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HASKELL LABORATORY REPORT NO. 379-81 [redacted]

Material Tested	Haskell No.	Other Codes
[redacted]	13,384	[redacted]

Study Initiated/Completed
8/8/80 - 9/5/80

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

SUBACUTE INHALATION TOXICITY STUDY IN RATS

I. Introduction:

The purpose of this study was to determine the effects of [redacted] on male rats after repeated inhalation exposure. A previous study determined an LC50 of [redacted] to be 35.3 mg/L.

II. Procedures:

A. Animals: Male Crl:CD® rats were housed 2/cage in 8" x 8" x 14" stainless steel, wire mesh cages and provided Purina Certified Rodent Chow® #5002 and water ad libitum. Rats were quarantined under these conditions and observed for general suitability for one week prior to testing.

B. Exposure Protocol: Groups of 10 rats, 8-weeks old and weighing between 240 and 271 grams were exposed to design concentration of 0, 1.0 and 3.0 mg/L of [redacted] Rats were exposed whole body, 6 hrs/day, 5 days/week for two weeks. All rats were weighed and observed daily through both the exposure period and a 14-day observation period (except weekends and holidays).

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C. Generation: Prior to test, the sample was liquified by heating it to approximately 100°C. A 50 cc plastic syringe wrapped with heating tape was loaded with liquified sample and placed on a syringe drive. The heated sample was sprayed onto the surface of a heated, round bottom flask. Dilution air swept the resultant vapor/aerosol mixture through heated glass connectors into the top of a 17 L glass chamber containing the test rats.

D. Analytical: Chamber samples were collected approximately every 1/2 hr by drawing a known volume of chamber atmosphere through 2 tandem midget impingers. The impingers contained acetone (ACS grade) as the trapping solvent.

Samples were analyzed using a Hewlett Packard #5730A gas chromatograph equipped with a flame ionization detector. A 6' x 1/4" glass column, packed with 20% neopentyl glycol sebacate on 60/80 mesh Chromosorb® PAW, was used to chromatograph the samples. Oven temperature was programmed to increase at 8°C per minute from an initial temperature of 90°C, until the third sample peak eluted. At this point the program was changed to 32°C/min until all other sample peaks eluted and the column was clear.

[redacted] concentrations were based on the second largest peak in the G.C. chromatograph. This peak is identified as [redacted] 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 dilutions of the test compound.

Chamber temperatures were monitored throughout each exposure.

E. Clinical Pathology[1]: After the 9th exposure and 13th observation day all test animals were individually housed in stainless steel metabolism cages for overnight (16-hour) urine collection. Water and ground Purina Certified Rodent Chow® #5002 were available ad libitum.

Urinalysis included a measure of the urine volume, osmolality, pH, and tests for sugar, blood, urobilinogen, bilirubin, protein, and acetone. The urine color and appearance were noted and sediment from pooled specimens examined microscopically. Fluoride concentration and total fluoride excreted in the urine were also measured.

Blood samples were taken from the rats' tails after the 10th exposure and the 14th observation day. Hematology measurements included: erythrocyte count, hemoglobin, platelet count, mean corpuscular volume, leukocyte count, and relative number of neutrophils, lymphocytes, eosinophils, monocytes, and basophils. Hematocrit, mean corpuscular hemoglobin and mean corpuscular hemoglobin concentration were calculated from these data.

Blood serum measurements included: alkaline phosphatase, glutamic-pyruvic transaminase, glutamic-oxalacetic transaminase, urea nitrogen, creatinine, and total protein.

F. Pathology[2]: After the 10th exposure, 5 rats from each group were selected at random and sacrificed for gross and histopathologic examination. Remaining rats were sacrificed on the 14th observation day for an identical examination. Organs and tissues examined included the ear pinna, skin, thymus†, mediastinal tissue, spleen†, bone marrow (sternum), heart†, trachea, lungs†, esophagus, stomach, small intestines (duodenum, jejunum, and ileum), large intestines (cecum and colon), liver†, kidneys†, testes†, epididymides, thyroids, adrenals, brain, eyes and any other tissues observed to be abnormal at necropsy.

G. Organ and Body Weight Analysis: Three parameters were calculated for each group and test groups were then compared with controls for statistical differences: mean body weight (Appendix I), mean organ weight†, and organ/body weight ratio (Appendix II). The mean body weights were also plotted and are attached as Figure I.

III. Results:

Chamber temperature was controlled at $\leq$ 30°C by placing dry ice on the top of the exposure chamber.

At times a cloud was visible in the high level chamber.

During exposure the generation nebulizer plugged repeatedly, presumably because the low boiling components evaporated readily while high boiling solids did not. This problem accounts for the high standard deviation and range for these exposure concentrations.

A. Exposure Data

	Design Level - 1.0 mg/L		
Exposure No.	Mean	S.D.	Range
1	0.82	0.46	0.21 - 1.62
2	0.59	0.30	0.26 - 1.09
3	0.49	0.34	0.14 - 1.09
4	1.48	0.41	0.93 - 2.31
5	0.96	0.65	0.45 - 2.33
6	0.97	0.12	0.77 - 1.18
7	0.86	0.45	0.20 - 1.40
8	1.01	0.35	0.64 - 1.74
9	0.89	0.64	0.19 - 2.07
10	1.11	0.59	0.60 - 2.48
Overall††	0.90	0.50	0.14 - 2.48

† Organs weighed at necropsy
†† Mean of all samples from 10 exposures

Exposure No.	Design Level - 3.0 mg/L Mean	S.D.	Range
1	1.56	0.83	0.40 - 3.37
2	2.90	1.18	1.40 - 5.34
3	2.13	0.71	1.14 - 3.43
4	3.34	1.84	1.25 - 6.84
5	3.53	1.73	1.30 - 5.59
6	3.01	0.78	1.82 - 4.74
7	3.21	1.10	1.71 - 4.46
8	3.02	1.53	1.77 - 6.46
9	2.78	1.11	1.20 - 4.08
10	3.13	1.19	1.90 - 5.13
Overall††	2.83	1.32	0.40 - 6.84

B. Clinical Observations: During exposure rats exposed to [redacted] at both levels had reduced response to sound, clear and red discharge from eyes and nose, moderate salivation, and slight dose-dependent weight loss. During the 14-day observation period no differences were observed between controls and treated rats.

C. Clinical Pathology[1]: After 10 exposures all test animals had significant dose-related increases in serum alkaline phosphatase activity and urinary fluoride excretion. Rats exposed at 2.8 mg/L also had elevated serum glutamic-pyruvic transaminase and glutamic-oxalacetic transaminase activities and excreted more dilute urine than controls.

After the 14-day observation period, remaining test rats had lower erythrocyte counts and higher urinary fluoride levels than controls. These rats also tended to have significantly less serum creatinine and excreted a more alkaline urine.

D. Pathology[2]: No gross findings related to the exposure were noted in any rat at necropsy.

Histologic findings showed mild hepatocyte and renal tubular epithelial damage after 10 exposures to [redacted] at 2.8 mg/L. However, the toxic effect was reversible following the 14-day observation period.

No compound related lesions were detected in rats exposed at 0.9 mg/L at either sacrifice.

E. Organ and Body Weight Analysis (Appendix I & II): The mean body weight of the high level rats was significantly lower than controls on test days 2 through 16 (Figure 1).

After 10 exposures significant dose dependent increases were noted in liver and kidney weights and organ/body weight ratios. Also noted were decreases in high level heart and lung weights, an increase in high level testis/body weight ratio, and increases in low level lung weight and lung/body weight ratio.

†† Mean of all samples from 10 exposures

Following the 14-day observation period high level liver and kidney absolute weights and organ/body weight ratios were still significantly increased. Other anomalies noted at the first sacrifice were no longer evident, however, there was an apparent dose-related increase in thymus-body weight ratio of exposed animals.

VI. Summary

Groups of 10 male Crl:CD® rats were exposed 6 hours/day, 5 days/week for 2 weeks to average concentrations of 0.9 or 2.8 mg/L of [redacted] in air. A control group was simultaneously exposed to air only.

During exposure, moderate salivation, clear to red discharges from eyes and nose, reduced response to sound, and slight dose dependent weight loss were noted in all exposed rats.

During the 14-day observation period there were no visible differences in appearance of controls and treated rats.

After 10 exposures test rats had significant dose-related increases in serum alkaline phosphatase activity and in urinary fluoride concentrations. Rats exposed at 2.8 mg/L also had elevated serum glutamic-pyruvic transaminase and glutamic-oxalacetic transaminase activities and excreted more dilute urine than controls.

Following a 14-day observation period both exposed groups had lower erythrocyte counts and excreted more fluoride than the controls. Treated rats also exhibited significant dose-dependent depression of serum creatinine levels and excreted more alkaline urine than controls.

No gross pathologic findings were noted upon necropsy during either sacrifice. Histologic findings in rats after 10 exposures to 2.8 mg/L were mild hepatocyte and renal tubular epithelial damage. This effect was determined to be reversible upon examination of rats after a 14-day observation period.

Mean body weights of high level rats were significantly lower on test days 2-16 than controls.

After 10 exposures significant dose dependent increases were noted in liver and kidney absolute weights and organ/body weight ratios. Also noted were decreases in high level heart and lung weights, an increase in high level testis/body weight ratio, and increases in low level lung absolute weights and lung/body weight ratios.

Liver and kidney absolute weights and organ/body weight ratios were still significantly increased after the 14-day observation period. Further, a dose-related increase in thymus/body weight ratios was observed at this time.

In conclusion, organ/body weight trends and clinical results are supported by pathologic lesions observed in the livers and kidneys of rats exposed to 2.8 mg/L. Some of these organ/body weight and clinical results were also observed in rats exposed to 0.9 mg/L however, pathologic lesions were not.

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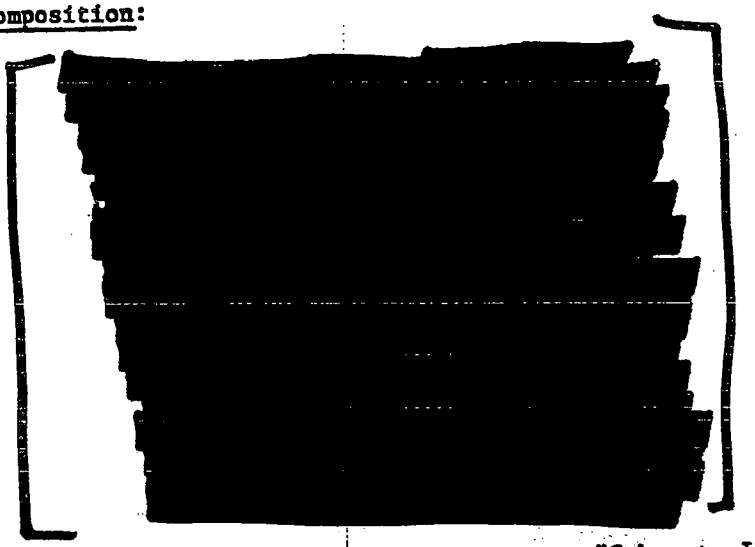
* Other Name: [redacted] 1647

Purity: [redacted]

Composition:



1 Matarese, Catherine C. and John R. Barnes, "Subacute Inhalation Toxicity of [redacted] H #13,384, Dec. 1, 1980.

2 Chen, Hans H. C., and William C. Krauss, [redacted] H #13,384, March 27, 1981.

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Report No. 379-81

[redacted] SUBACUTE BODY WEIGHT CURVE

[redacted] HW 13384

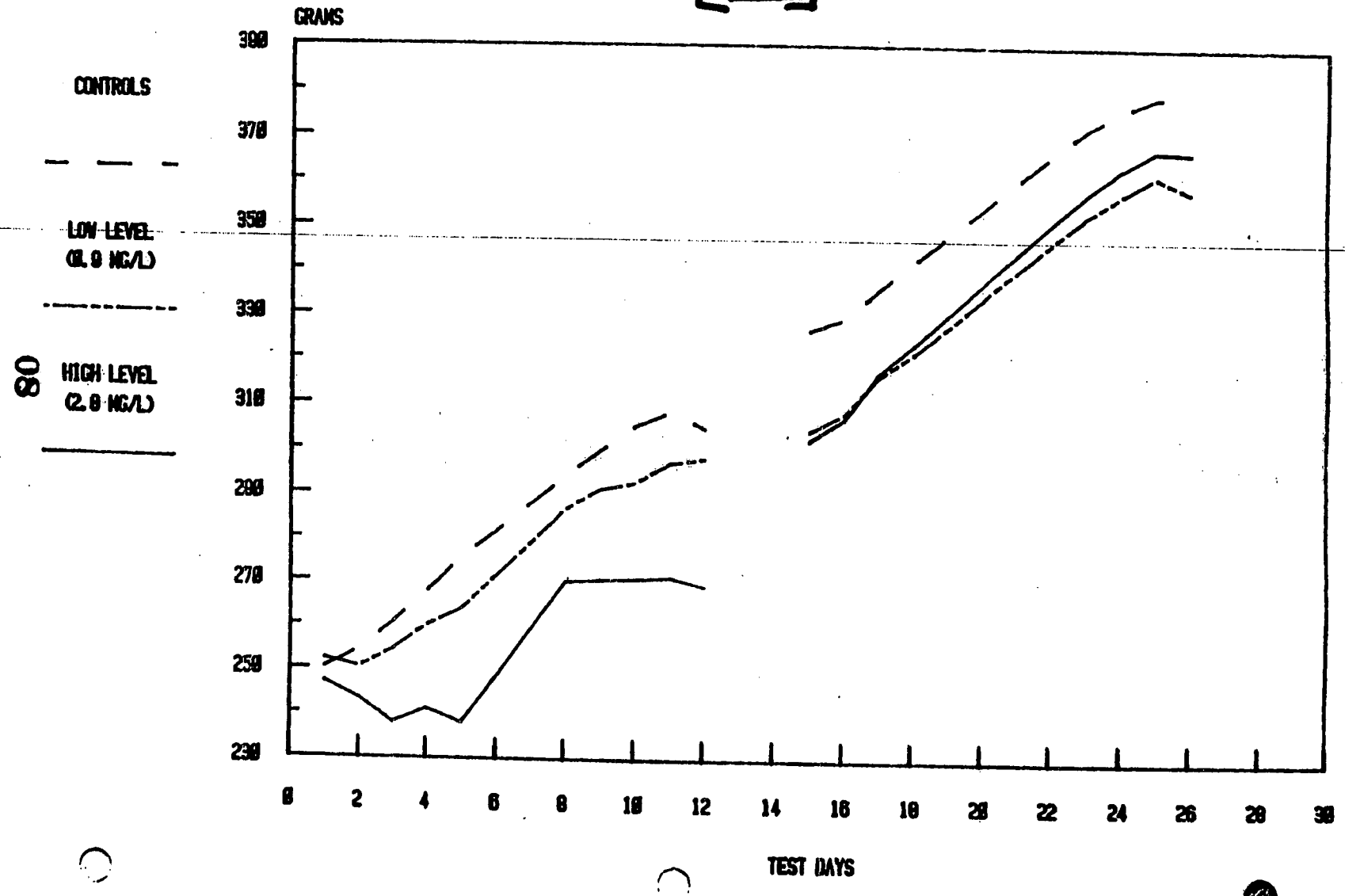

FIGURE 1

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APPENDIX I

[redacted] 2-WK SUBACUTE BODY WEIGHT SUMMARY TABLE
[redacted] H# 13384

INTERVALS IN TEST DAYS

GROUP	1.	2.	3.	4.	5.
CONTROLS	250.3000	254.3000	260.7000	267.6000	275.6000
LOW LEVEL(0.9 MG/L)	252.2000	250.4000	254.4000	260.0000	263.9000+
HIGH LEVEL(2.8 MG/L)	247.1000	243.4000#	237.9000#	241.0000#	238.0000#
F RATIO(1)	0.961	4.853*	14.322*	15.110*	23.503*
LSD(2)	7.6295	7.2744	9.0276	10.2276	11.5168
DUNNETT(3)	8.6267	8.2253	10.2076	11.5645	13.0222
WMS(4)	69.1333	62.8481	96.7926	124.2370	157.5296

INTERVALS IN TEST DAYS

GROUP	8.	9.	10.	11.	12.
CONTROLS	293.9000	299.6000	305.0000	308.2000	304.5000
LOW LEVEL(0.9 MG/L)	286.4000	291.0000	292.5000	296.9000	297.9000
HIGH LEVEL(2.8 MG/L)	269.9000#	270.3000#	270.5000#	270.9000#	268.9000#
F RATIO(1)	9.013*	12.590*	14.786*	15.449*	12.032*
LSD(2)	11.8674	12.3165	13.1806	14.1200	15.8424
DUNNETT(3)	13.4186	13.9264	14.9035	15.9657	17.9132
WMS(4)	167.2667	180.1667	206.3333	236.7926	298.0852

INTERVALS IN TEST DAYS

GROUP	15.	16.	17.	18.	23.
CONTROLS	326.4000	329.0000	335.4000	341.6000	371.8000
LOW LEVEL(0.9 MG/L)	304.0000+	307.8000	316.0000	321.0000	352.2000
HIGH LEVEL(2.8 MG/L)	302.0000#	306.6000+	316.6000	323.0000	357.2000
F RATIO(1)	4.525*	3.287	1.904	2.129	1.029
LSD(2)	19.6221	21.4144	24.6323	23.9906	30.9420
DUNNETT(3)	22.5148	24.5713	28.2636	27.5273	35.5035
WMS(4)	202.7667	241.5000	319.5333	303.1000	504.2000

INTERVALS IN TEST DAYS

GROUP	24.	25.	26.
CONTROLS	376.0000	379.0000	379.8000
LOW LEVEL(0.9 MG/L)	357.0000	361.2000	357.8000
HIGH LEVEL(2.8 MG/L)	362.8000	367.0000	366.6000
F RATIO(1)	0.873	0.796	1.191
LSD(2)	32.1076	31.3495	31.2636
DUNNETT(3)	36.8409	35.9711	35.8725
WMS(4)	542.9000	517.5667	514.7333

(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

[redacted] 2-WK SUBACUTE-ORGAN & BODY WEIGHT MEAN ABSOLUTE DATA
H# 13384 RATS SACRIFICED AFTER 10 EXPOSURES

GROUP	FINAL WGT.	HEART	LUNGS	LIVER	SPLEEN
CONTROLS	302.2000	.9840	1.6960	10.4620	.6620
LOW LEVEL(0.9 MG/L)	306.4000	1.0120	2.0220#	14.4160#	.6080
HIGH LEVEL(2.8 MG/L)	262.4000#	.8360#	1.5260+	15.1900#	.5540
F RATIO(1)	8.622*	7.260*	26.709*	16.309*	1.542
LSD(2)	25.4811	1082	.1503	1.9349	.1340
DUNNETT(3)	29.2375	.1241	.1724	2.2202	.1538
WMS(4)	341.9333	.0062	.0119	1.9717	.0095

GROUP	KIDNEY	TESTIS	THYMUS
CONTROLS	2.3680	2.9480	.6620
LOW LEVEL(0.9 MG/L)	2.61[illegible]	3.1820	.7460
HIGH LEVEL(2.8 MG/L)	2.7580+	3.1440	.5780
F RATIO(1)	2.445	1.488	5.209*
LSD(2)	.3890	.3172	.1134
DUNNETT(3)	.4463	.3639	.1301
WMS(4)	.0797	.0530	.0068

(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 RELATIVE DATA
H# 13384 RATS SACRIFICED AFTER 10 EXPOSURES

GROUP	HEART	LUNGS	LIVER	SPLEEN	KIDNEY
CONTROLS	.3253	.5621	3.4518	.2195	.7833
LOW LEVEL(0.9 MG/L)	.3304	.6600#	4.7067#	.1982	.8542
HIGH LEVEL(2.8 MG/L)	.3199	.5821	5.7825#	.2125	1.0573#
F RATIO(1)	.208	19.896*	94.735*	.458	8.090*
LSD(2)	.0356	.0357	.3693	.0495	.1540
DUNNETT(3)	.0409	.0410	.4237	.0568	.1767
WMS(4)	.0007	.0007	.0718	.0013	.0125

GROUP	TESTIS	THYMUS
CONTROLS	.9788	.2197
LOW LEVEL(0.9 MG/L)	1.0391	.2434
HIGH LEVEL(2.8 MG/L)	1.2077#	.2187
F RATIO(1)	5.192*	2.110
LSD(2)	.1605	.0296
DUNNETT(3)	.1841	.0340
WMS(4)	.0136	.0005

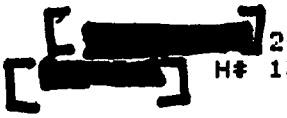
(1) Ratio of among- to within-group variation--one-factor analysis of variance.
(2) Least significant difference--given a significant (alpha=0.05) F ratio, any two means differing by more than the LSD are significantly different with a variable-wise false positive (alpha) error rate of 0.05.
(3) Dunnett 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.

2-WK SUBACUTE-ORGAN & BODY WEIGHT MEAN ABSOLUTE DATA
H# 13384 RATS SACRIFICED AFTER 14 DAY OBSERVATION PERIOD

GROUP	FINAL WGT.	HEART	LUNGS	LIVER	SPLEEN
CONTROLS	379.8000	1.1660	2.3280	14.5760	.7420
LOW LEVEL(0.9 MG/L)	357.8000	1.1700	2.1160	14.5280	.7320
HIGH LEVEL(2.8 MG/L)	366.6000	1.1940	2.1760	18.2100#	.8020
F RATIO(1)	1.191	.116	1.252	6.798*	.657
LSD(2)	31.2636	.1371	.3009	2.4960	.1439
DUNNETT(3)	35.8725	.1573	.3453	2.8639	.1651
WMS(4)	514.7333	.0099	.0477	3.2809	.0109

GROUP	KIDNEY	TESTIS	THYMUS
CONTROLS	2.7040	3.2840	.6800
LOW LEVEL(0.9 MG/L)	2.6400	3.2880	.7560
HIGH LEVEL(2.8 MG/L)	3.2940#	3.2460	.8300
F RATIO(1)	14.986*	.099	2.190
LSD(2)	.2870	.2273	.1562
DUNNETT(3)	.3293	.2608	.1792
WMS(4)	.0434	.0272	.0128

(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.



2-WK SUBACUTE-ORGAN/BODY WEIGHT MEAN RELATIVE DATA
H# 13384 RATS SACRIFICED AFTER 14 DAY OBSERVATION PERIOD

GROUP	HEART	LUNGS	LIVER	SPLEEN	KIDNEY
CONTROLS	.3072	.6119	3.8263	.1954	.7140
LOW LEVEL(0.9 MG/L)	.3269	.5918	4.0587	.2040	.7398
HIGH LEVEL(2.8 MG/L)	.3265	.5934	4.9587#	.2194	.9008#
F RATIO(1)	1.077	.396	20.982*	1.109	10.391*
LSD(2)	.0333	.0547	.4023	.0356	.0968
DUNNETT(3)	.0383	.0627	.4617	.0409	.1110
WMS(4)	.0006	.0016	.0852	.0007	.0049

GROUP	TESTIS	THYMUS
CONTROLS	.8660	.1793
LOW LEVEL(0.9 MG/L)	.9205	.2112+
HIGH LEVEL(2.8 MG/L)	.8910	.2248#
F RATIO(1)	.710	5.144*
LSD(2)	.0998	.0318
DUNNETT(3)	.1145	.0365
WMS(4)	.0052	.0005

(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.