WHITE EAGLE TOXICOLOGY LABORATORIES

2003 Lower State Road, Doylestown, Pennsylvania 18901
Telephone: (215) 348-3868
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Study Title

Guinea Pig Dermal Sensitization - Modified Buehler Method
with H-21891

Laboratory Project ID

Haskell Laboratory Project ID 504-96

Data Requirement

EEC 92/69 - Annex V - Part B.6 (1992) - 93/21 (1993),
OECD Guidelines for Testing of Chemicals, No. 406,
Skin Sensitization, adopted July 17, 1992
U.S. EPA Pesticide Assessment Guidelines Subdivision F,
Hazard Evaluation: Human and Domestic Animals, Series 81-6, 1982;
EPA Health Effects Testing Guidelines - Subpart E -
Specific Organ/Tissue Toxicity, September 1985, 40 CFR Part 798,
and MAFF Japan, Testing Guidelines for Toxicology Studies,
Dermal Sensitization Study, 59 NohSan No. 4200, 1985

Study Completed On

20 September 1996

Performing Laboratory

White Eagle Toxicology Laboratories
2003 Lower State Road
Doylestown, Pennsylvania 18901

Author

George E. Moore, B.S.

for

E.I. du Pont de Nemours and Company
Haskell Laboratory for Toxicology and Industrial Medicine
Elkton Road, P.O. Box 50
Newark, Delaware 19714

Study Monitor

Gregory S. Ladics, Ph.D.

Medical Research No.

[redacted]

White Eagle Project No.

[redacted]

THIS PAGE RESERVED FOR SPECIFIC COUNTRY REQUIREMENTS

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GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

White Eagle Project Number: [redacted]
Sponsor: E. I. du Pont de Nemours and Company
Test Article: H-21891
Test: Guinea Pig Dermal Sensitization - Modified Buehler Method
Protocol Number: [redacted] (Rev. 04/96)

This study was carried out in compliance with the Principles of Good Laboratory Practice (GLP) as promulgated by the following agencies:

U.S. Environmental Protection Agency FIFRA as stated in the Federal Register, 40 CFR, Part 160, August 17, 1989.

U.S. Environmental Protection Agency TSCA as stated in the Federal Register, 40 CFR, Part 792, August 17, 1989.

OECD Principles of Good Laboratory Practice (c(81)30(Final), Annex 2)

MAFF Japan Good Laboratory Practice Standards (59 NohSan No. 3850)

The study was conducted in compliance with the following exception:

Treatment solutions were not analyzed for concentration, uniformity or stability of the test and control articles. The procedures used by trained personnel to prepare the treatment solutions insured:

a) The accuracy of concentration because the test article diluent (vehicle) and positive control diluents were accurately measured with graduated devices. The test article was weighed on a balance accurate to at least 2 places. The positive control article was weighed on an analytical balance accurate to at least 3 places.

b) Uniformity because all solutions were mixed prior to administration to the test system; and

c) Stability because treatment solutions were prepared just prior to addition to the test system.

George E. Moore — 9/20/96

George E. Moore, B.S.
Study Director

Date

Sponsor: DuPont Specialty Chemicals
E.I. du Pont de Nemours and Company
Haskell Laboratory for Toxicology and Industrial Medicine
Elkton Road, P.O. Box 50
Newark, DE 19714

GENERAL INFORMATION

Test Material

Substance Tested: H-21891 [redacted]

Synonyms/Other Codes: [redacted]

CAS Registry Number: [redacted]

Haskell No.: 21891

Composition: [redacted]

Purity: [redacted]

Major Impurities: [redacted]

Stability: The test substance appears to be stable under the conditions of the study; no evidence of instability was observed.

Physical Form: Tan liquid

Sponsor: DuPont Specialty Chemicals
E.I. du Pont de Nemours and Company
Barley Mill Plaza
Wilmington, Delaware 19880-0038

GENERAL INFORMATION

Positive Control Material

Material Tested:	1-chloro-2,4-dinitrobenzene
Purity:	Approximately 100% (supplied by Eastman Kodak Co.)
Synonyms:	DNCB
Other Codes:	Eastman Kodak Company, Lot #A11R
CAS Registry No.:	97-00-7 (supplied by Eastman Kodak Co.)
Stability:	In the absence of visible evidence to the contrary, the test material was assumed to be stable under the conditions of administration.
Study Initiated/Completed:	06/03/96 - 09/20/96
In-Life Phase Start - Completion:	06/18/96 - 07/19/96

Guinea Pig Dermal Sensitization - Modified Buehler Method
with H-21891

Summary

The potential of H-21891 to produce contact dermal sensitization in guinea pigs was assessed by the Modified Buehler Method. Twenty (20) test article animals were induced and challenged by occluded application of the test article to clipped intact guinea pig skin. The same procedures were carried out on contemporaneous control groups, except that the test article was replaced by the vehicle (saline) or positive control (1-chloro-2,4-dinitrobenzene).

No reaction to very faint redness was observed on the test article animals during the induction phase of the study. No reaction to very faint redness was observed at the test article naive site (right side) approximately twenty-four and forty-eight hours after the challenge application.

No reaction was observed on any of the vehicle control animals after any of the induction applications. Twenty-four and forty-eight hours after the challenge application, no reaction was observed at either the vehicle or test article sites.

No reaction to faint redness was observed for the positive control animals during the induction phase of the study. No reaction to faint redness was observed at 24 hours after the challenge and no redness to moderate redness at 48 hours after the challenge.

No reaction was observed for the positive control naive animals at twenty-four and forty-eight hours after the challenge application of the positive control article.

It was concluded therefore, that, under the study conditions utilized, repeated administration of H-21891 did not produce delayed contact hypersensitivity in guinea pigs. It was also concluded that the DNCB, when induced at 0.1% w/v in 50% ethanol: 0.9% saline and challenged at 0.07% w/v in acetone, produced delayed contact hypersensitivity in albino guinea pigs.

On the basis of the nature of effects observed in this study and according to the guide for the labeling of dangerous substances published in the Official Journal of the European Communities (EEC Directive 93/21), H-21891 is not a skin sensitizer.

Guinea Pig Dermal Sensitization - Modified Buehler Method
with H-21891

Summary (continued)

George E Moore 9-20-96

George E. Moore, B.S. Date
Study Director

Reviewed/Audited by:

Diane T. Cook 9-20-96

Diane T. Cook, B.S. Date
Quality Assurance Manager

White Eagle Project Number: [redacted]
Sponsor Study Number: HLO 504-96
Test Article: H-21891
Study Initiation: 06/03/96

Quality Assurance Statement

Inspections	Dates			
Protocol	06/05/96			
Procedures	06/05/96	06/27/96	07/17/96	07/19/96
Raw Data	08/07/96	08/08/96	08/16/96	09/05/96
Draft Report	08/21/96	08/22/96	09/05/96	09/06/96
Final Report	09/20/96			

Reports to Study Director and Management

06/14/96	08/16/96	08/27/96	09/06/96	09/20/96

This review was completed on 09/20/96 and released by:

Diane T. Cook, B.S.
Quality Assurance Manager

TABLE OF CONTENTS

TABLE OF CONTENTS (continued)

WHITE EAGLE TOXICOLOGY LABORATORIES

2003 Lower State Road, Doylestown, Pennsylvania 18901
Telephone: (215) 348-3868
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Protocol Number:	[redacted] (Rev. 04/96)
White Eagle Project Number:	[redacted]
Testing Facility:	White Eagle Toxicology Laboratories 2003 Lower State Road Doylestown, PA 18901
Sponsor:	E. I. du Pont de Nemours and Company Haskell Laboratory for Toxicology and Industrial Medicine Elkton Road, P.O. Box 50 Newark, Delaware 19714
Test Article:	H-21891
Test Article Description:	Tan liquid
Test Article Received:	05/29/96
Vehicle Control Article:	Normal Saline Solution (Vedco Inc., St. Joseph, MO 64504 Lot #5080419, Exp. 8/98)
Vehicle Control Description:	Clear liquid
Positive Control Article:	1-chloro-2, 4-dinitrobenzene (Eastman Kodak Co., Rochester, N.Y. Lot #A11R, Exp. 12/26/98)
Positive Control Description:	Yellow crystal
Positive Control Vehicles:	Ethanol (95%)/Acetone (99.7% pure)
Positive Control Vehicles: Description:	Clear liquids
Dose-Rangefinding Initiated:	06/05/96
In-Life Starting Date:	06/18/96
In-life Completion Date:	07/19/96
Test:	Guinea Pig Dermal Sensitization - Modified Buehler Method*

* Ritz, H.L. and Buehler, E.V., Planning, Conduct, and Interpretation of Guinea Pig Sensitization Patch Tests, current Concepts in Cutaneous Toxicity, Drill & Lazar editors, Academic Press 1980.

Guinea Pig Dermal Sensitization - Modified Buehler Method

Objective of Test: To assess the contact dermal sensitization potential of the test article.

Testing Guidelines: The methods employed in the testing of this test article were similar to those described in the EEC 92/69 Annex V, Part B.6 (1992) 93/21 (1993); the OECD Guidelines for Testing of Chemicals, No. 406, Skin Sensitization, adopted July 17, 1992; U.S. EPA Pesticide Assessment Guidelines, Subdivision F, Hazard Evaluation: Human & Domestic Animals, Series 81-6, 1982; EPA Health Effects Testing Guidelines - Subpart E - Specific Organ/Tissue Toxicity, September 1985, 40 CFR Part 798, and the MAFF Japan Testing Guidelines for Toxicology Studies, Dermal Sensitization Study, 59 NohSan No. 4200, 1985.

Good Laboratory Practice: This study was conducted in compliance with the U.S. EPA FIFRA Good Laboratory Practice Standards (40 CFR 160), U.S. EPA TSCA Good Laboratory Practice Standards (40 CFR 792), OECD Principles of Good Laboratory Practice (c(81)30(Final), Annex 2), and MAFF Japan Good Laboratory Practice Standards (59 NohSan No. 3850).

Justification for Test System: The laboratory albino guinea pig was the system of choice since it is required in the EPA, OECD, EEC and MAFF Japan guidelines for this type of study and has a long history of use in dermal sensitization studies.

Justification for Route of Administration: The specific route of administration (dermal) is required in the testing guidelines used for this study.

IACUC Review: The Protocol for this study was reviewed by White Eagle's Institutional Animal Care and Use Committee (IACUC), and complies with acceptable standards for animal welfare and humane care.

Animals: The adult male Hartley guinea pig, weighing 457 to 557 grams, was used for this study. The animals were obtained from Ace Animals Inc., Boyertown, PA 19512, (U.S.D.A. License #23-B-009). Screen animals weighed between 366 and 465 grams. Animals (except screen animals) were weighed prior to initiation, weekly (6-8 days), and after the final dermal evaluation.

The animals (except for screens) were randomized into groups using computer generated random numbers. The initial body weights (Day -1) were statistically evaluated by Analysis of Variance (ANOVA) using CSS: Statistica, 3.1 copyright, Statsoft Inc. 1991.

The animals were housed and maintained in accordance with standards set forth in the Guide for the Care and Use of Laboratory Animals (National Institutes of Health Publication No. 86-23). The guinea pigs were acclimated to the laboratory for at least 7 days prior to the initial induction application.

Animals: (continued)

The animals were individually identified by color coding with solvent-resistant markers (Blue, Red, White and Green). Each cage was identified with a cage card displaying the project number, date initiated, animal numbers, group, sex and responsible technician's initials. Clinical observations were performed daily during the study. All test animals were euthanized with an intraperitoneal injection of sodium pentobarbital at the conclusion of the study.

Husbandry Conditions:

Temperature: 18°C - 27°C (65°F - 80°F)

Relative Humidity, 42 - 71 %

Light: 12 hour light/dark cycle

Diet: Purina Certified Guinea Pig Diet #5026 and tap water were provided ad libitum. Based on our current knowledge, no contaminants are known to be in this diet or water which might be expected to interfere with the objectives of the study.

Caging: Stainless steel cages with elevated wire mesh flooring, 1 guinea pig/cage.

Dose Range-finding:

Prior to initiation of the study, the irritation potential was determined. Two groups of four unexposed animals were exposed to two concentrations of the test material by the patching technique described in the induction stage. In this test, both sides of the animal were closely clipped and one group was exposed to the test article as supplied (100%) and at a 75% concentration in 0.9% saline and a second group was exposed to concentrations of 50% and 25% in 0.9% saline. The test site was wiped with deionized water following the removal of the patch, examined and scored for irritation according to the Scale for Evaluation of Dermal Irritation (Interpretation of Results section) at 24 and 48 hours from the initial patch application.

No reaction was observed at the 100% (as supplied), 75%, 50% or 25% sites. The induction and challenge applications were performed using the test article, as supplied.

Method of Study Performance:

Test Article Group

Induction Stage:

A group of twenty (20) guinea pigs was closely clipped over the induction site (approximately 1.5 X 1.5 inches) on their left flank during the day prior to the day of initiation and repeated as necessary.

Based on the range-finding results, the induction applications were performed using the test article, as supplied. A 0.5 mL portion of the test article, was applied to a 25 mm HillTop Chamber™ (HillTop Research Inc., Cincinnati, OH 45242) on a piece of dental dam (approximately 2 inches x 2 inches). After positioning the chamber and occlusive dental dam onto the prepared skin test site, plastic wrap was wrapped snugly around the trunk of the animal and overwrapped with Elastikon™.

Method of Study
Performance:
(continued)

Induction Stage:
(continued) Test Article Group (continued)

Approximately six hours later, the chamber was removed, and the test site was wiped with deionized water. The test site was examined following removal of the patch and scored for irritation according to the Scale for Evaluation of Dermal Irritation (Interpretation of Results section) at approximately 24 and 48 hours from the initial patch application. The procedure was performed once a week for three weeks (7-day intervals), for a total of three, six-hour applications with the test article.

Vehicle Control Group

A group of ten (10) guinea pigs was closely clipped over the induction site (approximately 1.5 X 1.5 inches) on their left flank during the day prior to the day of initiation and repeated as necessary.

A 0.5 mL portion of 0.9% saline was applied to a 25 mm HillTop Chamber™ on a piece of dental dam (approximately 2 inches x 2 inches). After positioning the chamber and occlusive dental dam onto the prepared skin test site, plastic wrap was wrapped snugly around the trunk of the animal and overwrapped with Elastikon™.

Approximately six hours later, the chamber was removed, and the test site was wiped with deionized water. The treated site was scored for irritation according to the Scale for Evaluation of Dermal Irritation (Interpretation of Results section) at approximately 24 and 48 hours from the initial patch application. The procedure was performed once a week for three weeks (7-day intervals), for a total of three, six-hour applications with the vehicle.

Positive Control

A group of five (5) guinea pigs was closely clipped over the induction site (approximately 1.5 X 1.5 inches) on their left flank during the day prior to the day of initiation and repeated as necessary.

A 0.5 mL portion of 0.1% 1-chloro-2,4-dinitrobenzene (DNCB) w/v in 50% ethanol: 0.9% saline was applied to a 25 mm HillTop Chamber™ on a piece of dental dam (approximately 2 inches x 2 inches). After positioning the chamber and occlusive dental dam onto the prepared skin test site, plastic wrap was wrapped snugly around the trunk of the animal and overwrapped with Elastikon™.

Method of Study Performance: (continued)

Induction Stage: (continued)

Positive Control (continued)

Approximately six hours later, the chamber was removed, and the test site was wiped with ethanol and then with deionized water. The test site was examined following removal of the patch and scored for irritation according to the Scale for Evaluation of Dermal Irritation (Interpretation of Results section) at approximately 24 and 48 hours after the initial patch application. The procedure was performed once a week for three weeks (7-day intervals), for a total of three, six-hour applications with DNCB.

Challenge Stage:

Test Article Group

After the third induction application, the animals were rested for approximately 15 days. At the termination of the rest period, a challenge application, using the procedures described previously, was applied to a naive challenge site (on the clipped right flank). Based on the range-finding results, the challenge application was performed using the test article, as supplied. The challenge application remained on for approximately 6 hours. Upon removal of the challenge application, the contact area was wiped with deionized water. Approximately 24 and 48 hours after the challenge application, the sites were examined for dermal irritation and signs of elicited sensitization, according to the Scale for Evaluation of Dermal Irritation (Interpretation of Results section).

Vehicle Control Group

After the third induction application, the animals were rested for approximately 15 days. At the termination of the rest period, using the procedures described previously, the vehicle control group was challenged with 0.9% saline on the clipped caudal left flank. The test article, as supplied, was placed on the naive clipped right flank.

The challenge application remained on for approximately 6 hours. Upon removal of the challenge application, the contact areas were wiped with deionized water. Approximately 24 and 48 hours after the challenge application, the sites were examined for dermal irritation and signs of elicited sensitization, according to the Scale for Evaluation of Dermal Irritation (Interpretation of Results section).

Method of Study Performance: (continued)

Challenge Stage: (continued)

Positive Control Group

After the third induction application, the animals were rested for approximately 15 days. At the termination of the rest period, a challenge application, 0.07% DNCB in acetone, using the procedures described previously, was applied to a naive challenge site (on the clipped right flank). At the time of challenge, a group of five naive animals was also treated with 0.07% DNCB in acetone, using procedures described previously. The challenge application for these groups remained on for approximately 6 hours. Upon removal of the challenge application, the contact area was wiped with acetone and then with deionized water. Approximately 24 and 48 hours after the challenge application, the sites were examined for dermal irritation and signs of elicited sensitization, according to the Scale for Evaluation of Dermal Irritation (Interpretation of Results section).

Statistical Analysis: Individual body weight percentage gain was statistically evaluated by Analysis of Variance (ANOVA), with the Neuman Keuls test applied in the event of a significant finding ($P<0.05$) for the data as a whole. Statistical analysis was performed using CSS: Statistica, 3.1 copyright, Statsoft Inc. 1991.

Mortality, Morbidity and Termination: No deaths occurred during the study. All animals were euthanized with an intraperitoneal injection of sodium pentobarbital at the conclusion of the study.

Data Retention: All raw data generated, all records accumulated during the performance of this study and a copy of the final report will be retained at White Eagle Toxicology Laboratories for a period of ten (10) years, after which time the data will be shipped to the Sponsor or, discarded upon written request from the Sponsor.

Personnel: The following is a list of personnel for this study:

George E. Moore, Study Director
Shashi P. Logani
Richard J. Hershman
Stanley Sekelewski
Lisa Wilkoski

Test Article and Positive Control Characterization: The Sponsor was responsible for test article characterization and stability determination prior to initiation of the study.

Details regarding the identity, strength, stability, purity and composition of the test article are in the possession of the Sponsor. Information supplied to White Eagle Toxicology Laboratories regarding characterization of the test article will be filed in the archives of White Eagle Toxicology Laboratories, Doylestown, Pennsylvania.

Details regarding the identity, strength, stability, purity, and composition of the positive control are provided by the Manufacturer and are on file at White Eagle Toxicology Laboratories, Doylestown, Pennsylvania. The positive control has been examined and has been found to be soluble in ethanol and acetone.

Mixtures or dilutions were not analyzed by White Eagle or the Sponsor.

Interpretation of Results: All non-eschar and non-necrotic test sites were scored on a scale of 0 to 3 as indicated in Table I.

TABLE I

Scale for Evaluation of Dermal Irritation

0	=	no reaction
0.5	=	very faint redness (usually nonconfluent)
1	=	faint redness (usually confluent)
2	=	moderate redness
3	=	severe redness with/without swelling

Note: 0.5 scores were recorded in the raw data as a +

Scores of 1 or greater in the test group are required to be indicative of sensitization. If scores of one (1) or greater are seen on the vehicle control animals, then the reactions of the test article group animals that exceed the most severe vehicle control reactions are considered to be positive scores. Incidence is reported as the number of positive animals in each group divided by the total number of animals tested in that group. Severity is reported as the sum of the test grades divided by the total number of animals tested in a given group determined for both 24 and 48 hours. Grades of + are equal to 0.5 for calculation of severity indices. All average grades are to be rounded off to the nearest tenth of a unit. A minimum of two out of five positive control animals must be shown to have elicited a positive reaction (Scores $\geq$ 1) to the positive control material to validate the test system for this particular sample.

Interpretation of Results: (continued)

If there is no evidence of sensitization in the study, it is concluded that the risk of sensitizing humans is minimal. If results are seen which indicate animals in the test group show significant responsiveness compared to the vehicle control group, it is concluded that subsequent exposure of humans to the material may involve substantial risk of sensitization. This rating scale is not fixed rigidly. Scientific evaluation of the data and judgement will determine whether the material will be classified as a sensitizer or not.

Results: Tables 2 through 7

Dose Range-finding

During the dose range-finding portion of the study, the test article, when applied dermally as supplied and at 75%, 50% and 25% concentrations in 0.9% saline did not produce any dermal irritation at 24 and 48 hours post application. The induction and challenge phases of the study were performed using 0.5 mL of the test article, as supplied.

Test Article Group - Induction and Challenge

All test animals appeared to be normal throughout the study. All animals exhibited an overall gain in body weight during the study (-1 through 32 days). One of twenty animals lost weight between Days 28 and 32. The water valve was functional and the animal had feed. A statistically significant difference was identified between the individual body weight percentage gains of the test article group and each of the other three groups of animals (Analysis of Variance).

No reaction to very faint redness was observed during the induction phase of the study. No reaction to very faint redness was observed at the test article naive site (right side) approximately twenty-four and forty-eight hours after the challenge application. The incidence of sensitization was 0/20.

Results:
(continued)

Vehicle Control Group

All vehicle control animals appeared normal and exhibited an overall gain in body weight during the observation period (-1 through 32 days). A statistically significant difference was identified between the individual body weight percentage gains of the vehicle control group and the test article group. There were no statistically significant differences identified between the individual body weight percentage gains of the vehicle control group of animals and any of the other two groups of animals (Analysis of Variance).

No reaction was observed on any animals after any of the induction applications as well as approximately 24 and 48 hours after the challenge application at either the vehicle or test article sites. Saline is not a sensitizer.

Positive Control Group

All positive control animals appeared normal and exhibited an overall gain in body weight during the observation period (-1 through 32 days). A statistically significant difference was identified between the individual body weight percentage gains of the positive control group and the test article group. There were no statistically significant differences identified between the individual body weight percentage gains of the positive control group of animals and any of the other two groups of animals (Analysis of Variance).

No reaction to faint redness was observed during the induction phase of the study. No reaction to faint redness was observed at 24 hours after the challenge and no redness to moderate redness 48 hours after the challenge. The incidence of sensitization was 3/5 and severity was 0.4 at 24 hours and 0.9 at 48 hours.

The positive control (1-chloro-2,4-dinitrobenzene), is a dermal sensitizer in this study.

Naive Positive Control Group

All positive control naive animals appeared normal and exhibited an overall gain in body weight during the observation period (-1 through 32 days). One of five animals lost weight between Days 28 and 32. The water valve was functional and the animal had feed. A statistically significant difference was identified between the individual body weight percentage gains of the positive control naive group and the test article group. There were no statistically significant differences identified between the individual body weight percentage gains of the positive control naive group of animals and any of the other groups of animals (Analysis of Variance).

Results:
(continued)

Naive Positive Control Group (continued)

No reaction was observed at twenty-four and forty-eight hours after the challenge application of the positive control article.

Positive Control - α-hexylcinnamaldehyde, tech, 85%

A second positive control was run at White Eagle Labs (Appendix D) in order to document the effect of a weak sensitizer in this test system.

Conclusion:

The test article, H-21891, when induced and challenged at a dose level of 0.5 mL of test article, as supplied, is not a dermal sensitizer in albino guinea pigs.

The positive control, 1-chloro-2,4-dinitrobenzene, when induced at a dose level of 0.1% w/v concentration in a 50% ethanol: 0.9% saline solution and challenged at a dose level of 0.07% w/v concentration in acetone is a dermal sensitizer in albino guinea pigs.

Signatures:

Submitted by White Eagle Toxicology Laboratories:

George C Moore 9-20-96

George E. Moore, B.S. Date
Study Director

Reviewed/Audited by:

Diane T. Cook 9-20-96

Diane T. Cook, B.S. Date
Quality Assurance Manager

TABLE 2

Dose Range-finding Scores

Screen #1 - Left Side
0.5 mL of H-21891

Temporary Animal Number	24 Hours	48 Hours
142	0	0
143	0	0
144	0	0
145	0	0

Screen #2 - Right Side
75% concentration of H-21891 in 0.9% saline

Temporary Animal Number	24 Hours	48 Hours
142	0	0
143	0	0
144	0	0
145	0	0

Body Weights (g)

Temporary Animal #142	393
Temporary Animal #143	380
Temporary Animal #144	380
Temporary Animal #145	366

TABLE 2 (continued)

Dose Range-finding Scores

Screen #3 - Left Side
50% concentration of H-21891 in 0.9% saline

Temporary Animal Number	24 Hours	48 Hours
146	0	0
147	0	0
148	0	0
149	0	0

Screen #4 - Right Side
25% concentration of H-21891 in 0.9% saline

Temporary Animal Number	24 Hours	48 Hours
146	0	0
147	0	0
148	0	0
149	0	0

Body Weights (g)

Temporary Animal #146	375
Temporary Animal #147	382
Temporary Animal #148	418
Temporary Animal #149	465

TABLE 3

Guinea Pig Dermal Irritation/Sensitization Scores

Test Article - H-21891
Induction

Study Animal Number	Application 1		Application 2		Application 3	
	24 Hrs	48 Hrs	24 Hrs	48 Hrs	24 Hrs	48 Hrs
1	0	0	0	0	0	0
2	0	0	0	+	+	0
3	0	0	+	0	0	0
4	0	0	0	0	0	0
5	0	0	+	0	0	0
6	0	0	0	0	0	0
7	0	0	0	0	0	+
8	0	0	+	0	0	0
9	0	0	+	0	0	0
10	0	0	0	0	+	+
11	0	0	0	0	0	0
12	0	0	0	0	0	0
13	0	0	+	+	+	+
14	0	0	0	0	0	0
15	0	0	0	0	0	0
16	0	0	+	0	0	0
17	0	0	0	0	0	0
18	0	0	0	0	+	+
19	0	0	0	0	0	0
20	0	0	+	0	0	0

Note: All induction applications were performed with 0.5 mL of the test article, as supplied.

TABLE 3(continued)

Guinea Pig Dermal Irritation/Sensitization Scores

Test Article - H-21891
Challenge

Study Animal Number	24 Hrs	48 Hrs
1	0	0
2	0	0
3	+	0
4	0	0
5	0	+
6	0	0
7	0	0
8	+	0
9	0	0
10	0	0
11	0	0
12	0	0
13	0	0
14	0	0
15	0	+
16	0	0
17	0	+
18	0	0
19	0	0
20	0	0

Note: The challenge application was performed with 0.5 mL of the test article, as supplied.

TABLE 4

Guinea Pig Dermal Irritation/Sensitization Scores

Vehicle Control

Study Animal Number	Hrs:	Induction - 0.9% Saline 1 24	1 48	2 24	2 48	3 24	3 48	Challenge H-21891 24	Challenge H-21891 48	Challenge 0.9% Saline 24	Challenge 0.9% Saline 48
21		0	0	0	0	0	0	0	0	0	0
22		0	0	0	0	0	0	0	0	0	0
23		0	0	0	0	0	0	0	0	0	0
24		0	0	0	0	0	0	0	0	0	0
25		0	0	0	0	0	0	0	0	0	0
26		0	0	0	0	0	0	0	0	0	0
27		0	0	0	0	0	0	0	0	0	0
28		0	0	0	0	0	0	0	0	0	0
29		0	0	0	0	0	0	0	0	0	0
30		0	0	0	0	0	0	0	0	0	0

Note: The challenge application of the test article sites was performed with 0.5 mL of the test article, as supplied.

TABLE 5

Guinea Pig Dermal Irritation/Sensitization Scores

Positive Control (DNCB) and Naive Positive Control Induction

Study Animal Number	Application 1		Application 2		Application 3		Challenge	
	24 Hrs	48 Hrs	24 Hrs	48 Hrs	24 Hrs	48 Hrs	24 Hrs	48 Hrs
31	0	+	1	+	1	1	1	1
32	0	0	+	0	+	1	+	0
33	+	+	1	1	1	1	0	2
34	0	0	1	1	1	1	+	1
35	0	0	1	1	1	1	0	+

Positive Control Naive

		Challenge	
		24 Hrs	48 Hrs
36	no induction applications	0	0
37	no induction applications	0	0
38	no induction applications	0	0
39	no induction applications	0	0
40	no induction applications	0	0

Note: All sites stained yellow; however, erythema could be scored.

Induction:
DNCB Preparation = 0.1% DNCB w/v in 50% ethanol (95% Clear Spring Grain Alcohol, Clearspring Distilling Co., Clearmont and Frankfort, KY and Cincinnati, OH, Lot #10465177, Exp. 6/18/97) and 0.9% Normal Saline Solution (Vedco Inc., St. Joseph, MO 64504, Lot #5080419, Exp. 8/98).

Challenge:
DNCB Preparation = 0.07% DNCB in acetone (Allied Signal Inc., Morristown, NJ, Lot #020795 Exp. 5/98).

TABLE 6

Daily Observations

Day -1:	All animals in all groups appeared to be normal.
Day 1:	All animals in all groups appeared to be normal.
Day 2:	All animals in all groups appeared to be normal.
Day 3:	All animals in all groups appeared to be normal.
Day 4:	All animals in all groups appeared to be normal.
Day 5:	All animals in all groups appeared to be normal.
Day 6:	All animals in all groups appeared to be normal.
Day 7:	All animals in all groups appeared to be normal.
Day 8:	All animals in all groups appeared to be normal.
Day 9:	All animals in all groups appeared to be normal.
Day 10:	All animals in all groups appeared to be normal.
Day 11:	All animals in all groups appeared to be normal.
Day 12:	All animals in all groups appeared to be normal.
Day 13:	All animals in all groups appeared to be normal.
Day 14:	All animals in all groups appeared to be normal.
Day 15:	All animals in all groups appeared to be normal.
Day 16:	All animals in all groups appeared to be normal.
Day 17:	All animals in all groups appeared to be normal.
Day 18:	All animals in all groups appeared to be normal.
Day 19:	All animals in all groups appeared to be normal.
Day 20:	All animals in all groups appeared to be normal.
Day 21:	All animals in all groups appeared to be normal.
Day 22:	All animals in all groups appeared to be normal.
Day 23:	All animals in all groups appeared to be normal.
Day 24:	All animals in all groups appeared to be normal.
Day 25:	All animals in all groups appeared to be normal.
Day 26:	All animals in all groups appeared to be normal.
Day 27:	All animals in all groups appeared to be normal.
Day 28:	All animals in all groups appeared to be normal.
Day 29:	All animals in all groups appeared to be normal.
Day 30:	All animals in all groups appeared to be normal.
Day 31:	All animals in all groups appeared to be normal.
Day 32:	All animals in all groups appeared to be normal.

TABLE 7

Individual Body Weights (g)

Test Article - H-21891

Study Animal Number	Initial†	Day 7	Day 14	Day 21	Day 28	Final Day 32	Change (-1 to 32)
1	530	586	641	694	756	771	+241
2	464	492	531	589	626	645	+181
3	517	558	588	638	658	682	+165
4	496	529	562	611	672	673	+177
5	538	577	611	657	699	709	+171
6	474	504	540	579	626	646	+172
7	507	540	571	623	665	679	+172
8	543	574	609	656	707	717	+174
9	481	518	561	603	629	641	+160
10	525	567	619	669	716	739	+214
11	508	535	597	616	681	650	+142
12	536	567	625	686	743	747	+211
13	473	499	550	601	623	634	+161
14	491	531	571	639	690	703	+212
15	524	554	620	678	714	727	+203
16	518	562	625	687	747	757	+239
17	496	539	591	645	709	717	+221
18	470	505	537	581	634	642	+172
19	512	560	595	647	683	688	+176
20	541	582	627	700	752	766	+225
Mean	507	544	589	640	687	697	+189
Std.Dev.	25	29	34	38	45	45	29

Vehicle Control - Saline

Study Animal Number	Initial†	Day 7	Day 14	Day 21	Day 28	Final Day 32	Change (-1 to 32)
21	501	541	586	640	669	676	+175
22	533	577	619	667	740	742	+209
23	464	526	570	640	718	730	+266
24	557	608	663	715	787	788	+231
25	489	542	589	640	721	726	+237
26	484	535	564	621	667	674	+190
27	521	583	624	689	747	753	+232
28	464	512	542	575	623	627	+163
29	537	582	626	661	697	715	+178
30	506	577	641	722	782	788	+282
Mean	506	558	602	657	715	722	+216
Std.Dev.	31	31	38	44	52	51	40

† = Body weights taken on Day -1 (the day before the first induction application of test article or vehicle).

TABLE 7(continued)

Individual Body Weights (g)

Positive Control (Dinitrochlorobenzene)

Study Animal Number	Initial†	Day 7	Day 14	Day 21	Day 28	Final Day 32	Change (-1 to 32)
31	499	547	598	636	697	703	+204
32	542	547	631	694	734	753	+211
33	528	581	642	696	768	769	+241
34	491	553	603	661	738	743	+252
35	457	525	615	685	745	756	+299
Mean	503	551	618	674	736	745	+241
Std.Dev.	33	20	19	26	26	25	38

Naive Positive Control (Dinitrochlorobenzene)

Study Animal Number	Initial†	Day 7	Day 14	Day 21	Day 28	Final Day 32	Change (-1 to 32)
36	514	567	635	703	764	770	+256
37	486	508	585	637	703	699	+213
38	522	565	622	684	745	761	+239
39	537	618	687	734	792	792	+255
40	477	535	577	634	677	684	+207
Mean	507	559	621	678	736	741	+234
Std.Dev.	25	41	44	43	46	47	23

† = Body weights taken on Day -1

APPENDIX A

COPY OF PROTOCOL COVER SHEET & PROTOCOL

PROTOCOL [redacted] (Rev. 04/96)

Guinea Pig Dermal Sensitization - Modified Buehler Method

Testing Facility:
White Eagle Toxicology Laboratories
2003 Lower State Road
Doylestown, PA 18901

Sponsor:
E.I. du Pont de Nemours and Company
Haskell Laboratory for Toxicology and
Industrial Medicine
Newark, DE 19714

2189/ mpa 5-29-96

Test Article: H-21665 (Tan Liquid)

White Eagle Project Number:	[redacted]	To be Completed by White Eagle
Test Article Description:	Tan Liquid	
Test Article Receipt Date:	5/29/96	
Proposed In-Life Starting Date:	6/24/96 @ 6/14/96	
Proposed In-Life Completion Date:	7/19/96	

REGULATORY COMPLIANCE INFORMATION

The Sponsor is responsible for test article characterization prior to initiation of the study:

1. The identity, strength, composition & purity of the Test Article has been documented by the Sponsor as of 5/17/96.
2. The Sponsor has labeled the Test Article container(s) with the name, expiration date, batch number, and where applicable storage conditions ☑ YES ☐ NO
3. The Test Article has been determined by the Sponsor to be stable under specific test and storage conditions as of 5/17/96.
4. If applicable, reserve samples of Test Article/Vehicle MIXTURES are to be RETURNED TO SPONSOR FOR ANALYSIS and STABILITY TESTING ☐ YES ☑ NO

EPA CONFIDENTIALITY STATEMENT

☑ A. Sponsor will include their own EPA Confidentiality Statement in the Final Report
☐ B. White Eagle will include standard EPA Confidentiality Statement in the Final Report
If "B", select one of the following:
☐ No Data Confidentiality Claims ☐ Data Confidentiality Claims

This study does not unnecessarily duplicate previous experiments nor is there an acceptable in-vitro method that can substitute for the use of animals.

PROTOCOL AMENDMENTS

PROTOCOL APPROVAL

Testing Facility:		Sponsor:	
George E Moore	6-3-96	Gregory S. Lalia	5/17/96
Study Director	Date	Sponsor Representative	Date

[redacted]

White Eagle Toxicology Laboratories

2003 Lower State Road Doylestown, Pennsylvania, 18901
Telephone: (215) 348-3868
Fax: (215) 348-5081

Protocol Number:

[redacted]

Protocol Name:

Guinea Pig Dermal Sensitization - Modified Buehler Method

WHITE EAGLE TOXICOLOGY LABORATORIES

2003 Lower State Road Doylestown, Pennsylvania, 18901
Telephone: (215) 348-3868
Fax: (215) 348-5081

Protocol Number:	[redacted] (Rev. 04/96) [redacted]
Project Number:	{to be completed by White Eagle Toxicology Laboratories}
Testing Facility:	White Eagle Toxicology Laboratories 2003 Lower State Road Doylestown, PA 18901
Sponsor:	E. I. du Pont de Nemours and Company Haskell Laboratory for Toxicology and Industrial Medicine Elkton Road, P.O. Box 50 Newark, DE 19714
Test Article:	{to be specified}
Test Article Description:	{to be specified}
Positive Control Article:	1-chloro-2, 4-dinitrobenzene (Eastman Kodak Co., Rochester, N.Y.)
Positive Control Description:	Yellow crystal
Positive Control Vehicles:	Ethyl Alcohol (95%) Acetone (99.7%)
Positive Control Vehicle Descriptions:	Clear liquids
Test Article Received:	{to be specified}
Proposed In-Life Starting Date:	{to be specified}
Proposed In-Life Completion Date:	{to be specified}
Test:	Guinea Pig Dermal Sensitization - Modified Buehler Method*
Objective of Test:	To assess the contact dermal sensitization potential of the test article.
Testing Guidelines:	The methods employed in the testing of this test article are similar to those described in the U.S. EPA Pesticide Assessment Guidelines, Subdivision F, Hazard Evaluation: Human & Domestic Animals, Series 81-6, 1982; EPA Health Effects Testing Guidelines - Subpart E - Specific Organ/Tissue Toxicity, September 1985, 40 CFR Part 798; the OECD Guidelines for Testing of Chemicals, No. 406, Skin Sensitization, adopted July 17, 1992; EEC 92/69 Annex V, Part B.6 (1992) 93/21 (1993); and the MAFF Japan Testing Guidelines for Toxicology Studies, Dermal Sensitization Study, 59 NohSan No. 4200, 1985.

*Ritz, H.L. and Buehler, E.V., Planning, Conduct, and Interpretation of Guinea Pig Sensitization Patch Tests, current Concepts in Cutaneous Toxicity, Drill & Lazar editors, Academic Press 1980.

Protocol Number: [redacted] (Rev. 04/96) Page 2 of 9

Guinea Pig Dermal Sensitization - Modified Buehler Method

[redacted]

Good Laboratory Statement: This study is conducted in compliance with the U.S. EPA FIFRA Good Laboratory Practice Standards (40 CFR 160), U.S. EPA TSCA Good Laboratory Practice Standards (40 CFR 792), OECD Principles of Good Laboratory Practice (c(81)30(Final), Annex 2), and MAFF Japan Good Laboratory Practice Standards (59 NohSan No. 3850).

Justification for Test System: The laboratory albino guinea pig is the system of choice since it is required in the EPA, EEC, OECD, and MAFF Japan guidelines for this type of study and has a long history of use in dermal sensitization studies.

Justification for Route of Administration: The specific route of administration (dermal) is required in the testing guidelines for this study.

Animals: The adult male Hartley guinea pig, weighing 300 to 550 grams, is used for this study. The animals are obtained from Davidson Mill Breeding Labs, Jamesburg, NJ 08831, U.S.D.A. License #22-A-003, Ace Animals Inc., Boyertown, PA 19512, U.S.D.A. License #23-B-009, Elm Hill Breeding Labs, Chelmsford, MA 01824, U.S.D.A. License #14-B-006 or Camm Research Lab Animals, Wayne, NJ 07470, U.S.D.A. License #22-005. The source of animals will be documented in the study records. The animals (except for screens) are weighed prior to initiation, weekly and upon completion of the study. The animals are randomized using computer generated random numbers into groups and the initial body weights (Day -1) are statistically evaluated by Analysis of Variance (ANOVA). Statistical analyses and random number generation are performed using CSS: Statistica, 3.1 copyright, Statsoft Inc. 1991. Re-challenge naive vehicle controls, if utilized, are not weighed prior to initiation of the study, but are weighed prior to re-challenge.

The animals are housed and maintained in accordance with standards set forth in the Guide for the Care and Use of Laboratory Animals (National Institutes of Health Publication No. 86-23). The guinea pigs are acclimated to the laboratory for at least 7 days prior to the initial induction application. Clinical observations are performed daily on all animals during the study.

The animals are individually identified by color coding with water- and/or solvent-resistant markers. Each cage is identified with a cage card displaying the project number, date initiated, animal numbers, group, sex, and responsible technician's initials.

IACUC Review: The Protocol for this study has been reviewed by White Eagle's Institutional Animal Care and Use Committee (IACUC) and complies with acceptable standards for animal welfare and humane care.

Protocol Number: [redacted] (Rev. 04/96) Page 3 of 9

Guinea Pig Dermal Sensitization - Modified Buehler Method

Husbandry Conditions: Temperature: 18°C - 26°C (64.4°F - 78.8°F) [redacted]

Relative Humidity, 50 ± 20%

Light: 12-hour light/dark cycle

Diet: Purina Certified Guinea Pig Diet #5026 and tap water are provided ad libitum. Based on our current knowledge, no contaminants are known to be in this diet or water which might be expected to interfere with the objectives of the study.

Caging: Stainless steel with elevated wire mesh flooring, 1-5 guinea pigs/cage.

Selection of Vehicle: If the test article is a solid, it is ground into a fine powder and the preparation of a homogenous paste, suspension or solution of the test article suitable for application will be prepared. If the test article is a liquid, the dilutions will be prepared with the appropriate vehicle.

Dose Rangefinding: Prior to initiation of the study, a series of screens is conducted in order to determine the irritation potential of the test article. Groups of 4 unexposed animals are weighed and exposed to different concentrations of the test material by the patching technique described in the induction stage. In this test, both sides of the animal are closely clipped and exposed to the various concentrations of the material (one concentration per side). The treated sites are scored for irritation using the scale included in Table I (Interpretation of Results) at approximately 24 and 48 hours. Concentrations at several intervals (if necessary) are utilized to determine the mildly irritating concentration of the test article and the highest non-irritating concentration. If the test article is a powder, then 0.5 g of test article is moistened (or mixed) with the appropriate vehicle (considered 100%) and applied to a 25 mm Hill Top Chamber™ (Hill Top Research Inc., Cincinnati, OH 45242). All concentrations of a powdered test article up to 50% are prepared as a percentage of test article in 0.5 g or 0.5 mL of solution, suspension, or mixture. Before beginning the study, the Sponsor is contacted and provided with results of the irritation screen. The Sponsor will then set the induction and challenge concentrations.

Method of Study Performance: Test Article Group

Induction Stage: A group of twenty (20) guinea pigs is closely clipped over the induction site on their left flank (approximately 1.5 X 1.5 inches) during the day prior to the day of initiation and repeated as necessary. If the induction site is moved, an additional area (sufficient for patching) is closely clipped at the new site.

Guinea Pig Dermal Sensitization - Modified Buehler Method

Method of Study Performance: [redacted]

Test Article Group (continued)

Induction Stage: (continued)

Based upon the range-finding results, a mildly irritating dose will be used for the induction stage. A 0.5 mL portion of a liquid test article or 0.5 g of a solid test article (moistened with the appropriate vehicle) is applied to a 25 mm Hill Top Chamber™ which is placed onto the test site and covered with a piece of dental dam (approximately 2 inches X 2 inches). After positioning the chamber and the occlusive dental dam onto the prepared skin test site, plastic material is wrapped snugly around the trunk of the animal and covered with elastic tape.

Approximately six hours later, the chamber is removed and the test site is wiped with an appropriate solvent. The treated site is scored for irritation, using the scale included in Table I, at approximately 24 and 48 hours from the initial application. The procedure is performed once a week for three weeks, for a total of three, six-hour applications with the test material. The interval between induction exposures may vary from 6 to 8 days. Should pronounced irritation be observed at the induction site, the site may be moved for the next induction application, with its relative location fully documented.

Vehicle Control Group

A group of ten (10) guinea pigs is closely clipped over the induction site towards the head on their left flank (approximately 1.5 X 1.5 inches) during the day prior to the day of initiation and repeated as necessary. If the induction site is moved, an additional area (sufficient for patching) is closely clipped at the new site.

A 0.5 mL portion of the vehicle (used for the test article group) is applied to a 25 mm Hill Top Chamber™ which is placed onto the test site and covered with a piece of dental dam (approximately 2 inches X 2 inches). After positioning the chamber and the occlusive dental dam onto the prepared skin test site, plastic material is wrapped snugly around the trunk of the animal and covered with elastic tape. If the test article does not require a vehicle (liquid dosed as supplied), saline will be used for induction and challenge control.

Approximately six hours later, the chamber is removed and the test site is wiped with an appropriate solvent. The treated site is scored for irritation according to the scale included in Table I at approximately 24 and 48 hours from the initial application. The procedure is performed once a week for three weeks, for a total of three, six-hour applications with the vehicle. The interval between induction exposures may vary from 6 to 8 days. Should pronounced irritation be observed at the induction site, the site may be moved for the next induction application, with its relative location fully documented.

Guinea Pig Dermal Sensitization - Modified Buehler Method

Method of Study Performance: [redacted]

Positive Control

A group of five (5) guinea pigs is closely clipped over the induction site on their left flank (approximately 1.5 X 1.5 inches) during the day prior to the day of initiation and repeated as necessary. If the induction site is moved, an additional area (sufficient for patching) is closely clipped at the new site.

A 0.5 mL portion of 0.1% 1-chloro-2,4-dinitrobenzene (DNCB) w/v in 50% ethanol: 0.9% saline is applied to a 25 mm Hill Top Chamber™ which is placed onto the test site and covered with a piece of dental dam (approximately 2" X 2"). After positioning the chamber and the occlusive dental dam onto the prepared skin test site, plastic material is wrapped snugly around the trunk of the animal and covered with elastic tape.

Approximately six hours later, the chamber is removed, and the test site is wiped with the appropriate solvent. The test site is scored for irritation according to the scale included in Table I at approximately 24 and 48 hours from the initial application. The procedure is performed once a week for three weeks, for a total of three, six-hour applications with DNCB. The interval between induction exposures may vary from 6 to 8 days. Should pronounced irritation be observed at the induction site, the site may be moved for the next induction application, with its relative location fully documented.

Challenge Stage: Test Material Group

After the third induction application, the animals are rested for approximately 14 to 16 days. At the termination of the rest period, a challenge application, using the procedures described previously, is applied to a challenge site (on the right flank). Based upon the range-finding results, the highest non-irritating dose will be used for the challenge phase. The challenge application remains on for approximately 6 hours. Upon removal of the challenge application, the contact area is wiped with an appropriate solvent. In the event of questionable findings, a rechallenge application of all animals may be performed on a second naive site no sooner than six days after primary challenge, along with a group of 5 naive animals taken from the colony pool, serving as vehicle controls. Dates of application and observations are recorded. Approximately 24 and 48 hours after the challenge (and rechallenge if necessary) application, the sites are examined for dermal irritation and/or signs of elicited sensitization, according to the scale included in Table I.

Guinea Pig Dermal Sensitization - Modified Buehler Method

Challenge Stage: Vehicle Control Group [redacted]

After the third induction application, the animals are rested for approximately 14 to 16 days. At the termination of the rest period, the vehicle control group is challenged with the vehicle on the left flank (caudal) and the test article on the right flank using the procedures described previously. The challenge application remains on for approximately 6 hours. Upon removal of the challenge application, the contact areas are wiped with an appropriate solvent. Dates of application and observations are recorded. Approximately 24 and 48 hours after the challenge application, the sites are examined for dermal irritation and/or signs of elicited sensitization according to the scale included in Table I.

Positive Control Group

After the third induction application, the animals are rested for approximately 14 to 16 days. At the termination of the rest period, 0.07% DNCB in acetone, using the procedures described previously, is applied to a challenge site (on the right flank). A group of five naive animals is also treated using the procedures described previously at the time of challenge. The challenge application for these groups remains on for approximately 6 hours. Upon removal of the challenge application, the contact area is wiped with an appropriate solvent. Dates of application and observations are recorded. Approximately 24 and 48 hours after the challenge application, the sites are examined for dermal irritation and/or signs of elicited sensitization according to the scale included in Table I.

Rechallenge: The Sponsor is notified of animals that are considered sensitized. Verbal instructions for rechallenge are given by the Sponsor. All animals considered sensitized can be rechallenged six days after primary challenge, but not before. The positive control group is not rechallenged. The animals are evaluated as described in "Interpretation of Results".

Data Retention: All raw data generated, all records accumulated during the performance of this study and a copy of the final report will be retained at White Eagle Toxicology Laboratories for a period of ten (10) years, after which time the data will be shipped to the Sponsor or, discarded upon written request from the Sponsor.

Test Article Characterization: The Sponsor will be responsible for test article characterization and stability determination prior to initiation of the study.

Details regarding the identity, strength, stability, purity, and composition of the test article will be provided by the Sponsor. Information supplied to White Eagle Toxicology Laboratories regarding characterization of the test article will be filed in the archives of White Eagle Toxicology, Doylestown, Pennsylvania.

Guinea Pig Dermal Sensitization - Modified Buehler Method [redacted]

Positive Control Characterization: Details regarding the identity, strength, stability, purity, and composition of the positive control are provided by the Manufacturer and are on file at White Eagle Toxicology Laboratories, Doylestown, Pennsylvania. The positive control has been examined and has been found to be soluble in ethanol and acetone.

Any mixtures or dilutions will not be analyzed by White Eagle or the Sponsor.

Statistical Analysis: Statistical Methods: Individual body weight percentage gain is statistically evaluated by Analysis of Variance (ANOVA), with the Neuman Keuls test applied in the event of a significant finding ($P<0.05$) for the data as a whole. Statistical analyses are performed using CSS: Statistica, 3.1 copyright, Statsoft Inc. 1991.

Mortality, Morbidity and Termination: All animals found dead during the observation period or euthanized in extremis are necropsied to establish, if possible, the cause of death or illness. All test animals are euthanized by CO_2 inhalation or IP pentobarbital at the completion of the study.

Protocol Changes: All changes in or revisions of the approved protocol and the reasons will be documented, signed by the study director, dated and maintained with the protocol.

Records Maintained: Records to be maintained include:
- irritation scores for screen animals
- irritation scores for redness and swelling (induction and challenge)
- daily clinical observations
- initial, weekly, and final body weights (including group means, standard deviation, and change between initial and final)
- test article preparation
- calculations and statistical analyses
- randomization scheme

Final Report: A final report is written which includes, but is not limited to, the items cited in U.S. EPA FIFRA Good Laboratory Practice Standards (40 CFR Part 160), Subpart J, Records and Reports, U.S. EPA TSCA Good Laboratory Practice Standards (40 CFR 792), Subpart J Records and Reports, OECD Principles of Good Laboratory Practice, c(81)30 Annex 2 (Final), Section II, Part 9.2, Content of the Final Report, and MAFF Japan Good Laboratory Practice Standards, 59 NohSan No. 3850, Chapter VIII, Report and Records.

Protocol Number: [redacted]v. 04/96) Page 8 of 9

Guinea Pig Dermal Sensitization - Modified Buehler Method [redacted]

Interpretation of Results: All non-eschar and non-necrotic test sites are scored on a scale of 0 to 3 as indicated in the following Table I. When edema is present with scores of +, 1, or 2 for erythema, it will be documented.

TABLE I

Scale for Evaluation of Dermal Irritation

0	=	no reaction
0.5	=	very faint redness (usually nonconfluent)
1	=	faint redness (usually confluent)
2	=	moderate redness
3	=	severe redness with/without swelling

Note: 0.5 scores will be recorded in the raw data as a +

Scores of 1 or greater in the test group are required to be indicative of sensitization. If scores of one (1) or greater are seen on the vehicle control animals, then the reactions of the test article group animals that exceed the most severe vehicle control reactions are considered to be positive scores. INCIDENCE is reported as the number of positive animals in each group divided by the total number of animals tested in that group. SEVERITY is reported as the sum of the test grades divided by the total number of animals tested in a given group determined for both 24 and 48 hours. Grades + are equal to 0.5 for calculation of severity indices. All average grades are to be rounded off to the nearest tenth of a unit. A minimum of two out of five positive control animals must be shown to have elicited a positive reaction (Scores $\geq$ one (1)) to the positive control material to validate the test system.

If there is no evidence of sensitization in the study, it is concluded that the risk of sensitizing humans is minimal. If results are seen which indicate animals in the test group show significant responsiveness compared to the vehicle control group, it is concluded that subsequent exposure of humans to the material may involve substantial risk of sensitization. This rating scale is not fixed rigidly.

Scientific evaluation of the data and judgement will be used to decide whether material will be classified as a sensitizer or not.

Protocol Number: [redacted] (Rev. 04/96) Page 9 of 9

Guinea Pig Dermal Sensitization - Modified Buehler Method

Signatures: Submitted by White Eagle Toxicology Laboratories: [redacted]

George E Moore 4-15-96

George E. Moore, B.S. Date
Study Director

Approved by:

Gregory S. Ladin 4/16/96

Sponsor Representative Date

APPENDIX B

DEVIATIONS FROM PROTOCOL

WHITE EAGLE TOXICOLOGY LABORATORIES

2003 Lower State Road, Doylestown, Pennsylvania 18901
Telephone: (215) 348-3868
Fax: (215) 348-5081

DEVIATIONS FROM PROTOCOL #1, #2, #3, #4, #5

White Eagle Project Number:
Protocol Number: [redacted] (Rev. 04/96)
Sponsor: E. I. du Pont de Nemours and Company
Test Article: H-21891
Test: Guinea Pig Dermal Sensitization - Modified Buehler Method
GLP Compliance: 40 CFR 160 40 CFR 792 OECD MAFF Japan

1) The Temperature during the In-Life phase of this study was 65°F-80°F instead of 64.4°F-78.8°F as stated in the protocol.

2) The Relative Humidity during the In-Life phase of this study was 46%-71% instead of 30%-70% as stated in the protocol.

3) One vehicle control group animal was 7 grams above the body weight range of 300 to 550 g as stated in the protocol.

The following are amendments to protocol which were not approved in writing until the completion of the study; therefore, they are presented as deviations.

4) Page 7, Final Report Section

Add: A second positive control (α-hexylcinnamaldehyde, tech, 85%) will be reported as an appendix.

Reason: Sponsor's Request

5) All positive control animals were wiped with the appropriate vehicle and then deionized water, instead of the appropriate vehicle only. The deionized water was used to remove the residual vehicle.

These deviations are not thought to have affected the integrity of this study.

Submitted by: George E Moore — 9-6-96
George E. Moore, B.S. — Date
Study Director

Reviewed by: [signature] — 9-6-96
Diane T. Cook, B.S. — Date
Quality Assurance Manager

Approved by: Gregory S. Ladics — 9/18/96
Sponsor Representative — Date

APPENDIX C

Guinea Pig Dermal Sensitization - Modified Buehler Method
with Positive Control (α-hexylcinnamaldehyde, tech, 85%)

WHITE EAGLE TOXICOLOGY LABORATORIES

2003 Lower State Road, Doylestown, Pennsylvania 18901
Telephone: (215) 348-3868
Fax: (215) 348-5081

Study Title

Guinea Pig Dermal Sensitization - Modified Buehler Method
with Positive Control (α-hexylcinnamaldehyde, tech, 85%)

Laboratory Project ID

Haskell Laboratory Project ID 306-96

Data Requirement

EEC 92/69 - Annex V - Part B.6 (1992) - 93/21 (1993),
OECD Guidelines for the Testing of Chemicals, No. 406,
Skin Sensitization, adopted July 17, 1992
U.S. EPA Pesticide Assessment Guidelines Subdivision F,
Hazard Evaluation: Human & Domestic Animals, Series 81-6, 1982
EPA Health Effects Testing Guidelines - Subpart E -
Specific Organ/Tissue Toxicity, September 1985, 40 CFR Part 798,
and MAFF Japan Testing Guidelines for Toxicology Studies,
Dermal Sensitization Study, 59 NohSan No. 4200, 1985

Study Completed On

26 June 1996

Contracting Laboratory

White Eagle Toxicology Laboratories
2003 Lower State Road
Doylestown, Pennsylvania 18901

Author

George E. Moore, B.S.

for

E.I. du Pont de Nemours and Company
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Study Monitor

Gregory S. Ladics, Ph.D.

Medical Research No.

[redacted]

White Eagle Project No.

[redacted]

Page 1 of 43

THIS PAGE RESERVED FOR SPECIFIC COUNTRY REQUIREMENTS

WHITE EAGLE TOXICOLOGY LABORATORIES

2003 Lower State Road, Doylestown, Pennsylvania 18901
Telephone: (215) 348-3868

GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

White Eagle Project Number: [redacted]
Sponsor: E. I. du Pont de Nemours and Company
Test Article: α-hexylcinnamaldehyde, tech, 85%
Test: Guinea Pig Dermal Sensitization - Modified Buehler Method
Protocol Number: [redacted] (Rev. 04/96)

This study was carried out in compliance with the Principles of Good Laboratory Practice (GLP) as promulgated by the following agencies:

U.S. Environmental Protection Agency FIFRA as stated in the Federal Register, 40 CFR, Part 160, August 17, 1989.

U.S. Environmental Protection Agency TSCA as stated in the Federal Register, 40 CFR, Part 792, August 17, 1989.

OECD Principles of Good Laboratory Practice (c(81)30(Final), Annex 2)

MAFF Japan Good Laboratory Practice Standards (59 NohSan No. 3850)

The study was conducted in compliance with the following exception:

Treatment solutions were not analyzed for concentration, uniformity or stability of the test and control articles. The procedures used by trained personnel to prepare the treatment solutions insured:

a) The accuracy of concentration because the test article diluent (vehicle) and positive control diluents were accurately measured with graduated devices. The positive control article was weighed on an analytical balance accurate to at least 0.001g. The test article was weighed on a balance accurate to at least 0.01g.

b) Uniformity because all solutions were mixed prior to administration to the test system; and

c) Stability because treatment solutions were prepared just prior to addition to the test system.

George E. Moore — 6-26-96

George E. Moore, B.S.
Study Director

Date

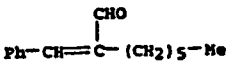
Sponsor: DuPont Agricultural Products
Haskell Laboratory for Toxicology and Industrial Medicine
Elkton Road, P.O. Box 50
Newark, DE 19714

E. I. du Pont de Nemours and Company - α-hexylcinnamaldehyde, tech, 85%

Guinea Pig Dermal Sensitization - Modified Buehler Method

GENERAL INFORMATION

Test Material

Substance Tested:	Octanal, 2-(phenylmethylene)-
Synonyms/Other Codes:	• HCA • Hexyl cinnamaldehyde • α-Hexylcinnamaldehyde • 2-Hexylcinnamaldehyde • Hexyl cinnamic aldehyde • α-Hexylcinnamic aldehyde • α-Hexylcinnamyl aldehyde • α-N-hexyl-beta-phenylacrolein • Octanal, 2-(phenylmethylene)- • Lot Number 10021 HF
CAS Registry Number:	101-86-0
Haskell No.:	21780
Purity:	85% (Supplied by Aldrich Chemical Co.; Milwaukee, WI 53233); Expiration date: 5/99
Major Impurities:	None known to be of toxicological significance at this time.
Stability:	The test substance appears to be stable under the conditions of the study; no evidence of instability was observed.
Physical Form:	Yellow liquid
Sponsor:	DuPont Agricultural Products E. I. du Pont de Nemours and Company Barley Mill Plaza Wilmington, Delaware 19880-0038
Structure:	
Study Initiated - Completed:	4/19/96 - 6/26/96
In-Life Phase Initiated - Completed:	4/30/96 - 5/31/96

E. I. du Pont de Nemours and Company - α-hexylcinnamaldehyde, tech, 85%

Guinea Pig Dermal Sensitization - Modified Buehler Method

Guinea Pig Dermal Sensitization - Modified Buehler Method with Positive Control (α-hexylcinnamaldehyde, tech, 85%)

Summary

The potential of α-hexylcinnamaldehyde, tech, 85% (HCA) to produce delayed contact hypersensitivity in guinea pigs was assessed by the Modified Buehler Method. Thirty (30) test article animals were induced and challenged by occluded application of HCA to clipped intact guinea pig skin. The same procedures were carried out on contemporaneous control groups except that the test article was replaced by vehicle.

No reaction to severe redness with or without swelling and eschar was observed during the induction phase of the 2.0% HCA group in ethanol. Approximately twenty-four hours and forty-eight hours after the challenge application, no reaction to severe redness with or without swelling was observed at the test article naive site (right side). The incidence of sensitization after the challenge application of 1.0% HCA in acetone was 8/10 and the severity was 1.4 at 24 hours and 1.1 at 48 hours.

No reaction to faint redness was observed during the induction phase of the 1.0% HCA group in ethanol. Approximately twenty-four hours and forty-eight hours after the challenge application, no reaction to moderate redness was observed at the test article naive site (right side). The incidence of sensitization after the challenge application of 0.75% HCA in acetone was 2/8 and the severity was 0.8 at 24 hours and 0.1 at 48 hours.

No reaction to faint redness was observed during the induction phase of the 0.5% HCA group in ethanol. Approximately twenty-four hours after the challenge application, no reaction to very faint redness was observed at the test article naive site (right side) and no reaction was observed at 48 hours. The incidence of sensitization after the challenge application of 0.25% HCA in acetone was 0/10.

No reaction to very faint redness was observed during the induction phase of the 2.0% HCA vehicle group. Very faint redness was noted for 1/10 animals at the test article site, approximately 24 hours after the challenge application. Other than this one test site, no reaction was observed approximately 24 and 48 hours after the challenge application of either the vehicle (acetone) or test article (1.5% HCA in acetone).

No reaction to very faint redness was observed during the induction phase of the 1.0% HCA vehicle group. Very faint redness was noted for 1/10 animals at the test article site, approximately 24 hours after the challenge application. Other than this one test site, no reaction was observed approximately 24 and 48 hours after the challenge application of either the vehicle (acetone) or test article (0.75% HCA in acetone).

No reaction was observed on any 0.5% HCA vehicle animals after any of the induction applications as well as approximately 24 and 48 hours after the challenge application of either the vehicle (acetone) or test article (1.0% HCA in acetone) sites.

White Eagle Project Number: [redacted] DuPont HLO 306-96

E. I. du Pont de Nemours and Company - α-hexylcinnamaldehyde, tech, 85%

Guinea Pig Dermal Sensitization - Modified Buehler Method

Guinea Pig Dermal Sensitization - Modified Buehler Method
with Positive Control (α-hexylcinnamaldehyde, tech, 85%)

Summary (continued)

It was concluded therefore, that, under the study conditions utilized, repeated administration of 2.0% and 1.0% α-hexylcinnamaldehyde, tech, 85% (HCA) in ethanol and challenged with 1.50% and 0.5% HCA in acetone, respectively, produced delayed contact hypersensitivity in guinea pigs.

On the basis of the nature of effects observed in this study and according to the guide for the labeling of dangerous substances published in the Official Journal of the European Communities (EEC Directive 93/21), α-hexylcinnamaldehyde, tech, 85% is a skin sensitizer.

George E Moore 6-26-96

George E. Moore, B.S. Date
Study Director

Reviewed/Audited by:

Diane T. Cook 6-26-96

Diane T. Cook, B.S. Date
Quality Assurance Manager

White Eagle Project Number: [redacted] DuPont HLO 306-96

E. I. du Pont de Nemours and Company - α-hexylcinnamaldehyde, tech, 85%

Guinea Pig Dermal Sensitization - Modified Buehler Method

White Eagle Project Number: [redacted]
Sponsor Study Number: 306-96
Test Article: α-hexylcinnamaldehyde, tech, 85% (HCA)
Study Initiation: 04/19/96

Quality Assurance Statement

Inspections	Dates		
Protocol	04/23/96		
Procedures	04/23/96	04/24/96	04/29/96
	05/01/96	05/14/96	
Raw Data	06/13/96	06/14/96	
Draft Report	06/14/96	06/17/96	
Final Report	06/26/96		

Reports to Study Director and Management

06/18/96 06/26/96

This review was completed on 06/26/96 and released by:

Diane T. Cook, B.S.
Quality Assurance Manager

White Eagle Project Number: [redacted] DuPont HLO 306-96

TABLE OF CONTENTS

TABLE OF CONTENTS (continued)

WHITE EAGLE TOXICOLOGY LABORATORIES

2003 Lower State Road, Doylestown, Pennsylvania 18901
Telephone: (215) 348-3868
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Protocol Number:	[redacted] (Rev. 04/96)
Project Number:	[redacted]
Testing Facility:	White Eagle Toxicology Laboratories 2003 Lower State Road Doylestown, PA 18901
Sponsor:	E. I. du Pont de Nemours and Company Haskell Laboratory for Toxicology and Industrial Medicine Elkton Road, P.O. Box 50 Newark, Delaware 19714
Test Article:	α-hexylcinnamaldehyde, tech, 85% (HCA), Lot # 10021 HF, Exp. date 5/99, Supplied by White Eagle Labs.
Test Article Description:	Clear liquid
Test Article Received:	05/01/94
Vehicle Control Articles:	Ethyl Alcohol (95%) (Clearspring Distilling Co., Clearmont & Frankfort, KY and Cincinnati, OH, Lot #09015145, Exp. 10/10/96) Acetone (99.7% pure) (Allied Signal Inc., Morristown, NJ 07962, Lot #020795, Exp. 5/98)
Vehicle Control Descriptions:	Clear liquids
Screens Initiated:	04/23/96
In-Life Study Initiated:	04/30/96
In-life Study Completed:	05/31/96
Test:	Guinea Pig Dermal Sensitization - Modified Buehler Method*
Objective of Test:	To validate the test system using a known sensitizer, α-hexylcinnamaldehyde, tech, 85% (HCA).

* Ritz, H.L. and Buehler, E.V., Planning, Conduct, and Interpretation of Guinea Pig Sensitization Patch Tests, current Concepts in Cutaneous Toxicity, Drill & Lazar editors, Academic Press 1980.

E. I. du Pont de Nemours and Company - α-hexylcinnamaldehyde, tech, 85%

Guinea Pig Dermal Sensitization - Modified Buehler Method

Testing Guidelines: The methods employed in the testing of this test article were similar to those described in the EEC 92/69 Annex V, Part B.6 1992 93/21 (1993); the OECD Guideline for Testing of Chemicals, No. 406, Skin Sensitization, adopted July 17, 1992; U.S. EPA Pesticide Assessment Guidelines, Subdivision F, Hazard Evaluation: Human & Domestic Animals, Series 81-6, 1982; EPA Health Effects Testing Guidelines - Subpart E - Specific Organ/Tissue Toxicity, September 1985, 40 CFR Part 798, and the MAFF Japan Testing Guidelines for Toxicology Studies, Dermal Sensitization Study, 59 NohSan No. 4200, 1985.

Good Laboratory Statement: This study was conducted in compliance with the U.S. EPA FIFRA Good Laboratory Practice Standards (40 CFR 160), U.S. EPA TSCA Good Laboratory Practice Standards (40 CFR 792), OECD Principles of Good Laboratory Practice (c(81)30(Final), Annex 2), and MAFF Japan Good Laboratory Practice Standards (59 NohSan No. 3850).

Justification for Test System: The laboratory albino guinea pig was the system of choice since it is required in the EPA, OECD, EEC and MAFF Japan guidelines for this type of study and has a long history of use in dermal sensitization studies.

Justification for Route of Administration: The specific route of administration (dermal) is required in the testing guidelines used for this study.

Animals: The adult male Hartley guinea pig, weighing 373 to 517 grams, was used for this study. The animals were obtained from Ace Animals Inc., Boyertown, PA 19512 U.S.D.A. License #23-B-009. Screen animals were weighed prior to initiation and all others were weighed prior to initiation, weekly (6-8 days), and upon completion of the study.

The animals were randomized into groups and the initial body weights (Day -1) were statistically evaluated by Analysis of Variance (ANOVA). Statistical analyses and random number generation were performed using CSS: Statistica, 3.1 copyright, Statsoft Inc. 1991.

The animals were housed and maintained in accordance with standards set forth in the Guide for the Care and Use of Laboratory Animals (National Institutes of Health Publication No. 86-23). The guinea pigs were acclimated to the laboratory for at least 7 days prior to the initial induction application. Clinical observations were performed daily on all animals during the study.

The animals were individually identified by color coding with water and/or solvent-resistant markers. Each cage was identified with a cage card displaying the project number, date initiated, animal numbers, group, sex and responsible technician's initials. All animals were euthanized with pentobarbital at the conclusion of the study.

E. I. du Pont de Nemours and Company - α-hexylcinnamaldehyde, tech, 85%

Guinea Pig Dermal Sensitization - Modified Buehler Method

IACUC Review: The Protocol for this study was reviewed by White Eagle's Institutional Animal Care and Use Committee (IACUC), and complies with acceptable standards for animal welfare and humane care.

Husbandry Conditions: Temperature: 18°C - 26°C (64°F - 78°F)

Relative Humidity, %: 24 - 88

Light: 12-hour light/dark cycle

Diet: Purina Certified Guinea Pig Diet #5026 and tap water were provided ad libitum. Based on our current knowledge, no contaminants are known to be in this diet or water which might be expected to interfere with the objectives of the study.

Caging: Stainless steel with elevated wire mesh flooring, 4-5 guinea pigs/cage.

Test Article Vehicle: Ethyl Alcohol (95%) was used as the test article vehicle for the induction phase of this study and acetone (99.7%) for the challenge phase of the study.

Dose Rangefinding: Historically, 5.0% and 2.5% HCA in 95% ethanol have produced no irritation to faint irritation after the first induction application and necrosis after the second or third induction application. Based on this steep dermal response, concentrations of 2.0%, 1.0% and 0.5% HCA in 95% ethanol were used for the induction phase of the study. Challenge concentrations were determined using two groups of 4 unexposed animals. The animals were weighed and exposed to concentrations of 1.50%, 0.75%, 0.50% and 0.25% HCA in acetone by the patching technique described in the induction phase.

Other than very faint redness noted for 1/4 animals (1.50% in acetone) at 24 hours, no reaction was observed for any of the four concentrations.

Challenge concentrations of 1.50%, 0.75% and 0.25% in acetone were chosen for the 2.0%, 1.0% and 0.5% in 95% ethanol induction application animals respectively.

White Eagle Project Number: [redacted] DuPont HLO 306-96

E. I. du Pont de Nemours and Company - α-hexylcinnamaldehyde, tech, 85%

Guinea Pig Dermal Sensitization - Modified Buehler Method

Method of Study Performance: Test Article Group

Induction Stage: Three groups of ten (10) guinea pigs were closely clipped over the induction site on their left flank (approximately 1.5 X 1.5 inches) during the day prior to the day of initiation and repeated as necessary.

Based upon historical data, dose levels of 2.0%, 1.0% and 0.5% w/v of HCA in 95% ethanol, were used for the induction stage. A 0.5 mL portion of the appropriate concentration of HCA in 95% ethanol was applied to a 25 mm Hill Top Chamber™ which was placed onto the test site and covered with a piece of dental dam (approximately 2 inches X 2 inches). After positioning the chamber and the occlusive dental dam onto the prepared skin test site, plastic wrap was wrapped snugly around the trunk of the animal and overwrapped with Elastikon™.

Approximately six hours later, the chamber was removed and the test site was wiped with ethanol and then deionized water. The treated site was scored for irritation, using the scale included in Table I, at approximately 24 and 48 hours from the initial application. The procedure was performed once a week for three weeks, for a total of three, six-hour applications with the appropriate concentration of HCA. The interval between induction exposures was 7 days.

Vehicle Control Group

Three groups of ten (10) guinea pigs were closely clipped over the induction site towards the head on their left flank (approximately 1.5 X 1.5 inches) during the day prior to the day of initiation and repeated as necessary.

A 0.5 mL portion of the vehicle (95% ethanol) was applied to a 25 mm Hill Top Chamber™ which was placed onto the test site and covered with a piece of dental dam (approximately 2 inches X 2 inches). After positioning the chamber and the occlusive dental dam onto the prepared skin test site, plastic wrap was wrapped snugly around the trunk of the animal and overwrapped with Elastikon™.

Approximately six hours later, the chamber was removed and the test site is wiped with deionized water. The treated site was scored for irritation according to the scale included in Table I at approximately 24 and 48 hours from the initial application. The procedure was performed once a week for three weeks, for a total of three, six-hour applications of 95% ethanol. The interval between induction exposures was 7 days.

E. I. du Pont de Nemours and Company - α-hexylcinnamaldehyde, tech, 85%

Guinea Pig Dermal Sensitization - Modified Buehler Method

Challenge Stage: Test Material Group

After the third induction application, the animals were rested for 15 days. At the termination of the rest period, a challenge application, using the procedures described previously, was applied to a challenge site (on the right flank). Based on the challenge dose rangefinding; animals induced with 2.0%, 1.0% or 0.5% HCA in 95% ethanol were challenged with 1.50%, 0.75% and 0.25% HCA in acetone respectively. The challenge application remained on for approximately 6 hours. Upon removal of the challenge application, the contact area was wiped with acetone and then deionized water. Dates of application and observations were recorded. Approximately 24 and 48 hours after the challenge application, the sites were examined for dermal irritation and/or signs of elicited sensitization, according to the scale included in Interpretation of Results (Table 1).

Vehicle Control Group

After the third induction application with the vehicle (95% ethanol), the 3 groups of ten animals were rested for 15 days. At the termination of the rest period, each of the 3 groups was challenged with acetone on the left flank (caudal). Each of the three groups also received either 1.50%, 0.75% or 0.25% HCA in acetone on the right flank. The challenge application remained on for approximately 6 hours. Upon removal of the challenge application, the contact areas were wiped with acetone and then deionized water. Dates of application and observations were recorded. Approximately 24 and 48 hours after the challenge application, the sites were examined for dermal irritation and/or signs of elicited sensitization according to the scale included in Interpretation of Results (Table 1).

Statistical Analysis: Individual body weight percentage gain was statistically evaluated by Analysis of Variance (ANOVA), with the Neuman Keuls test applied in the event of a significant finding ($P<0.05$) for the data as a whole. Statistical analyses were performed using CSS: Statistica, 3.1 copyright, Statsoft Inc. 1991.

Mortality, Morbidity and Termination: Animals #15 and #45 (1.0% HCA test article group) were found dead during the induction phase of the study (Days 14 and 16, respectively). All other animals were euthanized at the completion of the study with IP injection of pentobarbital.

Data Retention: All raw data generated, all records accumulated during the performance of this study and a copy of the final report will be retained at White Eagle Toxicology Laboratories for a period of ten (10) years, after which time the data will be shipped to the Sponsor or, discarded upon written request from the Sponsor.

E. I. du Pont de Nemours and Company - α-hexylcinnamaldehyde, tech, 85%

Guinea Pig Dermal Sensitization - Modified Buehler Method

Personnel: The following is a list of personnel for this study:

George E. Moore, Study Director
Shashi P. Logani
Richard J. Hershman
Stanley Sekelewski
Lisa Wilkoski

Positive Control Characterization: Details regarding the identity, strength, stability, purity, and composition of the positive control are provided by the Manufacturer and are on file at White Eagle Toxicology Laboratories, Doylestown, Pennsylvania. The positive control has been examined and has been found to be soluble in ethanol and acetone.

Any mixtures or dilutions were not analyzed by White Eagle or the Sponsor.

Interpretation of Results: All non-eschar and non-necrotic test sites were scored on a scale of 0 to 3 as indicated in Table 1.

TABLE 1

Scale for Evaluation of Dermal Irritation

0	=	no reaction
0.5	=	very faint redness (usually nonconfluent)
1	=	faint redness (usually confluent)
2	=	moderate redness
3	=	severe redness with/without swelling

Note: 0.5 scores were recorded in the raw data as a +

Scores of 1 or greater in the test group are required to be indicative of sensitization. If scores of one (1) or greater are seen on the vehicle control animals, then the reactions of the test article group animals that exceed the most severe vehicle control reactions are considered to be positive scores. Incidence is reported as the number of positive animals in each group divided by the total number of animals tested in that group. Severity is reported as the sum of the test grades divided by the total number of animals tested in a given group determined for both 24 and 48 hours. Grades of + are equal to 0.5 for calculation of severity indices. All average grades are to be rounded off to the nearest tenth of a unit. A minimum of two out of ten animals in a group must be shown to have elicited a positive reaction (Scores $\geq$ 1) to HCA to validate the test system.

E. I. du Pont de Nemours and Company - α-hexylcinnamaldehyde, tech, 85%

Guinea Pig Dermal Sensitization - Modified Buehler Method

Results: Tables 2 through 12

Test Article Group - Induction and Challenge

2.0% HCA

All test animals appeared to be normal throughout the study. All animals exhibited an overall gain in body weight during the study (-1 through 32 days). One of ten animals exhibited a loss of body weight between Days 14 and 21 and 3/10 animals exhibited a loss of body weight between Days 29 and 32. The water valves were functional and the animals had feed. There were no statistically significant differences identified between the individual body weight percentage gains of the test article group and any of the other five groups of animals (Analysis of Variance).

No reaction to severe redness with or without swelling and eschar was observed during the induction phase of the study. Approximately twenty-four hours and forty-eight hours after the challenge application, no reaction to severe redness with or without swelling was observed at the test article naive site (right side). The incidence of sensitization after the challenge application of 1.5% HCA in acetone was 8/10 and the severity was 1.4 at 24 hours and 1.1 at 48 hours.

1.0% HCA

Two animals (#15 and #45) were found dead during the induction phase of the study (Days 14 and 16 respectively). All other test animals appeared to be normal throughout the study. All surviving animals exhibited an overall gain in body weight during the study (-1 through 32 days). Three of the eight remaining animals exhibited a loss of body weight between Days 29 and 32. The water valves were functional and the animals had feed. There were no statistically significant differences identified between the individual body weight percentage gains of the test article group and any of the other five groups of animals (Analysis of Variance).

No reaction to faint redness was observed during the induction phase of the study. Approximately twenty-four hours after the challenge application, no reaction to moderate redness was observed at the test article naive site (right side) and no reaction to very faint redness was observed at approximately 48 hours. The incidence of sensitization after the challenge application of 0.75% HCA in acetone was 2/8 and the severity was 0.8 at 24 hours and 0.1 at 48 hours.

E. I. du Pont de Nemours and Company - α-hexylcinnamaldehyde, tech, 85%

Guinea Pig Dermal Sensitization - Modified Buehler Method

Results:
(continued)

Test Article Group - Induction and Challenge (continued)

0.5% HCA

All test animals appeared to be normal throughout the study. All animals exhibited an overall gain in body weight during the study (-1 through 32 days). One of ten animals exhibited a loss of body weight between Days 29 and 32. The water valve was functional and the animal had feed. There were no statistically significant differences identified between the individual body weight percentage gains of the test article group and any of the other five groups of animals (Analysis of Variance).

No reaction to faint redness was observed during the induction phase of the study. Approximately twenty-four hours after the challenge application, no reaction to very faint redness was observed at the test article naive site (right side) and no reaction at forty-eight hours. The incidence of sensitization after the challenge application of 0.25% HCA in acetone was 0/10.

Vehicle Control Group - Induction and Challenge

2.0% HCA

All vehicle control animals appeared normal and exhibited an overall gain in body weight during the observation period (-1 through 32 days). One of ten animals lost weight between Days 29 and 32. The water valve was functional and the animal had feed. There were no statistically significant differences identified between the individual body weight percentage gains of the vehicle control group of animals and any of the other five groups of animals (Analysis of Variance).

No reaction to very faint redness was observed during the induction phase of the 2.0% HCA vehicle group. Very faint redness was noted for 1/10 animals at the test article site, approximately 24 hours after the challenge application. Other than this one test site, no reaction was observed approximately 24 and 48 hours after the challenge application of either the vehicle (acetone) or test article (1.5% HCA in acetone).

1.0% HCA

All vehicle control animals appeared normal and exhibited an overall gain in body weight during the observation period (-1 through 32 days). One of ten animals lost weight between Days 21 and 29 and 1/10 animals between Days 29 and 32. The water valves were functional and the animals had feed. There were no statistically significant differences identified between the individual body weight percentage gains of the vehicle control group of animals and any of the other five groups of animals (Analysis of Variance).

E. I. du Pont de Nemours and Company - α-hexylcinnamaldehyde, tech, 85%

Guinea Pig Dermal Sensitization - Modified Buehler Method

Results:
(continued)

Vehicle Control Group - Induction and Challenge (continued)

1.0% HCA (continued)

No reaction to very faint redness was observed during the induction phase of the 1.0% HCA vehicle group. Very faint redness was noted for 1/10 animals at the test article site, approximately 24 hours after the challenge application. Other than this one test site, no reaction was observed approximately 24 and 48 hours after the challenge application of either the vehicle (acetone) or test article (0.75% HCA in acetone).

0.5% HCA

All vehicle control animals appeared normal and exhibited an overall gain in body weight during the observation period (-1 through 32 days). One of ten animals lost weight between Days -1 and 7. The water valve was functional and the animal had feed. There were no statistically significant differences identified between the individual body weight percentage gains of the vehicle control group of animals and any of the other five groups of animals (Analysis of Variance).

No reaction was observed on any 0.5% HCA vehicle animals after any of the induction applications as well as approximately 24 and 48 hours after the challenge application of either the vehicle (acetone) or test article (1.0% HCA in acetone) sites.

Conclusion: The test article, α-hexylcinnamaldehyde, tech, 85% (HCA), when induced with concentrations of 2.0% or 1.0% in ethanol and challenged with 1.5% and 0.75% HCA in acetone respectively, appears to be a dermal sensitizer in albino guinea pigs.

White Eagle Project Number: [redacted] DuPont HLO 306-96

E. I. du Pont de Nemours and Company - α-hexylcinnamaldehyde, tech, 85%

Guinea Pig Dermal Sensitization - Modified Buehler Method

Signatures: Submitted by White Eagle Toxicology Laboratories:

George E Moore 6-26-96
George E. Moore, B.S. Date
Study Director

Reviewed/Audited by:

Diane T. Cook 6-26-96
Diane T. Cook, B.S. Date
Quality Assurance Manager

E. I. du Pont de Nemours and Company - α-hexylcinnamaldehyde, tech, 85%

Guinea Pig Dermal Sensitization - Modified Buehler Method

TABLE 2

Challenge Screens

Screen #1 - Left Side
1.50% concentration in Acetone

Animal Number	24 Hours †	48 Hours †
61	0	0
62	0	0
63	0	0
64	+	0

Screen #2 - Right Side
0.75% concentration in Acetone

Animal Number	24 Hours	48 Hours
61	0	0
62	0	0
63	0	0
64	0	0

Body Weights (g)

Animal #61	415
Animal #62	393
Animal #63	409
Animal #64	401

† = Site stained light pale yellow; however, erythema could be scored

E. I. du Pont de Nemours and Company - α-hexylcinnamaldehyde, tech, 85%

Guinea Pig Dermal Sensitization - Modified Buehler Method

TABLE 2 (continued)

Challenge Screens

Screen #3 - Left Side
0.50% concentration in Acetone

Animal Number	24 Hours	48 Hours
65	0	0
66	0	0
67	0	0
68	0	0

Screen #4 - Right Side
0.25% concentration in Acetone

Animal Number	24 Hours	48 Hours
65	0	0
66	0	0
67	0	0
68	0	0

Body Weights (g)

Animal #65	377
Animal #66	400
Animal #67	380
Animal #68	373

White Eagle Project Number: [redacted] DuPont HLO 306-96

E. I. du Pont de Nemours and Company - α-hexylcinnamaldehyde, tech, 85%

Guinea Pig Dermal Sensitization - Modified Buehler Method

TABLE 3

2.0% HCA

Guinea Pig Dermal Irritation/Sensitization Scores

Animal Number	Reading - Hours After Application Number 1		2		3		Challenge	
	24	48	24	48	24	48	24 Hrs*	48 Hrs
17	0	0	1	1	S	S	3	2
18	+	0	1	1	S	S	2	+
27	0	0	0	0	+	+	1	1
31	0	0	0	0	+	+	0	0
32	0	0	+	0	3	3	1	+
35	0	0	+	0	S	S	3	3
41	0	0	1	0	3	3	1	2
47	+	0	2	+	S	S	2	1
55	0	0	1	0	2	2	+	0
57	0	0	0	0	1	1	+	1

* Readings are performed approximately 24 hours after the challenge application

S = Eschar

Note: All 6-hour exposures.

Induction applications - 2.0% HCA in 95% ethanol
Challenge application - 1.5% HCA in acetone

E. I. du Pont de Nemours and Company - α-hexylcinnamaldehyde, tech, 85%

Guinea Pig Dermal Sensitization - Modified Buehler Method

TABLE 4

2.0% HCA - Vehicle Control

Guinea Pig Dermal Irritation/Sensitization Scores

Animal Number	Reading - Hours After Application Number 1		2		3		Challenge Acetone		Challenge 1.5% HCA	
	24	48	24	48	24	48	24 Hrs*	48 Hrs	24 Hrs	48 Hrs
3	0	0	0	0	0	0	0	0	+	0
10	0	0	0	0	0	0	0	0	0	0
16	0	0	0	0	0	0	0	0	0	0
22	0	0	0	0	0	0	0	0	0	0
23	0	0	0	0	0	0	0	0	0	0
28	0	0	0	0	0	0	0	0	0	0
30	0	0	+	0	0	+	0	0	0	0
34	0	0	0	0	0	0	0	0	0	0
37	0	0	0	0	0	0	0	0	0	0
56	0	0	0	0	0	0	0	0	0	0

* Readings are performed approximately 24 hours after the challenge application

Note: All 6-hour exposures.

Induction applications - 95% ethanol
Challenge application - 1.5% HCA in acetone
Challenge application - Acetone

White Eagle Project Number: [redacted] DuPont HLO 306-96

E. I. du Pont de Nemours and Company - α-hexylcinnamaldehyde, tech, 85%

Guinea Pig Dermal Sensitization - Modified Buehler Method

TABLE 5

1.0% HCA

Guinea Pig Dermal Irritation/Sensitization Scores

Animal Number	Reading - Hours After Application Number						Challenge	
	1		2		3		24 Hrs*	48 Hrs
	24	48	24	48	24	48		
4	0	0	+	0	1	1	+	0
5	0	0	0	0	0	0	+	0
7	0	0	0	0	0	0	+	0
11	0	0	0	0	0	0	+	0
12	0	0	+	0	+	+	+	+
15	0	0	0	0	FD			
42	0	0	+	0	0	0	0	0
45	0	0	0	0	FD			
48	0	0	0	0	+	+	2	+
59	0	0	0	0	0	0	2	0

* Readings are performed approximately 24 hours after the challenge application

FD = Animal #15 and #45 were found dead on Days 14 and 16 of the study, respectively.

Note: All 6-hour exposures.

Induction applications - 1.0% HCA in 95% ethanol
Challenge application - 0.75% HCA in acetone

White Eagle Project Number: [redacted] DuPont HLO 306-96

E. I. du Pont de Nemours and Company - α-hexylcinnamaldehyde, tech, 85%

Guinea Pig Dermal Sensitization - Modified Buehler Method

TABLE 6

1.0% HCA - Vehicle Control

Guinea Pig Dermal Irritation/Sensitization Scores

Animal Number	Reading - Hours After Application Number 1		2		3		Challenge Acetone		Challenge 0.75% HCA	
	24	48	24	48	24	48	24 Hrs*	48 Hrs	24 Hrs	48 Hrs
1	0	0	0	0	0	0	0	0	0	0
9	0	0	0	0	0	0	0	0	0	0
20	0	0	0	0	0	0	0	0	0	0
36	0	0	0	0	0	0	0	0	+	0
43	0	0	0	0	0	0	0	0	0	0
46	0	0	+	0	0	0	0	0	0	0
49	0	0	0	0	0	0	0	0	0	0
50	0	0	0	0	0	0	0	0	0	0
51	0	0	0	0	0	0	0	0	0	0
54	0	0	0	0	0	0	0	0	0	0

* Readings are performed approximately 24 hours after the challenge application

Note: All 6-hour exposures.

Induction applications - 95% ethanol
Challenge application - 0.75% HCA in acetone
Challenge application - Acetone

White Eagle Project Number: [redacted] DuPont HLO 306-96

E. I. du Pont de Nemours and Company - α-hexylcinnamaldehyde, tech, 85%

Guinea Pig Dermal Sensitization - Modified Buehler Method

TABLE 7

0.5% HCA

Guinea Pig Dermal Irritation/Sensitization Scores

Animal Number	Reading - Hours After Application Number 1		2		3		Challenge 24 Hrs*	48 Hrs
	24	48	24	48	24	48		
2	0	0	0	0	0	0	0	0
13	0	0	0	0	+	+	0	0
19	0	0	0	0	+	+	0	0
33	0	0	0	0	1	1	0	0
38	0	0	0	0	0	0	0	0
39	0	0	0	0	0	0	0	0
40	0	0	0	0	0	0	0	0
44	0	0	0	0	+	+	+	0
53	0	0	0	0	+	+	0	0
58	0	0	0	0	0	0	0	0

* Readings are performed approximately 24 hours after the challenge application

Note: All 6-hour exposures.

Induction applications - 0.5% HCA in 95% ethanol
Challenge application - 0.25% HCA in acetone

White Eagle Project Number: [redacted] DuPont-HLO-306-96

E. I. du Pont de Nemours and Company - α-hexylcinnamaldehyde, tech, 85%

Guinea Pig Dermal Sensitization - Modified Buehler Method

TABLE 8

0.5% HCA - Vehicle Control

Guinea Pig Dermal Irritation/Sensitization Scores

Animal Number	Reading - Hours After Application Number 1		2		3		Challenge Acetone		Challenge 0.25% HCA	
	24	48	24	48	24	48	24 Hrs*	48 Hrs	24 Hrs	48 Hrs
6	0	0	0	0	0	0	0	0	0	0
8	0	0	0	0	0	0	0	0	0	0
14	0	0	0	0	0	0	0	0	0	0
21	0	0	0	0	0	0	0	0	0	0
24	0	0	0	0	0	0	0	0	0	0
25	0	0	0	0	0	0	0	0	0	0
26	0	0	0	0	0	0	0	0	0	0
29	0	0	0	0	0	0	0	0	0	0
52	0	0	0	0	0	0	0	0	0	0
60	0	0	0	0	0	0	0	0	0	0

* Readings are performed approximately 24 hours after the challenge application

Note: All 6-hour exposures.

Induction applications - 95% ethanol
Challenge application - 0.25% HCA in acetone
Challenge application - Acetone

E. I. du Pont de Nemours and Company - α-hexylcinnamaldehyde, tech, 85%

Guinea Pig Dermal Sensitization - Modified Buehler Method

TABLE 9

Daily Observations

Day -1:	All animals in all groups appeared to be normal.
Day 1:	All animals in all groups appeared to be normal.
Day 2:	All animals in all groups appeared to be normal.
Day 3:	All animals in all groups appeared to be normal.
Day 4:	All animals in all groups appeared to be normal.
Day 5:	All animals in all groups appeared to be normal.
Day 6:	All animals in all groups appeared to be normal.
Day 7:	All animals in all groups appeared to be normal.
Day 8:	All animals in all groups appeared to be normal.
Day 9:	All animals in all groups appeared to be normal.
Day 10:	All animals in all groups appeared to be normal.
Day 11:	All animals in all groups appeared to be normal.
Day 12:	All animals in all groups appeared to be normal.
Day 13:	All animals in all groups appeared to be normal.
Day 14:	Animal #15 (1.0% HCA test article group) was found dead. No gross abnormalities were noted at necropsy. All other animals in all groups appeared to be normal.
Day 15:	All animals in all groups appeared to be normal.
Day 16:	Animal #45 (1.0% HCA test article group) was found dead. Other than green liquid in the gastrointestinal tract, no gross abnormalities were noted at necropsy. All other animals in all groups appeared to be normal.
Day 17:	All animals in all groups appeared to be normal.
Day 18:	All animals in all groups appeared to be normal.
Day 19:	All animals in all groups appeared to be normal.
Day 20:	All animals in all groups appeared to be normal.
Day 21:	All animals in all groups appeared to be normal.
Day 22:	All animals in all groups appeared to be normal.
Day 23:	All animals in all groups appeared to be normal.
Day 24:	All animals in all groups appeared to be normal.
Day 25:	All animals in all groups appeared to be normal.
Day 26:	All animals in all groups appeared to be normal.
Day 27:	All animals in all groups appeared to be normal.
Day 28:	All animals in all groups appeared to be normal.
Day 29:	All animals in all groups appeared to be normal.
Day 30:	All animals in all groups appeared to be normal.
Day 31:	All animals in all groups appeared to be normal.
Day 32:	All animals in all groups appeared to be normal.

E. I. du Pont de Nemours and Company - α-hexylcinnamaldehyde, tech, 85%

Guinea Pig Dermal Sensitization - Modified Buehler Method

TABLE 10

Individual Body Weights (g)

Test Article - 2.0% HCA

Animal Number	Initial†	Day 7	Day 14	Day 21	Day 29	Final Day 32	Change (-1 to 32)
17	456	514	564	621	702	689	+233
18	435	494	550	606	689	689	+254
27	480	564	628	610	679	677	+197
31	460	510	575	635	709	699	+239
32	437	499	552	689	758	763	+326
35	470	553	622	670	750	771	+301
41	432	505	571	626	715	717	+285
47	474	571	645	707	777	790	+316
55	420	476	522	569	624	637	+217
57	483	548	618	703	763	789	+306
Mean	455	523	585	644	717	722	+267
Std.Dev.	22	33	41	46	47	53	45

Vehicle Control - Ethanol/Acetone

Animal Number	Initial†	Day 7	Day 14	Day 21	Day 29	Final Day 32	Change (-1 to 32)
3	476	546	606	661	715	702	+226
10	423	456	520	569	645	653	+230
16	501	550	622	671	765	777	+276
22	429	508	585	649	737	742	+313
23	450	525	612	683	770	776	+326
28	444	521	569	619	673	673	+229
30	498	568	650	710	787	795	+297
34	462	549	629	684	741	752	+290
37	463	523	573	648	706	711	+248
56	469	495	559	617	678	687	+218
Mean	462	524	593	651	722	727	+265
Std.Dev.	26	32	39	41	47	49	40

† = Body weights taken on Day -1 (the day before the first induction application of test article or vehicle).

E. I. du Pont de Nemours and Company - α-hexylcinnamaldehyde, tech, 85%

Guinea Pig Dermal Sensitization - Modified Buehler Method

TABLE 11

Individual Body Weights (g)

Test Article - 1.0% HCA

Animal Number	Initial†	Day 7	Day 14	Day 21	Day 29	Final Day 32	Change (-1 to 32)
4	459	549	613	685	741	729	+270
5	479	538	629	697	785	811	+332
7	442	494	538	568	641	638	+196
11	406	475	517	580	639	626	+220
12	434	502	559	619	694	716	+282
15	469	537	*				
42	433	498	552	596	650	651	+218
45	424	500	563	*			
48	517	598	648	712	763	766	+249
59	475	570	647	711	792	807	+332
Mean	454	526	585	646	713	718	+262
Std.Dev.	32	39	50	61	65	74	51

Vehicle Control - Ethanol/Acetone

Animal Number	Initial†	Day 7	Day 14	Day 21	Day 29	Final Day 32	Change (-1 to 32)
1	475	531	607	662	716	735	+260
9	455	501	556	641	724	728	+273
20	426	482	540	599	581	582	+156
36	479	559	639	712	776	809	+330
43	407	462	507	564	615	618	+211
46	465	547	614	590	697	721	+256
49	469	518	590	603	666	667	+198
50	433	468	519	554	627	627	+194
51	480	537	614	677	721	731	+251
54	453	563	632	636	718	704	+251
Mean	454	517	582	624	684	692	+238
Std.Dev.	25	37	48	51	60	68	49

† = Body weights taken on Day -1 (the day before the first induction application of test article or vehicle).

* = Animals #15 and #45 were found dead on Days 14 and 16 respectively

White Eagle Project Number: [redacted] DuPont HLO 306-96

E. I. du Pont de Nemours and Company - α-hexylcinnamaldehyde, tech, 85%

Guinea Pig Dermal Sensitization - Modified Buehler Method

TABLE 12

Individual Body Weights (g)

Test Article - 0.5% HCA

Animal Number	Initial†	Day 7	Day 14	Day 21	Day 29	Final Day 32	Change (-1 to 32)
2	458	521	577	652	723	731	+273
13	385	440	482	542	599	603	+218
19	442	527	569	656	714	733	+291
33	464	529	603	685	752	788	+324
38	451	519	595	662	737	759	+308
39	462	521	586	654	709	686	+224
40	413	462	500	564	623	639	+226
44	481	560	630	684	739	739	+258
53	507	580	632	708	754	792	+285
58	474	560	618	693	763	770	+296
Mean	454	522	579	650	711	724	+270
Std.Dev.	35	43	51	55	56	63	37

Vehicle Control - Ethanol/Acetone

Animal Number	Initial†	Day 7	Day 14	Day 21	Day 29	Final Day 32	Change (-1 to 32)
6	455	533	608	670	754	773	+318
8	411	479	549	614	685	701	+290
14	472	560	627	701	766	776	+304
21	431	517	590	653	705	716	+285
24	396	490	568	618	705	704	+308
25	421	505	573	628	691	688	+267
26	499	591	680	759	825	845	+346
29	451	519	554	581	631	588	+137
52	516	508	559	610	651	644	+128
60	411	484	547	613	686	687	+276
Mean	446	519	586	645	710	712	+266
Std.Dev.	40	35	42	53	57	72	74

† = Body weights taken on Day -1 (the day before the first induction application of test article or vehicle).

E. I. du Pont de Nemours and Company - α-hexylcinnamaldehyde, tech, 85%

Guinea Pig Dermal Sensitization - Modified Buehler Method

APPENDIX A

COPY OF PROTOCOL COVERSHEET AND PROTOCOL

PROTOCOL [redacted] (Rev. 04/96)

Guinea Pig Dermal Sensitization - Modified Buehler Method

Testing Facility:
White Eagle Toxicology Laboratories
2003 Lower State Road
Doylestown, PA 18901

Sponsor:
E.I. du Pont de Nemours and Company
Haskell Laboratory for Toxicology and
Industrial Medicine
Newark, DE 19714

Test Article: a-Hexylcinnamaldehyde, Tech, 85%

White Eagle Project Number:	[redacted]	To be Completed by White Eagle
Test Article Description:	Clear liquid	
Test Article Receipt Date:	5/1/94	
Proposed In-Life Starting Date:	4-23-96	
Proposed In-Life Completion Date:	5-30-96	

MGM 4/19/96

EPA CONFIDENTIALITY STATEMENT

☒ A. Sponsor will include their own EPA Confidentiality Statement in the Final Report
☐ B. White Eagle will include standard EPA Confidentiality Statement in the Final Report

If "B", select one of the following:
☐ No Data Confidentiality Claims ☐ Data Confidentiality Claims

This study does not unnecessarily duplicate previous experiments nor is there an acceptable in-vitro method that can substitute for the use of animals.

PROTOCOL AMENDMENTS

Prior to signing, an a- and tech, 85% were added to the Test article name - per phone with sponsor
GE Moore 4/19/96

PROTOCOL APPROVAL

Testing Facility:		Sponsor:	
George E Moore	4-19-96	Virginia S. Salvin	4/19/96
Study Director	Date	Sponsor Representative	Date

White Eagle Toxicology Laboratories

2003 Lower State Road Doylestown, Pennsylvania, 18901
Telephone: (215) 348-3868
Fax: (215) 348-5081

[redacted]

Protocol Number:

[redacted]

Protocol Name:

Guinea Pig Dermal Sensitization - Modified Buehler Method

WHITE EAGLE TOXICOLOGY LABORATORIES

2003 Lower State Road, Doylestown, Pennsylvania 18901
Telephone: (215) 348-3868
Fax: (215) 348-5081

Protocol Number:	[redacted] Rev. 04/96) [redacted]
Project Number:	(to be completed by White Eagle Toxicology Laboratories)
Testing Facility:	White Eagle Toxicology Laboratories 2003 Lower State Road Doylestown, Pennsylvania 18901
Sponsor:	E. I. du Pont de Nemours and Company Haskell Laboratory for Toxicology and Industrial Medicine Elkton Road, P.O. Box 50 Newark, DE 19714
Test Article:	Positive Control (a-hexylcinnamaldehyde, tech, 85%)
Test Article Description:	Clear liquid
Test Article Received:	Supplied by White Eagle Toxicology Laboratories
Vehicle Control Articles:	Ethyl Alcohol (95%) Acetone (99.7%)
Vehicle Control Descriptions:	Clear liquids
Proposed In-Life Starting Date:	(to be specified)
Proposed In-Life Completion Date:	(to be specified)
Test:	Guinea Pig Dermal Sensitization - Modified Buehler Method*
Objective of Test:	To validate the test system using a known sensitizer, a-hexylcinnamaldehyde (HCA).
Testing Guidelines:	The methods employed in the testing of this test article are similar to those described in the U.S. EPA Pesticide Assessment Guidelines, Subdivision F, Hazard Evaluation: Human & Domestic Animals, Series 81-6, 1982; EPA Health Effects Testing Guidelines - Subpart E - Specific Organ/Tissue Toxicity, September 1985, 40 CFR Part 798; the OECD Guidelines for Testing of Chemicals, No. 406, Skin Sensitization, adopted July 17, 1992; EEC 92/69 Annex V, Part B.6 (1992), 93/21 (1993); and the MAFF Japan Testing Guidelines for Toxicology Studies, Dermal Sensitization Study, 59 NohSan No. 4200, 1985.

*Ritz, H.L. and Buehler, E.V., Planning, Conduct, and Interpretation of Guinea Pig Sensitization Patch Tests, current Concepts in Cutaneous Toxicity, Drill & Lazar editors, Academic Press 1980.

[redacted]

Good Laboratory Statement: This study is conducted in compliance with the U.S. EPA FIFRA Good Laboratory Practice Standards (40 CFR 160), U.S. EPA TSCA Good Laboratory Practice Standards (40 CFR 792), OECD Principles of Good Laboratory Practice (c(81)30(Final), Annex 2), and MAFF Japan Good Laboratory Practice Standards (59 NohSan No. 3850).

Justification for Test System: The laboratory albino guinea pig is the system of choice since it is required in the EPA, EEC, OECD, and MAFF Japan guidelines for this type of study and has a long history of use in dermal sensitization studies.

Justification for Route of Administration: The specific route of administration (dermal) is required in the testing guidelines for this study.

Animals: The adult male Hartley guinea pig, weighing 300 to 550 grams, is used for this study. The animals are obtained from Davidson Mill Breeding Labs, Jamesburg, NJ. 08831 U.S.D.A. License #22-A-003, Ace Animals Inc., Boyertown, PA 19512 U.S.D.A. License #23-B-009, Elm Hill Breeding Labs, Chelmsford, MA 01824 U.S.D.A. License #14-B-006 or Camm Research Lab Animals, Wayne, NJ 07470 U.S.D.A. License #22-005. The source of animals will be documented in the study records. The animals (except for screens) are weighed prior to initiation, weekly and upon completion of the study. The animals are randomized using computer generated random numbers into groups and the initial body weights (Day -1) are statistically evaluated by Analysis of Variance (ANOVA). Statistical analyses and random number generation are performed using CSS: Statistica, 3.1 copyright, Statsoft Inc. 1991. Re-challenge naive vehicle controls, if utilized, are not weighed prior to initiation of the study, but are weighed prior to re-challenge.

The animals are housed and maintained in accordance with standards set forth in the Guide for the Care and Use of Laboratory Animals (National Institutes of Health Publication No. 86-23). The guinea pigs are acclimated to the laboratory for at least 7 days prior to the initial induction application. Clinical observations are performed daily on all animals during the study.

The animals are individually identified by color coding with water- and/or solvent-resistant markers. Each cage is identified with a cage card displaying the study number, date initiated, animal number(s), group, sex, and responsible technician's initials.

IACUC Review: The Protocol for this study has been reviewed by White Eagle's Institutional Animal Care and Use Committee (IACUC) and complies with acceptable standards for animal welfare and humane care.

Protocol Number: [redacted](Rev. 04/96) Page 3 of 7

Guinea Pig Dermal Sensitization - Modified Buehler Method [redacted]

Husbandry Conditions:

Temperature: 18°C - 26°C (64.4°F - 78.8°F)

Relative Humidity: 50 ± 20 %

Light: 12-hour light/dark cycle

Diet: Purina Certified Guinea Pig Diet #5026 and tap water are provided ad libitum. Based on our current knowledge, no contaminants are known to be in this diet or water which might be expected to interfere with the objectives of the study.

Caging: Stainless steel with elevated wire mesh flooring, 1-5 guinea pigs/cage.

Test Article Vehicle: Ethyl Alcohol (95%) will be used as the test article vehicle for the induction phase of this study and acetone (99.7%) for the challenge phase of this study.

Dose Rangefinding: Historically, 5.0% and 2.5% HCA in 95% ethanol have produced no irritation to faint irritation after the first induction application and necrosis after the second or third induction application. Based on this steep dermal response, concentrations of 2.0%, 1.0% and 0.5% will be used for the induction phase of this study. Two groups of 4 unexposed animals will be weighed and exposed to different challenge concentrations of HCA in acetone by the patching technique described in the induction stage. Concentrations of 1.50%, 0.75%, 0.50% and 0.25% in acetone will be utilized to determine non-irritating levels to be used as challenge concentrations.

Method of Study Performance: Test Article Group

Induction Stage: Three groups of ten (10) guinea pigs are closely clipped over the induction site on their left flank (approximately 1.5 X 1.5 inches) during the day prior to the day of initiation and repeated as necessary. If the induction site is moved, an additional area (sufficient for patching) is closely clipped at the new site.

Based upon historical data, dose levels of 2.0%, 1.0% and 0.5% w/v of HCA in 95% ethanol, will be used for the induction stage. A 0.5 mL portion of the appropriate concentration of HCA in 95% ethanol is applied to a 25 mm Hill Top Chamber™ which is placed onto the test site and covered with a piece of dental dam (approximately 2 inches X 2 inches). After positioning the chamber and the occlusive dental dam onto the prepared skin test site, plastic material is wrapped snugly around the trunk of the animal and overwrapped with elastic tape.

Induction Stage: (continued)

Approximately six hours later, the chamber is removed and the test site is wiped with an appropriate solvent. The treated site is scored for irritation, using the scale included in Table I, at approximately 24 and 48 hours from the initial application. The procedure is performed once a week for three weeks, for a total of three, six-hour applications with the appropriate concentration of HCA. The interval between induction exposures may vary from 6 to 8 days. Should pronounced irritation be observed at the induction site, the site may be moved for the next induction application, with its relative location fully documented.

Vehicle Control Group

Three groups of ten (10) guinea pigs are closely clipped over the induction site towards the head on their left flank (approximately 1.5 X 1.5 inches) during the day prior to the day of initiation and repeated as necessary. If the induction site is moved, an additional area (sufficient for patching) is closely clipped at the new site.

A 0.5 mL portion of the vehicle (95% ethanol) is applied to a 25 mm Hill Top Chamber™ which is placed onto the test site and covered with a piece of dental dam (approximately 2 inches X 2 inches). After positioning the chamber and the occlusive dental dam onto the prepared skin test site, plastic material is wrapped snugly around the trunk of the animal and overwrapped with elastic tape.

Approximately six hours later, the chamber is removed and the test site is wiped with an appropriate solvent. The treated site is scored for irritation according to the scale included in Table I at approximately 24 and 48 hours from the initial application. The procedure is performed once a week for three weeks, for a total of three, six-hour applications of 95% ethanol. The interval between induction exposures may vary from 6 to 8 days. Should pronounced irritation be observed at the induction site, the site may be moved for the next induction application, with its relative location fully documented.

Challenge Stage: Test Material Group

After the third induction application, the animals are rested for approximately 14 to 16 days. At the termination of the rest period, a challenge application in acetone, using the procedures described previously, is applied to a challenge site (on the right flank). The challenge application remains on for approximately 6 hours. Upon removal of the challenge application, the contact area is wiped with an appropriate solvent. In the event of questionable findings, a rechallenge application of all animals is performed on a second naive site no sooner than six days after primary challenge, along with a group of 5 naive animals taken from the colony pool, serving as vehicle controls. Dates of application and observations are recorded. Approximately 24 and 48 hours after the challenge (and rechallenge if necessary) application, the sites are examined for dermal irritation and/or signs of elicited sensitization, according to the scale included in Table I.

Guinea Pig Dermal Sensitization - Modified Buehler Method

Challenge Stage: Vehicle Control Group [redacted]

After the third induction application, the animals are rested for approximately 14 to 16 days. At the termination of the rest period, the vehicle control group is challenged with acetone on the left flank (caudal) and the appropriate concentration of HCA in acetone on the right flank using the procedures described previously. The challenge application remains on for approximately 6 hours. Upon removal of the challenge application, the contact areas are wiped with an appropriate solvent. Dates of application and observations are recorded. Approximately 24 and 48 hours after the challenge application, the sites are examined for dermal irritation and/or signs of elicited sensitization according to the scale included in Table I.

Rechallenge: If the study is not positive, the Sponsor is notified and the test animals are rechallenged 6 to 8 days after the primary challenge.

Data Retention: All raw data generated, all records accumulated during the performance of this study and a copy of the final report will be retained at White Eagle Toxicology Laboratories for a period of ten (10) years, after which time the data will be shipped to the Sponsor or, discarded upon written request from the Sponsor.

Positive Control Characterization: Details regarding the identity, strength, stability, purity, and composition of the positive control are provided by the Manufacturer and are on file at White Eagle Toxicology Laboratories, Doylestown, Pennsylvania. The positive control has been examined and has been found to be soluble in ethanol and acetone.

Any mixtures or dilutions will not be analyzed by White Eagle or the Sponsor.

Statistical Analysis: Statistical Methods: Individual body weight percentage gain is statistically evaluated by Analysis of Variance (ANOVA), with the Neuman Keuls test applied in the event of a significant finding ($P<0.05$) for the data as a whole. Statistical analyses are performed using CSS: Statistica, 3.1 copyright, Statsoft Inc. 1991.

Mortality, Morbidity and Termination: All animals found dead during the observation period or euthanized in extremis are necropsied to establish, if possible, the cause of death or illness. All test animals are euthanized by CO_2 inhalation or IP pentobarbital at the completion of the study.

Protocol Changes: All changes in or revisions of the approved protocol and the reasons will be documented, signed by the study director, dated and maintained with the protocol.

Protocol Number: [redacted] (Rev. 04/96) Page 6 of 7

Guinea Pig Dermal Sensitization - Modified Buehler Method

Records Maintained: Records to be maintained include: [redacted]
- irritation scores for screen animals
- irritation scores for redness and swelling (induction and challenge)
- daily clinical observations
- initial, weekly, and final body weights (including group means, standard deviation, and change between initial and final)
- HCA preparation
- calculations and statistical analyses
- randomization scheme

Final Report: A final report is written which includes, but is not limited to, the items cited in U.S. EPA FIFRA Good Laboratory Practice Standards (40 CFR Part 160), Subpart J, Records and Reports, U.S. EPA TSCA Good Laboratory Practice Standards (40 CFR 792), Subpart J Records and Reports, OECD Principles of Good Laboratory Practice, c(81)30 Annex 2 (Final), Section II, Part 9.2, Content of the Final Report, and MAFF Japan Good Laboratory Practice Standards, 59 NohSan No. 3850, Chapter VIII, Report and Records.

Interpretation of Results: All non-eschar and non-necrotic test sites are scored on a scale of 0 to 3 as indicated in the following Table I. When edema is present with scores of +, 1, or 2 for erythema, it will be documented.

TABLE I

Scale for Evaluation of Dermal Irritation

0	=	no reaction
0.5	=	very faint redness (usually nonconfluent)
1	=	faint redness (usually confluent)
2	=	moderate redness
3	=	severe redness with/without swelling

Note: 0.5 scores will be recorded in the raw data as a +

Scores of 1 or greater in the test group are required to be indicative of sensitization. If scores of one (1) or greater are seen on the vehicle control animals, then the reactions of the test article group animals that exceed the most severe vehicle control reactions are considered to be positive scores. INCIDENCE is reported as the number of positive animals in each group divided by the total number of animals tested in that group. SEVERITY is reported as the sum of the test grades divided by the total number of animals tested in a given group determined for both 24 and 48 hours. Grades + are equal to 0.5 for calculation of severity indices. All average grades are to be rounded off to the nearest tenth of a unit. A minimum of two out of ten positive control animals must be shown to have elicited a positive reaction (Scores $\geq$ one (1)) to the positive control material to validate the test system.

Protocol Number: [redacted] (Rev. 04/96) Page 7 of 7

Guinea Pig Dermal Sensitization - Modified Buehler Method [redacted]

Signatures: Submitted by White Eagle Toxicology Laboratories:

George E. Moore 4-15-96

George E. Moore, B.S. Date
Study Director

Approved by:

Gregory S. Ladin 4/16/96

Sponsor Representative Date

E. I. du Pont de Nemours and Company - α-hexylcinnamaldehyde, tech, 85%

Guinea Pig Dermal Sensitization - Modified Buehler Method

APPENDIX B

DEVIATIONS FROM PROTOCOL

DuPont HLO 306-96

WHITE EAGLE TOXICOLOGY LABORATORIES

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Telephone: (215) 348-3868
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DEVIATIONS FROM PROTOCOL #1, #2, #3

White Eagle Project Number: [redacted]
Protocol Number: [redacted] (Rev. 04/96)
Sponsor: E. I. du Pont de Nemours and Company
Test Article: α-hexylcinnamaldehyde, tech, 85%
Test: Guinea Pig Dermal Sensitization - Modified Buehler Method
GLP Compliance: 40 CFR 160 40 CFR 792 OECD MAFF Japan

1) The Relative Humidity during the In-Life phase of this study was 24%-88% instead of 30%-70% as stated in the protocol.

2) Due to a Holiday, animal body weights were taken on Day 29 (week 5) instead of Day 28 (week 4).

The following is an amendment to protocol which was not approved in writing until the completion of the study; therefore, it is presented as a deviation.

3) Protocol Cover Sheet and Protocol
Change From: a-hexylcinnamaldehyde, tech, 85%
Change To: α-hexylcinnamaldehyde, tech, 85%

These deviations are not thought to have affected the integrity of this study.

Submitted by: George E. Moore — 6-26-96
George E. Moore, B.S. — Date
Study Director

Reviewed by: [signature] — 6-26-96
Diane T. Cook, B.S. — Date
Quality Assurance Manager

Approved by: Gregory S. Ladics — 6/27/96
Sponsor Representative — Date