

PRODUCT

Stalosan F

STUDY TITLE

Primary Skin Irritation Study in Rabbits

DATA REQUIREMENT

U.S. EPA Health Effects Test Guidelines, OPPTS 870.2500 (1998)

AUTHOR

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STUDY COMPLETED ON

March 10, 2005

PERFORMING LABORATORY

Product Safety Laboratories

2394 Highway 130

Dayton, New Jersey 08810

LABORATORY STUDY NUMBER

16451

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



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

3/10/05
Date

Submitter:

Signature

Date

Sponsor:

Signature

Date



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	5/19/04 ¹ , 2/11/05	5/19/04, 2/14/05
In-process inspection: <i>24 hour scoring</i>	12/22/04	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, 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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PRIMARY SKIN IRRITATION STUDY IN RABBITS

PROTOCOL NO.: P326

AGENCY: EPA (FIFRA)

STUDY NUMBER: 16451

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 21 - 24, 2004

NOTEBOOK NO.: 04-94: pages 274-279

1. PURPOSE

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

2. SUMMARY

A primary skin irritation test was conducted with rabbits to determine the potential for Stalosan F to produce irritation after a single topical application. Under the conditions of this study, the test substance is classified as non-irritating to the skin.

Five-tenths of a gram of the test substance was moistened with distilled water and 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 (see Table 3).

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.

All animals appeared active and healthy. There were no signs of gross toxicity, dermal irritation, adverse pharmacologic effects or abnormal behavior.

The incidence and severity of irritation are detailed below:

Time After Patch Removal	Incidence of Irritation	
	Erythema	Edema
1 hour	0/3	0/3
24 hours	0/3	0/3
48 hours	0/3	0/3
72 hours	0/3	0/3

Time After Patch Removal	Severity of Irritation – Mean Score
1 hour	0
24 hours	0
48 hours	0
72 hours	0

The Primary Dermal Irritation Index (PDII) calculated for this test substance was 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. In order to insure adequate contact with the skin, the sample was applied as a dry paste (60% w/w mixture in distilled water). Documentation of the methods of synthesis, fabrication, or derivation of the test substance is retained by the Sponsor.

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: 2 Males and 1 Female. The female assigned to test was nulliparous and non-pregnant.

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, 2004.

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: 20-22°C

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

4.A.4 Acclimation Period: 6 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 16451, 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. On the day of dosing, but prior to application, the animals were examined for health and the skin checked for any abnormalities. Three healthy animals without pre-existing skin irritation were selected for test.

B. Application of Test Substance

Prior to application, the test substance was moistened with distilled water to achieve a dry paste by preparing a 60% w/w mixture. Five-tenths of a gram of the test substance (0.83 g of the test mixture) was placed on a 1-inch x 1-inch, 4-ply gauze pad and applied to one 6 cm² intact dose site on each animal. 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 were gently cleansed of any residual test substance.

C. Evaluation of Test Sites

Individual dose sites were scored according to the Draize scoring system¹ (see Table 3) at approximately 1, 24, 48, and 72 hours 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:

<u>PDII</u>	<u>Classification</u>
0	Non-irritating
> 0 - 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

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.

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 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 870.2500 (1998)

7. QUALITY ASSURANCE

The final report was audited for agreement with the raw data records and for compliance with the protocol, Product Safety 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 skin irritation scores are presented in Table 1. A summary of primary skin irritation scores used for calculation of Primary Dermal Irritation Index is presented in Table 2. The Draize Primary Skin Irritation Scoring System is presented in Table 3.

All animals appeared active and healthy. There were no signs of gross toxicity, dermal irritation, adverse pharmacologic effects or abnormal behavior.



The Primary Dermal Irritation Index for Stalosan F is 0.

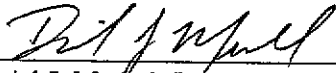
11. CONCLUSION

Under the conditions of this study, Stalosan F is classified as non-irritating to the skin.

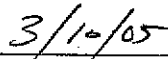
SIGNATURES

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



Date



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



Date

TABLE 1: INDIVIDUAL SKIN IRRITATION SCORES

ERYTHEMA/EDEMA

Animal No.	Sex	Hours After Patch Removal			
		1	24	48	72
13356	M	0/0	0/0	0/0	0/0
13357	F	0/0	0/0	0/0	0/0
13358	M	0/0	0/0	0/0	0/0
Total		0/0	0/0	0/0	0/0
Mean		0.0/0.0	0.0/0.0	0.0/0.0	0.0/0.0

TABLE 2: SUMMARY OF PRIMARY SKIN IRRITATION SCORES¹

	Hours			
	1	24	48	72
Erythema	0	0	0	0
Edema	0	0	0	0
TOTAL (PDI)²	0	0	0	0

Primary Dermal Irritation Index (PDII): $\frac{\text{PDI for 1, 24, 48 and 72 hours}}{4} = 0$

Classification: Non-irritating

CLASSIFICATION SYSTEM³

<u>PDII</u>	<u>Classification</u>
0	Non-irritating
> 0 - 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 U.S. EPA Addendum 3 on data reporting to pesticide assessment guidelines; Dermal Irritation, January 1988.

TABLE 3: PRIMARY SKIN IRRITATION SCORING SYSTEM

<u>Evaluation of Skin Reactions</u>	<u>Value</u>
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	4