psl product safety labs
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PRODUCT

Axenohl

STUDY TITLE

Dermal Sensitization Study in Guinea Pigs (Buehler Method)

DATA REQUIREMENT

Health Effects Test Guidelines, OPPTS 870.2600 (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 8114

EPL Study Number 331S09

CERTIFIED COPY
T Williamson 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 WOOLSON (Name) STUDY MONITOR (Title)

Edwin A Woolson (Signature) 10/22/99 (Date)

GOOD LABORATORY PRACTICE STATEMENT

Axenohl

This study meets the requirements of 40 CFR Part 160 EPA (FIFRA) with the following exceptions:

1. The stability, characterization, identity and verification of the test substance concentration as received and tested are the responsibility of the study sponsor.

2. The stability, uniformity of mixture and verification of concentration of DNCB in its carriers were not determined.

Study Director: George E Moore

George E. Moore, B.S.

10/21/99

Date

Submitter: _____

Signature

_____

Date

Sponsor: _____

Signature

_____

Date

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DERMAL SENSITIZATION STUDY IN GUINEA PIGS (BUEHLER METHOD)

PROTOCOL NO.:	P328
AGENCY:	EPA (FIFRA)
PSL STUDY NUMBER:	8114
EPL STUDY NUMBER:	331S09
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 9-October 8, 1999
NOTEBOOK NO.:	99-60; pages 92-108

1. PURPOSE

To determine the potential for Axenohl to elicit a skin sensitization reaction.

2. SUMMARY

A dermal sensitization test was conducted with guinea pigs to determine the potential for Axenohl to produce sensitization after repeated topical applications.

A 75% w/w solution of the test substance in distilled water was topically applied to twenty healthy test guinea pigs, once each week for a three week induction period. Twenty-seven days after the first induction dose, a challenge dose of the test substance at its highest non-irritating concentration (50% w/w solution in distilled water) was applied to a naive site on each guinea pig. A naive control group (ten animals) was maintained under the same environmental conditions and treated with the test substance at challenge only. Approximately 24 and 48 hours after each induction and challenge dose, the animals were scored for erythema.

A summary of the incidence and severity of the sensitization response noted after challenge is described below:

	Sensitization Response Indices			
	Incidence[1]		Severity[2]	
	Hours		Hours	
	24	48	24	48
Test Animals	0/20	0/20	0.4	0.35
Test Naive Control Animals	0/10	0/10	0.3	0.25

Based on the results of this study, the test substance is not considered to be a contact sensitizer. Historical data (See Section 7) indicating a positive response to 0.04% DNCB in acetone validates the test system used in this study.

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. 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: 34

3.B.2 Number of Groups: 3

3.B.3 Number of Animals per Group:
Preliminary Irritation Group: 4

[1] Animals with scores greater than 0.5.

[2] Sum of the erythema scores divided by the number of animals evaluated.

Test Group: 20
Test Naive Control Group: 10

3.B.4 Sex: Male and Female

3.B.5 Species/Strain: Guinea pigs/Hartley albino

3.B.6 Age/Bodyweight: Preliminary Irritation Group: Young adult
Test Group: Young adult/males 242-316 grams and females 251-340 grams at experimental start

3.B.7 Source: Received from Davidson's Mill Farms, South Brunswick, NJ on July 16 and 30, 1999 (Preliminary Irritation Group) and September 3, 1999 (Test Group)

4. METHODS

A. Husbandry

4.A.1 Housing: The animals were group 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: 18-26 °C

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

4.A.4 Acclimation Period: 6 days

4.A.5 Food: Pelleted Purina Guinea Pig Chow #5025

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: Each guinea pig was marked with a color code and given a sequential animal number assigned to study 8114, which constituted unique identification.

5. PROCEDURE

A. Preliminary Irritation Testing

A group of animals was used to determine the highest non-irritating concentration (HNIC) of test substance prior to the challenge dose. The fur was removed by clipping (Oster model #A2-small) the dorsal area and flanks of each guinea pig. This area was divided into four test sites (two sites on each side of the midline) on each animal. The test substance was applied neat (100%) and also diluted with distilled water to yield concentrations of 75%, 50% and 25% w/w. Each concentration was applied to a test site using an occlusive 25 mm Hill Top Chamber®. The sites were wrapped with non-allergenic Durapore adhesive tape. After 6 hours of exposure, the chambers were removed. Twenty-

four hours after application, each site was evaluated for local reactions (erythema) according to the scoring system described in Section 5.E.

From these results, the HNIC (the highest concentration that produced responses in 4 guinea pigs no more severe than two scores of 0.5 and two scores of zero) was established and used for challenge. Based on these findings (See Table 1), the HNIC selected for the challenge phase was a 50% w/w solution in distilled water.

B. Preparation and Selection of Animals

On the day before initiation, the fur of a group of animals was removed by clipping the dorsal area and flanks. After clipping and prior to initiation, the animals were weighed and the skin was checked for any abnormalities. Only healthy animals without pre-existing skin irritation were selected for test. Animals were reclipped prior to each dose.

C. Induction Phase

Once each week for three weeks, four-tenths of a milliliter of a 75% w/w solution of the test substance in distilled water was applied to the left side of each test animal using an occlusive 25 mm Hill Top Chamber®. The chambers were secured in place and wrapped with non-allergenic Durapore adhesive tape to avoid dislocation of the chambers and to minimize loss of the test substance. After the 6-hour exposure period, the chambers were removed and the test sites were gently wiped with water and a clean towel to remove any residual test substance. Twenty-four and 48 hours after each induction application, readings were made of local reactions (erythema) according to the scoring system described in Section 5.E. (See Table 4). Due to the severity of irritation and development of eschar following the second induction, the dose site of all animals were relocated to an adjacent, naive area for the third induction.

D. Challenge Phase

Twenty-seven days after the first induction dose, four-tenths of a milliliter of a 50% w/w solution of the test substance in distilled water was applied to a naive site on the right side of each animal as a challenge dose, using the procedures described above. These sites were evaluated for a sensitization response (erythema) at 24 and 48 hours after the challenge application according to the system described in Section 5.E. (See Table 6).

In addition to the test animals, 10 guinea pigs from the same shipment were maintained under identical environmental conditions and were treated with the HNIC of the test substance at challenge only. These animals constituted the "naive" group.

E. Scoring System

0 - no reaction
0.5 - very faint erythema, usually non-confluent
1 - faint erythema usually confluent
2 - moderate erythema
3 - severe erythema with or without edema

F. Bodyweights

Individual bodyweights of the animals were recorded prior to initiation and again on the day after challenge (See Table 2).

6. EVALUATION

In order to evaluate the sensitization response, two indices were used; one for incidence and one for severity in both test and positive control animals.

The incidence index was calculated to evaluate the incidence of erythema (sensitization response) 24 and 48 hours after challenge according to the following formula:

$$\text{Incidence Index} = \frac{\text{Number of erythema scores} > 0.5}{\text{Number of animals evaluated}}$$

The severity index (sensitization produced) at 24 and 48 hours after challenge was calculated using the following formula:

$$\text{Severity Index} = \frac{\text{Sum of erythema scores}}{\text{Number of animals evaluated}}$$

The following criteria were used to classify the test substance as a potential contact sensitizer (Ritz & Buehler, 1980):

A. At the 24 hour and/or 48 hour scoring interval, 15% or more of the test animals exhibit a positive response (scores > 0.5) in the absence of similar results in the naive control group.

B. The positive reaction must persist to 48 hours in at least one test animal.

7. VALIDATION - POSITIVE CONTROL

The procedures used in this study were validated using 1-Chloro-2,4-Dinitrobenzene (DNCB), as a positive control substance. From May 20, 1999 through June 18, 1999 a Buehler sensitization test was conducted with DNCB using Hartley strain albino guinea pigs from Davidson's Mills Farm following induction and challenge procedures identical to those described in Section 5. The results obtained from this testing are presented in Section 13 and Tables 1, 3, 5 and 7.

8. 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 870.2600 (1998).

9. REFERENCES

M. Robinson, T. Nusair, E. Fletcher, H. Ritz, A Review of the Buehler Guinea Pig Skin Sensitization Test And Its Use in a Risk Assessment Process for Human Skin Sensitization. *Toxicol*, 1990;61:91-107.

H. Ritz, E. Buehler; Planning, Conduct, and Interpretation of Guinea Pig Sensitization Patch Tests. in V.A. Drill and P. Lazar (Eds.), *Current Concepts in Cutaneous Toxicity*, Academic Press, New York, 1980, p. 25.

10. 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.

11. DEVIATIONS FROM FINAL PROTOCOL

None

12. 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.

13. RESULTS

Induction Phase

Test Animals (applied as a 75% w/w solution in distilled water): Very faint to severe erythema (0.5-3) was noted at all test sites during the induction phase. Due to the severity of irritation and development of eschar following the second induction, all sites were relocated to an adjacent, naive area for the third induction. Following the third induction, eschar, desquamation and/or moderate to severe erythema were observed at all test sites.

Historical Positive Control Animals (0.08% DNCB in 80% aqueous ethanol): Very faint to severe erythema (0.5-3) was noted at all positive control sites during the induction phase. Overall, the incidence and severity of irritation increased with each successive application.

Challenge Phase

Test Animals (applied as a 50% w/w solution in distilled water): Very faint erythema (0.5) was noted at sixteen test sites 24 hours after challenge. Irritation persisted at fourteen of these sites through 48 hours.

Test Naive Control Animals (applied as a 50% w/w solution in distilled water): Very faint erythema (0.5) was noted at six test naive control sites 24 hours after challenge. Irritation persisted at five of these sites through 48 hours.

Historical Positive Control Animals (0.04% DNCB in acetone): Eight of ten positive control animals exhibited signs of a sensitization response (faint to moderate erythema [1-2]) 24 and/or 48 hours after challenge.

Historical Positive Naive Control Animals (0.04% DNCB in acetone): Very faint erythema (0.5) was noted at two positive naive control sites 24 hours after challenge. Irritation cleared from both affected sites within 48 hours.

14. CONCLUSION

Based on these findings and on the evaluation system used, Axenohl is not considered to be a contact sensitizer.

Historical data (See Section 7) indicating a positive response to 0.04% DNCB in acetone validates the test system used in this study.

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

Daniel Markley
Principal Toxicology Technician

Oct 21, 1999
Date

TABLE 1: PRELIMINARY IRRITATION TESTING SCORES FOR DETERMINATION OF HNIC[1] (TEST SUBSTANCE)

	Concentration (%)[2]			
Animal No.	100	75	50	25
1803	2	1	0.5	0
2234	3	2	0	0
2235	3	2	0.5	0
2236	2	1	0	0

[1] HNIC - Highest Non-Irritating Concentration

[2] Four-tenths of a milliliter of the test substance was applied as w/w solution in distilled water.

TABLE 1 (cont.) PRELIMINARY IRRITATION TESTING SCORES FOR DETERMINATION OF HNIC[1] (POSITIVE CONTROL-DNCB)

Historical Data

	Concentration (%)[2]			
Animal No.	**0.05**	**0.04**	**0.03**	**0.02**
9676	0.5	0.5	0	0
9677	0.5	0	0	0
9678	0.5	0.5	0.5	0
9679	0.5	0	0	0

[1] HNIC - Highest Non-Irritating Concentration

[2] Four-tenths of a milliliter of DNCB was applied as w/w solutions in acetone.

TABLE 2: INDIVIDUAL BODYWEIGHTS (TEST SUBSTANCE)

Animal No. / Test	Sex	Initial (g)	Day After Challenge (g)
1898	M	300	497
1899	M	294	533
1900	M	282	527
1901	M	277	545
1902	M	259	554
1903	M	242	494
1904	M	270	500
1905	M	299	577
1906	M	249	548
1907	M	268	555
1908	F	293	517
1909	F	301	501
1910	F	281	476
1911	F	276	475
1912	F	269	468
1913	F	305	468
1914	F	301	386
1915	F	279	468
1916	F	295	486
1917	F	251	497

TABLE 2 (cont.): INDIVIDUAL BODYWEIGHTS (TEST SUBSTANCE)

Animal No. / Naive Control	Sex	Initial (g)	Day After Challenge (g)
1918	F	340	476
1919	F	326	456
1920	F	310	455
1921	F	294	435
1922	M	316	481
1923	M	259	467
1924	M	269	448
1925	M	300	497
1926	M	250	480
1927	M	268	491

TABLE 3: INDIVIDUAL BODYWEIGHTS (POSITIVE CONTROL-DNCB)

Historical Data

Animal No. / Positive Control	Sex	Initial (g)	Day After Challenge (g)
781	M	428	544
782	M	372	609
783	M	393	568
784	M	406	579
785	M	379	544
786	M	413	600
787	M	404	660
788	M	415	581
789	M	406	618
790	M	423	668
Naive Control			
791	M	411	536
792	M	408	591
793	M	408	551
794	M	386	639
795	M	407	599

TABLE 4: INDUCTION PHASE SKIN REACTION SCORES (TEST SUBSTANCE)

Induction Number	1		2		3	
Concentration[1]	75%		75%		75%[2]	
Amount Applied (milliliter)	0.4		0.4		0.4	
Hours[3]	24	48	24	48	24	48
Animal No.						
1898	0.5	1	3[4]	3[4]	3	3[4]
1899	1	1	3[4]	3[4]	3	3[4]
1900	1	1	3[4]	3[4]	3	3[4]
1901	0	0	3[4]	3[4]	3	3[4]
1902	2	2	3[4]	3[4]	3	3[4]
1903	1	1	3[4]	3[4]	2	3
1904	0	0.5	3[4]	3[4]	3	3[4]
1905	0.5	1	3	3	3	3[4]
1906	0.5	1	3[4]	3[4]	3	3[4]
1907	1	1	3[4]	3[4]	2[5]	2[5]
1908	0.5	0.5	3[4]	3[4]	3	3[4]
1909	1	1	3[4]	3[4]	3	3[4]
1910	2	2	3[4]	3[4]	2[5]	2[5]
1911	1	1	3[4]	3[4]	3	3[4]
1912	0.5	1	3[4]	3[4]	2[5]	2[5]
1913	0.5	0.5	3	3	3	3[4]
1914	1	1	3[4]	3[4]	3	3[4]
1915	1	1	3[4]	3[4]	3	3[4]
1916	0.5	0.5	3[4]	3[4]	3	3[4]
1917	0.5	0.5	3[4]	3[4]	3	3[4]

[1] Four-tenths of a milliliter of the test substance was applied as a 75% w/w solution in distilled water.

[2] Due to the severity of irritation and development of eschar following the previous induction, the dose site of all animals was relocated to an adjacent, naive site.

[3] Hours after induction dose.

[4] Eschar

[5] Desquamation

TABLE 5: INDUCTION PHASE SKIN REACTION SCORES (POSITIVE CONTROL-DNCB)

Historical Data

Induction Number	1		2		3	
Concentration[1]	0.08%		0.08%		0.08%	
Amount Applied (ml)	0.4		0.4		0.4	
Hours[2]	24	48	24	48	24	48
Animal No.						
781	0.5	1	2	2	2	2
782	1	1	2	2	2	2
783	0.5	0.5	2	2	3	3
784	0.5	1	2	1	2	2
785	0.5	0.5	2	2	2	2
786	0.5	0.5	2	2	2	3
787	0.5	0.5	2	2	3	3
788	1	1	2	1	3	3
789	0	0	2	2	2	2
790	0.5	0.5	2	2	2	2

[1] The positive control (DNCB) was applied as a 0.08% w/w solution in 80% aqueous ethanol.

[2] Hours after induction dose.

TABLE 6: CHALLENGE PHASE SKIN REACTION SCORES (TEST SUBSTANCE[1])

Animal No.	Hours[2]	
Test	24	48
1898	0.5	0
1899	0	0
1900	0.5	0.5
1901	0.5	0.5
1902	0.5	0.5
1903	0.5	0.5
1904	0	0
1905	0	0
1906	0.5	0.5
1907	0	0
1908	0.5	0.5
1909	0.5	0.5
1910	0.5	0
1911	0.5	0.5
1912	0.5	0.5
1913	0.5	0.5
1914	0.5	0.5
1915	0.5	0.5
1916	0.5	0.5
1917	0.5	0.5

[1] Four-tenths of a milliliter of the test substance was applied as a 50% w/w solution in distilled water.

[2] Hours after challenge dose.

TABLE 6 (cont.): CHALLENGE PHASE SKIN REACTION SCORES (TEST SUBSTANCE[1])

Animal No.	Hours[2]	
Naive Control	24	48
1918	0.5	0.5
1919	0	0
1920	0	0
1921	0.5	0
1922	0.5	0.5
1923	0	0
1924	0.5	0.5
1925	0.5	0.5
1926	0.5	0.5
1927	0	0

[1] Four-tenths of a milliliter of the test substance was applied as a 50% w/w solution in distilled water.

[2] Hours after challenge dose.

TABLE 7: CHALLENGE PHASE SKIN REACTION SCORES (POSITIVE CONTROL-DNCB[1])

Historical Data

Animal No.	Hours[2]	
Test	**24**	**48**
781	0.5	0.5
782	1	1
783	2	2
784	1	1
785	1	0.5
786	2	2
787	1	2
788	2	2
789	0.5	0.5
790	2	2
Naive Control		
791	0	0
792	0	0
793	0.5	0
794	0	0
795	0.5	0

[1] DNCB was applied as a 0.04% w/w solution in acetone.

[2] Hours after challenge dose.

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
10/7/99	Challenge bodyweight
10/8/99	48 hour scoring @ challenge
10/21/99	Raw data
10/21/99	Draft report
10/21/99	Final report

Findings reported to: Study Director 10/21/99

Management 10/21/99

Frank Fielder, B.S.
Quality Assurance Supervisor