TRADE SECRET

Study Title

[redacted] Acute Oral Toxicity - Fixed Dose Method

Laboratory Project ID: DuPont-6711

TEST GUIDELINE: OECD Guidelines for Testing of Chemicals
Section 4: Health Effects, No. 420 (1992)

AUTHOR: Carol Finlay, B.A.

STUDY COMPLETED ON: September 17, 2001

PERFORMING LABORATORY: E. I. du Pont de Nemours and Company
Haskell Laboratory for Health and Environmental Sciences
Elkton Road, P.O. Box 50
Newark, Delaware 19714-0050

[redacted]

PAGE RESERVED FOR SPECIFIC COUNTRY REQUIREMENTS

GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

This study was conducted in compliance with U.S. EPA TSCA (40 CFR part 792) Good Laboratory Practice Standards, which are consistent with the OECD Principles of Good Laboratory Practice (as revised in 1997) published in ENV/MC/CHEM(98)17 except for the items documented below. The items listed do not impact the validity of the study.

The test substance was characterized after initiation of this study. Although the characterization (ISO 9001) was not performed under Good Laboratory Practice Standards, the accuracy of the data is considered sufficient for the purposes of this study.

The dosing preparations used in the study were not analyzed for stability, homogeneity, or accuracy of concentration. The procedures used by trained staff to prepare the dosing preparations ensured:

- the accuracy of concentration because the test substance was weighed on an analytical balance accurate to 3 decimal places and the vehicle in which it was suspended was accurately measured in a flask graduated in 1 mL increments,
- homogeneity because the mixtures were stirred prior to dosing and while portions were removed for dose administration, and
- stability because the time between dose preparation and administration was kept to a minimum (less than 1 hour).

Applicant / Sponsor: Telomer Research Program
Arlington, Virginia
U.S.A.

Study Director: Carol Finlay 17-Sept-2001
Carol Finlay
Staff Scientist
Date

Company Sanitized. Does not contain TSCA CBI

QUALITY ASSURANCE STATEMENT

Haskell Sample Number(s):

24691

Dates of Inspections:

Conduct: May 17, 2001

Records, Reports: April 5, 2001; September 9, 10, 2001

Dates Findings Reported to:

Study Director: September 10, 2001

Management: September 13, 2001

Reported by: Joseph C Hamill 17-Sep-2001

Joseph C. Hamill
Sr. Quality Assurance Auditor

Date

Company Sanitized. Does not contain TSCA CBI

CERTIFICATION

We, the undersigned, declare that this report provides an accurate evaluation of data obtained from this study.

Pathology Evaluations Reported by: Lisa J. Lewis — 14-Sep-2001
Lisa J. Lewis
Laboratory Technician
Date

Pathology Evaluations Reviewed by: G. Tracy Makovec — 14-Sep-2001
G. Tracy Makovec, D.V.M.
Diplomate A.C.V.P.
Date

Reviewed by: James C. Mackay II — 14-Sep-2001
James C. Mackay II
Associate Scientist
Date

Issued by Study Director: Carol Finlay — 17-Sept-2001
Carol Finlay, B.A.
Staff Scientist
Date

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STUDY INFORMATION

9th Collective Nomenclature: [redacted]

Synonyms/Codes: [redacted]

Haskell Number: 24691

CAS Registry Number: [redacted]

Composition: [redacted]

Purity: [redacted]

Known Impurities: [redacted]

Physical Characteristics: White to yellow wax

Stability: The test substance appeared to be stable under the conditions of the study; no evidence of instability was observed.

Sponsor: Telomer Research Program
1200 South Hayes Street
Arlington, Virginia 22202-5050
U.S.A.

Study Initiated/Completed: April 2, 2001 / (see report cover page)

In-Life Initiated/Completed: April 25, 2001 / May 17, 2001

STUDY PERSONNEL

Study Director: Carol Finlay, B.A.
Management: Judith C. Stadler, Ph.D., D.A.B.T.
Primary Technician: James C. Mackay II

Pathologist: G. Tracy Makovec, D.V.M.
Management: Steven R. Frame, D.V.M., Ph.D.
Pathology Report by: Lisa J. Lewis

Toxicology Report Preparation: Wanda F. Dinbokowitz

Laboratory Veterinarian: William Singleton, D.V.M., A.C.L.A.M.

SUMMARY

Single doses of [redacted] were administered by intragastric intubation to fasted male and female rats. In the preliminary sighting study, a single dose of [redacted] was administered to fasted female rats at a dose of 500 or 2000 mg/kg. Since no toxicity was seen in these rats, the limit dose of 2000 mg/kg was selected for the main study.

In the main study, the test substance was administered to a group of 5 fasted male rats and a group of 5 fasted female rats at a dose of 2000 mg/kg. The rats were observed for mortality, body weight effects, and clinical signs of toxicity for 14 days after dosing. The rats were necropsied to detect grossly observable evidence of organ or tissue damage or dysfunction.

No deaths occurred during the study. The rats exhibited no clinical signs of toxicity. No significant body weight losses occurred after dosing. No gross lesions were present in the rats at necropsy.

Under the conditions of this study, the minimum lethal dose was greater than 2000 mg/kg.

According to the guidance provided in the OECD testing guideline, [redacted] is a compound which does not present a significant acute toxic risk if swallowed.

INTRODUCTION

The purpose of this study was to assess the acute oral toxicity of [redacted] when administered by oral gavage to male and female rats. The dose selected for the main study, 2000 mg/kg, was derived from a preliminary sighting study. In the sighting study, no toxicity was observed in rats dosed at 500 or 2000 mg/kg.

MATERIALS AND METHODS

A. Test Guideline

The study design complies with the following testing guideline:

- Organisation for Economic Co-Operation and Development (OECD) (1992). 420 Acute Oral Toxicity - Fixed Dose Method. *Guideline for Testing of Chemicals*.

B. Test Substance

The test substance [redacted] was supplied by the sponsor as a white to yellow wax. The test substance appeared to be stable under the conditions of the study. No evidence of instability, such as a change in color, was observed.

C. Animal Husbandry

Male and female Crl:CD®(SD)IGS BR rats were received from Charles River Laboratories, Inc., Raleigh, North Carolina. Rats were housed singly in suspended, stainless steel, wire-mesh cages. Each rat was assigned a unique identification number which was recorded on a card affixed to the cage. The rats were tail-marked, using a water-insoluble marker, with the last 3 digits of the animal number. PMI Nutrition International, Inc. Certified Rodent LabDiet® 5002 and water were available *ad libitum* except as noted in section E. Dosing.

As specified in the Haskell Laboratory animal health and environmental monitoring program, the following procedures are performed periodically to ensure that contaminant levels are below those that would be expected to impact the scientific integrity of the study:

- Water samples are analyzed for total bacterial counts, and the presence of coliforms, lead, and other contaminants.
- Feed samples are analyzed for total bacterial, spore and fungal counts.

- Samples from freshly washed cages and cage racks are analyzed to ensure adequate sanitation by the cagewashers.

Certified animal feed is used, guaranteed by the manufacturer to meet specified nutritional requirements and not to exceed stated maximum concentrations of key contaminants, including specified heavy metals, aflatoxin, chlorinated hydrocarbons, and organophosphates. The presence of these contaminants below the maximum concentration stated by the manufacturer would not be expected to impact the integrity of the study.

The animal health and environmental monitoring program is administered by the attending laboratory animal veterinarian. Evaluation of these data did not indicate any conditions that affected the validity of the study.

Rats were quarantined, weighed, and observed for general health for 6 days. Animal rooms were maintained on a timer-controlled, 12-hour light/12-hour dark cycle. Environmental conditions of the rooms were targeted for a temperature of 23 ± 1°C and relative humidity of 50 ± 10%. Excursions outside these ranges were of small magnitude and/or brief duration and did not adversely affect the validity of the study.

D. Test Substance Preparation

The test substance was melted and stirred in a water bath (approximately 75-80°C) for approximately 30 minutes to assure uniformity before each suspension was prepared.

E. Dosing

1. Sighting Study

A single oral dose of [redacted] melted and suspended in 0.5% aqueous methylcellulose, was administered by intragastric intubation to a fasted female rat at a dose of 500 mg/kg. Since toxicity was not seen at this dose, another fasted female rat was dosed at 2000 mg/kg. The rats were fasted approximately 17 or 18 hours prior to dosing with food being returned to the rats approximately 3 hours after dosing. The rats were approximately 9 weeks old on the day of dosing. The individual dose volumes were calculated using the suspension concentration and the fasted body weight obtained prior to dosing. The rats were dosed at a volume of 10 mL per kg of body weight. The dosing suspensions were stirred prior to and throughout the dosing procedure.

2. Main Study

Single oral doses of [redacted] melted and suspended in 0.5% aqueous methylcellulose, were administered by intragastric intubation to a group of 5 fasted male rats and a group of 5 fasted female rats at a dose of 2000 mg/kg. The rats were fasted approximately 18 hours prior to dosing with food being returned to the rats approximately 3.5 hours after dosing. The rats were approximately 9 weeks old on the day of dosing. Individual dose volumes

were calculated using the suspension concentration and the fasted body weights obtained prior to dosing. The rats were dosed at a volume of 10 mL per kg of body weight. The dosing suspension was stirred prior to and throughout the dosing procedure.

F. Observations

1. Sighting Study

Observations for mortality and signs of illness, injury, or abnormal behavior were made daily throughout the study. The rats were observed for clinical signs of toxicity twice on the day of dosing and once each day thereafter. The rats were weighed on test days –1, 0 (day of dosing), 1, and 7. On test day 7, the rats were euthanized by carbon dioxide asphyxiation.

2. Main Study

Observations for mortality and signs of illness, injury, or abnormal behavior were made daily throughout the study. The rats were observed for clinical signs of toxicity twice on the day of dosing and once each day thereafter. The rats were weighed on test days –1, 0 (day of dosing), 1, 2, 3, 7, and 14. On test day 14, the rats were euthanized and necropsied to detect grossly observable evidence of organ or tissue damage or dysfunction. The rats were euthanized by carbon dioxide asphyxiation followed by exsanguination.

RESULTS AND DISCUSSION

In-life Toxicology

A. Sighting Study

1. Dose Information and Mortality Summary

No deaths occurred. The dose information and the mortality data are summarized in the table below.

DOSE (mg/kg)	FASTED BODY WEIGHT (g)	SUSPENSION CONCENTRATION (mg/mL)	DOSE VOLUME (mL)	MORTALITY
FEMALE				
500	217.4	50	2.2	No
2000	213.7	200	2.1	No

2. Body Weights
(Appendix A)

The rats exhibited no body weight losses after dosing.

3. Clinical Signs
(Appendix B)

The rats exhibited no clinical signs of toxicity.

B. Main Study

1. Dose Information and Mortality Summary

No deaths occurred. The dose information and the mortality data are summarized in the table below.

DOSE (mg/kg)	AVERAGE FASTED BODY WEIGHT (g)	SUSPENSION CONCENTRATION (mg/mL)	AVERAGE DOSE VOLUME (mL)	MORTALITY RATIO
MALE				
2000	249.6	200	2.5	0/5
FEMALE				
2000	210.3	200	2.1	0/5

2. Body Weights
(Appendix A)

The rats exhibited no significant body weight losses after dosing.

3. Clinical Signs
(Appendix B)

The rats exhibited no clinical signs of toxicity. The end of the tail was missing from one male rat during the study.

Pathology Evaluations

A. Gross Observations

No gross lesions were present in the rats.

CONCLUSION

Under the conditions of this study, the minimum lethal dose was greater than 2000 mg/kg. According to the guidance provided in the OECD testing guideline [redacted] is a compound which does not present a significant acute toxic risk if swallowed.

RECORDS AND SAMPLE STORAGE

Specimens (if applicable), raw data, and the final report will be retained at Haskell Laboratory, Newark, Delaware, or at Iron Mountain Records Management, Wilmington, Delaware.

APPENDICES

APPENDIX A

Individual Body Weights

INDIVIDUAL BODY WEIGHTS OF FEMALE RATS FROM SIGHTING STUDY

DOSE: 500 mg/kg

Animal Number	Day -1[a]	Day 0[b]	Day 1	Day 7
648018	236.7	217.4	227.6	251.0

DOSE: 2000 mg/kg

Animal Number	Day -1[a]	Day 0[b]	Day 1	Day 7
648020	231.1	213.7	216.0	235.0

[a] Weight before fasting
[b] Fasted body weight

INDIVIDUAL BODY WEIGHTS OF MALE RATS FROM MAIN STUDY

DOSE: 2000 mg/kg

Animal Number	Day -1[a]	Day 0[b]	Day 1	Day 2	Day 3	Day 7	Day 14
648472	274.0	254.1	269.1	295.0	305.0	325.0	383.9
648473	276.0	254.8	272.6	292.9	307.1	328.5	387.4
648474	257.0	235.6	255.4	272.3	282.3	299.9	344.7
648475	268.0	247.7	271.7	287.8	295.4	310.4	349.7
648476	278.0	255.7	276.4	297.0	306.1	325.1	367.8

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[redacted]

[a] Weight before fasting
[b] Fasted body weight

INDIVIDUAL BODY WEIGHTS OF FEMALE RATS FROM MAIN STUDY

DOSE: 2000 mg/kg

Animal Number	Day -1[a]	Day 0[b]	Day 1	Day 2	Day 3	Day 7	Day 14
648488	230.0	213.5	226.6	240.6	248.9	261.3	274.4
648489	230.0	214.5	226.4	245.3	240.7	246.9	264.9
648490	230.0	211.7	226.9	249.5	252.4	251.2	261.8
648491	222.0	201.3	217.1	229.4	236.3	247.3	272.7
648492	229.0	210.5	221.6	238.9	242.3	244.8	257.7

[a] Weight before fasting
[b] Fasted body weight

APPENDIX B

Individual Clinical Observations and Mortality Records

INDIVIDUAL CLINICAL OBSERVATIONS AND MORTALITY RECORDS IN FEMALE RATS FROM SIGHTING STUDY

DOSE: 500 mg/kg

Sex	Animal	Observation	Days
F	648018	General observation, No Abnormality Detected	0-7
		Sacrificed by design	7

DOSE: 2000 mg/kg

Sex	Animal	Observation	Days
F	648020	General observation, No Abnormality Detected	0-7
		Sacrificed by design	7

INDIVIDUAL CLINICAL OBSERVATIONS AND MORTALITY RECORDS IN MALE RATS FROM MAIN STUDY

DOSE: 2000 mg/kg

Sex	Animal	Observation	Days
M	648472	General observation, No Abnormality Detected	-1-14
		Sacrificed by design	14
M	648473	General observation, No Abnormality Detected	-1-14
		Sacrificed by design	14
M	648474	General observation, No Abnormality Detected	-1-4
		End of tail missing	5-14
		Sacrificed by design	14
M	648475	General observation, No Abnormality Detected	-1-14
		Sacrificed by design	14
M	648476	General observation, No Abnormality Detected	-1-14
		Sacrificed by design	14

INDIVIDUAL CLINICAL OBSERVATIONS AND MORTALITY RECORDS IN FEMALE RATS FROM MAIN STUDY

DOSE: 2000 mg/kg

Sex	Animal	Observation	Days
F	648488	General observation, No Abnormality Detected	-1-14
		Sacrificed by design	14
F	648489	General observation, No Abnormality Detected	-1-14
		Sacrificed by design	14
F	648490	General observation, No Abnormality Detected	-1-14
		Sacrificed by design	14
F	648491	General observation, No Abnormality Detected	-1-14
		Sacrificed by design	14
F	648492	General observation, No Abnormality Detected	-1-14
		Sacrificed by design	14

APPENDIX C

Individual Animal Gross Observations

INDIVIDUAL ANIMAL GROSS OBSERVATIONS IN MALE RATS FROM MAIN STUDY

LESIONS	LESION INCIDENCE ANIMAL NUMBER TREATMENT	2000 mg/kg
GENERAL ORGAN		(5)
NO ABNORMALITY DETECTED		648472 648473 648474 648475 648476

Figures in parentheses is the number of animals grossly examined for this tissue
The absence of a number indicates the finding specified was not identified

INDIVIDUAL ANIMAL GROSS OBSERVATIONS IN FEMALE RATS FROM MAIN STUDY

LESIONS	LESION INCIDENCE ANIMAL NUMBER TREATMENT	 2000 mg/kg
GENERAL ORGAN		(5)
NO ABNORMALITY DETECTED		648488 648489 648490 648491 648492

Figures in parentheses is the number of animals grossly examined for this tissue
The absence of a number indicates the finding specified was not identified