



product safety labs

PRODUCT

Axenohl

STUDY TITLE

Acute Oral Toxicity Study in Rats - Limit Test

DATA REQUIREMENT

Health Effects Test Guidelines, OPPTS 870.1100 (1998)

AUTHOR

George E. Moore, B.S.

STUDY COMPLETED ON

October 21, 1999

PERFORMING LABORATORY

Product Safety Labs

725 Cranbury Road

East Brunswick, New Jersey 08816

LABORATORY PROJECT IDENTIFICATION NUMBER

PSL Study Number 8133

EPL Study Number 331S05

CERTIFIED COPY

George E. Moore *10/25/99*
Signature Date

STATEMENT OF NO DATA CONFIDENTIALITY CLAIMS

No claim of confidentiality is made for any information contained in this study on the basis of its falling within the scope of FIFRA 10 (d) (1) (A), (B) or (C).

Company: **EPL BIOANALYTICAL SERVICES, INC.**

Company Agent: EDWIN A. WATSON STUDY MONITOR
Name Title

Edwin A. Watson 10/22/99
Signature Date

GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

Axenohl

This study meets the requirements of 40 CFR Part 160 EPA (FIFRA) with the following exception: The stability, characterization, identity and verification of the test substance concentration as received and tested are the responsibility of the study sponsor.

Study Director:

George E Moore
George E. Moore, B.S.

10/21/99
Date

Submitter:

Signature

Date

Sponsor:

Edwin A. Woodson
Signature *Monita*

10/22/99
Date

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ACUTE ORAL TOXICITY STUDY IN RATS - LIMIT TEST

PROTOCOL NO.: P320

AGENCY: EPA (FIFRA)

PSL STUDY NUMBER: 8133

EPL STUDY NUMBER: 331S05

SPONSOR: EPL BIOANALYTICAL SERVICES, INC.
P.O. Box 109
395 N. Memorial Pkwy
Harristown, IL 62537

TEST SUBSTANCE IDENTIFICATION: Axenohl
Lot #8995

TEST SUBSTANCE DESCRIPTION: Clear liquid

DATE RECEIVED: September 9, 1999

PSL REFERENCE NO.: E90909-1R

DATE OF PROTOCOL APPROVAL: September 14, 1999

DATES OF TEST: September 16-30, 1999

NOTEBOOK NO.: 99-60; pages 109-125

1. PURPOSE

To provide information on health hazards likely to arise from a short-term exposure to Axenohl by the oral route.

2. SUMMARY

An acute oral toxicity test was conducted with rats to determine the potential for Axenohl to produce toxicity from a single dose via the oral route. Based on the results of this study, the single dose acute oral LD₅₀ of the test substance is greater than 5,000 mg/kg of bodyweight.

Five thousand milligrams of the test substance per kilogram of bodyweight was administered to ten healthy rats by oral gavage. The animals were observed for mortality, signs of gross toxicity, and behavioral changes at least once daily for 14 days. Bodyweights were recorded prior to administration and again on Days 7 and 14 (termination). Necropsies were performed on all animals at terminal sacrifice.

All animals survived and appeared active and healthy throughout the study. With the exception of one male that exhibited a loss in bodyweight between Days 7 and 14, all animals gained bodyweight over the 14-day observation period. There were no signs of gross toxicity, adverse pharmacologic effects or abnormal behavior. Gross necropsy findings at terminal sacrifice were unremarkable.

3. MATERIALS

A. Test Substance

The test substance identified as Axenohl, Lot #8995 was received on September 9, 1999 and was further identified with PSL Reference Number E90909-1R. The test substance was a clear liquid and was stored at room temperature. The sample was administered as received. Documentation of the methods of synthesis, fabrication, or derivation of the test substance is retained by ETI H₂O, Lake City, FL.

Characterization of the test substance provided to Product Safety Labs by the sponsor was:

Composition: 2438 ppm Ag⁺
21% citric acid
2.0% SLS
78% water

pH: 1.84

Solubility: Soluble in water

Stability: Test substance is expected to be stable for the duration of testing

Expiration Date: September 2000

B. Animals

3.B.1 Number of Animals: 10

3.B.2 Sex: 5 males and 5 females

3.B.3 Species/Strain: Rat/Sprague-Dawley derived, albino

3.B.4 Age/Bodyweight: Young adult/males 195-214 grams and females 172-184 grams at experimental start

3.B.5 Source: Received from Ace Animals, Inc., Boyertown, PA on September 3, 1999

4. METHODS

A. Husbandry

4.A.1 Housing: The animals were singly housed in suspended stainless steel caging with mesh floors which conform to the size recommendations in the most recent *Guide for the Care and Use of Laboratory Animals DHEW (NIH)*. Litter paper was placed beneath the cage and was changed at least three times per week.

4.A.2 Animal Room: Temperature Range: 20-22°C

- 4.A.3 Photoperiod: 12 hour light/dark cycle
- 4.A.4 Acclimation Period: 13 days
- 4.A.5 Food: Purina Rodent Chow #5012
- 4.A.6 Water: Filtered tap water was supplied *ad libitum* by an automatic water dispensing system.
- 4.A.7 Contaminants: There were no known contaminants reasonably expected to be found in the food or water at levels which would have interfered with the results of this study. Analyses of the food and water are conducted at least once a year and the records are kept on file at Product Safety Labs. The dates of the most recent analyses are presented in Appendix A.

B. Identification:

- 4.B.1 Cage: Each cage was identified with a cage card indicating at least the study number and identification and sex of the animals.
- 4.B.2 Animal: A number was allocated to each rat on receipt and a stainless steel ear tag bearing this number was attached to the rat. This number, together with a sequential animal number assigned to study 8133, constituted unique identification.

5. PROCEDURE

A. Selection of Animals

Prior to dosing, a group of animals was fasted for approximately 17 hours by removing feed from their cages. During the fasting period, the rats were examined for health and weighed (initial). Ten (five male and five female) healthy rats were selected for test.

B. Dose Calculations

Individual doses were calculated based on the initial bodyweights, taking into account the specific gravity (determined by PSL) of the test substance.

C. Dosing

Each animal received 5,000 mg/kg of the test substance, administered using a stainless steel ball-tipped gavage needle attached to an appropriate syringe. After administration, each animal was returned to its designated cage. Feed was replaced approximately 3.25 hours after dosing. The day of administration was considered Day zero of the study.

D. Bodyweights

Individual bodyweights of the animals were recorded prior to test substance administration (initial) and again on Days 7 and 14 (termination) (See Table 1).

E. Cage-Side Observations

The animals were observed for mortality, signs of gross toxicity, and behavioral changes at 1 and 3 hours post-dosing and at least once daily thereafter for 14 days. Observations included gross

evaluation of skin and fur, eyes and mucous membranes, respiratory, circulatory, autonomic and central nervous systems, somatomotor activity and behavior pattern. Particular attention was directed to observation of tremors, convulsions, salivation, diarrhea and coma (See Table 2).

F. Necropsy

All rats were euthanized via CO₂ inhalation on Day 14. Gross necropsies were performed on all animals. Tissues and organs of the thoracic and abdominal cavities were examined (See Table 3).

6. STUDY CONDUCT

This study was conducted at Product Safety Labs, 725 Cranbury Road, East Brunswick, NJ 08816, to comply with the good laboratory practices as defined in 40 CFR 160: U.S. EPA Good Laboratory Practice Standards: Pesticide Programs (FIFRA) and in accordance with Health Effects Test Guidelines, OPPTS 870.1100 (1998).

7. QUALITY ASSURANCE

The final report was audited for agreement with the raw data records and for compliance with the protocol, Product Safety Labs Standard Operating Procedures and appropriate Good Laboratory Practice Standards. Dates of inspections and audits performed during the study, and the dates of reporting of the inspection and audit findings to the Study Director and Facility Management are presented in the Quality Assurance Statement.

8. DEVIATIONS FROM FINAL PROTOCOL

None

9. RECORDS TO BE MAINTAINED

A copy of this signed report, together with the protocol and all raw data generated at Product Safety Labs, is retained in the Product Safety Labs Archives.

10. RESULTS

All animals survived and appeared active and healthy throughout the study. With the exception of one male that exhibited a loss in bodyweight between Days 7 and 14, all animals gained bodyweight over the 14-day observation period. There were no signs of gross toxicity, adverse pharmacologic effects or abnormal behavior. No gross abnormalities were noted for the animals when necropsied at the conclusion of the 14-day observation period.

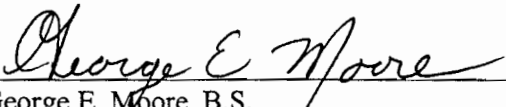
11. CONCLUSION

The single dose acute oral LD₅₀ of Axenohl is greater than 5,000 mg/kg of bodyweight.


SIGNATURES

Axenohl

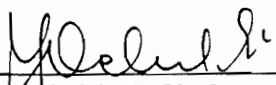
We the undersigned declare that the methods, results and data contained in this report faithfully reflect the procedures used and raw data collected during the study.


George E. Moore, B.S.
Study Director

Oct 21, 1999
Date


Gary Wnorowski, B.A.
Laboratory Manager

Oct 21, 1999
Date


Jacek Ochalski, D.V.M.
Principal Toxicology Technician

Oct. 21. 1999
Date

TABLE 1: INDIVIDUAL BODYWEIGHTS AND DOSES

| Animal No. | Sex | Bodyweight (g) | | | Dose ¹ |
|------------|-----|----------------|-------|--------|-------------------|
| | | Initial | Day 7 | Day 14 | mL |
| 9717 | M | 202 | 275 | 347 | 0.94 |
| 9718 | M | 195 | 280 | 366 | 0.91 |
| 9719 | M | 208 | 272 | 354 | 0.97 |
| 9720 | M | 214 | 285 | 371 | 1.0 |
| 9721 | M | 212 | 283 | 269 | 0.99 |
| 9722 | F | 175 | 226 | 247 | 0.82 |
| 9723 | F | 172 | 210 | 239 | 0.80 |
| 9724 | F | 172 | 205 | 240 | 0.80 |
| 9725 | F | 184 | 210 | 244 | 0.86 |
| 9726 | F | 182 | 214 | 250 | 0.85 |

¹ Administered as received. Specific Gravity - 1.074 g/ml.

TABLE 2: INDIVIDUAL CAGE-SIDE OBSERVATIONS

| <u>Animal Number</u> | <u>Findings</u> | <u>Day of Occurrence</u> |
|----------------------|--------------------|--------------------------|
| <u>MALES</u> | | |
| 9717 - 9721 | Active and healthy | 0-14 |
| <u>FEMALES</u> | | |
| 9722 - 9726 | Active and healthy | 0-14 |

TABLE 3: INDIVIDUAL NECROPSY OBSERVATIONS

| <u>Animal Number</u> | <u>Tissues</u> | <u>Findings</u> |
|----------------------|--------------------|------------------------|
| <u>MALES</u> | | |
| 9717 - 9721 | All tissues/organs | No gross abnormalities |
| <u>FEMALES</u> | | |
| 9722 - 9726 | All tissues/organs | No gross abnormalities |

APPENDIX A: FEED AND WATER ANALYSES

Animal feed analysis independently performed on March 10, 1999 for the presence of the following contaminants:

| | |
|--------------------|-------------------------|
| Aldrin | Ethyl Parathion |
| BHC | Heptachlor |
| Chlordane | Heptachlor Epoxide |
| DDD | Hexachlorobenzene - HCB |
| DDE | Lindane |
| DDT | Malathion |
| Diazinon | Methoxychlor |
| Dieldrin | Methyl Parathion |
| Endosulfan I & II | Mirex |
| Endosulfan Sulfate | Parathion |
| Endrin | PCB |
| Endrin aldehyde | Toxaphene |
| Ethion | |

LABORATORY: WOODSON-TENENT LABORATORIES
345 Adams Avenue
P.O. Box 2135
Memphis, TN 38101

Water analysis performed as of February 10, 1999 for NJDEPE Safe Drinking Water Act parameters.

LABORATORIES: NEW JERSEY LABORATORIES
NJDEPE LAB I.D. #15001
A.A. Labs Division
222 Easton Avenue
New Brunswick, NJ 08901

SILLIKER LABORATORIES
OF NEW JERSEY, INC.
400 South Avenue
Garwood, NJ 07027

Results of feed and water analysis for possible contaminants: Acceptable; none detected or within regulatory standards.

QUALITY ASSURANCE STATEMENT

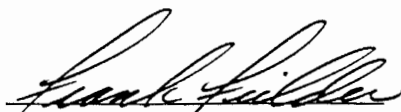
The Quality Assurance Unit randomly selects intervals for QA inspections prior to study initiation. Records of the findings of these inspections are kept on file. The summary below provides verification of statements made in the final report section that addresses Quality Assurance audits.

Inspections were made of:

| <u>DATE</u> | <u>PROCEDURE INSPECTED</u> |
|-----------------|----------------------------|
| 9/20/99 | Day 4 In-life observations |
| 9/23/99 | Day 7 bodyweights |
| 10/20/99 | Raw data |
| 10/20/99 | Draft report |
| <u>10/21/99</u> | Final report |

Findings reported to: Study Director 10/20/99

Management 10/21/99


Frank Fielder, B.S.
Quality Assurance Supervisor