psl product safety labs
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PRODUCT

Axenohl

STUDY TITLE

Acute Dermal Toxicity Study in Rats - Limit Test

DATA REQUIREMENT

Health Effects Test Guidelines, OPPTS 870.1200 (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 8111

EPL Study Number 331S06

CERTIFIED COPY

Signature Date 10/25/99

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 WOOLSON — Name

STUDY MONITOR — Title

Edwin A Woolson — Signature

10/22/99 — 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 Waalkes
Signature Monitor

10/22/99
Date

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ACUTE DERMAL TOXICITY STUDY IN RATS - LIMIT TEST

PROTOCOL NO.:	P322
AGENCY:	EPA (FIFRA)
PSL STUDY NUMBER:	8111
EPL STUDY NUMBER:	331S06
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 4, 1999
DATES OF TEST:	September 14-28, 1999
NOTEBOOK NO.:	99-60; pages 63-79

1. PURPOSE

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

2. SUMMARY

An acute dermal toxicity test was conducted with rats to determine the potential for Axenohl to produce toxicity from a single topical application. Based on the results of this study, the single dose acute dermal LD_{50} of the test substance is greater than 5,000 mg/kg of bodyweight.

Five thousand milligrams per kilogram of bodyweight of the test substance was applied to the skin of ten healthy rats for 24 hours. 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 application and again on Days 7 and 14 (termination). Necropsies were performed on all animals at terminal sacrifice.

All animals survived, gained weight and appeared active and healthy. 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 applied as received. Documentation of the methods of synthesis, fabrication, or derivation of the test substance is retained by ETI H_2O, 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: Rats/Sprague-Dawley derived, albino

3.B.4 Age/Bodyweight: Young adult/males 232-262 grams and females 202-218 grams at experimental start

3.B.5 Source: Received from Ace Animals, Inc., Boyertown, PA on August 31, 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: 20-24°C

4.A.3 Photoperiod: 12 hour light/dark cycle

4.A.4 Acclimation Period: 14 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 animal. This number, together with a sequential animal number assigned to study 8111, constituted unique identification.

5. PROCEDURE

A. Preparation and Selection of Animals

On the day prior to application, a group of animals was prepared by clipping (Oster model #A5-small) the dorsal area and the trunk. After clipping and prior to application, the animals were examined for health, weighed (initial) and the skin checked for any abnormalities. 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. Application of Test Substance

Five thousand mg/kg of bodyweight of the test substance was applied evenly over a dose area of approximately 2 inches x 3 inches (approximately 10% of the body surface) and covered with a 2 inch x 3 inch, 4-ply gauze pad. The gauze pad and entire trunk of each animal were then wrapped with 3 inch Durapore tape to avoid dislocation of the pad and to minimize loss of the test substance. The rats were then returned to their designated cages. The day of application was considered Day zero of the study.

After 24 hours of exposure to the test substance, the pads were removed and the test sites gently wiped with water and a clean towel to remove any residual test substance.

D. Bodyweights

Individual bodyweights of the animals were recorded prior to test substance application (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 5.75 hours after application 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 CO_2 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 870.1200 (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, gained weight and appeared active and healthy. There were no signs of gross toxicity, adverse pharmacologic effects or abnormal behavior. No gross abnormalities were noted for the animals necropsied at the conclusion of the 14-day observation period.

11. CONCLUSION

The single dose acute dermal LD_{50} 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

Christopher Day, B.S.
Principal Toxicology Technician

10/21/99
Date

TABLE 1: INDIVIDUAL BODYWEIGHTS AND DOSES

Animal No.	Sex	Bodyweight (g)			Dose[1]
		Initial	Day 7	Day 14	ML
9317	M	262	335	376	1.2
9318	M	232	289	309	1.1
9319	M	246	310	345	1.1
9320	M	257	327	362	1.2
9321	M	240	327	372	1.1
9322	F	218	231	257	1.0
9323	F	202	215	237	0.94
9324	F	211	223	245	0.98
9325	F	212	227	247	0.99
9326	F	207	211	227	0.96

[1] Applied as received. Specific Gravity - 1.074 g/ml.

TABLE 2: INDIVIDUAL CAGE-SIDE OBSERVATIONS

Animal Number	Findings	Day of Occurrence
MALES		
9317 - 9321	Active and healthy	0-14
FEMALES		
9322 - 9326	Active and healthy	0-14

TABLE 3: INDIVIDUAL NECROPSY OBSERVATIONS

Animal Number	Tissues	Findings
MALES		
9317 - 9321	All tissues/organs	No gross abnormalities
FEMALES		
9322 - 9326	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:

DATE	PROCEDURE INSPECTED
9/24/99	Test substance dispensing records
9/28/99	Terminal bodyweights
10/20/99	Raw data
10/20/99	Draft report
10/21/99	Final report

Findings reported to: Study Director 10/20/99

Management 10/21/99

Frank Fielder, B.S.
Quality Assurance Supervisor