psl product safety labs
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## PRODUCT

Axenohl

## STUDY TITLE

Primary Skin Irritation Study in Rabbits

## DATA REQUIREMENT

Health Effects Test Guidelines, OPPTS 870.2500 (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 8113

EPL Study Number 331S08

CERTIFIED COPY
T C Wadson 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 WADLSON (Name) STUDY MONITOR (Title)

Edwin A Wadlson (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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# PRIMARY SKIN IRRITATION STUDY IN RABBITS

| | |
|---|---|
| **PROTOCOL NO.:** | P326 |
| **AGENCY:** | EPA (FIFRA) |
| **PSL STUDY NUMBER:** | 8113 |
| **EPL STUDY NUMBER:** | 331S08 |
| **SPONSOR:** | EPL BIOANALYTICAL SERVICES, INC.<br>P.O. Box 109<br>395 N. Memorial Pkwy<br>Harristown, IL 62537 |
| **TEST SUBSTANCE IDENTIFICATION:** | Axenohl<br>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 23-October 3, 1999 |
| **NOTEBOOK NO.:** | 99-60; pages 87-91 |

## 1. PURPOSE

To provide information on the skin irritation likely to arise from a single topical exposure to Axenohl.

## 2. SUMMARY

A primary skin irritation test was conducted with rabbits to determine the potential for Axenohl to produce irritation after a single topical application. Based on the results of this study, the test substance is classified as slightly irritating to the skin.

Five-tenths of a milliliter of the test substance, as received, was applied to the skin of three healthy rabbits for 4 hours. Following exposure, dermal irritation was evaluated by the method of Draize *et al*[1].

[1] Draize, J.H., Woodward, G. and Calvery, H.O. Methods for the study of irritation and toxicity of substances applied topically to the skin and mucous membranes. *J. Pharmacol. Exp. Ther.* 1944; 82:377-390.

Twenty-four hours after patch removal, well-defined erythema was observed at all three treated sites. Very slight edema was also evident in two rabbits. The overall incidence and severity of irritation decreased with time. All animals were free from irritation by Day 10 (study termination).

The incidence, severity and reversibility of irritation are detailed below:

| | Incidence of Irritation | |
|---|---|---|
| **Time After Patch Removal** | **Erythema** | **Edema** |
| **1 hour** | 3/3 | 2/3 |
| **24 hours** | 3/3 | 2/3 |
| **48 hours** | 3/3 | 0/3 |
| **72 hours** | 2/3 | 0/3 |
| **Day 7** | 2/3 | 0/3 |
| **Day 10** | 0/3 | 0/3 |

| Time After Patch Removal | Severity of Irritation – Mean Score |
|---|---|
| **1 hour** | 2.4 |
| **24 hours** | 2.7 |
| **48 hours** | 1.0 |
| **72 hours** | 0.7 |
| **Day 7** | 0.7 |
| **Day 10** | 0.0 |

The Primary Dermal Irritation Index (PDII) calculated for this test substance was 1.7.

## 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: 3

3.B.2 Sex: 2 males and 1 female

3.B.3 Species/Strain: Rabbit/New Zealand albino

3.B.4 Age: Adult

3.B.5 Source: Received from Davidson's Mill Farm, South Brunswick, NJ on September 15, 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: 18-22°C

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

4.A.4 Acclimation Period: 8 days

4.A.5 Food: Pelleted Purina Rabbit Chow #5326

4.A.6 Water: Filtered tap water was supplied *ad-libitum* by 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 rabbit 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 8113, constituted unique identification.

## 5. PROCEDURE

### A. Preparation and Selection of Animals

On the day before 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 and the skin checked for any abnormalities. Only healthy animals without pre-existing skin irritation were selected for test.

### B. Application of Test Substance

Five-tenths of a milliliter of the test substance was applied to one 6 $cm^2$ intact dose site on each animal and covered with a 1 inch x 1 inch, 4-ply gauze pad. The pad and entire trunk of each animal were then wrapped with semi-occlusive 3 inch Micropore tape to avoid dislocation of the pad. Elizabethan collars were placed on each rabbit and they were returned to their designated cages.

After 4 hours of exposure to the test substance, the pads and collars were removed and the test sites gently wiped with water and a clean towel to remove any residual test substance.

### C. Evaluation of Test Sites

Individual dose sites were scored according to the Draize scoring system (Table 3)[1] at approximately 1, 24, 48 and 72 hours and at 7 and 10 days after patch removal.

The classification of irritancy was obtained by adding the average erythema and edema scores for the 1, 24, 48 and 72 hour scoring intervals and dividing by the number of evaluation intervals (4).

The resulting Primary Dermal Irritation Index (PDII) was classified as follows:

| PDII | Classification |
|---|---|
| < 0.5 | Non-irritating |
| 0.5 - 2.0 | Slightly irritating |
| 2.1 - 5.0 | Moderately irritating |
| > 5.0 | Severely irritating |

### D. Cage-Side Observations

The animals were observed for signs of gross toxicity and behavioral changes at least once daily during the test period. 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.

## 6. STUDY CONDUCT

This study was conducted at Product Safety Labs, 725 Cranbury Road, East Brunswick, New Jersey 08816 in compliance with good laboratory practices as defined in 40 CFR 160: U.S. EPA Good

[1] Draize, J.H., Woodward, G. and Calvery, H.O. Methods for the study of irritation and toxicity of substances applied topically to the skin and mucous membranes. *J. Pharmacol. Exp. Ther.* 1944; 82:377-390.

Laboratory Practice Standards: Pesticide Programs (FIFRA) and in accordance with Health Effects Test Guidelines, OPPTS 870.2500 (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 appeared active and healthy. Apart from the dermal irritation noted below, there were no other signs of gross toxicity, adverse pharmacologic effects or abnormal behavior.

Twenty-four hours after patch removal, well-defined erythema was observed at all three treated sites. Very slight edema was also evident in two rabbits. The overall incidence and severity of irritation decreased with time. All animals were free from irritation by Day 10 (study termination).

The Primary Dermal Irritation Index for Axenohl is 1.7.

## 11. CONCLUSION

Based on the classification system used, Axenohl is classified as slightly irritating to the skin.

## 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

George E. Moore, B.S.
Study Director

Oct 21, 1999
Date

Gary Wnorowski

Gary Wnorowski, B.A.
Laboratory Manager

Oct 21, 1999
Date

Daniel Markley

Daniel Markley
Principal Toxicology Technician

Oct 21, 1999
Date

## TABLE 1: INDIVIDUAL SKIN IRRITATION SCORES

### ERYTHEMA/EDEMA

| Animal No. | Sex | Hours After Patch Removal | | | | Days | |
|---|---|---|---|---|---|---|---|
| | | 1 | 24 | 48 | 72 | 7 | 10 |
| 9558 | M | 2/1 | 2/1 | 1/0 | 0/0 | 0/0 | 0/0 |
| 9559 | F | 2/1 | 2/0 | 1/0 | 1/0 | 1/0 | 0/0 |
| 9560 | M | 1/0 | 2/1 | 1/0 | 1/0 | 1/0 | 0/0 |
| **Total** | | 5/2 | 6/2 | 3/0 | 2/0 | 2/0 | 0/0 |
| **Mean** | | 1.7/0.7 | 2.0/0.7 | 1.0/0.0 | 0.7/0.0 | 0.7/0.0 | 0.0/0.0 |

## TABLE 2: SUMMARY OF PRIMARY SKIN IRRITATION SCORES[1]

| | Hours | | | | Days | |
|---|---|---|---|---|---|---|
| | 1 | 24 | 48 | 72 | 7 | 10 |
| **Erythema** | 1.7 | 2.0 | 1.0 | 0.7 | 0.7 | 0.0 |
| **Edema** | 0.7 | 0.7 | 0.0 | 0.0 | 0.0 | 0.0 |
| **TOTAL (PDI)[2]** | 2.4 | 2.7 | 1.0 | 0.7 | 0.7 | 0.0 |

$$\text{Primary Dermal Irritation Index (PDII)} : \frac{\text{PDI for 1, 24, 48 and 72 hours}}{4} = 1.7$$

Classification: Slightly irritating

CLASSIFICATION SYSTEM[3]

| PDII | Classification |
|---|---|
| < 0.5 | Non-irritating |
| 0.5 - 2.0 | Slightly irritating |
| 2.1 - 5.0 | Moderately irritating |
| > 5.0 | Severely irritating |

[1] Average values for three rabbits.

[2] PDI = Average Erythema + Average Edema

[3] Health Effects Test Guidelines, OPPTS 870.2500 (1998).

## TABLE 3: PRIMARY SKIN IRRITATION SCORING SYSTEM

| Evaluation of Skin Reactions | Value |
|---|---|
| Erythema and eschar formation: | |
| No erythema | 0 |
| Very slight erythema (barely perceptible) | 1 |
| Well-defined erythema | 2 |
| Moderate to severe erythema | 3 |
| Severe erythema (beet redness) to slight eschar formation (injuries in depth) | 4 |
| Edema formation: | |
| No edema | 0 |
| Very slight edema (barely perceptible) | 1 |
| Slight edema (edges of area well defined by definite raising) | 2 |
| Moderate edema (raised approximately 1 millimeter) | 3 |
| Severe edema (raised more than 1 millimeter and extending beyond the area of exposure) | 4 |

## 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 INSPECTIONS 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 | 24 hour scoring |
| 9/24/99 | Day 1 In-life observations |
| 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