MULTIDOSE RANGE-FINDING STUDY IN RATS

B180

FINAL REPORT

HAZLETON

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FINAL REPORT

Submitted to

Lorillard
Greensboro, North Carolina

October 16, 1985

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HAZLETON
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OFFICE OF QUALITY ASSURANCE

Project Title: Multidose Range-Finding Study in Rats

Project No.: 642-285

Quality Assurance review of the final report was conducted according to the procedures described in the standard operating procedures of the Report Review Section of the Office of Quality Assurance, and according to the general requirements of the Good Laboratory Practice regulations that were issued on December 22, 1978, by the Food and Drug Administration for compliance on and after June 20, 1979. The final report review was conducted and the findings were reported to management and to the study director on the following dates:

Final Report Review	Findings Reported	Reviewer
9/26/85	9/30/85	D. Hitzelberg

Frederick G. Snyder — 9/30/85
Director — Date
Office of Quality Assurance

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SUBJECT: Multidose Range-Finding Study in Rats
Project No. 642-285

We, the undersigned, hereby declare that the work was performed under our supervision, according to the procedures herein described.

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SPONSOR: Lorillard DATE: October 16, 1985

MATERIAL: B180

SUBJECT: FINAL REPORT
Multidose Range-Finding Study in Rats
Project No. 642-285

SUMMARY

The test material, B180, was evaluated for the maximum tolerated dose (MTD) level in male and female rats. Based upon the findings of this study, the MTD in male and female rats was estimated to be 625 mg/kg.

INTRODUCTION

This study was designed to determine the maximum tolerated dose (MTD) level of B180 following repeated daily oral doses to rats for four days. The Single Dose Range-Finding Study was initiated on July 31, 1985 and was terminated August 4, 1985. The Multidose Range-Finding Study was initiated on August 21, 1985 and was terminated on August 25, 1985.

TEST MATERIAL

The test material, B180, a pale yellow liquid, was received

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from the sponsor on June 19, 1985, and was stored in an amber jar under refrigeration. Corn oil (Duke's® Pure Corn Oil, The C. F. Sauer Company, Lot No. 52500), a yellow liquid stored at room temperature, was received on April 30, 1985, and was used as the vehicle. A value of 100% active ingredient was assumed for purposes of dosage calculations. Information on stability and methods of synthesis, as well as data on composition or other characteristics which define the test material are on file with the sponsor.

TEST ANIMALS

Sprague-Dawley rats were received from Charles River Breeding Laboratories, Inc., Kingston, New York. Animals were randomly housed upon receipt via computer generated random numbers and subsequently assigned to this study following the acclimation period. Two rats of each sex were assigned to the Single Dose Range-Finding Study and six rats of each sex to the Multidose Range-Finding Study and subsequently to groups. The initial body weights of the males ranged from 190.7 to 214.9 grams, and the initial body weights of the females ranged from 144.0 to 159.1 grams in the Single Dose Range-Finding Study. In the Multidose Range-Finding Study, the initial body weights of the males ranged from 105.4 to 161.5 grams, and the initial body weights of the females ranged from 114.8 to 137.7 grams.

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The rats were identified uniquely by ear tags and housed individually in elevated wire-mesh cages. Commercial rodent ration (Purina Rodent Laboratory Chow® #5001) and tap water were available ad libitum. During the Single Dose Range-Finding Study, the temperature in the animal room was 72°F and the humidity ranged from 60-78%. In the Multidose Range-Finding Study, the temperature in the animal room was 74°F and the humidity ranged from 61-72%. A 12-hour light/dark cycle was maintained daily. The rats were acclimated to laboratory conditions for a minimum of one week prior to initiation of treatment. Rats were used in this study because they have historically been used in safety evaluation studies and are required by appropriate regulatory agencies.

METHODS

Groups and Dosage Levels

Single Dose Range-Finding Study

Two animals of each sex were assigned to the following group.

Group	No of Animals		Dosage Level
	Males	Females	mg/kg
1	2	2	5000

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Multidose Range-Finding Study

Two animals of each sex were assigned to each of the following groups.

Group	No of Animals		Dosage Level
	Males	Females	mg/kg
1	2	2	500
2	2	2	1000
3	2	2	2500

Compound Preparation and Administration

The required amount of compound for each level was weighed into pre-calibrated glass beakers using an appropriate electronic balance. Corn oil was added to each beaker to bring the mixture to the desired volume. The mixtures were stirred for approximately five minutes on a magnetic stirrer and were stirred while dosing. During the Multidose Range-Finding phase, the test material mixtures were stored under refrigeration and brought to room temperature before dosing. Each rat received the appropriate amount of test material by gavage. Single Dose Range-Finding animals received one dose and Multidose Range-Finding animals received one dose daily for four consecutive days. The test material was administered by gavage because of the characteristics of the test compound.

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Observations and Records

Single Dose Range-Finding Study

All of the rats were observed for signs of toxic and pharmacologic effects at one and six hours postdose, and at least once daily thereafter to Day 5. Mortality/moribundity was recorded twice daily. Individual body weights were recorded immediately prior to dosing.

Multidose Range-Finding Study

All of the rats were observed for signs of toxic and pharmacologic effects at one and four hours postdose on Day 1, twice daily on Days 2 and 3, at one and six hours postdose on Day 4 and on Day 5 (24 hours following the Day 4 dose). Mortality/moribundity was recorded twice daily. Individual body weights were recorded immediately prior to the first dose.

Sacrifice and Gross Pathology

Single Dose Range-Finding Study

At termination (Day 5), all surviving rats were sacrificed by carbon dioxide asphyxiation and discarded. Animals which died on study were also discarded.

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Multidose Range-Finding Study

At termination (Day 5), all surviving rats were sacrificed by carbon dioxide asphyxiation and discarded.

Raw Data and Final Report Storage

All raw data and the final report are stored in the archives of Hazleton Laboratories America, Inc.

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RESULTS

Dosing Volumes

Individual dosing volumes for the Single Dose and Multidose Range-Finding Studies are presented in Tables 1A and 1B, respectively.

Mortality

Single Dose Range-Finding Study

Deaths occurring in the animals dosed at 5000 mg/kg consisted of one female rat on Day 1, six hours postdose.

Multidose Range-Finding Study

No deaths occurred during the Multidose Range-Finding Study.

Clinical Observations and Body Weights

Single Dose Range-Finding Study

A summary of clinical observations is presented in Table 2A. Initial body weights are presented in Table 3A.

A variety of commonly noted clinical signs was observed in both male and female rats during the study.

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Multidose Range-Finding Study

A summary of clinical observations is presented in Table 2B. Initial body weights are presented in Table 3B.

A variety of commonly noted clinical signs was observed in the Groups 2 and 3 males and in the Groups 1-3 females, with the severity increasing with increasing dose. The Group 1 males appeared normal throughout the study.

Conclusions

Based upon the presence of two incidences of gross toxicity (slight depression and/or depression) in both males and females dosed at 1000 mg/kg of body weight, the MTD was estimated to be 625 mg/kg of body weight in male and female rats.

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Table 1A
Dosing Volumes
Multidose Range-Finding Study in Rats
Single Dose Range-Finding Study

Animal Number	Group	Sex	Dosage Level mg/kg	Dosage Volume ml
D96967	1	M	5000	2.1
D96968	1	M	5000	1.9
D96979	1	F	5000	1.4
D96980	1	F	5000	1.6

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Table 1B
Dosing Volumes*
Multidose Range-Finding Study in Rats
Multidose Range-Finding Study

Animal Number	Group	Sex	Dosage Level mg/kg	Dosage Volume ml
D97433	1	M	500	6.0
D97434	1	M	500	6.4
D97458	1	F	500	4.4
D97459	1	F	500	5.6
D97435	2	M	1000	4.4
D97436	2	M	1000	6.4
D97460	2	F	1000	5.6
D97461	2	F	1000	5.2
D97437	3	M	2500	6.0
D97438	3	M	2500	6.4
D97462	3	F	2500	4.8
D97463	3	F	2500	5.6

* The dosage volume for each animal is a total of four equal doses.

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Table 2A
Summary of Clinical Observations*
Multidose Range-Finding Study in Rats - Single Dose Range-Finding Study
Group 1 - 5000 mg/kg

	Observation Intervals									
	Days									
	1		2		3		4		5	
OBSERVATION	1 Hour	6 Hours	a.m.	p.m.	a.m.	p.m.	a.m.	p.m.	a.m.	p.m.
Males										
Normal	0/2	0/2	0/2	0/2	0/2	1/2	1/2	1/2	1/2	1/2
Prostrate	2/2	1/2	0/2	0/2	0/2	0/2	0/2	0/2	0/2	0/2
Depression	0/2	1/2	0/2	0/2	0/2	0/2	0/2	0/2	0/2	0/2
Slight depression	0/2	0/2	2/2	1/2	0/2	0/2	0/2	0/2	0/2	0/2
Soft feces	0/2	0/2	2/2	1/2	0/2	0/2	0/2	0/2	0/2	0/2
Ataxia	0/2	1/2	0/2	0/2	0/2	0/2	0/2	0/2	0/2	0/2
Rough coat	0/2	1/2	2/2	2/2	2/2	0/2	1/2	1/2	1/2	1/2
Urine stains	0/2	1/2	1/2	1/2	1/2	1/2	0/2	0/2	0/2	0/2
Females										
Normal	0/2	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	1/1
Prostrate	2/2	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1
Depression	0/2	1/1	1/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1
Slight depression	0/2	0/1	0/1	1/1	1/1	0/1	0/1	0/1	0/1	0/1
Ataxia	0/2	1/1	1/1	1/1	0/1	0/1	0/1	0/1	0/1	0/1
Rough coat	0/2	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	0/1
Urine stains	0/2	1/1	1/1	1/1	0/1	0/1	0/1	0/1	0/1	0/1
Hunched	0/2	1/1	1/1	1/1	0/1	0/1	0/1	0/1	0/1	0/1

* Numbers indicate the number of rats exhibiting the finding over the number of rats examined.

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Table 2B
Summary of Clinical Observations*
Multidose Range-Finding Study in Rats - Multidose Range-Finding Study
Males

OBSERVATION	Dose 1 Day 1 Hours Postdose 1	Dose 1 Day 1 Hours Postdose 4	Dose 2 Day 2 a.m.	Dose 2 Day 2 p.m.	Dose 3 Day 3 a.m.	Dose 3 Day 3 p.m.	Dose 4 Day 4 Hours Postdose 1	Dose 4 Day 4 Hours Postdose 6	Dose 4 Day 4 Hours Postdose 24
			Group 1 - 500 mg/kg						
Normal	2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2
			Group 2 - 1000 mg/kg						
Normal	0/2	0/2	0/2	0/2	1/2	2/2	2/2	2/2	2/2
Slight depression	2/2	2/2	1/2	0/2	0/2	0/2	0/2	0/2	0/2
Rough coat	2/2	2/2	2/2	2/2	0/2	0/2	0/2	0/2	0/2
Soft feces	1/2	2/2	1/2	0/2	0/2	0/2	0/2	0/2	0/2
Urine stains	2/2	2/2	1/2	1/2	1/2	0/2	0/2	0/2	0/2
			Group 3 - 2500 mg/kg						
Normal	0/2	0/2	0/2	0/2	0/2	0/2	2/2	2/2	2/2
Depression	2/2	2/2	1/2	1/2	0/2	0/2	0/2	0/2	0/2
Slight depression	0/2	0/2	1/2	1/2	1/2	0/2	0/2	0/2	0/2
Ataxia	2/2	0/2	0/2	0/2	0/2	0/2	0/2	0/2	0/2
Rough coat	2/2	2/2	2/2	2/2	2/2	2/2	0/2	0/2	0/2
Soft feces	2/2	2/2	1/2	1/2	1/2	0/2	0/2	0/2	0/2
Urine stains	2/2	2/2	2/2	1/2	1/2	0/2	0/2	0/2	0/2

* Numbers indicate the number of rats exhibiting the finding over the number of rats examined.

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Table 2B - Continued
Summary of Clinical Observations*
Multidose Range-Finding Study in Rats - Multidose Range-Finding Study
Females

OBSERVATION	Dose 1 Day 1 Hours Postdose 1	Dose 1 Day 1 Hours Postdose 4	Dose 2 Day 2 a.m.	Dose 2 Day 2 p.m.	Dose 3 Day 3 a.m.	Dose 3 Day 3 p.m.	Dose 4 Day 4 Hours Postdose 1	Dose 4 Day 4 Hours Postdose 6	Dose 4 Day 4 Hours Postdose 24
Group 1 - 500 mg/kg									
Normal	1/2	1/2	1/2	1/2	1/2	1/2	1/2	1/2	2/2
Soft feces	1/2	1/2	0/2	0/2	0/2	0/2	0/2	0/2	0/2
Urine stains	0/2	1/2	1/2	1/2	1/2	1/2	1/2	1/2	0/2
Group 2 - 1000 mg/kg									
Normal	0/2	0/2	0/2	1/2	1/2	2/2	2/2	2/2	2/2
Depression	0/2	1/2	0/2	0/2	0/2	0/2	0/2	0/2	0/2
Slight depression	2/2	1/2	0/2	0/2	0/2	0/2	0/2	0/2	0/2
Rough coat	2/2	2/2	2/2	0/2	0/2	0/2	0/2	0/2	0/2
Soft feces	0/2	2/2	0/2	0/2	0/2	0/2	0/2	0/2	0/2
Urine stains	2/2	2/2	2/2	1/2	1/2	0/2	0/2	0/2	0/2
Group 3 - 2500 mg/kg									
Normal	0/2	0/2	0/2	0/2	0/2	0/2	1/2	2/2	2/2
Depression	0/2	2/2	2/2	1/2	0/2	0/2	0/2	0/2	0/2
Slight depression	2/2	0/2	0/2	1/2	2/2	1/2	0/2	0/2	0/2
Rough coat	2/2	2/2	2/2	2/2	2/2	2/2	0/2	0/2	0/2
Soft feces	0/2	0/2	1/2	1/2	1/2	0/2	0/2	0/2	0/2
Urine stains	2/2	2/2	2/2	2/2	2/2	0/2	1/2	0/2	0/2
Hunched	0/2	0/2	0/2	1/2	0/2	0/2	0/2	0/2	0/2
Red stains on nose and/or eyes	0/2	0/2	0/2	0/2	1/2	1/2	0/2	0/2	0/2

* Numbers indicate the number of rats exhibiting the finding over the number of rats examined.

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Table 3A
Initial Body Weights
Multidose Range-Finding Study in Rats
Single Dose Range-Finding Study

Animal Number	Sex	Initial Body Weight (grams)
	Group 1 - 5000 mg/kg	
D96967	M	214.9
D96968	M	190.7
D96979	F	144.0
D96980	F	159.1

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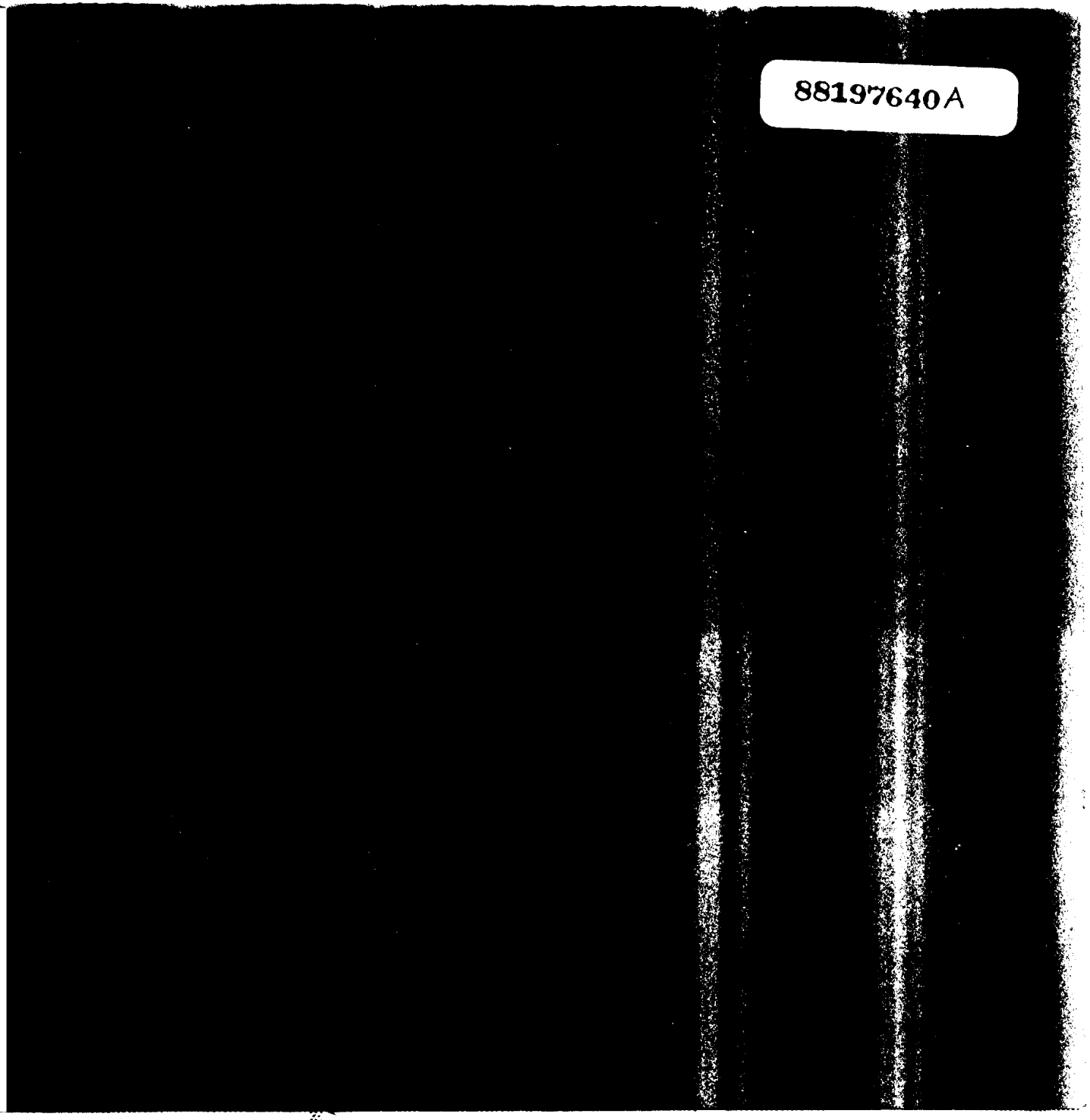
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Table 3B
Initial Body Weights
Multidose Range-Finding Study in Rats
Multidose Range-Finding Study

Animal Number	Sex	Initial Body Weight (grams)
	Group 1 - 500 mg/kg	
D97433	M	147.1
D97434	M	161.5
D97458	F	114.8
D97459	F	136.3
	Group 2 - 1000 mg/kg	
D97435	M	105.4
D97436	M	159.0
D97460	F	137.5
D97461	F	133.9
	Group 3 - 2500 mg/kg	
D97437	M	153.9
D97438	M	158.7
D97462	F	120.2
D97463	F	137.7

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