 COMPLETE REPORT IN MR FOLDER.

FOR DU PONT USE ONLY

Copies to: [redacted]

E. I. du Pont de Nemours and Company
Haskell Laboratory for Toxicology and Industrial Medicine
Elkton Road, Newark, Delaware 19711

HASKELL LABORATORY REPORT NO. 88-81 [redacted]

Material Tested
[redacted]

Haskell No.
13,383

[redacted]

Study Initiated/Completed
5/5/80-5/30/80

Material Submitted by
[redacted]
Chemicals & Pigments Department
Jackson Laboratory

SUBACUTE INHALATION TOXICITY STUDY OF [redacted] ON RATS

Introduction: The purpose of this study was to observe the effects on male rats of repeated inhalation exposure to [redacted] vapors. In a previous study [redacted] the LC50 for [redacted] was determined to be 107 mg/l. Design concentrations for this study are 0 mg/l (Group I), 1.0 mg/l (Group II), and 10.0 mg/l (Group III) of [redacted] in air.

Procedure:

A. Animals

Male Crl:CD® rats were housed in pairs in stainless steel wire mesh cages. Purina Certified Rodent Chow #5002 and water were available ad libitum. All rats were held under these conditions for a 1 week pre-test period to insure suitability.

B. Exposure Protocol

Groups of 10 rats weighing 234.0 to 245.0 grams were exposed to atmospheres containing [redacted] (either 1.0 mg/l - Group II or 10.0 mg/l - Group III) 6 hours/day, 5 days/week for 2 weeks. Control rats (Group I) weighing 234.0 to 242.0 grams were simultaneously exposed to air only. All rats were weighed and observed daily (except weekends and holidays) throughout the exposure period and a 14-day recovery period.

C. Generation

Atmospheres of the test material were generated by syringe driving the compound through a Spraying Systems nebulizer. The syringe and nebulizer were heated to a temperature of approximately 70°C. The aerosolized material sprayed onto the heated (50°C) surface of a 3-neck round bottom flask. Houseline air swept the evaporated material into 20 liter glass exposure chambers.

D. Analytical

Chamber samples were collected at approximately 30-minute intervals. A known volume of chamber atmosphere was drawn through 2 tandem midget impingers containing FC-113 as a trapping solvent. These samples were analyzed using a Hewlett Packard Model 5710 gas chromatograph equipped with a flame ionization detector. The column used was a 6' x 1/4" O.D. glass coil packed with 20% S.E. 30 on 80/100 mesh Chromosorb® W-HP. The G.C. oven temperature was programmed with an initial 2 minute hold at 50°C, increased at 32°/minute to 200°C, and held there for 2 minutes.

G.C. analysis was based on the [redacted] component. This peak was identified on the basis of the G.C. peak height area and the reported sample composition. Sample concentrations were determined by comparison with standards prepared by dilution of the test compound. Chamber concentrations were expressed as the equivalent [redacted] concentration (mg/l).

Oxygen and chamber temperature were monitored throughout each exposure.

E. Clinical Measurements

Clinical laboratory measurements were made on urine samples collected overnight following the 9th exposure and the 13th day of recovery. Blood samples were taken from the rats' tails after the 10th exposure and the 14th day of recovery. Detailed clinical chemistry procedures and indices are outlined in the Clinical Chemistry report (1).

F. Pathology

After the 10th exposure, 5 rats from each group were selected at random and sacrificed for gross and histopathological examination. Remaining rats were sacrificed on the 14th day of recovery for an identical examination. Pathological procedures and indices are included in the Pathology report (2).

G. Organ and Body Weight Analysis

Organ weights and organ-to-body weight ratios were determined for the heart, liver, lungs, kidneys, spleen, testes, and thymus (Appendices I and II).

Results During exposures, low boiling components evaporated readily while high boiling solids plugged the generation apparatus. This problem accounts for the high standard deviation and range for these exposure concentrations. Detailed data is listed in following Tables.

GROUP II

Design Level - 1.0 mg/l

Exposure No.	Average (mg/l)	S.D.	Range
1	2.02	2.56	0.57-7.36
2	1.77	2.10	0.43-7.24
3	0.99	0.90	0.23-2.74
4	1.44	2.39	0.14-8.09
5	1.91	2.05	0.54-6.23
6	1.11	1.67	0.16-5.31
7	3.58	3.17	0.08-8.26
8	0.97	0.57	0.27-2.25
9	0.[illegible]	0.70	0.14-2.17
10	0.78	0.89	0.0-2.68

GROUP III

Design Level - 10.0 mg/l

Exposure No.	Average (mg/l)	S.D.	Range
1	14.6	12.3	3.7-48.50
2	6.7	5.1	1.8-16.0
3	9.4	5.2	2.3-17.3
4	7.8	5.2	2.9-19.8
5	7.3	1.6	4.6-9.8
6	8.9	4.1	4.8-16.1
7	11.0	5.0	4.0-21.4
8	12.3	10.1	4.6-40.6
9	8.0	4.3	2.2-16.2
10	9.8	2.8	5.7-17.3

The overall averages were 1.5 $\pm$ 1.9 (Group II) and 9.6 $\pm$ 6.4 (Group III).

Chamber temperature was $\leq$28°C during all exposures. Chamber oxygen was $\geq$20% during all exposures.

Clinical Observations: During exposure, Group II rats exhibited a reduced response to sound. Group III rats showed a reduced response to sound and rapid breathing. Rapid breathing, however, was observed only after high concentration excursions.

After one exposure, mild weight loss was observed in Group I and Group II rats followed by normal rate of weight gain. Following 2 exposures, Group III rats exhibited moderate to severe weight loss followed by recovery of normal rate of weight gain.

One Group III rat was found dead prior to the second exposure. Another Group III rat died during the second exposure.

Clinical Chemistry: No effects were seen in Group II rats after exposure 10 or 14 days recovery. Group III rats tended to have slightly lower urea nitrogen and total protein. Total protein was still depressed in Group III rats 14 days later. Clinical results are detailed in the Clinical Report.

Pathology: No treatment-related gross or microscopic pathologic lesions were observed in any animals sacrificed at design intervals. Of the 2 animals which died during the test period, tissues of only 1 were saved for pathologic examination. Death of this animal was attributed to severe pulmonary edema and was concluded to have been treatment-related. Pathologic results are detailed in the Pathology Report.

Organ and Body Weight Analysis: Mean body weights of Group II rats were similar to weights of controls during the exposure period (Fig. 1). Mean body weights of Group III rats were significantly lower than controls from exposure 2 through 5. Through the remainder of the exposure and recovery period, rate of weight gain was not significantly different between all groups.

A comparison of absolute organ weights and relative organ-to-body weight ratios between Group II and controls showed no significant differences following the 10th exposure. Mean liver-to-body weight ratios of Group III rats were significantly higher in rats sacrificed after 10 exposures.

Following the 14-day observation period heart weight and heart-to-body weight ratio of Group II rats were significantly higher than controls. Kidney-to-body weight ratios of Group II rats was also higher. Kidney weight of Group III rats was significantly higher than controls. Although "significantly" different, lack of dose-related trends in these effects makes them difficult to evaluate.

Summary: Groups of 10 male Crl:CD® rats were exposed 6 hours/day, 5 days/week for 2 weeks to concentrations of either 1.5 or 9.6 mg/l of [redacted] in air. A control group was simultaneously exposed to air only.

During exposures, reduced response to sound was observed in all test rats. Rapid breathing was observed in Group III rats following high concentration excursions.

Following exposure, clinical signs of Group II rats were indistinguishable from controls. Group III rats exhibited severe weight loss following 2 exposures - normal weight gain thereafter. Two Group III rats died - 1 found dead on day 2 and the other died during the second exposure.

Clinical chemistry measurements of Group II rats were indistinguishable from controls. After 10 exposures, Group III rats had slightly lower values for urea nitrogen and total protein. The total protein was also lower in Group III rats following the 14-day recovery period.

No treatment-related gross or microscopic pathologic lesions were observed in any animals sacrificed at design intervals. Of the 2 animals which died during the test period, tissues of only 1 were saved for pathologic examination. Death of this animal was attributed to severe pulmonary edema and was concluded to have been treatment related.

Mean body weights of Group II rats were similar to the controls throughout the test period. Group III rats mean body weight was significantly lower than the controls on days 2 through 5 of the exposure period. The rate of weight gain of Group III rats was similar to the controls through the remainder of the test period.

No significant differences were observed in organ/body weights of Group II rats sacrificed after 10 exposures. Mean liver weight of Group III rats was significantly higher than controls after 10 exposures.

[redacted]

Company Sanitized. Does not contain TSCA CBI

(1) Walter B. Koniecki and J. R. Barnes, "Subacute Inhalation of [redacted] Clinical Laboratory Report," [redacted] H-13,383, September 9, 1980.

(2) Raymond M. Everett and William C. Krauss, Haskell Pathology Report No. 46-80, [redacted], H-13,383, October 24, 1980.

Report by: Stephen D. Nash
Stephen D. Nash
Technician

Approved by: Gerald L. Kennedy
Gerald L. Kennedy
Chief, Acute Investigations Section

SDN:vlm
Study Director: R. L. Ferenz
Date Issued: February 24, 1981
[redacted]
Report No. 88-81

Company Sanitized. Does not contain TSCA CBI

[redacted] SUBACUTE WGT. GROWTH CURVE

[redacted] H# 13383

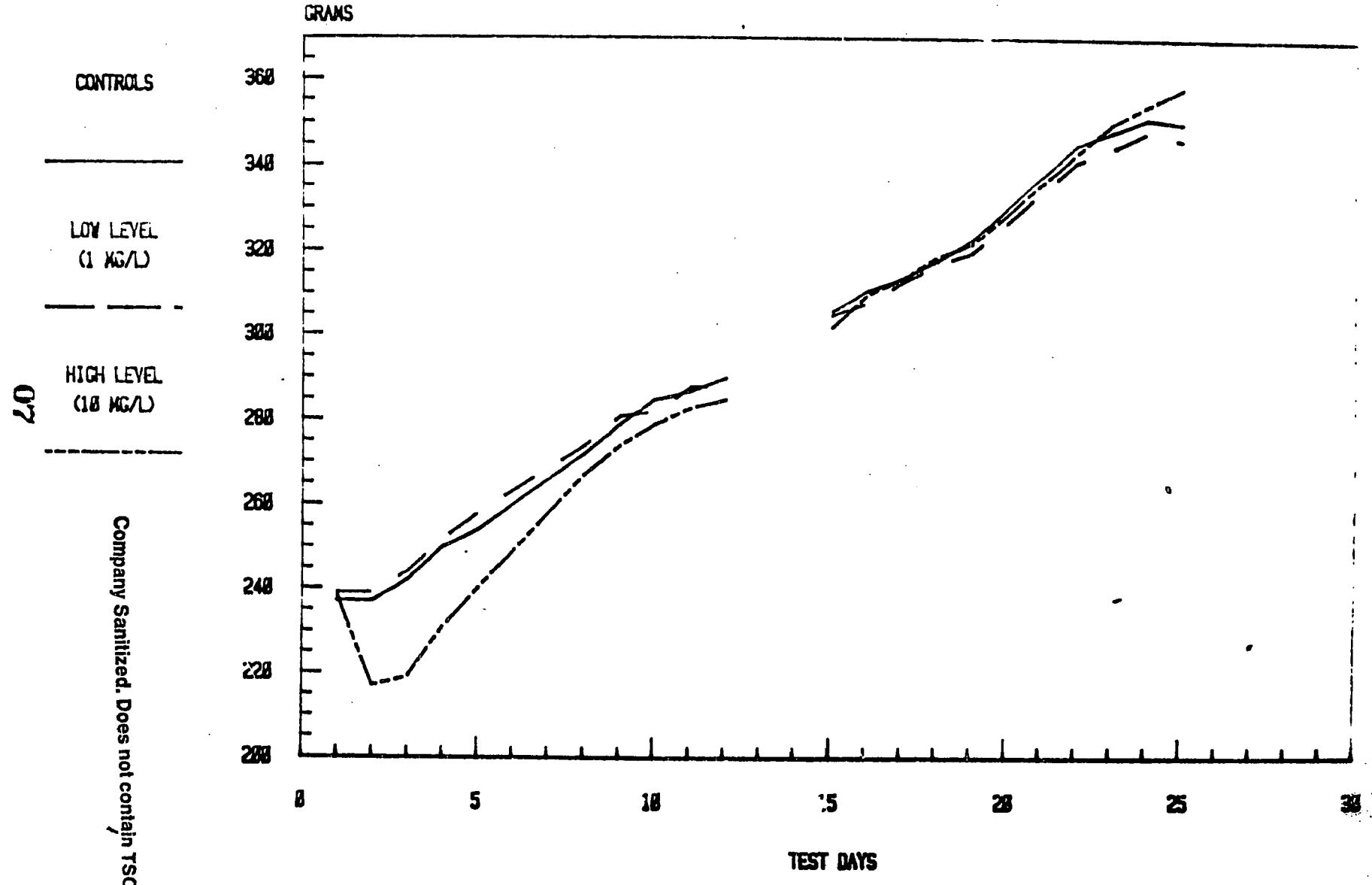

FIGURE I

07

Company Sanitized. Does not contain TSCA CB

APPENDIX I

Company Sanitized. Does not contain TSCA CBI

[redacted] 2-WK SUBACUTE BODY WEIGHT SUMMARY
[redacted] H# 13383

INTERVALS IN TEST DAYS

GROUP	1	2	3	4	5
CONTROLS	236.8000	236.8000	241.7000	249.8000	254.4000
LOW LEVEL(1 MG/L)	238.8000	239.3000	244.2000	251.9000	257.8000
HIGH LEVEL(10 MG/L)	238.3750	217.2500*	218.5000*	231.3750*	240.3750*
F RATIO(1)	0.980	28.678*	15.391*	13.270*	11.955*
LSD(2)	3.2029	6.3334	10.1449	8.7071	7.5188
DUNNETT(3)	3.6547	7.2268	11.5758	9.9352	8.5794
WMS(4)	11.1630	43.6480	111.9880	82.4950	61.5150

INTERVALS IN TEST DAYS

GROUP	8	9	10	11	12
CONTROLS	272.1000	279.3000	284.7000	286.5000	289.7000
LOW LEVEL(1 MG/L)	274.4000	281.1000	282.4000	287.8000	288.2000
HIGH LEVEL(10 MG/L)	266.8750	274.3750	279.2500	282.5000	285.2500
F RATIO(1)	2.182	2.099	0.924	1.155	1.181
LSD(2)	7.3814	6.7810	8.1072	7.2455	5.9008
DUNNETT(3)	8.4226	7.7375	9.2508	8.2675	6.7331
WMS(4)	59.2870	50.0350	71.5200	57.1240	37.8880

INTERVALS IN TEST DAYS

GROUP	15	16	17	18	19
CONTROLS	305.8000	310.8000	314.2000	317.8000	322.6000
LOW LEVEL(1 MG/L)	305.0000	308.0000	312.6000	317.2000	319.6000
HIGH LEVEL(10 MG/L)	302.0000	309.7500	314.2500	318.7500	322.0000
F RATIO(1)	0.152	0.099	0.042	0.026	0.118
LSD(2)	15.4624	14.5384	14.4937	14.5830	14.8427
DUNNETT(3)	17.7716	16.7116	16.6602	16.7628	17.0613
WMS(4)	113.8909	100.6864	100.0682	101.3045	104.9455

Company Sanitized. Does not contain TSCA CBI

GROUP	INTERVALS IN TEST DAYS 23	24	25	26
CONTROLS	345.0000	347.6000	351.2000	350.2000
LOW LEVEL(1 MG/L)	340.6000	343.8000	348.4000	345.8000
HIGH LEVEL(10 MG/L)	343.2500	349.5000	353.5000	357.5000
F RATIO(1)	0.105	0.167	0.129	0.623
LSD(2)	22.0943	22.0643	21.8383	22.7104
DUNNETT(3)	25.3969	25.3624	25.1026	26.1051
WMS(4)	232.5409	231.9091	227.1818	245.6909

(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.

APPENDIX II

Company Sanitized. Does not contain TSCA CBI

[redacted] 2-WK SUBACUTE ORGAN/BODY WEIGHT MEAN ABSOLUTE DATA
H# 13383 RATS SACRIFICED AFTER 10 EXPOSURES

GROUP	FINAL WGT.	HEART	LUNGS	LIVER	SPLEEN
CONTROLS	289.8000	1.0260	1.6880	11.1920	.6700
LOW LEVEL(1.0 MG/L)	286.4000	.9680	1.5820	10.6140	.5620
HIGH LEVEL(10.0 MG/L)	284.5000	1.0700	1.8300	12.1525	.6525
F RATIO(1)	1.197	2.434	1.225	3.676	2.994
LSD(2)	7.6042	.1009	.3423	1.2297	.1075
DUNNETT(3)	8.7409	.1160	.3935	1.4135	.1236
WMS(4)	27.5455	.0049	.0558	.7203	.0055

GROUP	KIDNEY	TESTIS	THYMUS
CONTROLS	2.3360	2.9040	.7740
LOW LEVEL(1.0 MG/L)	2.3380	3.0260	.7580
HIGH LEVEL(10.0 MG/L)	2.5775	3.0250	.7425
F RATIO(1)	2.716	.337	.051
LSD(2)	.2527	.3845	.2144
DUNNETT(3)	.2905	.4420	.2465
WMS(4)	.0304	.0704	.0219

(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.

[redacted] 2-WK SUBACUTE ORGAN/BODY WEIGHT MEAN RELATIVE DATA
H# 13383 RATS SACRIFICED AFTER 10 EXPOSURES

GROUP	HEART	LUNGS	LIVER	SPLEEN	KIDNEY
CONTROLS	.3541	.5828	3.8602	.2311	.8059
LOW LEVEL(1.0 MG/L)	.3381	.5528	3.7049	.1960	.8157
HIGH LEVEL(10.0 MG/L) #	.3765	.6423	4.2743+	.2295	.9064
F RATIO(1)	2.391	1.341	4.614*	3.016	4.085*
LSD(2)	.0380	.0190	.4133	.0364	.0822
DUNNETT(3)	.0437	.1368	.4751	.0418	.0945
WMS(4)	.0007	.0068	.0814	.0006	.0032

GROUP	TESTIS	THYMUS
CONTROLS	1.0029	.2675
LOW LEVEL(1.0 MG/L)	1.0560	.2649
HIGH LEVEL(10.0 MG/L)	1.0628	.2607
F RATIO(1)	.633	.019
LSD(2)	.1302	.0758
DUNNETT(3)	.1497	.0872
WMS(4)	.0081	.0027

(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.

[redacted] 2-WK SUBACUTE ORGAN/BODY WEIGHT MEAN ABSOLUTE DATA
H# 13383 RATS SACRIFICED AFTER 14 DAY OBSERVATION PERIOD

GROUP	FINAL WGT.	HEART	LUNGS	LIVER	SPLEEN
CONTROLS	350.2000	1.1000	2.0420	12.8760	.6760
LOW LEVEL(1 MG/L)	345.8000	1.2300+	2.0460	12.9560	.6440
HIGH LEVEL(10 MG/L)	357.5000	1.1950	2.1750	13.4075	.7200
F RATIO(1)	.623	2.792	.355	.143	.853
LSD(2)	22.7104	.1298	.3811	2.2753	.1257
DUNNETT(3)	26.1051	.1492	.4380	2.6155	.1445
WMS(4)	245.6909	.0080	.0692	2.4662	.0075

GROUP	KIDNEY	TESTIS	THYMUS
CONTROLS	2.5700	3.1000	.7580
LOW LEVEL(1 MG/L)	2.7780	3.1580	.7000
HIGH LEVEL(10 MG/L)	2.8425#	3.3175	.7825
F RATIO(1)	3.729	1.407	.428
LSD(2)	.2308	.2864	.2016
DUNNETT(3)	.2653	.3292	.2317
WMS(4)	.0254	.0391	.0194

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.

[redacted] 2-WK SUBACUTE ORGAN/BODY WEIGHT MEAN RELATIVE DATA
H# 13383 RATS SACRIFICED AFTER 14 DAY OBSERVATION PERIOD

GROUP	HEART	LUNGS	LIVER	SPLEEN	KIDNEY
CONTROLS	.3143	.5829	3.6726	.1931	.7331
LOW LEVEL(1 MG/L)	.3559#	.5920	3.7401	.1862	.8056+
HIGH LEVEL(10 MG/L)	.3340	.6071	3.7480	.2009	.7957
F RATIO(1)	4.933*	.153	.060	.507	3.482
LSD(2)	.0304	.0949	.5339	.0315	.0675
DUNNETT(3)	.0349	.1091	.6137	.0362	.0776
WMS(4)	.0004	.0043	.1358	.0005	.0022

GROUP	TESTIS	THYMUS
CONTROLS	.8861	.2170
LOW LEVEL(1 MG/L)	.9144	.2011
HIGH LEVEL(10 MG/L)	.9290	.2187
F RATIO(1)	.579	.306
LSD(2)	.0890	.0554
DUNNETT(3)	.1023	.0636
WMS(4)	.0038	.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.