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PRODUCT

Stalosan F

STUDY TITLE

Primary Eye Irritation Study in Rabbits

DATA REQUIREMENT

U.S. EPA Health Effects Test Guidelines, OPPTS 870.2400 (1998)

AUTHOR

Daniel J. Merkel, B.S.

STUDY COMPLETED ON

March 10, 2005

PERFORMING LABORATORY

Product Safety Laboratories

2394 Highway 130

Dayton, New Jersey 08810

LABORATORY STUDY NUMBER

16450

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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: **ARCH ANGEL LLC**

Company Agent:

Name	Title
Signature	Date

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GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

Stalosan F

This study meets the requirements of 40 CFR Part 160: U.S. EPA (FIFRA) with the following exception: Specific information related to the stability, characterization, identity and verification of the test substance concentration as received and tested is the responsibility of the study Sponsor (see Test Substance section).

Study Director: _____
Daniel J. Merkel, B.S.
Product Safety Laboratories

3/10/05
Date

Submitter: _____
Signature

_____
Date

Sponsor: _____
Signature

_____
Date

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QUALITY ASSURANCE STATEMENT

The Product Safety Laboratories' Quality Assurance Unit reviewed this study for adherence to PSL's Standard Operating Procedures, the study protocol, and all applicable GLP standards. This final report was found to be an accurate representation of the work conducted. Records of QA findings are kept on file. The summary below provides verification of statements made in the final report section that addresses Quality Assurance audits.

QA activities for this study:

QA Activity	Date Conducted	Date Findings Reported To Study Director And Management
Protocol review	2/19/04[1], 2/11/05	2/19/04, 2/14/05
In-process inspection: *24 hour scoring*	12/28/04	2/14/05
Raw data audit	2/11/05	2/14/05
Draft report review	2/11/05	2/14/05
Final report review	3/10/05	3/10/05

Louise N. Caruso

Louise N. Caruso, B.S.
Quality Assurance Auditor
Product Safety Laboratories

[1] PSL's "generic" protocol used for this study was reviewed by the Quality Assurance group on this date.

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TABLE OF CONTENTS

PSL PRODUCT SAFETY LABORATORIES

PRIMARY EYE IRRITATION STUDY IN RABBITS

PROTOCOL NO.:	P324
AGENCY:	EPA (FIFRA)
STUDY NUMBER:	16450
SPONSOR:	ARCH ANGEL LLC 636 Hampshire, Suite 208 Quincy, IL 62301
TEST SUBSTANCE IDENTIFICATION:	Stalosan F Lot #1 Batch 63
TEST SUBSTANCE DESCRIPTION:	Pinkish powder
DATE RECEIVED:	November 8, 2004
PSL REFERENCE NO.:	041108-3D
STUDY INITIATION DATE:	November 15, 2004
DATES OF TEST:	December 27 - 30, 2004
NOTEBOOK NO.:	04-94: pages 265-273

1. PURPOSE

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

2. SUMMARY

A primary eye irritation test was conducted with rabbits to determine the potential for Stalosan F to produce irritation from a single instillation via the ocular route. Under the conditions of this study, the test substance is classified as mildly irritating to the eye.

Prior to its use in the study, the test substance was ground to a powder. One-tenth of a milliliter (0.04 grams) of the ground test substance 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] (see Table 2).

[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 was noted for any treated eye during the study. One hour after test substance instillation, all three treated eyes exhibited iritis and conjunctivitis. The overall incidence and severity of irritation decreased with time. All animals were free of ocular irritation within 72 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	3/3	3/3
24 hours	0/3	0/3	3/3
48 hours	0/3	0/3	3/3
72 hours	0/3	0/3	0/3

Time Post Instillation	Severity of Irritation – Mean Score
1 hour	15.0
24 hours	6.0
48 hours	4.0
72 hours	0

3. MATERIALS

A. Test Substance

The test substance, identified as Stalosan F, Lot #1 Batch 63, was received on November 8, 2004 and was further identified with PSL Reference Number 041108-3D. The test substance was a pinkish powder and was stored at room temperature. Prior to use, the test substance was ground with a mortar and pestle. Documentation of the methods of synthesis, fabrication, or derivation of the test substance is retained in Denmark.

The following information related to the characterization of the test substance was provided by the Sponsor unless otherwise noted:

Composition: not given

pH: 3.5 (as a 1% w/w solution) [1]

Solubility: Slightly soluble in water.

[1] As determined by Product Safety Laboratories (from PSL study numbers 16446 and 16445 for the active ingredient and pH, respectively).

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

Expiration Date: Not applicable.

B. Animals

3.B.1 Number of Animals: 3

3.B.2 Sex: Male.

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

3.B.4 Age: Young adult.

3.B.5 Source: Received from Robinson Services, Inc. Clemmons, NC on December 15, 1004.

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: 20-23°C

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

4.A.4 Acclimation Period: 12 days

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

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 Laboratories.

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 animal.

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 16450, constituted unique identification.

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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 an ultraviolet light source, the eyes were checked for gross abnormalities according to the "Scale for Scoring Ocular Lesions" (see Table 2). Three healthy animals without pre-existing ocular irritation were selected for test.

B. Instillation

Prior to use, the test substance was ground with a mortar and pestle. One-tenth of a milliliter (0.04 grams) of the ground test substance 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*.[1] (see Table 2) 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 classify the test substance by the system of Kay and Calandra[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.

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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.

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6. STUDY CONDUCT

This study was conducted at Product Safety Laboratories, 2394 Highway 130, Dayton, New Jersey 08810. The primary technician for this study was Michelle DeCinque. This study was conducted to comply with the Good Laboratory Practice (GLP) regulations as defined in:

- 40 CFR 160: U.S. EPA GLP Standards: Pesticide Programs (FIFRA)

and in accordance with:

- U.S. EPA 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 Laboratories 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. FINAL REPORT AND RECORDS TO BE MAINTAINED

The original, signed final report will be forwarded to the Sponsor. A copy of this signed report, together with the protocol and all raw data generated at Product Safety Laboratories, is maintained in the Product Safety Laboratories Archives. PSL will maintain these records for a period of at least five years. After this time, the Sponsor will be offered the opportunity to take possession of the records or will be charged an archiving fee for continued archiving by PSL.

10. RESULTS

Individual eye irritation scores are presented in Table 1. The Draize Scale for Scoring Eye Lesions is presented in Table 2. The Kay and Calandra scheme for classifying eye irritants is presented in Table 3.

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 was noted for any treated eye during the study. One hour after test substance instillation, all three treated eyes exhibited iritis and conjunctivitis. The overall incidence and severity of irritation decreased with time. All animals were free of ocular irritation within 72 hours.

The Maximum Mean Total Score of Stalosan F is 15.0.

11. CONCLUSION

Under the conditions of this study, Stalosan F is classified as mildly irritating to the eye.

SIGNATURES

Stalosan F

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.

Daniel J. Merkel, B.S.
Study Director
Product Safety Laboratories

3/10/05
Date

Gary Wnorowski, B.A., M.B.A. JD 3/10/05
President
Product Safety Laboratories

3/10/05
Date

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TABLE 1: INDIVIDUAL SCORES FOR OCULAR IRRITATION

	Rabbit No.: 13406 (Male)				Rabbit No.: 13407 (Male)				Rabbit No.: 13408 (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	1	0	0	0	1	0	0	0	1	0	0	0
Ax5	5	0	0	0	5	0	0	0	5	0	0	0
III. Conjunctivae												
A. Redness	2	2	2	0	2	1	1	0	2	2	2	0
B. Chemosis	1	1	0	0	1	0	0	0	1	0	0	0
C. Discharge	2	1	1	0	2	1	0	0	2	1	0	0
(A+B+C)x2	10	8	6	0	10	4	2	0	10	6	4	0
Total	15	8	6	0	15	4	2	0	15	6	4	0

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

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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[2]
Easily discernible translucent areas, details of iris slightly obscured ..... 2[2]
Opalescent areas, no details of iris visible, size of pupil barely discernible ..... 3[2]
Opaque, iris invisible ..... 4[2]

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[2]
No reaction to light, hemorrhage, gross destruction (any or all of these) ..... 2[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[2]
Diffuse beefy red ..... 3[2]

B. Chemosis

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

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.

[2] These scores represent a positive response.

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