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HASKELL LABORATORY REPORT NO. 205-81 [REDACTED]

| <u>Material Tested</u>                          | <u>Haskell No.</u> | <u>Other Codes/Synonyms</u> |
|-------------------------------------------------|--------------------|-----------------------------|
| Octanoic acid, pentadecafluoro-, ammonium salt* | 12,037             | C-8<br>[REDACTED]           |

Study Initiated/Completed  
2/18/80 - 5/23/80

Material Submitted by  
[REDACTED]  
Polymer Products Department  
Washington Works

SUBACUTE INHALATION TOXICITY OF  
PENTADECAFLUOROOCTANOIC ACID, AMMONIUM SALT

Introduction: Pentadecafluorooctanoic acid, ammonium salt (C-8, [REDACTED]) has previously been shown to be highly toxic to rats by inhalation (ALC - 0.8 mg/l, [REDACTED]) and to cause a significant enlargement of the liver. An inhalation subacute study [REDACTED] performed at 11 and 83 mg/m<sup>3</sup> caused liver degeneration, enlarged livers and increases in liver enzymes at both concentrations. A recent subacute dermal study with rats [REDACTED] at 20, 200, and 2,000 mg/kg/day for 10 days had similar findings at all doses along with elevated blood fluoride throughout the 54 day test.

The purpose of this subacute inhalation study with rats at 0, 1, 8, and 80 mg/m<sup>3</sup> was three fold:

1. attempt to achieve a "no observable effect" concentration
2. correlate liver pathology, blood organofluoride and blood serum enzyme changes with dose and time
3. hold animals for prolonged observation to determine reversibility of effects.

General Protocol

Six-week old male Crl:CD® rats were housed 2/cage in stainless-steel wire mesh cages and provided Purina Certified Rodent Chow® #5002 and water ad libitum. Prior to exposure rats were quarantined for 10 days to assure they exhibited no overt signs of disease and a normal rate of growth.

Rats were tested at 7-8 weeks of age - weighing 240-279 g. Design concentrations of C-8 were 0, 1, 8, and 80 mg/m<sup>3</sup> for Groups I, II, III, and IV, respectively. Three groups of 24 rats were exposed to dust atmospheres

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of C-8 (II, III, and IV), while an identical control group (I) was simultaneously exposed to air only. Group I and II were contained in one high velocity hood, Group III and IV in another. Group I was handled with clean gloves during each loading and unloading. All groups were restrained in wire mesh holders and exposed head-only in 50 l glass chambers for 6 hr/day, 5 days/wk for 2 wks (excluding weekends).

During exposure rats were observed and clinical signs noted. Post-exposure, rats were weighed and observed daily for 14 recovery days, then weighed and observed twice/wk through 84 days recovery. By design 5 rats/group (until fewer than 5 remained) were sacrificed at 0, 14, 28, 42, and 84 days recovery, for a total of 96 test days.

#### Generation Procedure

Dust atmospheres of C-8 were generated by passing air through a glass generator (2 or 3 stages, depending on desired concentration). An electric motor and steel rod with plastic paddles agitated the first 2 stages, if necessary to achieve the desired concentration.

#### Analytical Procedure

Two types of analytical methods were used to monitor C-8 concentrations during exposures.

For the high concentration ( $80 \text{ mg/m}^3$ ), chamber atmosphere concentrations were primarily determined by gravimetric analysis. Known volumes of chamber air were drawn through preweighed Gelman glass fiber filters, Type AE, 47 mm at 2 l/min. Samples were taken once per hr. for 30 to 50 minutes. Concentrations were determined from weight gain (mg) of the filter/l of chamber air. During each exposure samples from this chamber were also analyzed by wet chemical analysis (below) in order to cross-check the methods. For the first six exposures 2 l samples were taken each hr. For the last 4 exposures two of the filters from the gravimetric analysis were chosen to be analyzed. The C-8 was eluted from the filter as described below.

For the intermediate and low concentrations, chamber atmosphere samples were collected once per hr (15 l for intermediate, 100 l for low) on Gelman glass fiber filters, but weight gain could not be measured. A wet chemical analytical method was used instead. This consisted of dissolving the C-8 from the filters into acidified aqueous methylene blue solution. A colored complex forms which is extracted into a nonpolar solvent (e.g., chloroform or methylene chloride). Concentrations of C-8/methylene blue complex were spectrophotometrically determined @ 650 nm and chamber concentrations were determined by comparing to standards prepared daily.

A Sierra Cascade Impactor Model #218K Eight Stage was used to determine particle size.

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### Clinical Chemistry Protocol

Following the tenth exposure (0 days recovery) and recovery days 14, 28, 42, and 84, blood was taken from the tails of 5 rats in each group. Measurements of alkaline phosphatase (AP), glutamic-oxaloacetic transaminase (GOT) and glutamic-pyruvic transaminase (GPT)<sub>2</sub> were made of the blood serum. Details are in the clinical pathology report.

### Pathology Protocol

Following the tenth exposure (0 days recovery) and recovery days 14, 28, 42, and 84, five rats from each group were sacrificed for pathologic evaluation. At necropsy the rats were examined grossly and selected tissues and organs were saved for microscopic evaluation. Weights of the lungs, heart, thymus, spleen, liver, testes, and kidneys<sub>3</sub> were obtained. Details of pathological indices are in the pathology report.

### Body Weight and Organ/Body Weight Analysis

Mean body weights were calculated and plotted to show growth curves of Groups I, II, III, and IV from 0 through 96 days on test.

At each sacrifice mean organ weights (listed above) and organ to body weight ratios were calculated.

### Procedure for Analysis of C-8 in Rat Blood

At necropsy approximately 8 to 10 ml of blood was collected (in heparinized tubes) from each rat by cardiac puncture. The blood was stored at 4°C until all samples were collected.

Blood samples from Groups I and IV were analyzed at each recovery period. Blood samples from Groups II and III were analyzed only at 0 and 28 recovery days.

Analysis of the blood was conducted by [REDACTED] Polymer Products Department, Experimental Station. Details appear in Appendix 4.

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## Results:

Exposure Time-Weighted-Average (TWA) concentrations and ranges are tabulated below. Comparison of the two analytical methods at the high level demonstrated that the 21 samples, taken for wet chemical analysis, are not representative of the 60-100 samples taken for gravimetric analysis. These samples tended to be lower (12 to 45%) than the gravimetric analysis. Direct wet chemical analysis of filters used for the gravimetric analysis showed that the two methods were comparable (wet method 6% lower than gravimetric).

| Exposure No. | Group II  |            | Group III |            | Group IV  |          |
|--------------|-----------|------------|-----------|------------|-----------|----------|
|              | TWA, mg/m | Range      | TWA, mg/m | Range      | TWA, mg/m | Range    |
| 1            | 0.1       | 0 - 0.2    | 6.7       | 0.5 - 10.5 | 110.0     | 17 - 167 |
| 2            | 0.4       | 0.02 - 1.2 | 10.7      | 5.5 - 21.9 | 83.5      | 44 - 148 |
| 3            | 1.5       | 0.4 - 2.8  | 9.0       | 2.4 - 16.5 | 79.5      | 23 - 136 |
| 4            | 1.0       | 0.7 - 1.3  | 8.9       | 5.6 - 13.3 | 67.8      | 45 - 115 |
| 5            | 1.6       | 0.7 - 3.0  | 10.0      | 2.1 - 24.9 | 95.1      | 63 - 158 |
| 6            | 1.4       | 0.2 - 2.2  | 7.3       | 1.4 - 9.3  | 88.6      | 61 - 160 |
| 7            | 1.2       | 0.5 - 1.8  | 8.1       | 4.1 - 15.1 | 78.1      | 61 - 97  |
| 8            | 0.9       | 0.5 - 1.4  | 7.5       | 1.7 - 15.0 | 89.1      | 62 - 122 |
| 9            | 1.0       | 0.5 - 2.0  | 7.6       | 0.3 - 11.3 | 77.4      | 72 - 86  |
| 10           | 0.4       | 0.1 - 0.7  | 1.3       | 0.7 - 3.2  | 75.1      | 67 - 94  |

Overall mean concentrations were:

| Group # | Conc. (relative) | Concentration, mg/m <sup>3</sup> + S.D. |
|---------|------------------|-----------------------------------------|
| II      | Low              | 1.0 + 0.5                               |
| III     | Intermediate     | 7.6 + 2.5                               |
| IV      | High             | 83.9 + 12.8                             |

Many attempts were made to determine particle size but the particles did not behave typically. The information obtained from the high concentration chamber suggests the particles were 1 to 2 u. The intermediate concentration chamber was analyzed during the 5th exposure. Mass median diameter was 3.8 u. These data indicate most of the particles generated were respirable.

## Clinical Signs

During exposures a high percentage of the rats had slight to mild nasal and ocular discharge, which appeared to be dose-related. After the third exposure one rat in Group IV was sacrificed "in extremis". During the fourth exposure one rat in Group IV died. Both animals were necropsied and tissues were saved for microscopic examination. Rats in Groups I, II, and III showed moderate weight loss for 1 to 3 days and sporadic weight loss throughout the remainder of the exposure and recovery periods. Three of 24 rats in Group IV had lung noise and all showed severe weight loss during the 12 day exposure period with sporadic weight loss throughout recovery.

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### Body Weight Analysis

Mean body weights were evaluated statistically (Appendix 1). Groups I & II were not significantly different. Group III was not significantly different from I and II until recovery day 5, when Group III weights were higher for 13 days. Group IV weights were significantly lower from test day 2 through 16 (Figure 1).

### Organ Weight Analysis

#### Organ/Body Weight Ratios

Relative organ to body weight ratios for each group after each recovery period are outlined in Table 1 and Appendix 3. The dose-related changes include increased liver, lungs and testes weights. Liver/body weight ratios are depicted graphically in Figure 2.

Other changes in organ/body weight ratios were found (Table 1). Their relationship to compound administration is unclear.

#### Mean Absolute Organ Weight Analysis

Mean absolute organ weight analysis (Appendix 2) demonstrated findings similar to organ/body weight ratios (Table 1). However, this analysis appeared to be more sensitive, in that it showed more dose-related changes in Group III, but this may be an artifact caused by an increase in Group III body weights (which cannot be explained) accompanied by a normal increase in organ weights.

Other changes in mean absolute organ weights were found (Table 1). Their relationship to compound administration is unclear.

### Clinical Laboratory Measurements

Elevated alkaline phosphatase (AP) activity was found in all groups exposed to C-8 after 10 exposures (0 recovery), but only Groups III and IV were significantly different from Group I. After 14 days recovery AP was elevated only in Group IV. After 28, 42, and 84 days recovery no differences were found.

### Pathology

#### Macroscopic

The morphologic changes observed at necropsy were varied. The only change considered to be compound-related was hepatic (i.e., heavy livers). This occurred in 1/24 Group I rats, 0/24 Group II rats, 5/24 Group III rats and 10/24 Group IV rats. This change was dose and recovery-time related. After 14 days recovery this change was not found in Group III and after 28 days was not found in Group IV.

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### Microscopic

Three compound-related histomorphologic changes were observed. All were hepatic. These include panlobular hepatocellular hypertrophy, centrilobular hepatocellular hypertrophy and hepatocellular necrosis. All three lesions were observed in Groups III and IV but not in Group II. One lesion was observed in Group I. The presence of hepatic lesions was dose and recovery-time related as outlined below. After 28 days recovery all changes are considered to be reversible in Group III and reversible by 42 days in Group IV.

### Number of Rats with Microscopic Hepatic Lesions

| <u>Recovery Day</u> | <u>Group I</u> | <u>Group II</u> | <u>Group III</u> | <u>Group IV</u> |
|---------------------|----------------|-----------------|------------------|-----------------|
| 0                   | 0/5            | 0/5             | 5/5              | 5/5             |
| 14                  | 1/5            | 0/5             | 1/5              | 4/5             |
| 28                  | 0/5            | 0/5             | 0/5              | 2/5             |
| 42                  | 0/5            | 0/5             | 1/5              | 0/4             |
| 84                  | 0/4            | 0/4             | 1/4              | 0/3             |

Cause of death could not be determined for the two rats that died or were sacrificed "in extremis" in Group IV.

### Blood Fluoride Analysis

Blood fluoride was elevated in all groups (including controls) 0 through 84 days recovery and was dose-related as shown on the table below. However, after 42 days recovery, blood fluoride in the controls was only 4 X above the limit of detectability (0.007 ug) while Group IV fluoride was 250 X above. After 84 days recovery the control blood fluoride was only 2 X above background, while Group IV was still 120 X above. The presence of C-8 in control blood was probably caused by cross-contamination during handling, exposure or housing.

### Average Concentration of C-8 in Rat Blood, ugF/g

| <u>Recovery Day</u> | <u>Group I</u> | <u>Group II</u> | <u>Group III</u> | <u>Group IV</u> |
|---------------------|----------------|-----------------|------------------|-----------------|
| 0                   | 5.3*           | 13              | 47               | 108             |
| 14                  | 0.28           | -               | -                | 10              |
| 28                  | 0.10           | 1.2             | 3.8              | 7.1             |
| 42                  | 0.032          | -               | -                | 1.8             |
| 84                  | 0.015          | -               | -                | 0.84            |

\* 1.4 excluding one high value

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## Summary

Pentadecafluorooctanoic acid, ammonium salt (C-8; [REDACTED]) was administered to male Crl:CD® rats by inhalation 6 hr/day, 5 days/wk for 2 wks at concentrations of 0 (Group I), 1.0 (Group II), 7.6 (Group III), and 83.9 mg/m<sup>3</sup> (Group IV).

Observations of clinical signs during exposures showed only slight signs of dose-related toxicity (nasal and ocular discharge). However, at the high concentration after 3-4 days on test one rat died during exposure and one rat was sacrificed "in extremis". Both of these rats were severely losing weight. Mortality was probably exposure-related although pathologic evaluation could not determine cause of death. The other rats in this group lost a significant amount of weight during the exposure period.

Body weight analysis demonstrated no significant differences between controls (Group I) and Group II. Group III body weights were significantly higher than controls from test days 17 through 33. Group IV body weights were significantly lower than controls from test days 2 through 16.

Organ to body weight ratios demonstrated a dose-related significant increase in lung, liver and testes weights after 0 recovery days. The liver/body weight ratios were significantly higher in Group IV through 26 days recovery. Mean absolute liver weights were significantly higher in Group III through 28 days recovery, but this may be an artifact caused by an unexplainable increase in Group III body weights accompanied by a normal increase in liver weights.

Clinical laboratory measurements demonstrated an increase in alkaline phosphatase (AP) in all groups exposed to C-8 after 10 exposures, which persisted in Group IV through 14 days recovery.

Pathologic findings included a macroscopic liver change (heavy livers) and three types of microscopic liver lesions in Groups III and IV. Both findings showed a dose-response relationship but were reversible by 28 days recovery (Group III) or 42 days recovery (Group IV).

Other changes were found (organ weights and pathologic lesions), but their relationship to compound administration is unclear.

Blood fluoride analysis clearly demonstrated a dose-related presence of C-8 in all groups including the controls. The amount of C-8 in blood decreased with time, but was detectable after 84 days recovery in both controls and Group IV.

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## Conclusions

Administration of C-8 by inhalation for 5 days a week for two weeks at either 0, 1.0, 7.6, or 83.9 mg/m<sup>3</sup> demonstrated the following:

- A no-effect concentration of 1 mg/m<sup>3</sup> for this study
- Exposure-related mortality at 83.9 mg/m<sup>3</sup>
- An adverse effect on body and liver weight at 83.9 mg/m<sup>3</sup>
- Elevated serum alkaline phosphatase at 7.6 and 83.9 mg/m<sup>3</sup>
- Macroscopic and microscopic liver pathology at 7.6 and 83.9 mg/m<sup>3</sup>
- Reversibility of toxic effects by 28 to 42 days recovery
- Persistent dose-related presence of C-8 in blood of all groups including controls
- Of the parameters examined, blood fluoride analysis proved to be the most sensitive and persistent indicator of C-8 exposure
- Exposure to C-8 is difficult to avoid as indicated by presence in controls

\* Composition: [REDACTED]

Contaminants: [REDACTED]

Synonyms: • C-8

- [REDACTED]
- Perfluorooctanoic acid, ammonium salt
- Ammonium perfluorooctanoate

1. Plastics Department Method, [REDACTED] Issued 10/9/69.

2. B. L. Moore and J. R. Barnes; "Inhalation Toxicity of [REDACTED] In Rats, [REDACTED]  
[REDACTED], H-12,037, Clinical Pathology Report, August 13, 1980.

3. R. M. Everett and W. C. Krauss; "Pathology Report No. [REDACTED] - [REDACTED]  
H-12,037, Sept. 10, 1980.

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**TABLE I**  
**CHANGES IN ORGAN/BODY WEIGHT RATIOS**  
**(CHANGES IN MEAN ABSOLUTE ORGAN WEIGHTS)**

| Organ   | Group | Recovery Day |       |       |       |       |
|---------|-------|--------------|-------|-------|-------|-------|
|         |       | 0            | 14    | 28    | 42    | 84    |
| Liver   | I     | 0 (0)        | 0 (0) | 0 (0) | 0 (0) | 0 (0) |
|         | II    | 0 (0)        | 0 (0) | 0 (0) | 0 (0) | 0 (0) |
|         | III   | + (+)        | 0 (+) | 0 (+) | 0 (0) | 0 (0) |
|         | IV    | + (+)        | + (+) | + (+) | 0 (0) | 0 (0) |
| Lungs   | I     | 0 (0)        | 0 (0) | 0 (0) | 0 (0) | 0 (0) |
|         | II    | 0 (0)        | 0 (0) | 0 (0) | 0 (0) | 0 (0) |
|         | III   | 0 (0)        | 0 (0) | 0 (0) | 0 (0) | 0 (0) |
|         | IV    | + (+)        | 0 (0) | 0 (0) | 0 (0) | 0 (0) |
| Kidneys | I     | 0 (0)        | 0 (0) | 0 (0) | 0 (0) | 0 (0) |
|         | II    | 0 (0)        | 0 (0) | + (0) | 0 (0) | + (0) |
|         | III   | 0 (+)        | 0 (0) | + (+) | 0 (0) | + (0) |
|         | IV    | 0 (0)        | 0 (0) | + (+) | 0 (0) | 0 (0) |
| Testes  | I     | 0 (0)        | 0 (0) | 0 (0) | 0 (0) | 0 (0) |
|         | II    | 0 (+)        | 0 (0) | 0 (0) | 0 (0) | 0 (0) |
|         | III   | 0 (+)        | 0 (0) | 0 (0) | 0 (0) | 0 (0) |
|         | IV    | + (+)        | 0 (+) | 0 (0) | 0 (0) | 0 (0) |
| Thymus  | I     | 0 (0)        | 0 (0) | 0 (0) | 0 (0) | 0 (0) |
|         | II    | - (0)        | 0 (0) | 0 (0) | 0 (0) | 0 (0) |
|         | III   | 0 (0)        | 0 (0) | 0 (0) | 0 (0) | 0 (0) |
|         | IV    | - (0)        | 0 (0) | 0 (0) | 0 (0) | 0 (0) |
| Spleen  | I     | 0 (0)        | 0 (0) | 0 (0) | 0 (0) | 0 (0) |
|         | II    | 0 (0)        | 0 (0) | 0 (0) | 0 (0) | + (0) |
|         | III   | 0 (0)        | 0 (0) | 0 (+) | 0 (0) | + (+) |
|         | IV    | 0 (0)        | 0 (0) | 0 (0) | 0 (0) | 0 (0) |
| Heart   | I     | 0 (0)        | 0 (0) | 0 (0) | 0 (0) | 0 (0) |
|         | II    | 0 (0)        | 0 (0) | 0 (0) | 0 (0) | + (0) |
|         | III   | 0 (0)        | 0 (0) | 0 (+) | 0 (0) | + (0) |
|         | IV    | 0 (0)        | 0 (0) | 0 (+) | 0 (0) | 0 (0) |

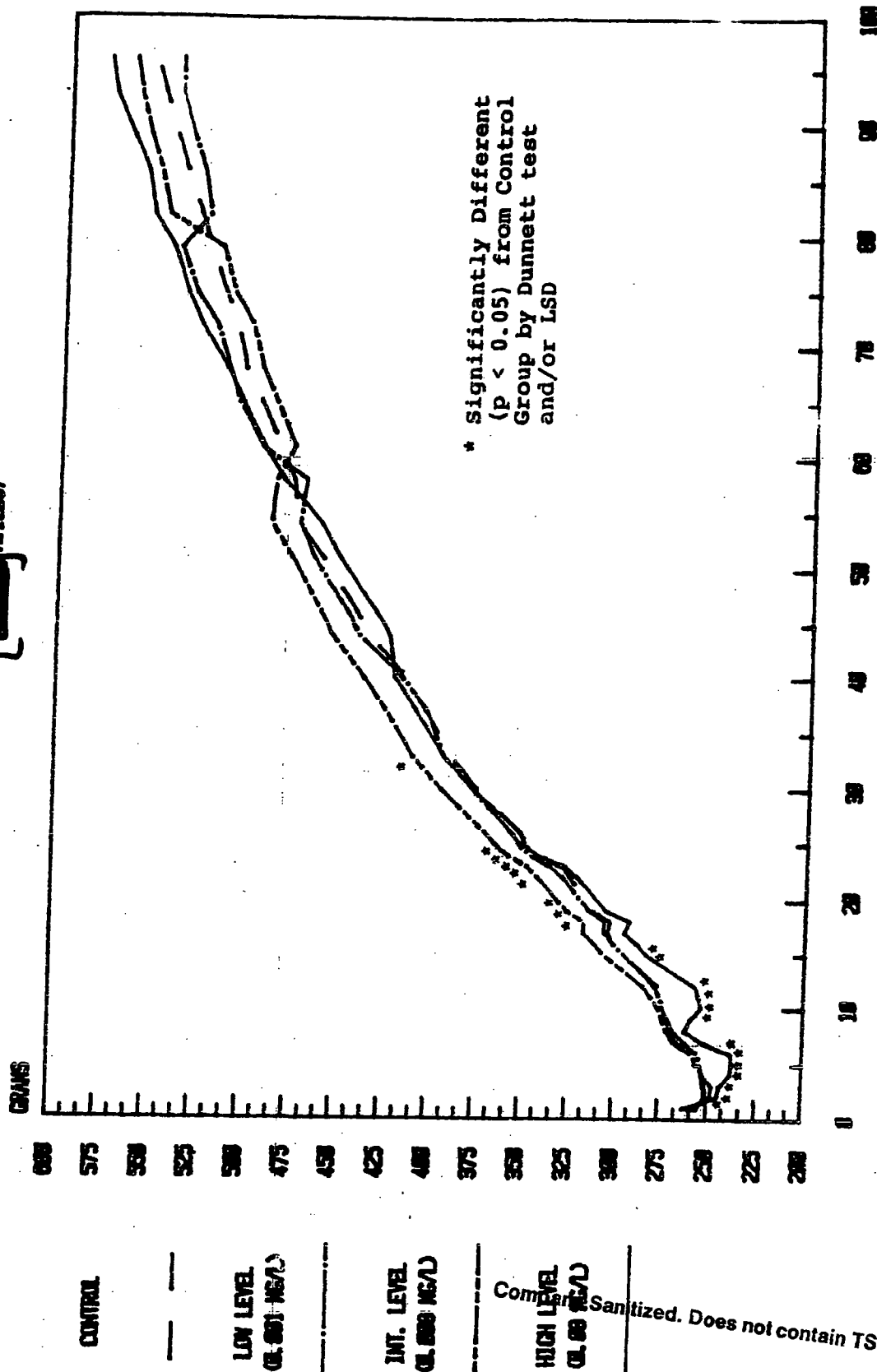
0 = no difference from control  
+ = increased † significantly different ( $p < 0.05$  from control group by  
- = decreased † Dunnett and/or LSD.

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FIGURE 1

# FC-143 BODY WEIGHT GROWTH CURVE

[REDACTED] 1412857



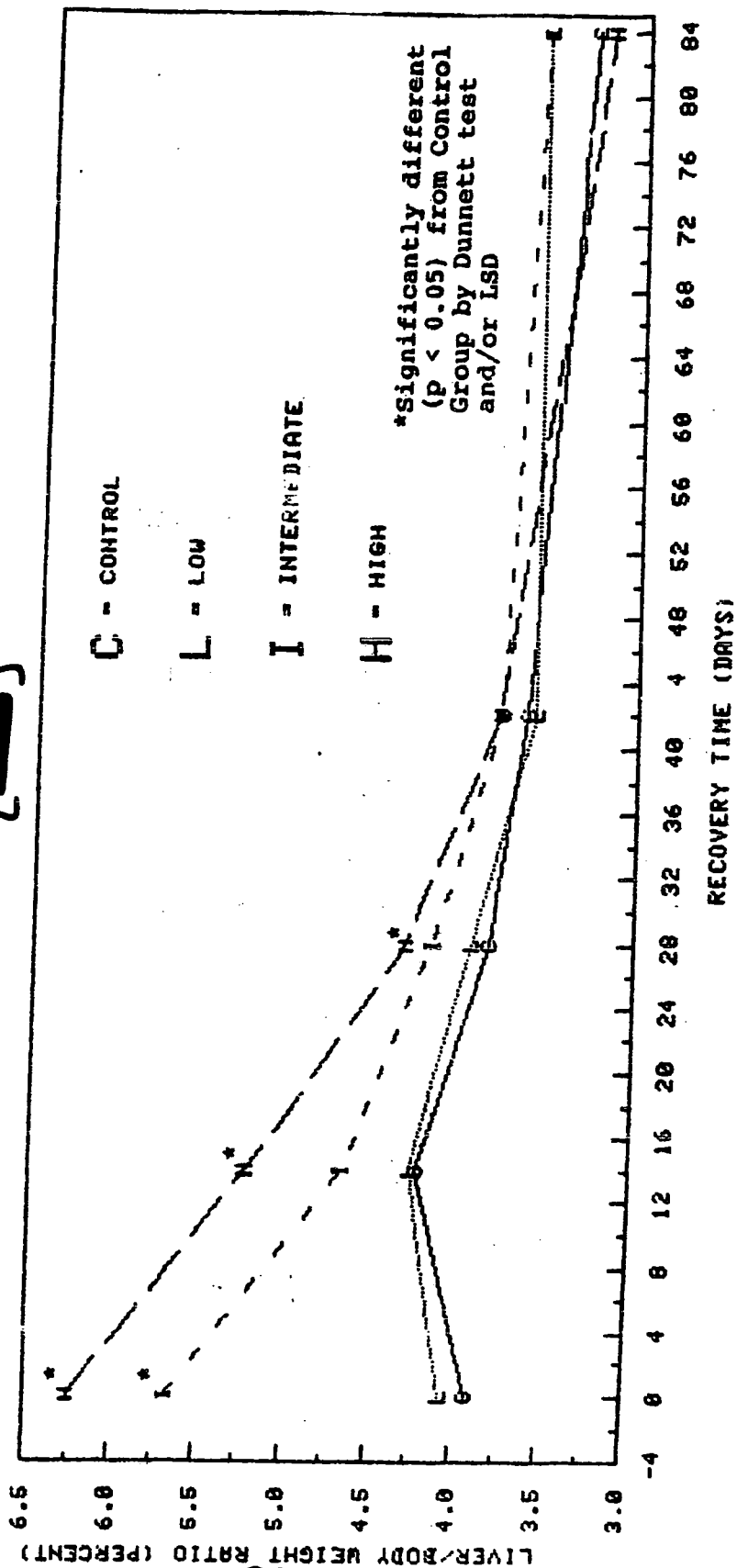
DAYS ON TEST

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Figure 2

# LIVER/BODY WEIGHT RATIO VS RECOVERY TIME



APPENDIX 1

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**BODY WEIGHT SUMMARY**

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**INTERVALS IN TEST DAYS**

| GROUP            | 1.        | 2.        | 3.        | 4.        | 5.        |
|------------------|-----------|-----------|-----------|-----------|-----------|
| CONTROLS         | 255.3333  | 251.0417  | 252.3750  | 253.0833  | 256.2917  |
| LOW (.001 MG/L)  | 256.2083  | 248.2500  | 247.1667  | 251.5000  | 254.0833  |
| INT (0.008 MG/L) | 259.3333  | 249.7500  | 251.3333  | 252.4583  | 255.9583  |
| HIGH (0.08MG/L)  | 262.2273+ | 243.7273+ | 243.4545+ | 238.0909+ | 236.7273+ |
| F RATIO(1)       | 2.276     | 2.050     | 3.339*    | 8.656*    | 12.351*   |
| LSD(2)           | 5.7974    | 6.1575    | 6.2306    | 6.7015    | 7.3751    |
| DUNNETT(3)       | 6.9919    | 7.4261    | 7.5143    | 8.0822    | 8.8946    |
| WMS(4)           | 99.5832   | 112.3369  | 115.0194  | 133.0623  | 161.1568  |

**INTERVALS IN TEST DAYS**

| GROUP            | 6.        | 7.        | 8.       | 9.        | 10.      |
|------------------|-----------|-----------|----------|-----------|----------|
| CONTROLS         | 259.0833  | 266.3750  | 270.0417 | 272.1667  | 273.8333 |
| LOW (.001 MG/L)  | 254.8333  | 261.9167  | 267.5833 | 270.5417  | 273.7500 |
| INT (0.008 MG/L) | 257.7500  | 267.7500  | 271.7917 | 274.4167  | 275.6667 |
| HIGH (0.08MG/L)  | 237.9091+ | 253.4091+ | 261.5909 | 258.7727+ | 253.954  |
| F RATIO(1)       | 12.264*   | 4.721*    | 2.094    | 4.698*    | 9.724*   |
| LSD(2)           | 7.7290    | 8.2386    | 8.5216   | 8.8888    | 9.0707   |
| DUNNETT(3)       | 9.3214    | 9.9360    | 10.2773  | 10.7202   | 10.9395  |
| WMS(4)           | 176.9943  | 201.1031  | 215.1563 | 234.0999  | 243.7791 |

**INTERVALS IN TEST DAYS**

| GROUP            | 11.       | 12.       |
|------------------|-----------|-----------|
| CONTROLS         | 277.0417  | 275.6250  |
| LOW (.001 MG/L)  | 274.6250  | 277.9583  |
| INT (0.008 MG/L) | 279.5833  | 283.5417  |
| HIGH (0.08MG/L)  | 254.8636+ | 256.5909+ |
| F RATIO(1)       | 11.849*   | 13.711*   |
| LSD(2)           | 9.0440    | 8.7298    |
| DUNNETT(3)       | 10.9073   | 10.5284   |
| WMS(4)           | 242.3445  | 225.7984  |

(1) RATIO OF AMONG- TO WITHIN-GROUP VARIATION--ONE-FACTOR ANALYSIS OF VARIANCE.

(2) LEAST SIGNIFICANT DIFFERENCE--GIVEN A SIGNIFICANT ( $\alpha=0.05$ ) F RATIO, ANY TWO MEANS DIFFERING BY MORE THAN THE LSD ARE SIGNIFICATLY DIFFERENT WITH A VARIABLE-WISE FALSE POSITIVE ( $\alpha$ ) ERROR RATE OF 0.05.

(3) DUNNETT TEST--ANY TREATMENT MEAN DIFFERING FROM THE CONTROL MEAN BY MORE THAN THE DUNNETT-STATISTIC-IS-SIGNIFICANTLY DIFFERENT FROM THE CONTROL MEAN--- WITH A VARIABLE-WISE FALSE POSITIVE ( $\alpha$ ) ERROR RATE OF 0.05.

(4) WITHIN-GROUP MEAN SQUARE.

+ SIGNIFICANTLY DIFFERENT ( $P<0.05$ ) FROM CONTROL GROUP BY LSD.

\* SIGNIFICANTLY DIFFERENT ( $P<0.05$ ) FROM CONTROL GROUP BY DUNNETT TEST AND LSD.

\* SIGNIFICANT AT THE 0.05 PROBABILITY LEVEL.

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APPENDIX 1 (cont'd)

BODY WEIGHT SUMMARY

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INTERVALS IN TEST DAYS

| GROUP            | 15.       | 16.       | 17.       | 18.       | 19.       |
|------------------|-----------|-----------|-----------|-----------|-----------|
| CONTROLS         | 295.4211  | 300.6316  | 303.5789  | 302.8947  | 311.7368  |
| LOW (.001 MG/L)  | 294.5789  | 301.0000  | 305.6842  | 305.8421  | 314.3158  |
| INT (0.008 MG/L) | 306.3684  | 311.1053  | 317.2105* | 317.2632* | 326.1053* |
| HIGH (0.08MG/L)  | 283.1765+ | 287.7059+ | 294.8235  | 292.2353  | 304.7647  |
| F RATIO(1)       | 5.756*    | 5.447*    | 5.240*    | 6.347*    | 4.494*    |
| LSD(2)           | 10.9620   | 11.3719   | 11.2223   | 11.3577   | 11.7205   |
| DUNNETT(3)       | 13.2205   | 13.7149   | 13.5345   | 13.6978   | 14.1352   |
| WMS(4)           | 280.0308  | 301.3677  | 293.4909  | 300.6151  | 320.1234  |

INTERVALS IN TEST DAYS

| GROUP            | 22.       | 23.       | 24.       | 25.       | 26.       |
|------------------|-----------|-----------|-----------|-----------|-----------|
| CONTROLS         | 323.7368  | 329.4211  | 342.5789  | 348.0526  | 350.7368  |
| LOW (.001 MG/L)  | 327.8421  | 335.0526  | 345.6316  | 351.8947  | 355.9474  |
| INT (0.008 MG/L) | 340.6842* | 346.5789* | 358.4737+ | 365.2105+ | 369.7368* |
| HIGH (0.08MG/L)  | 320.0000  | 326.8235  | 341.8824  | 348.8824  | 351.235   |
| F RATIO(1)       | 4.094*    | 3.609*    | 2.432     | 2.459     | 3.017*    |
| LSD(2)           | 12.5176   | 13.0048   | 14.0325   | 14.4293   | 14.4640   |
| DUNNETT(3)       | 15.0966   | 15.6841   | 16.9236   | 17.4021   | 17.4440   |
| WMS(4)           | 365.1474  | 394.1240  | 458.8793  | 485.1951  | 487.5339  |

(1) RATIO OF AMONG- TO WITHIN-GROUP VARIATION--ONE-FACTOR ANALYSIS OF VARIANCE.

(2) LEAST SIGNIFICANT DIFFERENCE--GIVEN A SIGNIFICANT (ALPHA=0.05) F RATIO, ANY TWO MEANS DIFFERING BY MORE THAN THE LSD ARE SIGNIFICANTLY DIFFERENT WITH A VARIABLE-WISE FALSE POSITIVE (ALPHA) ERROR RATE OF 0.05.

(3) DUNNETT TEST--ANY TREATMENT MEAN DIFFERING FROM THE CONTROL MEAN BY MORE THAN THE DUNNETT STATISTIC IS SIGNIFICANTLY DIFFERENT FROM THE CONTROL MEAN WITH A VARIABLE-WISE FALSE POSITIVE (ALPHA) ERROR RATE OF 0.05.

(4) WITHIN-GROUP MEAN SQUARE.

+ SIGNIFICANTLY DIFFERENT (P<0.05) FROM CONTROL GROUP BY LSD.

\* SIGNIFICANTLY DIFFERENT (P<0.05) FROM CONTROL GROUP BY DUNNETT TEST AND LSD.

\* SIGNIFICANT AT THE 0.05 PROBABILITY LEVEL.

Company Sanitized. Does not contain TSCA OBI

APPENDIX 1 (cont'd)

BODY WEIGHT SUMMARY  
H# 12037

INTERVALS IN TEST DAYS

| GROUP            | 30.      | 33.       | 37.      | 40.      |
|------------------|----------|-----------|----------|----------|
| CONTROLS         | 375.0000 | 388.5000  | 403.2143 | 414.4286 |
| LOW (.001 MG/L)  | 376.8571 | 392.7857  | 401.9286 | 416.1429 |
| INT (0.008 MG/L) | 393.9286 | 410.3571+ | 424.0000 | 435.9286 |
| HIGH (0.08MG/L)  | 376.6667 | 393.1667  | 407.6667 | 420.4167 |
| F RATIO(1)       | 1.687    | 1.750     | 1.736    | 1.418    |
| LSD(2)           | 19.8178  | 21.1564   | 22.3711  | 23.8561  |
| DUNNETT(3)       | 23.9589  | 25.5772   | 27.0457  | 28.6409  |
| WMS(4)           | 653.2662 | 744.4948  | 832.4391 | 946.6198 |

(1) RATIO OF AMONG- TO WITHIN-GROUP VARIATION--ONE-FACTOR ANALYSIS OF VARIANCE.

(2) LEAST SIGNIFICANT DIFFERENCE--GIVEN A SIGNIFICANT ( $\alpha=0.05$ ) F RATIO, ANY TWO MEANS DIFFERING BY MORE THAN THE LSD ARE SIGNIFICATLY DIFFERENT WITH A VARIABLE-WISE FALSE POSITIVE ( $\alpha$ ) ERROR RATE OF 0.05.

(3) DUNNETT TEST--ANY TREATMENT MEAN DIFFERING FROM THE CONTROL MEAN BY MORE THAN THE DUNNETT STATISTIC IS SIGNIFICANTLY DIFFERENT FROM THE CONTROL MEAN WITH A VARIABLE-WISE FALSE POSITIVE ( $\alpha$ ) ERROR RATE OF 0.05.

(4) WITHIN-GROUP MEAN SQUARE.

SIGNIFICANTLY DIFFERENT ( $P<0.05$ ) FROM CONTROL GROUP BY LSD.

\* SIGNIFICANTLY DIFFERENT ( $P<0.05$ ) FROM CONTROL GROUP BY DUNNETT TEST AND LSD.

[REDACTED] BODY WEIGHT SUMMARY  
[REDACTED] H# 12037

## INTERVALS IN TEST DAYS

| GROUP            | 44.      | 46.      | 51.       | 54.       | 58.       |
|------------------|----------|----------|-----------|-----------|-----------|
| CONTROLS         | 433.7778 | 440.2222 | 459.3333  | 473.1111  | 475.5000  |
| LOW (.001 MG/L)  | 438.8889 | 445.0000 | 463.8889  | 471.7778  | 468.7500  |
| INT (0.008 MG/L) | 452.8889 | 459.4444 | 476.4444  | 486.5556  | 485.0000  |
| HIGH (0.08MG/L)  | 423.1429 | 430.2857 | 450.2857  | 460.2857  | 481.0000  |
| F RATIO(1)       | 1.284    | 1.250    | 0.821     | 0.664     | 0.114     |
| LSD(2)           | 30.9153  | 30.8398  | 34.1047   | 37.2445   | 66.8715   |
| DUNNETT(3)       | 37.8438  | 37.7513  | 41.7479   | 45.5914   | 83.8552   |
| WMS(4)           | 962.4064 | 957.7069 | 1171.2180 | 1396.8032 | 1704.1591 |

(1) RATIO OF AMONG- TO WITHIN-GROUP VARIATION--ONE-FACTOR ANALYSIS OF VARIANCE.

(2) LEAST SIGNIFICANT DIFFERENCE--GIVEN A SIGNIFICANT ( $\alpha=0.05$ ) F RATIO, ANY TWO MEANS DIFFERING BY MORE THAN THE LSD ARE SIGNIFICANTLY DIFFERENT WITH A VARIABLE-WISE FALSE POSITIVE ( $\alpha$ ) ERROR RATE OF 0.05.

(3) DUNNETT TEST--ANY TREATMENT MEAN DIFFERING FROM THE CONTROL MEAN BY MORE THAN THE DUNNETT STATISTIC IS SIGNIFICANTLY DIFFERENT FROM THE CONTROL MEAN WITH A VARIABLE-WISE FALSE POSITIVE ( $\alpha$ ) ERROR RATE OF 0.05.

(4) WITHIN-GROUP MEAN SQUARE.

+ SIGNIFICANTLY DIFFERENT ( $P<0.05$ ) FROM CONTROL GROUP BY LSD.

\* SIGNIFICANTLY DIFFERENT ( $P<0.05$ ) FROM CONTROL GROUP BY DUNNETT TEST AND LSD.

Company Sanitized. Does not contain TSCA CBI.

APPENDIX 1 (cont'd)

BODY WEIGHT SUMMARY  
H# 12037

INTERVALS IN TEST DAYS

| GROUP            | 61.       | 65.       | 68.       | 72.       | 75.       |
|------------------|-----------|-----------|-----------|-----------|-----------|
| CONTROLS         | 483.2500  | 494.2500  | 503.0000  | 507.7500  | 514.5000  |
| LOW (.001 MG/L)  | 476.2500  | 486.0000  | 494.0000  | 500.2500  | 510.0000  |
| INT (0.008 MG/L) | 493.0000  | 503.7500  | 512.0000  | 519.0000  | 529.5000  |
| HIGH (0.08MG/L)  | 491.6667  | 504.3333  | 513.0000  | 526.6667  | 535.0000  |
| F RATIO(1)       | 0.133     | 0.174     | 0.148     | 0.220     | 0.209     |
| LSD(2)           | 67.8778   | 70.2745   | 72.5293   | 70.3120   | 80.0436   |
| DUNNETT(3)       | 85.1171   | 88.1225   | 90.9500   | 95.6934   | 100.3727  |
| WMS(4)           | 1755.8333 | 1882.0152 | 2004.7273 | 2219.2879 | 2441.6364 |

INTERVALS IN TEST DAYS

| GROUP            | 79.       | 82.       | 86.       | 89.       | 93.       |
|------------------|-----------|-----------|-----------|-----------|-----------|
| CONTROLS         | 523.2500  | 528.7500  | 536.7500  | 543.2500  | 549.7500  |
| LOW (.001 MG/L)  | 517.2500  | 523.5000  | 528.2500  | 533.7500  | 541.2500  |
| INT (0.008 MG/L) | 538.5000  | 545.5000  | 551.0000  | 557.0000  | 563.2500  |
| HIGH (0.08MG/L)  | 543.0000  | 554.0000  | 558.3333  | 565.6667  | 576.3333  |
| F RATIO(1)       | 0.224     | 0.278     | 0.241     | 0.231     | 0.252     |
| LSD(2)           | 79.6470   | 82.4204   | 84.8947   | 90.1867   | 92.6716   |
| DUNNETT(3)       | 99.8754   | 103.3532  | 105.4559  | 113.0919  | 116.2079  |
| WMS(4)           | 2417.5000 | 2588.7954 | 2746.5606 | 3099.6516 | 3272.8106 |

INTERVALS IN TEST DAYS

| GROUP            | 96.       |
|------------------|-----------|
| CONTROLS         | 554.7500  |
| LOW (.001 MG/L)  | 541.0000  |
| INT (0.008 MG/L) | 566.0000  |
| HIGH (0.08MG/L)  | 578.6667  |
| F RATIO(1)       | 0.284     |
| LSD(2)           | 91.5977   |
| DUNNETT(3)       | 114.8613  |
| WMS(4)           | 3197.4016 |

Company Sanitized. Does not contain TSCA CBI

APPENDIX 1 (cont'd)

- (1) RATIO OF AMONG- TO WITHIN-GROUP VARIATION--ONE-FACTOR ANALYSIS OF VARIANCE.
  - (2) LEAST SIGNIFICANT DIFFERENCE--GIVEN A SIGNIFICANT ( $\alpha=0.05$ ) F RATIO, ANY TWO MEANS DIFFERING BY MORE THAN THE LSD ARE SIGNIFICANTLY DIFFERENT WITH A VARIABLE-WISE FALSE POSITIVE ( $\alpha$ ) ERROR RATE OF 0.05.
  - (3) DUNNETT TEST--ANY TREATMENT MEAN DIFFERING FROM THE CONTROL MEAN BY MORE THAN THE DUNNETT STATISTIC IS SIGNIFICANTLY DIFFERENT FROM THE CONTROL MEAN WITH A VARIABLE-WISE FALSE POSITIVE ( $\alpha$ ) ERROR RATE OF 0.05.
  - (4) WITHIN-GROUP MEAN SQUARE.
- + SIGNIFICANTLY DIFFERENT ( $P<0.05$ ) FROM CONTROL GROUP BY LSD.
- # SIGNIFICANTLY DIFFERENT ( $P<0.05$ ) FROM CONTROL GROUP BY DUNNETT TEST AND LSD.

Company Sanitized. Does not contain TSCA CBI

APPENDIX 2

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Company Sanitized. Does not contain TSCA CBI

**APPENDIX 2**  
**ORGAN/BODY WEIGHT MEAN ABSOLUTE DATA**  
**H# 12037 RATS SACRIFICED AFTER 10 EXPOSURES**

| GROUP                  | FINAL WGT | HEART  | LUNGS   | LIVER    | SPLEEN |
|------------------------|-----------|--------|---------|----------|--------|
| CONTROLS               | 266.8000  | .9420  | 1.4340  | 10.5340  | .5380  |
| LOW LEVEL(0.001MG/L)   | 284.0000+ | 1.0000 | 1.6220  | 11.5680  | .6480  |
| INT. LEVEL(0.008MG/L)  | 281.0000  | .9700  | 1.5680  | 15.9760# | .6880  |
| + HIGH LEVEL(0.08MG/L) | 262.8000  | .9000  | 1.6720+ | 16.4300# | .4320  |
| F RATIO(1)             | 3.964*    | 1.404  | 1.761   | 14.424*  | 5.736* |
| LSD(2)                 | 15.6916   | .1076  | .2316   | 2.3757   | .1444  |
| DUNNETT(3)             | 19.4673   | .1335  | .2874   | 2.9473   | .1791  |
| WMS(4)                 | 136.9750  | .0064  | .0298   | 3.1397   | .0116  |

| GROUP                 | KIDNEY  | TESTIS  | THYMUS  |
|-----------------------|---------|---------|---------|
| CONTROLS              | 2.3020  | 2.7580  | .6140   |
| LOW LEVEL(0.001MG/L)  | 2.4860  | 3.0280+ | .5540   |
| INT. LEVEL(0.008MG/L) | 2.5840+ | 3.0140+ | .6640   |
| HIGH LEVEL(0.08MG/L)  | 2.4200  | 3.0180+ | .4100#  |
| F RATIO(1)            | 1.648   | 2.982   | 10.293* |
| LSD(2)                | .2765   | .2277   | .1027   |
| DUNNETT(3)            | .3430   | .2825   | .1275   |
| WMS(4)                | .0425   | .0288   | .0059   |

- (1) Ratio of among- to within-group variation--one-factor analysis of variance.  
(2) Least significant difference--given a significant (alpha=0.05) F ratio, any two means differing by more than the LSD are significantly different with a variable-wise false positive (alpha) error rate of 0.05.  
(3) Dunnett test--Any treatment mean differing from the control mean by more than the Dunnett statistic is significantly different from the control mean with a variable-wise false positive (alpha) error rate of 0.05.  
(4) Within-group Mean Square.  
+ Significantly different (P<0.05) from control group by LSD.  
# Significantly different (P<0.05) from control group by Dunnett test and LSD.  
\* Significant at the 0.05 probability level.

Company Sanitized. Does not contain TSCA CBI

**APPENDIX 2 (cont'd)**  
**ORGAN/BODY WEIGHT MEAN ABSOLUTE DATA**  
**H# 12037 RATS SACRIFICED AFTER 14 DAY OBSERVATION PERIOD**

| GROUP                | FINAL WGT | HEART  | LUNGS  | LIVER    | SPLEEN |
|----------------------|-----------|--------|--------|----------|--------|
| CONTROLS             | 337.6000  | 1.1820 | 1.8820 | 14.2860  | .7280  |
| LOW LEVEL(0.001MG/L) | 349.6000  | 1.2900 | 1.8320 | 14.8880  | .7900  |
| INT LEVEL(0.008MG/L) | 362.2000  | 1.2940 | 2.0080 | 16.9420# | .8580  |
| HIGH LEVEL(0.08MG/L) | 354.0000  | 1.1860 | 1.7560 | 18.3940# | .7100  |
| F RATIO(1)           | 1.400     | 1.745  | 1.360  | 7.343*   | 1.839  |
| LSD(2)               | 25.9962   | .1416  | .2722  | 2.0922   | .1483  |
| DUNNETT(3)           | 32.2516   | .1757  | .3377  | 2.5936   | .1840  |
| WMS(4)               | 375.9500  | .0112  | .0412  | 2.4351   | .0122  |

| GROUP                | KIDNEY | TESTIS  | THYMUS |
|----------------------|--------|---------|--------|
| CONTROLS             | 2.7860 | 3.0120  | .8780  |
| LOW LEVEL(0.001MG/L) | 2.8920 | 3.2100  | .7980  |
| INT LEVEL(0.008MG/L) | 2.9800 | 3.0740  | .9300  |
| HIGH LEVEL(0.08MG/L) | 2.9320 | 3.3620+ | .7420  |
| F RATIO(1)           | .825   | 1.934   | 1.453  |
| LSD(2)               | .2725  | .3252   | .2075  |
| DUNNETT(3)           | .3381  | .4159   | .2574  |
| WMS(4)               | .0413  | .0625   | .0240  |

- (1) Ratio of among- to within-group variation--one-factor analysis of variance.  
(2) Least significant difference--given a significant ( $\alpha=0.05$ ) F ratio, any two means differing by more than the LSD are significantly different with a variable-wise false positive ( $\alpha$ ) error rate of 0.05.  
(3) Dunnett test--Any treatment mean differing from the control mean by more than the Dunnett statistic is significantly different from the control mean with a variat -wise false positive ( $\alpha$ ) error rate of 0.05.  
(4) Within-group Mean Square.  
+ Significantly different ( $P<0.05$ ) from control group by LSD.  
# Significantly different ( $P<0.05$ ) from control group by Dunnett test and LSD.  
\* Significant at the 0.05 probability level.

Company Sanitized. Does not contain TSCA CBI

**APPENDIX 2 (CONT'D)**  
**ORGAN/BODY WEIGHT MEAN ABSOLUTE DATA**  
**HY 12037 RATS SACRIFICED AFTER 28 DAY OBSERVATION PERIOD**

| GROUP                | FINAL WGT | HEART   | LUNGS  | LIVER    | SPLEEN |
|----------------------|-----------|---------|--------|----------|--------|
| CONTROLS             | 408.0000  | 1.2620  | 1.9360 | 15.6660  | .7400  |
| LOW LEVEL(0.001MG/L) | 400.6000  | 1.3780  | 2.1260 | 15.8460  | .8400  |
| INT LEVEL(0.008MG/L) | 438.6000  | 1.4600+ | 1.9360 | 18.2420+ | .9140+ |
| HIGH LEVEL(0.08MG/L) | 441.4000  | 1.4600+ | 2.1580 | 19.0520# | .8680  |
| F RATIO(1)           | 2.330     | 2.557   | 1.367  | 4.940*   | 1.907  |
| LSD(2)               | 40.9799   | .1756   | .3068  | 2.2972   | .1598  |
| DUNNETT(3)           | 50.8407   | .2179   | .3806  | 2.8500   | .1983  |
| WMS(4)               | 934.2250  | .0172   | .0524  | 2.9357   | .0142  |

| GROUP                | KIDNEY  | TESTIS | THYMUS |
|----------------------|---------|--------|--------|
| CONTROLS             | 2.8640  | 3.2320 | .7040  |
| LOW LEVEL(0.001MG/L) | 3.0460  | 3.5160 | .8220  |
| INT LEVEL(0.008MG/L) | 3.3580# | 3.4420 | .9100  |
| HIGH LEVEL(0.08MG/L) | 3.4720# | 3.3720 | .8140  |
| F RATIO(1)           | 6.384*  | .949   | 1.087  |
| LSD(2)               | .3319   | .3721  | .2427  |
| DUNNETT(3)           | .4117   | .4617  | .3011  |
| WMS(4)               | .0613   | .0770  | .0328  |

- (1) Ratio of among- to within-group variation--one-factor analysis of variance.  
(2) Least significant difference--given a significant (alpha=0.05) F ratio, any two means differing by more than the LSD are significantly different with a variable-wise false positive (alpha) error rate of 0.05.  
(3) Dunnett test--Any treatment mean differing from the control mean by more than the Dunnett statistic is significantly different from the control mean with a variable-wise false positive (alpha) error rate of 0.05.  
(4) Within-group Mean Square.  
+ Significantly different (P<0.05) from control group by LSD.  
# Significantly different (P<0.05) from control group by Dunnett test and LSD.  
\* Significant at the 0.05 probability level.

Company Sanitized. Does not contain TSCA CBI

**ORGAN/BODY WEIGHT MEAN ABSOLUTE DATA**  
**HF 12037 RATS SACRIFICED AFTER 42 DAY OBSERVATION PERIOD**

| GROUP                | FINAL WGT | HEART  | LUNGS  | LIVER   | SPLEEN |
|----------------------|-----------|--------|--------|---------|--------|
| CONTROLS             | 481.6000  | 1.4720 | 2.0560 | 17.5420 |        |
| LOW LEVEL(0.001MG/L) | 483.8000  | 1.4760 | 2.1520 | 17.3360 | .8760  |
| INT LEVEL(0.008MG/L) | 493.0000  | 1.4750 | 2.3625 | 18.6275 | .8840  |
| HIGH LEVEL(0.08MG/L) | 453.0000  | 1.4000 | 2.0550 | 17.2750 | .9675  |
|                      |           |        |        |         | .8000  |
| F RATIO(1)           | .825      |        |        |         |        |
| LSD(2)               | 55.0991   | .407   | 1.439  | .453    | 1.064  |
| DUNNETT(3)           | 68.5913   | .1709  | .3537  | 2.7410  | .1912  |
| WMS(4)               | 1466.5714 | .2127  | .4403  | 3.4122  | .2380  |
|                      |           | .0141  | .0604  | 3.6293  | .0177  |

| GROUP                | KIDNEY | TESTIS | THYMUS |
|----------------------|--------|--------|--------|
| CONTROLS             | 3.0880 | 3.4880 | .8740  |
| LOW LEVEL(0.001MG/L) | 3.4360 | 3.5620 | .8280  |
| INT LEVEL(0.008MG/L) | 3.2075 | 2.8575 | .8450  |
| HIGH LEVEL(0.08MG/L) | 3.2325 | 3.6625 | .8375  |
| F RATIO(1)           | 1.136  | 1.555  | .061   |
| LSD(2)               | .4353  | .8484  | .2558  |
| DUNNETT(3)           | .5419  | 1.0561 | .3184  |
| WMS(4)               | .0915  | .3477  | .0316  |

- (1) Ratio of among- to within-group variation--one-factor analysis of variance.
- (2) Least significant difference--given a significant (alpha=0.05) F ratio, any two means differing by more than the LSD are significantly different with a variable-wise false positive (alpha) error rate of 0.05.
- (3) Dunnett test--Any treatment mean differing from the control mean by more than the Dunnett statistic is significantly different from the control mean with a variable-wise false positive (alpha) error rate of 0.05.
- (4) Within-group Mean Square.
- + Significantly different (P<0.05) from control group by LSD.
- \* Significantly different (P<0.05) from control group by Dunnett test and LSD.

Company Sanitized. Does not contain TSCA CBI

| GROUP                | FINAL WGT | HEART  | LUNGS  | LIVER   | SPLEEN  |
|----------------------|-----------|--------|--------|---------|---------|
| CONTROLS             | 554.7500  | 1.4850 | 2.2150 | 18.3800 | .7775   |
| LOW LEVEL(0.001MG/L) | 541.0000  | 1.6475 | 2.1875 | 19.4525 | .9325   |
| INT LEVEL(0.008MG/L) | 566.0000  | 1.6675 | 2.4175 | 20.4775 | 1.1100# |
| HIGH LEVEL(0.08MG/L) | 578.6667  | 1.6067 | 2.2700 | 18.5267 | .8633   |
| F RATIO(1)           | .284      | .863   | 1.345  | .558    | 4.975*  |
| LSD(2)               | 91.5977   | .2852  | .2865  | 4.1112  | .2035   |
| DUNNETT(3)           | 114.8613  | .3576  | .3592  | 5.1553  | .2552   |
| WMS(4)               | 3197.4016 | .0310  | .0313  | 6.4411  | .0158   |

| GROUP                | KIDNEY | TESTIS | THYMUS |
|----------------------|--------|--------|--------|
| CONTROLS             | 3.3975 | 3.4575 | .6425  |
| LOW LEVEL(0.001MG/L) | 3.7675 | 3.5950 | .5925  |
| INT LEVEL(0.008MG/L) | 3.9375 | 3.8175 | .6625  |
| HIGH LEVEL(0.08MG/L) | 3.6967 | 3.6000 | .6533  |
| F RATIO(1)           | 1.462  | .691   | .333   |
| LSD(2)               | .6042  | .5797  | .1734  |
| DUNNETT(3)           | .7576  | .7270  | .2175  |
| WMS(4)               | .1391  | .1281  | .0115  |

- (1) Ratio of among- to within-group variation--one-factor analysis of variance.  
 (2) Least significant difference--given a significant ( $\alpha=0.05$ ) F ratio, any two means differing by more than the LSD are significantly different with a variable-wise false positive ( $\alpha$ ) error rate of 0.05.  
 (3) Dunnett test--Any treatment mean differing from the control mean by more than the Dunnett statistic is significantly different from the control mean with a variable-wise false positive ( $\alpha$ ) error rate of 0.05.  
 (4) Within-group Mean Square.  
 + Significantly different ( $P<0.05$ ) from control group by LSD.  
 # Significantly different ( $P<0.05$ ) from control group by Dunnett test and LSD.  
 \* Significant at the 0.05 probability level.

Company Sanitized. Does not contain TSCA CBI

APPENDIX B

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Company Sanitized. Does not contain TSCA CBI

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**ORGAN/BODY WEIGHT MEAN RELATIVE DATA**  
**H# 12037 RATS SACRIFICED AFTER 10 EXPOSURES**

| GROUP                 | HEART | LUNGS  | LIVER   | SPLEEN | KIDNEY |
|-----------------------|-------|--------|---------|--------|--------|
| CONTROLS              | .3534 | .5371  | 3.9259  | .2032  | .8635  |
| LOW LEVEL(0.001MG/L)  | .3519 | .5698  | 4.0695  | .2269  | .8750  |
| INT. LEVEL(0.008MG/L) | .3446 | .5589  | 5.6781# | .2454  | .9188  |
| HIGH LEVEL(0.08MG/L)  | .3424 | .6365# | 6.2513# | .1644  | .9211  |
| F RATIO(1)            | .277  | 2.920  | 24.180* | 4.059* | 1.132  |
| LSD(2)                | .0308 | .0752  | .7078   | .0520  | .0835  |
| DUNNETT(3)            | .0382 | .0933  | .8781   | .0645  | .1036  |
| WMS(4)                | .0005 | .0031  | .2787   | .0015  | .0039  |

| GROUP                 | TESTIS  | THYMUS  |
|-----------------------|---------|---------|
| CONTROLS              | 1.0366  | .2300   |
| LOW LEVEL(0.001MG/L)  | 1.0661  | .1950+  |
| INT. LEVEL(0.008MG/L) | 1.0751  | .2361   |
| HIGH LEVEL(0.08MG/L)  | 1.1482+ | .1563#  |
| F RATIO(1)            | 2.238   | 10.410* |
| LSD(2)                | .0950   | .0342   |
| DUNNETT(3)            | .1179   | .0424   |
| WMS(4)                | .0050   | .0007   |

- (1) Ratio of among- to within-group variation--one-factor analysis of variance.  
 (2) Least significant difference--given a significant ( $\alpha=0.05$ ) F ratio, any two means differing by more than the LSD are significantly different with a variable-wise false positive ( $\alpha$ ) error rate of 0.05.  
 (3) Dunnett test--Any treatment mean differing from the control mean by more than the Dunnett statistic is significantly different from the control mean with a variable-wise false positive ( $\alpha$ ) error rate of 0.05.  
 (4) Within-group Mean Square.  
 + Significantly different ( $P<0.05$ ) from control group by LSD.  
 # Significantly different ( $P<0.05$ ) from control group by Dunnett test and LSD.  
 \* Significant at the 0.05 probability level.

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## APPENDIX 3 (cont'd)

ORGAN/BODY WEIGHT MEAN RELATIVE DATA  
 H# 12037 RATS SACRIFICED AFTER 14 DAY OBSERVATION PERIOD

| GROUP                | HEART | LUNGS | LIVER   | SPLEEN | KIDNEY |
|----------------------|-------|-------|---------|--------|--------|
| CONTROLS             | .3504 | .5574 | 4.2285  | .2155  | .8241  |
| LOW LEVEL(0.001MG/L) | .3689 | .5247 | 4.2593  | .2266  | .8278  |
| INT LEVEL(0.008MG/L) | .3574 | .5541 | 4.6752  | .2374  | .8237  |
| HIGH LEVEL(0.08MG/L) | .3359 | .4965 | 5.2236* | .2000  | .8301  |
| F RATIO(1)           | 1.192 | 1.473 | 5.105*  | 1.385  | .019   |
| LSD(2)               | .0379 | .0705 | .6170   | .0407  | .0671  |
| DUNNETT(3)           | .0470 | .0874 | .7655   | .0505  | .0833  |
| WMS(4)               | .0008 | .0028 | .2118   | .0009  | .0025  |

| GROUP                | TESTIS | THYMUS |
|----------------------|--------|--------|
| CONTROLS             | .8922  | .2598  |
| LOW LEVEL(0.001MG/L) | .9201  | .2278  |
| INT LEVEL(0.008MG/L) | .8506  | .2563  |
| HIGH LEVEL(0.08MG/L) | .9527  | .2104  |
| F RATIO(1)           | 1.555  | 1.696  |
| LSD(2)               | .1041  | .0543  |
| DUNNETT(3)           | .1291  | .0674  |
| WMS(4)               | .0060  | .0016  |

- (1) Ratio of among- to within-group variation--one-factor analysis of variance.  
 (2) Least significant difference--given a significant ( $\alpha=0.05$ ) F ratio, any two means differing by more than the LSD are significantly different with a variable-wise false positive ( $\alpha$ ) error rate of 0.05.  
 (3) Dunnett test--Any treatment mean differing from the control mean by more than the Dunnett statistic is significantly different from the control mean with a variable-wise false positive ( $\alpha$ ) error rate of 0.05.  
 (4) Within-group Mean Square.  
 + Significantly different ( $P<0.05$ ) from control group by LSD.  
 \* Significantly different ( $P<0.05$ ) from control group by Dunnett test and LSD.  
 \* Significant at the 0.05 probability level.

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**ORGAN/BODY WEIGHT MEAN RELATIVE DATA**  
**H# 12037 RATS SACRIFICED AFTER 28 DAY OBSERVATION PERIOD**

| GROUP                | HEART  | LUNGS | LIVER   | SPLEEN | KIDNEY |
|----------------------|--------|-------|---------|--------|--------|
| CONTROLS             | .3094  | .4739 | 3.8374  | .1812  | .7023  |
| LOW LEVEL(0.001MG/L) | .3432+ | .5323 | 3.9509  | .2094  | .7618+ |
| INT LEVEL(0.008MG/L) | .3338  | .4439 | 4.1562  | .2073  | .7666+ |
| HIGH LEVEL(0.08MG/L) | .3311  | .4886 | 4.3190# | .1969  | .7873# |
| F RATIO(1)           | 2.060  | 2.576 | 3.800*  | 1.769  | 3.408* |
| LSD(2)               | .0298  | .0687 | .3295   | .0291  | .0593  |
| DUNNETT(3)           | .0370  | .0853 | .4087   | .0361  | .0735  |
| WMS(4)               | .0005  | .0026 | .0604   | .0005  | .0020  |

| GROUP                | TESTIS | THYMUS |
|----------------------|--------|--------|
| CONTROLS             | .7949  | .1724  |
| LOW LEVEL(0.001MG/L) | .8799  | .2061  |
| INT LEVEL(0.008MG/L) | .7873  | .2065  |
| HIGH LEVEL(0.08MG/L) | .7646  | .1840  |
| F RATIO(1)           | 2.540  | .981   |
| LSD(2)               | .0950  | .0512  |
| DUNNETT(3)           | .1178  | .0635  |
| WMS(4)               | .0050  | .0015  |

- (1) Ratio of among- to within-group variation--one-factor analysis of variance.  
(2) Least significant difference--given a significant ( $\alpha=0.05$ ) F ratio, any two means differing by more than the LSD are significantly different with a variable-wise false positive ( $\alpha$ ) error rate of 0.05.  
(3) Dunnett test--Any treatment mean differing from the control mean by more than the Dunnett statistic is significantly different from the control mean with a variable-wise false positive ( $\alpha$ ) error rate of 0.05.  
(4) Within-group Mean Square.  
+ Significantly different ( $P<0.05$ ) from control group by LSD.  
# Significantly different ( $P<0.05$ ) from control group by Dunnett test and LSD.  
\* Significant at the 0.05 probability level.

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**ORGAN/BODY WEIGHT MEAN RELATIVE DATA**  
**HP 12037 RATS SACRIFICED AFTER 42 DAY OBSERVATION PERIOD**

| GROUP                | HEART | LUNGS | LIVER  | SPLEEN | KIDNEY |
|----------------------|-------|-------|--------|--------|--------|
| CONTROLS             | .3067 | .4286 | 3.6410 | .1825  | .6442  |
| LOW LEVEL(0.001MG/L) | .3065 | .4464 | 3.5886 | .1829  | .7144  |
| INT LEVEL(0.008MG/L) | .2993 | .4795 | 3.7843 | .1959  | .6512  |
| HIGH LEVEL(0.08MG/L) | .3098 | .4571 | 3.7997 | .1753  | .7127  |
| F RATIO(1)           | .112  | .620  | .688   | .640   | 1.447  |
| LSD(2)               | .0383 | .0817 | .3839  | .0309  | .0974  |
| DUNNETT(3)           | .0476 | .1017 | .4779  | .0383  | .1213  |
| WMS(4)               | .0007 | .0032 | .0712  | .0003  | .0046  |

| GROUP                | TESTIS | THYMUS |
|----------------------|--------|--------|
| CONTROLS             | .7257  | .1811  |
| LOW LEVEL(0.001MG/L) | .7417  | .1719  |
| INT LEVEL(0.008MG/L) | .5846  | .1710  |
| HIGH LEVEL(0.08MG/L) | .8095  | .1848  |
| F RATIO(1)           | 2.219  | .171   |
| LSD(2)               | .1830  | .0492  |
| DUNNETT(3)           | .2278  | .0613  |
| WMS(4)               | .0162  | .0012  |

- (1) Ratio of among- to within-group variation--one-factor analysis of variance.  
(2) Least significant difference--given a significant ( $\alpha=0.05$ ) F ratio, any two means differing by more than the LSD are significantly different with a variable-wise false positive ( $\alpha$ ) error rate of 0.05.  
(3) Dunnett test--Any treatment mean differing from the control mean by more than the Dunnett statistic is significantly different from the control mean with a variable-wise false positive ( $\alpha$ ) error rate of 0.05.  
(4) Within-group Mean Square.  
+ Significantly different ( $P<0.05$ ) from control group by LSD.  
# Significantly different ( $P<0.05$ ) from control group by Dunnett test and LSD.

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[REDACTED] H# 12037 ORGAN/BODY WEIGHT MEAN RELATIVE DATA  
RATS SACRIFICED AFTER 24 DAY OBSERVATION PERIOD

| GROUP                | HEART  | LUNGS | LIVER  | SPLEEN | KIDNEY |
|----------------------|--------|-------|--------|--------|--------|
| CONTROLS             | .2668  | .4015 | 3.3153 | .1399  | .6119  |
| LOW LEVEL(0.001MG/L) | .3046# | .4054 | 3.5910 | .1723+ | .6960# |
| INT LEVEL(0.008MG/L) | .2946+ | .4276 | 3.5955 | .1964# | .6955# |
| HIGH LEVEL(0.08MG/L) | .2787  | .3965 | 3.2076 | .1507  | .6424  |
| F RATIO(1)           | 5.485* | .487  | 3.609* | 7.572* | 7.540* |
| LSD(2)               | .0229  | .0617 | .3160  | .0290  | .0485  |
| DUNNETT(3)           | .0288  | .0774 | .3963  | .0364  | .0609  |
| WMS(4)               | .0002  | .0015 | .0381  | .0003  | .0009  |

| GROUP                | TESTIS | THYMUS |
|----------------------|--------|--------|
| CONTROLS             | .6215  | .1153  |
| LOW LEVEL(0.001MG/L) | .6640  | .1102  |
| INT LEVEL(0.008MG/L) | .6779  | .1171  |
| HIGH LEVEL(0.08MG/L) | .6269  | .1155  |
| F RATIO(1)           | 1.276  | .085   |
| LSD(2)               | .0774  | .0327  |
| DUNNETT(3)           | .0970  | .0411  |
| WMS(4)               | .0023  | .0004  |

- (1) Ratio of among- to within-group variation--one-factor analysis of variance.  
 (2) Least significant difference--given a significant ( $\alpha=0.05$ ) F ratio, any two means differing by more than the LSD are significantly different with a variable-wise false positive ( $\alpha$ ) error rate of 0.05.  
 (3) Dunnett test--Any treatment mean differing from the control mean by more than the Dunnett statistic is significantly different from the control mean with a variable-wise false positive ( $\alpha$ ) error rate of 0.05.  
 (4) Within-group Mean Square.  
 + Significantly different ( $P<0.05$ ) from control group by LSD.  
 # Significantly different ( $P<0.05$ ) from control group by Dunnett test and LSD.  
 \* Significant at the 0.05 probability level.

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APPENDIX 4

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WILMINGTON, DELAWARE 19898

POLYMER PRODUCTS DEPARTMENT  
EXPERIMENTAL STATION

ANALYTICAL REPORT

January 26, 1981

G. L. KENNEDY  
CENTRAL RESEARCH AND DEVELOPMENT DEPARTMENT  
HASKELL LAB

DETERMINATION OF PERFLUOROOCTANOIC ACID IN RAT BLOOD SAMPLES  
(JOB No. 800-598, 802-5477, 800-5562; PRAL Nos. 80-60270-376, 80-67459-485,  
80-67751-758, 80-68604-674)

As part of your [redacted] inhalation subacute test, a series of rat blood samples has been analyzed for perfluorooctanoate (C<sub>8</sub>, acid and salts). A listing of the 107 samples received 6/80, with identification numbers and current status, is given in Table I. As requested, only the group of 66 samples (plus blanks) indicated there was included in these first analyses. The remaining 26 samples have been dried for storage under the same conditions as the others, but not analyzed at this time.

The analyses were done using the gas chromatographic method developed here last year for specific determination of perfluorooctanoic acid in blood. As we discussed in our meeting on 1/8/81, this method should give values for total C<sub>8</sub> comparable to those of non-specific organic fluorine determination for samples of this type, as well as providing information on the distribution of C<sub>8</sub> isomers which was not previously been available.

A. Comparison of Analytical Methods

A comparison of the GC analysis with the Wickbold Torch analysis for total organic fluorine was recently made with a series of test samples from Parkersburg, and the results found to agree within experimental uncertainty when corrected for recovery of C<sub>8</sub>. Details of the comparison are discussed in a letter from [redacted] to [redacted] 1/23/81.

Seven of these rat blood samples were also sent to [redacted] at Jackson Lab for Wickbold Torch analysis. Results are given in Table II and Figure 1. As noted in the table, recovery of C<sub>8</sub> by the Wickbold Torch procedure

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There's a world of things we're doing something about

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has been determined to be ~83% in recent work, and results have been corrected by this factor for the plot in Figure 1. Overall agreement seems satisfactory, although based on this limited data the extent to which the 0.8-0.9 ppm F in the blank sample (80-67484) could represent a variable contribution to the others cannot be determined.

For the subacute skin test blood samples analyzed at Jackson Lab early in 1980, a correction for C<sub>8</sub> recovery in the 3M Parr Bomb method should also be made before comparison with these GC values. According to [REDACTED] the recovery at that time should have been 92±5%, as reported in the method published by 3M (Analytical Biochemistry 87, 545 [1978]).

#### B. Total Concentration of C<sub>8</sub>

Results are given in Table III for overall C<sub>8</sub> concentration (straight-chain plus four branched isomers), calculated as ppm fluoride to facilitate comparison with non-specific analyses. As noted there, none of the six "test blank" samples were found to contain detectable C<sub>8</sub>, while all except two of the Group I controls did contain measurable amounts.

Average concentrations are plotted against recovery time in Figure 2. For comparison with the earlier dermal study an calculation of half-times, linear least square lines have been drawn through the points up to 42 days (although it is not clear that this is the best overall fit). For all four groups, half-time (log 2/slope) can be seen to be about 8 days.

#### C. Contribution from Branched C<sub>8</sub> Isomers

Chromatograms obtained for three rat blood samples as well as for standards prepared from [REDACTED] and [REDACTED] are shown in Figure 3. (The [REDACTED] used here was the same as that used in your inhalation study.)

As discussed in the method, ECD response is greater for the four branched isomers (peaks 1,2,4 and 5) than for the straight-chain material (peak 3), and their concentrations were estimated using relative response factors as described there. Since the values calculated for the individual isomers are very approximate an attempt has not been made to tabulate them separately, but the percentage of straight-chain C<sub>8</sub> calculated for each sample is shown in Table IV. As noted there, a corresponding value for [REDACTED] standard would be ~74%, and for [REDACTED] standard ~94%. With a few exceptions, values for Sacrifice 1 are lower (greater contribution from branched isomers) than in the later sacrifice, although never as low as that for [REDACTED].

The pattern shown in Figure 3c is also fairly typical for samples containing a high percentage of straight-chain C<sub>8</sub>, with the major isomer contribution coming from peak #5 and a measurable contribution from peak #1

as well. Thus far, the only isomer to which a structure has been assigned is #5, which appears to be [REDACTED] (by comparison of GC and NMR data). Fluorine-19 NMR suggests that the others are [REDACTED] along the chain, but the position has been determined.

S. S. Stafford  
S. S. Stafford

Attachments  
fmc

Key Words:

Blood analysis

[REDACTED]  
Perfluorooctanoate

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**TABLE I**  
**Identification of Rat Blood Samples from [REDACTED] Inhalation Subacute Test (a)**

| <u>Sacrifice</u>           | <u>Group</u>               | <u>Tube #</u> | <u>Rat #</u> | <u>PRAI No.,<br/>As Received</u> | <u>PRAI No.,<br/>Dried</u> | <u>Status (b)</u> |   |
|----------------------------|----------------------------|---------------|--------------|----------------------------------|----------------------------|-------------------|---|
| 1<br>0 Days<br>2/29/80     | I (control)                | 1             | 270322       |                                  |                            |                   |   |
|                            |                            | 2             | 270323       | 80-60270                         | 80-67459                   | A                 |   |
|                            |                            | 3             | 270326       | 80-60271                         | 80-67460                   | A                 |   |
|                            |                            | 4             | 270327       | 80-60272                         | 80-68604                   | A                 |   |
|                            |                            | 5             | 270330       | 80-60273                         | 80-68605                   | A                 |   |
|                            |                            |               |              | 80-60274                         | 80-68606                   | A                 |   |
|                            | II (1 mg/m <sup>3</sup> )  | 6             | 270638       | 80-60275                         |                            |                   |   |
|                            |                            | 7             | 270639       | 80-60276                         | 80-67461                   | A                 |   |
|                            |                            | 8             | 270640       | 80-60277                         | 80-67462                   | A                 |   |
|                            |                            | 9             | 270641       | 80-60278                         | 80-68607                   | A                 |   |
|                            |                            | 10            | 270642       | 80-60279                         | 80-68608                   | A                 |   |
|                            |                            |               |              |                                  | 80-68609                   | A                 |   |
|                            | III (8 mg/m <sup>3</sup> ) | 11            | 270678       |                                  |                            |                   |   |
|                            |                            | 12            | 270679       | 80-60280                         | 80-67463                   |                   |   |
|                            |                            | 13            | 270682       | 80-60281                         | 80-67464                   | A                 |   |
| 14                         |                            | 270683        | 80-60282     | 80-68610                         | A                          |                   |   |
| 15                         |                            | 270684        | 80-60283     | 80-68611                         | A                          |                   |   |
|                            |                            |               | 80-60284     | 80-68612                         | A                          |                   |   |
| IV (80 mg/m <sup>3</sup> ) | 16                         | 270720        |              |                                  |                            |                   |   |
|                            | 17                         | 270721        | 80-60285     | 80-67465                         |                            |                   |   |
|                            | 18                         | 270724        | 80-60286     | 80-67466                         | A                          |                   |   |
|                            | 19                         | 270725        | 80-60287     | 80-68613                         | A                          |                   |   |
|                            | 20                         | 270728        | 80-60288     | 80-68614                         | A                          |                   |   |
|                            |                            |               | 80-60289     | 80-68615                         | A                          |                   |   |
| 2<br>14 Days<br>3/14/80    | I                          | 1             | 270330       | 80-60290                         |                            |                   |   |
|                            |                            | 2             | 270332       | 80-60291                         | 80-67467                   | L                 |   |
|                            |                            | 3             | 270333       | 80-60292                         | 80-67468                   | L                 |   |
|                            |                            | 4             | 270334       | 80-60293                         | 80-67751                   | A                 |   |
|                            |                            | 5             | 270335       | 80-60294                         | 80-68616                   | A                 |   |
|                            | II                         |               |              |                                  | 80-60294                   | 80-68617          | A |
|                            |                            | 6             | 270643       |                                  |                            |                   |   |
|                            |                            | 7             | 270644       | 80-60295                         | 80-68618                   |                   |   |
|                            |                            | 8             | 270645       | 80-60296                         | 80-68619                   | D                 |   |
|                            |                            | 9             | 270648       | 80-60297                         | 80-68620                   | D                 |   |
|                            | III                        | 10            | 270649       | 80-60298                         | 80-68621                   | D                 |   |
|                            |                            |               |              | 80-60299                         | 80-68622                   | D                 |   |
|                            |                            | 11            | 270685       |                                  |                            |                   |   |
|                            |                            | 12            | 270686       | 80-60300                         |                            |                   |   |
|                            |                            | 13            | 270687       | 80-60301                         | 80-68623                   | D                 |   |
|                            | IV                         | 14            | 270688       | 80-60302                         | 80-68624                   | D                 |   |
|                            |                            | 15            | 270689       | 80-60303                         | 80-68625                   | D                 |   |
|                            |                            |               |              | 80-60304                         | 80-68626                   | D                 |   |
|                            |                            |               |              |                                  | 80-68627                   | D                 |   |
|                            |                            | 16            | 270302       |                                  |                            |                   |   |
| 17                         | 270730                     | 80-60305      | 80-67469     |                                  |                            |                   |   |
| 18                         | 270731                     | 80-60306      | 80-67470     | L                                |                            |                   |   |
| 19                         | 270732                     | 80-60307      | 80-67752     | L                                |                            |                   |   |
| 20                         | 270733                     | 80-60308      | 80-68628     | A                                |                            |                   |   |
|                            |                            | 80-60309      | 80-68629     | A                                |                            |                   |   |

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TABLE I (con't)

| <u>Sacrifice</u>        | <u>Group</u> | <u>Tube #</u> | <u>Rat #</u> | <u>PRAL No.,<br/>As Received</u> | <u>PRAL No.,<br/>Dried</u> | <u>Status (b)</u> |
|-------------------------|--------------|---------------|--------------|----------------------------------|----------------------------|-------------------|
| 3<br>28 Days<br>3/28/80 | I            | 1             | 270336       | 80-60310                         | 80-67471                   | L                 |
|                         |              | 2             | 270337       | 80-60311                         | 80-67472                   | L                 |
|                         |              | 3             | 270356       | 80-60312                         | 80-67753                   | A                 |
|                         |              | 4             | 270357       | 80-60313                         | 80-68630                   | A                 |
|                         |              | 5             | 270358       | 80-60314                         | 80-68631                   | A                 |
|                         | II           | 6             | 270652       | 80-60315                         | 80-67473                   | L                 |
|                         |              | 7             | 270653       | 80-60316                         | 80-67474                   | L                 |
|                         |              | 8             | 270656       | 80-60317                         | 80-67754                   | A                 |
|                         |              | 9             | 270657       | 80-60318                         | 80-68632                   | A                 |
|                         |              | 10            | 270658       | 80-60319                         | 80-68633                   | A                 |
|                         | III          | 11            | 270690       | 80-60320                         | 80-67475                   | L                 |
|                         |              | 12            | 270691       | 80-60321                         | 80-67476                   | L                 |
|                         |              | 13            | 270692       | 80-60322                         | 80-67755                   | A                 |
|                         |              | 14            | 270693       | 80-60323                         | 80-68634                   | A                 |
|                         |              | 15            | 270694       | 80-60324                         | 80-68635                   | A                 |
|                         | IV           | 16            | 270734       | 80-60325                         | 80-67477                   | L                 |
|                         |              | 17            | 270735       | 80-60326                         | 80-67478                   | L                 |
|                         |              | 18            | 270738       | 80-60327                         | 80-67756                   | A                 |
|                         |              | 19            | 270739       | 80-60328                         | 80-68636                   | A                 |
|                         |              | 20            | 270744       | 80-60329                         | 80-68637                   | A                 |
| 4<br>42 Days<br>4/11/80 | I            | 1             | 270359       | 80-60330                         | 80-67479                   | A                 |
|                         |              | 2             | 270384       | 80-60331                         | 80-67480                   | A                 |
|                         |              | 3             | 270385       | 80-60332                         | 80-68638                   | A                 |
|                         |              | 4             | 270414       | 80-60333                         | 80-68639                   | A                 |
|                         |              | 5             | 270415       | 80-60334                         | 80-68640                   | A                 |
|                         | II           | 6             | 270659       | 80-60335                         | 80-68641                   | D                 |
|                         |              | 7             | 270662       | 80-60336                         | 80-68642                   | D                 |
|                         |              | 8             | 270663       | 80-60337                         | 80-68643                   | D                 |
|                         |              | 9             | 270668       | 80-60338                         | 80-68644                   | D                 |
|                         |              | 10            | 270669       | 80-60339                         | 80-68645                   | D                 |
|                         | III          | 11            | 270695       | 80-60340                         | 80-68646                   | N                 |
|                         |              | 12            | 270698       | 80-60341                         | 80-68647                   | D                 |
|                         |              | 13            | 270699       | 80-60342                         | 80-68648                   | D                 |
|                         |              | 14            | 270702       | 80-60343                         | 80-68649                   | N                 |
|                         |              | 15            | 270703       | 80-60344                         | 80-68650                   | D                 |
|                         | IV           | 16            | 270745       | 80-60345                         | 80-67481                   | A                 |
|                         |              | 17            | 270296       | 80-60346                         | 80-67482                   | A                 |
|                         |              | 18            | 270297       | 80-60347                         | 80-68651                   | A                 |
|                         |              | 19            | 270310       | 80-60348                         | 80-68652                   | A                 |
| 5<br>14 Days<br>5/23/80 | I            | 1             | 270444       | 80-60349                         | 80-68653                   | A                 |
|                         |              | 2             | 270445       | 80-60350                         | 80-68654                   | A                 |
|                         |              | 3             | 270474       | 80-60351                         | 80-67757                   | A                 |
|                         |              | 4             | 270475       | 80-60352                         | 80-68655                   | A                 |

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TABLE I (con't)

| <u>Sacrifice</u>               | <u>Group</u>         | <u>Tube #</u> | <u>Rat #</u> | <u>PRAL No.<br/>As Received</u> | <u>PRAL No.,<br/>Dried</u> | <u>Status (b)</u>  |
|--------------------------------|----------------------|---------------|--------------|---------------------------------|----------------------------|--------------------|
| 5                              | II                   | 5             | 270670       |                                 |                            |                    |
|                                |                      | 6             | 270671       | 80-60353                        | 80-68656                   | D                  |
|                                |                      | 7             | 270674       | 80-60354                        | 80-68657                   | D                  |
|                                |                      | 8             | 270675       | 80-60355                        | 80-68658                   | D                  |
|                                | III                  |               |              | 80-60356                        | 80-68659                   | D                  |
|                                |                      | 9             | 270716       |                                 |                            |                    |
|                                |                      | 10            | 270717       | 80-60357                        | 80-68660                   | D                  |
|                                |                      | 11            | 270718       | 80-60358                        | 80-68661                   | D                  |
|                                |                      | 12            | 270719       | 80-60359                        | 80-68662                   | D                  |
|                                | IV                   |               |              | 80-60360                        | 80-68663                   | D                  |
|                                |                      | 13            | 270311       |                                 |                            |                    |
|                                |                      | 14            | 270312       | 80-60361                        | 80-67768                   | A                  |
|                                |                      |               |              | 80-60362                        | 80-68664                   | A                  |
|                                |                      | 15            | 270313       | 80-60363                        | 80-68665                   | A                  |
|                                | <u>"Test Blanks"</u> |               |              |                                 |                            |                    |
| <u>Normal control rats (c)</u> |                      |               |              |                                 |                            |                    |
|                                |                      | 278363        | 80-60364     |                                 |                            | D (7/80)           |
|                                |                      |               | 80-60365     |                                 |                            | D (8/80)           |
|                                |                      | 278358        | 80-60366     |                                 |                            | D (7/80)           |
|                                |                      | 278357        | 80-60367     |                                 |                            | D (8/80)           |
|                                |                      | 278356        | 80-60368     | 80-67759                        |                            | A                  |
|                                |                      | 278355        | 80-60369     | 80-67484                        |                            | A                  |
|                                |                      |               | 80-60370     | 80-67485                        |                            | Stored as received |
|                                |                      | 278354        | 80-60371     | 80-67483                        |                            | L                  |
|                                |                      | 278353        | 80-60372     | 80-68670                        |                            | A                  |
|                                |                      | 278352        | 80-60373     | 80-68671                        |                            | A                  |
|                                |                      | 278351        | 80-60374     | 80-68672                        |                            | A                  |
|                                |                      | 278350        | 80-60375     | 80-68673                        |                            | A                  |
|                                |                      | 272994        | 80-60376     | 80-68674                        |                            | D                  |

- (a) As the samples were no longer homogeneous at the time of analysis (due to clotting), each entire sample was lyophilized and assigned a second PRAL No. before taking portions to analyze.
- (b) Current Status: A, Samples analyzed 12/80-1/81, results reported here  
 D, Samples lyophilized for further storage, but not analyzed at this time  
 N, No blood collected, empty tubes received  
 L, Samples lost during lyophilization (one group of 13, due to loss of vacuum in the apparatus). For this reason, the groups analyzed for sacrifices 2 and 3 contain only 3 rats each (Table III)
- (c) Blanks included with the samples submitted 6/80. One of these was included with each group of samples during preparation and analysis as a procedural blank.

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TABLE II  
Comparison of Ca/GC and Wickbold Torch Analyses of Rat Blood Samples (a)

| <u>PRAL No.</u> | <u>Sacrifice-Tube</u> | <u>GC Analysis,<br/>[Ca], ug F/g blood</u>               | <u>Wickbold Torch Analysis,<br/>Total Fluoride, ug F/g blood<br/>Uncorrected <math>\times 1/0.83(b)</math></u> |      |
|-----------------|-----------------------|----------------------------------------------------------|----------------------------------------------------------------------------------------------------------------|------|
| 80-67461        | 1-6                   | 7.7                                                      | 6.9                                                                                                            | 8.3  |
| 80-67462        | 1-7                   | 13.1                                                     | 10.5                                                                                                           | 12.7 |
| 80-67752        | 2-18                  | 11.4                                                     | 9.5                                                                                                            | 11.4 |
| 80-67756        | 3-18                  | 8.6                                                      | 9.0                                                                                                            | 10.8 |
| 80-67481        | 4-16                  | 1.3                                                      | 1.8                                                                                                            | 2.2  |
| 80-67460        | 1-2                   | 1.1                                                      | 1.1                                                                                                            | 1.3  |
| 80-67484        | Test blank            | <0.007<br>(none detected<br>above reagent<br>background) | 0.76                                                                                                           | 0.92 |

(a) Analyses on duplicate portions of the lyophilized blood samples. GC analyses as described in Table III, 12/1/80-12/8/80. Wickbold Torch analyses by [redacted] Jackson Lab, 12/31/80-1/7/81, [redacted]

(b) Recovery of [redacted] in the Wickbold Torch procedure is currently ~83%, is determined in recent work by [redacted]

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0.10

Concentration of [REDACTED] in Rat Blood Samples, GC Determination (a,b)

| Sacrifice  | Group I (control) |                         | Group II (1 mg/m <sup>3</sup> ) |                         | Group III (8 mg/m <sup>3</sup> ) |                         | Group IV (80 mg/m <sup>3</sup> ) |                         |
|------------|-------------------|-------------------------|---------------------------------|-------------------------|----------------------------------|-------------------------|----------------------------------|-------------------------|
|            | Tube #            | [Cr], $\mu\text{g F/R}$ | Tube #                          | [Cr], $\mu\text{g F/R}$ | Tube #                           | [Cr], $\mu\text{g F/R}$ | Tube #                           | [Cr], $\mu\text{g F/R}$ |
| 1, 0 Days  | 1                 | 0.68                    | 6                               | 7.7                     | 11                               | 53                      | 16                               | 131                     |
|            | 2                 | 1.1                     | 7                               | 13.                     | 12                               | 28                      | 17                               | 124                     |
|            | 3                 | 21.                     | 8                               | 17.                     | 13                               | 37                      | 18                               | 89                      |
|            | 4                 | 3.3                     | 9                               | 20.                     | 14                               | 61                      | 19                               | 86                      |
|            | 5                 | 0.53                    | 10                              | 9.4                     | 15                               | 57                      | 20                               | 110                     |
|            | Ave.              | 5.3                     |                                 | 13.                     |                                  | 47                      |                                  | 108                     |
| 2, 14 Days | 3                 | 0.30                    |                                 |                         |                                  |                         | 18                               | 11                      |
|            | 4                 | 0.43                    |                                 |                         |                                  |                         | 19                               | 10                      |
|            | 5                 | 0.12                    |                                 |                         |                                  |                         | 20                               | 8.5                     |
|            | Ave.              | 0.28                    |                                 |                         |                                  |                         |                                  | 10                      |
| 3, 28 Days | 3                 | 0.086                   | 8                               | 0.97                    | 13                               | 4.2                     | 18                               | 8.6                     |
|            | 4                 | 0.11                    | 9                               | 1.5                     | 14                               | 4.6                     | 19                               | 5.8                     |
|            | 5                 | 0.11                    | 10                              | 0.04                    | 15                               | 2.6                     | 20                               | 6.9                     |
|            | Ave.              | 0.10                    |                                 | 1.2                     |                                  | 3.8                     |                                  | 7.1                     |
| 4, 42 Days | 1                 | 0.010                   |                                 |                         |                                  |                         | 16                               | 1.3                     |
|            | 2                 | 0.037                   |                                 |                         |                                  |                         | 17                               | 3.6                     |
|            | 3                 | 0.058                   |                                 |                         |                                  |                         | 18                               | 1.2                     |
|            | 4                 | 0.038                   |                                 |                         |                                  |                         | 19                               | 1.1                     |
|            | 5                 | 0.017                   |                                 |                         |                                  |                         |                                  |                         |
|            | Ave.              | 0.032                   |                                 |                         |                                  |                         |                                  | 1.8                     |
| 5, 84 Days | 1                 | 0.029                   |                                 |                         |                                  |                         | 13                               | 0.14                    |
|            | 2                 | <.007 (c)               |                                 |                         |                                  |                         | 14                               | 1.3                     |
|            | 3                 | 0.021                   |                                 |                         |                                  |                         | 15                               | 1.1                     |
|            | 4                 | <.007 (c)               |                                 |                         |                                  |                         |                                  |                         |
|            | Ave.              | 0.015                   |                                 |                         |                                  |                         |                                  | 0.84                    |

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TABLE III (con't)

- (a) Procedure as described in the method "Determination of [REDACTED] in Blood Gas, Chromatographic Method", 1/2/81, using the capillary column GC analysis and [REDACTED] as calibration standard. Lower limit for quantitation 0.007 ppm F. Estimated uncertainty  $\pm 10\%$  relative standard deviation over most of the range, somewhat higher at the lowest concentrations.
- (b) For the six "test blank" samples listed as analyzed in Table I, [Cg]  $< 0.007$  ppm F (none detected above reagent background). (PRAL Nos. 80-60368-69, 80-60372-75).
- (c) Values of 0.005 (sample 5-2) and 0.003 (sample 5-4) were used to calculate the average, but quantitation cannot really be done at these levels, and it is questionable whether 5-4 contained any Cg above background.

Percent Straight-Chain [REDACTED] in Rat Blood Samples (a)

TABLE IV

| Sacrifice     | Group I (control) |            | Group II (1 mg/m <sup>3</sup> ) |         | Group III (8 mg/m <sup>3</sup> ) |         | Group IV (80 mg/m <sup>3</sup> ) |         |
|---------------|-------------------|------------|---------------------------------|---------|----------------------------------|---------|----------------------------------|---------|
|               | Tube #            | Percent    | Tube #                          | Percent | Tube #                           | Percent | Tube #                           | Percent |
| 1,<br>0 Days  | 1                 | 85         | 6                               | 82      | 11                               | 89      | 16                               | 88      |
|               | 2                 | 84         | 7                               | 80      | 12                               | 92      | 17                               | 88      |
|               | 3                 | 96         | 8                               | 85      | 13                               | 90      | 18                               | 87      |
|               | 4                 | 91         | 9                               | 88      | 14                               | 85      | 19                               | 84      |
|               | 5                 | 86         | 10                              | 81      | 15                               | 86      | 20                               | 86      |
| 2,<br>14 Days | 3                 | 93         |                                 |         |                                  |         | 18                               | 93      |
|               | 4                 | 92         |                                 |         |                                  |         | 19                               | 92      |
|               | 5                 | 91         |                                 |         |                                  |         | 20                               | 91      |
| 3,<br>28 Days | 3                 | not        | 8                               | 93      | 13                               | 96      | 18                               | 94      |
|               | 4                 | cal-       | 9                               | 91      | 14                               | 95      | 19                               | 92      |
|               | 5                 | culated(b) | 10                              | 87      | 15                               | 96      | 20                               | 93      |
| 4,<br>42 Days | 1                 | not        |                                 |         |                                  |         | 16                               | 94      |
|               | 2                 |            |                                 |         |                                  |         | 17                               | 96      |
|               | 3                 | cal        |                                 |         |                                  |         | 18                               | 95      |
|               | 4                 |            |                                 |         |                                  |         | 19                               | 94      |
|               | 5                 | culated(b) |                                 |         |                                  |         |                                  |         |
| 5,<br>84 Days | 1                 | not        |                                 |         |                                  |         | 13                               | 97      |
|               | 2                 | cal-       |                                 |         |                                  |         | 14                               | 94      |
|               | 3                 | culated(b) |                                 |         |                                  |         | 15                               | 93      |
|               | 4                 |            |                                 |         |                                  |         |                                  |         |

(a) Calculated as described in the method, for peak #3 in the group of five C<sub>8</sub> isomer peaks seen in Figure 3b.

The same calculation for spiked blood standards (10 ppm F) gave average values of 74% straight-chain for [REDACTED], 94% for [REDACTED]

(b) A calculation of branched isomer concentrations was not attempted for samples with [C<sub>8</sub>] < 0.1 ppm, due to the small size of the secondary peaks and interference from the reagents.

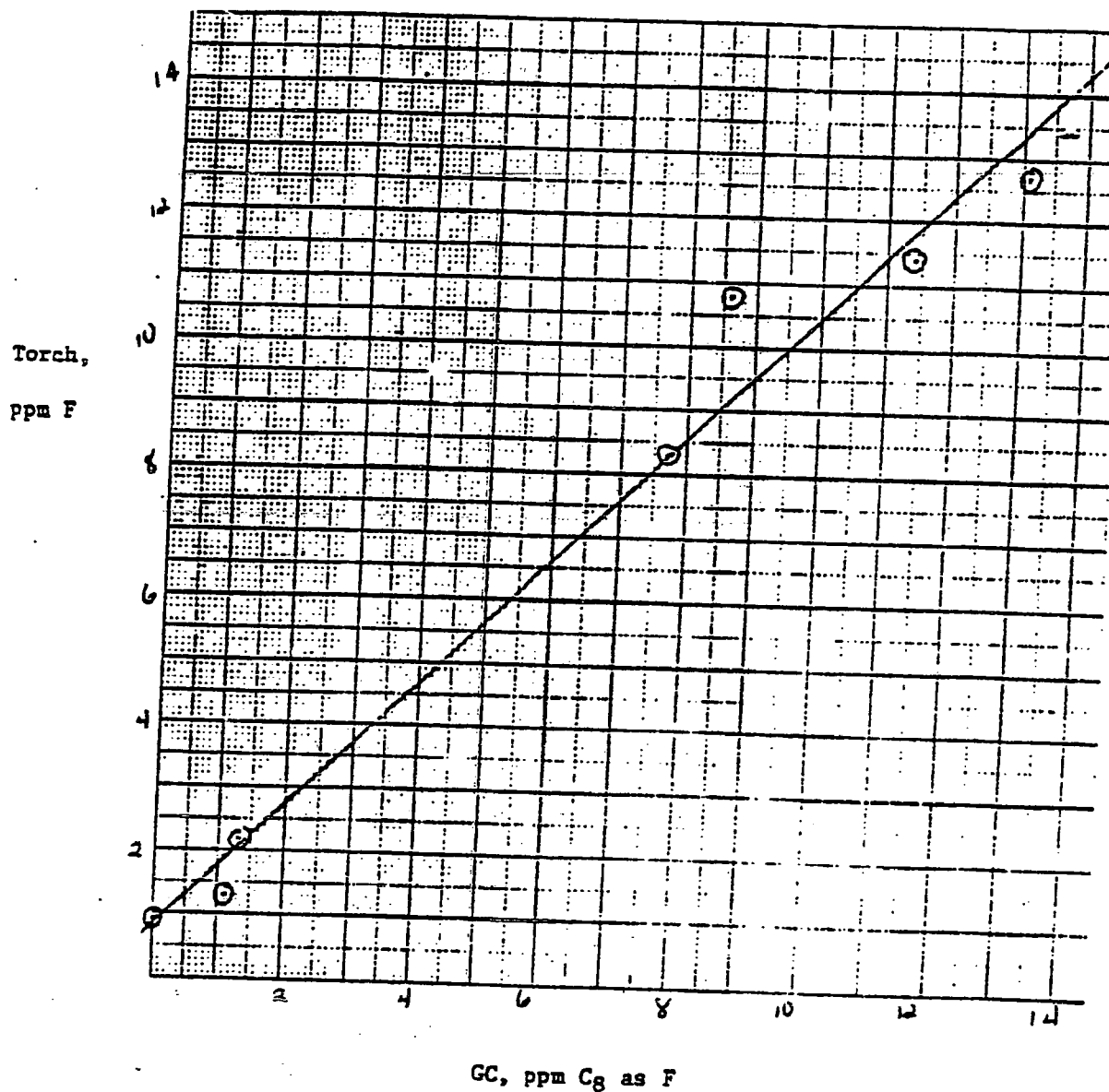
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Figure 1 - Comparison of C<sub>8</sub>/GC and Wickbold Torch Analyses of Rat Blood Samples

Data from Table II, using Torch values corrected for 83% recovery of

Linear Least Squares Fit: Torch = 0.90 + 0.96 (GC)



014

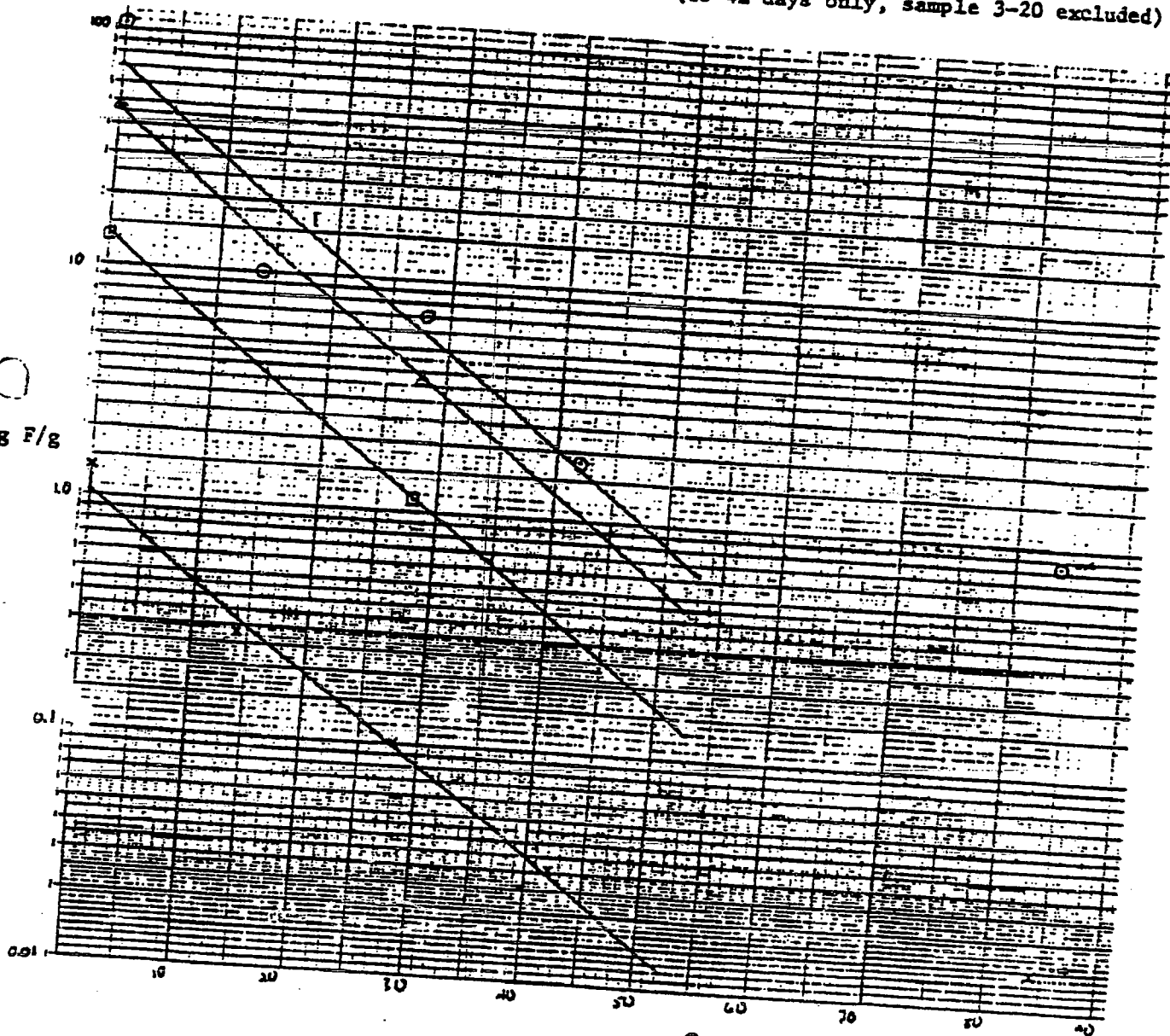
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Figure 2 - Concentration of  $C_8$  in Rat Blood Samples vs. Recovery Time

Data from Table III, Average Values by GC

- ⊙ = Group IV  $\log C = 1.9 - 0.039 t$  (to 42 days only)
- △ = Group III  $\log C = 1.7 - 0.039 t$
- = Group II  $\log C = 1.1 - 0.038 t$
- X = Group I  $\log C = 0.078 - 0.038 t$  (to 42 days only, sample 3-20 excluded)



Recovery Time, Days

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0.15