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June 18, 1999

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Dear Sir or Madam:

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Enclosed are the final results of sensitization studies for three substances: maleic anhydride (CPS&T 98-007), [ ], and [ ] sponsored by [ ] All three substances tested positive for sensitization in standard guinea pig assays. In addition, animals that responded positive to [ ] also responded positive to the near dry crumb of a nitrile polymer [ ] containing this material. Also included is final results of sensitization studies which showed that animals sensitized to [ ] did not elicit any sensitization reactions to [ ] the nitrile polymer pellets.

The following person should be contacted if you have any questions.

[ ]

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Sincerely,

[ ]

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Final Report

Dermal Sensitization Study of [ ] and [ ] in Guinea Pigs - Closed Patch Technique

PREPARED FOR:
[ ]

COVANCE STUDY NUMBER:
81203100

ISSUE DATE:
May 14, 1999

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COVANCE
THE DEVELOPMENT SERVICES COMPANY

Sponsor:

[ ]

FINAL REPORT

Study Title:

Dermal Sensitization Study of [ ]
in Guinea Pigs - Closed Patch Technique
(EPA/OECD Guidelines)

Author:

Steven M. Glaza

Study Completion Date:

May 14, 1999

Performing Laboratory:

Covance Laboratories Inc.
3301 Kinsman Boulevard
Madison, WI 53704

Laboratory Project Identification:

Covance 81203100

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COMPLIANCE STATEMENT

Dermal Sensitization Study of [ ]
in Guinea Pigs - Closed Patch Technique
(EPA/OECD Guidelines)

This study was conducted in accordance with the following Good Laboratory Practice Standards with the exception that analysis of the test material mixtures for concentration, homogeneity/solubility, and stability was not conducted:

United States Environmental Protection Agency TSCA:
Good Laboratory Practice Standards, 40 CFR 792

Steven M. Glaza
Study Director
Acute Studies
Covance Laboratories Inc.

5-14-99
Date

Sponsor's Representative

Date

QUALITY ASSURANCE STATEMENT

This report has been reviewed by the Quality Assurance Unit of Covance Laboratories Inc., in accordance with the Environmental Protection Agency (EPA) Good Laboratory Practice Standards, 40 CFR 792. The following inspections were conducted and findings reported to the study director and study director management. Written status reports of inspections and findings are issued to Covance management according to standard operating procedures.

Inspection Dates			Date Reported to Study Director and
From	To	Phase	Study Director Management
12/21/98	12/21/98	Protocol Review	12/21/98
01/26/99	01/26/99	Test Article Preparation	01/26/99
03/22/99	03/22/99	Data Review	03/22/99
03/22/99	03/22/99	Report Review	03/22/99

Representative, Quality Assurance Unit

5.14.99
Date

STUDY IDENTIFICATION

Dermal Sensitization Study of [ ]
in Guinea Pigs - Closed Patch Technique
(EPA/OECD Guidelines)

Test Material	[ ]
Sponsor	[ ]
Sponsor's Representative	[ ]
Study Monitor	**Robert W. Kapp, Jr., PhD** **Biotox** **2711 Buford Road, Suite 338** **Richmond, VA 23235** **Telephone No.: 804.320.2800** **Facsimile No.: 804.320.8274**
Study Director	**Steven M. Glaza** **Covance Laboratories Inc.** Telephone No.: 608.241.7292 Facsimile No.: 608.242.7936
Testing Facility	Covance Laboratories Inc. 3301 Kinsman Boulevard Madison, WI 53704

Study Timetable

Study Initiation Date	December 23, 1998
Experimental (In-life) Start Date	December 29, 1998
In-life End Date	February 1, 1999
Experimental Termination Date	February 1, 1999
Study Completion Date	May 14, 1999

KEY PERSONNEL

Acute Studies

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CONTENTS

COMPLIANCE STATEMENT 2

QUALITY ASSURANCE STATEMENT 3

STUDY IDENTIFICATION 4

KEY PERSONNEL 5

SUMMARY 8

OBJECTIVE 8

TEST AND VEHICLE CONTROL MATERIALS 8
Test Material Identification 8
Test Material Purity and Stability 9
Test Material Storage and Retention 9
Vehicle Control Material Identification 9
Vehicle Control Material Purity and Stability 9
Vehicle Control Material Storage and Retention 9

TEST SYSTEM 10
Test Animal 10
Housing 10
Animal Diet 10
Group Assignments 10
Justification for Species Selection 11

PROCEDURES 11
Induction Phase 11
Challenge Phase 11
Reason for Route of Administration 12
Observations 12
Termination 12
Evaluation of Challenge Responses 12
Statistical Evaluation 12
Location of Raw Data, Records, and Final Report 13

RESULTS/DISCUSSION 13
Clinical Observations and Body Weights 13
Dermal Reactions to [redacted] 13
Positive Control Report 13

CONCLUSION 14

SIGNATURE 14

REFERENCES 14

TABLE
Buehler Sensitization Scoring Scale 15
1 Individual Body Weights (g) 16
2 Individual Clinical Signs 18
3 Individual Dermal Reactions 20

APPENDIX A 22
Protocol Deviation 23
Protocol 24

APPENDIX B 36
Positive Control Report 37

SUMMARY

The delayed contact hypersensitivity potential of [ ] was evaluated in albino guinea pigs. The test material, [ ], was administered as a 15% w/v mixture in mineral oil to each animal in the test group during the three-application induction phase of the study. Faint to strong erythema reactions (scores of 1.0 to 3.0) were observed in all 20 test animals while very faint (nonsensitization) erythema reactions (score of 0.5) were seen in three of the 10 vehicle control animals when [ ] was administered as a 1% w/v mixture in mineral oil at challenge. The other test material, [ ], when administered as a 0.4-g dose (moistened with 0.3 mL of sterile water) at challenge did not elicit any dermal reactions in the animals sensitized to [ ]. Based on these results, [ ] is considered to be a dermal sensitizer in guinea pigs. The other test material, [ ] did not elicit any sensitization reactions in animals sensitized to [ ].

OBJECTIVE

The objective of this study was to assess the delayed contact hypersensitivity potential of two test materials in guinea pigs.[1,2]

All procedures used in this study were in compliance with the Animal Welfare Act, the Guide for the Care and Use of Laboratory Animals, and the Office of Protection from Research Risks. In the opinion of the Sponsor and study director, the study did not unnecessarily duplicate any previous work. All procedural times presented in this report fall within the acceptable ranges as specified in Covance standard operating procedure (SOP).

TEST AND VEHICLE CONTROL MATERIALS

Test Material Identification

The test materials were identified as [ ], Lot No. 17367-72-6 (induction and challenge phases) and [ ], Lot No. 222 (challenge phase only) and

described as a colorless, viscous liquid and opaque, near-white plastic pellets, respectively.

Test Material Purity and Stability

The Sponsor assumes responsibility for purity and stability determinations (including under test conditions).

Test Material Storage and Retention

The test material, [ ], was stored refrigerated and protected from direct light. A reserve sample of [ ] was taken and will be retained in a freezer set to maintain a temperature of -10 to -30°C in accordance with Covance SOP. The other test material, [ ] was stored at room temperature. A reserve sample of [ ] was taken and will be retained at room temperature in accordance with Covance SOP. The unused portion of [ ] will be returned to the Sponsor after issuance of the final report according to Covance SOP. There was no remaining [ ] after completion of the study.

Vehicle Control Material Identification

The vehicle control material used in this study was mineral oil, Mallinckrodt, Lot No. 6357 KVTA.

Vehicle Control Material Purity and Stability

The purity and stability of the mineral oil are the responsibility of Covance and were deemed adequate for the conduct of this study.

Vehicle Control Material Storage and Retention

The mineral oil was stored at room temperature. A reserve sample of the vehicle control material was taken and will be retained for 10 years in a freezer set to maintain a

temperature of -10 to -30°C in accordance with Covance SOP. Any unused vehicle control material will be retained by Covance for future tests until the indicated expiration date is reached.

TEST SYSTEM

Test Animal

Young adult albino guinea pigs of the Crl:(HA)BR strain were procured from Charles River Laboratories, Inc., Kingston, New York, on December 22, 1998.

Housing

After receipt, the animals were acclimated for a period of at least 7 days. During acclimation and throughout the study, the animals were individually housed in suspended, stainless steel cages. Environmental controls for the animal room were set to maintain a temperature of 18° to 26°C, a relative humidity of 30 to 70%, and a 12-hour light/12-hour dark cycle. In cases where variations from these conditions existed, they were documented and considered to have had no adverse effect on the study outcome.

Animal Diet

The animals were provided continuous access to Certified Guinea Pig Diet #5026, PMI Nutrition International, and water. The diet is routinely analyzed by the manufacturer for nutritional components and environmental contaminants. Samples of the water are periodically analyzed. There were no known contaminants in the diet or water at levels that would be expected to interfere with or affect the results of the study.

Group Assignments

Thirty healthy, acclimated male albino guinea pigs, weighing from 352 to 411 g and approximately 4 weeks of age, were divided into two groups consisting of a test group of 20 animals and a vehicle control group of 10 animals. The animals were identified by animal number and corresponding ear tag throughout the study.

Justification for Species Selection

Historically, the albino guinea pig has been the animal of choice for skin sensitization studies.

PROCEDURES

Induction Phase

On each day of test material application, the hair was removed from the back of each animal with electric clippers. The [ ] test material mixture (15% w/v mixture in mineral oil) was applied to each animal in the test group by placing 0.4 mL on an adhesive patch (Hill Top Chamber®, 25-mm diameter) and placing the patch on the induction site along the dorsal anterior left quadrant. The patch was covered with dental dam and overwrapped with Elastoplast® tape. The dressing remained in place for a period of 6 hours after which it was removed. Any residual material was then removed from the application site using liquid Ivory® soap mixed with water, rinsed with water, and dried with disposable paper towels. The vehicle control animals were treated with a 0.4-mL volume of mineral oil in the same manner. The animals in the test and vehicle control groups received one application per week for 3 weeks for a total of three applications, with the third induction application for the 20 animals in the test group being applied slightly posterior to the initial induction site on these animals.

Challenge Phase

Two weeks following the administration of the third induction dose, a challenge dose of 0.4 mL of a 1% w/v mixture of [ ] in mineral oil and a 0.4-g dose of [ ] (ground material moistened with approximately 0.3 mL of sterile water) were administered along the dorsal anterior right quadrant of the test group animals in the same manner as during the induction phase of the study. In addition, a naive area on the left side of each test group animal was treated with a 0.4-mL dose of mineral oil in the same manner as [ ] At this time, the 10 vehicle control animals were also treated with challenge applications of the test and vehicle control materials in the same manner as the animals in the test group.

Reason for Route of Administration

Historically, the dermal route has been the route of choice for determining delayed contact hypersensitization.

Observations

On the day of the 24-hour examination following the challenge applications, the application sites of the respective animals were depilated by applying Neet® depilatory. After approximately 10 to 20 minutes, the depilatory was washed from the application sites.

The respective application sites were examined and scored for dermal reactions according to the Buehler[3] scoring scale at 24 and 48 hours following the induction and challenge applications.

Clinical observations were conducted daily throughout the study. Body weights were determined before the initial test material administration and at termination of the in-life phase.

Termination

At termination of the in-life phase, all animals were euthanized and discarded.

Evaluation of Challenge Responses

Determination of sensitization was based on the dermal reactions to the challenge dose of the test materials. Grades of 1.0 or greater in the test animals may indicate evidence of sensitization, provided grades of less than 1.0 are seen in the vehicle control animals.

Statistical Evaluation

No statistical evaluations were required by the protocol.

Location of Raw Data, Records, and Final Report

The raw data, records, and an original signed copy of the final report will be retained in the archives of Covance in accordance with Covance SOP.

RESULTS/DISCUSSION

Clinical Observations and Body Weights

Individual body weights are in Table 1 and individual clinical signs are in Table 2. All animals in both groups appeared normal and exhibited body weight gain throughout the study.

Dermal Reactions to [ ]

Individual dermal reactions for the test and vehicle control animals are presented in Table 3. Moderate to strong erythema reactions were elicited from all 20 test animals to the test material, [ ], when administered as a 15% w/v mixture in mineral oil during the induction phase of the study. Subcutaneous hemorrhaging, blanching, desquamation, and/or necrotic appearing areas were also observed within the application sites of 19 test animals during the induction phase. Faint to strong erythema reactions (scores of 1.0 to 3.0) were elicited from all 20 animals in the test group following the challenge application of [ ], applied as a 1% w/v mixture in mineral oil. Very faint erythema reactions (score of 0.5) were observed in three of the 10 vehicle control animals following the challenge application of [ ] as a 1% w/v mixture in mineral oil. None of the test or vehicle control animals reacted to the challenge application of [ ] or the vehicle control material.

Positive Control Report

A study report detailing the results of sensitization testing of α-hexylcinnamaldehyde (a known skin sensitizer) using the same sensitization method is presented in Appendix B of this report. This positive control study was conducted within 6 months of the conduct of this study.

CONCLUSION

Based on the results obtained, [ ] is considered to be a dermal sensitizer in guinea pigs when tested by the closed patch technique. [ ] did not elicit any sensitization reactions in animals sensitized to [ ].

SIGNATURE

Steven M. Glaza
Study Director
Acute Studies

5-14-99
Date

REFERENCES

1. **United States Environmental Protection Agency; Prevention, Pesticides and Toxic Substances; "OPPTS 870.2600 Skin Sensitization"; *Health Effects Test Guidelines* (August 1998).**

2. "Skin Sensitisation," *Organisation for Economic Cooperation and Development Guidelines for Testing of Chemicals*, Section 4, Health Effects, Number 406, Paris Cedex (July 17, 1992).

3. Buehler, E. V. and Ritz, H. L., "Planning, Conduct, and Interpretation of Guinea Pig Sensitization Patch Tests," *Current Concepts in Cutaneous Toxicity*, p. 28 (1980).

Buehler Sensitization Scoring Scale

No reaction	0.0
Very faint erythema, usually nonconfluent	0.5
Faint erythema, usually confluent	1.0
Moderate erythema	2.0
Strong erythema, with or without edema	3.0

Table 1

Individual Body Weights (g)

Animal Number	Predose	Terminal
	Test Group	
E16953	382	671
E16954	371	645
E16955	364	650
E16956	368	633
E16957	360	563
E16958	393	707
E16959	364	652
E17012	388	428
E16961	378	688
E16962	392	671
E17011	379	608
E16964	383	613
E16965	373	630
E16966	372	551
E16967	373	670
E16968	377	644
E16969	357	561
E16970	399	576
E16971	357	533
E16972	369	626

Table 1 (Continued)

Individual Body Weights (g)

Animal Number	Predose	Terminal
Vehicle Control Group		
E16973	373	598
E16974	361	604
E16975	378	651
E16976	366	671
E16977	352	554
E16978	385	727
E16979	397	681
E16980	411	698
E16981	377	704
E16982	407	724

Table 2

Individual Clinical Signs

Animal Number	Observation	Day																			
		1-16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35
		Test Group																			
E16953	Normal appearance	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
E16954	Normal appearance	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
E16955	Normal appearance	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
E16956	Normal appearance	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
E16957	Normal appearance	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
E16958	Normal appearance	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
E16959	Normal appearance	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
E17012	Normal appearance	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
E16961	Normal appearance	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
E16962	Normal appearance	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
E17011	Normal appearance	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
E16964	Normal appearance	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
E16965	Normal appearance	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
E16966	Normal appearance	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
E16967	Normal appearance	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
E16968	Normal appearance	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
E16969	Normal appearance	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
E16970	Normal appearance	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
E16971	Normal appearance	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
E16972	Normal appearance	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓

✓ Condition existed.

Table 2 (Continued)

Individual Clinical Signs

Animal Number	Observation	Day 1-16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35
Vehicle Control Group																					
E16973	Normal appearance	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
E16974	Normal appearance	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
E16975	Normal appearance	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
E16976	Normal appearance	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
E16977	Normal appearance	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
E16978	Normal appearance	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
E16979	Normal appearance	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
E16980	Normal appearance	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
E16981	Normal appearance	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
E16982	Normal appearance	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓

✓ Condition existed.

Table 3

Individual Dermal Reactions

Animal Number	Induction Phase* Dose #1 Hour 24	Dose #1 Hour 48	Dose #2 Hour 24	Dose #2 Hour 48	Dose #3** Hour 24	Dose #3** Hour 48	Challenge Application [ ] Hour 24	[ ] Hour 48	[ ] Hour 24	[ ] Hour 48	Mineral Oil Hour 24	Mineral Oil Hour 48
Test Group												
E16953	1.0	0.5	3.0[a]	3.0[a]	3.0[a]	3.0[n]	2.0	2.0	0.0	0.0	0.0	0.0
E16954	0.5	0.5	3.0[a]	2.0[a]	1.0	1.0[d]	2.0	2.0[a]	0.0	0.0	0.0	0.0
E16955	1.0	1.0	3.0[a]	3.0[a]	2.0	2.0	2.0	2.0	0.0	0.0	0.0	0.0
E16956	1.0	0.5	3.0[a]	3.0[a]	2.0[a]	3.0[a]	2.0	1.0	0.0	0.0	0.0	0.0
E16957	0.5	0.5	3.0[a]	3.0[a]	2.0[a]	2.0[a]	1.0	1.0	0.0	0.0	0.0	0.0
E16958	0.5	0.0	2.0[a]	2.0[a]	2.0	2.0	2.0	1.0	0.0	0.0	0.0	0.0
E16959	1.0	0.5	3.0[a]	3.0[a,b]	3.0[a]	3.0[n]	1.0	0.5	0.0	0.0	0.0	0.0
E17012	1.0	0.5	3.0[a,b]	3.0[n]	2.0	2.0[a]	2.0	1.0	0.0	0.0	0.0	0.0
E16961	2.0	2.0	3.0[a]	3.0[a]	2.0	2.0	3.0	2.0	0.0	0.0	0.0	0.0
E16962	1.0	1.0	3.0[a]	3.0[a]	2.0	2.0[a]	1.0	1.0	0.0	0.0	0.0	0.0
E17011	0.5	0.5	3.0[a]	3.0[a]	1.0	1.0	2.0	2.0	0.0	0.0	0.0	0.0
E16964	0.5	0.5	3.0[a]	3.0[a]	2.0	2.0	2.0	2.0	0.0	0.0	0.0	0.0
E16965	1.0	0.5	3.0[a]	3.0[a]	2.0[a]	2.0[a]	2.0	2.0	0.0	0.0	0.0	0.0
E16966	1.0	0.5	3.0[a]	3.0[a]	2.0[a]	2.0[a]	2.0	2.0	0.0	0.0	0.0	0.0
E16967	0.5	0.0	2.0	3.0	2.0	2.0	2.0	1.0	0.0	0.0	0.0	0.0
E16968	0.5	0.5	2.0[a]	3.0[a]	1.0	1.0	1.0	0.5	0.0	0.0	0.0	0.0
E16969	1.0	1.0	3.0[a]	3.0[a]	2.0[a]	3.0[n]	3.0	3.0[a]	0.0	0.0	0.0	0.0
E16970	1.0	0.5	3.0[a]	3.0[a]	2.0	2.0	1.0	0.5	0.0	0.0	0.0	0.0
E16971	1.0	0.5	3.0[a,b]	3.0[a,b]	2.0[a]	2.0[a]	2.0	2.0	0.0	0.0	0.0	0.0
E16972	0.5	0.5	3.0[a]	3.0[a]	3.0[a]	3.0[a]	2.0	1.0	0.0	0.0	0.0	0.0

* Treated with a 15% w/v mixture of [ ] in mineral oil.
** Induction sites moved slightly posterior to the initial induction site.
† Administered as a 1% w/v concentration in mineral oil.
‡ Administered as a 0.4-g dose (moistened with sterile water).
a Subcutaneous hemorrhaging.
b Blanching.
d Desquamation.
n Necrotic appearing area.

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Table 3 (Continued)

Individual Dermal Reactions

Animal Number	Induction Phase*						Challenge Application					
	Dose #1		Dose #2		Dose #3		[ ]		[ ]		Mineral Oil	
	Hour		Hour		Hour		Hour		Hour		Hour	
	24	48	24	48	24	48	24	48	24	48	24	48
Vehicle Control Group												
E16973	0.0	0.0	0.0	0.0	0.0	0.0	0.5	0.5	0.0	0.0	0.0	0.0
E16974	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
E16975	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
E16976	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
E16977	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
E16978	0.0	0.0	0.0	0.0	0.0	0.0	0.5	0.5	0.0	0.0	0.0	0.0
E16979	0.0	0.0	0.0	0.0	0.0	0.0	0.5	0.0	0.0	0.0	0.0	0.0
E16980	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
E16981	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
E16982	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

* Treated with a 15% w/v mixture of [ ] in mineral oil.
† Administered as a 1% w/v concentration in mineral oil.
‡ Administered as a 0.4-g dose (moistened with sterile water).

APPENDIX A

Protocol Deviation
Protocol

Protocol Deviation

Protocol	Actual Procedure
Page 8, Treatment Procedures, Dose Administration. Second Paragraph, Last Sentence. Each site will be treated with one of the following materials: 1. [ ](0.4 mL of a 1% w/v mixture in mineral oil) - right side, 2. [ ](0.4 g dose moistened with deionized water) - right side, 3. Mineral oil (0.4 mL dose) - left side.	The 0.4 g dose of test material, [ ], was moistened with sterile water instead of deionized water as stated in the protocol.

This deviation is not considered to have had an adverse effect on the outcome of the study.

COVANCE.
THE DEVELOPMENT SERVICES COMPANY

Sponsor:

[ ]

PROTOCOL TP2008

Study Title:

Dermal Sensitization Study of [ ] and [ ] in Guinea Pigs - Closed Patch Technique (EPA/OECD Guidelines)

Date:

December 23, 1998

Performing Laboratory:

Covance Laboratories Inc.
3301 Kinsman Boulevard
Madison, Wisconsin 53704

Laboratory Project Identification:

Covance 81203100

Study
Dermal Sensitization Study in Guinea Pigs - Closed Patch Technique
(EPA/OECD Guidelines)

Purpose
To assess the delayed contact hypersensitivity potential of two test materials in guinea pigs.

Sponsor
[ ]

Sponsor's Representative
[ ]

Study Monitor
Robert W. Kapp, Jr., PhD
Biotox
2711 Buford Road, Suite 338
Richmond, VA 23235
Telephone: 804.320.2800
Facsimile: 804.320.8274

Study Director
Steven M. Glaza
Covance Laboratories Inc.
Telephone 608.241.7292
Facsimile 608.242.7936

Testing Facility
Covance Laboratories Inc.
3301 Kinsman Boulevard
Madison, WI 53704

Proposed Study Timetable

Experimental Start Date	Week of December 28, 1998
Experimental Termination Date	Week of January 25, 1999
Draft Report Date	Week of March 1, 1999

Regulatory Compliance
This study will be conducted in accordance with the following Good Laboratory Practice Regulations/Standards, with the exception that analysis of the test and control material mixtures for concentration, homogeneity/solubility and stability will not be conducted:

[ ] Conduct as a Nonregulated Study
[ ] 21 CFR 58 (FDA)
[ ] 40 CFR 160 (EPA-FIFRA)
[X] 40 CFR 792 (EPA-TSCA)
[ ] C(97)186/Final (OECD)
[ ] 59 NohSan No. 3850 (Japanese MAFF)
[ ] Ordinance 21 (Japanese MOHW)

Animal Care and Use Statement
All procedures in this protocol are in compliance with the Animal Welfare Act, the Guide for the Care and Use of Laboratory Animals, and the Office of Protection from Research Risks. In the opinion of the Sponsor and Study Director, the study does not unnecessarily duplicate any previous work.

Quality Assurance
The protocol, study conduct, and the final report will be audited by the Covance Quality Assurance Unit.

Test Materials

Identification

1. [ ] (Induction and challenge phases)
2. [ ] (Challenge phase only)

Physical Description

1. Colorless, viscous liquid
2. Opaque, near-white plastic pellets

Purity and Stability

The Sponsor assumes responsibility for purity and stability determinations (including under test conditions).

Storage Conditions

[ ] protected from light in a refrigerator set to maintain a temperature of 2 to 8°C.

[ ] room temperature

Reserve Samples

Reserve samples of each batch/lot of the test materials will be taken. The reserve sample for [ ] will be stored at Covance in a freezer set to maintain a temperature of -20°C ±10°. The reserve sample for [ ] will be stored at room temperature. The reserve samples will be maintained as outlined in the Record Retention section.

Disposition

The unused test materials will be returned to the Sponsor after issuance of the final report, unless otherwise directed by the Sponsor.

Vehicle Control Material

Identification
Mineral oil

Physical Description
Colorless liquid

Purity and Stability
The purity and stability of this control material are considered to be adequate for the conduct of this study based on the study director's opinion.

Storage Conditions
Room temperature

Reserve Samples
Reserve samples of each batch/lot of the vehicle control material will be taken and stored at Covance in a freezer set to maintain a temperature of -20°C ±10°. The reserve samples will be maintained at Covance for 10 years then discarded.

Disposition
Any unused vehicle control material will be discarded after the indicated expiration date is reached.

Animals

Species
Guinea Pig

Strain and Strain
Crl:(HA)BR, Charles River Laboratories Inc.

Age at Initiation of Treatment
Young adult

Weight at Initiation of Treatment
350 to 550 g

Number and Sex
30 of any sex

Identification
Individual numbered ear tag

Husbandry

Housing
Individually, in suspended, screen-bottom stainless steel cages

Diet
Certified Guinea Pig Diet #5026 (PMI Nutrition International), *ad libitum*. The diet is routinely analyzed by the manufacturer for nutritional components and environmental contaminants.

Water
Ad libitum from an automatic system. Samples of the water are periodically analyzed for specified microorganisms and environmental contaminants.

Contaminants
There are no known contaminants in the diet or water at levels that would be expected to interfere with this study.

Environment
Environmental controls for the animal room will be set to maintain a temperature of 18° to 26°C, a relative humidity of 50% ±20%, and a 12-hour light/12-hour dark cycle.

Acclimation
At least 7 days

Selection of Test Animals

Based on health and body weight according to Covance SOPs. An adequate number of extra animals will be purchased so that no animal in obviously poor health is placed on test.

Justification for Species Selection

Historically, the albino guinea pig has been the animal of choice for skin sensitization studies.

Treatment Procedures

Experimental Design

Thirty acclimated animals weighing between 350 and 550 g will be placed into the following groups:

Group	No. of Animals
Group 1 - Test	20
Group 2 - Vehicle Control	10

The test mixture concentrations to be utilized in this study were chosen based on previous sensitization studies with these materials. All test mixtures used in the study will be stored at room temperature until administered.

Dose Administration

Before each application the back of each animal will be clipped free of hair with an electric clipper. The test material [ ] will be applied to one area on each test animal by placing 0.4 mL of the test material as a 15% w/v mixture in mineral oil on an adhesive patch (Hill Top Chamber®, 25-mm diameter) and placing the patch on the induction site along the dorsal anterior left quadrant. The patch will be covered with dental dam and secured with an overwrap of Elastoplast® tape. The dressing will remain in place for approximately 6 hours. After the six hour exposure period, the patches and any residual material will be removed using water or an

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Business Information

appropriate substance and disposable paper towels. The vehicle control animals will be treated with mineral oil alone in the same manner. The test and vehicle control animals will receive one application each week for 3 weeks for a total of three applications. Each application will be made on the same site during the induction phase unless moderate to severe irritation is observed following the first or second induction application. The induction site may then be moved slightly posterior to the initial induction site.

Two weeks after the administration of the third induction dose, a challenge dose will be applied to the animals in the test and vehicle control groups. Three naïve sites will be selected on each animal (one on the left side and two on the right side). Each site will be treated with one of the following materials:

1. [ ] (0.4 mL of a 1% w/v mixture in mineral oil) - right side
2. [ ] (0.4 g dose moistened with deionized water) - right side
3. Mineral oil (0.4 mL dose) - left side

Application of the materials will be in the same manner as during the induction phase of the study.

Reason for Route of Administration
Historically, the dermal route has been the route of choice for determining delayed contact hypersensitization.

Observation of Animals

Clinical Observations
Daily clinical signs will be recorded.

Body Weights
Before the initial test material administration and at the termination of the experimental phase.

Reading of Dermal Reactions
The respective application sites will be examined and scored for dermal irritation according to the scoring scale in Attachment 1 at approximately 24 and 48 hours after the induction and challenge applications.

On the day of the 24-hour examination following the challenge applications, the respective challenge sites will be depilated by applying Neet® for approximately 10-20 minutes then washed off with water. The 24-hour examination must take place at least 2 hours after removal of the depilatory.

Pathology
Any animals dying during the study, or sacrificed in a moribund condition, will be subjected to an abbreviated macroscopic necropsy examination and all abnormalities will be recorded. After necropsy, the animals will be discarded and no tissues will be saved. At termination of the experimental phase, surviving animals will be sacrificed and discarded.

Statistical Evaluation
No statistical evaluations are required.

Report
A final report including the items listed below will be submitted.

Description of the test and vehicle control materials
Description of the test system
Procedures
Dates of experimental initiation and termination
Summary table showing the induction and challenge responses
Any special observations that were recorded
Determination of sensitization are based upon reactions to the challenge dose. Grades of 1 or greater in the test animals to a particular test material may indicate evidence of sensitization, provided grades of less than one are seen in the vehicle control animals to the same material. If grades of one or greater are noted in the vehicle control animals, then the reactions in the test animals to a particular material that exceed the most severe respective vehicle control reaction are considered sensitization reactions.

A positive control report from periodic validation of the test method, which has been conducted within six months of the study, will be presented as an appendix in the final report.
Macroscopic pathology findings (if applicable)

Record Retention
All raw data, documentation, records, test material reserve samples, protocol, protocol amendments (if applicable), and the final report generated as a result of this study will be archived in the storage facility of Covance for a period of one year following signing of the final report. One year after signing of the final report, all of the aforementioned materials may be sent to the Sponsor, and a return fee will be charged. The Sponsor may elect to have the materials retained in the Covance archives for an additional period of time, for which Covance will charge a storage fee. If the Sponsor chooses to have Covance dispose of the materials, a disposal fee will be charged. All raw data stored on magnetic media will be retained by Covance.

The following supporting records will be retained at Covance but will not be archived with the study data:

Animal receipt/acclimation records
Water analysis records
Animal room maintenance and environmental records
Refrigerator, freezer and room temperature records
Positive control data
Feed analysis records
Instrument calibration and maintenance records

PROTOCOL APPROVAL

[redacted]

Date 1/30/99

Robert W. Kapp, Jr., PhD
Study Monitor
Biotox

Date 1/16/99

Steven M. Glaza
Study Director
Acute Studies
Covance Laboratories Inc.

Date 12-23-98

(TP2008.ST4)

Attachment 1

Buehler Sensitization Scoring Scale

No reaction	0
Very faint erythema, usually nonconfluent	0.5
Faint erythema, usually confluent	1.0
Moderate erythema	2.0
Strong erythema, with or without edema	3.0

Buehler, E.V. and Ritz, H. L., "Planning, Conduct, and Interpretation of Guinea Pig Sensitization Patch Tests, "Current Concepts in Cutaneous Toxicity, p. 28 (1980)

APPENDIX B

Positive Control Report

COVANCE
THE DEVELOPMENT SERVICES COMPANY

Sponsor:

Covance Laboratories Inc.
Madison, WI

FINAL REPORT

Study Title:

Dermal Sensitization Study of Alpha-Hexylcinnamaldehyde in Guinea Pigs - Closed Patch Technique

Data Requirement:

OPPTS 870.2600

Author:

Steven M. Glaza

Study Completion Date:

February 16, 1999

Performing Laboratory:

Covance Laboratories Inc.
3301 Kinsman Boulevard
Madison, WI 53704

Laboratory Project Identification:

Covance 81103945

Page 1 of 20

STATEMENT OF NO DATA CONFIDENTIALITY CLAIMS

Dermal Sensitization Study of Alpha-Hexylcinnamaldehyde
in Guinea Pigs - Closed Patch Technique

No claim of confidentiality is made for any information contained in this study on the basis of its falling within the scope of the United States Environmental Protection Agency Federal Insecticide, Fungicide, and Rodenticide Act §10 (d) (1) (A), (B), or (C).

Covance Laboratories Inc.
Company

Steven M. Glaza
Company Agent

2-16-99
Date

Study Director
Title

Steven M. Glaza
Signature

COMPLIANCE STATEMENT

Dermal Sensitization Study of Alpha-Hexylcinnamaldehyde
in Guinea Pigs - Closed Patch Technique

This study was conducted in accordance with the following Good Laboratory Practice Regulations/Standards with the exception that analysis of the test material mixtures for concentration, homogeneity/solubility, and stability was not conducted:

United States Food and Drug Administration Good Laboratory Practice Regulations, 21 CFR 58

United States Environmental Protection Agency FIFRA: Good Laboratory Practice Standards, 40 CFR 160

United States Environmental Protection Agency TSCA: Good Laboratory Practice Standards, 40 CFR 792

Organisation for Economic Cooperation and Development Principles of Good Laboratory Practice, C(97)186(Final)

Japanese Ministry of Agriculture, Forestry and Fisheries, Notification No. 59 NohSan 3850

Japanese Ministry of Health and Welfare, Ordinance 21

Management
Covance Laboratories Inc.

2-16-99
Date

Steven M. Glaza
Study Director
Acute Studies
Covance Laboratories Inc.

2-16-99
Date

QUALITY ASSURANCE STATEMENT

This report has been reviewed by the Quality Assurance Unit of Covance Laboratories Inc., in accordance with the Food and Drug Administration (FDA) Good Laboratory Practice Regulations, 21 CFR 58, the Organisation for Economic Cooperation and Development (OECD) Principles of Good Laboratory Practice, C(97)186(Final), the Environmental Protection Agency (EPA) Good Laboratory Practice Standards, 40 CFR 160, the Environmental Protection Agency (EPA) Good Laboratory Practice Standards, 40 CFR 792, the Japanese Ministry of Agriculture, Forestry, and Fisheries (MAFF) Good Laboratory Practice Standards, 59 NohSan No. 3850, and the Japanese Ministry of Health and Welfare (MOHW), Good Laboratory Practice Standards, Ordinance 21. The following inspections were conducted and findings reported to the study director and study director management. Written status reports of inspections and findings are issued to Covance management according to standard operating procedures.

Inspection Dates			Date Reported to Study Director and
From	To	Phase	Study Director Management
11/21/98	11/21/98	Protocol Review	11/23/98
12/16/98	12/16/98	Animal Observation	12/16/98
02/04/99	02/04/99	Data Review	02/04/99
02/04/99	02/04/99	Report Review	02/04/99

Susan K. Swatch
Representative, Quality Assurance Unit

2-16-99
Date

STUDY IDENTIFICATION

Dermal Sensitization Study of Alpha-Hexylcinnamaldehyde
in Guinea Pigs - Closed Patch Technique

Test Material	Alpha-Hexylcinnamaldehyde
Sponsor	Covance Laboratories Inc. 3301 Kinsman Boulevard Madison, WI 53704
Study Director	Steven M. Glaza Covance Laboratories Inc. P.O. Box 7545 Madison, WI 53707-7545 Telephone No.: 608.241.7292 Facsimile No.: 608.242.7936
Test Facility	Covance Laboratories Inc. 3301 Kinsman Boulevard Madison, WI 53704
Study Timetable	
Study Initiation Date	November 25, 1998
Experimental (In-life) Start Date	December 1, 1998
In-life End Date	January 4, 1999
Experimental Termination Date	January 4, 1999
Study Completion Date	February 16, 1999

KEY PERSONNEL

Acute Studies

Steven M. Glaza
Study Director

Steven R. Sorenson
Study Coordinator

Rose M. Bridge
Administrative Supervisor

Toxicology Operations

Jeffrey B. Hicks
In-life Supervisor

Quality Assurance

Nancy M. Centanni
Manager

Laboratory Animal Medicine

Donna J. Clemons, DVM
Diplomate, ACLAM
Supervisor

CONTENTS

STATEMENT OF NO DATA CONFIDENTIALITY CLAIMS 2

COMPLIANCE STATEMENT 3

QUALITY ASSURANCE STATEMENT 4

STUDY IDENTIFICATION 5

KEY PERSONNEL 6

SUMMARY 9

OBJECTIVE 9

TEST MATERIAL 10
- Identification 10
- Purity and Stability 10
- Storage and Retention 10

TEST SYSTEM 10
- Test Animal 10
- Housing 10
- Animal Diet 11
- Group Assignments 11
- Justification for Species Selection 11

PROCEDURES 11
- Induction Phase 11
- Challenge Phase 12
- Reason for Route of Administration 12
- Observations 12
- Termination 12

Evaluation of Challenge Responses....13
Statistical Evaluation....13
Location of Raw Data, Records, and Final Report....13

RESULTS/DISCUSSION....13
Clinical Observations and Body Weights....13
Dermal Reactions to HCA at Challenge....13

CONCLUSION....14

SIGNATURE....14

REFERENCES....15

TABLE
Buehler Sensitization Scoring Scale....16
1 Individual Body Weights (g)....17
2 Individual Clinical Signs....18
3 Individual Dermal Reactions at Challenge....19

PROTOCOL DEVIATION....20

SUMMARY

The delayed contact hypersensitivity potential of Alpha-Hexylcinnamaldehyde (HCA) was evaluated in albino guinea pigs. The test material was administered as a 2.5% w/v mixture in ethanol to each animal in the test group during the three-application induction phase of the study. During the challenge phase of the study, HCA was administered to each animal in the test and naive/irritation control groups at concentrations of 1.0, 2.5, and 5.0% w/v in acetone (three sites per animal).

Very faint erythema reactions (scores of 0.5) were observed in nine of the 10 test animals while no erythema reactions were seen in any of the five naive/irritation control animals when HCA was administered as a 1.0% w/v mixture in acetone at challenge. Very faint to faint erythema reactions (scores of 0.5 to 1.0) were observed in nine of the 10 test animals while no erythema reactions were seen in any of the five naive/irritation control animals when HCA was administered as a 2.5% w/v mixture in acetone at challenge. Very faint to moderate erythema reactions (scores of 0.5 to 2.0) were observed in all 10 test animals while no erythema reactions were seen in any of the five naive/irritation control animals when HCA was administered as a 5.0% w/v mixture in acetone at challenge. In the test group, nine reactions to the 1.0% w/v mixture, nine reactions to the 2.5% w/v mixture, and all ten reactions to the 5.0% w/v mixture exceeded the highest reaction in the naive/irritation control group to the respective HCA mixture. Based on these results, HCA was shown to be a dermal sensitizer in guinea pigs.

OBJECTIVE

The objective of this study was to assess the delayed contact hypersensitivity potential of HCA in guinea pigs.[1,2,3,4]

All procedures used in this study were in compliance with the Animal Welfare Act, the Guide for the Care and Use of Laboratory Animals, and the Office of Protection from Research Risks. In the opinion of the Sponsor and study director, the study did not unnecessarily duplicate any previous work. The dose levels, method, frequency, and duration of administration utilized in this study were chosen based on the requirements of

the regulatory test guidelines. All procedural times presented in this report fall within the acceptable ranges as specified in Covance standard operating procedure (SOP).

TEST MATERIAL

Identification

The test material was identified as alpha-hexylcinnamaldehyde [HCA (Aldrich Chemical Company, Lot No. 10021HF)] and described as an amber liquid.

Purity and Stability

The purity and stability of this reagent grade material were the responsibility of Covance and were deemed adequate for the conduct of this study. The certificate of analysis will be retained in the study file.

Storage and Retention

The test material was stored at room temperature. A reserve sample of the test material was taken and will be retained for 10 years in a freezer set to maintain a temperature of -10 to -30°C in accordance with Covance SOP. Any unused test material will be retained for future tests until the indicated expiration date is reached.

TEST SYSTEM

Test Animal

Young adult albino guinea pigs of the Crl:(HA)BR strain were procured from Charles River Laboratories, Inc., Kingston, New York, on November 17, 1998.

Housing

After receipt, the animals were acclimated for a period of at least 7 days. During acclimation and throughout the study, the animals were individually housed in suspended, stainless steel cages. Environmental controls for the animal room were set to maintain a temperature of 18 to 26°C, a relative humidity of 30 to 70%, and a 12-hour light/12-hour

dark cycle. In cases where variations from these conditions existed, they were documented and considered to have had no adverse effect on the study outcome.

Animal Diet

The animals were provided continuous access to Certified Guinea Pig Diet #5026 (PMI Nutrition International) and water. The diet is routinely analyzed by the manufacturer for nutritional components and environmental contaminants. Samples of the water are periodically analyzed. There were no known contaminants in the diet or water at levels that would be expected to interfere with or affect the results of the study.

Group Assignments

Fifteen healthy, acclimated male albino guinea pigs, weighing from 401 to 531 g and approximately 6 to 8 weeks of age, were divided into two groups consisting of a test group of 10 animals and a naive control group of five animals. The animals were identified by animal number and corresponding ear tag throughout the study.

Justification for Species Selection

Historically, the albino guinea pig has been the animal of choice for skin sensitization studies.

PROCEDURES

Induction Phase

On each day of test material application, the hair was removed from the back of each animal in the test group with electric clippers. The test material was applied to each animal in the test group by placing 0.3 mL of a 2.5% w/v mixture of HCA in ethanol on an adhesive patch (Hill Top Chamber®, 25-mm diameter) and placing the patch on the induction site along the dorsal anterior left quadrant. The patch was covered with dental dam and overwrapped with Elastoplast® tape. The dressing remained in place for a period of 6 hours after which it was removed. Any residual material was then removed from the application sites using water and disposable paper towels. The animals in the test group received one application per week for 3 weeks for a total of three applications.

The naive/irritation control animals were not treated during this phase of the study. All test material mixtures used in the study were stored at room temperature until administered.

Challenge Phase

Two weeks following the administration of the third induction dose, a challenge dose of HCA at concentrations of 1.0, 2.5, and 5.0% w/v in acetone was administered on three respective challenge sites (0.3 mL volume/site) along the right side of the test group animals in the same manner as during the induction phase of the study. At this time the five naive/irritation control animals (previously untreated) were also treated in the same manner with a challenge application of the same three test material concentrations.

Reason for Route of Administration

Historically, the dermal route has been the route of choice for determining delayed contact hypersensitization.

Observations

On the day of the 24-hour examination following the challenge application, the application sites of the respective animals were depilated by applying Neet® depilatory. After approximately 10 to 20 minutes, the depilatory was washed from the application sites. The 24-hour examination occurred at least 2 hours after removal of the depilatory.

The respective application sites were examined and scored for dermal reactions according to the Buehler[5] scoring scale at 24 and 48 hours following the challenge application.

Clinical observations were conducted daily throughout the study. Body weights on the study animals were determined before the initial test material administration and at termination of the in-life phase.

Termination

At termination of the in-life phase, all animals were euthanized and discarded.

Evaluation of Challenge Responses

Determination of sensitization was based on the dermal reactions to the challenge dose. Grades of 1.0 or greater in the test animals indicate evidence of sensitization, provided grades of less than 1.0 are seen in the naive/irritation control animals.

Statistical Evaluation

No statistical evaluations were required by the protocol.

Location of Raw Data, Records, and Final Report

The raw data, records, and an original signed copy of the final report will be retained in the archives of Covance for 10 years in accordance with Covance SOP.

RESULTS/DISCUSSION

Clinical Observations and Body Weights

Individual body weights are in Table 1 and individual clinical signs are in Table 2. All animals in both groups appeared normal and exhibited body weight gain throughout the study.

Dermal Reactions to HCA at Challenge

Individual dermal reactions at challenge for the test and naive/irritation control animals are presented in Table 3.

1.0% w/v Mixture of HCA in Acetone

Animals in the test group exhibited very faint erythema reactions (scores of 0.5) at nine of the 10 challenge sites treated with the 1.0% w/v mixture of HCA in acetone at challenge. A pinpoint area of subcutaneous hemorrhaging was also observed at one of these challenge sites. None of the five animals in the naive/irritation control group exhibited any erythema reaction at the sites treated with the 1.0% w/v mixture. Nine of the reactions in the test group exceeded the highest irritation control reaction during the challenge phase of the study.

2.5% w/v Mixture of HCA in Acetone

Animals in the test group exhibited very faint (score of 0.5 in six animals) to faint (score of 1.0 in three animals) erythema reactions at the challenge sites treated with the 2.5% w/v mixture of HCA in acetone. None of the animals in the naive/irritation control group exhibited any erythema reaction at the sites treated with the same 2.5% w/v mixture. Nine of the challenge reactions in the test group exceeded the highest irritation control reaction during the challenge phase of the study.

5.0% w/v Mixture of HCA in Acetone

Animals in the test group exhibited very faint to moderate erythema reactions (score of 0.5 in one animal, 1.0 in six animals, and 2.0 in three animals) at the challenge sites treated with the 5.0% w/v mixture of HCA in acetone. A pinpoint area of subcutaneous hemorrhaging was also observed at one of these challenge sites. None of the animals in the naive/irritation control group exhibited any erythema reaction at any of the five sites treated with the same 5.0% w/v mixture. All of the reactions in the test group exceeded the highest irritation control reaction during the challenge phase of the study.

CONCLUSION

Based on the results obtained, this test material, HCA was shown to be a dermal sensitizer in guinea pigs when tested by the closed patch technique.

SIGNATURE

Steven M. Glaza
Study Director
Acute Studies

2-16-99
Date

REFERENCES

1. United States Environmental Protection Agency; Prevention, Pesticides and Toxic Substances; "OPPTS 870.2600 Skin Sensitization"; *Health Effects Test Guidelines* (August 1998).

2. "Dermal Sensitization Study," *Guidance on Toxicology Study Data for Application of Agricultural Chemical Registration*, Ministry of Agriculture Forestry and Fisheries, 59 NohSan No. 4200, January 28, 1985.

3. "Skin Sensitization Studies," *1990 Guidelines for Toxicity Studies of Drugs Manual*, First Edition, Ch. 7, pp. 75-80, YAKUJI NIPPO, LTD. (1991).

4. "Skin Sensitisation," *Organisation for Economic Cooperation and Development Guidelines for Testing of Chemicals*, Section 4. Health Effects, Number 406, Paris Cedex (July 17, 1992).

5. Buehler, E. V. and Ritz, H. L., "Planning, Conduct, and Interpretation of Guinea Pig Sensitization Patch Tests," *Current Concepts in Cutaneous Toxicity*, p. 28 (1980).

Buehler Sensitization Scoring Scale

No reaction	0.0
Very faint erythema, usually nonconfluent	0.5
Faint erythema, usually confluent	1.0
Moderate erythema	2.0
Strong erythema, with or without edema	3.0

Table 1

Individual Body Weights (g)

Animal Number	Predose	Terminal
	Test Group	
E16807	525	748
E16808	502	804
E16727	434	743
E16810	479	749
E16811	520	810
E16728	404	668
E16813	522	774
E16814	531	799
E16729	401	643
E16816	475	711
	Irritation Control Group	
E16809	468	722
E16812	521	806
E16815	465	724
E16730	436	709
E16731	424	676

Table 2

Individual Clinical Signs

Animal Number	Observation	Day 1-19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35
Test Group																		
E16807	Normal appearance	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
E16808	Normal appearance	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
E16727	Normal appearance	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
E16810	Normal appearance	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
E16811	Normal appearance	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
E16728	Normal appearance	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
E16813	Normal appearance	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
E16814	Normal appearance	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
E16729	Normal appearance	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
E16816	Normal appearance	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Irritation Control Group																		
E16809	Normal appearance	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
E16812	Normal appearance	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
E16815	Normal appearance	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
E16730	Normal appearance	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
E16731	Normal appearance	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓

✓ Condition existed.

Table 3

Individual Dermal Reactions at Challenge

Animal Number	Test Material Concentration (% w/v in Acetone)					
	1.0%		2.5%		5.0%	
	Hour		Hour		Hour	
	24	48	24	48	24	48
Test Group						
E16807	0.5	0.0	0.5	0.5	0.5	1.0
E16808	0.5	0.5	0.5	0.5	1.0	1.0
E16727	0.5	0.5	1.0	1.0	2.0	2.0
E16810	0.0	0.0	0.0	0.0	0.5	0.5
E16811	0.5	0.0	1.0	0.5	2.0	1.0
E16728	0.0	0.5	0.5	0.5	1.0	1.0
E16813	0.5	0.5	0.5	0.5	1.0	1.0
E16814	0.5[p]	0.5	1.0	1.0	2.0	2.0
E16729	0.5	0.0	0.5	0.5	0.5	1.0
E16816	0.5	0.0	0.5	0.5	1.0[p]	1.0
Irritation Control Group						
E16809	0.0	0.0	0.0	0.0	0.0	0.0
E16812	0.0	0.0	0.0	0.0	0.0	0.0
E16815	0.0	0.0	0.0	0.0	0.0	0.0
E16730	0.0	0.0	0.0	0.0	0.0	0.0
E16731	0.0	0.0	0.0	0.0	0.0	0.0

p Pinpoint areas of subcutaneous hemorrhaging.

PROTOCOL DEVIATION

Protocol	Actual Procedure
Page 4, Experimental Design, Animals, Weight at Initiation of Treatment. 300 to 500 g	Six animals weighed between 502 and 531 g at initiation.

This deviation is not considered to have had an adverse effect on the outcome of the study.

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COVANCE
THE DEVELOPMENT SERVICES COMPANY

Sponsor:

[ ]

FINAL REPORT

Study Title:

Dermal Sensitization Study of [ ]
in Guinea Pigs - Closed Patch Technique
(EPA/OECD Guidelines)

Author:

Steven M. Glaza

Study Completion Date:

May 13, 1999

Performing Laboratory:

Covance Laboratories Inc.
3301 Kinsman Boulevard
Madison, WI 53704

Laboratory Project Identification:

Covance 80901389

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COMPLIANCE STATEMENT

Dermal Sensitization Study of [ ]
in Guinea Pigs - Closed Patch Technique
(EPA/OECD Guidelines)

This study was conducted in accordance with the following Good Laboratory Practice Standards with the exception that analysis of the test and control material mixtures for concentration, homogeneity/solubility, and stability was not conducted:

United States Environmental Protection Agency TSCA:
Good Laboratory Practice Standards, 40 CFR 792

Steven M. Glaza
Study Director
Acute Studies
Covance Laboratories Inc.

5-13-99
Date

Sponsor's Representative

Date

QUALITY ASSURANCE STATEMENT

This report has been reviewed by the Quality Assurance Unit of Covance Laboratories Inc., in accordance with the Environmental Protection Agency (EPA) Good Laboratory Practice Standards, 40 CFR 792. The following inspections were conducted and findings reported to the study director and study director management. Written status reports of inspections and findings are issued to Covance management according to standard operating procedures.

Inspection Dates			Date Reported to Study Director and
From	To	Phase	Study Director Management
09/27/98	09/27/98	Protocol Review	09/28/98
11/05/98	11/05/98	Animal Observation	11/05/98
12/03/98	12/03/98	Protocol Amendment Review	12/03/98
02/08/99	02/08/99	Data Review	02/08/99
02/08/99	02/08/99	Report Review	02/08/99

Randy Lester
Representative, Quality Assurance Unit

5·13·99
Date

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STUDY IDENTIFICATION

Dermal Sensitization Study of [ ]
in Guinea Pigs - Closed Patch Technique
(EPA/OECD Guidelines)

Test Material	[ ]
Sponsor	[ ]
Sponsor's Representative	[ ]
Study Monitor	**Robert W. Kapp, Jr., PhD** **Biotox** **300 Arboretum Place, Suite 140** **Richmond, VA 23236** **Telephone No.: 804.320.2800** **Facsimile No.: 804.320.8274**
Study Director	**Steven M. Glaza** **Covance Laboratories Inc.** **Telephone No.: 608.241.7292** **Facsimile No.: 608.242.7936**
Testing Facility	Covance Laboratories Inc. 3301 Kinsman Boulevard Madison, WI 53704
Study Timetable	
Study Initiation Date	September 28, 1998
Experimental (In-life) Start Date	October 5, 1998
In-life End Date	December 4, 1998
Experimental Termination Date	December 4, 1998
Study Completion Date	May 13, 1999

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CONTENTS

COMPLIANCE STATEMENT 2

QUALITY ASSURANCE STATEMENT 3

STUDY IDENTIFICATION 4

KEY PERSONNEL 5

SUMMARY 8

OBJECTIVE 8

TEST AND VEHICLE CONTROL MATERIALS 9
- Test Material Identification 9
- Test Material Purity and Stability 9
- Test Material Storage and Retention 9
- Vehicle Control Material Identification 9
- Vehicle Control Material Purity and Stability 9
- Vehicle Control Material Storage and Retention 10

TEST SYSTEM 10
- Test Animal 10
- Housing 10
- Animal Diet 10
- Group Assignments 11
- Justification for Species Selection 11

PROCEDURES 11
- Irritation Screening Study 11
- Definitive Study 12
- Reason for Route of Administration 13
- Observations 13
- Termination 13
- Evaluation of Challenge Responses 13
- Statistical Evaluation 14
- Location of Raw Data, Records, and Final Report 14

RESULTS/DISCUSSION 14
Irritation Screening Study 14
Definitive Study 14

CONCLUSION 15

SIGNATURE 16

REFERENCES 16

TABLE
Buehler Sensitization Scoring Scale 17
1 Individual Body Weights and Dermal Reactions - Irritation Screening Study 18
2 Individual Body Weights (g) - Definitive Study 19
3 Individual Clinical Signs - Definitive Study 21
4 Individual Dermal Reactions - Definitive Study 23

APPENDIX A 25
Protocol TP2008 26
Protocol Amendment No. 1 39

APPENDIX B 41
Positive Control Report 42

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SUMMARY

The delayed contact hypersensitivity potential of [ ] was evaluated in albino guinea pigs. The test material was administered as a 5% w/v mixture in mineral oil to each animal in the test group during the three-application induction phase of the study. The test material produced very faint to moderate erythema reactions (eight animals with scores of 0.5 and 12 animals with scores of 1.0 to 2.0) in all 20 test animals when administered as a 0.5% w/v mixture in mineral oil at the initial challenge phase. Pinpoint areas of subcutaneous hemorrhaging were also observed in two of these animals. All of the initial challenge reactions to [ ] (scores of 0.5 to 2.0) in the test group exceeded the highest reaction in the vehicle control group (scores of 0.0). During the second challenge phase, the test material identified as [ ] which was formed by combining [ ] and Dowfax 8390 (35%) within 4 hours of administration was applied to the animals in the test and vehicle control groups. Application of [ ] as a 25% w/v mixture in deionized water elicited very faint erythema reactions (scores of 0.5) in 15 of the 20 test animals and nine of the 10 vehicle control animals. None of the second challenge reactions to the application of [ ] are considered to be significant enough to be considered sensitization reactions.

Based on these results, [ ] is considered to be a dermal sensitizer in guinea pigs. Application of [ ] to animals previously exposed to [ ] did not elicit any reactions indicative of sensitization.

OBJECTIVE

The objective of this study was to assess the delayed contact hypersensitivity potential of a test material in guinea pigs.[1,2]

All procedures used in this study were in compliance with the Animal Welfare Act, the Guide for the Care and Use of Laboratory Animals, and the Office of Protection from Research Risks. In the opinion of the Sponsor and study director, the study did not unnecessarily duplicate any previous work. All procedural times presented in this report fall within the acceptable ranges as specified in Covance standard operating procedure (SOP).

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TEST AND VEHICLE CONTROL MATERIALS

Test Material Identification

The test material was identified as [ ] and was described as a white crystalline solid. An additional material, identified as [ ] was described as a white liquid and was used during the second challenge phase of this study. [ ] was formed by combining [ ] and Dowfax 8390 (35%) according to the Sponsor's instructions within 4 hour of administration to the animals.

Test Material Purity and Stability

The Sponsor assumes responsibility for purity and stability determinations (including under test conditions).

Test Material Storage and Retention

Test material [ ] was stored at room temperature. A reserve sample of this test material was taken and will be retained at room temperature in accordance with Covance SOP. The prepared test material identified as [ ] and its component, Dowfax 8390 (35%), were stored at room temperature, while the other test material component [ ] was stored refrigerated. Any unused portion of this prepared test material was discarded after dose administration. Any unused portion of each test material supplied by the Sponsor will be returned to the Sponsor after issuance of the final report according to Covance SOP.

Vehicle Control Material Identification

The vehicle control material used in this study was mineral oil.

Vehicle Control Material Purity and Stability

The purity and stability of the mineral oil are the responsibility of Covance and were deemed adequate for the conduct of this study.

Vehicle Control Material Storage and Retention

The mineral oil was stored at room temperature. A reserve sample of the vehicle control material was taken and will be retained for 10 years in a freezer set to maintain a temperature of -10 to -30°C in accordance with Covance SOP. Any unused vehicle control material will be retained by Covance for future tests until the indicated expiration date is reached.

TEST SYSTEM

Test Animal

Young adult albino guinea pigs of the Crl:(HA)BR strain were procured from Charles River Laboratories, Inc., Kingston, New York, on September 15 and October 6, 1998, for the irritation screening study and on October 13, 1998, for the definitive study.

Housing

After receipt, the animals were acclimated for a period of at least 7 days. During acclimation and throughout the study, the animals were individually housed in suspended, stainless steel cages. Environmental controls for the animal room were set to maintain a temperature of 18° to 26°C, a relative humidity of 30 to 70%, and a 12-hour light/12-hour dark cycle. In cases where variations from these conditions existed, they were documented and considered to have had no adverse effect on the study outcome.

Animal Diet

The animals were provided continuous access to Certified Guinea Pig Diet #5026, PMI Nutrition International, and water. The diet is routinely analyzed by the manufacturer for nutritional components and environmental contaminants. Samples of the water are periodically analyzed. There were no known contaminants in the diet or water at levels that would be expected to interfere with or affect the results of the study.

Group Assignments

Thirty-eight healthy, acclimated male albino guinea pigs, weighing from 377 to 508 g and approximately 5 to 7 weeks of age, were identified by animal number and corresponding ear tag and divided into the following treatment groups:

Treatment Group	Number of Animals
Irritation screening study	4
Additional screening study	4
Test	20
Vehicle control (common to Covance Study No. 80901386)	10

Justification for Species Selection

Historically, the albino guinea pig has been the animal of choice for skin sensitization studies.

PROCEDURES

Irritation Screening Study

Initially, an irritation screening study using four animals was conducted to determine the irritation threshold of the test material. The test material was administered at concentrations of 0.1, 1, 10, and 20% w/v in mineral oil with each animal receiving all four concentrations of the test material. The appropriate test material concentrations, in the amount of 0.4 mL, were applied to adhesive patches (Hill Top Chamber®, 25-mm diameter). The patches were then placed on four shaved sites (two on the right side and two on the left side) on each animal, covered with an overlapping strip of dental dam, and overwrapped with Elastoplast® tape. The patches remained in place for 6 hours after which they were removed. Any residual material was then removed from the application sites using water and disposable paper towels. The application sites were observed for dermal reactions at 24 and 48 hours after test material application.

Due to the level of irritation observed in the initial irritation screening study, a second irritation screening study was conducted. Four additional animals were treated with the

test material at concentrations of 0.5, 1, 5, and 10% w/v in mineral oil in the same manner as the first irritation screening study.

Definitive Study

Based on the results of the irritation screening studies, the test material was administered as a 5% w/v mixture in mineral oil for each application in the induction phase and as a 0.5% w/v mixture in mineral oil for the initial challenge application. All test material mixtures used in the irritation screening or definitive phases of the study were stored at room temperature until administered.

Induction Phase. On each day of test material application, the hair was removed from the back of each animal with electric clippers. The test material mixture was applied to each animal in the test group by placing 0.4 mL on an adhesive patch (Hill Top Chamber®, 25-mm diameter) and placing the patch on the induction site along the dorsal anterior left quadrant. The patch was covered with dental dam and overwrapped with Elastoplast® tape. The dressing remained in place for a period of 6 hours after which it was removed. Any residual material was then removed from the application site using water and disposable paper towels. The vehicle control animals were treated with a 0.4-mL volume of mineral oil in the same manner. The animals in the test and vehicle control groups received one application per week for 3 weeks for a total of three applications, with the third induction application for the 20 animals in the test group being applied slightly posterior to the initial induction site on these animals.

Initial Challenge Phase. Two weeks following the administration of the third induction dose, a challenge dose at a volume of 0.4 mL of a 0.5% w/v mixture of the test material in mineral oil was administered along the dorsal anterior right quadrant of the animals in the test group in the same manner as during the induction phase of the study. In addition, a naive area on the left side of each animal in the test group was treated with a 0.4-mL dose of mineral oil in the same manner as the test material. At this time, the 10 vehicle control animals were also treated with a challenge application of the test and vehicle control material in the same manner as the animals in the test group.

Second Challenge Phase. A second challenge was conducted 12 days after the initial challenge application. A new naive site was selected on each test and vehicle control animal. Each animal in the test and vehicle control groups received a 0.4-mL dose of the

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test material identified as [ ] as a 25% w/v mixture in deionized water. The second challenge dose was conducted in the same manner as the initial challenge dose with the exception that application of mineral oil alone was not required.

Reason for Route of Administration

Historically, the dermal route has been the route of choice for determining delayed contact hypersensitization.

Observations

On the day of the 24-hour examination following the irritation screening and challenge applications, the application sites of the respective animals were depilated by applying Neet® depilatory. After approximately 10 to 20 minutes, the depilatory was washed from the application sites.

The respective application sites were examined and scored for dermal reactions according to the Buehler[3] scoring scale at 24 and 48 hours following the irritation screening, induction, and challenge applications.

Clinical observations were conducted daily throughout the study. Body weights on the irritation screening animals were determined only on the day of treatment. Body weights on the definitive study animals were determined before the initial test material administration and at termination of the in-life phase.

Termination

At termination of the respective in-life phase for each group, all animals were euthanized and discarded.

Evaluation of Challenge Responses

Determination of sensitization was based on the dermal reactions to the challenge dose. Grades of 1.0 or greater in the test animals may indicate evidence of sensitization, provided grades of less than 1.0 are seen in the vehicle control animals.

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Statistical Evaluation

No statistical evaluations were required by the protocol.

Location of Raw Data, Records, and Final Report

The raw data, records, and an original signed copy of the final report will be retained in the archives of Covance in accordance with Covance SOP.

RESULTS/DISCUSSION

Irritation Screening Study

Individual body weights and dermal reactions are in Table 1. Moderate erythema reactions (with subcutaneous hemorrhaging at two of the four sites) were observed at the sites treated with the 20% w/v mixture of test material in mineral oil. Faint to strong erythema reactions (with subcutaneous hemorrhaging at three of the eight sites) were seen at the sites treated with the 10% w/v mixture of test material in mineral oil. Faint erythema reactions were seen at the sites treated with the 5% w/v mixture of test material in mineral oil. Faint to moderate erythema reactions were seen at three of the eight sites treated with the 1% w/v mixture of test material in mineral oil. Subcutaneous hemorrhaging was also observed at one of the sites treated with this concentration. No dermal irritation was observed at the sites treated with the 0.5 and 0.1% w/v mixtures. All animals appeared normal during this phase of the study.

Definitive Study

Clinical Observations and Body Weights. Individual body weights are in Table 2 and individual clinical signs are in Table 3. All animals in both groups appeared normal and exhibited body weight gain throughout the study.

Dermal Reactions to [redacted] Individual dermal reactions for the test and vehicle control animals are presented in Table 4. Moderate to strong erythema reactions were elicited from all 20 test animals to the test material administered as a 5% w/v mixture in mineral oil during the induction phase of the study. Subcutaneous hemorrhaging, blanching, and/or necrotic appearing areas were also observed within the application sites of 19 of the 20 test animals during the induction phase. Very faint to

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moderate erythema reactions (eight animals with scores of 0.5 and 12 animals with scores of 1.0 to 2.0) were elicited from all 20 animals in the test group following the initial challenge application of the test material as a 0.5% w/v mixture in mineral oil. Pinpoint areas of subcutaneous hemorrhaging were also observed in two of these animals. None of the vehicle control animals reacted to the initial challenge application of the test material as a 0.5% w/v mixture in mineral oil. None of the test or vehicle control animals reacted to the vehicle control material. All reactions to [ ] in the test group (scores of 0.5 to 2.0) exceeded the highest reaction in the vehicle control group (score of 0.0) during the initial challenge phase of the study. Very faint erythema reactions (scores of 0.5) were observed in 15 of the 20 animals in the test group and nine of the 10 animals in the vehicle control group following the application of [ ] as a 25% w/v mixture in deionized water during the second challenge phase. None of the reactions in the test group to the challenge application of [ ] are significant enough to be considered sensitization reactions.

Positive Control Report. **A study report detailing the results of sensitization testing of α-hexylcinnamaldehyde (a known skin sensitizer) using the same sensitization method is presented in Appendix B of this report. This positive control study was conducted within 6 months of the conduct of this study.**

CONCLUSION

Based on the results obtained, this test material, [ ] is considered to be a dermal sensitizer in guinea pigs when tested by the closed patch technique. Application of CPS & T 98-006 in animals previously exposed to [ ] did not result in any reactions indicative of sensitization.

SIGNATURE

Steven M. Glaza
Study Director
Acute Studies

5-13-99
Date

REFERENCES

1. United States Environmental Protection Agency; Prevention, Pesticides and Toxic Substances; "OPPTS 870.2600 Skin Sensitization"; *Health Effects Test Guidelines* (August 1998).

2. "Skin Sensitisation," *Organisation for Economic Cooperation and Development Guidelines for Testing of Chemicals*, Section 4, Health Effects, Number 406, Paris Cedex (July 17, 1992).

3. Buehler, E. V. and Ritz, H. L., "Planning, Conduct, and Interpretation of Guinea Pig Sensitization Patch Tests," *Current Concepts in Cutaneous Toxicity*, p. 28 (1980).

Buehler Sensitization Scoring Scale

No reaction	0.0
Very faint erythema, usually nonconfluent	0.5
Faint erythema, usually confluent	1.0
Moderate erythema	2.0
Strong erythema, with or without edema	3.0

Table 1

Individual Body Weights and Dermal Reactions - Irritation Screening Study

Initial Irritation Screening

Animal Number	Initial Body Weight (g)	Dermal Reactions: Test Material Concentration (% w/v in Mineral Oil) 0.1% Hour 24	0.1% Hour 48	1% Hour 24	1% Hour 48	10% Hour 24	10% Hour 48	20% Hour 24	20% Hour 48
E16231	508	0.0	0.0	0.0	0.0	2.0[a]	2.0[a]	2.0	1.0
E16232	480	0.0	0.0	1.0	1.0	2.0	2.0	2.0	2.0[a]
E16233	466	0.0	0.0	0.0	0.0	2.0	2.0	2.0	2.0
E16234	458	0.0	0.0	0.0	0.0	1.0	1.0	2.0	2.0[a]

a Subcutaneous hemorrhaging.

Additional Irritation Screening

Animal Number	Initial Body Weight (g)	Dermal Reactions: Test Material Concentration (% w/v in Mineral Oil) 0.5% Hour 24	0.5% Hour 48	1% Hour 24	1% Hour 48	5% Hour 24	5% Hour 48	10% Hour 24	10% Hour 48
E16481	397	0.0	0.0	1.0	0.5	1.0	1.0	1.0	2.0[a]
E16482	377	0.0	0.0	2.0[a]	2.0[a]	1.0	1.0	2.0	2.0
E16483	440	0.0	0.0	0.0	0.0	1.0	1.0	1.0	1.0
E16426	412	0.0	0.0	0.0	0.0	1.0	1.0	3.0[a]	3.0[a]

a Subcutaneous hemorrhaging.

Comment: All animals appeared normal throughout the irritation screening study.

Table 2

Individual Body Weights (g) - Definitive Study

Animal Number	Predose	Terminal
	Test Group	
E16521	397	687
E16522	452	818
E16523	439	770
E16524	425	743
E16541	423	838
E16526	425	791
E16527	407	725
E16528	415	630
E16529	449	800
E16530	478	827
E16531	415	733
E16532	440	691
E16533	422	711
E16534	406	716
E16535	393	711
E16536	404	745
E16537	430	756
E16538	420	728
E16539	429	725
E16540	420	815

Table 2 (Continued)

Individual Body Weights (g) - Definitive Study

Animal Number	Predose	Terminal
	Vehicle Control Group	
E16571	426	716
E16572	402	702
E16573	428	765
E16574	416	727
E16575	443	763
E16576	417	748
E16577	435	688
E16578	395	620
E16579	403	673
E16580	427	722

Table 3

Individual Clinical Signs - Definitive Study

Animal Number	Observation	Day																			
		1-25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44
		Test Group																			
E16521	Normal appearance	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
E16522	Normal appearance	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
E16523	Normal appearance	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
E16524	Normal appearance	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
E16541	Normal appearance	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
E16526	Normal appearance	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
E16527	Normal appearance	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
E16528	Normal appearance	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
E16529	Normal appearance	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
E16530	Normal appearance	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
E16531	Normal appearance	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
E16532	Normal appearance	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
E16533	Normal appearance	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
E16534	Normal appearance	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
E16535	Normal appearance	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
E16536	Normal appearance	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
E16537	Normal appearance	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
E16538	Normal appearance	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
E16539	Normal appearance	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
E16540	Normal appearance	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓

✓ Condition existed.

Table 3 (Continued)

Individual Clinical Signs - Definitive Study

Animal Number	Observation	Day																			
		1-25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44
Vehicle Control Group																					
E16571	Normal appearance	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
E16572	Normal appearance	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
E16573	Normal appearance	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
E16574	Normal appearance	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
E16575	Normal appearance	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
E16576	Normal appearance	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
E16577	Normal appearance	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
E16578	Normal appearance	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
E16579	Normal appearance	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
E16580	Normal appearance	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓

✓ Condition existed.

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Table 4

Individual Dermal Reactions - Definitive Study

Animal Number	Induction Phase† Dose #1 Hour 24	Dose #1 Hour 48	Dose #2 Hour 24	Dose #2 Hour 48	Dose #3 Hour 24	Dose #3 Hour 48	Challenge Application Initial [redacted] Hour 24**	Initial [redacted] Hour 48**	Initial Mineral Oil Hour 24	Initial Mineral Oil Hour 48	Second [redacted] Hour 24	Second [redacted] Hour 48
Test Group												
E16521*	1.0	1.0	2.0[a]	3.0[n]	2.0[a]	3.0[n]	2.0	1.0	0.0	0.0	0.5	0.5
E16522*	2.0	2.0	2.0[a]	2.0[a]	2.0[a]	2.0[a]	2.0	2.0	0.0	0.0	0.5	0.0
E16523*	2.0	2.0	3.0[n]	3.0[n]	2.0	3.0	0.5	0.5	0.0	0.0	0.5	0.0
E16524*	0.5	0.5	2.0	2.0[a]	2.0	2.0[a]	0.5	0.0	0.0	0.0	0.5	0.0
E16541*	1.0	0.5	1.0	1.0	2.0[a]	3.0[n]	0.5	0.0	0.0	0.0	0.5	0.0
E16526*	1.0	0.5	1.0	1.0	1.0	2.0[a]	2.0	2.0	0.0	0.0	0.5	0.5
E16527*	1.0	1.0	2.0[a]	2.0[a]	2.0[a]	2.0[a]	1.0	0.5	0.0	0.0	0.5	0.5
E16528*	1.0	0.5	2.0[a]	2.0[a]	2.0[a]	3.0[n]	2.0[p]	2.0[p]	0.0	0.0	0.5	0.5
E16529*	1.0	1.0	2.0[a]	2.0[a]	2.0[a]	2.0[a]	0.5	0.0	0.0	0.0	0.5	0.0
E16530*	1.0	1.0	2.0[a]	2.0[a]	2.0	2.0	2.0[p]	2.0[p]	0.0	0.0	0.5	0.5
E16531*	1.0	1.0	2.0[a]	2.0[a]	2.0[a]	2.0[a]	1.0	0.5	0.0	0.0	0.5	0.5
E16532*	0.5	0.5	2.0[a]	3.0[n]	2.0[a]	2.0[a]	1.0	0.5	0.0	0.0	0.5	0.5
E16533*	1.0	1.0	2.0[a]	2.0[a]	1.0	2.0[a]	0.5	0.0	0.0	0.0	0.5	0.5
E16534*	1.0	1.0	3.0[a]	3.0[n]	1.0	2.0[a]	0.5	0.0	0.0	0.0	0.0	0.0
E16535*	1.0	1.0	2.0[a,b]	2.0[a,b]	2.0	2.0[a]	2.0	1.0	0.0	0.0	0.0	0.0
E16536*	1.0	1.0	2.0[a]	2.0[a]	3.0[n]	3.0[n]	1.0	0.5	0.0	0.0	0.5	0.5
E16537*	0.5	1.0	3.0[n]	3.0[n]	2.0	2.0[a]	1.0	1.0	0.0	0.0	0.5	0.5
E16538*	1.0	0.5	2.0[a]	2.0[a]	1.0	2.0	0.5	0.0	0.0	0.0	0.0	0.0
E16539*	1.0	1.0	3.0	3.0	1.0	2.0	2.0	2.0	0.0	0.0	0.0	0.0
E16540*	1.0	1.0	2.0[a,b]	2.0[a,b]	1.0	2.0	0.5	0.0	0.0	0.0	0.0	0.0

† Treated with a 5% w/v mixture of [redacted] in mineral oil.
†† Administered as a 0.5% w/v concentration in mineral oil.
‡ Administered as a 25% w/v concentration in deionized water.
* Induction site on this animal was moved slightly posterior to the initial induction site for Induction Dose #3.
** Scores were based on the limited irritation that was present at only 30 to 50% of the test site, with the exception of E16528 in which the score was based on the reaction present at 100% of the test site.
a Subcutaneous hemorrhaging.
b Blanching.
n Necrotic appearing areas.
p Pinpoint areas of subcutaneous hemorrhaging.

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Table 4 (Continued)

Individual Dermal Reactions - Definitive Study

Animal Number	Induction Phase†						Challenge Application					
							Initial				Second	
	Dose #1		Dose #2		Dose #3		[redacted]		Mineral Oil		[redacted]	
	Hour		Hour		Hour		Hour		Hour		Hour	
	24	48	24	48	24	48	24	48	24	48	24	48
Vehicle Control Group												
E16571	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.5	0.5
E16572	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.5	0.5
E16573	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.5	0.5
E16574	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.5	0.0
E16575	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.5	0.5
E16576	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.5	0.5
E16577	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.5	0.0
E16578	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.5	0.5
E16579	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.5	0.5
E16580	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

† Treated with mineral oil.
†† Administered as a 0.5% w/v concentration in mineral oil.
‡ Administered as a 25% w/v concentration in deionized water.

APPENDIX A

Protocol TP2008
Protocol Amendment No. 1

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COVANCE
THE DEVELOPMENT SERVICES COMPANY

Sponsor:

[ ]

PROTOCOL TP2008

Study Title:

**Dermal Sensitization Study of [ ] in Guinea Pigs-
Closed Patch Technique
(EPA/OECD Guidelines)**

Date:

September 28, 1998

Performing Laboratory:

Covance Laboratories Inc.
3301 Kinsman Boulevard
Madison, Wisconsin 53704

Laboratory Project Identification:

Covance 80901389

Study
Dermal Sensitization Study of in Guinea Pigs - Closed Patch Technique
(EPA/OECD Guidelines)

Purpose
To assess the delayed contact hypersensitivity potential of a test material in guinea pigs.

Sponsor

[redacted]

Sponsor's Representative

[redacted]

Study Monitor
Robert W. Kapp, Jr., PhD
Biotox
300 Arboretum Place, Suite 140
Richmond, VA 23236
Telephone: 804.320.2800
Facsimile: 804.320.8274

Study Director
Steven M. Glaza
Covance Laboratories Inc.
Telephone 608.241.7292
Facsimile 608.242.7936

Testing Facility
Covance Laboratories Inc.
3301 Kinsman Boulevard
Madison, WI 53704

Proposed Study Timetable

Experimental Start Date	Week of October 5 , 1998
Experimental Termination Date	Week of November 16, 1998
Draft Report Date	Week of December 21, 1998

Regulatory Compliance
This study will be conducted in accordance with the following Good Laboratory Practice Regulations/Standards, with the exception that analysis of the test material mixtures for concentration, homogeneity/solubility and stability will not be conducted:

[ ] Conduct as a Nonregulated Study
[ ] 21 CFR 58 (FDA)
[ ] 40 CFR 160 (EPA-FIFRA)
[X] 40 CFR 792 (EPA-TSCA)
[ ] C(97)186/Final (OECD)
[ ] 59 NohSan No. 3850 (Japanese MAFF)
[ ] Ordinance 21 (Japanese MOHW)

Animal Care and Use Statement
All procedures in this protocol are in compliance with the Animal Welfare Act, the Guide for the Care and Use of Laboratory Animals, and the Office of Protection from Research Risks. In the opinion of the Sponsor and Study Director, the study does not unnecessarily duplicate any previous work.

Quality Assurance
The protocol, study conduct, and the final report will be audited by the Covance Quality Assurance Unit.

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Test Material

Identification

[ ]

Physical Description
White, crystalline solid

Purity and Stability
The Sponsor assumes responsibility for purity and stability determinations (including under test conditions).

Storage Conditions
Room temperature

Reserve Samples
Reserve samples of each batch/lot of the test material will be taken and stored at Covance at room temperature. The reserve samples will be maintained as outlined in the Record Retention section.

Disposition
Any unused test material will be returned to the Sponsor after issuance of the final report, unless otherwise directed by the Sponsor.

Safety Precautions
As required by Covance Standard Operating Procedures (SOPs) and policies

Vehicle Control Material

Identification
Mineral oil

Physical Description
Clear, colorless liquid

Purity and Stability
The purity and stability of this vehicle control material are considered to be adequate for the conduct of this study.

Storage Conditions
Room temperature

Reserve Samples
Reserve samples of each batch/lot of the vehicle control materials will be taken and stored at Covance in a freezer set to maintain a temperature of -20°C ±10°. The reserve samples will be maintained at Covance for ten years then discarded.

Disposition
Any unused vehicle control material will be discarded after the indicated expiration date is reached.

Safety Precautions
As required by Covance Standard Operating Procedures (SOPs) and policies

Animals

Species
Guinea Pig

Strain and Strain
Crl:(HA)BR, Charles River Laboratories Inc.

Age at Initiation of Treatment
Young adult

Weight at Initiation of Treatment
350 to 550 g

Number and Sex
34 of any sex

Identification
Individual numbered ear tag

Husbandry

Housing
Individually, in suspended, screen-bottom stainless steel cages

Diet
Certified Guinea Pig Diet #5026 (PMI Nutrition International), *ad libitum*. The diet is routinely analyzed by the manufacturer for nutritional components and environmental contaminants.

Water
Ad libitum from an automatic system. Samples of the water are periodically analyzed for specified microorganisms and environmental contaminants.

Contaminants
There are no known contaminants in the diet or water at levels that would be expected to interfere with this study.

Environment
Environmental controls for the animal room will be set to maintain a temperature of 18° to 26°C, a relative humidity of 50% ±20%, and a 12-hour light/12-hour dark cycle.

Acclimation
At least 7 days

Selection of Test Animals
Based on health and body weight according to Covance SOPs. An adequate number of extra animals will be purchased so that no animal in obviously poor health is placed on test.

Justification for Species Selection

Historically, the albino guinea pig has been the animal of choice for skin sensitization studies.

Treatment Procedures

Experimental Design

Thirty-eight acclimated animals weighing between 350 and 550 g will be placed into the following groups:

Group	No. of Animals
Irritation Screening	4
Group 1 - Test	20
Group 2 - Vehicle Control	10

Irritation Screen

An irritation screening study of four guinea pigs will be conducted. The dosing procedure will be in the same manner as the definitive study. Four application sites will be selected on each animal. Each site will be treated with 0.4 mL of one of the following test material concentrations: 0.1%, 1.0%, 10% and 20% w/v in mineral oil. Certain concentrations may not be administered based on the ability to formulate and administer. The mild to moderately irritating concentration will be used in the induction phase of the definitive study. The highest nonirritating concentration will be used for the challenge application.

If appropriate concentrations are not found, additional testing using new animals will be employed.

All test mixtures used in the irritation screen or definitive phases of the study will be stored at room temperature until administered.

Dose Administration (Definitive Study)

Before each application the back of each animal will be clipped free of hair with an electric clipper. The test material will be applied to one area on each test animal by placing 0.4 mL of the test material on an adhesive patch (Hill Top Chamber®, 25-mm diameter) and placing the patch on the induction site along the dorsal anterior left quadrant. The patch will be covered with dental dam and secured with an overwrap of Elastoplast® tape. The dressing will remain in place for approximately 6 hours, and the residual material will be removed using water or an appropriate substance and disposable paper towels. The vehicle control animals will be treated with mineral oil in the same manner. The test and vehicle control animals will receive one application each week for 3 weeks for a total of three applications. Each application will be made on the same site during the induction phase unless moderate to severe irritation is observed following the first or second induction application. The induction site may then be moved slightly posterior to the initial induction site.

Two weeks after the administration of the third induction dose, a challenge dose, at a volume of 0.4 mL of the highest nonirritating concentration, will be administered to the test group along the dorsal anterior right quadrant and in the same manner as during the induction phase of the study. In a similar manner, a naïve area on the left side of each animal will be treated with a 0.4 mL dose of mineral oil. At this time, the 10 vehicle control animals will also be treated with a challenge application of the test material and mineral oil.

The study will terminate after the 48 hour challenge application scoring unless equivocal responses are observed. If equivocal responses are observed in the initial challenge application, the animals in the test group and a new group of 10 control animals may be given a second challenge as determined by the study director. The second challenge will occur 1 to 2 weeks after the initial challenge application.

Reason for Route of Administration

Historically, the dermal route has been the route of choice for determining delayed contact hypersensitization.

Observation of Animals

Clinical Observations
Daily clinical signs will be recorded.

Body Weights
Before test material administration (Irritation Screen and Definitive Study animals) and at the termination of the experimental phase (Definitive Study animals only).

Reading of Dermal Reactions
The respective application sites will be examined and scored for dermal irritation, according to the scoring scale in Attachment 1, at approximately 24 and 48 hours after the irritation screen, induction, and challenge applications.

On the day of the 24-hour examination, following the irritation screen and challenge applications, the respective test sites will be depilated by applying Neet® for approximately 10-20 minutes, then washed off with water. The 24-hour examination must take place at least 2 hours after removal of the depilatory.

Pathology
Any animals dying during the study, or sacrificed in a moribund condition, will be subjected to an abbreviated gross necropsy examination and all abnormalities will be recorded. After necropsy, the animals will be discarded and no tissues will be saved. At termination of the respective experimental phase, surviving animals will be sacrificed and discarded.

Statistical Evaluation
No statistical evaluations are required.

Report

A final report including the items listed below will be submitted.

Description of the test material
Description of the test system
Procedures
Dates of experimental initiation and termination
Summary table showing the induction and challenge responses
Any special observations that were recorded
Determination of sensitization based upon reactions to the challenge dose. Grades of 1 or greater in the test animals indicate evidence of sensitization, provided grades of less than one are seen in the vehicle control animals. If grades of one or greater are noted in the vehicle control animals, then the reactions of test animals exceeding the most severe vehicle control reactions are considered sensitization reactions.

A positive control report from periodic validation of the test method, which has been conducted within six months of the study, will be presented as an appendix in the final report.
Gross pathology findings (if applicable)

Record Retention

All raw data, documentation, records, reserve samples, protocol, protocol amendments (if applicable), and the final report generated as a result of this study will be archived in the storage facility of Covance for a period of one year following signing of the final report. One year after signing of the final report, all of the aforementioned materials may be sent to the Sponsor, and a return fee will be charged. The Sponsor may elect to have the materials retained in the Covance archives for an additional period of time, for which Covance will charge a storage fee. If the Sponsor chooses to have Covance dispose of the materials, a disposal fee will be charged. All raw data stored on magnetic media will be retained by Covance.

The following supporting records will be retained at Covance but will not be archived with the study data:

Animal receipt/acclimation records
Water analysis records
Animal room maintenance and environmental records
Refrigerator, freezer and room temperature records
Positive control data
Feed analysis records
Instrument calibration and maintenance records

PROTOCOL APPROVAL

[ ]

Oct 1, 1998
Date

Robert W. Kapp, Jr. PhD
Study Monitor
Biotox

9/30/98
Date

Steven M. Glaza
Study Director
Acute Studies
Covance Laboratories Inc.

9-28-98
Date

(TP2008.ST4)

Attachment 1

Buehler Sensitization Scoring Scale

No reaction	0
Very faint erythema, usually nonconfluent	0.5
Faint erythema, usually confluent	1.0
Moderate erythema	2.0
Strong erythema, with or without edema	3.0

Buehler, E.V. and Ritz, H. L., "Planning, Conduct, and Interpretation of Guinea Pig Sensitization Patch Tests, "Current Concepts in Cutaneous Toxicity, p. 28 (1980)

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COVANCE
THE DEVELOPMENT SERVICES COMPANY

PROTOCOL AMENDMENT NO. 1

Study Title:

Dermal Sensitization Study of [redacted] in Guinea Pigs - Closed Patch Technique (EPA/OECD Guidelines)

Covance 80901389

Sponsor

[redacted]

Sponsor's Representative

[redacted]

Testing Facility

Covance Laboratories Inc.
3301 Kinsman Boulevard
Madison, WI 53704

Study Monitor

Robert W. Kapp, Jr., PhD
Biotox

Study Director

Steven M. Glaza
Covance Laboratories

This amendment modifies the following portion of the protocol:

Effective October 14, 1998

1. **Page 7, Irritation Screen.** Based on the results of the initial irritation screen, a second irritation screening study is to be conducted. Add the following as the second paragraph in this section:

   A second irritation screen is to be conducted in the same manner as the initial irritation screen by treating four additional animals with the test material at concentrations of 0.5, 1, 5, and 10% w/v in mineral oil.

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Effective November 24, 1998

2. **Page 8, Dose Administration (Definitive Study).** Per discussion with the Sponsor, a rechallenge is to be conducted in this study. Add the following as the last paragraph in this section:

   A second challenge will be conducted in this study by treating the animals in the test group and the initial vehicle control animals with a 25% w/v mixture of the material identified as [ ] in deionized water. Naïve sites are to be used on each animal. The second challenge dose will be conducted in the same manner as the initial challenge dose with the exception that application of deionized water alone is not required.

AMENDMENT APPROVAL

[ ]

12/15/98
Date

Robert W. Kapp, Jr., PhD
Study Monitor
Biotox

12/14/98
Date

Steven M. Glaza
Study Director
Acute Studies
Covance Laboratories Inc.

12-4-98
Date

APPENDIX B

Positive Control Report

COVANCE.
THE DEVELOPMENT SERVICES COMPANY

Sponsor:

Covance Laboratories Inc.
Madison, Wisconsin

FINAL REPORT

Study Title:

Dermal Sensitization Study of Hexylcinnamaldehyde in Guinea Pigs - Closed Patch Technique

Data Requirement:

EPA Guideline 81-6

Author:

Steven M. Glaza

Study Completion Date:

September 1, 1998

Performing Laboratory:

Covance Laboratories Inc.
3301 Kinsman Boulevard
Madison, Wisconsin 53704

Laboratory Study Identification:

Covance 80504850

STATEMENT OF NO DATA CONFIDENTIALITY CLAIMS

Dermal Sensitization Study of Hexylcinnamaldehyde
in Guinea Pigs - Closed Patch Technique

No claim of confidentiality is made for any information contained in this study on the basis of its falling within the scope of the United States Environmental Protection Agency Federal Insecticide, Fungicide, and Rodenticide Act §10 (d) (1) (A), (B), or (C).

Covance Laboratories Inc.
Company

Steven M. Glaza
Company Agent

9-1-98
Date

Study Director
Title

Steven M. Glaza
Signature

COMPLIANCE STATEMENT

Dermal Sensitization Study of Hexylcinnamaldehyde
in Guinea Pigs - Closed Patch Technique

This study was conducted in accordance with the following Good Laboratory Practice Regulations/Standards with the exception that analysis of the test material mixtures for concentration, homogeneity/solubility, and stability was not conducted:

United States Food and Drug Administration Good Laboratory Practice Regulations, 21 CFR 58

United States Environmental Protection Agency FIFRA: Good Laboratory Practice Standards, 40 CFR 160

United States Environmental Protection Agency TSCA: Good Laboratory Practice Standards, 40 CFR 792

Organisation for Economic Cooperation and Development Principles of Good Laboratory Practice, C(97)186(Final)

Japanese Ministry of Agriculture, Forestry and Fisheries, Notification No. 59 NohSan 3850

Japanese Ministry of Health and Welfare, Ordinance 21

Management
Covance Laboratories Inc.

9/1/98
Date

Steven M. Glaza
Study Director
Acute Studies
Covance Laboratories Inc.

9-1-98
Date

QUALITY ASSURANCE STATEMENT

This report has been reviewed by the Quality Assurance Unit of Covance Laboratories Inc., in accordance with the Environmental Protection Agency (EPA) Good Laboratory Practice Standards, 40 CFR 160, the Organisation for Economic Cooperation and Development (OECD) Principles of Good Laboratory Practice, C(97)186(Final), the Environmental Protection Agency (EPA) Good Laboratory Practice Standards, 40 CFR 792, the Food and Drug Administration (FDA) Good Laboratory Practice Regulations, 21 CFR 58, the Japanese Ministry of Agriculture, Forestry, and Fisheries (MAFF) Good Laboratory Practice Standards, 59 NohSan No. 3850, and the Japanese Ministry of Health and Welfare (MOHW), Good Laboratory Practice Standards, Ordinance 21. The following inspections were conducted and findings reported to the study director and study director management. Written status reports of inspections and findings are issued to Covance management according to standard operating procedures.

Inspection Dates			Date Reported to Study Director and
From	To	Phase	Study Director Management
06/01/98	06/01/98	Protocol Review	06/01/98
06/16/98	06/16/98	Test Article Administration	06/16/98
08/23/98	08/24/98	Data Review	08/26/98
08/23/98	08/24/98	Report Review	08/26/98

Susan K. Swatch — Representative, Quality Assurance Unit

9-1-98 — Date

STUDY IDENTIFICATION

Dermal Sensitization Study of Hexylcinnamaldehyde
in Guinea Pigs - Closed Patch Technique

Test Material	Hexylcinnamaldehyde
Sponsor	Covance Laboratories Inc. 3301 Kinsman Boulevard Madison, WI 53704
Study Director	Steven M. Glaza Covance Laboratories Inc. Telephone No.: 608.241.7292 Facsimile No.: 608.242.7936
Test Facility	Covance Laboratories Inc. 3301 Kinsman Boulevard Madison, WI 53704
Study Timetable	
Study Initiation Date	June 1, 1998
Experimental (In-life) Start Date	June 2, 1998
In-life End Date	July 10, 1998
Experimental Termination Date	July 10, 1998
Study Completion Date	September 1, 1998

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CONTENTS

STATEMENT OF NO DATA CONFIDENTIALITY CLAIMS 2

COMPLIANCE STATEMENT 3

QUALITY ASSURANCE STATEMENT 4

STUDY IDENTIFICATION 5

KEY PERSONNEL 6

SUMMARY 9

OBJECTIVE 9

TEST MATERIAL 10
- Identification 10
- Purity and Stability 10
- Storage and Retention 10
- Safety Precautions 10

TEST SYSTEM 10
- Test Animal 10
- Housing 11
- Animal Diet 11
- Group Assignments 11
- Justification for Species Selection 12

PROCEDURES 12
- Irritation Screening Study 12
- Definitive Study 12
- Reason for Route of Administration 13
- Observations 13
- Termination 14

Evaluation of Challenge Responses....14
Statistical Evaluation....14
Location of Raw Data, Records, and Final Report....14

RESULTS/DISCUSSION....14
Irritation Screening Study....14
Definitive Study....15

CONCLUSION....16

SIGNATURE....16

REFERENCES....17

TABLE

Buehler Sensitization Scoring Scale....18
1 Individual Body Weights and Dermal Reactions - Irritation Screening Study....19
2 Individual Body Weights (g) - Definitive Study....20
3 Individual Clinical Signs - Definitive Study....21
4 Individual Dermal Reactions at Challenge - Definitive Study....22

SUMMARY

The delayed contact hypersensitivity potential of hexylcinnamaldehyde (HCA) was evaluated in albino guinea pigs. The test material was administered as a 2.5% w/v mixture in ethanol to each animal in the test group during the three-application induction phase of the study. During the challenge phase of the study, HCA was administered to each animal in the test and irritation control groups at three concentrations of 1.0%, 2.5%, and 5.0% w/v in acetone (three sites per animal).

Very faint to moderate erythema reactions (scores of 0.5 to 2.0) were observed in seven of the 10 test animals while only a very faint erythema reaction (score of 0.5) was seen in one of the five irritation control animals when HCA was administered as a 1.0% w/v mixture in acetone at challenge. Very faint to moderate erythema reactions (scores of 0.5 to 2.0) were observed in eight of the 10 test animals while very faint erythema reactions (scores of 0.5) were seen two of the five irritation control animals when HCA was administered as a 2.5% w/v mixture in acetone at challenge. Very faint to moderate erythema reactions (scores of 0.5 to 2.0) were observed in eight of the 10 test animals while very faint to faint erythema (scores of 0.5 to 1.0) reactions were seen in two of the five irritation control animals when HCA was administered as a 5.0% w/v mixture in acetone at challenge. In the test group, four reactions to the 1.0% w/v mixture, seven reactions to the 2.5% w/v mixture, and five reactions to the 5.0% w/v mixture exceeded the highest reaction in the irritation control group to the respective HCA mixture. Based on these results, HCA is considered to be a dermal sensitizer in guinea pigs.

OBJECTIVE

The objective of this study was to assess the delayed contact hypersensitivity potential of HCA in guinea pigs.[1,2,3,4,5]

All procedures used in this study were in compliance with the Animal Welfare Act and/or the Guide for the Care and Use of Laboratory Animals and the Office of Protection from Research Risks. In the opinion of the Sponsor and study director, the study did not unnecessarily duplicate any previous work. The dose levels, method, frequency, and duration of administration utilized in this study were chosen based on the requirements of

the regulatory test guidelines. All procedural times presented in this report fall within the acceptable ranges as specified in Covance standard operating procedure (SOP).

TEST MATERIAL

Identification

The test material was identified as α-hexylcinnamaldehyde [HCA (Aldrich Chemical Company, Lot No. 10021HF)] and described as a clear, yellow liquid.

Purity and Stability

The purity and stability of this reagent grade material were the responsibility of Covance and were deemed adequate for the conduct of this study. The certificate of analysis will be retained in the study file.

Storage and Retention

The test material was stored at room temperature. A reserve sample of the test material was taken and will be retained for 10 years in a freezer set to maintain a temperature of -20°C ±10° in accordance with Covance SOP. Any unused test material will be retained for future tests until the indicated expiration date is reached.

Safety Precautions

The test material handling procedures were according to Covance SOPs and policies.

TEST SYSTEM

Test Animal

Young adult albino guinea pigs of the Crl:(HA)BR strain were procured from Charles River Laboratories, Inc., on May 18, 1998 (Portage, Michigan facility) for the irritation screening study and on May 26, 1998 (Kingston, New York facility) for the definitive study.

Housing

After receipt, the animals were acclimated for a period of at least 7 days. During acclimation and throughout the study, the animals were individually housed in suspended, stainless steel cages. Environmental controls for the animal room were set to maintain a temperature of 18° to 26°C, a relative humidity of 50% ±20%, and a 12-hour light/12-hour dark cycle. In cases where variations from these conditions existed, they were documented and considered to have had no adverse effect on the study outcome.

Animal Diet

The animals were provided continuous access to Certified Guinea Pig Diet #5026 (PMI Nutrition International) and water. The diet is routinely analyzed by the manufacturer for nutritional components and environmental contaminants. Samples of the water are periodically analyzed. There were no known contaminants in the diet or water at levels that would be expected to interfere with or affect the results of the study.

Group Assignments

Twenty-three healthy, acclimated male albino guinea pigs, weighing from 406 to 498 g and approximately 6 weeks of age, were divided into the following treatment groups:

Treatment Group	Number of Animals
Irritation screen	8*
Definitive study	
Test (HCA)	10
Irritation control	5

* Two subgroups of four animals each.

The animals were identified by animal number and corresponding ear tag throughout the study.

Justification for Species Selection
Historically, the albino guinea pig has been the animal of choice for skin sensitization studies.

PROCEDURES

Irritation Screening Study
An irritation screening study using eight animals was conducted to determine the irritation threshold of the test material. One subgroup of four animals was administered the test material undiluted and at concentrations of 10%, 25%, and 50% w/v in ethanol and a second subgroup of four animals was administered the test material at concentrations of 0.5%, 1.0%, 2.5%, and 5.0% w/v in ethanol with each animal in both groups receiving all four concentrations of test material. The appropriate test material concentrations, in the amount of 0.3 mL, were applied to adhesive patches (Hill Top Chamber®, 25-mm diameter). The patches were then placed on four shaved sites (two on the right side and two on the left side) on each animal, covered with an overlapping strip of dental dam, and overwrapped with Elastoplast® tape. The patches remained in place for 6 hours after which they were removed. Any residual material was then removed from the application sites using water and disposable paper towels. The application sites were observed for dermal reactions at 24 and 48 hours after test material application.

Definitive Study
Based on the results of the irritation screening study, the test material was administered as a 2.5% w/v mixture in ethanol for each application in the induction phase and at concentrations of 1.0%, 2.5%, and 5.0% w/v in acetone for the challenge application. All test material mixtures used in the irritation screening or definitive phases of the study were stored at room temperature until administered.

Induction Phase. On each day of test material application, the hair was removed from the back of each animal in the test group with electric clippers. The test material was applied to each animal in the test group by placing 0.3 mL of the 2.5% w/v mixture on an adhesive patch (Hill Top Chamber®, 25-mm diameter) and placing the patch on the induction site along the dorsal anterior left quadrant. The patch was covered with dental dam and overwrapped with Elastoplast® tape. The dressing remained in place for a

period of 6 hours after which it was removed. Any residual material was then removed from the application sites using water and disposable paper towels. The animals in the test group received one application per week for 3 weeks for a total of three applications. Due to the moderate irritation present at the induction site of these test animals, the second induction dose was applied to an induction site slightly posterior to the initial site. The irritation control animals were not treated during this phase of the study.

Challenge Phase. Two weeks following the administration of the third induction dose, a challenge dose of HCA at concentrations of 1.0, 2.5%, and 5.0% w/v in acetone was administered on three respective challenge sites (0.3 mL volume/site) along the dorsal anterior right quadrant of the test group animals in the same manner as during the induction phase of the study. At this time the five irritation control animals (previously untreated) were also treated in the same manner with a challenge application of the same three test material concentrations.

Reason for Route of Administration
Historically, the dermal route has been the route of choice for determining delayed contact hypersensitization.

Observations
On the day of the 24-hour examination following the irritation screening and challenge applications, the application sites of the respective animals were depilated by applying Neet® depilatory. After approximately 10 to 20 minutes, the depilatory was washed from the application sites. The 24-hour examination occurred at least 2 hours after removal of the depilatory.

The respective application sites were examined and scored for dermal reactions according to the Buehler[6] scoring scale at 24 and 48 hours following the irritation screening and challenge applications.

Clinical observations were conducted daily throughout the study. Body weights on the irritation screening animals were determined only on the day of treatment. Body weights on the definitive study animals were determined before the initial test material administration and at termination of the in-life phase.

Termination
At termination of the respective in-life phase for each group, all animals were euthanized and discarded.

Evaluation of Challenge Responses
Determination of sensitization was based on the dermal reactions to the challenge dose. Grades of 1.0 or greater in the test animals indicate evidence of sensitization, provided grades of less than 1.0 are seen in the irritation control animals. If reactions in the irritation control group are 1.0 or greater, then only those reactions in the test group that exceed the highest irritation control reaction are considered to be sensitization reactions.

Statistical Evaluation
No statistical evaluations were required by the protocol.

Location of Raw Data, Records, and Final Report
The raw data, records, and an original signed copy of the final report will be retained in the archives of Covance for 10 years in accordance with Covance SOP.

RESULTS/DISCUSSION

Irritation Screening Study
Individual body weights and dermal reactions are in Table 1. No dermal irritation was observed in the animals treated with the 0.5% w/v concentration of HCA in ethanol. Only very faint erythema reactions (scores of 0.5) were observed at two of the four test sites treated with the 1.0% w/v mixture. Very faint to strong erythema reactions (scores of 0.5 to 3.0) were seen at three of the four test sites treated with the 2.5 w/v mixture. Subcutaneous hemorrhaging and possible necrotic areas were also observed at one of the four test sites treated with this mixture. Strong erythema reactions (scores of 3.0) with possible necrotic areas were observed at all four test sites treated with the 5.0%, 10%, 25%, and 50% w/v mixtures. Subcutaneous hemorrhaging was also observed at two of the four test sites treated with the 5.0% w/v mixture and at one of the four test sites treated with the 50% w/v mixture. Very faint to moderate erythema reactions (scores of

0.5 to 2.0) were observed at three of the four test sites treated with the undiluted test material. The decrease in irritation with the undiluted test material is possibly related to a decrease in absorbency of this concentration into the skin of the animals. All animals appeared normal during this phase of the study.

Definitive Study

Clinical Observations and Body Weights. Individual body weights are in Table 2 and individual clinical signs are in Table 3. All animals in both groups appeared normal and exhibited body weight gain throughout the study with the exception of one animal in the test group (E15037) which exhibited few feces on Days 20 through 23 and appeared thin on Days 21 and 22.

Dermal Reactions to HCA. Individual dermal reactions at challenge for the test and irritation control animals are presented in Table 4.

1.0% w/v Mixture of HCA in Acetone

Animals in the test group exhibited very faint to moderate erythema reactions (scores of 0.5 to 2.0) at seven of the 10 test sites treated with the 1.0% w/v mixture of HCA in acetone at challenge. One of the five animals in the irritation control group exhibited a very faint erythema reaction (score of 0.5) at the site treated with the same 1.0% w/v mixture. Four of the reactions in the test group exceeded the highest irritation control reaction during the challenge phase of the study.

2.5% w/v Mixture of HCA in Acetone

Animals in the test group exhibited very faint to moderate erythema reactions (scores of 0.5 to 2.0) at eight of the 10 test sites treated with the 2.5% w/v mixture of HCA in acetone. Animals in the irritation control group exhibited very faint erythema reactions (scores of 0.5) at two of the five test sites treated with the same 2.5% w/v mixture. Seven of the reactions in the test group exceeded the highest irritation control reaction during the challenge phase of the study.

5.0% w/v Mixture of HCA in Acetone

Animals in the test group exhibited very faint to moderate erythema reactions (scores of 0.5 to 2.0) at eight of the 10 test sites treated with the 5.0% w/v mixture of HCA in acetone. Animals in the irritation control group exhibited very faint to faint erythema reactions (scores of 0.5 to 1.0) at two of the five test sites treated with the same 5.0% w/v mixture. Five of the reactions in the test group exceeded the highest irritation control reaction during the challenge phase of the study.

CONCLUSION

Based on the results obtained, this test material, HCA is considered to be a dermal sensitizer in guinea pigs when tested by the closed patch technique.

SIGNATURE

Steven M. Glaza
Study Director
Acute Studies

9-1-98
Date

REFERENCES

1. Hitch, R. K., "Dermal Sensitization Study," *Pesticide Assessment Guidelines, Subdivision F, Hazard Evaluation: Human and Domestic Animals*, U.S. Environmental Protection Agency Office of Pesticide and Toxic Substances, Series 81-6, pp. 59-62 (November 1982).

2. "Dermal Sensitization," *Health Effects Test Guidelines*, 40 CFR 798.4100 (1989).

3. "Dermal Sensitization Study," *Guidance on Toxicology Study Data for Application of Agricultural Chemical Registration*, Ministry of Agriculture Forestry and Fisheries, 59 NohSan No. 4200, January 28, 1985.

4. "Skin Sensitization Studies," *1990 Guidelines for Toxicity Studies of Drugs Manual*, First Edition, Ch. 7, pp. 75-80, YAKUJI NIPPO, LTD. (1991).

5. "Skin Sensitisation," *Organisation for Economic Cooperation and Development Guidelines for Testing of Chemicals*, Section 4. Health Effects, Number 406, Paris Cedex (July 17, 1992).

6. Buehler, E. V. and Ritz, H. L., "Planning, Conduct, and Interpretation of Guinea Pig Sensitization Patch Tests," *Current Concepts in Cutaneous Toxicity*, p. 28 (1980).

Buehler Sensitization Scoring Scale

No reaction	0
Very faint erythema, usually nonconfluent	0.5
Faint erythema, usually confluent	1.0
Moderate erythema	2.0
Strong erythema, with or without edema	3.0

Table 1

Individual Body Weights and Dermal Reactions - Irritation Screening Study

Animal Number	Body Weight (g)	Dermal Reactions: Test Material Concentration (% w/v in Ethanol) 0.5% Hour 24	0.5% Hour 48	1.0% Hour 24	1.0% Hour 48	2.5% Hour 24	2.5% Hour 48	5.0% Hour 24	5.0% Hour 48
E14944	425	0.0	0.0	0.0	0.0	1.0	1.0	2.0[a]	3.0[n]
E14945	460	0.0	0.0	0.5	0.0	0.0	0.0	3.0[n]	3.0[n]
E14946	427	0.0	0.0	0.0	0.0	2.0[a]	3.0[n]	2.0[a]	3.0[n]
E14947	419	0.0	0.0	0.5	0.0	0.5	0.0	3.0[n]	3.0[n]

a Subcutaneous hemorrhaging.
n Possible necrotic areas.

Animal Number	Body Weight (g)	Dermal Reactions: Test Material Concentration (% w/v in Ethanol) 10% Hour 24	10% Hour 48	25% Hour 24	25% Hour 48	50% Hour 24	50% Hour 48	100% Hour 24	100% Hour 48
E14940	419	3.0[n]	3.0[n]	3.0[n]	3.0[n]	3.0[n]	3.0[n]	0.0	0.0
E14941	429	3.0[n]	3.0[n]	3.0[n]	3.0[n]	3.0[n]	3.0[n]	0.5	0.0
E14942	423	3.0[n]	3.0[n]	3.0[n]	3.0[n]	2.0[a]	3.0[n]	0.5	0.5
E14943	406	3.0[n]	3.0[n]	3.0[n]	3.0[n]	3.0[n]	3.0[n]	2.0	2.0

a Subcutaneous hemorrhaging.
n Possible necrotic areas.

Comment: All animals appeared normal throughout the irritation screening study.

Table 2

Individual Body Weights (g) - Definitive Study

Animal Number	Predose	Terminal
	Test Group	
E15032	449	645
E15033	457	667
E15034	444	673
E15035	442	721
E15036	477	705
E15037	473	620
E15038	435	697
E15039	453	670
E15040	425	675
E15041	498	704
	Irritation Control Group	
E15042	492	791
E15043	459	670
E15044	461	694
E15045	450	627
E15046	482	712

Table 3

Individual Clinical Signs - Definitive Study

Animal Number	Observation	Day 1-16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32
Test Group																		
E15032	Normal appearance	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
E15033	Normal appearance	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
E15034	Normal appearance	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
E15035	Normal appearance	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
E15036	Normal appearance	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
E15037	Normal appearance	✓	✓	✓	✓	-	-	-	-	✓	✓	✓	✓	✓	✓	✓	✓	✓
	Few feces	-	-	-	-	✓	✓	✓	✓	-	-	-	-	-	-	-	-	-
	Thin appearance	-	-	-	-	-	✓	✓	-	-	-	-	-	-	-	-	-	-
E15038	Normal appearance	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
E15039	Normal appearance	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
E15040	Normal appearance	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
E15041	Normal appearance	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Irritation Control Group																		
E15042	Normal appearance	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
E15043	Normal appearance	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
E15044	Normal appearance	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
E15045	Normal appearance	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
E15046	Normal appearance	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓

✓ Condition existed.
- Condition not evident.

Table 4

Individual Dermal Reactions at Challenge - Definitive Study

Animal Number	Test Material Concentration (% w/v in Acetone)					
	1.0%		2.5%		5.0%	
	Hour		Hour		Hour	
	24	48	24	48	24	48
Test Group						
E15032	0.5	0.5	1.0	1.0	2.0	2.0
E15033	0.5	1.0	0.5	2.0	1.0	2.0
E15034	0.0	0.0	0.0	0.5	0.0	0.0
E15035	0.5	0.5	1.0	1.0	1.0	1.0
E15036	1.0	1.0	2.0	2.0	2.0	2.0
E15037	0.0	0.0	0.0	0.0	0.5	0.0
E15038	0.5	0.0	1.0	1.0	1.0	0.5
E15039	1.0	2.0	2.0	2.0	2.0	2.0
E15040	0.0	1.0	1.0	2.0	2.0	2.0
E15041	0.0	0.0	0.0	0.0	0.0	0.0
Irritation Control Group						
E15042	0.0	0.0	0.0	0.0	0.0	0.0
E15043	0.0	0.0	0.0	0.0	0.0	0.0
E15044	0.5	0.0	0.0	0.5	0.0	0.0
E15045	0.0	0.0	0.0	0.0	1.0	0.5
E15046	0.0	0.0	0.0	0.5	0.5	0.0

Covance is an independent, publicly held company operating in over 15 countries and over 30 offices worldwide, with headquarters in Princeton, New Jersey, USA.

Covance is the marketing name for Covance Inc. and its subsidiaries around the world, the principal ones of which are listed on this page. The use of "Covance" in this report refers to one or more of these and other subsidiaries.

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Final Report

Dermal Sensitization Study of [ ] in Guinea Pigs - Closed Patch Technique

PREPARED FOR:
[ ]

COVANCE STUDY NUMBER:
80901388

ISSUE DATE:
May 7, 1999

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COVANCE
THE DEVELOPMENT SERVICES COMPANY

Sponsor:

[ ]

FINAL REPORT

Study Title:

Dermal Sensitization Study of [ ] in Guinea Pigs - Closed Patch Technique (EPA/OECD Guidelines)

Author:

Steven M. Glaza

Study Completion Date:

May 7, 1999

Performing Laboratory:

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Laboratory Project Identification:

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Business Information

COMPLIANCE STATEMENT

Dermal Sensitization Study of [ ]
in Guinea Pigs - Closed Patch Technique
(EPA/OECD Guidelines)

This study was conducted in accordance with the following Good Laboratory Practice Standards with the exception that analysis of the prepared test material and the test material mixtures for concentration, homogeneity/solubility, and stability was not conducted:

United States Environmental Protection Agency TSCA:
Good Laboratory Practice Standards, 40 CFR 792

Steven M. Glaza
Study Director
Acute Studies
Covance Laboratories Inc.

5-7-99
Date

Sponsor's Representative

Date

QUALITY ASSURANCE STATEMENT

This report has been reviewed by the Quality Assurance Unit of Covance Laboratories Inc., in accordance with the Environmental Protection Agency (EPA) Good Laboratory Practice Standards, 40 CFR 792. The following inspections were conducted and findings reported to the study director and study director management. Written status reports of inspections and findings are issued to Covance management according to standard operating procedures.

Inspection Dates			Date Reported to Study Director and
From	To	Phase	Study Director Management
09/27/98	09/27/98	Protocol Review	09/28/98
10/30/98	10/30/98	Animal Observation	10/30/98
10/07/98	10/07/98	Test Article Preparation	10/16/98
12/03/98	12/03/98	Protocol Amendment Review	12/03/98
01/15/99	01/15/99	Data Review	01/15/99
01/15/99	01/15/99	Report Review	01/15/99

Representative, Quality Assurance Unit

5·7·99
Date

STUDY IDENTIFICATION

Dermal Sensitization Study of [ ]
in Guinea Pigs - Closed Patch Technique
(EPA/OECD Guidelines)

Test Material	[ ]
Sponsor	[ ]
Sponsor's Representative	[ ]
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Study Timetable

Study Initiation Date	September 28, 1998
Experimental (In-life) Start Date	October 7, 1998
In-life End Date	November 26, 1998
Experimental Termination Date	November 26, 1998
Study Completion Date	May 7, 1999

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CONTENTS

COMPLIANCE STATEMENT 2

QUALITY ASSURANCE STATEMENT 3

STUDY IDENTIFICATION 4

KEY PERSONNEL 5

SUMMARY 8

OBJECTIVE 8

TEST AND VEHICLE CONTROL MATERIALS 9
Test Material Identification 9
Test Material Purity and Stability 9
Test Material Storage and Retention 9
Vehicle Control Material Identification 9
Vehicle Control Material Purity and Stability 10
Vehicle Control Material Storage and Retention 10

TEST SYSTEM 10
Test Animal 10
Housing 10
Animal Diet 10
Group Assignments 11
Justification for Species Selection 11

PROCEDURES 11
Preparation of Test Material 11
Irritation Screening Study 12
Definitive Study 12
Reason for Route of Administration 13
Observations 13
Termination 14
Evaluation of Challenge Responses 14
Statistical Evaluation 14
Location of Raw Data, Records, and Final Report 14

RESULTS/DISCUSSION 14

Irritation Screening Study 14
Definitive Study 15

CONCLUSION 16

SIGNATURE 16

REFERENCES 17

TABLE
Buehler Sensitization Scoring Scale 18
1 Individual Body Weights and Dermal Reactions - Irritation Screening Study 19
2 Individual Body Weights (g) - Definitive Study 20
3 Individual Clinical Signs - Definitive Study 22
4 Individual Dermal Reactions - Definitive Study 24

APPENDIX A 26
Protocol TP2008 27
Protocol Amendment No. 1 40

APPENDIX B 42
Positive Control Report 43

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SUMMARY

The delayed contact hypersensitivity potential of [ ] was evaluated in albino guinea pigs. The test material was administered undiluted to each animal in the test group during the three-application induction phase of the study. The test material produced very faint to faint erythema reactions (scores of 0.5 to 1.0) in 17 of the 20 animals in the test group and very faint erythema reactions (scores of 0.5) in six of the 10 vehicle control animals when administered as a 50% w/v mixture in deionized water at the initial challenge phase. One of the initial challenge reactions (score of 1.0) in the test group exceeded the highest vehicle control reaction (score of 0.5). Very faint to moderate erythema reactions (scores of 0.5 to 2.0) were observed in 19 of the 20 animals in the test group and very faint to faint erythema reactions (scores of 0.5 to 1.0) were seen in all 10 additional naive control animals when the test material was administered as a 25% w/v mixture in deionized water at the second challenge phase. At least six of the animals in the test group are considered to have exhibited an overall greater response to the undiluted test material during the second challenge phase when compared to the responses seen in the control group. Also during the second challenge phase, a 0.4-g dose of the test material identified as [ ] that had been moistened with deionized water was applied to the animals in the test and additional naive control groups. Application of [ ] did not elicit any dermal reactions during the second challenge phase.

Based on these results, [ ] is considered to be a dermal sensitizer in guinea pigs. [ ] did not elicit any sensitization reactions in the animals previously exposed to [ ]

OBJECTIVE

The objective of this study was to assess the delayed contact hypersensitivity potential of a test material in guinea pigs.[1,2]

All procedures used in this study were in compliance with the Animal Welfare Act, the Guide for the Care and Use of Laboratory Animals, and the Office of Protection from Research Risks. In the opinion of the Sponsor and study director, the study did not

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unnecessarily duplicate any previous work. All procedural times presented in this report fall within the acceptable ranges as specified in Covance standard operating procedure (SOP).

TEST AND VEHICLE CONTROL MATERIALS

Test Material Identification

The test material was identified as [ ] and was formed by combining [ ] and Dowfax 8390 (35%) according to the Sponsor's instructions within 4 hours of administration to the animals. The prepared test material [ ] was described as an opaque, white liquid. An additional material, identified as [ ] and described as a white powder, was used during the second challenge phase of this study.

Test Material Purity and Stability

The Sponsor assumes responsibility for purity and stability determinations (including under test conditions) of the test material components and of [ ].

Test Material Storage and Retention

The test material component, [ ] was stored refrigerated and protected from direct light, while the other test material component, Dowfax 8390 (35%), and the prepared test material [ ] were stored at room temperature. A reserve sample of each prepared batch of test material [ ] was taken and will be retained at room temperature in accordance with Covance SOP. Any unused portion of the prepared test material was discarded after dose administration. Any unused portion of the test material components and [ ] supplied by the Sponsor will be returned to the Sponsor after issuance of the final report according to Covance SOP.

Vehicle Control Material Identification

The vehicle control material used in this study was deionized water (Covance in-house supply).

Vehicle Control Material Purity and Stability

The purity and stability of the deionized water are the responsibility of Covance and were deemed adequate for the conduct of this study.

Vehicle Control Material Storage and Retention

The deionized water was stored at room temperature. A reserve sample of the vehicle control material was taken and will be retained for 10 years in a freezer set to maintain a temperature of -10 to -30°C in accordance with Covance SOP. Any unused vehicle control material will be retained by Covance for future tests until the indicated expiration date is reached.

TEST SYSTEM

Test Animal

Young adult albino guinea pigs of the Crl:(HA)BR strain were procured from Charles River Laboratories, Inc., Kingston, New York, on September 15, 1998, for the irritation screening study and on October 6 and November 17, 1998, for the definitive study.

Housing

After receipt, the animals were acclimated for a period of at least 7 days. During acclimation and throughout the study, the animals were individually housed in suspended, stainless steel cages. Environmental controls for the animal room were set to maintain a temperature of 18° to 26°C, a relative humidity of 30 to 70%, and a 12-hour light/12-hour dark cycle. In cases where variations from these conditions existed, they were documented and considered to have had no adverse effect on the study outcome.

Animal Diet

The animals were provided continuous access to Certified Guinea Pig Diet #5026, PMI Nutrition International, and water. The diet is routinely analyzed by the manufacturer for nutritional components and environmental contaminants. Samples of the water are periodically analyzed. There were no known contaminants in the diet or water at levels that would be expected to interfere with or affect the results of the study.

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Group Assignments

Initially, 34 healthy, acclimated male albino guinea pigs, weighing from 353 to 498 g and approximately 5 to 7 weeks of age, were identified by animal number and corresponding ear tag and divided into the following treatment groups:

Treatment Group	Number of Animals
Irritation screening study	4
Test	20
Initial vehicle control (common to Covance Study No. 80901391)	10

In addition, a new naive control group of 10 healthy, acclimated male albino guinea pigs was added to the study on Day 42 and utilized for the second challenge phase. These animals were identified by animal number and corresponding ear tag, weighed from 446 to 499 g, and were approximately 5 weeks of age on Day 42.

Justification for Species Selection

Historically, the albino guinea pig has been the animal of choice for skin sensitization studies.

PROCEDURES

Preparation of Test Material

On each day of test material administration, the test material, [ ], was formed by adding 1.43 g of Dowfax 8390 (35%) to 43.77 g of deionized water and heating to approximately 60°C. A 5-g subsample of [ ] was added to the mixture while maintaining the temperature at approximately 60°C. After the [ ] was melted and completely mixed, the pH of the mixture was adjusted to approximately 6.2 with NaOH solution. Each test material mixture was allowed to cool to room temperature and used within 4 hours of preparation.

Irritation Screening Study

An irritation screening study using four animals was conducted to determine the irritation threshold of the prepared test material. The prepared test material was administered undiluted and at concentrations of 25, 50, and 75% w/v in deionized water with each animal receiving all four concentrations of the test material. The appropriate test material concentrations, in the amount of 0.4 mL, were applied to adhesive patches (Hill Top Chamber®, 25-mm diameter). The patches were then placed on four shaved sites (two on the right side and two on the left side) on each animal, covered with an overlapping strip of dental dam, and overwrapped with Elastoplast® tape. The patches remained in place for 6 hours after which they were removed. Any residual material was then removed from the application sites using water and disposable paper towels. The application sites were observed for dermal reactions at 24 and 48 hours after test material application.

Definitive Study

Based on the results of the irritation screening study, the test material was administered undiluted for each application in the induction phase and as a 50% w/v mixture in deionized water for the initial challenge application. All dilutions (mixtures) of the prepared test material used in the irritation screening or definitive phases of the study were stored at room temperature until administered.

Induction Phase. On each day of test material application, the hair was removed from the back of each animal with electric clippers. The undiluted test material was applied to each animal in the test group by placing 0.4 mL on an adhesive patch (Hill Top Chamber®, 25-mm diameter) and placing the patch on the induction site along the dorsal anterior left quadrant. The patch was covered with dental dam and overwrapped with Elastoplast® tape. The dressing remained in place for a period of 6 hours after which it was removed. Any residual material was then removed from the application site using water and disposable paper towels. The initial vehicle control animals were treated with a 0.4-mL volume of deionized water in the same manner. The animals in the test and initial vehicle control groups received one application per week for 3 weeks for a total of three applications, with the third induction application for the 20 animals in the test group being applied slightly posterior to the initial induction site on these animals.

Initial Challenge Phase. Two weeks following the administration of the third induction dose, a challenge dose at a volume of 0.4 mL of a 50% w/v mixture of the test material in

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deionized water was administered along the dorsal anterior right quadrant of the animals in the test group in the same manner as during the induction phase of the study. In addition, a naive area on the left side of each animal in the test group was treated with a 0.4-mL dose of deionized water in the same manner as the test material. At this time, the 10 vehicle control animals were also treated with a challenge application of the test and vehicle control materials in the same manner as the animals in the test group.

Second Challenge Phase. A second challenge was conducted 13 days after the initial challenge application. Two new naive sites were selected on each test animal. In addition, 10 new naive control animals were selected. Each animal in the test and additional naive control groups received a 0.4-mL dose of a 25% w/v mixture of the test material in deionized water. In addition, each animal received a 0.4-g dose (moistened with approximately 0.8 mL of deionized water) of the test material identified as [ ]. The second challenge dose was conducted in the same manner as the initial challenge dose with the exception that application of deionized water alone was not required. The initial vehicle control animals were not treated during the second challenge phase of the study.

Reason for Route of Administration

Historically, the dermal route has been the route of choice for determining delayed contact hypersensitization.

Observations

On the day of the 24-hour examination following the irritation screening and challenge applications, the application sites of the respective animals were depilated by applying Neet® depilatory. After approximately 10 to 20 minutes, the depilatory was washed from the application sites.

The respective application sites were examined and scored for dermal reactions according to the Buehler[3] scoring scale at 24 and 48 hours following the irritation screening, induction, and challenge applications.

Clinical observations were conducted daily throughout the study. Body weights on the irritation screening animals were determined only on the day of treatment. Body weights

on the definitive study animals were determined before the initial test material administration and at termination of the in-life phase.

Termination

At termination of the respective in-life phase for each group, all animals were euthanized and discarded.

Evaluation of Challenge Responses

Determination of sensitization was based on the dermal reactions to the challenge dose. Grades of 1.0 or greater in the test animals may indicate evidence of sensitization, provided grades of less than 1.0 are seen in the vehicle control animals. If reactions in the naive control group are 1.0 or greater, then only those reactions in the test group that exceed the highest naive control reaction are considered to be sensitization reactions.

Statistical Evaluation

No statistical evaluations were required by the protocol.

Location of Raw Data, Records, and Final Report

The raw data, records, and an original signed copy of the final report will be retained in the archives of Covance in accordance with Covance SOP.

RESULTS/DISCUSSION

Irritation Screening Study

Individual body weights and dermal reactions are in Table 1. Very faint to faint erythema reactions were observed at three of the sites treated with the undiluted test material. Pinpoint areas of subcutaneous hemorrhaging were also observed at two of these sites. Very faint to moderate erythema reactions were seen at two of the sites treated with the 75% w/v mixture of test material in deionized water. Subcutaneous hemorrhaging (some

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areas pinpoint in size) was also observed at these two sites. No dermal irritation was observed at the sites treated with the 25 or 50% w/v mixtures. All animals appeared normal during this phase of the study.

Definitive Study

Clinical Observations and Body Weights. Individual body weights are in Table 2 and individual clinical signs are in Table 3. All animals in all three groups appeared normal throughout the study and exhibited body weight gain with the exception of two animals in the additional naive control group (one animal exhibited an insignificant weight loss of 4 g from Day 42 to Day 44 and one animal weighed the same on Days 42 and 44.).

Dermal Reactions to [ ] Individual dermal reactions for all three groups are presented in Table 4. Faint to strong erythema reactions were elicited from all 20 test animals to the undiluted test material during the induction phase of the study. Subcutaneous hemorrhaging was also observed within the application sites of 10 test animals during the induction phase. Very faint to faint erythema reactions (scores of 0.5 to 1.0) were elicited from 17 of the 20 animals in the test group following the initial challenge application of the test material as a 50% w/v mixture in deionized water. Pinpoint areas of subcutaneous hemorrhaging were also observed within the test sites of seven animals in the test group at the initial challenge. Very faint erythema reactions (scores of 0.5) were observed in six of the initial vehicle control animals. Pinpoint areas of subcutaneous hemorrhaging were also observed with the test sites of one animal in the initial vehicle control group. None of test or initial vehicle control animals reacted to the challenge application of the vehicle control material. One reaction in the test group (score of 1.0) exceeded the highest vehicle control reaction (score of 0.5) during the initial challenge phase of the study.

Very faint to moderate erythema reactions (scores of 0.5 to 2.0) were observed in 19 of the 20 test animals following the second challenge application of the test material as a 25% w/v mixture of the test material in deionized water. Pinpoint areas of subcutaneous hemorrhaging were also observed within four of these second challenge sites. Very faint to faint erythema reactions (scores of 0.5 to 1.0) were observed in all 10 of the additional naive control animals following the second challenge application of the test material as a 25% w/v mixture in deionized water. No other dermal irritation was observed. Six reactions in the test group (scores of 1.0 or 2.0 at both scoring intervals) exceeded the

highest vehicle control reaction (score of 1.0 at the 48 hour interval only) in the additional control group during the second challenge phase of the study. None of the test or additional naive control animals reacted to the 0.4-g dose of [ ] administered during the second challenge phase.

Positive Control Report. A study report detailing the results of sensitization testing of α-hexylcinnamaldehyde (a known skin sensitizer) using the same sensitization method is presented in Appendix B of this report. This positive control study was conducted within 6 months of the conduct of this study.

CONCLUSION

Based on the results obtained, this test material, [ ] is considered to be a dermal sensitizer in guinea pigs when tested by the closed patch technique. Application of [ ] did not result in any sensitization reactions in animals previously exposed to [ ]

SIGNATURE

Steven M. Glaza
Study Director
Acute Studies

5-7-99
Date

REFERENCES

1. United States Environmental Protection Agency; Prevention, Pesticides and Toxic Substances; "OPPTS 870.2600 Skin Sensitization"; *Health Effects Test Guidelines* (August 1998).

2. "Skin Sensitisation," *Organisation for Economic Cooperation and Development Guidelines for Testing of Chemicals*, Section 4, Health Effects, Number 406, Paris Cedex (July 17, 1992).

3. Buehler, E. V. and Ritz, H. L., "Planning, Conduct, and Interpretation of Guinea Pig Sensitization Patch Tests," *Current Concepts in Cutaneous Toxicity*, p. 28 (1980).

Buehler Sensitization Scoring Scale

No reaction	0.0
Very faint erythema, usually nonconfluent	0.5
Faint erythema, usually confluent	1.0
Moderate erythema	2.0
Strong erythema, with or without edema	3.0

Table 1

Individual Body Weights and Dermal Reactions - Irritation Screening Study

Animal Number	Initial Body Weight (g)	Dermal Reactions							
		Test Material Concentration (% w/v in Deionized Water)							
		25%		50%		75%		100%*	
		Hour		Hour		Hour		Hour	
		24	48	24	48	24	48	24	48
E16247	491	0.0	0.0	0.0	0.0	0.5[p]	0.5[p]	0.0	0.0
E16248	498	0.0	0.0	0.0[e]	0.0	0.0	0.0	1.0[p]	1.0[p]
E16249	495	0.0	0.0	0.0	0.0	2.0[a]	2.0[a]	1.0[p]	1.0[p]
E16250	479	0.0	0.0	0.0	0.0	0.0	0.0	0.5	0.5

a Subcutaneous hemorrhaging.
e Irritation at margin of test site.
p Pinpoint areas of subcutaneous hemorrhaging.
* Prepared material applied undiluted.

Comment: All animals appeared normal throughout the irritation screening study.

Table 2

Individual Body Weights (g) - Definitive Study

Animal Number	Predose	Terminal
	Test Group	
E16427	425	688
E16428	432	722
E16429	392	751
E16430	359	589
E16484	416	793
E16432	386	716
E16433	403	709
E16434	404	751
E16435	411	780
E16436	408	723
E16437	388	648
E16438	353	626
E16439	391	746
E16440	381	671
E16441	373	611
E16442	418	721
E16443	420	763
E16444	374	744
E16445	392	599
E16446	411	710

Table 2 (Continued)

Individual Body Weights (g) - Definitive Study

Animal Number	Predose	Terminal
Initial Vehicle Control Group		
E16447	373	540
E16448	401	576
E16449	404	660
E16450	404	616
E16451	397	673
E16452	400	547
E16453	386	600
E16454	368	597
E16455	392	673
E16456	380	664
Additional Naive Control Group		
E16787	488	496
E16788	479	492
E16789	456	460
E16790	467	476
E16791	446	462
E16792	464	475
E16793	481	477†
E16794	471	479
E16795	485	490
E16796	499	499

† Denotes weight loss from previous weighing.

Table 3

Individual Clinical Signs - Definitive Study

Animal Number	Observation	Day																			
		1-25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44
		Test Group																			
E16427	Normal appearance	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
E16428	Normal appearance	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
E16429	Normal appearance	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
E16430	Normal appearance	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
E16484	Normal appearance	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
E16432	Normal appearance	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
E16433	Normal appearance	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
E16434	Normal appearance	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
E16435	Normal appearance	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
E16436	Normal appearance	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
E16437	Normal appearance	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
E16438	Normal appearance	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
E16439	Normal appearance	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
E16440	Normal appearance	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
E16441	Normal appearance	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
E16442	Normal appearance	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
E16443	Normal appearance	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
E16444	Normal appearance	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
E16445	Normal appearance	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
E16446	Normal appearance	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓

✓ Condition existed.

Table 3 (Continued)

Individual Clinical Signs - Definitive Study

Animal Number	Observation	Day 1-25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44
Initial Vehicle Control Group																					
E16447	Normal appearance	✓	✓	✓	✓	✓	✓	✓	*	*	*	*	*	*	*	*	*	*	*	*	*
E16448	Normal appearance	✓	✓	✓	✓	✓	✓	✓	*	*	*	*	*	*	*	*	*	*	*	*	*
E16449	Normal appearance	✓	✓	✓	✓	✓	✓	✓	*	*	*	*	*	*	*	*	*	*	*	*	*
E16450	Normal appearance	✓	✓	✓	✓	✓	✓	✓	*	*	*	*	*	*	*	*	*	*	*	*	*
E16451	Normal appearance	✓	✓	✓	✓	✓	✓	✓	*	*	*	*	*	*	*	*	*	*	*	*	*
E16452	Normal appearance	✓	✓	✓	✓	✓	✓	✓	*	*	*	*	*	*	*	*	*	*	*	*	*
E16453	Normal appearance	✓	✓	✓	✓	✓	✓	✓	*	*	*	*	*	*	*	*	*	*	*	*	*
E16454	Normal appearance	✓	✓	✓	✓	✓	✓	✓	*	*	*	*	*	*	*	*	*	*	*	*	*
E16455	Normal appearance	✓	✓	✓	✓	✓	✓	✓	*	*	*	*	*	*	*	*	*	*	*	*	*
E16456	Normal appearance	✓	✓	✓	✓	✓	✓	✓	*	*	*	*	*	*	*	*	*	*	*	*	*
Additional Naive Control Group																					
E16787	Normal appearance	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	✓	✓	✓
E16788	Normal appearance	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	✓	✓	✓
E16789	Normal appearance	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	✓	✓	✓
E16790	Normal appearance	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	✓	✓	✓
E16791	Normal appearance	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	✓	✓	✓
E16792	Normal appearance	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	✓	✓	✓
E16793	Normal appearance	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	✓	✓	✓
E16794	Normal appearance	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	✓	✓	✓
E16795	Normal appearance	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	✓	✓	✓
E16796	Normal appearance	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	✓	✓	✓

✓ Condition existed.
* Animal not on test during this phase of the study.

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Table 4

Individual Dermal Reactions - Definitive Study

Animal Number	Induction Phase† Dose #1 Hour 24	48	Dose #2 Hour 24	48	Dose #3 Hour 24	48	Challenge Application Initial [redacted] Hour 24	48	Initial Deionized Water Hour 24	48	Second [redacted] Hour 24	48	Second [redacted] Hour 24	48
Test Group														
E16427**	0.0	0.0	2.0[a]	2.0[a]	2.0	2.0	0.5	0.0	0.0	0.0	1.0	1.0	0.0	0.0
E16428**	0.0	0.0	2.0	2.0	1.0	2.0	0.5[p]	0.0[p]	0.0	0.0	0.0	0.0	0.0	0.0
E16429**	0.0	0.0	2.0[a]	2.0[a]	1.0	1.0	0.5[p]	0.0[p]	0.0	0.0	0.5	0.5	0.0	0.0
E16430**	0.0	0.0	2.0[a]	2.0[a]	1.0	1.0	0.5	0.0	0.0	0.0	0.5	0.5	0.0	0.0
E16484**	0.0	0.0	2.0	2.0[a]	1.0	2.0	0.5	0.0	0.0	0.0	0.5	0.5	0.0	0.0
E16432**	0.0	0.0	2.0[a]	3.0[a]	2.0	2.0[a]	0.5[p]	0.0[p]	0.0	0.0	0.5	1.0	0.0	0.0
E16433**	0.0	0.0	2.0	2.0	2.0	1.0	0.5	0.0	0.0	0.0	0.5	0.5	0.0	0.0
E16434**	0.0	0.0	2.0	3.0[a]	1.0	1.0	1.0	0.0	0.0	0.0	0.5[p]	0.5[p]	0.0	0.0
E16435**	0.0	0.0	2.0[a]	3.0[a]	1.0	1.0	0.5[p]	0.0[p]	0.0	0.0	0.5[p]	1.0	0.0	0.0
E16436**	0.0	0.0	1.0	2.0	1.0	1.0	0.0	0.0	0.0	0.0	0.5	0.5	0.0	0.0
E16437**	0.0	0.0	2.0	2.0[a]	0.5	1.0	0.0	0.0	0.0	0.0	0.5	0.5	0.0	0.0
E16438**	0.0	0.0	1.0	1.0	0.5	0.5	0.0	0.0	0.0	0.0	0.5	0.5	0.0	0.0
E16439**	0.0	0.0	2.0	2.0	0.5	0.5	0.5	0.0	0.0	0.0	0.5	0.5	0.0	0.0
E16440**	0.0	0.0	2.0	2.0	1.0	1.0	0.5	0.0	0.0	0.0	1.0	1.0	0.0	0.0
E16441**	0.0	0.0	2.0	2.0	1.0	1.0	0.5	0.0	0.0	0.0	2.0	2.0	0.0	0.0
E16442**	0.0	0.0	2.0	2.0	0.5	0.5	0.5[p]	0.0[p]	0.0	0.0	0.5	1.0	0.0	0.0
E16443**	0.0	0.0	2.0[a]	2.0[a]	1.0	1.0	0.5	0.0	0.0	0.0	1.0	1.0	0.0	0.0
E16444**	0.0	0.0	2.0	3.0	1.0	1.0	0.5	0.0	0.0	0.0	0.5	0.5	0.0	0.0
E16445**	0.0	0.0	2.0	3.0	0.5	0.0	0.5[p]	0.0[p]	0.0	0.0	1.0[p]	1.0[p]	0.0	0.0
E16446**	0.0	0.0	0.5	2.0[a]	0.5	0.0	0.5[p]	0.0[p]	0.0	0.0	1.0[p]	1.0[p]	0.0	0.0

† Treated with undiluted[redacted]
†† Administered as a 50% w/v concentration in deionized water.
‡ Administered as a 25% w/v concentration in deionized water.
* Administered as a 4-g dose (moistened with deionized water).
** Induction site on this animal was moved slightly posterior to the initial induction site for Induction Dose #3.
a Subcutaneous hemorrhaging.
p Pinpoint areas of subcutaneous hemorrhaging.

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Table 4 (Continued)

Individual Dermal Reactions - Definitive Study

Animal Number	Induction Phase† Dose #1 Hour 24	48	Dose #2 Hour 24	48	Dose #3 Hour 24	48	Challenge Application Initial [redacted] Hour 24	48	Initial Deionized Water Hour 24	48	Second [redacted] Hour 24	48	Second [redacted] Hour 24	48
Initial Vehicle Control Group														
E16447	0.0	0.0	0.0	0.0	0.0	0.0	0.5	0.0	0.0	0.0	**	**	**	**
E16448	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	**	**	**	**
E16449	0.0	0.0	0.0	0.0	0.0	0.0	0.5	0.0	0.0	0.0	**	**	**	**
E16450	0.0	0.0	0.0	0.0	0.0	0.0	0.5	0.0	0.0	0.0	**	**	**	**
E16451	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	**	**	**	**
E16452	0.0	0.0	0.0	0.0	0.0	0.0	0.5	0.0	0.0	0.0	**	**	**	**
E16453	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	**	**	**	**
E16454	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	**	**	**	**
E16455	0.0	0.0	0.0	0.0	0.0	0.0	0.5	0.0	0.0	0.0	**	**	**	**
E16456	0.0	0.0	0.0	0.0	0.0	0.0	0.5p	0.0p	0.0	0.0	**	**	**	**
Additional Naive Control Group														
E16787	**	**	**	**	**	**	**	**	**	**	0.5	0.5	0.0	0.0
E16788	**	**	**	**	**	**	**	**	**	**	0.5	0.5	0.0	0.0
E16789	**	**	**	**	**	**	**	**	**	**	0.0	0.5	0.0	0.0
E16790	**	**	**	**	**	**	**	**	**	**	0.0	0.5	0.0	0.0
E16791	**	**	**	**	**	**	**	**	**	**	0.5	1.0	0.0	0.0
E16792	**	**	**	**	**	**	**	**	**	**	0.5	0.5	0.0	0.0
E16793	**	**	**	**	**	**	**	**	**	**	0.5	0.5	0.0	0.0
E16794	**	**	**	**	**	**	**	**	**	**	0.5	0.5	0.0	0.0
E16795	**	**	**	**	**	**	**	**	**	**	0.5	0.5	0.0	0.0
E16796	**	**	**	**	**	**	**	**	**	**	0.0	0.5	0.0	0.0

† Treated with deionized water.
†† Administered as a 50% w/v concentration in deionized water.
‡ Administered as a 25% w/v concentration in deionized water.
* Administered as a 4-g dose (moistened with deionized water).
** Animal not on test during this phase of the study.
p Pinpoint areas of subcutaneous hemorrhaging.

APPENDIX A

Protocol TP2008
Protocol Amendment No. 1

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COVANCE.
THE DEVELOPMENT SERVICES COMPANY

Sponsor:

[ ]

PROTOCOL TP2008

Study Title:

Dermal Sensitization Study of [ ] in Guinea Pigs-
Closed Patch Technique
(EPA/OECD Guidelines)

Date:

September 28, 1998

Performing Laboratory:

Covance Laboratories Inc.
3301 Kinsman Boulevard
Madison, Wisconsin 53704

Laboratory Project Identification:

Covance 80901388

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Study
Dermal Sensitization Study of in Guinea Pigs - Closed Patch Technique
(EPA/OECD Guidelines)

Purpose
To assess the delayed contact hypersensitivity potential of a test material in guinea pigs.

Sponsor

[redacted]

Sponsor's Representative

[redacted]

Study Monitor
Robert W. Kapp, Jr., PhD
Biotox
300 Arboretum Place, Suite 140
Richmond, VA 23236
Telephone: 804.320.2800
Facsimile: 804.320.8274

Study Director
Steven M. Glaza
Covance Laboratories Inc.
Telephone 608.241.7292
Facsimile 608.242.7936

Testing Facility
Covance Laboratories Inc.
3301 Kinsman Boulevard
Madison, WI 53704

Proposed Study Timetable

Experimental Start Date	Week of October 5, 1998
Experimental Termination Date	Week of November 16, 1998
Draft Report Date	Week of December 21, 1998

Regulatory Compliance
This study will be conducted in accordance with the following Good Laboratory Practice Regulations/Standards, with the exception that analysis of the test material mixtures for concentration, homogeneity/solubility and stability will not be conducted:

[ ] Conduct as a Nonregulated Study
[ ] 21 CFR 58 (FDA)
[ ] 40 CFR 160 (EPA-FIFRA)
[X] 40 CFR 792 (EPA-TSCA)
[ ] C(97)186/Final (OECD)
[ ] 59 NohSan No. 3850 (Japanese MAFF)
[ ] Ordinance 21 (Japanese MOHW)

Animal Care and Use Statement
All procedures in this protocol are in compliance with the Animal Welfare Act, the Guide for the Care and Use of Laboratory Animals, and the Office of Protection from Research Risks. In the opinion of the Sponsor and Study Director, the study does not unnecessarily duplicate any previous work.

Quality Assurance
The protocol, study conduct, and the final report will be audited by the Covance Quality Assurance Unit.

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Test Material

Identification
[ ] (prepared by Covance on each day of treatment)

Physical Description
Opaque, white liquid (once prepared)

Purity and Stability
The Sponsor assumes responsibility for purity and stability determinations (including under test conditions).

Storage Conditions
Room temperature

Reserve Samples
A reserve sample of each prepared batch of test material will be taken and stored at Covance at room temperature. The reserve samples will be maintained as outlined in the Record Retention section.

Disposition
Any unused test material will be returned to the Sponsor after issuance of the final report, unless otherwise directed by the Sponsor.

Safety Precautions
As required by Covance Standard Operating Procedures (SOPs) and policies

Vehicle Control Material

Identification
Deionized water

Physical Description
Colorless liquid

Purity and Stability
The purity and stability of this control material is considered to be adequate for the conduct of this study.

Storage Conditions
Room temperature

Reserve Samples
Reserve samples of each batch of the vehicle control material will be taken and stored at Covance in a freezer set to maintain a temperature of -20°C ±10°. The reserve samples will be maintained at Covance for 10 years then discarded.

Disposition
Any unused vehicle control material will be discarded after the indicated expiration date is reached.

Safety Precautions
As required by Covance Standard Operating Procedures (SOPs) and policies

Animals

Species
Guinea Pig

Strain and Strain
Crl:(HA)BR, Charles River Laboratories Inc.

Age at Initiation of Treatment
Young adult

Weight at Initiation of Treatment
350 to 550 g

Number and Sex
34 of any sex

Identification
Individual numbered ear tag

Husbandry

Housing
Individually, in suspended, screen-bottom stainless steel cages

Diet
Certified Guinea Pig Diet #5026 (PMI Nutrition International), *ad libitum*. The diet is routinely analyzed by the manufacturer for nutritional components and environmental contaminants.

Water
Ad libitum from an automatic system. Samples of the water are periodically analyzed for specified microorganisms and environmental contaminants.

Contaminants
There are no known contaminants in the diet or water at levels that would be expected to interfere with this study.

Environment
Environmental controls for the animal room will be set to maintain a temperature of 18° to 26°C, a relative humidity of 50% ±20%, and a 12-hour light/12-hour dark cycle.

Acclimation
At least 7 days

Selection of Test Animals
Based on health and body weight according to Covance SOPs. An adequate number of extra animals will be purchased so that no animal in obviously poor health is placed on test.

Justification for Species Selection
Historically, the albino guinea pig has been the animal of choice for skin sensitization studies.

Treatment Procedures

Experimental Design
Thirty-four acclimated animals weighing between 350 and 550 g will be placed into the following groups:

Group	No. of Animals
Irritation Screening	4
Group 1 - Test	20
Group 2 - Vehicle Control	10

The vehicle control group will be common to this study and one other [ ] sensitization study (Covance Study No. 80901391).

Test Material Preparation
The test material [ ] will be prepared fresh on each day of administration from the following components:

Ingredient	Weight Required (g)
Deionized water	43.77
Dowfax 8390 (35%)	1.43
CPS & T 98-005	5.00
NaOH (approx. 10% soln.)	to pH = 6.2

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Procedure

Mix together the Dowfax and deionized water and heat on a heat/stir plate to approximately 60°C. Add the [ ] and mix on a stir plate while maintaining the temperature at approximately 60°C. After the [ ] has melted and been completely mixed, adjust the pH of the mixture to approximately 6.2 with the NaOH solution. Cool the mixture to room temperature and use within four hours of preparation. Mix as needed to maintain uniformity.

Irritation Screen

An irritation screening study of four guinea pigs will be conducted. The dosing procedure will be in the same manner as the definitive study. Four application sites will be selected on each animal. Each site will be treated with 0.4 mL of one of the following concentrations of the prepared test material: 25%, 50%, 75% w/v in deionized water or 100% (undiluted). The mild to moderately irritating concentration will be used in the induction phase of the definitive study. The highest nonirritating concentration will be used for the challenge application.

If a nonirritating concentration is not found, additional testing using new animals will be employed.

All test mixtures used in the irritation screen or definitive phases of the study will be stored at room temperature until administered.

Dose Administration (Definitive Study)

Before each application the back of each animal will be clipped free of hair with an electric clipper. The test material will be applied to one area on each test animal by placing 0.4 mL of the test material on an adhesive patch (Hill Top Chamber®, 25-mm diameter) and placing the patch on the induction site along the dorsal anterior left quadrant. The patch will be covered with dental dam and secured with an overwrap of Elastoplast® tape. The dressing will remain in place for approximately 6 hours, and the residual material will be removed using water or an appropriate substance and disposable paper towels. The vehicle control animals will be treated with deionized water in the same manner. The test and vehicle control animals will receive one application each week for 3 weeks for a total of three applications. Each application will be made on the same site during the induction phase unless moderate to severe

irritation is observed following the first or second induction application. The induction site may then be moved slightly posterior to the initial induction site.

Two weeks after the administration of the third induction dose, a challenge dose, at a volume of 0.4 mL of the highest nonirritating concentration, will be administered to the test group along the dorsal anterior right quadrant and in the same manner as during the induction phase of the study. In a similar manner, a naïve area on the left side of each animal will be treated with a 0.4 mL dose of deionized water. At this time, the 10 vehicle control animals will also be treated with a challenge application of the test and control materials.

The study will terminate after the 48 hour challenge application scoring unless equivocal responses are observed. If equivocal responses are observed in the initial challenge application, the animals in the test group and a new group of 10 control animals may be given a second challenge as determined by the study director. The second challenge will occur 1 to 2 weeks after the initial challenge application.

Reason for Route of Administration
Historically, the dermal route has been the route of choice for determining delayed contact hypersensitization.

Observation of Animals

Clinical Observations
Daily clinical signs will be recorded.

Body Weights
Before test material administration (Irritation Screen and Definitive Study animals) and at the termination of the experimental phase (Definitive Study animals only).

Reading of Dermal Reactions
The respective application sites will be examined and scored for dermal irritation, according to the scoring scale in Attachment 1, at approximately 24 and 48 hours after the irritation screen, induction, and challenge applications.

On the day of the 24-hour examination, following the irritation screen and challenge applications, the respective test sites will be depilated by applying Neet® for approximately 10-20 minutes, then washed off with water. The 24-hour examination must take place at least 2 hours after removal of the depilatory.

Pathology

Any animals dying during the study, or sacrificed in a moribund condition, will be subjected to an abbreviated gross necropsy examination and all abnormalities will be recorded. After necropsy, the animals will be discarded and no tissues will be saved. At termination of the respective experimental phase, surviving animals will be sacrificed and discarded.

Statistical Evaluation

No statistical evaluations are required.

Report

A final report including the items listed below will be submitted.

Description of the test material
Description of the test system
Procedures
Dates of experimental initiation and termination
Summary table showing the induction and challenge responses
Any special observations that were recorded
Determination of sensitization based upon reactions to the challenge dose. Grades of 1 or greater in the test animals indicate evidence of sensitization, provided grades of less than one are seen in the vehicle control animals. If grades of one or greater are noted in the vehicle control animals, then the reactions of test animals exceeding the most severe vehicle control reactions are considered sensitization reactions.

A positive control report from periodic validation of the test method, which has been conducted within six months of the study, will be presented as an appendix in the final report.
Gross pathology findings (if applicable)

Record Retention

All raw data, documentation, records, reserve samples, protocol, protocol amendments (if applicable), and the final report generated as a result of this study will be archived in the storage facility of Covance for a period of one year following signing of the final report. One year after signing of the final report, all of the aforementioned materials may be sent to the Sponsor, and a return fee will be charged. The Sponsor may elect to have the materials retained in the Covance archives for an additional period of time, for which Covance will charge a storage fee. If the Sponsor chooses to have Covance dispose of the materials, a disposal fee will be charged. All raw data stored on magnetic media will be retained by Covance.

The following supporting records will be retained at Covance but will not be archived with the study data:

Animal receipt/acclimation records
Water analysis records
Animal room maintenance and environmental records
Refrigerator, freezer and room temperature records
Positive control data
Feed analysis records
Instrument calibration and maintenance records

PROTOCOL APPROVAL

[ ]

Oct 1, 1997
Date

Robert W. Kapp, Jr., PhD
Study Monitor
Biotox

9/30/98
Date

Steven M. Glaza
Study Director
Acute Studies
Covance Laboratories Inc.

9-28-98
Date

(TP2008.ST4)

Attachment 1

Buehler Sensitization Scoring Scale

No reaction	0
Very faint erythema, usually nonconfluent	0.5
Faint erythema, usually confluent	1.0
Moderate erythema	2.0
Strong erythema, with or without edema	3.0

Buehler, E.V. and Ritz, H. L., "Planning, Conduct, and Interpretation of Guinea Pig Sensitization Patch Tests, "Current Concepts in Cutaneous Toxicity, p. 28 (1980)

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COVANCE
THE DEVELOPMENT SERVICES COMPANY

PROTOCOL AMENDMENT NO. 1

Study Title:

Dermal Sensitization Study of [redacted] in Guinea Pigs - Closed Patch Technique (EPA/OECD Guidelines)

Covance 80901388

Sponsor

[redacted]

Testing Facility

Covance Laboratories Inc.
3301 Kinsman Boulevard
Madison, WI 53704

Sponsor's Representative

[redacted]

Study Monitor

Robert W. Kapp, Jr., PhD
Biotox

Study Director

Steven M. Glaza
Covance Laboratories

This amendment modifies the following portion of the protocol:

Effective November 23, 1998

1. **Page 8, Dose Administration (Definitive Study).** Per discussion with the Sponsor, a rechallenge is to be conducted in this study. Add the following as the last paragraph in this section:

   A second challenge will be conducted in this study by treating the animals in the test group and ten additional naïve control animals with a 25% w/v mixture of the test material in deionized water. In addition, each animal is to receive a challenge application of the material identified as [redacted]. This material will be applied as a 0.4 g dose that has been moistened with deionized water. Naïve sites for each application are to be used on each animal. The second challenge dose will be conducted in the same manner as the initial challenge dose with the exception that application of deionized water alone is not required. The initial irritation control animals are to be terminated after completion of the initial challenge phase.

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AMENDMENT APPROVAL

[_____]

12/15/98
Date

_____
Robert W. Kapp, Jr., PhD
Study Monitor
Biotox

12/14/98
Date

_____
Steven M. Glaza
Study Director
Acute Studies
Covance Laboratories Inc.

12-4-98
Date

APPENDIX B

Positive Control Report

COVANCE
THE DEVELOPMENT SERVICES COMPANY

Sponsor:

Covance Laboratories Inc.
Madison, Wisconsin

FINAL REPORT

Study Title:

Dermal Sensitization Study of Hexylcinnamaldehyde in Guinea Pigs - Closed Patch Technique

Data Requirement:

EPA Guideline 81-6

Author:

Steven M. Glaza

Study Completion Date:

September 1, 1998

Performing Laboratory:

Covance Laboratories Inc.
3301 Kinsman Boulevard
Madison, Wisconsin 53704

Laboratory Study Identification:

Covance 80504850

STATEMENT OF NO DATA CONFIDENTIALITY CLAIMS

Dermal Sensitization Study of Hexylcinnamaldehyde
in Guinea Pigs - Closed Patch Technique

No claim of confidentiality is made for any information contained in this study on the basis of its falling within the scope of the United States Environmental Protection Agency Federal Insecticide, Fungicide, and Rodenticide Act §10 (d) (1) (A), (B), or (C).

Covance Laboratories Inc.
Company

Steven M. Glaza
Company Agent

9-1-98
Date

Study Director
Title

Steven M. Glaza
Signature

COMPLIANCE STATEMENT

Dermal Sensitization Study of Hexylcinnamaldehyde
in Guinea Pigs - Closed Patch Technique

This study was conducted in accordance with the following Good Laboratory Practice Regulations/Standards with the exception that analysis of the test material mixtures for concentration, homogeneity/solubility, and stability was not conducted:

United States Food and Drug Administration Good Laboratory Practice Regulations, 21 CFR 58

United States Environmental Protection Agency FIFRA: Good Laboratory Practice Standards, 40 CFR 160

United States Environmental Protection Agency TSCA: Good Laboratory Practice Standards, 40 CFR 792

Organisation for Economic Cooperation and Development Principles of Good Laboratory Practice, C(97)186(Final)

Japanese Ministry of Agriculture, Forestry and Fisheries, Notification No. 59 NohSan 3850

Japanese Ministry of Health and Welfare, Ordinance 21

Management
Covance Laboratories Inc.

9/1/98
Date

Steven M. Glaza
Study Director
Acute Studies
Covance Laboratories Inc.

9-1-98
Date

QUALITY ASSURANCE STATEMENT

This report has been reviewed by the Quality Assurance Unit of Covance Laboratories Inc., in accordance with the Environmental Protection Agency (EPA) Good Laboratory Practice Standards, 40 CFR 160, the Organisation for Economic Cooperation and Development (OECD) Principles of Good Laboratory Practice, C(97)186(Final), the Environmental Protection Agency (EPA) Good Laboratory Practice Standards, 40 CFR 792, the Food and Drug Administration (FDA) Good Laboratory Practice Regulations, 21 CFR 58, the Japanese Ministry of Agriculture, Forestry, and Fisheries (MAFF) Good Laboratory Practice Standards, 59 NohSan No. 3850, and the Japanese Ministry of Health and Welfare (MOHW), Good Laboratory Practice Standards, Ordinance 21. The following inspections were conducted and findings reported to the study director and study director management. Written status reports of inspections and findings are issued to Covance management according to standard operating procedures.

Inspection Dates			Date Reported to Study Director and
From	To	Phase	Study Director Management
06/01/98	06/01/98	Protocol Review	06/01/98
06/16/98	06/16/98	Test Article Administration	06/16/98
08/23/98	08/24/98	Data Review	08/26/98
08/23/98	08/24/98	Report Review	08/26/98

Representative, Quality Assurance Unit

9-1-98
Date

STUDY IDENTIFICATION

Dermal Sensitization Study of Hexylcinnamaldehyde
in Guinea Pigs - Closed Patch Technique

Test Material	Hexylcinnamaldehyde
Sponsor	Covance Laboratories Inc. 3301 Kinsman Boulevard Madison, WI 53704
Study Director	Steven M. Glaza Covance Laboratories Inc. Telephone No.: 608.241.7292 Facsimile No.: 608.242.7936
Test Facility	Covance Laboratories Inc. 3301 Kinsman Boulevard Madison, WI 53704
Study Timetable	
Study Initiation Date	June 1, 1998
Experimental (In-life) Start Date	June 2, 1998
In-life End Date	July 10, 1998
Experimental Termination Date	July 10, 1998
Study Completion Date	September 1, 1998

KEY PERSONNEL

Acute Studies

Steven M. Glaza
Study Director

Steven R. Sorenson
Study Coordinator

Jeffrey B. Hicks
In-life Supervisor

Rose M. Bridge
Administrative Supervisor

Toxicology Support

Kathleen R. Myers
Manager

Quality Assurance

Nancy M. Centanni
Manager

Laboratory Animal Medicine

Donna J. Clemons, DVM
Diplomate, ACLAM
Supervisor

CONTENTS

STATEMENT OF NO DATA CONFIDENTIALITY CLAIMS 2

COMPLIANCE STATEMENT 3

QUALITY ASSURANCE STATEMENT 4

STUDY IDENTIFICATION 5

KEY PERSONNEL 6

SUMMARY 9

OBJECTIVE 9

TEST MATERIAL 10
- Identification 10
- Purity and Stability 10
- Storage and Retention 10
- Safety Precautions 10

TEST SYSTEM 10
- Test Animal 10
- Housing 11
- Animal Diet 11
- Group Assignments 11
- Justification for Species Selection 12

PROCEDURES 12
- Irritation Screening Study 12
- Definitive Study 12
- Reason for Route of Administration 13
- Observations 13
- Termination 14

Evaluation of Challenge Responses 14
Statistical Evaluation 14
Location of Raw Data, Records, and Final Report 14

RESULTS/DISCUSSION 14
Irritation Screening Study 14
Definitive Study 15

CONCLUSION 16

SIGNATURE 16

REFERENCES 17

TABLE
Buehler Sensitization Scoring Scale 18
1 Individual Body Weights and Dermal Reactions - Irritation Screening Study 19
2 Individual Body Weights (g) - Definitive Study 20
3 Individual Clinical Signs - Definitive Study 21
4 Individual Dermal Reactions at Challenge - Definitive Study 22

SUMMARY

The delayed contact hypersensitivity potential of hexylcinnamaldehyde (HCA) was evaluated in albino guinea pigs. The test material was administered as a 2.5% w/v mixture in ethanol to each animal in the test group during the three-application induction phase of the study. During the challenge phase of the study, HCA was administered to each animal in the test and irritation control groups at three concentrations of 1.0%, 2.5%, and 5.0% w/v in acetone (three sites per animal).

Very faint to moderate erythema reactions (scores of 0.5 to 2.0) were observed in seven of the 10 test animals while only a very faint erythema reaction (score of 0.5) was seen in one of the five irritation control animals when HCA was administered as a 1.0% w/v mixture in acetone at challenge. Very faint to moderate erythema reactions (scores of 0.5 to 2.0) were observed in eight of the 10 test animals while very faint erythema reactions (scores of 0.5) were seen two of the five irritation control animals when HCA was administered as a 2.5% w/v mixture in acetone at challenge. Very faint to moderate erythema reactions (scores of 0.5 to 2.0) were observed in eight of the 10 test animals while very faint to faint erythema (scores of 0.5 to 1.0) reactions were seen in two of the five irritation control animals when HCA was administered as a 5.0% w/v mixture in acetone at challenge. In the test group, four reactions to the 1.0% w/v mixture, seven reactions to the 2.5% w/v mixture, and five reactions to the 5.0% w/v mixture exceeded the highest reaction in the irritation control group to the respective HCA mixture. Based on these results, HCA is considered to be a dermal sensitizer in guinea pigs.

OBJECTIVE

The objective of this study was to assess the delayed contact hypersensitivity potential of HCA in guinea pigs.[1,2,3,4,5]

All procedures used in this study were in compliance with the Animal Welfare Act and/or the Guide for the Care and Use of Laboratory Animals and the Office of Protection from Research Risks. In the opinion of the Sponsor and study director, the study did not unnecessarily duplicate any previous work. The dose levels, method, frequency, and duration of administration utilized in this study were chosen based on the requirements of

the regulatory test guidelines. All procedural times presented in this report fall within the acceptable ranges as specified in Covance standard operating procedure (SOP).

TEST MATERIAL

Identification

The test material was identified as α-hexylcinnamaldehyde [HCA (Aldrich Chemical Company, Lot No. 10021HF)] and described as a clear, yellow liquid.

Purity and Stability

The purity and stability of this reagent grade material were the responsibility of Covance and were deemed adequate for the conduct of this study. The certificate of analysis will be retained in the study file.

Storage and Retention

The test material was stored at room temperature. A reserve sample of the test material was taken and will be retained for 10 years in a freezer set to maintain a temperature of -20°C ±10° in accordance with Covance SOP. Any unused test material will be retained for future tests until the indicated expiration date is reached.

Safety Precautions

The test material handling procedures were according to Covance SOPs and policies.

TEST SYSTEM

Test Animal

Young adult albino guinea pigs of the Crl:(HA)BR strain were procured from Charles River Laboratories, Inc., on May 18, 1998 (Portage, Michigan facility) for the irritation screening study and on May 26, 1998 (Kingston, New York facility) for the definitive study.

Housing

After receipt, the animals were acclimated for a period of at least 7 days. During acclimation and throughout the study, the animals were individually housed in suspended, stainless steel cages. Environmental controls for the animal room were set to maintain a temperature of 18° to 26°C, a relative humidity of 50% ±20%, and a 12-hour light/12-hour dark cycle. In cases where variations from these conditions existed, they were documented and considered to have had no adverse effect on the study outcome.

Animal Diet

The animals were provided continuous access to Certified Guinea Pig Diet #5026 (PMI Nutrition International) and water. The diet is routinely analyzed by the manufacturer for nutritional components and environmental contaminants. Samples of the water are periodically analyzed. There were no known contaminants in the diet or water at levels that would be expected to interfere with or affect the results of the study.

Group Assignments

Twenty-three healthy, acclimated male albino guinea pigs, weighing from 406 to 498 g and approximately 6 weeks of age, were divided into the following treatment groups:

Treatment Group	Number of Animals
Irritation screen	8*
Definitive study	
Test (HCA)	10
Irritation control	5

* Two subgroups of four animals each.

The animals were identified by animal number and corresponding ear tag throughout the study.

Justification for Species Selection
Historically, the albino guinea pig has been the animal of choice for skin sensitization studies.

PROCEDURES

Irritation Screening Study
An irritation screening study using eight animals was conducted to determine the irritation threshold of the test material. One subgroup of four animals was administered the test material undiluted and at concentrations of 10%, 25%, and 50% w/v in ethanol and a second subgroup of four animals was administered the test material at concentrations of 0.5%, 1.0%, 2.5%, and 5.0% w/v in ethanol with each animal in both groups receiving all four concentrations of test material. The appropriate test material concentrations, in the amount of 0.3 mL, were applied to adhesive patches (Hill Top Chamber®, 25-mm diameter). The patches were then placed on four shaved sites (two on the right side and two on the left side) on each animal, covered with an overlapping strip of dental dam, and overwrapped with Elastoplast® tape. The patches remained in place for 6 hours after which they were removed. Any residual material was then removed from the application sites using water and disposable paper towels. The application sites were observed for dermal reactions at 24 and 48 hours after test material application.

Definitive Study
Based on the results of the irritation screening study, the test material was administered as a 2.5% w/v mixture in ethanol for each application in the induction phase and at concentrations of 1.0%, 2.5%, and 5.0% w/v in acetone for the challenge application. All test material mixtures used in the irritation screening or definitive phases of the study were stored at room temperature until administered.

Induction Phase. On each day of test material application, the hair was removed from the back of each animal in the test group with electric clippers. The test material was applied to each animal in the test group by placing 0.3 mL of the 2.5% w/v mixture on an adhesive patch (Hill Top Chamber®, 25-mm diameter) and placing the patch on the induction site along the dorsal anterior left quadrant. The patch was covered with dental dam and overwrapped with Elastoplast® tape. The dressing remained in place for a

period of 6 hours after which it was removed. Any residual material was then removed from the application sites using water and disposable paper towels. The animals in the test group received one application per week for 3 weeks for a total of three applications. Due to the moderate irritation present at the induction site of these test animals, the second induction dose was applied to an induction site slightly posterior to the initial site. The irritation control animals were not treated during this phase of the study.

Challenge Phase. Two weeks following the administration of the third induction dose, a challenge dose of HCA at concentrations of 1.0, 2.5%, and 5.0% w/v in acetone was administered on three respective challenge sites (0.3 mL volume/site) along the dorsal anterior right quadrant of the test group animals in the same manner as during the induction phase of the study. At this time the five irritation control animals (previously untreated) were also treated in the same manner with a challenge application of the same three test material concentrations.

Reason for Route of Administration

Historically, the dermal route has been the route of choice for determining delayed contact hypersensitization.

Observations

On the day of the 24-hour examination following the irritation screening and challenge applications, the application sites of the respective animals were depilated by applying Neet® depilatory. After approximately 10 to 20 minutes, the depilatory was washed from the application sites. The 24-hour examination occurred at least 2 hours after removal of the depilatory.

The respective application sites were examined and scored for dermal reactions according to the Buehler[6] scoring scale at 24 and 48 hours following the irritation screening and challenge applications.

Clinical observations were conducted daily throughout the study. Body weights on the irritation screening animals were determined only on the day of treatment. Body weights on the definitive study animals were determined before the initial test material administration and at termination of the in-life phase.

Termination

At termination of the respective in-life phase for each group, all animals were euthanized and discarded.

Evaluation of Challenge Responses

Determination of sensitization was based on the dermal reactions to the challenge dose. Grades of 1.0 or greater in the test animals indicate evidence of sensitization, provided grades of less than 1.0 are seen in the irritation control animals. If reactions in the irritation control group are 1.0 or greater, then only those reactions in the test group that exceed the highest irritation control reaction are considered to be sensitization reactions.

Statistical Evaluation

No statistical evaluations were required by the protocol.

Location of Raw Data, Records, and Final Report

The raw data, records, and an original signed copy of the final report will be retained in the archives of Covance for 10 years in accordance with Covance SOP.

RESULTS/DISCUSSION

Irritation Screening Study

Individual body weights and dermal reactions are in Table 1. No dermal irritation was observed in the animals treated with the 0.5% w/v concentration of HCA in ethanol. Only very faint erythema reactions (scores of 0.5) were observed at two of the four test sites treated with the 1.0% w/v mixture. Very faint to strong erythema reactions (scores of 0.5 to 3.0) were seen at three of the four test sites treated with the 2.5 w/v mixture. Subcutaneous hemorrhaging and possible necrotic areas were also observed at one of the four test sites treated with this mixture. Strong erythema reactions (scores of 3.0) with possible necrotic areas were observed at all four test sites treated with the 5.0%, 10%, 25%, and 50% w/v mixtures. Subcutaneous hemorrhaging was also observed at two of the four test sites treated with the 5.0% w/v mixture and at one of the four test sites treated with the 50% w/v mixture. Very faint to moderate erythema reactions (scores of

0.5 to 2.0) were observed at three of the four test sites treated with the undiluted test material. The decrease in irritation with the undiluted test material is possibly related to a decrease in absorbency of this concentration into the skin of the animals. All animals appeared normal during this phase of the study.

Definitive Study

Clinical Observations and Body Weights. Individual body weights are in Table 2 and individual clinical signs are in Table 3. All animals in both groups appeared normal and exhibited body weight gain throughout the study with the exception of one animal in the test group (E15037) which exhibited few feces on Days 20 through 23 and appeared thin on Days 21 and 22.

Dermal Reactions to HCA. Individual dermal reactions at challenge for the test and irritation control animals are presented in Table 4.

1.0% w/v Mixture of HCA in Acetone

Animals in the test group exhibited very faint to moderate erythema reactions (scores of 0.5 to 2.0) at seven of the 10 test sites treated with the 1.0% w/v mixture of HCA in acetone at challenge. One of the five animals in the irritation control group exhibited a very faint erythema reaction (score of 0.5) at the site treated with the same 1.0% w/v mixture. Four of the reactions in the test group exceeded the highest irritation control reaction during the challenge phase of the study.

2.5% w/v Mixture of HCA in Acetone

Animals in the test group exhibited very faint to moderate erythema reactions (scores of 0.5 to 2.0) at eight of the 10 test sites treated with the 2.5% w/v mixture of HCA in acetone. Animals in the irritation control group exhibited very faint erythema reactions (scores of 0.5) at two of the five test sites treated with the same 2.5% w/v mixture. Seven of the reactions in the test group exceeded the highest irritation control reaction during the challenge phase of the study.

5.0% w/v Mixture of HCA in Acetone

Animals in the test group exhibited very faint to moderate erythema reactions (scores of 0.5 to 2.0) at eight of the 10 test sites treated with the 5.0% w/v mixture of HCA in acetone. Animals in the irritation control group exhibited very faint to faint erythema reactions (scores of 0.5 to 1.0) at two of the five test sites treated with the same 5.0% w/v mixture. Five of the reactions in the test group exceeded the highest irritation control reaction during the challenge phase of the study.

CONCLUSION

Based on the results obtained, this test material, HCA is considered to be a dermal sensitizer in guinea pigs when tested by the closed patch technique.

SIGNATURE

Steven M. Glaza
Study Director
Acute Studies

9-1-98
Date

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Buehler Sensitization Scoring Scale

No reaction	0
Very faint erythema, usually nonconfluent	0.5
Faint erythema, usually confluent	1.0
Moderate erythema	2.0
Strong erythema, with or without edema	3.0

Table 1

Individual Body Weights and Dermal Reactions - Irritation Screening Study

Animal Number	Body Weight (g)	Dermal Reactions: Test Material Concentration (% w/v in Ethanol) 0.5% Hour 24	0.5% Hour 48	1.0% Hour 24	1.0% Hour 48	2.5% Hour 24	2.5% Hour 48	5.0% Hour 24	5.0% Hour 48
E14944	425	0.0	0.0	0.0	0.0	1.0	1.0	2.0[a]	3.0[n]
E14945	460	0.0	0.0	0.5	0.0	0.0	0.0	3.0[n]	3.0[n]
E14946	427	0.0	0.0	0.0	0.0	2.0[a]	3.0[n]	2.0[a]	3.0[n]
E14947	419	0.0	0.0	0.5	0.0	0.5	0.0	3.0[n]	3.0[n]

a Subcutaneous hemorrhaging.
n Possible necrotic areas.

Animal Number	Body Weight (g)	Dermal Reactions: Test Material Concentration (% w/v in Ethanol) 10% Hour 24	10% Hour 48	25% Hour 24	25% Hour 48	50% Hour 24	50% Hour 48	100% Hour 24	100% Hour 48
E14940	419	3.0[n]	3.0[n]	3.0[n]	3.0[n]	3.0[n]	3.0[n]	0.0	0.0
E14941	429	3.0[n]	3.0[n]	3.0[n]	3.0[n]	3.0[n]	3.0[n]	0.5	0.0
E14942	423	3.0[n]	3.0[n]	3.0[n]	3.0[n]	2.0[a]	3.0[n]	0.5	0.5
E14943	406	3.0[n]	3.0[n]	3.0[n]	3.0[n]	3.0[n]	3.0[n]	2.0	2.0

a Subcutaneous hemorrhaging.
n Possible necrotic areas.

Comment: All animals appeared normal throughout the irritation screening study.

Table 2

Individual Body Weights (g) - Definitive Study

Animal Number	Predose	Terminal
	Test Group	
E15032	449	645
E15033	457	667
E15034	444	673
E15035	442	721
E15036	477	705
E15037	473	620
E15038	435	697
E15039	453	670
E15040	425	675
E15041	498	704
	Irritation Control Group	
E15042	492	791
E15043	459	670
E15044	461	694
E15045	450	627
E15046	482	712

Table 3

Individual Clinical Signs - Definitive Study

Animal Number	Observation	Day																
		1-16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32
Test Group																		
E15032	Normal appearance	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
E15033	Normal appearance	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
E15034	Normal appearance	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
E15035	Normal appearance	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
E15036	Normal appearance	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
E15037	Normal appearance	✓	✓	✓	✓	-	-	-	-	✓	✓	✓	✓	✓	✓	✓	✓	✓
	Few feces	-	-	-	-	✓	✓	✓	✓	-	-	-	-	-	-	-	-	-
	Thin appearance	-	-	-	-	-	✓	✓	-	-	-	-	-	-	-	-	-	-
E15038	Normal appearance	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
E15039	Normal appearance	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
E15040	Normal appearance	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
E15041	Normal appearance	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Irritation Control Group																		
E15042	Normal appearance	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
E15043	Normal appearance	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
E15044	Normal appearance	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
E15045	Normal appearance	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
E15046	Normal appearance	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓

✓ Condition existed.
- Condition not evident.

Table 4

Individual Dermal Reactions at Challenge - Definitive Study

	Test Material Concentration (% w/v in Acetone)					
	1.0%		2.5%		5.0%	
Animal	Hour		Hour		Hour	
Number	24	48	24	48	24	48
			Test Group			
E15032	0.5	0.5	1.0	1.0	2.0	2.0
E15033	0.5	1.0	0.5	2.0	1.0	2.0
E15034	0.0	0.0	0.0	0.5	0.0	0.0
E15035	0.5	0.5	1.0	1.0	1.0	1.0
E15036	1.0	1.0	2.0	2.0	2.0	2.0
E15037	0.0	0.0	0.0	0.0	0.5	0.0
E15038	0.5	0.0	1.0	1.0	1.0	0.5
E15039	1.0	2.0	2.0	2.0	2.0	2.0
E15040	0.0	1.0	1.0	2.0	2.0	2.0
E15041	0.0	0.0	0.0	0.0	0.0	0.0
			Irritation Control Group			
E15042	0.0	0.0	0.0	0.0	0.0	0.0
E15043	0.0	0.0	0.0	0.0	0.0	0.0
E15044	0.5	0.0	0.0	0.5	0.0	0.0
E15045	0.0	0.0	0.0	0.0	1.0	0.5
E15046	0.0	0.0	0.0	0.5	0.5	0.0

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Final Report

Dermal Sensitization Study of [ ]in Guinea Pigs - Closed Patch Technique

PREPARED FOR:
[ ]

COVANCE STUDY NUMBER:
80901386

ISSUE DATE:
May 7, 1999

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COVANCE.
THE DEVELOPMENT SERVICES COMPANY

Sponsor:

[ ]

FINAL REPORT

Study Title:

Dermal Sensitization Study of [ ] in Guinea Pigs - Closed Patch Technique (EPA/OECD Guidelines)

Author:

Steven M. Glaza

Study Completion Date:

May 7, 1999

Performing Laboratory:

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COMPLIANCE STATEMENT

Dermal Sensitization Study of [ ]
in Guinea Pigs - Closed Patch Technique
(EPA/OECD Guidelines)

This study was conducted in accordance with the following Good Laboratory Practice Standards with the exception that analysis of the test and control material mixtures for concentration, homogeneity/solubility, and stability was not conducted:

United States Environmental Protection Agency TSCA:
Good Laboratory Practice Standards, 40 CFR 792

Steven M. Glaza
Study Director
Acute Studies
Covance Laboratories Inc.

5-7-99
Date

Sponsor's Representative

Date

QUALITY ASSURANCE STATEMENT

This report has been reviewed by the Quality Assurance Unit of Covance Laboratories Inc., in accordance with the Environmental Protection Agency (EPA) Good Laboratory Practice Standards, 40 CFR 792. The following inspections were conducted and findings reported to the study director and study director management. Written status reports of inspections and findings are issued to Covance management according to standard operating procedures.

Inspection Dates			Date Reported to Study Director and
From	To	Phase	Study Director Management
09/27/98	09/27/98	Protocol Review	09/28/98
11/05/98	11/05/98	Test Article Preparation	11/05/98
12/03/98	12/03/98	Protocol Amendment Review	12/03/98
01/13/99	01/13/99	Protocol Amendment Review	01/13/99
01/29/99	02/01/99	Data Review	02/01/99
01/29/99	02/01/99	Report Review	02/01/99

Randy Leety
Representative, Quality Assurance Unit

5-7-99
Date

STUDY IDENTIFICATION

Dermal Sensitization Study of [ ]
in Guinea Pigs - Closed Patch Technique
(EPA/OECD Guidelines)

Test Material	[ ]
Sponsor	[ ]
Sponsor's Representative	[ ]
Study Monitor	**Robert W. Kapp, Jr., PhD** **Biotox** **300 Arboretum Place, Suite 140** **Richmond, VA 23236** **Telephone No.: 804.320.2800** **Facsimile No.: 804.320.8274**
Study Director	**Steven M. Glaza** **Covance Laboratories Inc.** Telephone No.: 608.241.7292 Facsimile No.: 608.242.7936
Testing Facility	Covance Laboratories Inc. 3301 Kinsman Boulevard Madison, WI 53704
Study Timetable	
Study Initiation Date	September 28, 1998
Experimental (In-life) Start Date	October 6, 1998
In-life End Date	December 4, 1998
Experimental Termination Date	December 4, 1998
Study Completion Date	May 7, 1999

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CONTENTS

COMPLIANCE STATEMENT 2

QUALITY ASSURANCE STATEMENT 3

STUDY IDENTIFICATION 4

KEY PERSONNEL 5

SUMMARY 8

OBJECTIVE 8

TEST AND VEHICLE CONTROL MATERIALS 9
Test Material Identification 9
Test Material Purity and Stability 9
Test Material Storage and Retention 9
Vehicle Control Material Identification 9
Vehicle Control Material Purity and Stability 9
Vehicle Control Material Storage and Retention 9

TEST SYSTEM 10
Test Animal 10
Housing 10
Animal Diet 10
Group Assignments 11
Justification for Species Selection 11

PROCEDURES 11
Irritation Screening Study 11
Definitive Study 12
Reason for Route of Administration 13
Observations 13
Termination 13
Evaluation of Challenge Responses 14
Statistical Evaluation 14
Location of Raw Data, Records, and Final Report 14

RESULTS/DISCUSSION 14
Irritation Screening Study 14
Definitive Study 14

CONCLUSION 16

SIGNATURE 16

REFERENCES 16

TABLE
Buehler Sensitization Scoring Scale 17
1 Individual Body Weights and Dermal Reactions - Irritation Screening Study 18
2 Individual Body Weights (g) - Definitive Study 20
3 Individual Clinical Signs - Definitive Study 22
4 Individual Dermal Reactions - Definitive Study 24

APPENDIX A 26
Protocol TP2008 27
Protocol Amendment No. 1 40
Protocol Amendment No. 2 43

APPENDIX B 45
Positive Control Report 46

SUMMARY

The delayed contact hypersensitivity potential of [ ] was evaluated in albino guinea pigs. The test material was administered as a 15% w/v mixture in mineral oil to each animal in the test group during the three-application induction phase of the study. The test material produced faint to moderate erythema reactions (scores of 1.0 to 2.0) in all 20 test animals when administered as a 1% w/v mixture in mineral oil at the initial challenge phase. All of the initial challenge reactions to [ ] (scores of 1.0 to 2.0) in the test group exceeded the highest reaction in the vehicle control group (scores of 0.0). During the second challenge phase, the test material identified as [ ] that had been moistened with deionized water was applied to the animals in the test and vehicle control groups. Application of [ ] elicited very faint erythema reactions (scores of 0.5) in nine of the 20 test animals but did not elicit any dermal reactions in the vehicle control animals. Although none of the challenge reactions in the test group to [ ] were to an intensity level typically associated with sensitization (ie. erythema grades of 1.0 or greater), the incidence of these mild reactions in the test group (45%) may be indicative of some cross-reactivity to this material in animals previously exposed to [ ].

Based on these results, [ ] is considered to be a dermal sensitizer in guinea pigs. A challenge application of [ ] to animals previously exposed to [ ] resulted in equivocal sensitization reactions.

OBJECTIVE

The objective of this study was to assess the delayed contact hypersensitivity potential of a test material in guinea pigs.[1,2]

All procedures used in this study were in compliance with the Animal Welfare Act, the Guide for the Care and Use of Laboratory Animals, and the Office of Protection from Research Risks. In the opinion of the Sponsor and study director, the study did not unnecessarily duplicate any previous work. All procedural times presented in this report fall within the acceptable ranges as specified in Covance standard operating procedure (SOP).

TEST AND VEHICLE CONTROL MATERIALS

Test Material Identification

The test material was identified as [ ] and was described as a colorless, viscous liquid. An additional material, identified as [ ] and described as a white powder, was used during the second challenge phase of this study.

Test Material Purity and Stability

The Sponsor assumes responsibility for purity and stability determinations (including under test conditions).

Test Material Storage and Retention

Test material [ ] was stored refrigerated and protected from direct light. A reserve sample of the test material was taken and will be retained in a freezer set to maintain a temperature of -10 to -30°C in accordance with Covance SOP. The test material identified as [ ] was stored at room temperature. A reserve sample of this test material was also obtained and will be retained at room temperature in accordance with Covance SOP. Any unused portion of each test material will be returned to the Sponsor after issuance of the final report according to Covance SOP.

Vehicle Control Material Identification

The vehicle control materials used in this study were acetone, 80% v/v ethanol in deionized water (prepared by Covance from in-house supply), and mineral oil.

Vehicle Control Material Purity and Stability

The purity and stability of these control materials are the responsibility of Covance and were deemed adequate for the conduct of this study.

Vehicle Control Material Storage and Retention

The acetone, 80% v/v ethanol in deionized water, and mineral oil were stored at room

temperature. Reserve samples of the vehicle control materials were taken and will be retained for 10 years in a freezer set to maintain a temperature of -10 to -30°C in accordance with Covance SOP. Any unused vehicle control material will be retained by Covance for future tests until the indicated expiration date is reached.

TEST SYSTEM

Test Animal

Young adult albino guinea pigs of the Crl:(HA)BR strain were procured from Charles River Laboratories, Inc., Kingston, New York, on September 15 and October 6, 1998, for the irritation screening study and on October 13, 1998, for the definitive study.

Housing

After receipt, the animals were acclimated for a period of at least 7 days. During acclimation and throughout the study, the animals were individually housed in suspended, stainless steel cages. Environmental controls for the animal room were set to maintain a temperature of 18° to 26°C, a relative humidity of 30 to 70%, and a 12-hour light/12-hour dark cycle. In cases where variations from these conditions existed, they were documented and considered to have had no adverse effect on the study outcome.

Animal Diet

The animals were provided continuous access to Certified Guinea Pig Diet #5026, PMI Nutrition International, and water. The diet is routinely analyzed by the manufacturer for nutritional components and environmental contaminants. Samples of the water are periodically analyzed. There were no known contaminants in the diet or water at levels that would be expected to interfere with or affect the results of the study.

Group Assignments

Forty-two healthy, acclimated male albino guinea pigs, weighing from 395 to 548 g and approximately 5 to 7 weeks of age, were identified by animal number and corresponding ear tag and divided into the following treatment groups:

Treatment Group	Number of Animals
Irritation screening study - ethanol vehicle	4
Irritation screening study - acetone vehicle	4
Additional screening study - mineral oil vehicle	4
Test	20
Vehicle control (common to Covance Study No. 80901389)	10

Justification for Species Selection

Historically, the albino guinea pig has been the animal of choice for skin sensitization studies.

PROCEDURES

Irritation Screening Study

Initially, an irritation screening study using eight animals (2 subgroups of four animals each) was conducted to determine the irritation threshold of the test material. The test material was administered undiluted and at concentrations of 25, 50, and 75% w/v in 80% v/v ethanol in deionized water to one subgroup and at concentrations of 25, 50, and 75% w/v in acetone and undiluted to the second subgroup with each animal receiving all four concentrations of the test material. The appropriate test material concentrations, in the amount of 0.4 mL, were applied to adhesive patches (Hill Top Chamber®, 25-mm diameter). The patches were then placed on four shaved sites (two on the right side and two on the left side) on each animal, covered with an overlapping strip of dental dam, and overwrapped with Elastoplast® tape. The patches remained in place for 6 hours after which they were removed. Any residual material was then removed from the application sites using water and disposable paper towels. The application sites were observed for dermal reactions at 24 and 48 hours after test material application.

Due to the level of irritation observed in the initial irritation screening study, a second irritation screening study was conducted. Four additional animals were treated with the test material at concentrations of 1, 5, 15, and 30% w/v in mineral oil in the same manner as the first irritation screening study.

Definitive Study

Based on the results of the irritation screening studies, the test material was administered as a 15% w/v mixture in mineral oil for each application in the induction phase and as a 1% w/v mixture in mineral oil for the initial challenge application. All test material mixtures used in the irritation screening or definitive phases of the study were stored at room temperature until administered.

Induction Phase. On each day of test material application, the hair was removed from the back of each animal with electric clippers. The test material mixture was applied to each animal in the test group by placing 0.4 mL on an adhesive patch (Hill Top Chamber®, 25-mm diameter) and placing the patch on the induction site along the dorsal anterior left quadrant. The patch was covered with dental dam and overwrapped with Elastoplast® tape. The dressing remained in place for a period of 6 hours after which it was removed. Any residual material was then removed from the application site using water and disposable paper towels. The vehicle control animals were treated with a 0.4-mL volume of mineral oil in the same manner. The animals in the test and vehicle control groups received one application per week for 3 weeks for a total of three applications, with the third induction application for the 20 test animals being applied slightly posterior to the initial induction site on these animals.

Initial Challenge Phase. Two weeks following the administration of the third induction dose, a challenge dose of 0.4 mL of a 1% w/v mixture of the test material in mineral oil was administered along the dorsal anterior right quadrant of the test group animals in the same manner as during the induction phase of the study. In addition, a naive area on the left side of each test group animal was treated with a 0.4-mL dose of mineral oil in the same manner as the test material. At this time, the 10 vehicle control animals were also treated with a challenge application of the test and vehicle control material in the same manner as the animals in the test group.

Second Challenge Phase. A second challenge was conducted 12 days after the initial challenge application. A new naive site was selected on each test and vehicle control animal. Each animal in the test and vehicle control groups received a 0.4-g dose (moistened with approximately 0.8 mL of deionized water) of the test material identified as [ ]. The second challenge dose was conducted in the same manner as the initial challenge dose with the exception that application of deionized water alone was not required.

Reason for Route of Administration

Historically, the dermal route has been the route of choice for determining delayed contact hypersensitization.

Observations

On the day of the 24-hour examination following the irritation screening and challenge applications, the application sites of the respective animals were depilated by applying Neet® depilatory. After approximately 10 to 20 minutes, the depilatory was washed from the application sites.

The respective application sites were examined and scored for dermal reactions according to the Buehler[3] scoring scale at 24 and 48 hours following the irritation screening, induction, and challenge applications.

Clinical observations were conducted daily throughout the study. Body weights on the irritation screening animals were determined only on the day of treatment. Body weights on the definitive study animals were determined before the initial test material administration and at termination of the in-life phase.

Termination

At termination of the respective in-life phase for each group, all animals were euthanized and discarded.

Evaluation of Challenge Responses

Determination of sensitization was based on the dermal reactions to the challenge dose. Grades of 1.0 or greater in the test animals may indicate evidence of sensitization, provided grades of less than 1.0 are seen in the vehicle control animals.

Statistical Evaluation

No statistical evaluations were required by the protocol.

Location of Raw Data, Records, and Final Report

The raw data, records, and an original signed copy of the final report will be retained in the archives of Covance in accordance with Covance SOP.

RESULTS/DISCUSSION

Irritation Screening Study

Individual body weights and dermal reactions are in Table 1. Faint to moderate erythema reactions were observed at the sites treated with the undiluted test material. Blanching was also observed at two of the eight sites treated with the undiluted test material. Moderate to strong erythema reactions were seen at the sites treated with the 25, 50, and 75% w/v mixtures of test material in 80% v/v ethanol/ deionized water. Subcutaneous hemorrhaging and/or blanching were also observed at these sites. Moderate to strong erythema reactions were seen at the sites treated with the 50 and 75% w/v mixtures of test material in acetone. Subcutaneous hemorrhaging and blanching were also observed at one of the sites treated with each of these concentrations. Moderate erythema reactions (including subcutaneous hemorrhaging at one site) were seen at the sites treated with the 25% w/v mixture of test material in acetone. Moderate erythema reactions (including subcutaneous hemorrhaging at one site) were seen at the sites treated with the 30% w/v mixture of test material in mineral oil. Very faint and/or faint erythema reactions were seen at the sites treated with the 5 and 15% w/v mixtures of test material in mineral oil. No irritation was observed at the sites treated with the 1% w/v mixture of the test material in mineral oil. All animals appeared normal during this phase of the study.

Definitive Study

Clinical Observations and Body Weights. Individual body weights are in Table 2 and individual clinical signs are in Table 3. All animals in both groups appeared normal and exhibited body weight gain throughout the study.

Dermal Reactions to [ ] Individual dermal reactions for the test and vehicle control animals are presented in Table 4. Strong erythema reactions were elicited from all 20 test animals to the test material administered as a 15% w/v mixture in mineral oil during the induction phase of the study. Subcutaneous hemorrhaging, blanching, desquamation, and/or necrotic appearing areas were also observed within the application sites of all 20 test animals during the induction phase. Faint to moderate erythema reactions (scores of 1.0 to 2.0) were elicited from all 20 animals in the test group following the initial challenge application of the test material as a 1% w/v mixture in mineral oil. No other dermal irritation was observed in these animals. None of the vehicle control animals reacted to the initial challenge application of the test material as a 1% w/v mixture in mineral oil. None of the test or vehicle control animals reacted to the vehicle control material. All reactions to [ ] in the test group (scores of 1.0 to 2.0) exceeded the highest reaction in the vehicle control group (score of 0.0) during the initial challenge phase of the study. Very faint erythema reactions (scores of 0.5) were observed in nine of the animals in the test group following the application of the 0.4-g dose of [ ] during the second challenge phase. None of the vehicle control animals reacted to the 0.4-g dose of [ ] administered during the second challenge phase. Although none of the challenge reactions in the test group to [ ] were to an intensity level typically associated with sensitization (ie. erythema grades of 1.0 or greater), the incidence of these mild reactions in the test group (45%) may be indicative of some cross-reactivity to this material in animals previously exposed to [ ]

Positive Control Report. A study report detailing the results of sensitization testing of α-hexylcinnamaldehyde (a known skin sensitizer) using the same sensitization method is presented in Appendix B of this report. This positive control study was conducted within 6 months of the conduct of this study.

CONCLUSION

Based on the results obtained, this test material, [ ] is considered to be a dermal sensitizer in guinea pigs when tested by the closed patch technique. A challenge application of [ ] in animals previously exposed to [ ] resulted in equivocal sensitization reactions.

SIGNATURE

Steven M. Glaza
Study Director
Acute Studies

5-7-99
Date

REFERENCES

1. **United States Environmental Protection Agency; Prevention, Pesticides and Toxic Substances; "OPPTS 870.2600 Skin Sensitization"; *Health Effects Test Guidelines*** (August 1998).

2. "Skin Sensitisation," *Organisation for Economic Cooperation and Development Guidelines for Testing of Chemicals*, Section 4, Health Effects, Number 406, Paris Cedex (July 17, 1992).

3. Buehler, E. V. and Ritz, H. L., "Planning, Conduct, and Interpretation of Guinea Pig Sensitization Patch Tests," *Current Concepts in Cutaneous Toxicity*, p. 28 (1980).

Buehler Sensitization Scoring Scale

No reaction	0.0
Very faint erythema, usually nonconfluent	0.5
Faint erythema, usually confluent	1.0
Moderate erythema	2.0
Strong erythema, with or without edema	3.0

Table 1

Individual Body Weights and Dermal Reactions - Irritation Screening Study

Initial Irritation Screening

Animal Number	Initial Body Weight (g)	Dermal Reactions: Test Material Concentration (% w/v in 80% v/v Ethanol/Deionized Water)							
		25%		50%		75%		100%	
		Hour		Hour		Hour		Hour	
		24	48	24	48	24	48	24	48
E16235	493	3.0[a,b]	3.0[a,b]	2.0[b]	2.0[b]	3.0[a,b]	3.0[a,b]	1.0	1.0
E16236	518	2.0[b]	3.0[a,b]	3.0[a,b]	3.0[a,b]	2.0[b]	2.0[b]	1.0	1.0
E16237	462	2.0[b]	2.0[b]	2.0	2.0	3.0[a,b]	3.0[a,b]	1.0	1.0
E16238	548	2.0[b]	2.0[b]	2.0[b]	2.0[a,b]	2.0	2.0	2.0	2.0

Animal Number	Initial Body Weight (g)	Dermal Reactions: Test Material Concentration (% w/v in Acetone)							
		25%		50%		75%		100%	
		Hour		Hour		Hour		Hour	
		24	48	24	48	24	48	24	48
E16239	469	2.0	2.0	2.0	2.0	3.0[a,b]	3.0[a,b]	2.0	2.0
E16240	533	2.0	2.0	3.0[a,b]	3.0[a,b]	2.0	2.0	2.0	2.0[b]
E16241	526	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0
E16242	508	2.0[a]	2.0[a]	2.0	2.0	2.0	2.0	2.0	2.0[b]

a Subcutaneous hemorrhaging.
b Blanching.

Comment: All animals appeared normal throughout the initial irritation screening study.

Table 1 (Continued)

Individual Body Weights and Dermal Reactions - Irritation Screening Study

Additional Irritation Screening

Animal Number	Initial Body Weight (g)	Dermal Reactions							
		Test Material Concentration (% w/v in Mineral Oil)							
		1%		5%		15%		30%	
		Hour		Hour		Hour		Hour	
		24	48	24	48	24	48	24	48
E16477	428	0.0	0.0	0.0	0.0	1.0	1.0	2.0	2.0
E16478	398	0.0	0.0	0.5	0.5	0.5	0.5	2.0	2.0
E16479	398	0.0	0.0	0.5	0.5	0.5	1.0	2.0	2.0
E16480	399	0.0	0.0	0.5	0.5	1.0	1.0	2.0[a]	2.0[a]

a Subcutaneous hemorrhaging.

Comment: All animals appeared normal throughout the additional irritation screening study.

Table 2

Individual Body Weights (g) - Definitive Study

Animal Number	Predose	Terminal
	Test Group	
E16551	433	761
E16552	415	701
E16553	407	734
E16554	455	808
E16555	435	695
E16556	451	833
E16557	405	721
E16558	410	680
E16559	415	727
E16560	417	679
E16561	462	862
E16562	410	720
E16563	426	673
E16564	438	742
E16565	438	706
E16566	436	722
E16567	401	705
E16568	402	756
E16569	412	591
E16550	432	771

Table 2 (Continued)

Individual Body Weights (g) - Definitive Study

Animal Number	Predose	Terminal
	Vehicle Control Group	
E16571	426	716
E16572	402	702
E16573	428	765
E16574	416	727
E16575	443	763
E16576	417	748
E16577	435	688
E16578	395	620
E16579	403	673
E16580	427	722

Table 3

Individual Clinical Signs - Definitive Study

Animal Number	Observation	Day																			
		1-25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44
		Test Group																			
E16551	Normal appearance	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
E16552	Normal appearance	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
E16553	Normal appearance	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
E16554	Normal appearance	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
E16555	Normal appearance	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
E16556	Normal appearance	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
E16557	Normal appearance	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
E16558	Normal appearance	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
E16559	Normal appearance	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
E16560	Normal appearance	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
E16561	Normal appearance	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
E16562	Normal appearance	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
E16563	Normal appearance	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
E16564	Normal appearance	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
E16565	Normal appearance	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
E16566	Normal appearance	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
E16567	Normal appearance	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
E16568	Normal appearance	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
E16569	Normal appearance	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
E16550	Normal appearance	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓

✓ Condition existed.

Table 3 (Continued)

Individual Clinical Signs - Definitive Study

Animal Number	Observation	Day 1-25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44
Vehicle Control Group																					
E16571	Normal appearance	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
E16572	Normal appearance	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
E16573	Normal appearance	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
E16574	Normal appearance	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
E16575	Normal appearance	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
E16576	Normal appearance	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
E16577	Normal appearance	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
E16578	Normal appearance	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
E16579	Normal appearance	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
E16580	Normal appearance	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓

✓ Condition existed.

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Table 4

Individual Dermal Reactions - Definitive Study

Animal Number	Induction Phase† Dose #1 Hour 24	Dose #1 Hour 48	Dose #2 Hour 24	Dose #2 Hour 48	Dose #3 Hour 24	Dose #3 Hour 48	Challenge Application Initial [ ] Hour 24	Initial [ ] Hour 48	Initial Mineral Oil Hour 24	Initial Mineral Oil Hour 48	Second [ ] Hour 24	Second [ ] Hour 48
Test Group												
E16551*	0.5	0.5	3.0	3.0	2.0[a]	2.0[a]	1.0	1.0	0.0	0.0	0.5	0.0
E16552*	1.0	1.0	3.0[a]	3.0[n]	2.0[a]	2.0[a]	2.0	2.0	0.0	0.0	0.0	0.0
E16553*	0.5	0.5	3.0[a]	3.0[n]	2.0	2.0[a]	2.0	2.0	0.0	0.0	0.5	0.5
E16554*	1.0	1.0	3.0[a]	3.0[n]	2.0[a]	2.0[a]	1.0	2.0	0.0	0.0	0.0	0.0
E16555*	1.0	1.0	3.0[a]	3.0[n]	2.0[a]	3.0[a]	2.0	2.0	0.0	0.0	0.5	0.0
E16556*	1.0	1.0	3.0	3.0	2.0[a]	2.0[a]	1.0	1.0	0.0	0.0	0.0	0.0
E16557*	2.0[a]	2.0[a]	3.0[a]	3.0[n]	2.0	2.0	2.0	2.0	0.0	0.0	0.5	0.5
E16558*	1.0	0.5	3.0	3.0	2.0	2.0[a]	2.0	1.0	0.0	0.0	0.0	0.0
E16559*	1.0	1.0	3.0	3.0	2.0[a]	3.0[a,b]	1.0	2.0	0.0	0.0	0.0	0.0
E16560*	1.0	1.0	3.0	3.0	2.0[a]	3.0[a]	2.0	2.0	0.0	0.0	0.0	0.0
E16561*	0.5	0.5	3.0[a]	3.0[n]	2.0	2.0	1.0	1.0	0.0	0.0	0.5	0.0
E16562*	1.0	0.5[d]	3.0[a]	3.0[n]	1.0	2.0	1.0	1.0	0.0	0.0	0.0	0.0
E16563*	0.5	0.5	3.0[a]	3.0[n]	1.0	2.0[a]	1.0	1.0	0.0	0.0	0.5	0.0
E16564*	0.5	0.5	3.0[a]	3.0[n]	1.0	2.0[a]	2.0	2.0	0.0	0.0	0.0	0.0
E16565*	0.5	0.5	2.0[a]	2.0[a]	2.0[a]	3.0[a]	1.0	1.0	0.0	0.0	0.0	0.0
E16566*	0.5	0.5	3.0[a]	3.0[n]	2.0[a]	2.0[a]	2.0	2.0	0.0	0.0	0.0	0.0
E16567*	0.5	1.0	3.0[a]	3.0[n]	2.0	2.0	2.0	2.0	0.0	0.0	0.5	0.5
E16568*	1.0	1.0	3.0[a]	3.0[n]	2.0[a]	3.0[a]	1.0	1.0	0.0	0.0	0.0	0.0
E16569*	0.5	0.5	3.0[a]	3.0[n]	2.0[a]	2.0[a]	2.0	2.0	0.0	0.0	0.5	0.5
E16550*	1.0	1.0	3.0[a]	3.0[n]	1.0	2.0	2.0	2.0	0.0	0.0	0.5	0.5

† Treated with a 15% w/v mixture of [ ] in mineral oil.
†† Administered as a 1% w/v concentration in mineral oil.
‡ Administered as a 0.4-g dose (moistened with deionized water).
* Induction site on this animal was moved slightly posterior to the initial induction site for Induction Dose #3.
a Subcutaneous hemorrhaging.
b Blanching.
d Desquamation.
n Necrotic appearing areas.

Table 4 (Continued)

Individual Dermal Reactions - Definitive Study

Animal Number	Induction Phase†						Challenge Application					
							Initial				Second	
	Dose #1		Dose #2		Dose #3		[redacted]		Mineral Oil		[redacted]	
	Hour		Hour		Hour		Hour		Hour		Hour	
	24	48	24	48	24	48	24	48	24	48	24	48
Vehicle Control Group												
E16571	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
E16572	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
E16573	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
E16574	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
E16575	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
E16576	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
E16577	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
E16578	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
E16579	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
E16580	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

† Treated with mineral oil.
†† Administered as a 1% w/v concentration in mineral oil.
‡ Administered as a 0.4-g dose (moistened with deionized water).

APPENDIX A

Protocol TP2008
Protocol Amendment No. 1
Protocol Amendment No. 2

COVANCE
THE DEVELOPMENT SERVICES COMPANY

Sponsor:

[ ]

PROTOCOL TP2008

Study Title:

**Dermal Sensitization Study of [ ] in Guinea Pigs-
Closed Patch Technique
(EPA/OECD Guidelines)**

Date:

September 28, 1998

Performing Laboratory:

Covance Laboratories Inc.
3301 Kinsman Boulevard
Madison, Wisconsin 53704

Laboratory Project Identification:

Covance 80901386

Study
Dermal Sensitization Study of in Guinea Pigs - Closed Patch Technique
(EPA/OECD Guidelines)

Purpose
To assess the delayed contact hypersensitivity potential of a test material in guinea pigs.

[**Sponsor**

Sponsor's Representative

]

Study Monitor
Robert W. Kapp, Jr., PhD
Biotox
300 Arboretum Place, Suite 140
Richmond, VA 23236
Telephone: 804.320.2800
Facsimile: 804.320.8274

Study Director
Steven M. Glaza
Covance Laboratories Inc.
Telephone 608.241.7292
Facsimile 608.242.7936

Testing Facility
Covance Laboratories Inc.
3301 Kinsman Boulevard
Madison, WI 53704

Proposed Study Timetable

Experimental Start Date	Week of October 5, 1998
Experimental Termination Date	Week of November 16, 1998
Draft Report Date	Week of December 21, 1998

Regulatory Compliance
This study will be conducted in accordance with the following Good Laboratory Practice Regulations/Standards, with the exception that analysis of the test and control material mixtures for concentration, homogeneity/solubility and stability will not be conducted:

[ ] Conduct as a Nonregulated Study
[ ] 21 CFR 58 (FDA)
[ ] 40 CFR 160 (EPA-FIFRA)
[X] 40 CFR 792 (EPA-TSCA)
[ ] C(97)186/Final (OECD)
[ ] 59 NohSan No. 3850 (Japanese MAFF)
[ ] Ordinance 21 (Japanese MOHW)

Animal Care and Use Statement
All procedures in this protocol are in compliance with the Animal Welfare Act, the Guide for the Care and Use of Laboratory Animals, and the Office of Protection from Research Risks. In the opinion of the Sponsor and Study Director, the study does not unnecessarily duplicate any previous work.

Quality Assurance
The protocol, study conduct, and the final report will be audited by the Covance Quality Assurance Unit.

Test Material

Identification

[ ]

Physical Description
Colorless, viscous liquid

Purity and Stability
The Sponsor assumes responsibility for purity and stability determinations (including under test conditions).

Storage Conditions
Protected from light in a refrigerator set to maintain a temperature of 2 to 8°C.

Reserve Samples
Reserve samples of each batch/lot of the test material will be taken and stored at Covance in a freezer set to maintain a temperature of -20°C ±10°. The reserve samples will be maintained as outlined in the Record Retention section.

Disposition
Any unused test material will be returned to the Sponsor after issuance of the final report, unless otherwise directed by the Sponsor.

Safety Precautions
As required by Covance Standard Operating Procedures (SOPs) and policies

Vehicle Control Materials

Identification
Acetone
80% ethanol in deionized water (prepared by Covance)

Physical Description
Colorless liquids

Purity and Stability
The purity and stability of these control materials are considered to be adequate for the conduct of this study.

Storage Conditions
Room temperature

Reserve Samples
Reserve samples of each batch/lot of the vehicle control materials will be taken and stored at Covance in a freezer set to maintain a temperature of -20°C ±10°. The reserve samples will be maintained at Covance for 10 years then discarded.

Disposition
Any unused vehicle control material will be discarded after the indicated expiration date is reached.

Safety Precautions
As required by Covance Standard Operating Procedures (SOPs) and policies

Animals

Species
Guinea Pig

Strain and Strain
Crl:(HA)BR, Charles River Laboratories Inc.

Age at Initiation of Treatment
Young adult

Weight at Initiation of Treatment
350 to 550 g

Number and Sex
38 of any sex

Identification
Individual numbered ear tag

Husbandry

Housing
Individually, in suspended, screen-bottom stainless steel cages

Diet
Certified Guinea Pig Diet #5026 (PMI Nutrition International), *ad libitum*. The diet is routinely analyzed by the manufacturer for nutritional components and environmental contaminants.

Water
Ad libitum from an automatic system. Samples of the water are periodically analyzed for specified microorganisms and environmental contaminants.

Contaminants
There are no known contaminants in the diet or water at levels that would be expected to interfere with this study.

Environment
Environmental controls for the animal room will be set to maintain a temperature of 18° to 26°C, a relative humidity of 50% ±20%, and a 12-hour light/12-hour dark cycle.

Acclimation
At least 7 days

Selection of Test Animals

Based on health and body weight according to Covance SOPs. An adequate number of extra animals will be purchased so that no animal in obviously poor health is placed on test.

Justification for Species Selection

Historically, the albino guinea pig has been the animal of choice for skin sensitization studies.

Treatment Procedures

Experimental Design

Thirty-eight acclimated animals weighing between 350 and 550 g will be placed into the following groups:

Group	No. of Animals
Irritation Screen - ethanol vehicle	4
Irritation Screen - acetone vehicle	4
Group 1 - Test	20
Group 2 - Vehicle Control	10

Irritation Screen

An irritation screening study of eight guinea pigs will be conducted. The dosing procedure will be in the same manner as the definitive study. Four application sites will be selected on each animal. Each site will be treated with 0.4 mL of one of the following test material concentrations: Subgroup 1 - 25%, 50%, 75% w/v in 80% ethanol in deionized water or undiluted; Subgroup 2 - 25%, 50%, 75% w/v in acetone or undiluted. Certain concentrations may not be administered based on the ability to formulate and administer. The mild to moderately irritating concentration using 80% ethanol as the vehicle will be used in the induction phase of the definitive study. The highest nonirritating concentration using acetone as the vehicle will be used for the challenge application.

If appropriate concentrations are not found, additional testing using new animals will be employed.

All test mixtures used in the irritation screen or definitive phases of the study will be stored at room temperature until administered.

Dose Administration (Definitive Study)
Before each application the back of each animal will be clipped free of hair with an electric clipper. The test material will be applied to one area on each test animal by placing 0.4 mL of the test material on an adhesive patch (Hill Top Chamber®, 25-mm diameter) and placing the patch on the induction site along the dorsal anterior left quadrant. The patch will be covered with dental dam and secured with an overwrap of Elastoplast® tape. The dressing will remain in place for approximately 6 hours, and the residual material will be removed using water or an appropriate substance and disposable paper towels. The vehicle control animals will be treated with 80% ethanol in the same manner. The test and vehicle control animals will receive one application each week for 3 weeks for a total of three applications. Each application will be made on the same site during the induction phase unless moderate to severe irritation is observed following the first or second induction application. The induction site may then be moved slightly posterior to the initial induction site.

Two weeks after the administration of the third induction dose, a challenge dose, at a volume of 0.4 mL of the highest nonirritating concentration in acetone, will be administered to the test group along the dorsal anterior right quadrant and in the same manner as during the induction phase of the study. In a similar manner, a naïve area on the left side of each animal will be treated with a 0.4 mL dose of acetone. At this time, the 10 vehicle control animals will also be treated with a challenge application of the test material and acetone.

The study will terminate after the 48 hour challenge application scoring unless equivocal responses are observed. If equivocal responses are observed in the initial challenge application, the animals in the test group and a new group of 10 control animals may be given a second challenge as determined by the study director. The second challenge will occur 1 to 2 weeks after the initial challenge application.

Reason for Route of Administration
Historically, the dermal route has been the route of choice for determining delayed contact hypersensitization.

Observation of Animals

Clinical Observations
Daily clinical signs will be recorded.

Body Weights
Before test material administration (Irritation Screen and Definitive Study animals) and at the termination of the experimental phase (Definitive Study animals only).

Reading of Dermal Reactions
The respective application sites will be examined and scored for dermal irritation, according to the scoring scale in Attachment 1, at approximately 24 and 48 hours after the irritation screen, induction, and challenge applications.

On the day of the 24-hour examination, following the irritation screen and challenge applications, the respective test sites will be depilated by applying Neet® for approximately 10-20 minutes, then washed off with water. The 24-hour examination must take place at least 2 hours after removal of the depilatory.

Pathology
Any animals dying during the study, or sacrificed in a moribund condition, will be subjected to an abbreviated gross necropsy examination and all abnormalities will be recorded. After necropsy, the animals will be discarded and no tissues will be saved. At termination of the respective experimental phase, surviving animals will be sacrificed and discarded.

Statistical Evaluation
No statistical evaluations are required.

Report
A final report including the items listed below will be submitted.

Description of the test material
Description of the test system
Procedures
Dates of experimental initiation and termination
Summary table showing the induction and challenge responses
Any special observations that were recorded
Determination of sensitization based upon reactions to the challenge dose. Grades of 1 or greater in the test animals indicate evidence of sensitization, provided grades of less than one are seen in the vehicle control animals. If grades of one or greater are noted in the vehicle control animals, then the reactions of test animals exceeding the most severe vehicle control reactions are considered sensitization reactions.

A positive control report from periodic validation of the test method, which has been conducted within six months of the study, will be presented as an appendix in the final report.
Gross pathology findings (if applicable)

Record Retention
All raw data, documentation, records, test material reserve samples, protocol, protocol amendments (if applicable), and the final report generated as a result of this study will be archived in the storage facility of Covance for a period of one year following signing of the final report. One year after signing of the final report, all of the aforementioned materials may be sent to the Sponsor, and a return fee will be charged. The Sponsor may elect to have the materials retained in the Covance archives for an additional period of time, for which Covance will charge a storage fee. If the Sponsor chooses to have Covance dispose of the materials, a disposal fee will be charged. All raw data stored on magnetic media will be retained by Covance.

The following supporting records will be retained at Covance but will not be archived with the study data:

Animal receipt/acclimation records
Water analysis records
Animal room maintenance and environmental records
Refrigerator, freezer and room temperature records
Positive control data
Feed analysis records
Instrument calibration and maintenance records

PROTOCOL APPROVAL

[ ]

Oct 1, 1997
Date

Robert W. Kapp, Jr., PhD
Study Monitor
Biotox

9/30/98
Date

Steven M. Glaza
Study Director
Acute Studies
Covance Laboratories Inc.

9-28-98
Date

(TP2008.ST4)

Attachment 1

Buehler Sensitization Scoring Scale

No reaction	0
Very faint erythema, usually nonconfluent	0.5
Faint erythema, usually confluent	1.0
Moderate erythema	2.0
Strong erythema, with or without edema	3.0

Buehler, E.V. and Ritz, H. L., "Planning, Conduct, and Interpretation of Guinea Pig Sensitization Patch Tests, "Current Concepts in Cutaneous Toxicity, p. 28 (1980)

Brackets Contains TSCA Confidential
Business Information

COVANCE
THE DEVELOPMENT SERVICES COMPANY

PROTOCOL AMENDMENT NO. 1

Study Title:

Dermal Sensitization Study of [redacted] in Guinea Pigs - Closed Patch Technique (EPA/OECD Guidelines)

Covance 80901386

Sponsor

[redacted]

Testing Facility

Covance Laboratories Inc.
3301 Kinsman Boulevard
Madison, WI 53704

Sponsor's Representative

[redacted]

Study Monitor

Robert W. Kapp, Jr., PhD
Biotox

Study Director

Steven M. Glaza
Covance Laboratories

This amendment modifies the following portion of the protocol:

Effective October 12, 1998

1. **Page 7, Irritation Screen.** Based on the results of the initial irritation screen, a second irritation screening study is to be conducted. Add the following as the second paragraph in this section:

   A second irritation screen is to be conducted in the same manner as the initial irritation screen by treating four additional animals with the test material at concentrations of 0.1, 1, 5, and 10% w/v in 80% ethanol. In addition, four additional animals are to be treated in the same manner with the test material at concentrations of 0.1, 1, 5, and 10% w/v in acetone.

Effective October 14, 1998

2. **Page 7, Irritation Screen.** Based on the results of the first and second irritation screens, a third irritation screening study is to be conducted. Add the following as the third paragraph in this section:

   A third irritation screen is to be conducted in the same manner as the initial irritation screens by treating four additional animals with the test material at concentrations of 1, 5, 15, and 30% w/v in mineral oil.

Effective November 24, 1998

3. **Page 9, Dose Administration (Definitive Study).** Per discussion with the Sponsor, a rechallenge is to be conducted in this study. Add the following as the last paragraph in this section:

   A second challenge will be conducted in this study by treating the animals in the test group and the initial vehicle control animals with a 0.4 g dose of the material identified as [redacted] moistened with deionized water. Naïve sites are to be used on each animal. The second challenge dose will be conducted in the same manner as the initial challenge dose with the exception that application of deionized water alone is not required.

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Business Information

AMENDMENT APPROVAL

[_____]

12/15/98
Date

_____

Robert W. Kapp, Jr., PhD
Study Monitor
Biotox

12/14/98
Date

_____

Steven M. Glaza
Study Director
Acute Studies
Covance Laboratories Inc.

12-4-98
Date

COVANCE
THE DEVELOPMENT SERVICES COMPANY

PROTOCOL AMENDMENT NO. 2

Study Title:

Dermal Sensitization Study of [ ] in Guinea Pigs - Closed Patch Technique (EPA/OECD Guidelines)

Covance 80901386

Sponsor
[ ]

Testing Facility
Covance Laboratories Inc.
3301 Kinsman Boulevard
Madison, WI 53704

Sponsor's Representative
[ ]

Study Monitor
Robert W. Kapp, Jr., PhD
Biotox

Study Director
Steven M. Glaza
Covance Laboratories

This amendment modifies the following portion of the protocol:

Effective January 13, 1999

1. **Protocol Amendment No. 1, Item No. 1.** Delete this item and procedure as it was not conducted due to a change in the vehicle used in preparing the test material mixtures.

2. **Protocol Amendment No. 1, Item No. 2.** The second item addressed in Amendment No. 1 incorrectly stated that a third irritation screening study was to be conducted. However, the second irritation screen addressed in first item of Amendment No. 1 was not conducted due to a change in the vehicle for preparing the test mixtures. Replace that originally stated with the following:

   A second irritation screen is to be conducted in the same manner as the initial irritation screen by treating four additional animals with the test material at concentrations of 1, 5, 15, and 30% w/v in mineral oil.

Brackets Contains TSCA Confidential
Business Information

3. **Page 8, Dose Administration (Definitive Study).** Due to the use of mineral oil as the vehicle in preparing the test material mixtures, replace the second paragraph with the following:

Two weeks after the administration of the third induction dose, a challenge dose, at a volume of 0.4 mL of the highest nonirritating concentration in mineral oil, will be administered to the test group along the dorsal anterior right quadrant and in the same manner as during the induction phase of the study. In a similar manner, a naïve area on the left side of each animal will be treated with a 0.4 mL dose of mineral oil. At this time, the 10 vehicle control animals will also be treated with a challenge application of the test material and mineral oil

AMENDMENT APPROVAL

[_____] 1/30/99
Date

_____ 1/16/99
Robert W. Kapp, Jr., PhD Date
Study Monitor
Biotox

_____ 1-13-99
Steven M. Glaza Date
Study Director
Acute Studies
Covance Laboratories Inc.

APPENDIX B

Positive Control Report

COVANCE
THE DEVELOPMENT SERVICES COMPANY

Sponsor:

Covance Laboratories Inc.
Madison, Wisconsin

FINAL REPORT

Study Title:

Dermal Sensitization Study of Hexylcinnamaldehyde in Guinea Pigs - Closed Patch Technique

Data Requirement:

EPA Guideline 81-6

Author:

Steven M. Glaza

Study Completion Date:

September 1, 1998

Performing Laboratory:

Covance Laboratories Inc.
3301 Kinsman Boulevard
Madison, Wisconsin 53704

Laboratory Study Identification:

Covance 80504850

STATEMENT OF NO DATA CONFIDENTIALITY CLAIMS

Dermal Sensitization Study of Hexylcinnamaldehyde
in Guinea Pigs - Closed Patch Technique

No claim of confidentiality is made for any information contained in this study on the basis of its falling within the scope of the United States Environmental Protection Agency Federal Insecticide, Fungicide, and Rodenticide Act §10 (d) (1) (A), (B), or (C).

Covance Laboratories Inc.
Company

Steven M. Glaza
Company Agent

9-1-98
Date

Study Director
Title

Steven M. Glaza
Signature

COMPLIANCE STATEMENT

Dermal Sensitization Study of Hexylcinnamaldehyde
in Guinea Pigs - Closed Patch Technique

This study was conducted in accordance with the following Good Laboratory Practice Regulations/Standards with the exception that analysis of the test material mixtures for concentration, homogeneity/solubility, and stability was not conducted:

United States Food and Drug Administration Good Laboratory Practice Regulations, 21 CFR 58

United States Environmental Protection Agency FIFRA: Good Laboratory Practice Standards, 40 CFR 160

United States Environmental Protection Agency TSCA: Good Laboratory Practice Standards, 40 CFR 792

Organisation for Economic Cooperation and Development Principles of Good Laboratory Practice, C(97)186(Final)

Japanese Ministry of Agriculture, Forestry and Fisheries, Notification No. 59 NohSan 3850

Japanese Ministry of Health and Welfare, Ordinance 21

Management
Covance Laboratories Inc.

9/1/98
Date

Steven M. Glaza
Study Director
Acute Studies
Covance Laboratories Inc.

9-1-98
Date

QUALITY ASSURANCE STATEMENT

This report has been reviewed by the Quality Assurance Unit of Covance Laboratories Inc., in accordance with the Environmental Protection Agency (EPA) Good Laboratory Practice Standards, 40 CFR 160, the Organisation for Economic Cooperation and Development (OECD) Principles of Good Laboratory Practice, C(97)186(Final), the Environmental Protection Agency (EPA) Good Laboratory Practice Standards, 40 CFR 792, the Food and Drug Administration (FDA) Good Laboratory Practice Regulations, 21 CFR 58, the Japanese Ministry of Agriculture, Forestry, and Fisheries (MAFF) Good Laboratory Practice Standards, 59 NohSan No. 3850, and the Japanese Ministry of Health and Welfare (MOHW), Good Laboratory Practice Standards, Ordinance 21. The following inspections were conducted and findings reported to the study director and study director management. Written status reports of inspections and findings are issued to Covance management according to standard operating procedures.

Inspection Dates			Date Reported to Study Director and
From	To	Phase	Study Director Management
06/01/98	06/01/98	Protocol Review	06/01/98
06/16/98	06/16/98	Test Article Administration	06/16/98
08/23/98	08/24/98	Data Review	08/26/98
08/23/98	08/24/98	Report Review	08/26/98

Representative, Quality Assurance Unit

9-1-98
Date

STUDY IDENTIFICATION

Dermal Sensitization Study of Hexylcinnamaldehyde
in Guinea Pigs - Closed Patch Technique

Test Material	Hexylcinnamaldehyde
Sponsor	Covance Laboratories Inc. 3301 Kinsman Boulevard Madison, WI 53704
Study Director	Steven M. Glaza Covance Laboratories Inc. Telephone No.: 608.241.7292 Facsimile No.: 608.242.7936
Test Facility	Covance Laboratories Inc. 3301 Kinsman Boulevard Madison, WI 53704
Study Timetable	
Study Initiation Date	June 1, 1998
Experimental (In-life) Start Date	June 2, 1998
In-life End Date	July 10, 1998
Experimental Termination Date	July 10, 1998
Study Completion Date	September 1, 1998

KEY PERSONNEL

Acute Studies

Steven M. Glaza
Study Director

Steven R. Sorenson
Study Coordinator

Jeffrey B. Hicks
In-life Supervisor

Rose M. Bridge
Administrative Supervisor

Toxicology Support

Kathleen R. Myers
Manager

Quality Assurance

Nancy M. Centanni
Manager

Laboratory Animal Medicine

Donna J. Clemons, DVM
Diplomate, ACLAM
Supervisor

CONTENTS

STATEMENT OF NO DATA CONFIDENTIALITY CLAIMS 2

COMPLIANCE STATEMENT 3

QUALITY ASSURANCE STATEMENT 4

STUDY IDENTIFICATION 5

KEY PERSONNEL 6

SUMMARY 9

OBJECTIVE 9

TEST MATERIAL 10
- Identification 10
- Purity and Stability 10
- Storage and Retention 10
- Safety Precautions 10

TEST SYSTEM 10
- Test Animal 10
- Housing 11
- Animal Diet 11
- Group Assignments 11
- Justification for Species Selection 12

PROCEDURES 12
- Irritation Screening Study 12
- Definitive Study 12
- Reason for Route of Administration 13
- Observations 13
- Termination 14

Evaluation of Challenge Responses....14
Statistical Evaluation....14
Location of Raw Data, Records, and Final Report....14

RESULTS/DISCUSSION....14
Irritation Screening Study....14
Definitive Study....15

CONCLUSION....16

SIGNATURE....16

REFERENCES....17

TABLE

Buehler Sensitization Scoring Scale....18
1 Individual Body Weights and Dermal Reactions - Irritation Screening Study....19
2 Individual Body Weights (g) - Definitive Study....20
3 Individual Clinical Signs - Definitive Study....21
4 Individual Dermal Reactions at Challenge - Definitive Study....22

SUMMARY

The delayed contact hypersensitivity potential of hexylcinnamaldehyde (HCA) was evaluated in albino guinea pigs. The test material was administered as a 2.5% w/v mixture in ethanol to each animal in the test group during the three-application induction phase of the study. During the challenge phase of the study, HCA was administered to each animal in the test and irritation control groups at three concentrations of 1.0%, 2.5%, and 5.0% w/v in acetone (three sites per animal).

Very faint to moderate erythema reactions (scores of 0.5 to 2.0) were observed in seven of the 10 test animals while only a very faint erythema reaction (score of 0.5) was seen in one of the five irritation control animals when HCA was administered as a 1.0% w/v mixture in acetone at challenge. Very faint to moderate erythema reactions (scores of 0.5 to 2.0) were observed in eight of the 10 test animals while very faint erythema reactions (scores of 0.5) were seen two of the five irritation control animals when HCA was administered as a 2.5% w/v mixture in acetone at challenge. Very faint to moderate erythema reactions (scores of 0.5 to 2.0) were observed in eight of the 10 test animals while very faint to faint erythema (scores of 0.5 to 1.0) reactions were seen in two of the five irritation control animals when HCA was administered as a 5.0% w/v mixture in acetone at challenge. In the test group, four reactions to the 1.0% w/v mixture, seven reactions to the 2.5% w/v mixture, and five reactions to the 5.0% w/v mixture exceeded the highest reaction in the irritation control group to the respective HCA mixture. Based on these results, HCA is considered to be a dermal sensitizer in guinea pigs.

OBJECTIVE

The objective of this study was to assess the delayed contact hypersensitivity potential of HCA in guinea pigs.[1,2,3,4,5]

All procedures used in this study were in compliance with the Animal Welfare Act and/or the Guide for the Care and Use of Laboratory Animals and the Office of Protection from Research Risks. In the opinion of the Sponsor and study director, the study did not unnecessarily duplicate any previous work. The dose levels, method, frequency, and duration of administration utilized in this study were chosen based on the requirements of

the regulatory test guidelines. All procedural times presented in this report fall within the acceptable ranges as specified in Covance standard operating procedure (SOP).

TEST MATERIAL

Identification

The test material was identified as α-hexylcinnamaldehyde [HCA (Aldrich Chemical Company, Lot No. 10021HF)] and described as a clear, yellow liquid.

Purity and Stability

The purity and stability of this reagent grade material were the responsibility of Covance and were deemed adequate for the conduct of this study. The certificate of analysis will be retained in the study file.

Storage and Retention

The test material was stored at room temperature. A reserve sample of the test material was taken and will be retained for 10 years in a freezer set to maintain a temperature of -20°C ±10° in accordance with Covance SOP. Any unused test material will be retained for future tests until the indicated expiration date is reached.

Safety Precautions

The test material handling procedures were according to Covance SOPs and policies.

TEST SYSTEM

Test Animal

Young adult albino guinea pigs of the Crl:(HA)BR strain were procured from Charles River Laboratories, Inc., on May 18, 1998 (Portage, Michigan facility) for the irritation screening study and on May 26, 1998 (Kingston, New York facility) for the definitive study.

Housing

After receipt, the animals were acclimated for a period of at least 7 days. During acclimation and throughout the study, the animals were individually housed in suspended, stainless steel cages. Environmental controls for the animal room were set to maintain a temperature of 18° to 26°C, a relative humidity of 50% ±20%, and a 12-hour light/12-hour dark cycle. In cases where variations from these conditions existed, they were documented and considered to have had no adverse effect on the study outcome.

Animal Diet

The animals were provided continuous access to Certified Guinea Pig Diet #5026 (PMI Nutrition International) and water. The diet is routinely analyzed by the manufacturer for nutritional components and environmental contaminants. Samples of the water are periodically analyzed. There were no known contaminants in the diet or water at levels that would be expected to interfere with or affect the results of the study.

Group Assignments

Twenty-three healthy, acclimated male albino guinea pigs, weighing from 406 to 498 g and approximately 6 weeks of age, were divided into the following treatment groups:

Treatment Group	Number of Animals
Irritation screen	8*
Definitive study	
Test (HCA)	10
Irritation control	5

* Two subgroups of four animals each.

The animals were identified by animal number and corresponding ear tag throughout the study.

Justification for Species Selection
Historically, the albino guinea pig has been the animal of choice for skin sensitization studies.

PROCEDURES

Irritation Screening Study
An irritation screening study using eight animals was conducted to determine the irritation threshold of the test material. One subgroup of four animals was administered the test material undiluted and at concentrations of 10%, 25%, and 50% w/v in ethanol and a second subgroup of four animals was administered the test material at concentrations of 0.5%, 1.0%, 2.5%, and 5.0% w/v in ethanol with each animal in both groups receiving all four concentrations of test material. The appropriate test material concentrations, in the amount of 0.3 mL, were applied to adhesive patches (Hill Top Chamber®, 25-mm diameter). The patches were then placed on four shaved sites (two on the right side and two on the left side) on each animal, covered with an overlapping strip of dental dam, and overwrapped with Elastoplast® tape. The patches remained in place for 6 hours after which they were removed. Any residual material was then removed from the application sites using water and disposable paper towels. The application sites were observed for dermal reactions at 24 and 48 hours after test material application.

Definitive Study
Based on the results of the irritation screening study, the test material was administered as a 2.5% w/v mixture in ethanol for each application in the induction phase and at concentrations of 1.0%, 2.5%, and 5.0% w/v in acetone for the challenge application. All test material mixtures used in the irritation screening or definitive phases of the study were stored at room temperature until administered.

Induction Phase. On each day of test material application, the hair was removed from the back of each animal in the test group with electric clippers. The test material was applied to each animal in the test group by placing 0.3 mL of the 2.5% w/v mixture on an adhesive patch (Hill Top Chamber®, 25-mm diameter) and placing the patch on the induction site along the dorsal anterior left quadrant. The patch was covered with dental dam and overwrapped with Elastoplast® tape. The dressing remained in place for a

period of 6 hours after which it was removed. Any residual material was then removed from the application sites using water and disposable paper towels. The animals in the test group received one application per week for 3 weeks for a total of three applications. Due to the moderate irritation present at the induction site of these test animals, the second induction dose was applied to an induction site slightly posterior to the initial site. The irritation control animals were not treated during this phase of the study.

Challenge Phase. Two weeks following the administration of the third induction dose, a challenge dose of HCA at concentrations of 1.0, 2.5%, and 5.0% w/v in acetone was administered on three respective challenge sites (0.3 mL volume/site) along the dorsal anterior right quadrant of the test group animals in the same manner as during the induction phase of the study. At this time the five irritation control animals (previously untreated) were also treated in the same manner with a challenge application of the same three test material concentrations.

Reason for Route of Administration
Historically, the dermal route has been the route of choice for determining delayed contact hypersensitization.

Observations
On the day of the 24-hour examination following the irritation screening and challenge applications, the application sites of the respective animals were depilated by applying Neet® depilatory. After approximately 10 to 20 minutes, the depilatory was washed from the application sites. The 24-hour examination occurred at least 2 hours after removal of the depilatory.

The respective application sites were examined and scored for dermal reactions according to the Buehler[6] scoring scale at 24 and 48 hours following the irritation screening and challenge applications.

Clinical observations were conducted daily throughout the study. Body weights on the irritation screening animals were determined only on the day of treatment. Body weights on the definitive study animals were determined before the initial test material administration and at termination of the in-life phase.

Termination
At termination of the respective in-life phase for each group, all animals were euthanized and discarded.

Evaluation of Challenge Responses
Determination of sensitization was based on the dermal reactions to the challenge dose. Grades of 1.0 or greater in the test animals indicate evidence of sensitization, provided grades of less than 1.0 are seen in the irritation control animals. If reactions in the irritation control group are 1.0 or greater, then only those reactions in the test group that exceed the highest irritation control reaction are considered to be sensitization reactions.

Statistical Evaluation
No statistical evaluations were required by the protocol.

Location of Raw Data, Records, and Final Report
The raw data, records, and an original signed copy of the final report will be retained in the archives of Covance for 10 years in accordance with Covance SOP.

RESULTS/DISCUSSION

Irritation Screening Study
Individual body weights and dermal reactions are in Table 1. No dermal irritation was observed in the animals treated with the 0.5% w/v concentration of HCA in ethanol. Only very faint erythema reactions (scores of 0.5) were observed at two of the four test sites treated with the 1.0% w/v mixture. Very faint to strong erythema reactions (scores of 0.5 to 3.0) were seen at three of the four test sites treated with the 2.5 w/v mixture. Subcutaneous hemorrhaging and possible necrotic areas were also observed at one of the four test sites treated with this mixture. Strong erythema reactions (scores of 3.0) with possible necrotic areas were observed at all four test sites treated with the 5.0%, 10%, 25%, and 50% w/v mixtures. Subcutaneous hemorrhaging was also observed at two of the four test sites treated with the 5.0% w/v mixture and at one of the four test sites treated with the 50% w/v mixture. Very faint to moderate erythema reactions (scores of

0.5 to 2.0) were observed at three of the four test sites treated with the undiluted test material. The decrease in irritation with the undiluted test material is possibly related to a decrease in absorbency of this concentration into the skin of the animals. All animals appeared normal during this phase of the study.

Definitive Study

Clinical Observations and Body Weights. Individual body weights are in Table 2 and individual clinical signs are in Table 3. All animals in both groups appeared normal and exhibited body weight gain throughout the study with the exception of one animal in the test group (E15037) which exhibited few feces on Days 20 through 23 and appeared thin on Days 21 and 22.

Dermal Reactions to HCA. Individual dermal reactions at challenge for the test and irritation control animals are presented in Table 4.

1.0% w/v Mixture of HCA in Acetone

Animals in the test group exhibited very faint to moderate erythema reactions (scores of 0.5 to 2.0) at seven of the 10 test sites treated with the 1.0% w/v mixture of HCA in acetone at challenge. One of the five animals in the irritation control group exhibited a very faint erythema reaction (score of 0.5) at the site treated with the same 1.0% w/v mixture. Four of the reactions in the test group exceeded the highest irritation control reaction during the challenge phase of the study.

2.5% w/v Mixture of HCA in Acetone

Animals in the test group exhibited very faint to moderate erythema reactions (scores of 0.5 to 2.0) at eight of the 10 test sites treated with the 2.5% w/v mixture of HCA in acetone. Animals in the irritation control group exhibited very faint erythema reactions (scores of 0.5) at two of the five test sites treated with the same 2.5% w/v mixture. Seven of the reactions in the test group exceeded the highest irritation control reaction during the challenge phase of the study.

5.0% w/v Mixture of HCA in Acetone

Animals in the test group exhibited very faint to moderate erythema reactions (scores of 0.5 to 2.0) at eight of the 10 test sites treated with the 5.0% w/v mixture of HCA in acetone. Animals in the irritation control group exhibited very faint to faint erythema reactions (scores of 0.5 to 1.0) at two of the five test sites treated with the same 5.0% w/v mixture. Five of the reactions in the test group exceeded the highest irritation control reaction during the challenge phase of the study.

CONCLUSION

Based on the results obtained, this test material, HCA is considered to be a dermal sensitizer in guinea pigs when tested by the closed patch technique.

SIGNATURE

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9-1-98
Date

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Buehler Sensitization Scoring Scale

No reaction	0
Very faint erythema, usually nonconfluent	0.5
Faint erythema, usually confluent	1.0
Moderate erythema	2.0
Strong erythema, with or without edema	3.0

Table 1

Individual Body Weights and Dermal Reactions - Irritation Screening Study

Animal Number	Body Weight (g)	Dermal Reactions: Test Material Concentration (% w/v in Ethanol) 0.5% Hour 24	0.5% Hour 48	1.0% Hour 24	1.0% Hour 48	2.5% Hour 24	2.5% Hour 48	5.0% Hour 24	5.0% Hour 48
E14944	425	0.0	0.0	0.0	0.0	1.0	1.0	2.0[a]	3.0[n]
E14945	460	0.0	0.0	0.5	0.0	0.0	0.0	3.0[n]	3.0[n]
E14946	427	0.0	0.0	0.0	0.0	2.0[a]	3.0[n]	2.0[a]	3.0[n]
E14947	419	0.0	0.0	0.5	0.0	0.5	0.0	3.0[n]	3.0[n]

a Subcutaneous hemorrhaging.
n Possible necrotic areas.

Animal Number	Body Weight (g)	Dermal Reactions: Test Material Concentration (% w/v in Ethanol) 10% Hour 24	10% Hour 48	25% Hour 24	25% Hour 48	50% Hour 24	50% Hour 48	100% Hour 24	100% Hour 48
E14940	419	3.0[n]	3.0[n]	3.0[n]	3.0[n]	3.0[n]	3.0[n]	0.0	0.0
E14941	429	3.0[n]	3.0[n]	3.0[n]	3.0[n]	3.0[n]	3.0[n]	0.5	0.0
E14942	423	3.0[n]	3.0[n]	3.0[n]	3.0[n]	2.0[a]	3.0[n]	0.5	0.5
E14943	406	3.0[n]	3.0[n]	3.0[n]	3.0[n]	3.0[n]	3.0[n]	2.0	2.0

a Subcutaneous hemorrhaging.
n Possible necrotic areas.

Comment: All animals appeared normal throughout the irritation screening study.

Table 2

Individual Body Weights (g) - Definitive Study

Animal Number	Predose	Terminal
	Test Group	
E15032	449	645
E15033	457	667
E15034	444	673
E15035	442	721
E15036	477	705
E15037	473	620
E15038	435	697
E15039	453	670
E15040	425	675
E15041	498	704
	Irritation Control Group	
E15042	492	791
E15043	459	670
E15044	461	694
E15045	450	627
E15046	482	712

Table 3

Individual Clinical Signs - Definitive Study

Animal Number	Observation	Day 1-16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32
							Test Group											
E15032	Normal appearance	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
E15033	Normal appearance	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
E15034	Normal appearance	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
E15035	Normal appearance	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
E15036	Normal appearance	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
E15037	Normal appearance	✓	✓	✓	✓	-	-	-	-	✓	✓	✓	✓	✓	✓	✓	✓	✓
	Few feces	-	-	-	-	✓	✓	✓	✓	-	-	-	-	-	-	-	-	-
	Thin appearance	-	-	-	-	-	✓	✓	-	-	-	-	-	-	-	-	-	-
E15038	Normal appearance	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
E15039	Normal appearance	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
E15040	Normal appearance	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
E15041	Normal appearance	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
							Irritation Control Group											
E15042	Normal appearance	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
E15043	Normal appearance	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
E15044	Normal appearance	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
E15045	Normal appearance	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
E15046	Normal appearance	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓

✓ Condition existed.
- Condition not evident.

Table 4

Individual Dermal Reactions at Challenge - Definitive Study

Animal Number	Test Material Concentration (% w/v in Acetone)					
	1.0%		2.5%		5.0%	
	Hour		Hour		Hour	
	24	48	24	48	24	48
Test Group						
E15032	0.5	0.5	1.0	1.0	2.0	2.0
E15033	0.5	1.0	0.5	2.0	1.0	2.0
E15034	0.0	0.0	0.0	0.5	0.0	0.0
E15035	0.5	0.5	1.0	1.0	1.0	1.0
E15036	1.0	1.0	2.0	2.0	2.0	2.0
E15037	0.0	0.0	0.0	0.0	0.5	0.0
E15038	0.5	0.0	1.0	1.0	1.0	0.5
E15039	1.0	2.0	2.0	2.0	2.0	2.0
E15040	0.0	1.0	1.0	2.0	2.0	2.0
E15041	0.0	0.0	0.0	0.0	0.0	0.0
Irritation Control Group						
E15042	0.0	0.0	0.0	0.0	0.0	0.0
E15043	0.0	0.0	0.0	0.0	0.0	0.0
E15044	0.5	0.0	0.0	0.5	0.0	0.0
E15045	0.0	0.0	0.0	0.0	1.0	0.5
E15046	0.0	0.0	0.0	0.5	0.5	0.0

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