE, MINNESOTA**

	<b>N</b>	<b>MEAN</b>	<b>TB/P SD</b>	<b>MEDIAN</b>	<b>RANGE</b>	<b>TEST#</b>
<b>BMI</b>						
<25	40	87	48.4	82	20-221	F=.60
25-30	58	87	85.0	64	22-624	p=.55
>30	17	88	36.1	87	28-158	
<b>AGE</b>						
<31	20	75	47.1	68	20-208	F=.83
31-40	48	96	91.0	79	22-624	p=.48
41-50	26	78	41.6	72	23-163	
51-60	19	73	34.4	63	34-158	
<b>Alcohol</b>						
<1oz/d	86	87	73.0	72	20-624	F=1.23
1-3oz/d	19	68	49.9	49	22-221	p=.27
missing	8	95	20.8	100	55-117	
<b>Tobacco</b>						
smoker	27	121	122.8	94	27-624	F=11.31
nonsmoker	84	73	38.7	66	22-208	p=.001
missing	2	60	56.8	60	20-100	
<b>TOTAL</b>	113					

#univariate Anova



**TABLE A4.1.20 ESTRADIOL TO PROLACTIN RATIO (E/P)  
BY BODY MASS INDEX, AGE, SMOKING AND DRINKING STATUS  
1990 PERFLUORO-CHEMICAL EFFECTS STUDY,  
3M CHEMOLITE PLANT, COTTAGE GROVE, MINNESOTA**

	<b>N</b>	<b>MEAN</b>	<b>E/P SD</b>	<b>MEDIAN</b>	<b>RANGE</b>	<b>TEST#</b>
<b>BMI</b>						
<25	40	4.7	2.80	4.4	1.1-13.8	F=.19
25-30	56	5.1	4.88	3.9	1.1-32.5	p=.82
>30	17	5.1	2.68	4.3	1.9-9.7	
<b>AGE</b>						
<31	20	4.2	2.20	4.1	1.1-9.0	F=1.09
31-40	48	5.7	5.18	4.6	1.1-32.5	p=.36
41-50	26	4.7	3.22	4.0	1.1-15.1	
51-60	19	4.3	2.00	3.7	2.2-9.6	
<b>Alcohol</b>						
<1oz/d	86	5.0	4.11	4.1	1.1-32.5	F=.59
1-3oz/d	19	4.2	2.94	3.1	1.1-13.0	p=.45
missing	8	6.5	4.15	4.6	3.0-15.1	
<b>Tobacco</b>						
smoker	27	7.2	6.67	5.2	1.1-32.5	F=12.21
nonsmoker	84	4.3	2.16	4.0	1.1-10.2	p=.001
missing	2	4.6	4.84	4.6	1.1-8.0	
<b>TOTAL</b>	113					

#univariate Anova

TABLE A4.1.21 FOLLICLE STIMULATING HORMONE TO PROLACTIN RATIO (FSH/P) BY BODY MASS INDEX, AGE, SMOKING AND DRINKING STATUS  
1990 PERFLUORO-CHEMICAL EFFECTS STUDY,  
3M CHEMOLITE PLANT, COTTAGE GROVE, MINNESOTA

	N	MEAN	FSH/P SD	MEDIAN	RANGE	TEST#
<b>BMI</b>						
<25	40	0.72	0.51	0.57	0.2-2.1	F=.22
25-30	56	0.79	0.52	0.65	0.1-2.6	.80
>30	17	0.74	0.58	0.57	0.2-2.6	
<b>AGE</b>						
<31	20	0.46	0.23	0.45	0.2-1.0	F=5.41
31-40	48	0.71	0.51	0.54	0.1-2.6	p=.002
41-50	26	0.88	0.50	0.73	0.2-2.1	
51-60	19	1.05	0.62	0.81	0.2-2.6	
<b>Alcohol</b>						
<1oz/d	86	0.81	0.57	0.66	0.2-2.1	F=3.18
1-3oz/d	19	0.57	0.32	0.50	0.1-2.6	p=.08
missing	8	0.66	0.31	0.60	0.2-2.6	
<b>Tobacco</b>						
smoker	27	1.00	0.57	0.79	0.3-2.6	F=7.90
nonsmoker	84	0.68	0.49	0.51	0.1-2.6	p=.006
missing	2	0.75	0.57	0.62	0.3-1.2	
<b>TOTAL</b>	113					

#univariate Anova

**APPENDIX 3**

**TABLES OF HORMONE RATIOS BY TOTAL SERUM FLUORIDE**

TABLE A4.2.1 HORMONE RATIOS BY TOTAL SERUM FLUORIDE:  
 ESTRADIOL/FREE TESTOSTERONE (E/TF)  
 ESTRADIOL/BOUND TESTOSTERONE (E/TB)  
 ESTRADIOL/THYROID STIMULATING HORMONE (E/TSH)  
 1990 PERFLUORO-CHEMICAL EFFECTS STUDY,  
 3M CHEMOLITE PLANT, COTTAGE GROVE, MINNESOTA

	N	MEAN	SD	MEDIAN	RANGE	TEST#
<b>E/TF</b>						
<b>TOTAL FLUORIDE ppm</b>						
<1	23	2.5	1.2	2.2	0.7-5.3	F=1.65
>=1-3	64	2.1	0.8	1.9	0.8-5.4	p=.16
>3-10	15	2.2	0.6	2.2	0.8-3.2	
>10-15	6	2.6	1.1	2.1	1.6-4.0	
>15-26	5	2.7	0.7	3	1.9-3.3	
<b>TOTAL</b>	<b>113</b>	<b>2.25</b>	<b>.92</b>	<b>2.1</b>	<b>0.7-5.4</b>	
<b>E/TB (X100)</b>						
<1	23	7.3	3.5	6.3	1.1-14.4	F=1.17
>=1-3	64	5.9	2.5	5.5	1.7-13.5	p=.33
>3-10	15	6.7	2.8	5.9	2.9-12.3	
>10-15	6	6.9	2.8	5.6	4.3-11.7	
>15-26	5	6.3	1.8	5.3	4.8-8.4	
<b>TOTAL</b>	<b>113</b>	<b>6.4</b>	<b>2.8</b>	<b>5.8</b>	<b>1.2-14.4</b>	
<b>E/TSH</b>						
<1	23	29.2	21.8	21.0	10.4-108.1	F=0.75
>=1-3	64	24.9	13.2	23.6	1.8-52.2	p=.56
>3-10	15	26.7	16.7	21.1	3.9-59.0	
>10-15	6	19.3	1.9	15.8	7.8-45.8	
>15-26	5	19.8	6.8	16.9	15.2-31.5	
<b>TOTAL</b>	<b>113</b>	<b>25.5</b>	<b>15.6</b>	<b>21.3</b>	<b>1.76-108.1</b>	

#univariate Anova

TABLE A4.2.2 HORMONE RATIOS BY TOTAL SERUM FLUORIDE:  
 BOUND TESTOSTERONE/FREE TESTOSTRONE (TB/TF)  
 BOUND TESTOSTRONE/FOLLICLE STIMULATING HORMONE (TB/FSH)  
 BOUND TESTOSTERONE/PROLACTIN (TB/P)  
 FREE TESTOSTERONE/PROLACTIN (TF/P)  
 1990 PERFLUORO-CHEMICAL EFFECTS STUDY,  
 3M CHEMOLITE PLANT, COTTAGE GROVE, MINNESOTA

	N	MEAN	SD	MEDIAN	RANGE	TEST#
<b>TOTAL FLUORIDE ppm</b>						
<b>TB/TF</b>						
<1	23	36.7	9.6	37.1	16.7-62.6	F=.94
>=1-3	64	36.8	9.6	35.6	19.2-58.8	P=.45
>3-10	15	34.5	7.0	33.3	25.0-43.6	
>10-15	6	38.3	7.6	38.9	29.3-47.6	
>15-26	5	43.6	10.6	39.9	36.9-62.4	<=10vs >10
TOTAL	113	36.8	9.2	37.0	16.7-62.6	T=2.10 P=.15
<b>TB/FSH</b>						
<1	23	148.3	85.6	120.4	56.7-411.0	F=.40
>=1-3	64	130.9	76.3	114.2	23.1-347.7	P=.81
>3-10	15	135.3	81.4	86.9	49.0-303.3	
>10-15	6	122.7	48.3	135.8	34.4-169.8	
>15-26	5	162.9	76.4	143.5	67.1-253.5	
TOTAL	113	136.1	77.1	120.0	23.1-411.0	
<b>TB/P</b>						
<1	23	82.1	43.2	67.5	19.8-205.5	F=.40
>=1-3	64	87.5	81.2	63.3	23.0-624.2	P=.81
>3-10	15	86.4	51.0	78.0	28.1-224.2	
>10-15	6	51.5	27.9	52.1	22.2-89.3	
>15-26	5	90.3	30.6	83.4	58.3-129.7	
TOTAL	113	84.5	67.3	70.7	19.8-624.2	
<b>TF/P</b>						
<1	23	2.34	1.46	2.01	.7-7.8	F=.52
>=1-3	64	2.38	1.97	1.88	.6-15.0	P=.72
>3-10	15	2.64	1.84	2.40	.8-8.1	
>10-15	6	1.40	0.82	1.35	.5-2.3	
>15-26	5	2.20	0.91	2.16	.9-3.5	
TOTAL	113	2.35	1.78	1.95	.5-15.0	

#univariate Anova

TABLE A4.2.3 HORMONE RATIOS BY TOTAL SERUM FLUORIDE:  
 ESTRADIOL/PROLACTIN (E/P)  
 THYROID STIMULATING HORMONE/PROLACTIN (TSH/P)  
 FOLLICLE STIMULATING HORMONE/PROLACTIN (FSH/P)  
 PROLACTIN/LUTENIZING HORMONE (P/LH)  
 1990 PERFLUORO-CHEMICAL EFFECTS STUDY,  
 3M CHEMOLITE PLANT, COTTAGE GROVE, MINNESOTA

	N	MEAN	SD	MEDIAN	RANGE	TEST#
<b>TOTAL FLUORIDE ppm</b>						
<b>E/P</b>						
<1	23	5.34	2.77	4.81	1.45-10.22	F=.64
>=1-3	64	4.75	4.38	3.77	1.09-32.50	p=.63
>3-10	15	5.87	4.65	4.76	1.87-17.89	
>10-15	6	3.13	1.08	3.45	1.13-4.07	
>15-26	5	5.55	1.69	6.17	3.11-7.09	
TOTAL	113	4.97	3.94	4.08	1.09-32.50	
<b>TSH/P</b>						
<1	23	0.22	0.11	0.21	0.07-0.49	F=.40
>=1-3	64	0.24	0.21	0.17	0.04-1.20	p=.81
>3-10	15	0.29	0.76	0.20	0.07-0.85	
>10-15	6	0.24	0.14	0.25	0.02-0.41	
>15-26	5	0.29	0.09	0.27	0.20-0.44	
TOTAL	113	0.24	0.20	0.19	0.02-1.20	
<b>FSH/P</b>						
<1	23	0.65	0.47	0.60	0.18-2.56	F=.89
>=1-3	64	0.80	0.52	0.66	0.15-2.17	p=.47
>3-10	15	0.67	0.68	0.57	0.15-2.58	
>10-15	6	0.52	0.36	0.47	0.13-1.04	
>15-26	5	0.66	0.36	0.47	0.33-1.13	
TOTAL	113	0.76	0.52	0.60	0.13-2.58	
<b>P/LH</b>						
<1	23	1.71	0.85	1.64	0.35-3.02	F=1.72
>=1-3	63	1.85	1.75	1.57	0.41-6.39	p=.15
>3-10	15	1.79	1.20	1.45	0.39-4.00	
>10-15	6	3.14	3.00	1.88	1.10-9.11	
>15-26	5	1.5	0.44	1.33	1.03-2.10	
TOTAL	112	1.87	1.29	1.62	0.35-9.11	

#univariate Anova

**TABLE A4.2.4 HORMONE RATIOS BY TOTAL SERUM FLUORIDE:  
 ESTRADIOL/LUTENIZING HORMONE (E/LH)  
 ESTRADIOL/FOLLICLLE STIMULATING HORMONE(E/FSH)  
 FOLLICLLE STIMULATING HORMONE/LUTENIZING HORMONE (FSH/LH)  
 1990 PERFLUORO-CHEMICAL EFFECTS STUDY,  
 3M CHEMOLITE PLANT, COTTAGE GROVE, MINNESOTA**

	<b>N</b>	<b>MEAN</b>	<b>SD</b>	<b>MEDIAN</b>	<b>RANGE</b>	<b>TEST#</b>
<b>TOTAL FLUORIDE ppm</b>						
			<b>E/LH</b>			
<1	23	8.64	4.73	8.28	1.51-18.81	F=.92
>=1-3	63	8.85	4.01	6.19	0.95-20.59	p=.45
>3-10	15	7.24	2.74	7.55	1.96-11.52	
>10-15	6	7.21	2.15	7.09	4.39-10.27	
>15-26	5	7.99	2.71	7.03	6.03-12.70	
<b>TOTAL</b>	<b>112</b>	<b>7.34</b>	<b>3.91</b>	<b>7.01</b>	<b>0.95-20.59</b>	
			<b>E/FSH</b>			
<1	23	10.27	6.85	8.69	1.36-33.12	F=1.00
>=1-3	64	7.71	5.98	6.10	1.30-29.60	p=.041
>3-10	15	7.74	3.26	6.89	3.71-12.42	
>10-15	6	8.04	4.24	8.27	3.09-15.30	
>15-26	5	10.32	6.60	7.03	5.45-21.50	
<b>TOTAL</b>	<b>113</b>	<b>8.37</b>	<b>5.81</b>	<b>6.85</b>	<b>1.30-33.12</b>	
			<b>FSH/LH</b>			
<1	23	0.91	0.30	0.89	0.42-1.48	F=.41
>=1-3	63	1.04	0.44	0.94	0.37-2.25	p=.73
>3-10	15	0.99	0.43	0.91	0.41-1.95	
>10-15	6	1.08	0.50	1.03	0.53-1.78	
>15-26	5	0.91	0.35	0.99	0.55-1.40	
<b>TOTAL</b>	<b>112</b>	<b>1.00</b>	<b>0.41</b>	<b>0.94</b>	<b>0.37-2.25</b>	

#univariate Anova

TABLE A4.2.5 HORMONE RATIOS BY TOTAL SERUM FLUORIDE:  
 FREE TESTOSTERONE/THYROID STIMULATING HORMONE (TF/TSH)  
 BOUND TESTOSTERONE/THYROID STIMULATING HORMONE (TB/TSH)  
 FREE TESTOSTERONE/LUTENIZING HORMONE (TF/LH)  
 BOUND TESTOSTERONE/LUTENIZING HORMONE (TB/LH)  
 1990 PERFLUORO-CHEMICAL EFFECTS STUDY,  
 3M CHEMOLITE PLANT, COTTAGE GROVE, MINNESOTA

	N	MEAN	SD	MEDIAN	RANGE	TEST#
<b>TOTAL FLUORIDE ppm</b>						
<b>TF/TSH</b>						
<1	23	12.6	8.5	9.5	4.5-35.1	F=.93
>=1-3	64	12.7	7.5	11.1	1.7-43.7	p=.45
>3-10	15	11.9	6.8	10.4	3.2-27.2	
>10-15	6	8.5	1.7	7.5	12.0-20.7	
>15-26	5	7.5	1.9	7.9	4.6-9.6	
TOTAL	113	12.1	7.5	9.9	1.7-43.7	
<b>TB/TSH</b>						
<1	23	456	330	320	170-1367	F=.54
>=1-3	64	479	363	370	51-2102	p=.70
>3-10	15	416	270	401	95-1185	
>10-15	6	334	296	226	87-900	
>15-26	5	314	49	317	247-370	
TOTAL	113	451	333	353	51-2102	
<b>TF/LH</b>						
<1	23	3.7	2.3	3.3	1.2-11.3	F=.28
>=1-3	64	3.5	1.8	3.2	0.6-9.1	p=.89
>3-10	15	3.3	1.0	3.3	1.2-5.6	
>10-15	6	3.1	1.33	2.89	1.4-4.6	
>15-26	5	3.0	0.8	3.4	1.9-3.9	
TOTAL	113	3.4	1.7	3.2	0.6-11.3	
<b>TB/LH</b>						
<1	23	127	66	125	39-298	F=.13
>=1-3	64	121	58	114	24-288	p=.93
>3-10	15	115	51	105	52-234	
>10-15	6	118	56	105	61-201	
>15-26	5	127	21	125	122-149	
TOTAL	113	122.1	57.3	118	24.3-298	

#univariate Anova



TABLE A4.2.6 HORMONE RATIOS BY TOTAL SERUM FLUORIDE:  
 THYROID STIMULATING HORMONE/FOLLICLE STIMULATING HORMONE (TSH/FSH)  
 THYROID STIMULATING HORMONE/LUTENIZING HORMONE (TSH/LH)  
 1990 PERFLUORO-CHEMICAL EFFECTS STUDY,  
 3M CHEMOLITE PLANT, COTTAGE GROVE, MINNESOTA

	N	MEAN	SD	MEDIAN	RANGE	TEST#
<b>TOTAL FLUORIDE ppm</b>						
<b>TSH/FSH</b>						
<1	23	0.42	0.24	0.40	0.08-0.89	F=0.23
>=1-3	64	0.40	0.38	0.29	0.06-2.34	p=.92
>3-10	15	0.44	0.33	0.35	0.06-1.20	
>10-15	6	0.49	0.25	0.49	0.19-0.80	
>15-26	5	0.49	0.17	0.45	0.27-0.68	
TOTAL	113	0.42	0.33	0.35	0.06-2.34	
<b>TSH/LH</b>						
<1	23	0.36	0.22	0.33	0.08-1.00	F=0.23
>=1-3	64	0.36	0.30	0.28	0.04-1.70	p=.92
>3-10	15	0.38	0.27	0.31	0.04-1.1	
>10-15	6	0.45	0.19	0.43	0.22-0.70	
>15-26	5	0.40	0.37	0.40	0.36-0.45	
TOTAL	113	0.37	0.26	0.31	0.04-1.70	
<b>TF/FSH</b>						
<1	23	4.40	3.27	3.7	1.5-15.6	F=.34
>=1-3	64	3.77	2.38	3.1	.7-11.1	p=.85
>3-10	15	3.78	1.84	3.2	1.7-7.3	
>10-15	6	3.42	1.63	3.9	.8-5.3	
>15-26	5	3.93	2.15	3.6	1.8-6.6	
TOTAL	113					

#univariate Anova

TABLE 4.1.78 LINEAR MULTIVARIATE REGRESSION MODEL OF FACTORS  
 PREDICTING THE HEMAGLOBIN AMONG 111 MALE WORKERS.  
 3M CHEMOLITE PLANT, COTTAGE GROVE, MINNESOTA

Variable	$\beta$	SE( $\beta$ )	p-value
Intercept	14.51	.67	.0001
Total Fluorine (ppm)*	-.002	.0009	.02
Alcohol #			
low (<1oz/day)	.22	.20	.27
nonresponse (NR)	.56	.33	.09
Age (years)	.001	.009	.88
BMI (kg/m <sup>2</sup> )	.01	.02	.65
Cigarettes/day	.01	.007	.20
Cigs/day X Fluorine <sup>2**</sup>	.0003	.0001	.0005
Estradiol (pg/ml)	.01	.006	.07

R<sup>2</sup>=.23

\*square transformation of total fluoride

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

\*\* Interaction term between cigarettes per day and square transformation of total fluoride

DuPont Fluoroproducts  
1007 Market Street  
Wilmington, DE 19898



DuPont Fluoroproducts

February 21, 1995

Dr. Lance L. Simpson  
Jefferson Clinical Center In Environ. Medicine  
Room 314-Jah, Jefferson Medical College  
1020 Locust St.  
Philadelphia, PA 19107

Dear Dr. Simpson:

As we discussed over the telephone recently, we are interested in establishing a corporate policy on medical surveillance for our employees, particularly for the blood monitoring of telomeric acid fluorides, including perfluorooctanoic acid (C8). We are concerned about the potential long-term human health effects of these materials considering they all appear to have long biological half-lives. Your expertise is necessary to assist us in the design, conduct and interpretation of a monitoring program. Specifically, we are interested in a blood monitoring program design which includes relevant test batteries. The outcome of this program would be to ensure that our industrial hygiene programs are adequate to protect our employees from any potential adverse effects.

In addition to the above, we would also like you to suggest certain studies that we could conduct that would allow us to utilize our large toxicological database in developing a human risk model. We would like to discuss this program in greater depth with you, outlining in some detail the existing toxicity data that we have collected, and define more completely our issues on this bleeding monitoring program.

Please contact me at your earliest convenience in order that we can facilitate this program. Thank you for your consideration.

Sincerely,

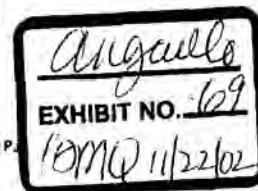
William J. Brock, Ph.D.  
Toxicology Consultant

WJB/jat

bcc: J. Walrath ✓

JW000048

EID098719



cc: W.J. Brock  
W.J. Vogler

February 10, 1997

TO: A.J. PLAYTIS - EP - WASHINGTON WORKS  
G.A. PLOEGER - EP WASHINGTON WORKS  
Y.L. POWER, M.D. - HR - WASHINGTON WORKS

*PERSONAL AND CONFIDENTIAL*

**WASHINGTON WORKS**  
**MORTALITY AND CANCER SURVEILLANCE RESULTS**

Attached is the final report on the updated mortality and cancer incidence surveillance results for Washington River Works. The final report incorporates the results of the recommendations we made to you in the draft report.

Feel free to contact Bill Brock (366-5213) or Bill Vogler (366-5448) should you have any questions.

I'll be leaving the DuPont Company effective today, February 10th. I've enjoyed working with you all and wish you the best.



JUDY WALRATH  
SENIOR EPIDEMIOLOGIST  
EPIDEMIOLOGY COMPETENCY  
HASKELL LAB, CR&D

Attachment

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**DuPont  
Epidemiology  
Surveillance  
Report**

**MORTALITY AND CANCER INCIDENCE SURVEILLANCE  
AT WASHINGTON WORKS**

Requester: Gerald A. Ploeger  
External Affairs/Litigation  
Washington Works

Judy Walrath, Ph.D.  
Senior Epidemiologist  
Haskell Laboratory  
Central Research & Development

Report Completion Date: 6/30/96

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**MORTALITY AND CANCER INCIDENCE SURVEILLANCE  
AT WASHINGTON WORKS**

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**SUMMARY**

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## INTERPRETATION OF EPIDEMIOLOGIC SURVEILLANCE DATA

Enclosed are tables for Washington Works showing (1) cancer incidence surveillance from 1956 through 1992 among active employees and (2) mortality surveillance from 1957 through 1993 among active employees and pensioners. For each specific cause, the number of observed cases/deaths (OBS) is compared to the number expected (EXP) based on the experience of the entire Company. Comparison is made by the ratio of the observed to the expected numbers of cases/deaths (OBS/EXP). Accompanying each table is descriptive text which summarizes the major findings.

Sources of Surveillance Data

Since 1956, cancer cases that occur among active employees in the U.S. are recorded in the DuPont Cancer Registry. Through 1988, cases have been reported to the Registry primarily by diagnoses entered on Accident and Health Insurance (A&H) claims and by death certificates that accompany life insurance claims filed by beneficiaries of deceased employees. Since 1988, insurance claims data are being used to ascertain diagnoses of cancer among active employees. Beginning in 1977, registry data sources were supplemented by Cancer Registry Report forms submitted by Company physicians. The Cancer Registry does not include cases diagnosed among employees whose cancer was first diagnosed after retirement or after employment termination due to reasons other than pension.

Deaths that occur among active and pensioned employees in the U.S. are recorded in the DuPont Mortality Registry that was initiated in 1957. Deaths are identified through life insurance claims filed by beneficiaries of deceased employees. Deaths that occur among employees terminated without pension are not included since there is no uniform mechanism for identification of these deaths.

Methods

To determine expected numbers of cases/deaths for the standardized analysis, cancer incidence and mortality rates for DuPont employees (and pensioners for mortality), specific for 5-year age categories, gender and payroll class (i.e., wage or salary roll), are computed for each cause category shown in the enclosed tables. Then, the Company-wide incidence/mortality rates are multiplied by the cumulative mid-year population of employees (and pensioners, where applicable) from each location, specific for age, gender and payroll class, over the entire study period. The sum of the products over all age groups is the expected number of cases or deaths.

Standardized analyses are preferred because they provide age-adjusted expected numbers and are based on actual plant populations. In isolated cases where accurate population data are not available, proportionate incidence or mortality analyses are presented. In these analyses, the observed distribution of cases/deaths by cause is compared with that expected derived from proportions which occur throughout the entire Company. Proportions can be misleading, however, as it is possible to have an unusual

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distribution of cases/deaths without there being any excess rate for any specific cause. For example, if a plant has a lower death rate from heart disease than the rate in the Company as a whole, the proportion of deaths from other causes would be inflated (compared to that of the entire Company) in order that proportions for all causes add up to 100 percent.

**Tests of Statistical Significance**

To test whether the observed to expected ratios (OBS/EXP) given in the tables differ significantly from 1.00, we determine the probability that the difference between the observed and expected numbers occurred by chance alone. This probability value is obtained from the Poisson probability distribution. The difference is considered statistically significant if the probability value is less than 0.10 using the two-tailed test. In the two-tailed test, statistically significant deficits as well as excesses are denoted. Statistical significance is tested only if either the observed or expected number of cases or deaths is four or more.

**Interpretation of Statistically Significant Results**

The designation of a statistically significant excess often suggests the need for further investigation to determine whether the excess may have occurred because of some agent at the plant. However, an excess may also occur because of environmental and other factors associated with increased risks, such as smoking, diet, alcohol, ethnic origin, socioeconomic status or genetic factors.

Chance alone may account for a statistically significant difference. When the level of statistical significance is set at 0.10, one should expect to find a statistically significant difference in about 10 out of every 100 comparisons due to chance alone, even when no specific causative factor is responsible.

The magnitude of the difference, expressed as the ratio of observed to expected numbers (OBS/EXP), must also be considered in data interpretation. The OBS/EXP ratio and its corresponding probability value should be considered together in assessment of the difference between an observed and expected number.

It may be that the observed number for a particular cause is greater than the expected number, but the difference is not statistically significant. In this instance, it does not necessarily follow that a particular agent at the plant may not be associated with the moderate excess. If the number of persons at the plant exposed to the agent is small, excess morbidity or mortality in that group would be difficult to detect because of dilution by data from the rest of the plant. Also, it may be too soon for effects of an agent to be manifested by excess morbidity or mortality.

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## CANCER INCIDENCE SURVEILLANCE - 1956-1992

Male Employees (Table 1)

Among male employees at Washington Works from 1956-1992, 149 cancer cases are reported and 132 expected. The total observed/expected case ratio is 1.25 for male salary and 1.07 for male wage employees.

Buccal cavity and pharynx

Cancers of the buccal, or oral, cavity and pharynx are statistically significantly elevated among male wage (9 cases and 3.1 expected) and all male employees (10 cases versus 4.6 expected). One case has been reported since the last surveillance update through 1989 and 0.3 would have been expected. Anatomic sites are lip (4 cases), other parts of mouth (1 case), oropharynx (1 case), pharynx (2 cases) tongue (1 case) and salivary gland (1 case).

Primary risk factors for oral cancers are use of smokeless tobacco, alcohol usage and tobacco smoking. Although several occupational groups have been reported to have an excess of this cancer (e.g. those employed as metal, textile or steel workers and plumbers and asbestos workers), occupational exposures are not suspected to play a major role in the etiology of this disease. An exception is that persons employed in occupations with prolonged exposure to sunlight have a greater risk of lip cancer.

Recommendations (and Results):

**Determine smoking histories, with particular attention to information on use of smokeless tobacco, if available.**

'Out of 10 cases, 7 smoked cigarettes for a number of years before diagnosis, 2 were nonsmokers and 1 case could not be determined. Information on the use of smokeless tobacco was not available.'

**Consider use of educational materials: on the adverse health effects from use of smokeless tobacco and importance of regular dental hygiene visits in screening oral cancers.**

'As part of our Wellness program, it is our current practice to distribute American Cancer Society literature on smokeless tobacco usage to anyone who is at risk according to their wellness appraisal. The site has also run several clinics for people who are attempting to quit smoking. The policy of only allowing smoking outdoors has also focused attention on its dangers.'

**Add work history of one additional case to previous work history review of 9 cases.**

'The 10 cases show no commonality of exposure. Also, for 2 cases the date of diagnosis is less than one year after they were hired, and for 2 others the diagnosis was within 5 years of hiring. This would make it unlikely that exposure at the site was the cause of the disease. Of the remaining 6 cases, 4 were cigarette smokers and 2 were nonsmokers.'

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Kidney and other urinary cancers

The category of kidney cancer and other urinary cancers is no longer statistically significant in male employees. No kidney cancer cases have been reported since 1989. At that time, examination of work histories of the 9 cases identified no commonality that would suggest a workplace causality.

Multiple myeloma

A three fold excess of multiple myeloma is seen among male employees (5 cases are observed versus 1.6 expected). An additional case was reported in 1990, which results in statistical significance. Among female employees, 2 cases were observed and less than .1 expected. Statistical significance, however, is only tested on four or more cases.

Suspected risk factors for multiple myeloma are familial history, exposure to ionizing radiation and increased age. Occupational groups that have been reported to have an increased risk of mortality from multiple myeloma are farmers, copper smelter workers exposed to arsenic, rubber workers, petroleum refinery workers and workers exposed to asbestos, plastics, cutting oil, wood dust and leather.

Recommendation (and Result)

**Work histories of all 7 cases were examined in late 1992, shortly after the last surveillance results on cancer incidence were issued. No unusual patterns were observed which would be indicative of a common workplace exposure. This work history review should be expanded and reassessed after inclusion of the one additional case.**

' The additional case does not change the previous conclusion of no commonality of exposure.'

Other lymphoma

A statistically significant excess of 'other lymphoma' continues among all male employees (8 cases versus 3.8 expected). One additional case has been reported since 1989 whereas 0.8 would have been expected. Seven cases are reported to be malignant lymphoma, not otherwise specified, and one is nodular lymphoma.

Occupational risk factors are difficult to determine from the literature since most studies report only on mortality and in mortality studies, deaths from all lymphatic cancers and leukemia are generally grouped together. Mortality from lymphomas has been reported to be elevated among chemists, rubber workers, petroleum refinery workers, and less consistently in several other occupational groups.

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**Recommendation (and Result)**

**Work histories of the 7 cases reported through 1989 had been examined and no job or work area clusters were noted. The work history of the one additional case should be determined and compared with those of the previously examined 7 work histories.**

'The additional case does not change the previous conclusion of no commonality of exposure. Also, one case was diagnosed less than half a year after to transfer to the site.'

**Leukemia**

The elevation in leukemia previously reported among male wage and all male employees is no longer statistically significant (9 cases are reported and 5.1 would be expected). A 1991 case-control study of leukemia at Washington Works found no association between work in any area at the plant and development of leukemia. We will continue to monitor for any additional cases.

**Malignant melanoma**

Incidence of malignant melanoma is elevated among men in the salary roll, with 8 cases reported and 3.8 expected. This finding was not present in the most recent surveillance report through 1989. Since then, 1 case has been reported and .55 would have been expected. Incidence is somewhat lower than expected in the wage roll, with 5 cases observed and 7.3 expected. This pattern follows reports in the general population, where non-manual workers are at greater risk than manual workers of developing malignant melanoma. The effect, however, does not appear to be related to occupational factors.

In the general population, the incidence of malignant melanoma has been rising rapidly. The major risk factor is believed to be excessive sunlight exposure, particularly in the first 20 years of life. Although an excess of malignant melanoma has been reported in several occupational epidemiology studies, no consistent patterns have been seen and no chemical etiologic agent is suspected.

**Recommendations (and Results)**

**Since malignant melanoma is the most deadly form of skin cancer, early diagnosis is a crucial factor for a favorable prognosis. We recommend distribution of literature available through the local branch of your American Cancer Society which describes characteristics of moles which may indicate the presence of malignant melanoma and the need for urgent professional medical consultation.**

'Our Wellness Consultant has prepared an employee training package on the dangers of skin cancer which will be used in the near future.'

**As a precautionary measure, we recommend examination of the work histories of the 8 salary roll cases to ensure that no jobs or work areas are more in common than would be expected.**

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**'These 8 cases do not appear to have any commonality of exposure. One individual worked for 5 years at Savannah River and another worked for 10 years at Sabine River. Both locations provide ample opportunity for exposure to strong sunlight. Two other cases were diagnosed 4-6 years after transfer to the site, which is shorter than the 10-20 year latency period typical for melanoma.'**

**Female Employees (Table 2)**

There are no statistically significant excesses or deficits in the cancer experience of female employees at Washington Works during 1956-1989. Overall, 20 cancer cases were reported and 14.9 expected; 7 of these cases were breast cancer, compared with 5.2 expected. In the wage roll, 10 cases were observed versus 5.8 expected, and in the salary roll, 10 cases versus 9.0 expected.

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TABLE 1 - Observed/Expected Cancer Incidence Results for Male Employees

OBSERVED AND EXPECTED CANCER INCIDENCE USING POPULATION BASED RATES - 07FEB96  
 WASHINGTON WORKS - (LOCATION CODE = 013)  
 1956 - 1992, MALES

TYPE OF CANCER	SALARY ROLL			WAGE ROLL			WAGE + SALARY		
	OBSERVED	EXPECTED	OBS/EXP	OBSERVED	EXPECTED	OBS/EXP	OBSERVED	EXPECTED	OBS/EXP
BUCCAL CAVITY & PHARYNX	1	1.57	0.63	9	3.07	2.93 *	10	4.64	2.15 *
ESOPHAGUS	0	0.54	0.00	0	1.37	0.00	0	1.92	0.00
STOMACH	0	0.87	0.00	0	1.71	0.00	0	2.57	0.00
SMALL AND LARGE INTESTINE	9	5.86	1.54	8	8.49	0.94	17	14.34	1.19
RECTUM	3	2.03	1.48	3	3.70	0.81	6	5.73	1.05
LIVER AND BILIARY PASSAGES	0	0.57	0.00	2	1.04	1.93	2	1.61	1.24
PANCREAS	1	1.26	0.79	2	2.29	0.88	3	3.55	0.85
PERITONEUM	0	0.12	0.00	1	0.17	5.72	1	0.29	3.42
UNSPEC. DIGESTIVE ORGANS	0	0.03	0.00	0	0.01	0.00	0	0.03	0.00
NOSE, NASAL CAVITIES, ETC.	1	0.15	6.76	0	0.63	0.00	1	0.78	1.29
LARYNX	1	0.75	1.34	3	2.34	1.28	4	3.08	1.30
LUNG, BRONCHUS, & TRACHEA	8	7.86	1.02	13	17.70	0.73	21	25.57	0.82
MEDIASTINUM & UNSPEC.	0	0.12	0.00	0	0.17	0.00	0	0.29	0.00
PLEURA	0	0.14	0.00	2	0.42	4.79	2	0.56	3.58
BREAST	0	0.11	0.00	0	0.10	0.00	0	0.21	0.00
PROSTATE	2	3.25	0.62	3	5.58	0.54	5	8.83	0.57
TESTIS	1	1.17	0.86	2	2.12	0.94	3	3.28	0.91
OTHER MALE GENITAL ORGANS	0	0.02	0.00	0	0.23	0.00	0	0.25	0.00
KIDNEY & OTHER URINARY	5	2.17	2.30	4	2.78	1.44	9	4.96	1.82
BLADDER	1	2.24	0.45	9	5.06	1.78	10	7.31	1.37
MALIGNANT MELANOMA	8	3.75	2.14 *	5	7.34	0.68	13	11.09	1.17
EYE	0	0.12	0.00	0	0.23	0.00	0	0.35	0.00
BRAIN & OTHER NERVOUS SYSTEM	1	1.60	0.62	3	3.29	0.91	4	4.90	0.82
THYROID	0	0.57	0.00	1	1.52	0.66	1	2.09	0.48
OTHER ENDOCRINE GLANDS	1	0.22	4.60	2	0.26	7.67	3	0.48	6.28
BONE	0	0.21	0.00	1	0.44	2.26	1	0.66	1.52
CONNECTIVE TISSUE	0	0.76	0.00	2	1.29	1.55	2	2.05	0.98
LYMPHOSARCOMA & RETICULOSARCOMA	2	1.33	1.50	0	1.40	0.00	2	2.74	0.73
HODGKIN'S DISEASE	2	0.99	2.01	2	2.40	0.83	4	3.39	1.18
OTHER LYMPHOMA	2	1.01	1.98	6	2.77	2.17	8	3.78	2.12 *
MULTIPLE MYELOMA	2	0.51	3.91	3	1.10	2.73	5	1.61	3.11 *
LEUKEMIA	3	1.68	1.79	6	3.38	1.77	9	5.06	1.78
MYCOSIS FUNGOIDES	0	0.12	0.00	0	0.00	-	0	0.12	0.00
OTHER HEMATOPOIETIC SYSTEM	1	0.18	5.57	0	0.23	0.00	1	0.40	2.47
OTHER & UNKNOWN	1	1.03	0.97	1	2.46	0.41	2	3.49	0.57
TOTAL ALL CAUSES	56	44.91	1.25	93	87.09	1.07	149	132.00	1.13

STAT SIGNIFICANT EXCESS (\*) / DEFICIT (#) AT 2-TAILED 0.10 LEVEL (CALCULATED ONLY WHERE EITHER OBS OR EXP GE 4)

NOTE: OTHER SKIN CANCER NOT INCLUDED IN THIS REPORT

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**TABLE 2 - Observed/Expected Cancer Incidence Results for Female Employees**

OBSERVED AND EXPECTED CANCER INCIDENCE USING POPULATION BASED RATES - 07FEB96  
 WASHINGTON WORKS - (LOCATION CODE = 013)  
 1956 - 1992, FEMALES

TYPE OF CANCER	SALARY ROLL			WAGE ROLL			WAGE + SALARY		
	OBSERVED	EXPECTED	OBS/EXP	OBSERVED	EXPECTED	OBS/EXP	OBSERVED	EXPECTED	OBS/EXP
BUCCAL CAVITY & PHARYNX	0	0.06	0.00	0	0.17	0.00	0	0.23	0.00
ESOPHAGUS	0	0.00	0.00	0	0.00	-	0	0.00	0.00
STOMACH	0	0.09	0.00	0	0.00	-	0	0.09	0.00
SMALL AND LARGE INTESTINE	0	0.42	0.00	0	0.20	0.00	0	0.62	0.00
RECTUM	0	0.13	0.00	0	0.14	0.00	0	0.27	0.00
LIVER AND BILIARY PASSAGES	0	0.02	0.00	0	0.12	0.00	0	0.14	0.00
PANCREAS	0	0.05	0.00	0	0.02	0.00	0	0.07	0.00
PERITONEUM	0	0.00	-	0	0.00	0.00	0	0.00	0.00
NOSE, NASAL CAVITIES, ETC.	0	0.01	0.00	0	0.00	-	0	0.01	0.00
LARYNX	0	0.04	0.00	0	0.01	0.00	0	0.05	0.00
LUNG, BRONCHUS, & TRACHEA	0	0.49	0.00	1	0.14	7.01	1	0.63	1.59
BREAST	4	3.23	1.24	3	1.99	1.51	7	5.22	1.34
CERVIX	0	1.53	0.00	4	1.53	2.62	4	3.06	1.31
OTHER FEMALE GENITAL ORGANS	0	1.03	0.00	0	0.46	0.00	0	1.49	0.00
KIDNEY & OTHER URINARY	0	0.03	0.00	0	0.17	0.00	0	0.19	0.00
BLADDER	0	0.07	0.00	0	0.02	0.00	0	0.09	0.00
MALIGNANT MELANOMA	2	0.44	4.59	1	0.22	4.48	3	0.66	4.55
EYE	0	0.01	0.00	0	0.00	-	0	0.01	0.00
BRAIN & OTHER NERVOUS SYSTEM	0	0.13	0.00	0	0.00	0.00	0	0.13	0.00
THYROID	1	0.31	3.23	0	0.06	0.00	1	0.37	2.74
OTHER ENDOCRINE GLANDS	0	0.00	-	0	0.06	0.00	0	0.06	0.00
BONE	0	0.08	0.00	0	0.00	-	0	0.08	0.00
CONNECTIVE TISSUE	0	0.09	0.00	1	0.07	13.39	1	0.16	6.22
LYMPHOSARCOMA & RETICULOSARCOMA	0	0.14	0.00	0	0.06	0.00	0	0.20	0.00
HODGKIN'S DISEASE	0	0.19	0.00	0	0.03	0.00	0	0.21	0.00
OTHER LYMPHOMA	0	0.14	0.00	0	0.19	0.00	0	0.32	0.00
MULTIPLE MYELOMA	2	0.09	22.81	0	0.00	-	2	0.09	22.81
LEUKEMIA	1	0.14	7.22	0	0.09	0.00	1	0.23	4.32
MYCOSIS FUNGOIDES	0	0.00	-	0	0.00	-	0	0.00	-
OTHER & UNKNOWN	0	0.10	0.00	0	0.06	0.00	0	0.17	0.00
TOTAL ALL CAUSES	10	9.03	1.11	10	5.82	1.72	20	14.85	1.35

STAT SIGNIFICANT EXCESS (\*) / DEFICIT (#) AT 2-TAILED 0.10 LEVEL (CALCULATED ONLY WHERE EITHER OBS OR EXP GE 4)

NOTE: OTHER SKIN CANCER NOT INCLUDED IN THIS REPORT

RL002156

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## CONFIDENTIAL

## MORTALITY SURVEILLANCE - 1957-1993

Male Employees and Pensioners (Table 3)

The overall mortality experience of male wage and all male employees at Washington Works continues to be statistically significantly lower than expected. For the time period 1957-1993, a total of 444 deaths are observed among all male employees and pensioners at the site and 494 would have been expected based on DuPont mortality rates (Table 3).

All malignant neoplasms

A statistically significant deficit of deaths is seen for all cancers combined among male wage (72 deaths versus 88.6 expected) and all male employees and pensioners (115 deaths versus 141.3 expected). None of the individual cancer cause of death categories show a statistically significant deficit in mortality.

Mortality from lymphatic and hematopoietic neoplasms, a category which is elevated in the incidence surveillance results, is close to expected.

Other heart disease

Mortality from "other heart disease" is elevated, with 17 deaths reported and 10.7 expected. This category has shown an excess in previous reports, but the ratio of observed to expected deaths has not been statistically significant until now. This residual grouping comprises non-specific 'heart failure' and other ill-defined descriptions and complications of heart disease; i.e. acute and chronic pulmonary disease, acute pericarditis, acute and subacute endocarditis, acute myocarditis, other disease of pericardium and endocardium, cardiomyopathy, conduction disorders and cardiac dysrhythmias. No occupational risk factors are suspected.

Arteriosclerosis

There is a statistically significant excess of deaths from arteriosclerosis among all male employees (4 deaths versus 1.3 expected). Arteriosclerosis, a condition characterized by thickening and loss of elasticity of arterial walls, has no known occupational risk factors. Years of death are 1977, 1978, 1992 and 1993, and ages at death are 50 and 78 (three).

Motor vehicle accidents

Motor vehicle accidental deaths were higher than expected in the male salary roll, with 8 deaths observed and 3.7 expected. Four of these deaths were recent, 2 in 1990 and 2 in 1993. There is a marked deficit of deaths from "other accidents" in the male wage roll.

Suicide

There is a marked deficit of suicide among male wage and all male employees.

**CONFIDENTIAL****Female Employees and Pensioners (Table 4)**

**Among female employees and pensioners, 16 deaths are observed and 11.9 expected.  
No unusual patterns are seen with respect to individual cause of death categories.**

RL002158



TABLE 3 - Observed/Expected Mortality Results for Male Employees

CAUSE OF DEATH	OBSERVED AND EXPECTED DEATHS USING POPULATION-BASED RATES - 14FEB96 WASHINGTON WORKS - (LOCATION CODE = 013)								
	SALARY ROLL			1957 - 1993, MALES WAGE ROLL			WAGE + SALARY		
	OBSERVED	EXPECTED	OBS/EXP	OBSERVED	EXPECTED	OBS/EXP	OBSERVED	EXPECTED	OBS/EXP
<b>MALIGNANT NEOPLASMS</b>									
BUCCAL CAVITY & PHARYNX	1	0.88	1.13	3	1.79	1.67	4	2.68	1.49
DIGESTIVE ORGANS	10	14.05	0.71	16	21.57	0.74	26	35.62	0.73
RESPIRATORY SYSTEM	16	16.76	0.95	24	32.92	0.73	40	49.68	0.81
BREAST	0	0.10	0.00	0	0.09	0.00	0	0.19	0.00
GENITAL ORGANS	4	4.24	0.94	3	6.50	0.46	7	10.74	0.65
URINARY ORGANS	1	3.07	0.33	5	4.21	1.19	6	7.28	0.82
LYMPHATIC, ETC.	5	6.14	0.81	10	9.59	1.04	15	15.73	0.95
OTHER & UNSPECIFIED	6	7.47	0.80	11	11.94	0.92	17	19.40	0.88
TOTAL MALIGNANT NEOPLASMS	43	52.70	0.82	72	88.62	0.81 #	115	141.32	0.81 #
<b>CEREBROVASCULAR DISEASE</b>	6	8.08	0.74	10	15.57	0.64	16	23.65	0.68
<b>DISEASES OF THE HEART</b>									
CHRONIC RHEUMATIC HEART DISEASE	2	0.73	2.74	2	0.80	2.50	4	1.53	2.61
ARTERIOSCLEROTIC HEART DISEASE	62	55.29	1.12	91	98.91	0.92	153	154.20	0.99
CHRONIC ENDOCARDITIS	1	0.32	3.15	0	0.60	0.00	1	0.91	1.09
HYPERTENSIVE HEART DISEASE	0	1.46	0.00	1	1.97	0.51	1	3.43	0.29
OTHER HEART DISEASE	17	10.67	1.59 *	20	19.38	1.03	37	30.06	1.23
<b>OTHER CARDIOVASCULAR DISEASE</b>									
RHEUMATIC FEVER	0	0.02	0.00	0	0.02	0.00	0	0.04	0.00
HYPERTENSION WITHOUT MENTION OF HEART	2	0.52	3.84	1	0.74	1.35	3	1.26	2.38
GENERALIZED ARTERIOSCLEROSIS	2	0.49	4.07	2	0.79	2.52	4	1.28	3.12 #
OTHER	7	3.74	1.87	5	5.65	0.88	12	9.39	1.28
<b>EXTERNAL CAUSES OF DEATH</b>									
MOTOR VEHICLE ACCIDENTS	8	3.74	2.14 *	10	15.58	0.64	18	19.32	0.93
SUICIDE	1	3.68	0.27	4	9.29	0.43 #	5	12.97	0.39 #
HOMICIDE	0	0.48	0.00	1	2.78	0.36	1	3.25	0.31
OTHER ACCIDENTS	4	3.69	1.08	4	10.59	0.38 #	8	14.28	0.56
<b>OTHER CAUSES</b>									
INFLUENZA	0	0.04	0.00	0	0.11	0.00	0	0.15	0.00
PNEUMONIA	5	2.72	1.84	4	5.53	0.72	9	8.24	1.09
NEPHRITIS & NEPHROSIS	0	0.96	0.00	1	2.15	0.47	1	3.10	0.32
TUBERCULOSIS OF RESPIRATORY SYSTEM	0	0.05	0.00	0	0.22	0.00	0	0.27	0.00
DIABETES MELLITUS	1	1.34	0.75	4	3.53	1.13	5	4.87	1.03
PEPTIC ULCER	0	0.46	0.00	0	0.71	0.00	0	1.17	0.00
APPENDICITIS	0	0.06	0.00	0	0.03	0.00	0	0.09	0.00
HERNIA & INTESTINAL OBSTRUCTION	0	0.28	0.00	1	0.36	2.81	1	0.64	1.57
CIRRHOSIS OF THE LIVER	3	2.07	1.45	2	3.73	0.54	5	5.80	0.86
EMPHYSEMA	1	1.26	0.79	4	2.56	1.56	5	3.83	1.31
SYMPTOMS & ILL-DEFINED CONDITIONS	1	1.66	0.60	2	3.27	0.61	3	4.93	0.61
RESIDUAL	13	15.75	0.83	24	26.84	0.89	37	42.60	0.87
UNSPECIFIED	0	0.09	0.00	0	0.89	0.00	0	0.98	0.00
<b>TOTAL ALL CAUSES</b>	<b>179</b>	<b>172.34</b>	<b>1.04</b>	<b>265</b>	<b>321.21</b>	<b>0.83 #</b>	<b>444</b>	<b>493.55</b>	<b>0.90 #</b>

STAT SIG EXCESS (\*) / DEFICIT (#) AT 2-TAILED 0.10 LEVEL (CALCULATED ONLY WHERE EITHER OBS OR EXP GE 4)

6S1Z00TR

TABLE 4 - Observed/Expected Mortality Results for Female Employees

CAUSE OF DEATH	OBSERVED AND EXPECTED DEATHS USING POPULATION-BASED RATES - 14FEB96 WASHINGTON WORKS - (LOCATION CODE = 013) 1957 - 1993, FEMALES								
	SALARY ROLL			WAGE ROLL			WAGE + SALARY		
	OBSERVED	EXPECTED	OBS/EXP	OBSERVED	EXPECTED	OBS/EXP	OBSERVED	EXPECTED	OBS/EXP
<b>MALIGNANT NEOPLASMS</b>									
BUCCAL CAVITY & PHARYNX	0	0.04	0.00	0	0.00	0.00	0	0.04	0.00
DIGESTIVE ORGANS	0	0.52	0.00	0	0.27	0.00	0	0.79	0.00
RESPIRATORY SYSTEM	0	0.56	0.00	0	0.16	0.00	0	0.72	0.00
BREAST	0	1.14	0.00	1	0.87	1.14	1	2.02	0.50
GENITAL ORGANS	0	0.48	0.00	0	0.09	0.00	0	0.57	0.00
URINARY ORGANS	0	0.07	0.00	0	0.18	0.00	0	0.25	0.00
LYMPHATIC, ETC.	1	0.47	2.14	0	0.23	0.00	1	0.70	1.44
OTHER & UNSPECIFIED	1	0.49	2.04	1	0.15	6.58	2	0.64	3.11
TOTAL MALIGNANT NEOPLASMS	2	3.77	0.53	2	1.96	1.02	4	5.74	0.70
<b>CEREBROVASCULAR DISEASE</b>	0	0.47	0.00	1	0.29	3.47	1	0.76	1.32
<b>DISEASES OF THE HEART</b>									
CHRONIC RHEUMATIC HEART DISEASE	0	0.10	0.00	0	0.00	-	0	0.10	0.00
ARTERIOSCLEROTIC HEART DISEASE	2	0.82	2.44	0	0.35	0.00	2	1.17	1.71
CHRONIC ENDOCARDITIS	0	0.01	0.00	0	0.00	-	0	0.01	0.00
HYPERTENSIVE HEART DISEASE	0	0.04	0.00	0	0.00	0.00	0	0.05	0.00
OTHER HEART DISEASE	1	0.27	3.66	0	0.20	0.00	1	0.47	2.12
<b>OTHER CARDIOVASCULAR DISEASE</b>									
RHEUMATIC FEVER	0	0.00	-	0	0.00	-	0	0.00	-
HYPERTENSION WITHOUT MENTION OF HEART	0	0.02	0.00	0	0.00	-	0	0.02	0.00
GENERALIZED ARTERIOSCLEROSIS	0	0.00	-	0	0.00	0.00	0	0.00	0.00
OTHER	0	0.10	0.00	0	0.03	0.00	0	0.13	0.00
<b>EXTERNAL CAUSES OF DEATH</b>									
MOTOR VEHICLE ACCIDENTS	1	0.59	1.69	1	0.38	2.64	2	0.97	2.06
SUICIDE	0	0.25	0.00	0	0.15	0.00	0	0.40	0.00
HOMICIDE	0	0.15	0.00	0	0.23	0.00	0	0.38	0.00
OTHER ACCIDENTS	0	0.13	0.00	0	0.05	0.00	0	0.18	0.00
<b>OTHER CAUSES</b>									
INFLUENZA	0	0.01	0.00	0	0.00	-	0	0.01	0.00
PNEUMONIA	0	0.11	0.00	0	0.01	0.00	0	0.12	0.00
NEPHRITIS & NEPHROSIS	0	0.01	0.00	0	0.03	0.00	0	0.03	0.00
TUBERCULOSIS OF RESPIRATORY SYSTEM	0	0.00	-	0	0.00	-	0	0.00	-
DIABETES MELLITUS	1	0.05	19.50	0	0.01	0.00	1	0.06	17.29
PEPTIC ULCER	0	0.00	-	0	0.00	0.00	0	0.00	0.00
APPENDICITIS	0	0.00	-	0	0.00	-	0	0.00	-
HERNIA & INTESTINAL OBSTRUCTION	0	0.00	-	0	0.00	-	0	0.00	-
CIRRHOSIS OF THE LIVER	0	0.06	0.00	0	0.01	0.00	0	0.07	0.00
EMPHYSEMA	0	0.05	0.00	1	0.00	-	1	0.05	18.87
SYMPTOMS & ILL-DEFINED CONDITIONS	1	0.11	8.98	0	0.03	0.00	1	0.15	6.89
RESIDUAL	2	0.79	2.54	1	0.17	6.05	3	0.95	3.15
UNSPECIFIED	0	0.01	0.00	0	0.06	0.00	0	0.06	0.00
<b>TOTAL ALL CAUSES</b>	<b>10</b>	<b>7.92</b>	<b>1.26</b>	<b>6</b>	<b>3.94</b>	<b>1.52</b>	<b>16</b>	<b>11.86</b>	<b>1.35</b>

RT002160

**CONFIDENTIAL****SUMMARY**

The first draft of this document contained recommendations for consideration by your site to follow-up on positive surveillance findings. The final surveillance report includes results of recommendations and follow-up actions taken by the site.

Results of these surveillance data were communicated to employees at the plant site.

RL002161



Richard E. Purdy  
12/03/98 11:53 AM

To: Georjean L. Adams/US-Corporate/3M/US@3M-Corporate  
cc:  
Subject: Risk to the environment due to the presence of PFOS

In the attached analysis of possible risk of ecological harm due to environmental levels of PFOS, I concluded there is a significant risk of harm. In addition to this, my review of the available data on the properties of PFOS, indicates that the sink for it in the environment is biota. We know of nothing that it has more affinity for than blood serum and liver components. Other persistent pollutants have a high affinity for sediment, but PFOS does not. So, PFOS can't be trapped and buried like they can. It likely continues to partition into biota from the other compartments in the environment; thus the levels we are seeing in eagles and other biota is likely to climb each year.

I believe all this taken together constitutes a significant risk that should be reported to EPA under TSCA 8e.



Pioneer Food Chain Risk Assessment of PFO

**Exhibit  
1533**

State of Minnesota v. 3M Co.,  
Court File No. 27-CV-10-28862

# **Pioneer Risk Assessment of Adverse Effects in Marine Mammals from PFOS in the Food Chain**

**Rich Purdy  
3 December 1998**

## **Introduction:**

PFOS (perfluorooctanesulfonate) was found in two of three populations of naïve rats--that is, rats not knowingly exposed to PFOS. The rats that had PFOS in their blood were fed food that contained fishmeal. Incomplete studies indicate that PFOS is in fishmeal. This is consistent with the observation that fledgling eagles that eat predominately fish contain PFOS in their blood.

Most fishmeal is made from menhaden (*Brevoortia tyrannus*)(Draft Report: Sources of Fish Food Constituents By John Giesy and Paul Mehrle, September 6, 1998). Menhaden eat plankton and are considered a second link in the US Atlantic coastal food chain. They are in turn eaten by other species of fish, which are in turn eaten by other fish, mammals and birds. The mass of menhaden is so great that their catch represents 40% of the US commercial finfish fisheries.

Since these fish appear to contain PFOS, it seemed prudent to calculate the amount of PFOS that might be transferred up the food chain and compare this value to a concentration that causes adverse effects. These calculations do not contain precise data so the evaluation is approximate. The purpose is to see whether predicted environmental concentrations are anywhere near concentrations that cause effects.

This assessment was organized and performed in accordance with the guidance given by EPA in Guidelines for Ecological Risk Assessment (EPA/630/R-95/002F April 1998). The process presented in these guidelines is a repetitive one where a risk assessment is updated or redone when new information is available. The new information can be generated by other processes or generation can be driven by the risk assessment.

## **Problem Formulation:**

A study of rats not purposely exposed to PFOS found that they had significant levels of it in their livers. The likely source was the fishmeal in their diet. If PFOS is in fish then other fish, fish eating mammals and birds are consuming PFOS. The concentrations found are not likely to cause toxic responses, but what about after biomagnification through a food chain?

The hypothesis to test: The concentration of PFOS in food of marine animals causes adverse effects.

It was decided to keep the problem narrow in this assessment in order to simplify the analysis. This is not to imply that this is the most important or only problem. In addition this is a pioneer and possibly first iteration of this assessment. Refinements in the analysis and more data will allow other iterations if this pioneer assessment indicates there may be a significant risk. The purpose of this pioneer assessment is to determine the magnitude of the risk and whether other iterations are warranted.

## Analysis

### Characterization of Exposure:

The task of this characterization is to estimate the concentration of PFOS in the food chain organisms that eat menhaden and the concentration in the animals comprising the next two steps in the food chain. Two examples of the many food chains possible are:

Menhaden -> cod -> seal -> killer whale

Menhaden -> carnivores fish 1 -> carnivores fish 2 -> seal

For the purposes of this analysis the biomagnification by fish and mammals is about the same. This is supported by the metabolic rates, daily consumption and growth rates published (Fugacity-Based Model of PCB Bioaccumulation in Complex Aquatic Food Webs, Jan Campfens and Donald Mackay in Environ. Sci. Technol. 31, 577-583(1997) and Wildlife Exposure Factors Handbook, EPA/600/R-93/187 December 1993). Based on the data in these references and recollection from other readings, an average biomagnification value is 9.

In an evaluation of PFOS in the livers of naïve rats an average level found in male and female 10-14 week rats was 0.09 mg/kg. The food label listed fishmeal as the fifth ingredient. According to labeling standards, fishmeal was less than 20% of the food. For this analysis it is assumed that fishmeal made up 16% of the food and that it was the only source of PFOS. This value could be too high which would mean the ultimate calculated tissue levels would be low, but the value cannot be too low by much because the most it could be is 19%. If an animal were to eat 100% fish, as many fish and sea mammals do, it should have about 6 times as much in its livers as these rats do. That level would be 0.56mg/kg (0.09mg/kg/0.16).

This is the estimated level in the liver, which is the organ that contains most of the PFOS. The blood contains the next highest level. Work with rats has shown that blood contains about 1/6 the amount of PFOS to be found in the liver. Assuming that the liver mass is 3% and blood is 8% the mass of an animal and that the concentration in other organs is insignificant in comparison to the levels in liver and blood, then the total body burden of PFOS in the first step of the food chain above menhaden is 0.062mg/kg (0.56mg/kg X 0.03 + (0.56mg/kg /6) X 0.08)

The level in the second food chain link then would be nine times this level or 0.56 mg/kg.

Using data on seals, the accumulated dose at time of whelping is calculated. It is assumed that 100% of PFOS consumed is retained. Data for killer whale food consumption sexual maturation was not immediately available, but they are assumed to be similar.

The mean time for sexual maturity for female harbor seals is 5.5 years and gestation is 11 months (Wildlife Exposure Factors Handbook, EPA/600/R-93/187 December 1993). So for up to 77 months before whelping a seal was assumed to be eating fish with the above calculated concentration of 0.56 mg/kg PFOS. Seals eat 6-8% of their weight per day in fish. They eat 13% their first year and 10% when gestating. For the ease of calculation it was assumed that an 80kg seal eats 8% or 6.4kg/day. This works out to 15 X 10<sup>3</sup> kg of fish (77months X 30days/month X 6.4kg/day). Assuming the fish a seal consumed contained 0.56mg/kg, then a seal would consume about 8.4 X 10<sup>3</sup> mg PFOS (0.56mg/kg X 15 X 10<sup>3</sup> kg). This works out to a cumulative dose before whelping of about 105mg/kg (8.4 X 10<sup>3</sup> mg/80kg)

This cumulative dose can be used for seals that are two food chain lengths above menhaden. This is probably not the norm. The average seal is probably one food chain length above menhaden. But this calculated cumulative dose probably represents that seen by populations of killer whales that eat seals.

### **Characterization of Ecological Effects:**

In a two generation rat study it was found that 34% of the pups born to animals dosed by gavage 1.6 mg/kg per day PFOS were born dead or died within four days of birth. For the purposes of this assessment rats are assumed to be an adequate test surrogate species for marine mammals. There is uncertainty both to whether marine mammals such as seals, sea lions, and killer whales are less sensitive or more sensitive than rats.

The lowest dose of 1.6mg/kg that caused an adverse effect was given for 6 weeks before mating, the week of mating and the 22 days of gestation for a total of 71 days. The cumulative dose up to the time of whelping is 113mg/kg.

It has been reported that natural populations of sea lions are experiencing significant reproductive dysfunction (Peter Ross, Environmental Contaminants and the Risk of Adverse Effects in Marine Mammals: An Overview, 1998 SETAC Annual Meeting). Also 70% of a ringed seal population was not reproducing. This is higher than the norm. Apparently some field biologists believe killer whale reproductions has declined, but there has been no scientific study to verify this.

### **Risk Characterization:**

The predicted cumulative dose of PFOS through two food chain links to sea mammals such as seals, sea lions, killer whales and porpoises was calculated to be about 105 mg/kg. This value is about the same as the calculated cumulative dose of 113 mg/kg PFOS that causes reproductive impairment to mammals. Thus there is a significant risk. There is not enough input data to calculate uncertainty in this risk. Such data is needed to reevaluate the degree of risk.

SCIENCE PUBLICATION STRATEGY

DEC 10 1998

Publication of scientific and technical information on the FC issue should follow a strategic plan so that key findings can be understood in the context of the published scientific literature. Under this strategy, the science needed to evaluate the safety of PFOS (i.e. the available occupational and toxicology studies) will be published -- or in press -- and thus available to be cited when the publication on serum levels in the general population is published. This will allow the serum level findings to be placed in an understandable, credible context which demonstrates that there is no medical or scientific basis to attribute any adverse health effects to 3M products. In this strategy, the analytical methodology will be published concurrently with the serum level findings.

The strategy is described as a series of steps with a timeline for each activity. The strategy begins with a brief summary of the scientific and technical studies published or publically available:

Key Studies and Reports Available

Ubel, F.A., and others, "Health status of plant workers exposed to fluorochemicals - a preliminary report," *American Industrial Hygiene Association Journal*, vol. 41, pages 584-589, 1980. (*Published study of 3M workers showing no ill health effects of occupational exposure to fluorochemicals.*)

Gilliland, F.D. and Mandel, J.S., "Mortality among employees in a PFOA production plant," *Journal of Occupational Medicine*, vol. 35, pages 950-954, 1993 (*Published study of 3M employees showing no increased mortality due to occupational exposure to PFOA.*).

Gilliland, F.D. and Mandel, J.S., "Serum perfluorooctanoic acid and hepatic enzymes, lipoproteins and cholesterol: a study of occupationally exposed men," *American Journal of Industrial Medicine*, vol. 29, pages 560-568, 1996 (*Published study of 115 3M employees showing no toxicity to the liver due to occupational exposure to PFOA.*).

Key B.D., and others, "Critical review: Fluorinated organics in the biosphere," *Environmental Science and Technology*, vol. 31, pages 2445-2454, 1997. (*PFOS is described as "important commercially as a surfactant and as a precursor of other fluorinated surfactants," as "resistant to biological attack," and as an inhibitor of "gap junction intercellular communication (GJIC) in rat liver epithelial cells cultured in vitro." The paper reports that "inhibition of GJIC has been implicated in tumor promotion during carcinogenesis, teratogenesis and reproductive dysfunction."*)

Reich, C., "Re: TSCA Section 8(e) — Perfluorooctane Sulfonate — Docket Numbers 8EHQ-1180-373; 8EHQ-1180-374; 8EHQ-0381-0394," 3M letter to Office of Toxic Substances, United States Environmental Protection Agency, May 15, 1998. (*This document, which will soon*

Exhibit  
1535

State of Minnesota v. 3M Co.,  
Court File No. 27-CV-10-28862

3MA00729193



SCIENCE PUBLICATION STRATEGY, page 2

*become publicly available through the TSCA 8e Office, reports the presence of very low (part per billion) levels of PFOS in blood sera samples for individuals with no known occupational exposure to fluorochemicals.)*

Olsen, G.W., "An epidemiologic investigation of reproductive hormones in men with occupational exposure to perfluorooctanoic acid," *Journal of Occupational and Environmental Medicine*, vol. 40, pages 614-622, 1998. (Study by 3M Medical Department showing no significant hormonal changes in 191 men occupational exposed to PFOA.).

Reich, C., "Re: TSCA 8(E) SUBSTANTIAL RISK NOTICE ON: N-Ethyl Perfluorooctyl sulfonamido ethanol and Perfluorooctane Sulfonate, Docket Numbers 8EHQ-1180-373; 8EHQ-1180-374; 8EHQ-0381-0394," 3M letter to Office of Toxic Substances, United States Environmental Protection Agency, September 14, 1998. (This document, which will become publicly available through the TSCA 8e Office, reported that PFOS, when administered to female rats at oral doses of 1.6 or 3.2 milligrams per kilogram body weight per day during pregnancy, significantly reduced pup survival. PFOS also reduced the average gain in body weight of the female rats during pregnancy, with the weight gain at the 3.2 milligrams per kilogram dose of only 87% of the control (no PFOS) rats.)

Strategy for Publication of Key Studies

1. The PFOS worker study, prepared by Dr. Jeff Mandel and others in the 3M Medical Department, is in final review before submission to an occupationally-focused medical journal. (This paper will report no adverse biological health effects from exposure to PFOS, based on medical monitoring of workers.) Comment: publication of this paper is key to demonstrating there is no medical or scientific basis to attribute any adverse health effects to exposure to PFOS.  
**Recommendations:**
  - 1) The journal should be selected on the basis of interest in the paper and ability to ensure peer review as quickly as possible.
  - 2) Target submission of the paper by December 15, 1998; acceptance for publication within three months of submission.
  - 3) With this plan, this key study could be cited as early as March 15, 1999.
2. PFOS mitochondria study, by Dr. Ken Wallace of the University of Minnesota School of Medicine in Duluth, is being prepared for submission to a peer-reviewed science journal. (Paper will demonstrate PFOS's mechanism of action on energy metabolism in a test tube (in vitro) system.) Comment: this paper will be useful for demonstrating a possible mechanism of toxicity of PFOS. However, without the toxicology studies discussed below, the findings are of limited utility for a safety assessment.

SCIENCE PUBLICATION STRATEGY, page 3

**Recommendations:**

- 1) **The journal should be selected on the basis of interest in the paper and ability to ensure peer review in a timely manner.**
- 2) **Target: submission of the paper by March 1999; acceptance for publication within six months of submission.**
- 3) **With this plan, this study could be cited by September 1999.**

3. The PFOS teratology study, conducted by the 3M Toxicology Department, has been completed. A manuscript of the results, possibly including blood level measurements, could be prepared for publication or presentation at a science conference. *(Paper will demonstrate that exposure to high doses of PFOS to pregnant animals does not cause birth defects in the offspring. The blood level measurements will allow correlations between doses administered in this study and blood levels in animals and humans.)*  
 Comment: since the study reports largely negative findings (no birth defects), it may be difficult to publish even with the blood level measurements. Consideration should be given to combining this study with the results of the 3M and published subchronic toxicity studies discussed below.

**Recommendations:**

- 1) **Dr. Chris Wilkinson, a well respected toxicologist with Jellinek, Schwartz & Connolly, Inc., in Washington, D.C., who has been briefed on the FC issue, should be hired to review the study and provide a recommendation on publication of a paper on the teratology study and blood level measurements.**
- 2) **Assuming that Dr. Wilkinson and the 3M Toxicology Department agree to submit a paper on the teratology study, Dr. Wilkinson should draft the paper, make final revisions based on 3M review and comments, and submit the paper for publication in a peer reviewed toxicology journal.**
- 3) **Target: one month for the recommendation and decision on publication. If the decision is made to proceed with publication, target is three months for completion of the draft paper, one month for 3M review and comment, one month for final revision and submission to a peer-reviewed toxicology journal and three months for acceptance.**
- 4) **With this plan, the teratology study could be cited as early as August 1999.**

4. PFOS subchronic toxicology studies, conducted by 3M or reported in the scientific literature, could be summarized and a manuscript prepared for publication. *(The paper would review what is known about the toxicity of PFOS from animal studies, prior to conduct of the current studies by 3M.)*

**Recommendations:**

- 1) **This paper should review, or at least cite, other published toxicity studies on PFOS in addition to the subchronic studies, i.e. all of the published toxicity studies discussed under "Summary of Toxicology Studies" in the "Current Summary" document.**

SCIENCE PUBLICATION STRATEGY, page 4

- 2) **Dr. Wilkinson should be hired to review the studies and draft a paper for publication.**
- 3) **Target: six months for completion of the draft paper, one month for 3M review and comment, one month for final revision and submission to a peer-reviewed toxicology journal and three months for acceptance.**
5. The analytical methods developed to allow specific detection of PFOS in serum levels with a low part per billion detection limit should be written up for publication in a peer reviewed analytical chemistry journal. *(This paper would need to contain data on PFOS levels in serum to document the utility and accuracy of the analytical method.)*  
**Recommendations:**
- 1) **Dr. Wilkinson should be asked to recommend an analytical chemist to prepare a paper for publication on the analytical methods.**
- 2) **Assuming that Dr. Wilkinson's recommendation is acceptable to the Analytical Department, the analytical chemist consultant should draft the paper with Dr. Wilkinson's assistance, make final revisions based on 3M review and comments, and submit the paper for publication in a peer reviewed analytical chemistry journal.**
- 3) **Target: one month for the recommendation and decision on the consultant. Once a decision is made on the consultant, the target is three months for completion of the draft paper, one month for 3M review and comment, one month for final revision and submission to a peer-reviewed toxicology journal and three to six months for acceptance.**
- 4) **With this plan, the analytical study could be cited as early as August 1999.**
6. Additional serum level data is needed to document blood levels of PFOS for publication of a peer reviewed science publication. *(This paper would document what is known about PFOS levels in serum and assess the safety of current exposure levels based on the worker study [paper #1 above], and the toxicology studies [papers 2-4 above] that have been completed. The paper would need to reference the analytical methods cited in paper #5).*  
**Recommendations:**
- 1) **A decisions should be made by the 3M Medical Department with the advise of the Legal Department and the Core Team as to what additional data is needed and a plan developed to generate the needed data.**
- 2) **The 3M Medical Department should supervise the collection of serum samples with analysis of PFOS levels by the Analytical Department or a contract laboratory approved by them.**
- 3) **Dr. Wilkinson should be hired to review the serum data and draft a paper for publication.**
- 4) **Target: finalize plans by January 1, 1999, three months to collect samples and analyze the data, three months for completion of the draft paper, one month for 3M review and comment, one month for final revision and submission to a peer-**

SCIENCE PUBLICATION STRATEGY, page 5

reviewed toxicology journal and three months for acceptance.

5) With this plan, the serum study could be cited as early as November 1999.

AR226-0264

**Pathology Review of Reported Tumorigenesis  
in a Two Year Study of FM-3924 in Rats**

**November 25, 1998**

**BY**

**Pathology Associates International  
6217 Centre Park Drive  
West Chester, Ohio 45069**

**Telephone (513) 779-9600  
Fax (513) 779-9603**

**G03099**

## **Pathology Review of Tumors Reported in Rats Given FM-3924**

**Introduction:** This report presents an independent assessment of tumorigenesis data obtained from a study to determine the chronic toxicity and carcinogenic potential of the fluorochemical FM-3924 in rats. The study, entitled "Two Year (Diet) Toxicity / Carcinogenicity Study of Fluorochemical FM-3924 in Rats," was sponsored by the 3M Company, St. Paul, Minnesota. Biophase procedures were conducted at Riker Laboratories, Inc., between April, 1981 and May, 1983 and were in compliance with FDA Good Laboratory Practice (GLP) guidelines. At Riker Laboratories, Leonard J. Sibinski, BA, served as the Study Director. Following in-life procedures, all major organs and tumors were processed into microslides and examined by Robert G. Geil, DVM, DACVP. Dr. Geil's findings and interpretations were incorporated into the final report for the study.

For the tumorigenesis review, the complete study report, including all relevant pathology data was forwarded to Pathology Associates International (PAI), West Chester, Ohio for examination by Richard H. Bruner, DVM, DACVP. The sponsor (3M) regarded original pathology interpretations by Dr. Geil as adequate, and examination of microscopic tissue sections was not included in the review process. Specific objectives of the review were to evaluate tumor data and to provide an opinion relative to the potential relationship of reported neoplasms with the test material based upon: 1. The incidence and morphology of observed tumors and associated proliferative and non-proliferative lesions. 2. A literature review to examine the biologic behavior and carcinogenetic potential of similar fluorochemicals, 3. Contemporary knowledge of tumor mechanistic data, especially with respect to possible epigenetic pathways, 4. Consideration of chronic toxicity, immunosuppression, hormonal modulation or ancillary biochemical interactions which may serve as modulating factors in the development of tumors in this study, and 5. Personal experience in evaluating rodent carcinogenesis bioassays.

### **Review Procedures and Findings**

**1. Reported tumorigenesis and ancillary pathologic changes for FM-3924:** Based upon reported pathology findings, the review pathologist concurred that treatment-related changes were present at both the 1-Year (interim) and 2-Year (terminal) sacrifices as well as in some unscheduled deaths. At the 1-Year interim sacrifice, treatment-related changes were generally restricted to the liver and were characterized by a dose-dependent increase in hepatocellular cytomegaly, vacuolation and necrosis along with slightly increased inflammatory cell infiltrates. Cytomegaly

and vacuolation were generally consistent with treatment-induced perturbation of liver cell metabolism resulting in hypertrophy via proliferation of peroxisomes and/or smooth endoplasmic reticulum (P-450 enzyme induction). Hepatocellular necrosis was largely attributed to chronic liver cell swelling with compromised metabolism and/or reduced blood perfusion (hypoxia).

Treatment-related microscopic findings in unscheduled deaths and animals continued until the terminal (2-Year) sacrifice included persistent hepatocellular cytomegaly and vacuolation in both sexes. Additionally, cystoid degeneration ("spongiosis hepatis") of the liver was significantly increased in high dose males. Most notably, hyperplastic nodules were increased in the liver of both sexes and hepatocellular adenomas and carcinomas were increased in females.

Based upon reported pathology data, dietary levels of 100 ppm FM-3924 for two years resulted in unequivocal hepatocellular cytomegaly, vacuolation and cystoid degeneration as well as increased hyperplastic nodules in both sexes and increased adenomas and carcinomas in high dose females. Although increased hepatocellular neoplasms were not reported in males, it is likely that some of the "hyperplastic nodules" (in both sexes) would be regarded as hepatocellular adenomas if contemporary diagnostic criteria were applied (1). It is noteworthy, also, that hepatic cytomegaly was observed in males given 10 and 30 ppm of the test material suggesting that a NOEL for hepatocytomegaly was not achieved for males assigned to this investigation

## **2. Literature review of the biologic behavior and carcinogenic potential of fluorochemicals**

The reviewer regarded the test material (N-ethylperfluorooctanesulfonamido ethanol) as a unique xenobiotic with unknown structure-activity relationships. A limited literature review was conducted to determine if the biological behavior, including tumorigenesis, of similar fluorochemicals had been reported. The literature review included a survey of rodent carcinogenesis bioassays completed by the National Toxicology Program (NTP) and select scientific journals and biological extracts. Although no carcinogenesis bioassays with "complex" fluorochemicals were discovered, several relevant reports concerning subchronic investigations were located. Additionally, several reports, including the NTP 2-year bioassay of sodium fluoride were available to provide perspectives on the biological effects of long-term fluoride exposure. Results of these studies are briefly summarized as follows:

1. Subchronic toxicity studies in rats with "complex" fluorochemicals have identified treatment-related hepatocellular hypertrophy (cytomegaly) similar to findings with FM-3924. In studies conducted by Van Rafelghem et al, a single intraperitoneal injection of perfluoro-*n*-decanoic acid (PFDA) in several rodent

species resulted in persistent hepatocellular swelling which, ultrastructurally, was characterized by peroxisomal proliferation (12).

In subchronic studies sponsored by the 3M Company, Griffith and Long administered ammonium perfluorooctanoate to rats, mice and monkeys via oral routes (3). At doses of 30 ppm or greater, rats displayed hepatocellular hypertrophy. This change was more prevalent and severe in males. Noteworthy was the observation that all mice given doses of 1000 ppm *ad libidum* and all monkeys given 100 mg/kg/day (gavage) died preterminally. Additionally, all monkeys given 30 mg/kg/day displayed anorexia, emesis, black stools, facial pallor, and prostration; and one monkey given 10 mg/kg/day displayed anorexia, black stools and facial pallor. Liver effects, however, were not observed in monkeys assigned to these studies. Furthermore, microbial assays using five *Salmonella* stains and one *Saccharomyces* strain, with and without metabolic activation, did not reveal mutagenic activity for the test material.

2. Review of studies relating to the chronic toxicity of fluoride and fluoride-containing compounds was limited to the NTP 2-year carcinogenesis bioassay of sodium fluoride and select environmental studies of fluoride in cattle. In the NTP study, treatment-related pathologic changes were not observed in the liver of rats given dietary concentrations of up to 175 ppm sodium fluoride in the drinking water for two years (11). Furthermore, in an extensive survey of cattle exposed to high environmental fluoride concentrations for lifetime periods, increased liver disease or neoplasia was not reported although many animals exhibited dental and skeletal changes typical of advanced fluorosis (9).

### **3. Mechanisms resulting in increased hepatocellular nodular hyperplasia and neoplasia in rats given FM-3924:**

Results of possible genotoxicity studies with FM-3924 were not provided to the review pathologist. It was reasoned that this complex fluorochemical probably was not strongly mutagenic and that most biologic effects were due to perturbation of liver cell metabolism, with peroxisomal proliferation and general disruption of multiple metabolic pathways. Accordingly, it is likely that the development of liver cell tumors (and nodular hyperplasia) was associated with epigenetic mechanisms, possibly including peroxisomal proliferation and genetic damage (ploidy) associated with oxidative stress and/or altered regulation of the cell cycle. Following publication of the final report for this study (1988), numerous journal articles have been published which identify carcinogenesis in rodents following exposure to non-genotoxic test materials. Subsets of these chemicals which induce liver cell tumors in rodents are characterized by antecedent hepatocytomegaly and peroxisomal proliferation (2,5,6,8).



**4. Effects of ancillary biochemical interactions which may have served as tumor promoters:**

Comments relative to all metabolic aberrations which may have influenced liver cell tumorigenesis in this study would be largely speculative. It should be noted, however, that hepatic toxicity was unequivocally linked with ingestion of FM-3924, and at high dose levels liver cell alterations had resulted in necrosis. Correspondingly, accelerated proliferation of hepatocytes would be expected to repair damaged tissue, and may have served as a tumor promoter (10). Although it should be emphasized that increased liver cell proliferation which may occur in rodents following exposure to many toxic materials does not invariably result in increased tumor formation, most pathologists agree that cell damage which promotes in increased mitotic activity may contribute to tumor formation (4,10,13).

**5. Personal opinion relative to a relationship between proliferative lesions and FM-3924 observed in this study:**

It is my opinion that distinct increases in hepatocellular neoplasms in high dose females, combined with increased hyperplastic nodules in both sexes are clear indicators that the test material should be regarded as a liver carcinogen in Sprague Dawley rats. Other proliferative lesions and neoplasms were considered to be spontaneous alterations or secondary to the systemic effects of altered hepatocellular metabolism (7). Based upon histomorphologic changes observed in this study, it is likely that epigenetic mechanisms (especially peroxisomal proliferation, oxidative stress and other factors which deregulate the cell cycle) were key factors in the development of hepatocellular proliferative lesions. Ancillary data which would support epigenetic pathways for tumorigenesis in this study would provide a rationale for selection of exposure thresholds in humans. Based upon reference literature available for the preparation of this review, liver damage, including carcinogenesis, has not been reported in humans exposed to test materials that promote hepatocytomegaly and neoplasia in rodents via peroxisomal proliferation and associated metabolic perturbations

**Additional Information which might Contribute to the Safety  
Assessment of FM-3924**

1. Mutagenesis assays or ancillary procedures to establish the genotoxic potential of the test material.
2. Cell proliferation studies to provide an index treatment-related increases in the cell cycle.
3. Ultrastructural analyses or contemporary analytical procedures to confirm the possible peroxisomal proliferation and ancillary metabolic perturbations.
4. Recovery studies to establish the persistence of liver cell effects following subchronic exposures to FM-3924.

**Conclusions:** Based upon review of the information available in the study report, it is my opinion that dietary FM-3924 for 2 years resulted in chronic liver changes (megalocytosis) in males at all dose levels (10, 30 and 100 ppm) and for females at the high dose concentration (100 ppm). At the high dose level, hyperplastic nodules were increased in both sexes and hepatocellular tumors (adenomas and carcinomas) were increased in females. Incidence values for liver proliferative lesions indicated that FM-3924 should be regarded as a liver carcinogen for Sprague Dawley rats under the conditions of this study. The presence of persistent, dose-dependent liver cell cytomegaly suggested that epigenetic mechanisms were causative for tumorigenesis in this study, and that safe exposure thresholds may be established providing that toxicokinetic and ancillary data do not indicate additional adverse effects such as reduced excretion and bioaccumulation.



Richard H. Bruner, DVM, DACVP

11/25/98  
Date

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28 March 1999

To: 3M

I resign my position as Environmental Specialist effective 6 April 1999. My resignation is prompted by my profound disappointment in 3M's handling of the environmental risks associated with the manufacture and use of perfluorinated sulfonates (PFOS)(CAS# 29081-56-9) and its precursors, such as ethyl FOSE alcohol (CAS #1691-99-2) and methyl FOSE alcohol (CAS #24448-09-7).

Perfluorooctanesulfonate is the most insidious pollutant since PCB. It is probably more damaging than PCB because it does not degrade, whereas PCB does; it is more toxic to wildlife; and its sink in the environment appears to be biota and not soil and sediment, as is the case with PCB.

I have worked within the system to learn more about this chemical and to make the company aware of the dangers associated with its continued use. But I have continually met roadblocks, delays, and indecision. For weeks on end I have received assurances that my samples would be analyzed soon--never to see results. There are always excuses and little is accomplished. I can illustrate with several examples.

- For more than twenty years 3M's ecotoxicologists have urged the company to allow testing to perform an ecological risk assessment on PFOS and similar chemicals. Since I have been assigned to the problem a year ago, the company has continued its hesitancy.
- Over a period of seven months I made frequent requests that ecological risk consultants be hired to help me plan toxicity testing, environmental sampling, chemical fate studies, and ecological risk procedure. I still have not received authorization even to bring people in to interview.
- I requested, very frequently, over a nine-month period, a sample of chemical to send out for fate property and ecotoxicity testing. Finally I was provided with one that apparently the division had had all along.
- I put together a pioneer risk assessment on PFOS that indicated a greater than 100% probability of harm to sea mammals, based on preliminary data on the concentration of PFOS in menhaden fish meal. The 8e committee told me that they would like to reconsider the assessment after we had a validated value for fishmeal. That analysis was given high priority by the committee. After three months the analysis is still not done--not because there were technical problems, but because management did not actually give the analysis high priority.
- 3M submitted a TSCA 8e last May. There is tremendous concern within EPA, the country, and the world about persistent bioaccumulative chemicals such as PFOS. Just before that submission we found PFOS in the blood of eaglets--eaglets still young enough that their only food consisted of fish caught in remote lakes by their parents. This finding indicates a widespread environmental contamination and food chain transfer and probable bioaccumulation and bio-magnification. This is a very significant finding that the 8e reporting rule was created to collect. 3M chose to

**Exhibit  
1001**

State of Minnesota v. 3M Co.,  
Court File No. 27-CV-10-28862

1001.0001

3MA00480715

report simply that PFOS had been found in the blood of animals, which is true but omits the most significant information.

- ◆ One of our customers, Griffin, has data on some of our chemicals. They developed this data for pesticide registration purposes. I started regularly asking for permission to visit Griffin and view the data last May. Their data can help us plan our studies of similar chemicals. It can also indicate if there is an unforeseen risk to certain biota or via certain exposure pathways. It was ten months before I was allowed to visit Griffin, at which time I did not get to see the data. I have to return another time to see it.
- 3M waited too long to tell customers about the widespread dispersal of PFOS in people and the environment. We knew before May of 1998, yet 3M did not start telling customers until January of 1999. I felt guilty about this and told customers I personally knew earlier. Still, it was not as early as it should have been. I kept waiting for 3M to do its duty, as I was continually assured that it would. Some of the customers have done risk assessments on the PFOS precursor they use. They assume there is not a background in the environment and in wildlife. Since there is a background, their risk assessments are inaccurate. Thus they can make inappropriate business decisions and not realize that their use of PFOS precursors contributes to an aggregate risk.
- 3M continues to make and sell these chemicals, though the company knows of an ecological risk assessment I did that indicates there is a better than 100% probability that perfluorooctansulfonate is biomagnifying in the food chain and harming sea mammals. This chemical is more stable than many rocks. And the chemicals the company is considering for replacement are just as stable and biologically available. The risk assessment I performed was simple, and not worst case. If worst case is used, the probability of harm exceeds 100,000%.
- 3M told those of us working on the fluorochemical project not to write down our thoughts or have email discussions on issues because of how our speculations could be viewed in a legal discovery process. This has stymied intellectual development on the issue, and stifled discussion on the serious ethical implications of decisions.

I have worked to the best of my ability within the system to see that the right actions are taken on behalf of the environment. At almost every step, I have been assured that action will be taken—yet I see slow or no results. I am told the company is concerned, but their actions speak to different concerns than mine. I can no longer participate in the process that 3M has established for the management of PFOS and precursors. For me it is unethical to be concerned with markets, legal defensibility and image over environmental safety.

Sincerely,

Rich Purdy

AR226-0145

RDT-5

# Draft Report

26-Week Capsule Toxicity Study with Perfluorooctane Sulfonic  
Acid Potassium Salt (PFOS; T-6295) in Cynomolgus Monkeys

PREPARED FOR:  
3M

COVANCE STUDY NUMBER:  
6329-223

VOLUME  
I of II

**002636**

**Sponsor:**

3M  
St. Paul, Minnesota

**FINAL REPORT**

**Study Title:**

26-Week Capsule Toxicity Study with Perfluorooctane Sulfonic Acid  
Potassium Salt (PFOS; T-6295) in Cynomolgus Monkeys

**Author:**

Peter J. Thomford, PhD

**Study Completion Date:**

To be determined

**Testing Facility:**

Covance Laboratories Inc.  
3301 Kinsman Boulevard  
Madison, Wisconsin 53704-2595

**Laboratory Study Identification:**

Covance 6329-223

**Sponsor Study Identification:**

3M Study No. T-6295.7

Volume I of II

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**002637**

**COMPLIANCE STATEMENT**

**26-Week Capsule Toxicity Study with Perfluorooctane Sulfonic Acid  
Potassium Salt (PFOS; T-6295) in Cynomolgus Monkeys**

All aspects of this study were in accordance with the Environmental Protection Agency  
Good Laboratory Practice Standards, 40 CFR 792.

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Peter J. Thomford, PhD  
Study Director  
Covance Laboratories Inc.

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Date

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Andrew M. Seacat, PhD  
Study Monitor  
3M

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Date



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**STUDY IDENTIFICATION**

**26-Week Capsule Toxicity Study with Perfluorooctane Sulfonic Acid  
Potassium Salt (PFOS; T-6295) in Cynomolgus Monkeys**

Test Material	Perfluorooctane Sulfonic Acid Potassium Salt (PFOS; T-6295)
Sponsor	3M Toxicology Services Building 220-2E-02, 3M Center St. Paul, Minnesota 55144-1000
Study Monitor	Andrew M. Seacat, PhD 3M 651.575.3161
Alternate Study Monitor	Marvin T. Case, DVM, PhD 3M Toxicology Services 651.733.5180
Study Location	Covance Laboratories Inc. 3301 Kinsman Boulevard Madison, Wisconsin 53704-2595
Study Director	Peter J. Thomford, PhD Covance Laboratories Inc. PO Box 7545 Madison, Wisconsin 53707-7545 608.241.7207
Study Timetable	
Study Initiation Date	August 20, 1998
In-Life (Experimental) Start Date	August 26, 1998
In-Life Termination Date	March 7, 2000
Experimental Termination Date	To be determined

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**KEY PERSONNEL**

**26-Week Capsule Toxicity Study with Perfluorooctane Sulfonic Acid  
Potassium Salt (PFOS; T-6295) in Cynomolgus Monkeys**

Study Director	Peter J. Thomford, PhD
Study Toxicologist	Dale Aldridge, BS
Study Coordinator	Elizabeth A. Disch, BA
Manager, Large Animal Toxicology	Sharon Dunn, LATG, AT
Supervisor, Dose Formulation	Dixie Bushee, BS, LATG
Associate Director, Laboratory Animal Medicine	Donna J. Clemons, DVM, MS Diplomate, ACLAM
Clinical Pathologist	Robert L. Hall, DVM, PhD Diplomate, ACVP (Clinical Pathology)
Supervisor, Clinical Pathology	Ronald Markevitch, BS, MT (ASCP)
Anatomic Pathologist	Robert A. Leedle, DVM, PhD Diplomate, ACVP
Supervisor, Anatomic Pathology	Laurie J. Schuller, BA, LAT
Supervisor, Anatomic Pathology	Kimberly W. Durland, BS, HT
Consultant	Stephen I. Bistner, DVM Diplomate, ACVO Veterinary Ophthalmologist

**002640**

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## ABSTRACT

The purpose of this study was to assess the effect of the test material, Perfluorooctane Sulfonic Acid Potassium Salt [PFOS; T-6295 (hereafter referred to as PFOS)] on critical enzyme levels, hormones, and other selected biochemical parameters when administered daily by oral capsule to cynomolgus monkeys for at least 26 weeks. The treatment period was followed by an approximate 52-week recovery period.

Male and female cynomolgus monkeys were assigned to four groups (six animals/sex in Groups 1, 3, and 4; four animals/sex in Group 2). Each group received dose preparations containing the vehicle, lactose, or 0.03, 0.15, or 0.75 mg of PFOS/kg of body weight/day (mg/kg/day). Two animals/group in Groups 1, 3, and 4 were in a recovery period and were not treated for at least 52 weeks following the 26 week treatment period.

Food was provided *ad libitum*, except when animals were fasted. Water was provided *ad libitum*. The animals were observed twice daily (a.m. and p.m.) for mortality and moribundity. At least once daily, animals were examined for abnormalities and signs of toxicity, and food consumption was assessed qualitatively. Ophthalmic examinations were done before initiation of treatment and during Weeks 26 and 52. Body weight data were recorded weekly before initiation of treatment, on Days -1 and 1, and weekly thereafter. Blood and urine samples were collected for clinical hematology, clinical chemistry, and urinalysis tests before initiation of treatment and at specified intervals during treatment and recovery. Blood was also collected for blood hormone and PFOS level determinations before, during, and after treatment at specified intervals. Feces and liver samples were also collected at specified intervals. On Day 155 (Week 23), one male given 0.75 mg/kg/day died, and on Day 179 (Week 26), one male given 0.75 mg/kg/day was sacrificed due to poor health. On Days 184 and 185 (Week 27), four animals/sex/group (Groups 1 through 3) and four females and two males (Group 4) were anesthetized, weighed, exsanguinated, and necropsied. At necropsy at the scheduled and unscheduled sacrifices, a serum sample was collected, macroscopic observations were recorded, selected organs were weighed, and selected tissues were collected and preserved. Microscopic examinations were done on tissues from each animal in the control and high-dose groups and selected tissues from animals in the low- and mid-dose groups. Tissues were also collected for palmitoyl CoA oxidase determination, cell proliferation evaluation, PFOS determination, and electron microscopy. Additionally, the bile was collected from the gallbladder, and the gallbladder was preserved. At the recovery sacrifice on Day 549, the remaining Group 4

animals were anesthetized, weighed, exsanguinated, and necropsied. Macroscopic observations were recorded and specified tissues and serum were collected. Remaining animals in Groups 1 and 3 were donated or transferred to a follow-up study, Covance 6329-268.

At all dose levels, clinical observations, ophthalmic observations, and palmitoyl CoA oxidase determinations do not appear to be affected by treatment with PFOS.

Two males given 0.75 mg/kg/day died during treatment. These deaths were preceded by some adverse clinical observations (constricted pupil, pale gums, abnormal feces, excessive salivation, labored respiration, dehydrated appearance, hypoactive, ataxic, recumbent, low food consumption) and appeared to be related to the administration of PFOS. When compared with animals given the control material, covariate adjusted mean body weights (CAM) for males given 0.75 mg/kg/day were slightly lower beginning at Week 21, and for females given 0.75 mg/kg/day CAM body weights were, in general, significantly lower beginning at Week 11. Similar decreases were not seen in the other treated groups; therefore, this finding is likely test material-related. Test material-related effects on body weights were not apparent during recovery. Low food consumption was noted sporadically for animals in the groups given the control material and 0.03 mg/kg/day throughout treatment. The incidence of low food consumption was generally higher in the groups given 0.15 or 0.75 mg/kg/day as compared to animals given the control material and appeared to be test material-related. During recovery, effects on food consumption were reversed.

Estradiol values were generally lower on Days 62, 91, and 182 in males given 0.75 mg/kg/day, although because of the variation in the data only the Day 182 value was significant. Estrone values were generally higher in all of the treated females on Days 37, 62, and 91, although because of the variation in the data none of these values were significantly different, and this difference was not apparent on Day 182. Triiodothyronine values were notably lower in both males and females given 0.15 and 0.75 mg/kg/day on Days 91 and 182. With the single exception of males given 0.15 mg/kg/day on Day 91, all values were significantly lower. During recovery were occasional instances in which the hormone values in treated groups differed slightly from those of controls, but those differences were not consistent over time or between sexes, were not clearly dose-related, and did not appear to be clearly related to the administration of the test material. Apparent differences in the sexual maturity of both males and females used in this study complicates the interpretation of the hormone data.



The only clinical pathology parameters considered related to the test material were lower total cholesterol for animals given 0.75 mg/kg/day and lower high density lipoprotein cholesterol for animals given 0.15 or 0.75 mg/kg/day. These effects were reversed within the first 5 and 9 weeks of recovery, respectively.

At the terminal sacrifice, increased liver weights, macroscopic observations of mottled liver, hepatocellular hypertrophy, and hepatocellular vacuolation in animals given 0.75 mg/kg/day were considered related to PFOS treatment. However, the microscopic examination liver biopsies taken during recovery did not indicate any test material-related findings and none of the macroscopic observations made at the recovery sacrifice were considered test material-related. There were no microscopic findings in the liver from the animals in the high-dose recovery group. This indicates that the hepatic test material-related effects were reversible.

Treatment with PFOS by oral capsule for at least 26 weeks is generally well-tolerated in male and female cynomolgus monkeys at doses up to 0.15 mg/kg/day. Clinical and pathological findings considered to be associated with the treatment of PFOS after at least 26 weeks of treatment were found to be reversible during a 52-week recovery period. Based on the data presented in this report, the no-observable-effect level is 0.03 mg/kg/day. Dose analyses (provided by the Sponsor) and electron microscopy results (provided by PAI) are forthcoming.

## **PURPOSE**

The purpose of this study was to assess the effect of the test material on critical enzyme levels, hormones, and other selected biochemical parameters when administered daily by capsule to cynomolgus monkeys for at least 26 weeks. The treatment period was followed by an approximate 52-week recovery period.

## **REGULATORY COMPLIANCE**

All aspects of this study were done in accordance with the Environmental Protection Agency Good Laboratory Practice Standards, 40 CFR 792.

## **TEST MATERIAL, VEHICLE, AND SOLVENT**

### **Test Material**

The test material, Perfluorooctane Sulfonic Acid Potassium Salt (PFOS; T-6295), Lot No. 217, is a white to off-white powder. It was received at Covance on September 4, 1997. The test material was stored at room temperature.

Information on synthesis methods, stability, purity, composition, or other characteristics that define the test material is on file with the Sponsor.

### **Vehicle**

The vehicle was lactose (Spectrum, New Brunswick, New Jersey), Lot No. NN0192 (expiration date February 13, 1999). It was received at Covance on March 30, 1998.

The vehicle was stored at room temperature.

Information on synthesis methods, purity, stability, composition, or other characteristics that define the vehicle is on file with the manufacturer.

### **Solvent**

The solvent was acetone (Spectrum, Gardena, California), Lot No. LH0253, (expiration date June 2000). It was received at Covance on June 23, 1997. The solvent was stored at room temperature.

Information on synthesis methods, composition, or other characteristics that define the solvent is on file with the manufacturer.

### **Gelatin Capsules**

Gelatin capsules, Size Nos. 2 (Lot No. 122932, expiration date June 12, 2003) and 4 (Lot No. 544043, expiration date August 1, 2002) were manufactured by Torpac Inc., (Fairfield, New Jersey). Lot No. 122932 was received at Covance on June 12, 1998, and Lot No. 544043 was received on September 1 and November 11, 1998. The capsules were stored at room temperature. A copy of the Certificate of Analysis provided by the manufacturer is maintained in the data.

### **Reserve (Archive) Samples**

A reserve sample (1 g) of each lot of the test material, vehicle, and each test material/lactose dilution was taken and stored at room temperature. These samples were transferred to the Sponsor after completion of the treatment phase (see Protocol Deviations).

### **Disposition**

Remaining test material will be retained at Covance for use in possible future studies.

## **TEST ANIMALS AND HUSBANDRY**

### **Animals**

Young adult to adult cynomolgus monkeys were obtained from Covance Research Products Inc. (Denver, Pennsylvania) on June 30, 1998. The animals weighed 2.4 to 4.4 kg at initiation of treatment.

### **Identification**

Each animal was assigned a permanent number upon arrival and identified with a collar tag before initiation of treatment. All data for an animal are recorded under this number.

### **Justification**

PFOS is a known hepatic peroxisome proliferator (PP) in the rat. When exposed to a PP, nonhuman primates (such as the cynomolgus monkey) respond similarly to humans (i.e., low to no hepatic response) and therefore are an appropriate human surrogate species.

### **Husbandry**

Animal Rooms 251 and 259 were used for this study. Environmental controls for the animal rooms were set to maintain 18 to 29°C, a relative humidity of 30 to 70%, and a 12-hour light/12-hour dark cycle. Variations from these conditions are documented in the data and are considered to have had no effect on the outcome of the study.

The animals were housed individually in suspended, stainless-steel cages.

Certified primate diet (#8726C, Harlan Teklad) was provided once or twice daily, unless otherwise specified. The lot numbers are recorded in the data. The diet is routinely analyzed by the manufacturer for nutritional components and environmental contaminants. Results of specified nutrient and contaminant analyses are on file with Covance-Madison. Fruits or additional supplements were provided, but did not require analysis.

Water was provided *ad libitum*. Samples of the water are analyzed for specified microorganisms and environmental contaminants. The results are on file with Covance-Madison.

There were no known contaminants in the diet or water at levels that would have interfered with this study.

**Acclimation**

Twenty-four males and 24 females were received on June 30, 1998, and acclimated in Animal Room 251 for 57 days before initiation of treatment. In general, animals in this shipment appeared healthy. During acclimation, the animals were examined for abnormalities indicative of health problems. In addition, three tuberculosis tests, a physical examination, and a fecal flotation for parasites were performed on each animal.

**PROCEDURES**

This study was conducted in accordance with the Protocol dated August 20, 1998, and Protocol Amendment Nos. 1, 2, and 3. The protocol, protocol amendments, and protocol deviations are in Appendix 1.

**Group Designations and Dosage Levels**

Selection of animals for the study was based on data collected during acclimation. Animals were assigned to treatment groups using a computerized blocking procedure designed to achieve body weight balance with respect to treatment group.

Group	Dose Level (mg/kg/day) <sup>a</sup>	Total Material Dose Level (mg/kg/day) <sup>b</sup>	Number of Animals	
			Males	Females
1	0 <sup>a</sup>	30 <sup>a</sup>	6 <sup>d</sup>	6 <sup>d</sup>
2	0.03	15 <sup>b</sup>	4	4
3	0.15	6 <sup>c</sup>	6 <sup>d</sup>	6 <sup>d</sup>
4	0.75	30 <sup>c</sup>	6 <sup>d</sup>	6 <sup>d</sup>

- a The control group (Group 1) received the equivalent amount of lactose in gelatin capsules as the total material administered to Group 4.
- b The low-dose (Group 2) received the test material diluted with lactose (1:499, w:w).
- c The mid-dose (Group 3) and high-dose (Group 4) groups received the test material diluted with lactose (1:39, w:w).
- d Two animals in Groups 1, 3, and 4 designated as recovery animals were treated for at least 26 weeks, then treatment was discontinued, and the animals were observed for reversibility, persistence, or delayed occurrence of toxic effects for at least 52 weeks posttreatment.

### **Dosing Procedures**

**Vehicle.** Dose levels were based on the vehicle as supplied for Group 1. For Group 1 dose preparations, the specified amount of lactose was weighed, transferred into gelatin capsules, and the top and bottom halves of each capsule were joined. Capsules were prepared at least once weekly.

**Test Material.** The test material/lactose preparations for Groups 2 through 4 were diluted once before initiation of treatment; capsules were prepared at least once weekly.

A specified amount of test material was weighed, placed into a labeled mixing container, and the appropriate volume of acetone was added. After stirring manually until the test material was dissolved, the required amount of lactose was weighed and transferred to the container. The components were mixed thoroughly using a spatula. The prepared test material dilution was stirred periodically while allowed to stand exposed to the air until the acetone had evaporated. Preparations were diluted to facilitate capsule preparation.

Samples of the finished mixture for dose analyses were taken directly from the container.

The dose preparations were stored at room temperature between capsule preparations. The appropriate amount of prepared test material was weighed and transferred into Size 2 (Days 1 through 8) or 4 (Days 9 through 184) gelatin capsules and the top and bottom halves of each capsule were joined. Size 4 capsules were used instead of Size 2 to better facilitate dose administration. Individual daily doses were based on the most recently recorded body weight, with the exception of doses given on days when body weight measurements were performed; on those days, the previous body weight was used.

All capsule preparations were stored at room temperature until used for dosing.

**Method of Administration.** Gelatin capsules were used to facilitate comparison with data from previous toxicology studies that used the oral route. Also, oral is the most likely route of exposure in humans. Partial or intact capsules were noted in the vomitus of several animals on occasion; however, this is not considered to have adversely affected the results of the study.

The dose preparations were administered orally in gelatin capsules once daily 7 days/week for at least 26 weeks (see Protocol Deviations for exceptions).

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### **Dose Analyses**

Homogeneity and stability analyses were the responsibility of the Sponsor.

Samples (approximately 1 g each) were taken from the top, middle, and bottom of the test material/lactose preparations on Day -15 for homogeneity analysis. Samples collected from the middle of the preparations were also designated for prestudy stability analysis. A set of samples (approximately 1 g each) were taken from the low- and high-dose test material/lactose preparations at the end of the treatment phase for test material content analysis.

All samples were stored at room temperature until sent under ambient conditions to the Sponsor for analysis. Results of dose analyses will be provided for inclusion in the final report.

### **Observation of Animals**

**Clinical Observations.** The animals were observed twice daily (a.m. and p.m.) for mortality and moribundity. Animals were also observed at least once daily (a.m.) for signs of poor health or abnormal behavior, and food consumption was assessed qualitatively; only abnormal findings were recorded. Once weekly and on the day of scheduled sacrifice, each animal was observed; abnormal findings or an indication of normal was recorded (see Protocol Deviations for exceptions). Additionally, postdose observations were recorded during treatment approximately 30 to 90 minutes after the last dose administration; only abnormal findings were recorded.

**Ophthalmology.** Ophthalmic examinations were done on each animal before initiation of treatment, before the scheduled terminal sacrifice during Week 26, and during Week 52 (see Protocol Deviations). The pupils were dilated with 1% Mydriacyl® and the anterior portion of the eye, optic media, and ocular fundus were examined with an indirect ophthalmoscope by a board-certified ophthalmologist.

**Body Weights.** Individual body weight data were recorded weekly before initiation of treatment, on Day -1, on the first day of treatment, and weekly thereafter. Body weights were also recorded for animals sacrificed at unscheduled intervals.

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### Clinical Pathology

Blood and urine samples were collected from each animal once before initiation of treatment (Day -27); on Days 37, 62, 91, 153, and 182 of treatment; and on Days 245, 271, 274, 322, 364, 456, and 546 during recovery (see Protocol Deviations). Animals were fasted overnight, and urine was collected overnight on wet ice before blood sampling; water was provided *ad libitum*. Blood was collected from the femoral vein. Potassium EDTA was the anticoagulant used for hematology tests; no anticoagulant was used for the chemistry tests. Blood samples were collected from the animal that was sacrificed at an unscheduled interval. Animals were bled in sequential order on Days 37, 62, and 91 and in random order at all other scheduled collections; this is not expected to have an impact on the clinical pathology results. The following were evaluated (see Protocol Deviations for exceptions).

#### Hematology

red blood cell (erythrocyte) count	differential blood cell count
hemoglobin	segmented neutrophil count
hematocrit	lymphocyte count
mean corpuscular volume	monocyte count
mean corpuscular hemoglobin	eosinophil count
mean corpuscular hemoglobin concentration	basophil count
platelet count	blood cell morphology
white blood cell (leukocyte) count	reticulocyte count

#### Clinical Chemistry

glucose	sorbitol dehydrogenase
urea nitrogen	creatine kinase
creatinine	calcium
total protein	inorganic phosphorus
albumin	sodium
globulin	potassium
total bilirubin	chloride
cholesterol	bile acids
triglycerides	amylase
alanine aminotransferase	lipase
alkaline phosphatase	pancreatic-specific amylase
aspartate aminotransferase	high density lipoprotein (HDL)
gamma glutamyltransferase	(effective with collection on Day 153)



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**Urinalysis**

volume (approximately 16 hours)	bilirubin
specific gravity	blood
pH	urobilinogen
protein	microscopic examination of sediment
glucose	appearance
ketones	

**Blood Hormone Determination**

Blood samples (approximately 5 mL) were collected from each animal three times before initiation of treatment (Days -50, -40, and -27); on Days 37, 62, 91, and 182 of treatment; and on Days 217, 245, 274, 322, 364, 458, and 549 during recovery. Animals were fasted overnight. Blood was collected from a femoral vein without using an anticoagulant. Samples were allowed to clot and centrifuged within 1 hour after collection; serum was harvested. The serum was divided into two approximately equal aliquots and stored in a freezer, set to maintain -60 to -80°C, until packed on dry ice and shipped to Ani Lytics Inc. for analysis of cortisol, testosterone, estradiol, estrone, estriol, thyroid stimulating hormone, total triiodothyronine, and total thyroxine. Beginning with the collection on Day 322 the samples were also analyzed for free triiodothyronine and free thyroxine.

**Serum PFOS Level Determination**

Blood samples (approximately 2 mL) were collected from each animal once before initiation of treatment (Day -27); during Weeks 1 (Day 7), 2, 4, 6, 8, 12, 16, 20, 24, and 26, and 27 (Day 183) of treatment; and during Weeks 27 (Days 184, 185, and 187), 28 (Day 190), 29 (Day 198), 30 (Day 204), 31 (Day 211), 35, 39, 43, 47, 51, 53, 57, 61, 65, 69, 73, 77, and 79 (see Protocol Deviations). Animals were fasted overnight and water was provided *ad libitum*. Blood was collected from a femoral vein without using an anticoagulant. Samples were centrifuged within 1 hour after collection and serum was harvested. Serum samples were stored in a freezer, set to maintain -60 to -80°C, until packed on dry ice and shipped to the Sponsor for analysis. Results will be reported separately.

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#### **Additional Serum Collection**

At the scheduled terminal necropsy and the necropsy of Animal No. I05506 (Group 4 male), blood samples (approximately 20 mL) were collected from the vena cava at the time of exsanguination. Samples were collected without using an anticoagulant and centrifuged within 1 hour of collection. Serum was harvested and stored in a freezer, set to maintain -60 to -80°C, until packed on dry ice and shipped to the Sponsor for possible future analysis.

An aliquot (0.8 ml) of the additional serum collection samples collected from all animals from Groups 1, 2, 3, and 4 sacrificed at the terminal necropsy were sent on dry ice by the Sponsor to AniLytics for total triiodothyronine, total thyroxin, free triiodothyronine, and free thyroxin determinations.

#### **Urine and Feces PFOS Level Determination**

Urine [at least 2 mL (see Protocol Deviations)] and feces (at least 5 g) were collected overnight on the first day of recovery (Day 184) and on Days 189, 216, 275, 321, and 366 during recovery. In addition, a 24-hour sample of urine and feces was collected before the completion of 52 weeks of recovery. Except for the first day of recovery, animals were not fasted. Samples were stored in a freezer set to maintain -10 to -30°C, until they were packed on dry ice and shipped to the Sponsor. The samples will be analyzed for PFOS. Results will be reported separately.

#### **Additional Fecal Samples**

During Week 23, a fresh fecal sample (up to 5 g) was collected from all animals in the control and high-dose groups. Samples were collected in white polypropylene containers after pans were cleaned in the morning to ensure that the fecal samples were not more than 6 hours old (see Protocol Deviations). Samples were packed on dry ice and shipped to the Mayo Clinic for analysis.

#### **Interim Liver Biopsy Samples**

A sample of liver (approximately 1 to 2 g) was collected by biopsy from animals in Group 4 only during recovery [Week 57 (Day 393), on the same day as the serum PFOS blood collection]. This sample was divided into four portions as follows.

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One subsample was preserved in 10% neutral-buffered formalin, embedded in paraffin, sectioned, stained with hematoxylin and eosin (duplicate slides were prepared), and examined microscopically.

The second subsample was flash-frozen in liquid nitrogen and stored in a freezer, set to maintain -60 to -80°C, until shipped to the Sponsor for analysis (see Protocol Deviations). Results will be reported separately.

The third subsample was processed to block stage for electron microscopic evaluation. The tissue blocks and a hematoxylin and eosin-stained slide for light microscopy were transferred to PAI. Tissues will be processed and evaluated by electron microscopy by PAI. A report will be provided by PAI for inclusion in the final report.

The fourth subsample was flash-frozen in liquid nitrogen and stored in a freezer, set to maintain -60 to -80°C, until transferred to the Sponsor for possible future analysis.

#### **Terminal Liver Biopsy Samples**

A sample of liver (approximately 1 g) was collected by biopsy from all animals in Group 3 during recovery [Week 80 (Day 554), one week after the serum PFOS blood collection (see Protocol Deviations)]. This sample was flash-frozen in liquid nitrogen and stored in a freezer, set to maintain -60 to -80°C, until shipped to the Sponsor for analysis. Results will be reported separately.

#### **Anatomic Pathology - Terminal Sacrifice**

**Necropsy.** A necropsy was done on Animal No. I05509 (Group 4 male) that died on Day 155 (Week 23) and Animal No. I05506 (Group 4 male) that was sacrificed in a moribund condition on Day 179 (Week 26). During Week 27 (Days 184 and 185) four animals/sex/group (Groups 1 through 3) and four females and two males (Group 4) were fasted overnight, anesthetized with ketamine and xylazine, weighed, bled for required tests, exsanguinated, and necropsied. Animals were necropsied in random order.

The necropsy included a macroscopic examination of the external surface of the body; all orifices; the cranial cavity; the external surface of the brain; the nasal cavity and paranasal sinuses; cervical tissues and organs; and the thoracic, abdominal, and pelvic cavities and viscera.

**Organ Weights.** At scheduled and unscheduled sacrifices, the following organs (when present) were weighed; paired organs were weighed separately.

adrenal (2)	ovary (2)
brain	pancreas
epididymis (2)	testis (2)
kidney (2)	thyroid (2) with parathyroid
liver	

Organ-to-body weight percentages and organ-to-brain weight ratios were calculated.

**Palmitoyl CoA Oxidase Determinations.** Representative samples of the right lateral lobe of liver were collected from each animal at the scheduled sacrifice, weighed, flash-frozen in liquid nitrogen, and stored in a freezer, set to maintain -60 to -80°C, until analyzed for palmitoyl CoA oxidase activity.

**Cell Proliferation Evaluation.** Representative samples of the left lateral lobe of the liver, left and right testes, and pancreas were collected and preserved in zinc formalin. A second set of tissues (representative samples of the left lateral lobe of the liver, left and right testes, and pancreas) preserved in formalin without zinc were also prepared. After fixation, samples were embedded in paraffin and shipped to Pathology Associates International (PAI) for proliferation cell nuclear antigen (PCNA) evaluation, including the examination of slides stained with hematoxylin and eosin (see Protocol Deviations). Results were provided by PAI for inclusion in the final report (Appendix 7).

**Liver PFOS Determination.** A section of liver (approximately 20 g) was collected from each animal at the scheduled sacrifice, weighed, flash-frozen in liquid nitrogen, and stored in a freezer, set to maintain -60 to -80°C, until shipped with plasma samples to the Sponsor. Results will be reported separately.

**Gallbladder and Bile Collection.** At the scheduled terminal sacrifice for each animal, bile was collected from the gallbladder, measured, transferred into a cryovial, and flash-frozen in liquid nitrogen. The gallbladder, once emptied, was weighed, and a section (approximately 4 to 5 mm) from the mid-portion was collected. The remaining gallbladder was placed in a cryovial and flash-frozen in liquid nitrogen. The bile and gallbladder samples were stored on dry ice until transferred to a freezer set to maintain

-60 to -80°C. Samples were packed on dry ice and shipped to the Sponsor for possible future analysis.

**Tissue Preservation.** The following tissues (when present) or representative samples were collected and preserved in 10% neutral-buffered formalin, unless otherwise specified (see Protocol Deviations).

adrenal (2)	ovary (2)
aorta	pancreas
brain	pituitary
cecum	prostate
cervix	rectum
colon	salivary gland [mandibular (2)]
duodenum	sciatic nerve
epididymis (2)	seminal vesicle (2)
esophagus	skeletal muscle (thigh)
eyes [(2) preserved in Davidson's fixative for all sacrificed animals]	skin
femur with bone marrow (articular surface of the distal end)	spinal cord (cervical, thoracic, and lumbar)
gallbladder	spleen
heart	sternum with bone marrow
ileum	stomach
jejunum	testis [(2) preserved in Bouin's solution for all sacrificed animals]
kidney (2)	thymus
lesions	thyroid (2) with parathyroid
liver	trachea
lung	urinary bladder
mammary gland	uterus
mesenteric lymph node	vagina

**Histopathology.** Tissues (as appropriate) were embedded in paraffin, sectioned, stained with hematoxylin and eosin, and examined microscopically from each animal in the control and high-dose groups (see Protocol Deviations for exceptions). In addition, liver and thymus for all animals in the low- and mid-dose groups and spinal cord gray matter from females in the low- and mid-dose groups were embedded in paraffin, sectioned, stained with hematoxylin and eosin, and examined microscopically. Other tissues, as appropriate, will be retained for possible future examination.

Bone marrow smears from the sternum of each animal at scheduled and unscheduled sacrifices were prepared, stained with Wright's stain, and retained for possible examination.

**Electron Microscopy.** A sample of the liver was collected from each animal at the scheduled terminal sacrifice. Tissues were processed into blocks and, along with a hematoxylin and eosin-stained slide, were shipped to PAI for analysis. Results will be provided for inclusion in the final report.

**Anatomic Pathology - Recovery Sacrifice**

**Termination.** Remaining animals in Group 1 were donated on Day 549 and remaining animals in Group 3 were transferred to Covance 6329-268 on Day 560. On Day 549, remaining animals in Group 4 were fasted overnight, anesthetized with ketamine and xylazine, weighed, exsanguinated, and necropsied.

The necropsy of the animals in Group 4 included a macroscopic examination of the external surface of the body; all orifices; the cranial cavity; the external surface of the brain; the nasal cavity and paranasal sinuses; cervical tissues and organs; and the thoracic, abdominal, and pelvic cavities and viscera.

**Liver Samples.** Samples of liver were collected from animals in Group 4 as follows.

One sample was preserved in 10% neutral-buffered formalin, embedded in paraffin, sectioned, stained with hematoxylin and eosin (duplicate slides were prepared), and examined microscopically.

The second sample was flash-frozen in liquid nitrogen and stored in a freezer, set to maintain -60 to -80°C, until shipped to the Sponsor for analysis. Results will be reported separately.

The third sample was processed to block stage for electron microscopic evaluation. The tissue blocks and a hematoxylin and eosin-stained slide for light microscopy were transferred to Pathology Associates International (PAI). Tissues will be processed and evaluated by electron microscopy by PAI. A report will be provided by PAI for inclusion in the final report.

**Additional Tissue and Serum Samples.** Samples of lung, kidney, spleen, thyroid, brain, abdominal fat, heart, (approximately 3 g each), and bile and serum (each as much as possible) were collected. These samples were flash-frozen in liquid nitrogen and stored in a freezer, set to maintain -60 to -80°C, until shipped to the Sponsor for possible future analysis.

### **Statistical Analyses**

Levene's test (Levene, 1960) was done to test for variance homogeneity. In the case of heterogeneity of variance at  $p \leq 0.05$ , transformations were used to stabilize the variance. Comparison tests took variance heterogeneity into consideration.

One-way analysis of variance [ANOVA (Winer, 1971a)] was used (if applicable) to analyze initial body weights, organ weights, palmitoyl CoA oxidase activities, continuous clinical pathology values, and blood hormone determinations. If the ANOVA was significant, Dunnett's t-test (Dunnett, 1964) was used for control versus treated group comparisons.

One-way analysis of covariance [ANCOVA (Winer, 1971b)] was used to analyze body weights, with initial body weights as the covariate. If the ANCOVA was significant, covariate-adjusted means were used for control versus treated group comparisons.

Group comparisons (Groups 2 through 4 versus Group 1) were evaluated at the 5.0%, two-tailed probability level. Only data collected on or after the first day of treatment were analyzed statistically. Statistical analyses were not performed on data collected during recovery.

### **Record Retention**

All raw data, documentation, records, protocol, and specimens generated as a result of this study will be archived in the storage facilities of Covance-Madison for a period of 1 year. One year after the submission of the final report, the Sponsor will determine the final disposition of the materials. All raw data stored on magnetic media and the protocol, study correspondence, and an original copy of the final report will be retained by Covance-Madison.

Within 1 year after submission of the final report, all of the aforementioned materials from the Sponsor's designees (Ani Lytics Inc., 3M E. T. & S, Mayo Clinic, and Pathology Associates International) will be sent to the Sponsor (Andrew Seacat, PhD, 3M) by the Sponsor's designees.

## RESULTS AND DISCUSSION

### Observation of Animals

**Clinical Observations.** Clinical observations are summarized in Tables 1, 2, and 3; individual data are presented in Appendix 2. Individual animal fate data are also presented in Appendix 2.

Animal Nos. I05506 and I05509 given 0.75 mg/kg/day (Group 4 males) did not survive to the scheduled terminal sacrifice. All other animals survived to the scheduled study termination. No clinical observations noted in the animals that survived to the terminal sacrifice or recovery were attributable to the administration of PFOS.

Animal No. I05509 (Group 4 male) died after dosing on Day 155 (Week 23). On Day 154 (Week 22) observations of constricted pupil in both eyes and pale gums were noted. Observations noted on Day 155 prior to dosing included few, mucoid, liquid, and black-colored feces and low food consumption. Approximately 15 minutes after dosing, the animal was observed as hypoactive with labored respiration and pale gums. This animal also appeared dehydrated and was cold to the touch. These observations persisted until approximately 30 minutes postdose when the animal was also noted as recumbent. Shortly thereafter, the animal died during an examination by a laboratory animal veterinarian. An enlarged liver was detected by palpation. The cause of death was determined to be pulmonary necrosis with severe acute inflammation.

On Day 179 (Week 26), Animal No. I05506 (Group 4 male) was sacrificed in a moribund condition. Low food consumption was noted on Day 178 (Week 26) and at the a.m. observation interval on Day 179. Approximately 5 to 10 minutes postdose on Day 179, the animal had excessive salivation, labored respiration, and hypoactive and ataxic behavior. With the exception of excessive salivation, these findings continued to be observed approximately 3 hours postdose. The cause of the moribund condition was not determined.



Two additional animals had noteworthy observations during treatment. One female in the group given the control material, Animal No. I05529, was examined by a laboratory animal veterinarian on Day 5 (Week 1) due to observations of dehydration, thin appearance, clear oral and nasal discharge, excessive salivation, and audible respiration. This animal was diagnosed with pneumonia and treated with Lactated Ringer's solution and antibiotics. This animal had recovered by Day 14 (Week 2). Animal No. I05534 (Group 4 female) was diagnosed with a tapeworm infection during Week 23 and was treated with praziquantel. Neither infection was test material-related.

Clinical observations during recovery were typical of laboratory primates.

**Ophthalmology.** Ophthalmic observations are summarized in Table 4; individual data are presented in Appendix 2.

There were no ophthalmic observations at the Week 26 or Week 52 examinations that were test material-related. Animal No. I05529 (Group 1 female) was noted as having increased myelination of the right optic nerve at the baseline and Week 52 ophthalmic examinations. Because this is a permanent, congenital condition, it was noted at the baseline and recovery examinations only and is not related to treatment with PFOS.

**Body Weights.** Body weight data are illustrated in Figures 1 and 2 and summarized in Tables 5 and 6; individual data are presented in Appendix 3.

Covariate-adjusted mean (CAM) body weights were slightly lower in males given 0.75 mg/kg/day when compared with males given the control material beginning at Week 21; the difference was significant at Weeks 23 and 27. In females given 0.75 mg/kg/day, CAM body weights were significantly lower at Weeks 11 through 16, 19 through 23, and 25 through 27 when compared with females given the control material. These decreases were likely test material-related.

Differences in body weights were not apparent during the recovery period.

**Food Consumption.** Food consumption data are summarized in Tables 1, 2, and 3 (Summary of Clinical Observations); individual data are included in the individual clinical observations in Appendix 2.

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Low food consumption was noted sporadically for animals in the groups given the control material and 0.03 mg/kg/day. The incidence of low food consumption was generally higher in the groups given 0.15 and 0.75 mg/kg/day as compared to animals given the control material and appeared to be test material-related. During recovery, instances of low food consumption were sporadic and were similar for animals in the control and treated groups.

### **Clinical Pathology**

Hematology, clinical chemistry, and urinalysis data are summarized in Tables 7 through 45; individual data are presented in Appendix 4.

Administration of PFOS was associated with moderately to markedly lower total cholesterol for males and females given 0.75 mg/kg/day and high density lipoprotein cholesterol for males and females given 0.15 or 0.75 mg/kg/day. During the treatment period, the effect on total cholesterol became progressively worse over time. The effect on cholesterol was reversed within 5 weeks of the end of treatment, and the effect on high density lipoprotein cholesterol was reversed within 9 weeks of the end of treatment. Of uncertain relationship to administration of PFOS was lower total bilirubin concentration for males given 0.75 mg/kg/day and higher serum bile acid concentration for males given 0.75 mg/kg/day. These potential effects of the test material were very mild, and neither was considered adverse.

### **Palmitoyl CoA Oxidase Determination**

Palmitoyl CoA oxidase determinations are summarized in Table 46; individual data are presented in Appendix 5.

Results of palmitoyl CoA oxidase determinations were not considered to be related to the test material.

### **Blood Hormone Determination**

Summary and Individual Blood Hormone Data are presented in Appendix 6.

Estradiol values were generally lower on Days 62, 91, and 182 in males given 0.75 mg/kg/day, although because of the variation in the data only the Day 182 value was significant. Estrone values were generally higher in all of the treated females on Days 37, 62, and 91, although because of the variation in the data none of these values were significantly different, and this difference was not apparent on Day 182. Triiodothyronine values were notably lower on Days 91 and 182 in both males and females given 0.15 or 0.75 mg/kg/day. With the single exception on Day 91 of males given 0.15 mg/kg/day, all values were significantly lower. There were several other instances in which the hormone values in treated groups differed from those of controls, but these differences were not consistent over time or between sexes, were not clearly dose-related, and did not appear to be related to the administration of the test material.

During recovery were occasional instances in which the hormone values in treated groups differed slightly from those of controls, but those differences were not consistent over time or between sexes, were not clearly dose-related, and did not appear to be clearly related to the administration of the test material.

Apparent differences in the sexual maturity of both males and females used in this study complicates the interpretation of the hormone data.

### **Anatomic Pathology**

Terminal body weights, absolute organ weights, organ-to-body weight percentages, and organ-to-brain weight ratios are summarized in Table 47; incidences of macroscopic and microscopic observations are summarized in Tables 48 through 51. Individual data are presented in Appendix 5.

Two of four males receiving 0.75 mg/kg/day (high dose) did not survive to the scheduled terminal sacrifice at Week 27. At the terminal sacrifice, females in the group receiving 0.75 mg/kg/day had increased absolute liver weight, liver-to-body weight percentages, and liver-to-brain weight ratios. In males, liver-to-body weight percentages were increased in the high-dose group compared to the control group. Absolute and relative liver weight increases were regarded as test material-related. Among the macroscopic observations, only "mottled" liver was considered test material-related. "Mottled" livers were observed in the two high-dose males and in one high-dose female. Of the two males not surviving until the scheduled terminal sacrifice, one had "mottled" and "large" liver.