

PRODUCT

Citrex

STUDY TITLE

Dermal Sensitization Study in Guinea Pigs (Buehler Method)

DATA REQUIREMENT

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

AUTHOR

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STUDY COMPLETED ON

June 13, 2005

PERFORMING LABORATORY

Product Safety Laboratories

2394 Highway 130

Dayton, New Jersey 08810

LABORATORY STUDY NUMBER

16827

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: **CITREX INC.**

Company Agent: _____
Name

Title

Signature


Date

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Company: **CITREX INC.**

Company Agent: Fernando Tamames President
Name Title

 2/7/04
Signature Date

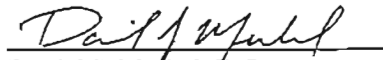
GOOD LABORATORY PRACTICE STATEMENT

Citrex

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

6/13/05
Date

Submitter:


Signature Fernando Tarnames

2/7/04
Date

Sponsor:


Signature Fernando Tarnames

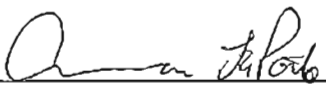
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Date

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	1/26/04 ¹ , 3/28/05	1/26/04, 3/28/05
In-process inspection: <i>24 hour scoring</i>	2/11/05	3/28/05
Raw data audit	3/28/05	3/28/05
Draft report review	3/28/05	3/28/05
Final report review	6/13/05	6/13/05



Annamarie LaPorte
 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: 16827

SPONSOR: CITREX INC.
1394 Coral Way
Miami, Florida 33145

TEST SUBSTANCE IDENTIFICATION: Citrex (see Section 11, Amendment #1)
Lot #1742

TEST SUBSTANCE DESCRIPTION: Yellow liquid with a slight citric odor

DATE RECEIVED: December 16, 2004

PSL REFERENCE NO.: 041216-2D

STUDY INITIATION DATE: January 20, 2005

DATES OF TEST: January 26 - February 25, 2005

NOTEBOOK NO.: 04-114: pages 139-139B, 140-155

1. PURPOSE

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

2. SUMMARY

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

The undiluted test substance 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 75% w/w mixture 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 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	0.08	0.00
Naive Control Animals	0/10	0/10	0.20	0.00

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 Citrex, Lot #1742, was received on December 16, 2004 and was further identified with PSL Reference Number 041216-2D. The test substance was a yellow liquid with a slight citric odor and was stored at room temperature. Documentation of the methods of synthesis, fabrication, or derivation of the test substance is retained at Siatag, Barcelona, Spain.

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

Composition (see Section 11, Amendment #2): Ascorbic Acid - 0.017803% (active ingredient)
Citric Acid - 0.03%
Lactic Acid - 0.04%
Sodium Chloride - 0.01%
Ammonium Propionate - 0.01%
Vegetable Glycerin - 0.88%

Solubility: Soluble in water.

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

¹ Animals with scores greater than 0.5.

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

- 3.B.3 Number of Animals per Group:
Preliminary Irritation Group: 4
Test Group: 20
Naive Control Group: 10
- 3.B.4 Sex: Male
- 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/325-416 grams at experimental start.
- 3.B.7 Source: Received from Elm Hill Breeding Labs, Chelmsford, MA on January 14 and February 4, 2005 (Preliminary Irritation Group) and January 21, 2005 (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: 18-23 °C
- 4.A.3 Photoperiod: 12-hour light/dark cycle
- 4.A.4 Acclimation Period: 6 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.

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 16827, 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 test substance was applied neat (100%) and also diluted with distilled water to yield w/w concentrations of 100%, 75%, 50%, and 25%. Each concentration was applied (0.4 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 75% w/w mixture 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 re-clipped prior to each dose.

C. Induction Phase

Once each week for three weeks, four-tenths of a milliliter of the undiluted test substance 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 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 75% w/w mixture of the test substance in distilled water (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. AMENDMENTS TO THE PROTOCOL

- 1) At the request of the Sponsor, the Product ID was changed to Citrex.
- 2) At the request of the Sponsor, the Composition section of the protocol was changed from

Active ingredient = Ascorbic acid 0.0017803%

to

Ascorbic Acid - 0.017803% (active ingredient)

Citric Acid - 0.03%

Lactic Acid – 0.04%
Sodium Chloride – 0.01%
Ammonium Propionate – 0.01%
Vegetable Glycerin – 0.88%

12. DEVIATIONS FROM FINAL PROTOCOL

None.

13. 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.

14. 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 (100% test substance): Very faint erythema (0.5) was noted for several 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 (75% w/w mixture of the test substance in distilled water): Very faint erythema (0.5) was noted for three of twenty test sites 24 hours following the challenge application. All animals were free of irritation by 48 hours.

Naive Control Animals (75% w/w mixture of the test substance in distilled water): Very faint erythema (0.5) was noted for four of ten naive control sites 24 hours following the challenge application. All animals were free of irritation by 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.

15. CONCLUSION

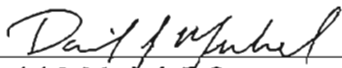
Based on these findings and on the evaluation system used, Citrex is not considered to be a contact sensitizer.

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).

SIGNATURES

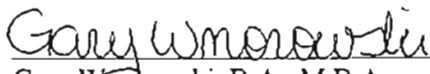
Citrex

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 6/13/05



Gary W. Monowski, B.A., M.B.A.
President
Product Safety Laboratories

Date 6/13/05

TABLE 1: PRELIMINARY IRRITATION TESTING SCORES FOR DETERMINATION OF HNIC¹ (TEST SUBSTANCE)

Animal No.	Sex	Concentration (%) ²			
		100	75	50	25
22894	M	0.5	0	0	0
22895	M	0.5	0.5	0	0
22896	M	0.5	0	0	0
22897	M	0	0	0	0

¹ HNIC - Highest Non-Irritating Concentration

² Four-tenths of a milliliter of the test substance was applied as received and as w/w mixtures in distilled water.

TABLE 2: INDIVIDUAL BODY WEIGHTS (TEST SUBSTANCE)

Test Substance Group

Animal No.	Sex	Initial (g)	Day After Challenge (g)
23020	M	369	537
23021	M	344	461
23022	M	360	573
23023	M	416	692
23024	M	372	632
23025	M	364	598
23026	M	363	584
23027	M	372	601
23028	M	371	579
23029	M	366	596
23030	M	353	547
23031	M	367	619
23032	M	354	555
23033	M	357	576
23034	M	370	603
23035	M	352	520
23036	M	370	513
23037	M	372	569
23038	M	373	581
23039	M	331	503

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

Naive Control Group

Animal No.	Sex	Initial (g)	Day After Challenge (g)
23040	M	352	571
23041	M	385	583
23042	M	364	610
23043	M	388	592
23044	M	325	587
23045	M	385	559
23046	M	329	518
23047	M	347	512
23048	M	342	575
23049	M	366	562

TABLE 3: INDIVIDUAL BODY WEIGHTS (POSITIVE CONTROL)

Historical Positive Control Validation Study¹

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	
	100%		100%		100%	
Concentration	100%		100%		100%	
Amount Applied (ml)	0.4		0.4		0.4	
Hours ¹	24	48	24	48	24	48
Animal No.						
23020	0	0	0	0	0	0
23021	0	0	0	0	0	0
23022	0	0	0	0	0	0
23023	0.5	0	0	0	0.5	0
23024	0	0	0	0	0	0
23025	0	0	0	0	0	0
23026	0	0	0	0	0	0
23027	0	0	0	0	0	0
23028	0.5	0	0	0	0	0
23029	0.5	0	0	0	0	0
23030	0	0	0.5	0	0	0
23031	0	0	0	0	0.5	0
23032	0	0	0	0	0	0
23033	0.5	0	0	0	0	0
23034	0.5	0	0	0	0	0
23035	0	0	0	0	0	0
23036	0	0	0	0	0	0
23037	0	0	0	0	0	0
23038	0	0	0	0	0	0
23039	0	0	0	0	0	0

¹ Hours after induction dose.

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

Historical Positive Control Validation Study¹

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 Group¹

Animal No.	Hours after Dosing	
	24	48
23020	0	0
23021	0	0
23022	0.5	0
23023	0	0
23024	0.5	0
23025	0	0
23026	0	0
23027	0	0
23028	0	0
23029	0	0
23030	0	0
23031	0	0
23032	0	0
23033	0	0
23034	0	0
23035	0	0
23036	0.5	0
23037	0	0
23038	0	0
23039	0	0

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

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

Naive Control Group¹

Animal No.	Hours after Dosing	
	24	48
23040	0.5	0
23041	0	0
23042	0	0
23043	0.5	0
23044	0	0
23045	0	0
23046	0	0
23047	0.5	0
23048	0.5	0
23049	0	0

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

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

Historical Positive Control Validation Study¹

Positive Control Group²

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 Group²

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.