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PRODUCT

Stalosan F

STUDY TITLE

Dermal Sensitization Study in Guinea Pigs (Buehler Method)

DATA REQUIREMENT

U.S. EPA Health Effects Test Guidelines, OPPTS 870.2600 (2003)

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

16452

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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 STATEMENT

Stalosan F

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

1. 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).

2. The stability, uniformity of mixture and verification of concentration of alpha-Hexylcinnamaldehyde Technical (HCA) in its carriers during Product Safety Laboratories historical positive control study were not determined.

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	8/11/04[1], 2/11/05	8/11/04, 2/14/05
In-process inspection: *48 hour scoring*	12/10/04	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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DERMAL SENSITIZATION STUDY IN GUINEA PIGS (BUEHLER METHOD)

PROTOCOL NO.:	P328
AGENCY:	EPA (FIFRA)
STUDY NUMBER:	16452
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:	November 22 - December 22, 2004
NOTEBOOK NO.:	04-94: pages 280-292

1. PURPOSE

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

2. SUMMARY

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

A 65%[1] w/w mixture of the ground test substance in mineral oil 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 (HNIC, determined in the preliminary irritation screen to be a 33% w/w mixture in

[1] The test substance, as received, was a powder. To enhance skin contact, the test substance was moistened with mineral oil prior to application. Concentrations in excess of 65% were not considered as they were too dry to ensure adequate contact with the skin.

mineral oil) 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 table summarizing the incidence and severity of the sensitization response noted after challenge is found below:

	Sensitization Response Indices			
	Incidence of Positive Response[1]		Severity[2]	
	Hours		Hours	
	24	48	24	48
Test Animals	0/20	0/20	0.15	0.08
Naive Control Animals	0/10	0/10	0.20	0.10

Based on the results of this study, the test substance is not considered to be a contact sensitizer. The positive response observed in the historical positive control validation study with alpha-Hexylcinnamaldehyde Technical (HCA) validates the test system used in this study (see Section 7).

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. Preliminary solubility testing conducted by PSL indicated that concentrations in excess of 65% were too dry to allow for adequate contact with the skin. 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) [3]

Solubility: Slightly soluble in water.

[1] Animals with scores greater than 0.5.

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

[3] 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: 34

3.B.2 Number of Groups: 3

3.B.3 Number of Animals per Group:
Preliminary Irritation Group: 4
Test Group: 20
Naive Control Group: 10

3.B.4 Sex: Male and Female. Females assigned to test were nulliparous and non-pregnant.

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

3.B.6 Age/Body weight: Preliminary Irritation Group: Young adult
Test and Naive Control Groups: Young adult/females 381-447 grams at experimental start.

3.B.7 Source: Received from Elm Hill Breeding Labs, Chelmsford, MA on November 12 and December 1, 2004 (Preliminary Irritation Group) and November 12, 2004 (Test and Naive Control Groups).

4. METHODS

A. Husbandry

4.A.1 Housing: The animals were group housed in suspended stainless steel caging with mesh floors or plastic perforated bottom caging 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: 19-23 °C

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

4.A.4 Acclimation Period: 10, 11, or 12 days

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

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.

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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: Each guinea pig was marked with a color code and given a sequential animal number assigned to study 16452, 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 the test substance prior to the challenge dose. The fur was removed by clipping (Oster model #A5-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 ground test substance was moistened with mineral oil to yield w/w concentrations of 65%[1], 49%, 33%, and 17%. Each concentration was applied (0.4 g or ml each) 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 and the test sites were gently cleansed of any residual test substance. Approximately 24 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. The HNIC selected for the challenge phase was a 33% w/w mixture in mineral oil.

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 re-clipped prior to each dose.

C. Induction Phase

Prior to use, the test substance was ground with a mortar and pestle. Once each week for three weeks, four-tenths of a gram of a 65%[1] w/w mixture of the ground test substance in mineral oil 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 cleansed of any residual test

[1] The test substance, as received, was a powder. To enhance skin contact, the test substance was moistened with mineral oil prior to application. Concentrations in excess of 65% were not considered as they were too dry to ensure adequate contact with the skin.

substance. Approximately 24 and 48 hours after each induction application, readings were made of local reactions (erythema) according to the scoring system described in Section 5.E.

D. Challenge Phase

Twenty-seven days after the first induction dose, four-tenths of a milliliter of a 33% w/w mixture (HNIC) of the ground test substance in mineral oil (HNIC) 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) approximately 24 and 48 hours after the challenge application according to the system described in Section 5.E.

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 control" 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

*Very faint erythema is not considered a positive reaction.

F. Body Weights

Individual body weights of the animals were recorded prior to initiation and again on the day after challenge.

6. EVALUATION

In order to evaluate the sensitization response at challenge, two indices were used: one for incidence and one for severity (Ritz, H. and Buehler, E., 1980) in the test and vehicle control animals.

The incidence index is the ratio of animals with erythema scores greater than 0.5 per number of animals evaluated, and is presented for both the 24 and 48-hour intervals after challenge evaluation as follows:

Incidence Index = Number of erythema scores greater than 0.5 / Number of animals evaluated

The severity index is the mean erythema score, and is calculated for both the 24 and 48-hour intervals after challenge evaluation according to 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 (Robinson, et al., 1990):

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 vehicle control group.

The positive reaction at the 24-hour interval must persist to 48 hours in at least one test animal.

7. HISTORICAL POSITIVE CONTROL VALIDATION STUDY

The procedures used in this study were validated using alpha-Hexylcinnamaldehyde Technical (HCA) as a positive control substance. The most recent validation, PSL Study #15590, was performed by Product Safety Laboratories and testing was completed on August 12, 2004. The raw data and report for this study are archived in Product Safety Laboratories Historical Data Notebook No. 02: pages 132-141. This test was conducted at the Dayton Facility with Hartley strain albino guinea pigs from Elm Hill Breeding Labs following induction and challenge procedures similar to those described in Section 5. The results obtained from this testing are presented in Section 13.

8. STUDY CONDUCT

This study was conducted at Product Safety Laboratories, 2394 Highway 130, Dayton, New Jersey 08810. The primary technician for this study was Anselmo Villagran, B.S. 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 870.2600 (2003)

9. REFERENCES

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

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

10. 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.

11. DEVIATIONS FROM FINAL PROTOCOL

None.

12. 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.

13. RESULTS

Preliminary irritation testing scores are presented in Table 1. Individual body weights for test, naive control, and historical positive control animals are presented in Tables 2 and 3, respectively. Induction and Challenge Phase skin reaction scores for test, naive control, and historical positive control animals are presented in Tables 4 through 7.

Induction Phase

Test Animals (65% w/w mixture of the test substance in mineral oil): Very faint to faint erythema (0.5-1) was noted at most test sites during the induction phase.

Historical Positive Control Animals (HCA applied undiluted): Very faint to faint erythema (0.5-1) was noted for all positive control test sites during the induction phase.

Challenge Phase

Test Animals (33% w/w mixture of the test substance in mineral oil): Very faint erythema (0.5) was noted at six of twenty test sites 24 hours following the challenge application. Similar irritation persisted at three sites through 48 hours.

Naive Control Animals (33% w/w mixture of the test substance in mineral oil): Very faint erythema (0.5) was noted at four of ten naive control sites 24 hours following the challenge application. Similar irritation persisted at two sites through 48 hours.

Historical Positive Control Animals (75% w/w mixture of HCA in mineral oil): Six of ten positive control animals exhibited signs of a sensitization response (faint erythema [1]) 24 and 48 hours after challenge.

Historical Naive Control Animals (75% w/w mixture of HCA in mineral oil): Very faint erythema (0.5) was noted for four of five positive control naive test sites 24 hours after challenge. Irritation persisted at two of these sites through 48 hours.

14. CONCLUSION

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

The positive response observed in the historical positive control validation study with alpha-Hexylcinnamaldehyde Technical (HCA) validates the test system used in this study (see Section 7).

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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

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

		Concentration (%)[2]			
Animal No.	**Sex**	**65%[3]**	**49**	**33**	**17**
21747	M	1	0.5	0	0
21748	M	0.5	0.5	0	0
21749	M	1	0.5	0	0
21750	M	0.5	0	0	0

[1] HNIC - Highest Non-Irritating Concentration

[2] Four-tenths of a gram or milliliter of the test substance was ground and applied as w/w mixtures in mineral oil.

[3] The test substance, as received, was a powder. To enhance skin contact, the test substance was moistened with mineral oil prior to application.

TABLE 2: INDIVIDUAL BODY WEIGHTS (TEST SUBSTANCE)

Test Substance Group

Animal No.	Sex	Initial (g)	Day After Challenge (g)
21906	F	424	577
21907	F	442	602
21908	F	431	604
21909	F	420	568
21910	F	401	538
21911	F	403	520
21912	F	394	535
21913	F	408	577
21914	F	410	606
21915	F	399	509
21916	F	412	543
21917	F	430	597
21918	F	399	547
21919	F	389	519
21920	F	380	493
21921	F	410	572
21922	F	398	512
21923	F	412	527
21924	F	392	557
21925	F	409	517

TABLE 2 (cont.): INDIVIDUAL BODY WEIGHTS (TEST SUBSTANCE)

Naive Control Group

Animal No.	Sex	Initial (g)	Day After Challenge (g)
21926	F	414	568
21927	F	412	555
21928	F	381	501
21929	F	432	615
21930	F	427	577
21931	F	418	572
21932	F	414	626
21933	F	447	611
21934	F	423	597
21935	F	401	548

TABLE 3: INDIVIDUAL BODY WEIGHTS (POSITIVE CONTROL)

Historical Positive Control Validation Study[1]

Positive Control Group

Animal No.	Sex	Initial (g)	Day After Challenge (g)
19934	M	499	612
19935	M	522	707
19936	M	469	727
19937	M	553	739
19938	M	513	657
19939	M	587	761
19940	F	443	566
19941	F	441	539
19942	F	441	539
19943	F	469	630

Naive Control Group

Animal No.	Sex	Initial (g)	Day After Challenge (g)
19944	F	503	621
19945	F	475	594
19946	M	559	674
19947	M	536	656
19948	M	584	756

[1] PSL Study #15590, performed by PSL and testing was completed on August 12, 2004.

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

Test Substance Group

Induction Number	1		2		3	
Concentration[1]	65%		65%		65%	
Amount Applied (g)	0.4		0.4		0.4	
Hours[2]	24	48	24	48	24	48
Animal No.						
21906	0	0	0	0	0	0
21907	0	0	0.5	0.5	0.5	0.5
21908	0	0	0	0	0	0
21909	1	0.5	0.5	0.5	0.5	0.5
21910	0.5	0.5	0	0	0	0
21911	0.5	0.5	0	0	0	0
21912	0	0	0	0	0	0
21913	0.5	0	0	0	0	0
21914	0.5	0	0.5	0.5	0.5	0
21915	0	0	0	0	0.5	0.5
21916	0.5	0	0.5	0.5	0	0
21917	0	0	0	0	0	0
21918	0	0	0	0	0.5	0.5
21919	0	0	0.5	0.5	0.5	0
21920	0	0	0	0	0	0
21921	1	0.5	0	0	0	0
21922	0	0	0	0	0.5	0
21923	0.5	0.5	0.5	0.5	0	0
21924	0.5	0.5	0.5	0.5	0.5	0
21925	0	0	0	0	0	0

[1] The ground test substance was applied as a 65% w/w mixture in mineral oil.

[2] Hours after induction dose.

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

Historical Positive Control Validation Study[1]

Positive Control Group

Induction Number	1		2		3	
Concentration[2]	Undiluted		Undiluted		Undiluted	
Amount Applied (ml)	0.4		0.4		0.4	
Hours[3]	24	48	24	48	24	48
Animal No.						
19934	0.5	0	0.5	0.5	0.5	0.5
19935	0.5	0	0.5	0.5	0.5	0.5
19936	0.5	0.5	1	1	1	1
19937	1	0.5	1	1	1	1
19938	0.5	0.5	1	1	1	0.5
19939	1	0.5	1	1	1	1
19940	0	0	1	1	1	1
19941	0.5	0.5	1	1	0.5	0.5
19942	0.5	0	1	1	1	1
19943	1	0.5	1	1	1	1

[1] PSL Study #15590, performed by PSL and testing was completed on August 12, 2004.

[2] Four-tenths of a milliliter of HCA was applied undiluted.

[3] Hours after induction dose.

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

Test Substance Group[1]

Animal No.	Hours after Dosing	
	24	48
21906	0.5	0
21907	0	0
21908	0	0
21909	0	0
21910	0.5	0
21911	0	0
21912	0	0
21913	0	0
21914	0.5	0.5
21915	0	0
21916	0	0
21917	0.5	0.5
21918	0	0
21919	0	0
21920	0.5	0.5
21921	0	0
21922	0.5	0
21923	0	0
21924	0	0
21925	0	0

[1] Four-tenths of a milliliter of a 33% w/w mixture of the ground test substance in mineral oil was applied.

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

Naive Control Group[1]

Animal No.	Hours after Dosing	
	24	48
21926	0	0
21927	0	0
21928	0	0
21929	0.5	0
21930	0.5	0.5
21931	0	0
21932	0.5	0
21933	0.5	0.5
21934	0	0
21935	0	0

[1] Four-tenths of a milliliter of a 33% w/w mixture of the ground test substance in mineral oil was applied.

TABLE 7: CHALLENGE PHASE SKIN REACTION SCORES (POSITIVE CONTROL)

Historical Positive Control Validation Study[1]

Positive Control Group[2]

Animal No.	Hours after Dosing	
	24	48
19934	0	0
19935	0.5	0.5
19936	1	1
19937	1	1
19938	0.5	0.5
19939	0.5	0.5
19940	1	1
19941	1	1
19942	1	1
19943	1	1

Naive Control Group[2]

Animal No.	Hours after Dosing	
	24	48
19944	0.5	0.5
19945	0.5	0
19946	0.5	0
19947	0	0
19948	0.5	0.5

[1] PSL Study #15590, performed by PSL and testing was completed on August 12, 2004.

[2] Four-tenths of a milliliter of a 75% w/w mixture of HCA in mineral oil was applied.