Project Number: WIL-12069

Sponsor: Great Lakes Chemical Corporation
P.O. Box 2200
West Lafayette, Indiana 47906

Acute Oral Toxicity (LD50)
Study in Albino Rats with CN-322

FINAL REPORT

Date of Issuance: March 10, 1986

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I. SUMMARY

Dosage levels for this LD50 study were selected based on the results of a range-finding study. In the range-finding study, one male and one female albino rat were fasted and dosed as described below at one of four dosage levels: 500, 1500, 2500, 4000 and 5000 mg/kg. The rats were observed for mortality only for seven days. None of the animals died, therefore, 5000 mg/kg was selected as the first dosage level in the LD50 study.

The test material CN-322 was orally administered via gastric intubation to one group of rats at a dosage level of 5000 mg/kg. The group consisted of five male and five female albino rats that had been fasted for approximately 19 hours prior to dosing. The test material was administered undiluted as received at a dose volume of 3.1 ml/kg.

The animals were observed for treatment-related effects on the day of dosing and for a subsequent fourteen day observation period. Mortality, clinical observations, body weights and gross necropsy findings were recorded.

The LD50 value of CN-322 was found to be greater than 5.0 g/kg for combined male and female mortality under the conditions of this study. None of the rats died. Clinical observations noted early in the study period included respiratory rales for most rats and hypersensitivity to touch for females only. In general, the rats appeared normal three days after dosing. There were no remarkable body weight changes during the study. All rats had no significant changes observed for all tissues examined at the terminal necropsy.

II. INTRODUCTION AND OBJECTIVE

This report presents the data from an "Acute Oral Toxicity (LD50) Study in Albino Rats with CN-322". The study, designated WIL-12069, was conducted in compliance with the Standard Operating Procedures of WIL Research Laboratories, Inc., the Good Laboratory Practice Regulations, the protocol, which was designed in compliance with the Environmental Protection Agency (EPA) guidelines for registering Industrial Chemicals in the U.S. (Pesticide Assessment Guidelines, Subdivision F, Hazard Evaluation: Humans and Domestic Animals, Section 81-1, issued November, 1982) and the Toxic Substances Control Act (TSCA) Health Effects Test Guidelines issued August, 1982 and the Organization for Economic Cooperation and Development (OECD) Guidelines for Testing of Chemicals, Section 401, adopted May 12, 1981 and protocol amendments with the following exception: On a two occasions the temperature was a few degrees below the protocol specified range. Final (day 7) body weights were not collected for the rats on the range-finding study. These deviations do not affect the scientific validity, integrity or objective of the study.

The objective of the study was to determine the acute median lethal dose (LD50) of CN-322 when administered once to fasted male and female albino rats.

The study initiated with dosing of the range-finding study on December 10, 1985 and concluded with terminal necropsy examinations on December 26, 1985. The study was conducted at WIL Research Laboratories, Inc., Ashland, Ohio.

III. EXPERIMENTAL DESIGN - MATERIALS AND METHODS

A. ANIMALS AND HOUSING

Sixty-three male and sixty-one female young adult Sprague-Dawley COBS® CD® rats were received from Charles River Breeding Laboratories, Inc., Portage, Michigan on November 14, 1985. Upon receipt each animal was observed by a qualified technician and weighed. Animals considered suitable for study use were identified uniquely by a Monel® metal ear tag displaying the animal number and acclimated to laboratory conditions for a twenty-six to twenty-eight day quarantine period. During this time the animals were observed twice daily for mortality and general changes in appearance and behavior. Animals used on this study were selected from this group of rats.

The test animals were housed individually in wire-mesh cages suspended above cage board. Purina® Certified Rodent Chow® #5002 and drinking water from on-site wells were provided ad libitum during the quarantine and study period except during an approximate 19-hour interval prior to dosing when food but not water was withheld. The basal diet utilized at WIL Research Laboratories, Inc., is a certified feed with appropriate analyses performed and provided by the manufacturer. Tap water supplying the facility is analyzed twice yearly according to Standard Operating Procedures. The results of these analyses are maintained at WIL Research Laboratories, Inc., and are available upon Sponsor request.

Throughout the quarantine and study period the animals were housed in an environmentally controlled room. Controls were set to maintain temperatures at 72° ± 4° F and relative humidity at greater than 40%. Light timers were set to provide fluorescent lighting for a 12-hour light/12-hour dark photoperiod. Temperature and humidity were recorded once daily.

B. ASSIGNMENT OF ANIMALS TO STUDY

On the day prior to dosing, thirteen male and ten female albino rats were selected arbitrarily from stock for possible use on the study. At this time the animals were examined for evidence of physical abnormalities and weighed.

On the day of study initiation the rats were again examined for evidence of physical abnormalities and weighed. Only healthy animals within the prescribed weight range were selected for study use. The rats weighed from 234 to 302 grams at study initiation.

C. TEST MATERIAL RECEIPT AND IDENTIFICATION

The test material CN-322 was received from Great Lakes Chemical Corporation, West Lafayete, Indiana on November 26, 1985 as follows:

LABEL IDENTIFICATION	LOT NUMBER	NO. CONTAINERS RECEIVED	GROSS WEIGHT	PHYSICAL DESCRIPTION
CN-322 Ref. No. 1223-30-D1 11/8/85	1223-30-D1	1 bottle	821 g	Amber viscous liquid

D. TEST MATERIAL PREPARATION AND DISPENSATION

A sufficient amount of the test material CN-322 was transferred from its original container into a labeled clear container that was covered and transported to the animal room for dosing.

E. TEST MATERIAL ADMINISTRATION

The undiluted test material was administered orally via gastric intubation. Individual doses were calculated based on the animals' fasted body weights taken just prior to dosing and the volume of 3.1 ml/kg. Doses were administered using a one or three milliliter syringe equipped with a 16-gauge stainless steel, snub-tipped dosing needle.

Dose volumes (ml/kg) were calculated based on the dosage level (expressed in g/kg) divided by the density of the test material (1.60 g/ml, as determined by the WIL Research Laboratories Inc. Pharmacy personnel) as follows:

DOSAGE LEVEL (g/kg)	DENSITY (g/ml)	DOSE VOLUME (ml/kg)
5.0	1.60	3.1

F. OBSERVATIONS

1. MORTALITY

The rats were observed for mortality at 1.0, 2.5 and 4.0 hours after dosing on Day 0 and twice daily thereafter (morning and afternoon) for the duration of the study.

2. CLINICAL OBSERVATIONS

The rats were observed for treatment-related effects at 1.0, 2.5 and 4.0 hours after dosing on Day 0 and once daily thereafter for the duration of the study.

3. BODY WEIGHTS

Body weights were obtained and recorded on the day prior to dosing and on study days 0, 7 and 14 (termination).

4. NECROPSY EXAMINATION

Gross necropsy examinations of the major organ systems of the thoracic and visceral cavities were performed on all rats that were sacrificed at study termination. Sacrifice was by carbon dioxide asphyxiation.

G. DATA RETENTION

All raw data generated during the conduct of this investigation, including that information stored and processed by the WIL Computer Data Management System, a copy of the final report and the test material retention sample are retained in the Archives at WIL Research Laboratories, Inc., Ashland, Ohio.

IV. RESULTS

A. OBSERVATIONS

1. MORTALITY

None of the rats died during the study.

2. CLINICAL OBSERVATIONS (Table 1)

Seven of the ten rats had respiratory rales on the day of dosing. Four females were hypersensitive to touch. One male had lethargy on day 0 and another male had hair loss on both forepaws throughout the study period. Except for the hair loss, all rats appeared normal by day 3 and for the duration of the study.

3. BODY WEIGHTS (Table 2)

There were no remarkable changes or differences observed in body weights during the study period.

4. NECROPSY (Table 3)

All rats had no significant changes observed for all tissues examined at the terminal necropsy.

V. DISCUSSION AND CONCLUSIONS

Based on the data obtained, the LD50 value of CN-322 was found to be greater than 5.0 g/kg when administered once orally via gastric intubation to fasted male and female albino rats.

The only clinical observation noted which could be attributed to administration of the test material was respiratory rales which was observed for seven rats on the day of dosing. Hypersensitivity to touch was noted for four females. Lethargy and hair loss were each observed for one male rat. All of the rats appeared essentially normal by day three and for the duration of the study.

There were no remarkable changes or differences observed in body weights during the study period. All rats had no significant changes observed for all tissues examined at the terminal necropsy.

Dennis J. Naas, B.S.
Study Director

3/10/86
Date

VI. PERSONNEL AND REPORT SUBMISSION

Study Supervisor: Jennifer Bassett
Assistant Section Head I

Report Prepared By:

Diana C. Bates
Diana C. Bates, M.A.
Report Writer I
3/5/86
Date

Reviewed By:

Mark D. Nemec
Mark D. Nemec, B. S.
Manager
Teratology and Reproduction
3/10/86
Date

James Laveglia
James Laveglia, Ph.D.
Vice President
Toxicology
3/7/86
Date

Approved and Submitted By:

Dennis J. Naas
Dennis J. Naas, B.S.
Study Director
3/10/86
Date

VII. QUALITY ASSURANCE UNIT STATEMENT

Acute Oral Toxicity (LD50) Study in Albino Rats with CN-322

Dates of Inspection(s)	Date(s) Findings Reported to Study Director
December 19, 1985	December 19, 1985
February 28, 1986	March 3, 1986
March 3, 1986	March 3, 1986

This study was inspected in accordance with the Good Laboratory Practice Regulations and the Standard Operating Procedures of WIL Research Laboratories, Inc. The study was conducted in compliance with Good Laboratory Practice Regulations, the Standard Operating Procedures of WIL Research Laboratories, Inc., the protocol and protocol amendments. To the best of the signatory's knowledge there were no significant deviations from the Good Laboratory Practice Regulations which affected the quality or integrity of the study. Quality Assurance findings, derived from the inspection(s) during the conduct of the study and from the inspection of the final report, are documented and have been reported to the study director. A status report is submitted to management monthly.

The raw data and a copy of the final report will be kept in the Archives at WIL Research Laboratories, Inc.

Ralph Anderson — 3/10/86

Ralph Anderson, B.S.
Director - Quality Assurance

Date

Acute Oral Toxicity (LD50) Study in Albino Rats with CN-322

TABLES 1-3

TABLE 1

PROJECT NO.:WIL-12069 ACUTE ORAL TOXICITY (LD50) STUDY IN ALBINO RATS WITH CN-322 PAGE 1

SPONSOR:GREAT LAKES CHEM. CORP. INCIDENCE OF CLINICAL OBSERVATIONS - DAYS POST DOSING

OBSERVATION	NO.	SEX	GRP	DAY POST DOSING 0 1 2 3 4 5 6 7 8 9 10 11 12 13 14
APPEARED NORMAL	25814	M	1	P P P P P P P P P P P P P P
	25822	M	1	
	25818	M	1	P P P P P P P P P P P P P P
	25807	M	1	P P P P P P P P P P P P P P
	25835	M	1	P P P P P P P P P P P P P P
	25887	F	1	P P P P P P P P P P P P P P
	25892	F	1	P P P P P P P P P P P P P P
	25893	F	1	P P P P P P P P P P P P P P
	25894	F	1	P P P P P P P P P P P P P P
	25897	F	1	P P P P P P P P P P P P P P
RESPIRATORY RALES	25814	M	1	P
	25818	M	1	P
	25835	M	1	P
	25892	F	1	P
	25893	F	1	P
	25894	F	1	P
	25897	F	1	P
HYPERSENSITIVE TO TOUCH	25887	F	1	P
	25892	F	1	P P P
	25894	F	1	P
	25897	F	1	P
HAIR LOSS RIGHT AND LEFT FOREPAWS	25822	M	1	P P P P P S P S P P S P P S S
LETHARGY	25818	M	1	P

1- 5000 MG/KG

GRADE CODE: P=PRESENT S=SLIGHT M=MODERATE V=SEVERE

SEX CODE: M=MALE F=FEMALE

TABLE 2

PROJECT NO.:WIL-12069 ACUTE ORAL TOXICITY (LD50) STUDY IN ALBINO RATS WITH CN-322

SPONSOR:GREAT LAKES CHEM. CORP. INDIVIDUAL BODY WEIGHTS (GRAMS)

MALE GROUP: 5000 MG/KG

DAY	-1	0	7	14
ANIMAL				
25814	306.0	264.0	290.0	331.0
25822	296.0	268.0	328.0	363.0
25818	320.0	294.0	326.0	351.0
25807	296.0	270.0	303.0	332.0
25835	331.0	302.0	337.0	364.0
MEAN	309.8	279.6	316.8	348.2
S.D.	15.4	17.2	19.5	16.1
N	5	5	5	5

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TABLE 2
ACUTE ORAL TOXICITY (LD50) STUDY IN ALBINO RATS WITH CN-322
INDIVIDUAL BODY WEIGHTS (GRAMS)

FEMALE GROUP: 5000 MG/KG

DAY	-1	0	7	14
ANIMAL				
25887	271.0	246.0	261.0	277.0
25892	273.0	251.0	279.0	280.0
25893	279.0	251.0	281.0	301.0
25894	260.0	235.0	260.0	294.0
25897	268.0	234.0	275.0	270.0
MEAN	270.2	243.4	271.2	284.4
S.D.	7.0	8.4	10.0	12.7
N	5	5	5	5

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TABLE 3
ACUTE ORAL TOXICITY (LD50) STUDY IN ALBINO RATS WITH CN-322
GROSS NECROPSY OBSERVATIONS INCIDENCE SUMMARY

	MALE	FEMALE
GROUP:	1	1
NUMBER OF ANIMALS IN DOSE GROUP	5	5
NUMBER OF ANIMALS TERMINALLY SACRIFICED	5	5
NO SIGNIFICANT CHANGES OBSERVED - ALL TISSUES	5	5

1- 5000 MG/KG