psl product safety labs
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PRODUCT

AXEN

STUDY TITLE

Primary Eye Irritation Study in Rabbits

DATA REQUIREMENT

Health Effects Test Guidelines, OPPTS 870.2400 (1998)

AUTHOR

George E. Moore, B.S.

STUDY COMPLETED ON

October 6, 1999

PERFORMING LABORATORY

Product Safety Labs

725 Cranbury Road

East Brunswick, New Jersey 08816

LABORATORY PROJECT IDENTIFICATION NUMBER

8115

CERTIFIED COPY
Signature 10/25/99
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 WOOLSON	STUDY MONITOR
Name	Title
Edwin A Woolson	10/22/99
Signature	Date

GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

AXEN

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.

10/6/99
Date

Submitter:

Signature

Date

Sponsor: Edwin a Woodson
Signature Monitor

10/22/99
Date

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PRIMARY EYE IRRITATION STUDY IN RABBITS

PROTOCOL NO.:	P324
AGENCY:	EPA (FIFRA)
STUDY NUMBER:	8115
SPONSOR:	EPL BIOANALYTICAL SERVICES, INC. P.O. Box 109 395 N. Memorial Parkway Harristown, IL 62537
TEST SUBSTANCE IDENTIFICATION:	AXEN Lot #8995A
TEST SUBSTANCE DESCRIPTION:	Clear liquid
DATE RECEIVED:	September 13, 1999
PSL REFERENCE NO.:	E90913-4R
DATE OF PROTOCOL APPROVAL:	September 4, 1999
DATES OF TEST:	September 20-23, 1999
NOTEBOOK NO.:	99-55; pages 126-132

1. PURPOSE

To provide information on the irritation likely to arise from a single instillation of AXEN into the eye.

2. SUMMARY

A primary eye irritation test was conducted with rabbits to determine the potential for AXEN to produce irritation from a single instillation via the ocular route. Based on the results of this study, the test substance is classified as practically non-irritating to the eye.

One-tenth of a milliliter of the test substance, as received, was instilled into the right eye of three healthy rabbits. The left eye remained untreated and served as a control. Ocular 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.

No corneal opacity or iritis was observed during the study. One hour after test substance instillation, two treated eyes exhibited conjunctivitis. The incidence and severity of irritation decreased thereafter. Ocular irritation cleared from the affected eyes within 24 hours.

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

Time Post Instillation	Incidence of Irritation		
	Corneal Opacity	Iritis	Conjunctivitis
1 hour	0/3	0/3	2/3
24 hours	0/3	0/3	0/3
48 hours	0/3	0/3	0/3
72 hours	0/3	0/3	0/3

Time Post Instillation	Severity of Irritation – Mean Score
1 hour	1.3
24 hours	0.0
48 hours	0.0
72 hours	0.0

3. MATERIALS

A. Test Substance

The test substance identified as AXEN, Lot #8995A was received on September 13, 1999 and was further identified with PSL Reference Number E90913-4R. The test substance was a clear liquid and was stored at room temperature. The sample was instilled as received. Documentation of the methods of synthesis, fabrication, or derivation of the test substance is retained by the sponsor.

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

Composition: 12 ppm Ag^{+}

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: 19-22°C

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

4.A.4 Acclimation Period: 5 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 8115, constituted unique identification.

5. PROCEDURE

A. Preparation and Selection of Animals

Prior to instillation, both eyes of a number of animals were examined using a fluorescein dye procedure. One drop of 2% ophthalmic fluorescein sodium was instilled into both eyes of each rabbit. The eyes were rinsed with physiological saline (0.9% NaCl) approximately 30 seconds after instillation of the fluorescein. Using a Blak-Ray® Lamp (compact 4-watt UV Lamp), the eyes were checked for gross abnormalities according to the "Scale for Scoring Ocular Lesions" (Table 2). Only healthy animals without pre-existing ocular irritation were selected for test.

B. Instillation

One-tenth of a milliliter of the test substance, as received, was instilled into the conjunctival sac of the right eye of each rabbit by pulling the lower lid away from the eyeball. The upper and lower lids were then gently held together for about one second before releasing, to minimize loss of the test substance. The other eye of each rabbit remained untreated with the test substance and served as a control. The rabbits were then returned to their designated cages.

C. Ocular Scoring

Ocular irritation was evaluated using a high-intensity white light (Mag Lite) in accordance with Draize *et al.* (Table 2)[1] at 1, 24, 48 and 72 hours post-instillation. The fluorescein dye evaluation procedure described in Section 5.A was used at 24 hours to verify the absence of corneal damage. Individual scores were recorded for each animal. In addition to observations of the cornea, iris and conjunctivae, any other observed lesions were noted. The average score for all rabbits at each scoring period was calculated to aid in data interpretation.

D. Classification of Eye Scores

The time interval with the highest mean score (Maximum Mean Total Score - MMTS) for all rabbits was used to further classify the test substance by the system of Kay and Calandra (Table 3)[2].

E. 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 to comply with 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 870.2400 (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.

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[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.

[2] Kay, J.H. and Calandra, J.C. Interpretation of eye irritation tests. *J. Soc. Cos. Chem.* 1962; 13:281-289.

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 eye irritation noted below, there were no other signs of gross toxicity, adverse pharmacologic effects or abnormal behavior.

No corneal opacity or iritis was observed during the study. One hour after test substance instillation, two treated eyes exhibited conjunctivitis. The incidence and severity of irritation decreased thereafter. Ocular irritation cleared from the affected eyes within 24 hours.

The Maximum Mean Total Score of AXEN is 1.3.

11. CONCLUSION

Based on the classification system used, AXEN is classified as practically non-irritating to the eye.

SIGNATURES

AXEN

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 — 10/6/99

George E. Moore, B.S.
Study Director

Date

Gary Wnorowski — Oct 6, 1999

Gary Wnorowski, B.A.
Laboratory Manager

Date

Daniel Markley — Oct 6, 1999

Daniel Markley
Principal Toxicology Technician

Date

TABLE 1: INDIVIDUAL SCORES FOR OCULAR IRRITATION

	Rabbit No.: 9537 (Male)				Rabbit No.: 9538 (Female)				Rabbit No.: 9539 (Male)			
	Hours				Hours				Hours			
	1	24	48	72	1	24	48	72	1	24	48	72
I. Cornea												
A. Opacity	0	0[1]	0	0	0	0[1]	0	0	0	0[1]	0	0
B. Area	4	4	4	4	4	4	4	4	4	4	4	4
(AxB)x5	0	0	0	0	0	0	0	0	0	0	0	0
II. Iris												
A. Values	0	0	0	0	0	0	0	0	0	0	0	0
Ax5	0	0	0	0	0	0	0	0	0	0	0	0
III. Conjunctivae												
A. Redness	1	0	0	0	0	0	0	0	1	0	0	0
B. Chemosis	0	0	0	0	0	0	0	0	0	0	0	0
C. Discharge	0	0	0	0	0	0	0	0	0	0	0	0
(A+B+C)x2	2	0	0	0	0	0	0	0	2	0	0	0
Total	2	0	0	0	0	0	0	0	2	0	0	0

[1] 2% fluorescein sodium used to verify the absence of corneal opacity

TABLE 2: SCALE FOR SCORING OCULAR LESIONS[1]

1. Cornea

A Opacity-degree of density (area most dense taken for reading)

No Opacity.....0
Scattered or diffuse area, details of iris clearly visible.....1*
Easily discernible translucent areas, details of iris slightly obscured.....2*
Opalescent areas, no details of iris visible, size of pupil barely discernible.....3*
Opaque, iris invisible.....4*

B. Area of cornea involved

One quarter (or less) but not zero.....1
Greater than one quarter, but less than half.....2
Greater than half, but less than three quarters.....3
Greater than three quarters, up to whole area.....4

A X B X 5 Total Maximum = 80

2. Iris

A Values

Normal.....0
Folds above normal, congestion, swelling, circumcorneal injection (any or all of these or combination of any thereof) iris still reacting to light (sluggish reaction is positive).....1*
No reaction to light, hemorrhage, gross destruction (any or all of these).....2*

A X 5 Total Maximum = 10

3. Conjunctivae

A Redness (refers to palpebral and bulbar conjunctivae excluding cornea and iris)

Vessels normal.....0
Vessels definitely injected above normal.....1
More diffuse, deeper crimson red, individual vessels not easily discernible.....2*
Diffuse beefy red.....3*

B. Chemosis

No swelling.....0
Any swelling above normal (includes nictitating membrane).....1
Obvious swelling with partial eversion of lids.....2*
Swelling with lids about half-closed.....3*
Swelling with lids about half-closed to completely closed.....4*

C. Discharge

No discharge.....0
Any amount different from normal (does not include small amounts observed in inner canthus of normal animals).....1
Discharge with moistening of the lids and hairs just adjacent to lids.....2
Discharge with moistening of the lids and hairs, and considerable area around the eye.....3

Score (A + B + C) X 2 Total Maximum = 20

Total Maximum Score: 110 represents the sum of all scores obtained for the cornea, iris and conjunctivae.

[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.

* These scores represent a positive response.

TABLE 3: CLASSIFICATION OF EYE IRRITATION SCORES

MMTS	Irritation Classification	Requirement For Maintenance of Classification[1]
0.0 - 0.5	non	Up to 0.5 at 1 hour with zeros at 24 hours; otherwise, increase one level
0.6 - 2.5	practically non	with zeros at 24 hours; otherwise, increase one level
2.6 - 15.0	minimally	with zeros at 48 hours; otherwise, increase one level
15.1 - 25.0	mildly	with zeros at 96 hours; otherwise, increase one level
25.1 - 50.0	moderately	with 7 day mean $\leq$ 20 and individual total scores $\leq$ 10 in at least 60% of the rabbits with no total score >30; otherwise, increase one level
50.1 - 80.0	severely	with 7 day mean $\leq$ 40 and individual total scores $\leq$ 30 in at least 60% of the rabbits with no total score > 60; otherwise, increase one level
80.1 - 100.0	extremely	with 7 day mean $\leq$ 80 and individual total scores $\leq$ 60 in at least 60% of the rabbits with no total score >100; otherwise, increase one level
100.1 - 110	maximally	with 7 day mean > 80 and individual total scores > 60 in at least 60% of the rabbits; otherwise, decrease one level

[1] Kay JH, and Calandra JC. Interpretation of eye irritation tests. *J Soc Cos Chem* 1962; 13:281-289.

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
9/21/99	24 hour scoring
9/22/99	Day 2 In-life observations
10/5/99	Raw data
10/5/99	Draft report
10/6/99	Final report

Findings reported to: Study Director 10/5/99

Management 10/6/99

Frank Fielder, B.S.
Quality Assurance Supervisor