

Study Title

Repeated Dose Oral Toxicity: 14-Day Feeding Study
with H-19683 in Male Mice

Author

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Performing Laboratory

E. I. du Pont de Nemours and Company
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[REDACTED]

Laboratory Project ID

Haskell Laboratory Report No. 325-95

REPEATED DOSE ORAL TOXICITY: 14-DAY FEEDING STUDY

WITH H-19683 IN MALE MICE

GENERAL INFORMATION

Substance Tested:

[REDACTED]

Synonyms/Codes:

• H-19683

[REDACTED]

Purity:

[REDACTED]

Physical Form:

[REDACTED]

Composition:

[REDACTED]

Contaminants:

[REDACTED]

C.A.S. Registry No.:

[REDACTED]

Sponsor:

DuPont Chemicals/DuPont Polymers
E. I. du Pont de Nemours and Company
Wilmington, Delaware

Study Initiated/Completed:

10/8/92 - 4/28/95

In-life

Study Initiated/Completed:

11/4/92 - 11/18/92

REPEATED DOSE ORAL TOXICITY: 14-DAY FEEDING STUDY
WITH H-19683 IN MALE MICE

SUMMARY

The purpose of this study was to evaluate the repeated dose oral toxicity of H-19683 when incorporated into nutritionally adequate chow and fed to male Crl:CD-1®BR mice for 14 consecutive days. Mice were fed diets containing 0, 3, 30, 300, or 3000 ppm H-19683. Mice were weighed and observed for clinical signs of toxicity once each week. Food consumption was not measured. At the end of the 2 week feeding period, all mice were euthanatized and necropsied. The livers were weighed, and liver weight/final body weight ratios were calculated.

No deaths occurred during the study. Other than a body weight effect in mice from the 3000 ppm dose group, no adverse clinical signs of toxicity were observed during the study. Significant decreases in mean body weight were observed in mice from the 3000 ppm dose group on test days 7 and 14. Statistically significant decreases in mean body weight gain were observed in this same dose group for the intervals 0-7 days on test, and overall.

Significant increases in mean absolute and relative liver weights were observed in mice from the 300 and 3000 ppm dose groups killed at the end of the 14-day feeding period.

The no observable adverse effect level (NOAEL) was determined to be 30 ppm. The NOAEL was based on the increases in mean absolute and relative liver weight observed in the 300 and 3000 ppm dose groups and the decreases in mean body weights and body weight gains observed in the 3000 ppm dose group.

Work by:

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REPEATED DOSE ORAL TOXICITY: 14-DAY FEEDING STUDY
WITH H-19683 IN MALE MICE

INTRODUCTION

The purpose of this study was to evaluate the repeated dose oral toxicity of H-19683 when incorporated into nutritionally adequate chow and fed to male Crl:CD®BR mice for 14 consecutive days. Mice were fed diets contained 0, 3, 30, 300, or 3000 ppm H-19683.

MATERIALS AND METHODS

A. Test Substance

The test substance, H-19683, was supplied by DuPont Specialty Chemicals, E. I. du Pont de Nemours and Company, Wilmington, Delaware. Purity was estimated to be [REDACTED]. Available information about the identity, purity, composition, stability, and toxicity of the test substance was filed with Haskell Laboratory Information Services by the Sponsor when the substance was submitted for testing. Upon receipt, the test substance was assigned a unique Haskell Number (H-19683).

B. Test Species

On 10/27/92, 30 male Crl:CD-1®(ICR)BR mice, born approximately 9/21/92, were received from Charles River Laboratories, Inc., Montreal, Canada. On 11/4/92, the body weights ranged from 24.4 to 29.6 grams. The Crl:CD-1®BR mouse was selected on the basis of extensive experience with this strain and its suitability with respect to hardiness, longevity, sensitivity, and low incidence of spontaneous diseases.

C. Animal Husbandry

All mice were housed singly in suspended stainless steel, wire-mesh cages throughout the study. Animal rooms were maintained on a 12-hour light/dark cycle and targeted at a temperature of $23 \pm 2^{\circ}\text{C}$ and a relative humidity of $50 \pm 10\%$.

Water was provided ad libitum from an automatic watering system. During the quarantine period (see Section D), all mice were fed (ground) Purina® Certified Rodent Chow® #5002. During the test period, all mice were fed the diet of their respective treatment group (see Section F).

Haskell Laboratory has an animal health monitoring program. This program is monitored and administered by the Laboratory Veterinarian. Water samples are periodically analyzed for total bacterial counts and for the presence of coliforms, lead, and other contaminants. Additionally, samples from freshly washed cages and cage racks are periodically analyzed to assure adequate sanitation by the cagewashers. Data from this program are maintained separately from study records. Animal feed is certified by the manufacturer to meet specified nutritional requirements and to be free of a list of specified contaminants. On the basis of these analyses, there is no evidence suggesting that contaminants were present in the feed or water in amounts which may have interfered with the results of this study.

D. Quarantine Period

Upon arrival at Haskell Laboratory, the mice were removed from shipping cartons and housed 3 per cage in suspended stainless steel, wire-mesh cages. During the quarantine period, each mouse was identified by color-code tail marking and a temporary identification number affixed to its cage. The animals were weighed 3 times during the quarantine period and observed daily for any gross signs of disease or injury.

On the basis of body weights and clinical observations, all mice were released from quarantine on 11/3/92 by the laboratory veterinarian.

E. Assignment to Groups and Study Start

After the quarantine period, 25 mice were selected for study use on the basis of adequate body weight gain and freedom from any clinical signs of disease or injury. The selected mice were divided by computerized, stratified randomization into 5 groups of 5 mice each, so there were no statistically significant differences among group body weight means. The order of mice within each group was randomized. Each group of mice was designated as either a control, low-, low-intermediate-, or high-concentration group.

After assignment to dosing groups, each mouse was assigned a 5-digit Haskell animal number. Each number was printed on a card affixed to the outside of the cage. Each mouse was also assigned a 3-digit identification number that was marked on the mouse's tail with water insoluble ink. The mice were approximately 44 days of age at study start. Mice not assigned to a test group were sacrificed by CO₂ asphyxiation.

F. Concentration Selection and Diet Administration

H-19683 was mixed in the diet at concentrations of 0 (control), 3, 30, 300, or 3000 ppm. These concentrations were selected so that the results of this study could be compared to the results obtained from an earlier study conducted with C-8.

During the test period, mice in each group were fed a diet of ground Purina® Certified Rodent Chow® #5002 that contained the following concentrations of H-19683:

<u>Group No.</u>	<u>Dietary Concentration^a</u>	<u># Rats/Group</u>
<u>Male</u>	<u>ppm</u>	
I	0 (Control)	5
III	3 (Low)	5
V	30 (Low-Intermediate)	5
VII	300 (Intermediate)	5
IX	3000 (High)	5

^a Weight/weight concentration of H-19683

G. Diet Preparation and Sampling

For each diet concentration, the appropriate amount of H-19683 was first added to a small amount of ground chow and mixed by hand until it appeared homogeneous. The hand-mixed portion was then added to the remainder of the ground chow weighed out for that diet in a Hobart low speed mixer and was mixed for 15 minutes. After mixing, the diet was visually inspected to ensure it was free of clumps. Control diets were also mixed for the same period of time. The diets for this study were prepared the day the study started and stored refrigerated until used. Samples for concentration, homogeneity, and stability were taken for analyses at a later date if deemed necessary by the study director.

H. Body Weights and Clinical Observations

All mice were weighed and observed for clinical signs of toxicity once each week throughout the study. Cageside examinations to detect moribund or dead mice were conducted twice daily.

I. Food Consumption, Food Efficiency, and Intake of H-19683

Food consumption was not determined during this study.

J. Necropsy

At the end of the 2-week feeding period, all mice were necropsied. The mice were euthanatized by chloroform anesthesia and exanguination. The livers were weighed at necropsy, and liver weight/final body weight ratios were calculated.

STATISTICAL ANALYSES

Body weights, body weight gains, and organ weights were analyzed by a one-way analysis of variance. When the statistic for differences among test group means (F statistic) was significant, pairwise comparisons between test and control groups were made with the Dunnett's test. Significance was judged at $\alpha = 0.05$.

RECORDS AND SAMPLE RETENTION

All original records will be retained at Haskell Laboratory or at the Records Management Center, E. I. du Pont de Nemours and Company, Wilmington, Delaware.

RESULTS

A. Diet Sample Analysis

All diets prepared appeared to be thoroughly mixed. No clumps or other signs of non-uniform mixing were observed. Therefore, diet samples taken at the time of diet preparation were discarded without analysis.

B. Body Weights and Body Weight Gains (Tables 1-2; Appendix I)

Statistically significant decreases in mean body weight of mice from the 3000 ppm dose group were observed on test days 7 and 14. Statistically significant decreases in mean body weight gains of this same group were observed for the interval 0-7 days on test and overall.

C. Clinical Observations

One mouse in the 3000 ppm dose group exhibited pallor of the ears on test days 7 and 14. No other clinical signs of toxicity were observed.

D. Necropsy (Tables 3,4; Appendix II)

Gross observations at necropsy revealed liver discoloration in one mouse from the 300 ppm dose group and in 4 mice from the 3000 ppm dose group. Significant increases in mean absolute and relative liver weights were observed in mice from the 300 and 3000 ppm dose groups. No other gross observations were noted.

CONCLUSIONS

Male mice were fed diets containing target concentrations of 0, 30, 300, or 3000 ppm H-19693 for 14 days. Significant body weight effects were observed in mice from the 3000 ppm dose group. Significant increases in mean absolute and relative liver weights were observed in mice from the 300 and 3000 ppm dose groups. The no observable adverse effect level observed during this study was 30 ppm.

TABLES

REPEATED ORAL DOSE TOXICITY: 14 DAY FEEDING STUDY WITH H 19683 IN MALE MICE

TABLE 1

MEAN BODY WEIGHTS OF MALE MICE

MEAN BODY WEIGHTS (g)

GROUP:	I	III	V	VII	IX
CONCENTRATION (PPM):	0	3	30	300	3000
DAYS ON TEST					
0	26.7(0.5)*	26.7(1.9)	27.2(1.1)	27.8(1.6)	27.8(0.6)
7	29.9(1.5)	30.6(1.5)	29.6(1.2)	29.8(1.2)	26.3(2.4)*
14	31.7(1.3)	31.9(1.3)	31.5(1.3)	31.0(1.3)	28.7(1.7)*

* Standard deviation is reported in parentheses

Statistical methods: One-way ANOV and Dunnett's tests were performed on weekly interval data. (See Statistical Analyses)

* Statistically significant difference from control at alpha = 0.05.

REPEATED ORAL DOSE TOXICITY: 14-DAY FEEDING STUDY WITH H-19683 IN MALE MICE

TABLE 2

MEAN BODY WEIGHT GAINS OF MALE MICE

MEAN BODY WEIGHT GAINS (g)

GROUP:	I	III	V	VII	IX
CONCENTRATION (PPM):	0	3	30	300	3000
DAYS ON TEST					
0-7	3.2(1.2) ^a	3.9(1.5)	2.3(1.1)	2.0(1.1)	-1.5(2.6)*
7-14	1.8(0.5)	1.4(0.6)	1.9(0.6)	1.2(0.4)	2.4(1.5)
0-14	5.0(0.9)	5.3(1.6)	4.2(1.2)	3.2(0.8)	0.9(2.0)*

^a Standard deviation is reported in parentheses

Statistical methods: One-way ANOVA and Dunnett's tests were performed on both weekly and summary interval data. (See Statistical Analyses)

* Statistically significant difference from control at alpha = 0.05.

**REPEATED DOSE ORAL TOXICITY: 14-DAY FEEDING
STUDY WITH H-19683 IN MALE MICE**

TABLE 3

MEAN FINAL BODY AND ORGAN WEIGHTS FROM MALE MICE

MEAN FINAL BODY WEIGHTS (grams)

GROUP	CONC. (PPM)	MEAN FINAL BODY WEIGHTS
I	0	31.7 (1.3)
III	3	31.9 (1.3)
V	30	31.5 (1.3)
VII	300	31.0 (1.3)
IX	3000	28.7 (1.7)#

MEAN ABSOLUTE ORGAN WEIGHTS (grams)

GROUP	CONC. (PPM)	LIVER
I	0	1.811 (0.112)
III	3	1.907 (0.154)
V	30	2.241 (0.344)
VII	300	2.871 (0.336)#
IX	3000	4.365 (0.685)#

MEAN RELATIVE ORGAN WEIGHTS (% of body weight)

GROUP	CONC. (PPM)	LIVER
I	0	5.7043 (.1324)
III	3	5.9781 (.5546)
V	30	7.0975 (.7963)
VII	300	9.2486 (.9889)#
IX	3000	15.1789 (1.920)#

Standard Deviation in parentheses

- Significantly different ($p < 0.05$) from control group by
Dunnett's Test

**REPEATED DOSE ORAL TOXICITY: 14-DAY FEEDING
STUDY WITH H-19683 IN MALE MICE**

TABLE 3 (continued)

MEAN FINAL BODY AND ORGAN WEIGHTS FROM MALE MICE

MEAN FINAL BODY WEIGHTS (grams)

GROUP	CONC. (PPM)	MEAN FINAL BODY WEIGHTS
I	0	31.7 (0.000)
III	3	31.9 (0.803)
V	30	31.5 (0.768)
VII	300	31.0 (0.444)
IX	3000	28.7 (0.002)#

TEST - HOMOGENEITY	0.008
TEST - TREND	0.002
BARTLETT'S TEST	0.972

MEAN ABSOLUTE ORGAN WEIGHTS (grams)

GROUP	CONC. (PPM)	LIVER
I	0	1.811 (0.000)
III	3	1.907 (0.697)
V	30	2.241 (0.092)
VII	300	2.871 (0.000)#
IX	3000	4.365 (0.000)#

TEST - HOMOGENEITY	0.000
TEST - TREND	0.000
BARTLETT'S TEST	0.011

Values in parentheses - P value of Student T Test comparison of treatment mean to control mean.

- Significantly different ($p < 0.05$) from control group by Dunnett's Test

Homogeneity - P value of F Test of whether group means are equal.

Trend - P value of F test of whether there is a dose-related change in group means.

Bartlett's Test - P value of Test of Homogeneity of Variance.

**REPEATED DOSE ORAL TOXICITY: 14-DAY FEEDING
STUDY WITH H-19683 IN MALE MICE**

TABLE 3 (continued)

MEAN FINAL BODY AND ORGAN WEIGHTS FROM MALE MICE

MEAN FINAL BODY WEIGHTS (grams)

GROUP	CONC. (PPM)	<u>MEAN FINAL BODY WEIGHTS</u>
I	0	31.7 (0.000)
III	3	31.9 (0.803)
V	30	31.5 (0.768)
VII	300	31.0 (0.444)
IX	3000	28.7 (0.002)*

TEST - HOMOGENEITY	0.008
TEST - TREND	0.002
BARTLETT'S TEST	0.972

MEAN RELATIVE ORGAN WEIGHTS (% of body weight)

GROUP	CONC. (PPM)	LIVER
I	0	5.7043 (0.000)
III	3	5.9781 (0.688)
V	30	7.0975 (0.051)
VII	300	9.2486 (0.000)*
IX	3000	15.1789 (0.000)*

TEST - HOMOGENEITY	0.000
TEST - TREND	0.000
BARTLETT'S TEST	0.001

Values in parentheses - P value of Student T Test comparison of treatment mean to control mean.

- Significantly different ($p < 0.05$) from control group by Dunnett's Test

Homogeneity - P value of F Test of whether group means are equal.

Trend - P value of F test of whether there is a dose-related change in group means.

Bartlett's Test - P value of Test of Homogeneity of Variance.

REPEATED ORAL DOSE TOXICITY: 14-DAY FEEDING STUDY WITH H-19683 IN MALE MICE

TABLE 4
INCIDENCES OF GROSS OBSERVATIONS IN MALE MICE

SITE/LESION:	GROUP DESIGNATION:		I		III		V		VII		IX	
	CONCENTRATION (PPM):		0		3		30		300		3000	
	NUMBER IN GROUP:		5		5		5		5		5	
LIVER			0		0		0		0		1	
LARGE DISCOLORATION			0		0		0		1		4	

NOTE: 0 INDICENCES REFLECT THE NUMBER OF ANIMALS WITH A GIVEN LESION.

APPENDIX I

INDIVIDUAL BODY WEIGHTS

**REPEATED DOSE ORAL TOXICITY: 14-DAY FEEDING
STUDY WITH H-19683 IN MALE MICE**

APPENDIX I

INDIVIDUAL BODY WEIGHTS (g) OF MALE MICE

GROUP I - 0 PPM

DAYS			
ON TEST:	0	7	14
ANIMAL #			
90620	26.4	30.4	31.5
90621	27.3	31.0	32.6
90622	27.1	31.5	33.4
90623	26.7	28.5	30.8
90624	26.2	28.3	30.3

GROUP III - 3 PPM

DAYS			
ON TEST:	0	7	14
ANIMAL #			
90625	28.2	30.7	31.6
90626	26.4	31.6	32.2
90627	29.0	32.1	33.6
90628	25.3	28.3	30.1
90629	24.4	30.2	32.2

GROUP V - 30 PPM

DAYS			
ON TEST:	0	7	14
ANIMAL #			
90630	26.3	30.2	31.7
90631	26.0	28.6	30.6
90632	28.7	31.3	33.5
90633	27.3	28.7	31.4
90634	27.8	29.0	30.1

REPEATED DOSE ORAL TOXICITY: 14-DAY FEEDING
STUDY WITH H-19683 IN MALE MICE

APPENDIX I (Continued)

INDIVIDUAL BODY WEIGHTS (g) OF MALE MICEGROUP VII - 300 PPM

DAYS
ON TEST: 0 7 14

ANIMAL

90635	28.6	29.9	31.0
90636	28.1	31.4	32.3
90637	29.6	30.4	32.1
90638	25.2	28.2	29.1
90639	27.6	29.2	30.7

GROUP IX - 3000 PPM

DAYS
ON TEST: 0 7 14

ANIMAL

90640	28.1	27.5	28.6
90641	28.7	26.7	28.3
90642	27.9	22.1	26.7
90643	27.1	27.0	28.4
90644	27.3	28.1	31.4

APPENDIX II

INDIVIDUAL ANIMAL FINAL BODY AND ORGAN WEIGHTS

Explanatory Notes

STD. DEV. = Standard Deviation

REPEATED DOSE ORAL TOXICITY: 14-DAY FEEDING
STUDY WITH H-19683 IN MALE MICE

APPENDIX II

INDIVIDUAL ANIMAL FINAL BODY AND ORGAN WEIGHTS

FINAL BODY AND ABSOLUTE ORGAN WEIGHTS (grams) FROM MALE MICEGROUP I - 0 PPM

ANIMAL NUMBER	FINAL	
	BODY	LIVER
90620	31.5	1.783
90621	32.6	1.903
90622	33.4	1.951
90623	30.8	1.705
90624	30.3	1.711
GROUP MEAN	31.7	1.811
STD. DEV.	1.3	0.112

GROUP III - 3 PPM

ANIMAL NUMBER	FINAL	
	BODY	LIVER
90625	31.6	2.098
90626	32.2	1.914
90627	33.6	1.946
90628	30.1	1.906
90629	32.2	1.669
GROUP MEAN	31.9	1.907
STD. DEV.	1.3	0.154

GROUP V - 30 PPM

ANIMAL NUMBER	FINAL	
	BODY	LIVER
90630	31.7	2.370
90631	30.6	1.909
90632	33.5	2.736
90633	31.4	2.265
90634	30.1	1.924
GROUP MEAN	31.5	2.241
STD. DEV.	1.3	0.344

REPEATED DOSE ORAL TOXICITY: 14-DAY FEEDING
STUDY WITH H-19683 IN MALE MICE

APPENDIX II (Continued)

INDIVIDUAL ANIMAL FINAL BODY AND ORGAN WEIGHTS

FINAL BODY AND ABSOLUTE ORGAN WEIGHTS (grams) FROM MALE MICEGROUP VII - 300 PPM

<u>ANIMAL NUMBER</u>	<u>FINAL</u>	
	<u>BODY</u>	<u>LIVER</u>
90635	31.0	2.886
90636	32.3	2.541
90637	32.1	3.306
90638	29.1	2.540
90639	30.7	3.082
GROUP MEAN	31.0	2.871
STD. DEV.	1.3	0.336

GROUP IX - 3000 PPM

<u>ANIMAL NUMBER</u>	<u>FINAL</u>	
	<u>BODY</u>	<u>LIVER</u>
90640	28.6	4.343
90641	28.3	5.014
90642	26.7	3.294
90643	28.4	4.266
90644	31.4	4.909
GROUP MEAN	28.7	4.365
STD. DEV.	1.7	0.685

REPEATED DOSE ORAL TOXICITY: 14-DAY FEEDING
STUDY WITH H-19683 IN MALE MICE

APPENDIX II (continued)

INDIVIDUAL ANIMAL FINAL BODY AND ORGAN WEIGHTS

RELATIVE ORGAN WEIGHTS (% of body weight) FROM MALE MICEGROUP I - 0 PPM

<u>ANIMAL NUMBER</u>	<u>LIVER</u>
90620	5.6603
90621	5.8374
90622	5.8413
90623	5.5357
90624	5.6469
GROUP MEAN	5.7043
STD. DEV.	0.1324

GROUP III - 3 PPM

<u>ANIMAL NUMBER</u>	<u>LIVER</u>
90625	6.6392
90626	5.9441
90627	5.7917
90628	6.3322
90629	5.1832
GROUP MEAN	5.9781
STD. DEV.	0.5546

GROUP V - 30 PPM

<u>ANIMAL NUMBER</u>	<u>LIVER</u>
90630	7.4763
90631	6.2386
90632	8.1672
90633	7.2134
90634	6.3920
GROUP MEAN	7.0975
STD. DEV.	0.7963

REPEATED DOSE ORAL TOXICITY: 14-DAY FEEDING
STUDY WITH H-19683 IN MALE MICE

APPENDIX II (continued)

INDIVIDUAL ANIMAL FINAL BODY AND ORGAN WEIGHTS

RELATIVE ORGAN WEIGHTS (% of body weight) FROM MALE MICEGROUP VII - 300 PPM

<u>ANIMAL NUMBER</u>	<u>LIVER</u>
90635	9.3097
90636	7.8669
90637	10.2991
90638	8.7285
90639	10.0391
GROUP MEAN	9.2486
STD. DEV.	0.9889

GROUP IX - 3000 PPM

<u>ANIMAL NUMBER</u>	<u>LIVER</u>
90640	15.1853
90641	17.7173
90642	12.3371
90643	15.0211
90644	15.6338
GROUP MEAN	15.1789
STD. DEV.	1.9204