BORRISTON
LABORATORIES, INC.

14-DAY SUBACUTE TOXICITY STUDY IN THE RAT WITH D-8

Borriston Project No. 1452(A)
FINAL REPORT

Submitted to:

LORILLARD
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Submitted by:

BORRISTON LABORATORIES, INC.

July 30, 1982

SPONSOR: Lorillard

INITIATION DATE: June 3, 1982
COMPLETION DATE: June 17, 1982
ISSUE DATE: July 30, 1982

MATERIAL: D-8

SUBJECT: 14-DAY SUBACUTE TOXICITY STUDY IN THE RAT WITH D-8
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SUMMARY

This study was designed to evaluate the subacute toxicity of orally administered D-8 in rats. Three groups of five male and five female rats each received D-8 by oral intubation for 14 consecutive days at dose levels of 1260, 2510, and 5010 mg/kg body weight (Groups 2, 3, and 4, respectively). A fourth group of five male and five female rats (Group 1) received distilled water each day and served as the control.

No signs of toxicity were noted among the D-8 treated animals; thus the maximum no effect level could not be determined, but is considered to be greater than 5010 mg/kg.

TEST ARTICLE

The test article, D-8, is a brown paste and was received from the sponsor on March 31, 1982. It was given the BRL code number 477, and stored in a cool dry area in the container in which it was received. All data which appropriately characterize the test article with respect to identity, strength, purity, composition, and stability under conditions of use are retained by the sponsor. The test article was used daily from June 3 through June 16, 1982. The test article was prepared on June 2, 1982 and June 9, 1982. Distilled water was used as the vehicle.

TEST ANIMALS

Twenty male and twenty female Sprague-Dawley albino rats, purchased from Charles River Breeding Laboratories (Wilmington, Massachusetts), were selected for this study. Prior to placement on the study, the animals were quarantined for at least seven days and observed twice daily for appearance and behavior. The animals were individually housed in suspended stainless steel cages with Purina® Rodent Laboratory Chow® #5001 and water was available ad libitum. At the end of the quarantine period, healthy animals were assigned to treatment groups using a computerized random number generator. Each animal assigned to the study received a unique permanent identification number and was ear-tagged with the last 4 digits of that number. Treatment groups were identified by color-coded cage cards placed on the outside of the animal cages. The male body weights ranged from 175 g to 216 g. The female body weights ranged from 126 g to 165 g.

EXPERIMENTAL DESIGN

The test article/vehicle suspensions were administered orally by intubation, once daily, for 14 consecutive days, to three groups of five male and five female rats at dose levels of 1260, 2510, and 5010 mg/kg. A fourth group (Group 1) of five male and five female rats received only the vehicle, distilled water, and served as the control group. A constant dosing volume of 10.0 ml/kg of body weight was used for all groups. All mixtures were thoroughly agitated just prior to and continually during dosing.

The test article in this study, D-8, was tested concurrently with another compound, D-9, and a common control group was used. The D-9 study was reported separately.

Observations for physical appearance, behavior, moribundity, pharmacotoxic signs, and mortality were performed on all animals three times daily throughout the study. Observations were made prior to dosing, one hour after dosing, and again in the afternoon after a minimum of five hours had elapsed. Body weights were recorded at Day 1 and Day 8, immediately prior to test article administration, and at termination.

All rats surviving on Day 15 were sacrificed by carbon dioxide asphyxiation. Complete necropsies were performed on all rats sacrificed and included observation of at least the following organs and tissues:

Brain/pituitary	Lungs	Pancreas
Thyroid/parathyroid	Liver	Kidneys
Esophagus/trachea	Spleen	Adrenals
Thymus	Stomach	Urinary Bladder
Heart	Intestines	Testes/Ovaries
		Uterus

Terminal body weights were recorded before sacrifice. At sacrifice, the liver and kidneys were weighed fresh.

The listing of key personnel participating in this study is presented in Appendix 1.

Statistics

Student's t-test at the 5% probability level was performed on body weights (Days 1, 8, and 15), mean change and terminal liver and kidney weights as well as relative weights of these organs.

Results

Mortality - No mortalities were observed during the study.

Clinical Signs - Table 1 summarizes the clinical signs observed in each animal with regard to nature, onset, and duration of each sign.

No treatment related clinical signs were observed during the study. Four males (Group 1, 820620 and 820623; Group 4, 820649, 820653) were observed to have a scab on the right side of the neck, one male (Group 3, 820643) had hair loss on the head. This was a result of the animals rubbing against the springs holding the feed jars in place. One male (Group 3, 820640) had a red discharge from the mouth immediately after dosing. This sign was believed to be produced by dosing trauma.

Body Weight - Mean body weights (Days 1, 8, and 15) and mean body weight changes are presented in Table 2. The mean body weights of all treated groups were similar to the controls on Days 1, 8, and 15 of study. No statistically significant difference was noted in body weight change during the study between the D-8 treated groups and the vehicle control animals.

Gross Pathology - Individual necropsy findings are presented in Table 3. No lesions or abnormalities were observed in any of the animals on study.

Organ Weights - Mean terminal body weights and mean absolute and relative organ weights are presented in Table 4. Mean absolute and relative weights of male and female livers and kidneys were comparable between the treated groups and the respective control group.

CONCLUSION

Based on the results of this study a maximum no effect level could not be determined, but is considered to be greater than 5010 mg/kg.

RAW DATA AND FINAL REPORT STORAGE

All raw data and final reports are stored in the archives at Borriston Laboratories, Inc., 5050 Beech Place, Temple Hills, Maryland 20748.

Sponsor: Dr. H.J. Minnemeyer

Submitted by: Vincent Picciriello Date: 7/30/82
V.J. Picciriello, Ph.D., D.A.B.T.

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TABLE 1

14-DAY SUBACUTE TOXICITY STUDY IN THE RAT WITH D-8

CLINICAL OBSERVATIONS

GROUP NO.	DOSE LEVEL (mg/kg)	ANIMAL NO.	SEX	CLINICAL SIGNS[b]	DAY:[a] 1	2	3	4	5	6	7	8	9	10	11	12	13	14
1	0	820620	M	Scab right side of neck									3	3	3	3	3	3
		820623	M	Scab right side of neck												3	3	3
3	2510	820640	M	Red discharge mouth											1			
		820643	M	Hair loss head			3	3	3	3	3	3	3	3	3			3
4	5010	820649	M	Scab right side of neck												3		
		820653	M	Scab right side of neck												3	3	

[a]Clinical signs were observed three times daily for each animal. Numerals indicate the total number of times the clinical signs were observed during each day.

[b]All other animals on study were observed as normal for the duration of the study.

TABLE 2

14-DAY SUBACUTE TOXICITY STUDY IN THE RAT WITH D-8

MEAN BODY WEIGHTS (g)

GROUP NO.	DOSE LEVEL (mg/kg)		MALES DAY 1	MALES DAY 8	MALES DAY 15	WEIGHT CHANGE DAYS 1-15	FEMALES DAY 1	FEMALES DAY 8	FEMALES DAY 15	WEIGHT CHANGE DAYS 1-15
1	0	$\bar{x}$	191	243	289	97	145	169	193	48
		S.D.	12	15	17	9	12	14	20	9
		N	5	5	5	5	5	5	5	5
2	1260	$\bar{x}$	197	248	298	100	137	164	186	49
		S.D.	10	13	18	17	6	10	13	8
		N	5	5	5	5	5	5	5	5
3	2510	$\bar{x}$	194	246	295	101	138	162	183	46
		S.D.	9	13	16	7	10	9	14	10
		N	5	5	5	5	5	5	5	5
4	5010	$\bar{x}$	205	267	316	111	138	165	190	52
		S.D.	13	19	25	14	7	6	7	10
		N	5	5	5	5	5	5	5	5

TABLE 3

14-DAY SUBACUTE TOXICITY STUDY IN THE RAT WITH D-8

GROSS PATHOLOGIC OBSERVATIONS

GROUP 1

OBSERVATION:	Dose Level: (mg/kg)	0									
	Sex:	M	M	M	M	M	F	F	F	F	F
	Animal No.:	820619	820620	820621	820622	820623	820624	820625	820626	820627	820628
No gross lesions		X	X	X	X	X	X	X	X	X	X

TABLE 3 (Continued)

14-DAY SUBACUTE TOXICITY STUDY IN THE RAT WITH D-8

GROUP 2

OBSERVATION:	Dose Level: (mg/kg)	1260									
	Sex:	M	M	M	M	M	F	F	F	F	F
	Animal No.:	820629	820630	820631	820632	820633	820634	820635	820636	820637	820638
No gross lesions		X	X	X	X	X	X	X	X	X	X

TABLE 3 (Continued)

14-DAY SUBACUTE TOXICITY STUDY IN THE RAT WITH D-8

GROSS PATHOLOGIC OBSERVATIONS

GROUP 3

OBSERVATION:	Dose Level: (mg/kg)	2510									
	Sex:	M	M	M	M	M	F	F	F	F	F
	Animal No.:	820639	820640	820641	820642	820643	820644	820645	820646	820647	820648
No gross lesions		X	X	X	X	X	X	X	X	X	X

TABLE 3 (Continued)

14-DAY SUBACUTE TOXICITY STUDY IN THE RAT WITH D-8

GROSS PATHOLOGIC OBSERVATIONS

GROUP 4

OBSERVATION:	Dose Level: (mg/kg)	5010									
	Sex:	M	M	M	M	M	F	F	F	F	F
	Animal No.:	820649	820650	820651	820652	820653	820654	820655	820656	820657	820658
No gross lesions		X	X	X	X	X	X	X	X	X	X

TABLE 4

14-DAY SUBACUTE TOXICITY STUDY IN THE RAT WITH D-8

MEAN TERMINAL BODY WEIGHTS AND ORGAN WEIGHTS

GROUP NO.	DOSE LEVEL (mg/kg)	SEX		MEAN TERMINAL WEIGHTS (g) BODY	LIVER	KIDNEYS	MEAN RELATIVE WEIGHTS (%) LIVER	KIDNEYS
1	0	M	$\bar{x}$	289	14.73	2.75	5.10	0.95
			S.D.	17	1.18	0.28	0.15	0.09
			N	5	5	5	5	5
2	1260	M	$\bar{x}$	298	15.17	2.85	5.08	0.96
			S.D.	18	1.55	0.21	0.23	0.04
			N	5	5	5	5	5
3	2510	M	$\bar{x}$	295	14.64	2.72	4.96	0.92
			S.D.	16	1.34	0.24	0.23	0.07
			N	5	5	5	5	5
4	5010	M	$\bar{x}$	316	15.85	2.96	5.02	0.94
			S.D.	25	1.24	0.32	0.26	0.08
			N	5	5	5	5	5

TABLE 4 (Continued)

14-DAY SUBACUTE TOXICITY STUDY IN THE RAT WITH D-8

MEAN TERMINAL BODY WEIGHTS AND ORGAN WEIGHTS

GROUP NO.	DOSE LEVEL (mg/kg)	SEX		MEAN TERMINAL WEIGHTS (g) BODY	LIVER	KIDNEYS	MEAN RELATIVE WEIGHTS (%) LIVER	KIDNEYS
1	0	F	$\bar{x}$	193	9.45	1.87	4.88	0.97
			S.D.	20	1.46	0.13	0.33	0.07
			N	5	5	5	5	5
2	1260	F	$\bar{x}$	186	9.45	1.91	5.07	1.03
			S.D.	13	0.96	0.18	0.18	0.07
			N	5	5	5	5	5
3	2510	F	$\bar{x}$	183	9.63	1.82	5.23	0.99
			S.D.	14	1.28	0.19	0.38	0.06
			N	5	5	5	5	5
4	5010	F	$\bar{x}$	190	9.70	1.82	5.10	0.95
			S.D.	7	0.45	0.17	0.25	0.07
			N	4	4	4	4	4

APPENDIX 1

KEY PERSONNEL

The following key personnel participated in integral aspects of this study:

V.J. Piccirillo	Study Director
W.C. Hartman	Technical Supervisor
T. Brewer	Technician
H. Robinson	Technician
C. DiCarlo	Technician
O. Gaines	Animal Caretaker
C. Lunchick	Report Writer
C. Ullman	Quality Assurance Officer

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