DuPont HLO-1998-00730

TRADE SECRET

Study Title

Acute Dermal Irritation/Corrosion Study with H-22482 in Rabbits

FINAL REPORT

Haskell Laboratory Project ID: HLO-1998-00730

Performing Laboratory Project ID: WIL-189161

Data Requirement

Organization for Economic Cooperation and Development (OECD), Part 404 (1992)

European Economic Community (EEC) 92/69 Annex V - Method B4 (1992)

Author

Tom G. Kern, B.S.

Study Completed On

January 15, 1998

Performing Laboratory
WIL Research Laboratories, Inc.
1407 George Road
Ashland, Ohio 44805-9281

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

[redacted]

Company Sanitized. Does not contain TSCA CBI

Acute Dermal Irritation/Corrosion
Study with H-22482 in Rabbits

SPECIFIC COUNTRY REQUIREMENTS

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Acute Dermal Irritation/Corrosion
Study with H-22482 in Rabbits

GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

This study was conducted in compliance with EPA FIFRA (40 CFR 160) and EPA TSCA (40 CFR 792) Good Laboratory Practice Standards, and OECD Principles of Good Laboratory Practice [C(81)30(Final), Annex 2] with the following exception:

Although H-22482 was characterized in a DuPont Laboratory, the test substance was not characterized according to Good Laboratory Practice. This deviation did not affect the validity of the study.

Submitter: E. I. du Pont de Nemours and Company

Sponsor: E. I. du Pont de Nemours and Company

Study Director: _____ Date 1/15/98
Tom G. Kern, B.S.
Group Supervisor - Acute Toxicology
WIL Research Laboratories, Inc.

Sponsoring Company
Representative: _____ _____
Registration Manager

Acute Dermal Irritation/Corrosion
Study with H-22482 in Rabbits

GENERAL INFORMATION

Substance Tested: [redacted]

Synonyms/Codes: [redacted]

Haskell Number: 22482

Composition: [redacted]

Known Impurities: [redacted]

CAS Registry Number: [redacted]

Stability: In the absence of visible evidence to the contrary, the test substance was assumed to be stable under the conditions of administration.

Sponsor: DuPont Specialty Chemicals
E. I. du Pont de Nemours and Company
Wilmington, Delaware 19898

Study Initiated-Completed: June 19, 1997 - January 15, 1998

In Life Phase
Initiated-Completed: June 20, 1997 - July 1, 1997

All raw data and the final report will be stored in the archives of WIL Research Laboratories, Inc., Ashland, Ohio, in the archives of Haskell Laboratory for Toxicology and Industrial Medicine, E. I. du Pont de Nemours and Company, Newark, Delaware, or at Iron Mountain, 200 Todds Lane, Wilmington, Delaware (formerly DuPont Records Management Center, Wilmington, Delaware).

Acute Dermal Irritation/Corrosion
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Study with H-22482 in Rabbits

SUMMARY:

The primary dermal irritation and corrosion potentials of H-22482 were evaluated in this study with New Zealand White rabbits.

The study consisted of single 0.5-gram doses of the test substance that were applied to the clipped, intact skin of three albino rabbits under semi-occlusive dressings. Initially, a single screen animal was dosed to evaluate the irritative/corrosive potential of the test substance. This single rabbit received three sequential applications of the test substance for three minutes, one hour, and four hours. No severe dermal irritation or corrosion was observed following any exposure to the screen animal. Based on these results, two additional rabbits received single four-hour exposures of the test substance. Dermal observations were made in accordance with the method of Draize (Appendix A) and corrosion evaluations were made immediately following unwrap of the three-minute and one-hour exposure sites. The four-hour exposure sites were observed for dermal irritation at 30-60 minutes and 24, 48, and 72 hours after patch removal and once daily through day 7 if irritation persisted.

For the screen animal, there was no evidence of corrosion following the three-minute, one-hour, and four-hour exposures. There was no dermal irritation for the three-minute and one-hour sites following unwrap.

The test substance induced erythema (score of 1 or 2) on all three of the four-hour exposure sites. There was no edema or other dermal findings. All irritation was reversible and completely subsided by day 7 or earlier.

Mean values were calculated for each animal separately by using numerical scores obtained from the quantitative evaluation of each dermal response (erythema and edema) observed in the rabbits at the 24-, 48-, and 72-hour observations (excluding the three-minute and one-hour exposures). These values are as follows:

Animal Number	Erythema	Edema
23581	1.67	0.00
23588	1.33	0.00
23591	0.00	0.00

Based on the overall average of the mean values for erythema and edema calculated according to EEC Commission Directive 93/21 relative to the general classification and labeling requirements for dangerous substances published in the Official Journal of the European Communities (EEC Directive 93/21), H-22482 is not classified as an irritant.

PERSONNEL AND REPORT SUBMISSION:

Key Personnel:

Jenny R. Randall	Biologist II - Acute Toxicology
Daniel W. Sved, Ph.D.	Director of Metabolism and Analytical Chemistry
Carney B. Jackson, D.V.M., D.A.C.V.P., D.A.C.V.P.M.	Assistant Director of Pathology and Veterinary Medicine

Report Prepared By:

Anita F. Wilmot, B.S. — 1/15/98 Date
Biologist II/Report Writer II - Acute Toxicology

Reviewed By:

Jonathan M. Hurley, B.S. — 1/15/98 Date
Senior Biologist - Acute Toxicology

Gary R. Kiplinger, B.S. — 1/15/98 Date
Manager of General Toxicology

Approved and Submitted By:

Tom G. Kern, B.S. — 1/15/98 Date
Study Director

Study Monitor:

Tracy A. Filliben — 3/12/98 Date
Toxicology Associate

QUALITY ASSURANCE UNIT STATEMENT:

Date(s) of Inspection(s)	Phase Inspected	Date(s) Findings Reported to Study Director	Date(s) Findings Reported to Management
6/20/97	Test Material Dispensation and Administration	6/20/97	7/29/97
7/24/97	Study Records (I-1)	7/24/97	8/29/97
7/24/97	Draft Report	7/24/97	8/29/97

This study was conducted and inspected in accordance with the Good Laboratory Practice Regulations, the Standard Operating Procedures of WIL Research Laboratories, Inc., and the protocol and protocol amendment(s), if any. Quality Assurance findings, derived from the inspection(s) during the conduct of the study and from the inspections of the raw data and draft report, are documented and have been reported to the Study Director. A status report is submitted to management monthly.

The raw data, the retention sample(s), if applicable, and the final report will be stored in the Archives at WIL Research Laboratories, Inc., or another location specified by the Sponsor.

Deborah L. Little
Manager of Quality Assurance

1/15/98
Date

OBJECTIVE AND INTRODUCTION:

The objective of the study was to determine the irritative and corrosive potentials of the test substance following a single exposure to the skin of albino rabbits.

The protocol was designed and the study was conducted in general compliance with the Organization for Economic Cooperation and Development (OECD) Guidelines for Testing of Chemicals, Section 404 and European Economic Communities (EEC) guidelines in the Official Journal of the European Communities, 92/69, B4 (1992).

The study was conducted in compliance with the U.S. EPA FIFRA and TSCA Good Laboratory Practice Standards (40 CFR Part 160 and 792, respectively) and the OECD Principles of Good Laboratory Practice [C(81) 30 (Final) Annex 2].

MATERIALS AND METHODS.

Study Title:	Acute Dermal Irritation/Corrosion Study with H-22482 in Rabbits
WIL Study No.:	WIL-189161
Experimental Start Date:	June 20, 1997
Experimental Termination Date:	July 1, 1997
Test Facility:	WIL Research Laboratories, Inc. Ashland, OH 44805-9281
Study 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
Study Director:	Tom G. Kern, B.S. Senior Group Supervisor - Acute Toxicology
Sponsor Representative:	Tracy A. Filliben Toxicology Associate

Test System:

Species:	Albino rabbit
Strain:	New Zealand White
Justification for Species Selection:	The New Zealand White rabbit is generally recognized as appropriate for primary skin irritation studies. Considerable historical control data have been accumulated with this species for this type of study.
Source:	Covance Research Products, Inc. Denver, PA
Number on Study:	One male and two females
Body Weight Range:	3189 to 3464 grams at initiation of dosing

Test System (continued):

Age at Start of Study:	Young adult
Method of Identification:	Eartag
Housing:	Individual suspended wire-mesh cages. The animals were maintained by the animal husbandry staff of WIL Research Laboratories, Inc., in accordance with Standard Operating Procedures.
Quarantine:	The animals were acclimated to laboratory conditions for a minimum of seven days prior to initiation of dosing.
Food and Water:	PMI Feeds, Inc.© Certified Rabbit LabDiet© 5322 was provided at approximately 150 grams per day. Analysis of feed was performed and provided by the manufacturer. Reverse osmosis-treated municipal water was provided *ad libitum*. Water was analyzed in accordance with Standard Operating Procedures. Contaminants were not present in animal feed or water at levels expected to interfere with the objective of this study. Results of analyses are available upon Sponsor request.
Environmental Conditions:	Animal room with controlled temperature (65.8-67.1°F), humidity (49.7-52.1%), and light (12 hours light/12 hours dark).

Test Substance Preparation:

Individual 0.5-gram doses of the test substance were weighed into plastic weigh boats that were covered, labeled, and transported to the animal room for dosing. Sufficient deionized water was dispensed for moistening the test substance.

Route and Rationale of Test Substance Administration:

The route of test substance administration was direct application to clipped, intact skin. This route of administration is standard for assessment of local dermal irritative/corrosive potential.

Method of Test Substance Administration:

On the day prior to dosing, the hair was removed from the backs and flanks of the rabbits using an Oster® small animal clipper. The clipped area on each animal constituted approximately 25% of the total body surface. The 0.5-g test sites, delineated with water-insoluble ink, were located lateral to the midline of the back on each rabbit. Approximately 0.1 milliliters of deionized water were added to the three-minute site of the screen animal. The other exposure sites were not moistened with deionized water as per protocol. The test substance was characterized as a semi-solid after preparation for dosing and with grease-like consistency after bandage removal. Moistening was considered to be unnecessary as the test substance was observed to have made good contact with skin upon unwrap. Each dose was applied to an area of skin approximately 6 cm^2 under a secured two-ply, one-inch square gauze patch that was overwrapped with gauze bandaging and secured with Dermiform® tape. The wrappings were used to retard evaporation and keep the test substance in close contact with the skin. Collars were applied to each animal for the one- and four-hour exposure periods to prevent ingestion of the test substance and/or wrappings. Only one test substance was applied to each rabbit.

Dosage Level(s)/Group(s)/Treatment Regimen:

The dosage level was 0.5 g/site. There was one group of three rabbits. Initially, a single animal was selected and treated with the test substance in order to minimize unnecessary use of additional rabbits in case the test substance was corrosive or severely irritating. One screen animal received three separate semi-occluded applications of the test substance for the three-minute, one-hour, and four-hour exposure periods. Following the three-minute and one-hour exposures, the patches were removed and the sites were immediately evaluated for corrosion and dermal irritation (see Appendix A for scoring criteria). No other observations were conducted on the three-minute and one-hour exposure sites. The four-hour exposure site was evaluated for dermal irritation at 30-60 minutes after patch removal and further observed in accordance with the protocol. As no corrosion or severe dermal irritation was observed on these sites, two additional animals subsequently received a single, four-hour, semi-occluded exposure. At the end of each exposure, the collars (if applicable) and bandages of each rabbit were removed and the sites were wiped with disposable paper towels moistened with tepid tap water.

OBSERVATIONS:

Mortality:

The rabbits were observed twice daily (morning and afternoon) for mortality for the duration of the study.

Dermal Observations:

The three-minute and one-hour dose sites were observed once immediately after bandage removal for the presence or absence of corrosion. Dermal irritation was also evaluated and graded in accordance with the method of Draize (Appendix A).

The four-hour application sites were observed for erythema, edema, and other dermal findings approximately 30-60 minutes and 24, 48, and 72 hours after patch removal and once daily through day 7 if irritation persisted. Dermal irritation was graded in accordance with the method of Draize (Appendix A). The adjacent areas of untreated skin were used for comparison. In order to facilitate dermal observations, the areas of application (four-hour site) of two rabbits were clipped free of hair after collecting the 72-hour dermal scores.

Clinical Observations:

The rabbits were observed for clinical signs of toxicity at each dermal evaluation (excluding the three-minute and one-hour exposures).

Body Weights:

Body weights were obtained and recorded on study day 0 (initiation) and at each animal's termination from the study.

Termination:

Upon termination, the rabbits were euthanized by intravenous injection of sodium pentobarbital solution and discarded.

DATA ANALYSIS AND INTERPRETATION OF RESULTS:

A. EEC Classification

The numerical values corresponding to each animal for erythema and edema were recorded at each observation period. Mean values for each dermal response (erythema and edema) were calculated for each animal separately from numerical scores obtained at the 24-, 48-, and 72-hour observations (excluding the three-minute and one-hour exposures). The results were interpreted according to EEC Commission Directive 93/21 relative to the general classification and labeling requirements for dangerous substances.

Interpretation according to Annex VI Skin Corrosion or Irritation Criteria:

1. Corrosive

The test substance will be considered to be corrosive if it produces full thickness destruction of the skin tissue on at least one animal during the test for skin irritation. The substance will be classified as corrosive and assigned the symbol "C" and the indication of danger "corrosive." Risk phrases shall be assigned in accordance with the following criteria:

R35 "CAUSES SEVERE BURNS"

If, when applied to healthy intact animal skin, full thickness destruction of skin tissue occurs as a result of up to three-minute exposure or if this result can be predicted.

R34 "CAUSES BURNS"

If, when applied to healthy intact animal skin, full thickness destruction of skin tissue occurs as a result of up to four hour exposure or if this can be predicted.

2. Irritant

The test substance will be classified as "IRRITANT" and will require the "Xi" symbol and the indication of danger "IRRITANT" in accordance with the following criteria:

R38 "IRRITATING TO SKIN"

If, when applied to healthy intact animal skin for up to four hours, significant inflammation is caused and is present 24 hours or more after the end of the exposure period. Inflammation is significant if the mean values of the scores for either erythema and eschar formation or edema formation corresponds to one or more of the following mean values calculated for each animal separately and has been observed in two or more animals:

DATA ANALYSIS AND INTERPRETATION OF RESULTS (continued):

Dermal Lesion	Mean Value
Erythema and eschar formation	2.0 or more
Edema	2.0 or more

All scores at each of the reading times (24, 48, and 72 hours) for an effect should be used in calculating the respective mean values.

Inflammation of the skin is also significant if it persists in at least two animals at the end of the observation time. Particular effects such as hyperplasia, scaling, discoloration, fissures, scabs, and alopecia should be taken into account.

RESULTS:

Mortality:

No deaths occurred during the study.

Dermal Observations (Tables 1 and 2):

For the screen animal, there was no evidence of corrosion following any of the three different exposure times (three-minute, one-hour, and four-hour exposures). There was no dermal irritation observed for the three-minute or one-hour exposures.

Erythema (score of 1 or 2) was noted on all three four-hour exposure sites at the 30-60 minute observation. Erythema totally cleared for one animal each at 24 hours, day 6, and day 7. There was no edema or other dermal findings.

Clinical Observations (Table 3):

No clinical signs of toxicity were observed during the study.

Body Weights (Table 3):

No remarkable changes or differences were noted in body weights during the study.

CONCLUSIONS:

Under the conditions of testing and according to the guidance provided in EEC Directive 93/21, H-22482 is not classified as an irritant.

Tom G. Kern, B.S.
Study Director

1/15/98
Date

Acute Dermal Irritation/Corrosion
Study with H-22482 in Rabbits

TABLES 1-3

TABLE 1

ACUTE DERMAL IRRITATION/CORROSION
STUDY WITH H-22482 IN RABBITS

INDIVIDUAL DERMAL SCORES

Erythema

Animal	Sex	Site	1 hr	24 hr	48 hr	72 hr	4d	5d	6d	7d
23581	M	A	1r	2r	2r	1r	1r	1r	1r	0r
23588	F	A	1r	2r	1r	1r	1r	1r	0	-
23591	F	A	2r	0r	0r	0r	-	-	-	-

Edema

Animal	Sex	Site	1 hr	24 hr	48 hr	72 hr	4d	5d	6d	7d
23581	M	A	0	0	0	0	0	0	0	0
23588	F	A	0	0	0	0	0	0	0	-
23591	F	A	0	0	0	0	-	-	-	-

hr = Hour d = Day M = Male F = Female
r = Residual test material present on the site
- = Dermal irritation previously subsided; animal was terminated from the study
A = Four-hour sites

EEC:

Animal Number	Erythema	Edema
23581	1.67	0.00
23588	1.33	0.00
23591	0.00	0.00

TABLE 2

ACUTE DERMAL IRRITATION/CORROSION
STUDY WITH H-22482 IN RABBITS

INCIDENCES OF DERMAL RESPONSE (SCORES)[a]

	Erythema[b]							
Score	1 hr	24 hr	48 hr	72 hr	4d	5d	6d	7d
0	0/3	1/3	1/3	1/3	0/2[b]	0/2[b]	1/2[c]	1/1[d]
1	2/3	0/3	1/3	2/3	2/2	2/2	1/2	0/1
2	1/3	2/3	1/3	0/3	0/2	0/2	0/2	0/1

	Edema[b]							
Score	1 hr	24 hr	48 hr	72 hr	4d	5d	6d	7d
0	3/3	3/3	3/3	3/3	2/2[b]	2/2[b]	2/2[c]	1/1[d]
1	0/3	0/3	0/3	0/3	0/2	0/2	0/2	0/1
2	0/3	0/3	0/3	0/3	0/2	0/2	0/2	0/1

[a]According to EPA test guidelines.
[b]Four-hour sites evaluated.
[c]Two rabbits were evaluated for dermal irritation since irritation for one animal had totally cleared.
[d]One remaining rabbit was evaluated for dermal irritation since dermal irritation had completely subsided for the other two animals.

hr = Hour
d = Day

TABLE 3

ACUTE DERMAL IRRITATION/CORROSION
STUDY WITH H-22482 IN RABBITS

INDIVIDUAL BODY WEIGHTS (GRAMS)

Group	Animal	Sex	Initiation (Day 0)	Termination (interval)
0.5 g/site	23581	M	3464	3668 (day 7)
	23588	F	3258	3279 (day 6)
	23591	F	3189	3283 (72 hours)

M = Male
F = Female

CLINICAL OBSERVATIONS

No clinical findings were noted for any animals during the study.

Acute Dermal Irritation/Corrosion
Study with H-22482 in Rabbits

APPENDIX A

Scoring Criteria for Dermal Reactions

APPENDIX A

ACUTE DERMAL IRRITATION/CORROSION STUDY WITH H-22482 IN RABBITS

SCORING CRITERIA FOR DERMAL REACTIONS

Evaluation of Dermal Reactions*

Value	Erythema and Eschar Formation
0	No erythema
1	Very slight erythema (barely perceptible, edges of area not well defined)
2	Mild erythema (pale red in color and edges definable)
3	Moderate to severe erythema (definite red in color and area well defined)
4	Severe erythema (beet or crimson red) to slight eschar formation (injuries in depth)

	Edema Formation
0	No edema
1	Very slight edema (barely perceptible, edges of area not well defined)
2	Mild edema (edges of area well defined by definite raising)
3	Moderate edema (raised approximately 1 mm)
4	Severe edema (raised more than 1 mm and extending beyond area of exposure)

*Draize, J.H., 1959. The Appraisal of the Safety of Chemicals in Foods, Drugs and Cosmetics. Dermal Toxicity, pp. 46-59. Assoc. of Food and Drug Officials of the U.S., Topeka, Kansas.

Acute Dermal Irritation/Corrosion
Study with H-22482 in Rabbits

APPENDIX B

Protocol

Study Number: WIL-187161 (WIL)

MASTER PROTOCOL COVER SHEET

Sponsor: E.I. du Pont de Nemours and Company

Master Protocol No.: E06 (Sponsor)

Sponsor Project No. [redacted]

A. Title of Study:

Acute Dermal Irritation/Corrosion Study in Albino Rabbits (Sponsor)

B. Protocol Modification:

1) II. PERSONNEL INVOLVED IN THE STUDY

A. Sponsor Representative

Name: Tracy A. Filliben (Sponsor)

Title: Toxicology Associate (Sponsor)

2) III. STUDY SCHEDULE DATA

A. Proposed Experimental Start Date: 6/20/97 (WIL)

B. Proposed Experimental Termination Date: 6/27/97 (WIL)

C. Proposed Audited Draft Report Date: 8/8/97 (WIL)

3) IV. TEST MATERIAL DATA

A. Identification: [redacted] (Sponsor)

B. Lot Number: [redacted] H-22482 (Sponsor)

C. Purity: [redacted] (Sponsor)

D. Stability: Stable (Sponsor)

E. Physical Description: [redacted] (WIL)

F. Storage Conditions: Ambient temperature (Sponsor)

G. Personnel Safety Data: Normal laboratory procedures. For complete available safety information see attached MSDS. (Sponsor)

H. CAS Number: [redacted] (Sponsor)

C. Reason for Protocol Modification:

1) Sponsor Representative is specified.

2) Proposed scheduling information is added to the protocol.

3) Test material data are added to the protocol.

Approved By:

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

Tracy A. Fillibeu
Sponsor Representative

6/13/97
Date

Prepared By.

WIL Research Laboratories, Inc.
Ashland, OH 44805-9281

Tom G. Kern, B.S.
Study Director

6/15/97
Date

PROTOCOL

Acute Dermal Irritation/Corrosion Study in Albino Rabbits

(OECD/EEC Guidelines)

Master Protocol No.: E06

For

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

By

WIL Research Laboratories, Inc.
Ashland, OH 44805-9281

June 4, 1997

I. OBJECTIVE OF STUDY

To determine the irritative and corrosive potential of the test material following a single exposure to the skin of albino rabbits.

This protocol has been designed and the study will be conducted in general compliance with the Organization for Economic Cooperation and Development (OECD) Guidelines for Testing of Chemicals, Section 404 and European Economic Communities (EEC) guidelines in the Official Journal of the European Communities, 92/69, B4 (1992).

The study will be conducted in compliance with the U.S. EPA FIFRA and TSCA Good Laboratory Practice Standards (40 CFR Parts 160 and 792, respectively) and the OECD Principles of Good Laboratory Practice [C(81) 30 (Final) Annex 2].

II. PERSONNEL INVOLVED IN THE STUDY

A. Sponsor Representative

To be specified by Master Protocol Cover Sheet

B. WIL Study Director

Tom G. Kern, B.S.
Group Supervisor - Acute Toxicology

C. WIL Deputy Director

Jonathan M. Hurley, B.S.
Senior Biologist - Acute Toxicology

D. Study Responsibilities

1. James L. Schardein, M.S., A.T.S.
   Director of Research
2. Christopher P. Chengelis, Ph.D., D.A.B.T.
   Senior Toxicologist
3. Jozef J.W.M. Mertens, Ph.D., D.A.B.T.
   Staff Toxicologist
4. Gary R. Kiplinger, B.S.
   Manager of General Toxicology

II. PERSONNEL INVOLVED IN THE STUDY (continued)

5. Ronald E. Wilson, B.S
Director of Informational Systems

6. Daniel W. Sved, Ph.D.
Director of Metabolism and Analytical Chemistry

7. Sally A. Keets, A.S.
Manager of Vivarium

8. Deborah L. Little
Manager of Quality Assurance

9. Kerin Clevidence, B.S.
Group Supervisor of Gross Pathology
and Developmental Toxicology Laboratory

10. Charlene M. Lindsey, M.A.
Manager of Technical Report Writing

11. Carney B. Jackson, D.V.M., D.A.C.V.P., D.A.C.V.P.M.
Assistant Director of Pathology and Veterinary Medicine

12. Jenny R. Randall
Biologist II - Acute Toxicology

III. STUDY SCHEDULE DATA

A. Proposed Experimental Start Date: To be added by Master Protocol Cover Sheet

B. Proposed Experimental Termination Date: To be added by Master Protocol Cover Sheet

C. Proposed Audited Draft Report Date: To be added by Master Protocol Cover Sheet

IV. TEST SUBSTANCE DATA

A. Identification: To be added by Master Protocol Cover Sheet

B. Lot Number: To be added by Master Protocol Cover Sheet

C. Purity: To be added by Master Protocol Cover Sheet

D. Stability: To be added by Master Protocol Cover Sheet

IV. TEST SUBSTANCE DATA (continued)

E. Physical Description:	To be documented by WIL Research Laboratories, Inc. and added by Master Protocol Cover Sheet
F. Storage Conditions:	To be added by Master Protocol Cover Sheet
G. Personnel Safety Data:	To be added by Master Protocol Cover Sheet

V. TEST SYSTEM

A. Species:	Albino rabbit
B. Strain:	New Zealand White
C. Source:	Covance Research Products, Inc. Denver, PA or Kalamazoo, MI
D. Number on Study:	Three animals (except as noted in section VIII. C. of this protocol).
E. Sex:	Males and/or females
F. Body Weight Range:	2.0 kg or greater at initiation of dosing.
G. Approximate Age:	Young adult
H. Identification System:	Each animal will be uniquely identified by a plastic eartag displaying the animal number. Individual cage cards will be affixed to each cage and will display the animal number, group and study number.
I. Justification for Selection.	This species and strain is generally recognized as appropriate for primary skin irritation studies. The experimental design uses the procedures and standards required by the current and proposed international guidelines.

VI SPECIFIC MAINTENANCE SCHEDULE

A. Animal Housing

The animals will be housed individually in suspended wire-mesh cages in an environmentally controlled room. Animals will be housed in clean cages elevated above cage-board or other suitable material that will be changed at least three times each week. The facilities at WIL Research Laboratories, Inc. are fully accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC International).

B. Environmental Conditions

Controls will be set to maintain temperature at 67 ± 4°F and relative humidity at approximately 30-70%. Fluorescent lighting controlled by light timers will provide illumination for a 12-hour light/dark photoperiod. Temperature and relative humidity will be recorded once daily.

C. Drinking Water

Reverse osmosis treated municipal water will be available *ad libitum*. Filters servicing the automatic watering system are changed regularly according to Standard Operating Procedures. Water supplying the laboratory is analyzed for contaminants according to Standard Operating Procedures. No contaminants known to be in the water are expected to interfere with the validity of the study.

D. Basal Diet

PMI Feeds, Inc.© Certified Rabbit LabDiet© 5322 will be offered at approximately 150 g/day during the study. Periodic analyses of the certified feed for the presence of heavy metals and pesticides are performed and provided by the manufacturer to ensure that none are present in concentrations that would be expected to affect the outcome of the study.

VII. EXPERIMENTAL DESIGN

A. Animal Receipt and Acclimation

Each animal will be inspected by a qualified technician upon receipt. Rabbits judged to be in good health and suitable as test animals will be acclimated to laboratory conditions for a minimum of seven days. All rabbits will be weighed initially and permanently identified with an eartag. During the acclimation period, each rabbit will be observed twice daily for changes in general appearance and behavior Prior to initiation of dosing, those animals judged to be suitable test subjects will be identified.

VII. EXPERIMENTAL DESIGN (continued)

B. Route and Rationale of Test Material Administration

The route of administration will be dermal in order to test the potential for dermal irritation and corrosion. The dermal route is a possible route of exposure in humans.

C. Body Weights

The body weight of each animal will be determined on study day 0 and at termination. Rabbits remaining on study beyond day 7 will be weighed at least once a week if the study is extended.

VIII. DOSAGE LEVELS AND TREATMENT REGIMEN

A. Treatment Levels

The same dose level will be used for all three animals (0.5 ml or 0.5 g per test site).

B. Treatment Groups

Following the acclimation period, three rabbits will be arbitrarily selected from available stock based upon health and a body weight of 2.0 kg or greater. No separate control group will be utilized; each animal will serve as its own control. All test sites will be left intact.

C. Initiation Procedure

If it is anticipated that the test substance may be severely irritating and/or corrosive, a single animal screen study will be dosed to evaluate the irritative/corrosive potential of the test substance. Three sites on this single animal will receive individual exposures of the test substance for three minutes, one hour, and four hours. The three-minute exposure site will be dosed and observed for the presence of severe irritation/corrosion immediately following the unwrap. If no serious skin reaction is present on the three-minute site, the one-hour exposure site will be dosed and observed for the presence of severe irritation/corrosion immediately following the unwrap. If no serious skin reaction is present on the one-hour site, the four-hour exposure site will be dosed and observed approximately 30-60 minutes after test substance removal.

If severe irritation (erythema, edema and/or eschar) is observed after any exposure period on the single animal screen, no further testing will be conducted without the specific written request of the Sponsor. If corrosion is observed after any exposure, the study will immediately be terminated. If neither a corrosive effect nor a severe irritant effect is observed after the four-hour exposure, the study will be completed using two additional animals, each receiving a single four-hour exposure.

VIII. DOSAGE LEVELS AND TREATMENT REGIMEN (continued)

D. Method of Administration

Approximately 24 hours prior to dermal applications, the back and flanks of each rabbit will be clipped free of hair with an Oster® small animal clipper. The clipped area on each animal will constitute approximately 25% of the total body surface area. Animals with dermal abnormalities or injuries will be excluded. The test sites will be located lateral to the midline of the back and delineated with four dots made with water-insoluble ink spaced approximately 2.5 centimeters apart (~6 cm^2) arranged in a square.

Approximately 0.5 ml of the undiluted test substance or 0.5 g of solid or semi-solid will be applied evenly to each test site covering an area at approximately 6 cm^2. Analysis of dosing solutions (if prepared) will be performed at the specific written request of the Sponsor (additional cost). Solid materials will be slightly moistened with water or other suitable vehicle (amount recorded) to form a thick paste. The amount of vehicle used will be documented in the study records.

Each test site will be immediately covered with a two-ply, approximately one-inch square gauze patch. The patches are secured in place with surgical porous tape. The trunk will then be wrapped with gauze bandaging that is secured with several wrappings of non-irritating tape. Collars will be applied to each animal for the one- and four-hour exposure periods to prevent ingestion of the test material and/or wrappings. For the purpose of this study, all test substance applied is assumed to be available for absorption by the test system. Only one test substance will be applied to each rabbit.

IX. OBSERVATIONS

After each exposure, the bandages will be removed and the residual test substance gently wiped off with disposable towels moistened with water (as thoroughly as possible without irritating the skin). Alternate moistening materials may be used (to be documented in study records) in order to remove residual test substance upon approval by the Study Director.

Approximately 30-60 minutes after test substance removal on four-hour exposure sites only, the test sites will be examined and the degree of erythema and edema recorded according to the Draize Technique (Appendix A). Additional examinations will be performed at approximately 24, 48 and 72 hours after patch removal. If irritation is present at 72 hours, scoring will continue daily up to seven days after dosing. Examination and recording of erythema and edema will only be conducted immediately upon bandage removal on the three-minute and one-hour exposure sites. The animals will also be observed for clinical signs at each dermal evaluation excluding the 3 and 60-minute exposures). The animals will be observed twice daily for mortality and signs of illness, injury, or abnormal behavior.

The areas of application will be clipped free of hair following the 72-hour evaluation, if necessary and as needed during the study to facilitate accurate dermal observations.

IX. OBSERVATIONS (continued)

Individual animals may be terminated if no irritation is present at the 72- hour or any subsequent observation. If irritation is present at the end of seven days, observation will continue daily until it subsides or is obviously irreversible up to 14 days (additional cost). The study need not normally exceed 14 days after application unless specifically requested and authorized by the Sponsor (additional cost).

X. GROSS PATHOLOGY

The animals will be euthanized by an intravenous injection of euthanasia solution upon termination and discarded. Collection of skin samples from the four-hour exposure sites may be collected and preserved in 10% neutral-buffered formalin for histopathological examination at the specific written request of the Sponsor (additional cost). A gross necropsy examination on major organ systems of the thoracic and visceral cavities will be conducted on all animals found dead or euthanized *in extremis*.

XI. DATA ANALYSIS AND INTERPRETATION OF RESULTS

A. EEC Classification

The numerical values corresponding to each animal for erythema and edema will be recorded at each observation period. Mean group scores will be calculated for each dermal response (erythema and edema) by summing the scores obtained from all animals at the 24-, 48- and 72-hour observations (excluding the 3 and 60 minute exposures). The three means corresponding to the three observation periods will be averaged and overall averages will be obtained for each dermal lesion. The results will be interpreted according to EEC Commission Directive 93/21 relative to the general classification and labeling requirements for dangerous substances.

Interpretation according to Annex VI Skin Corrosion or Irritation Criteria:

1. Corrosive

The test substance will be considered to be corrosive if it produces full thickness destruction of the skin tissue on at least one animal during the test for skin irritation. The substance will be classified as corrosive and assigned the symbol "C" and the indication of danger "corrosive." Risk phrases shall be assigned in accordance with the following criteria:

R35 "CAUSES SEVERE BURNS"

If, when applied to healthy intact animal skin, full thickness destruction of skin tissue occurs as a result of up to three minute exposures or if this result can be predicted.

XI. DATA ANALYSIS AND INTERPRETATION OF RESULTS (continued)

R34 "CAUSES BURNS"

If, when applied to healthy intact animal skin, full thickness destruction of skin tissue occurs as a result of up to four hours exposure or if this can be predicted.

2. Irritant

The test substance will be classified as "IRRITANT" and will require the "Xi" symbol and the indication of danger "IRRITANT" in accordance with the following criteria:

R38 "IRRITATING TO SKIN"

If, when applied to healthy intact animal skin for up to four hours, significant inflammation is caused and is present 24 hours or more after the end of the exposure period. Inflammation is significant if the mean values of the scores for either erythema and eschar formation or edema formation corresponds to one or more of the following mean values:

Dermal Lesion	Mean Score
Erythema and eschar formation	2.0 or more
Edema	2.0 or more

All scores at each of the reading times (24, 48, and 72 hours) for an effect will be used in calculating the respective mean values.

Inflammation of the skin is also significant if it persists in at least two animals at the end of the observation time. Particular effects such as hyperplasia, scaling, discoloration, fissures, scabs, and alopecia should be taken into account.

XII. REPORT

The final report will include the following summary, objective, test substance identification and receipt, methods, individual dermal observations, mortality, individual body weights, a table summarizing mean values for each rabbit, gross necropsy findings (if applicable), and classification of the test substance based on dermal irritation properties. One copy of the audited draft report will be provided approximately six weeks following completion of the study. Two copies of the report will be provided upon finalization.

XIII. RECORDS

All original raw data records (as defined by the applicable GLPs and WIL SOPs) generated by WIL Research Laboratories, Inc. will be collected and maintained by WIL Research Laboratories, Inc.

XIV. WORK PRODUCT

Sponsor will have title to all documentation records, raw data, slides, specimens, or other work product generated during the performance of the study. All work product including raw paper data, magnetically encoded records and specimens will be retained at no charge for a period of six months following issuance of the final report in the Archives at WIL Research Laboratories, Inc. Thereafter, WIL Research Laboratories will return all work product to Haskell Laboratory for storage All work product will be stored in compliance with regulatory requirements.

XV. QUALITY ASSURANCE

The study will be audited by the WIL Quality Assurance Unit while in progress to assure compliance with Good Laboratory Practice regulations and adherence to the protocol and to WIL Standard Operating Procedures. The raw data and draft report will be audited by the WIL Quality Assurance Unit prior to submission to the Sponsor to assure that the final report accurately describes the conduct and the findings of the study.

XVI. PROTOCOL MODIFICATION

This Master Protocol shall be used for multiple studies. A Master Protocol Cover Sheet shall be prepared for each study which will use this design. The Master Protocol and Master Protocol Cover Sheet shall constitute the protocol for an individual study. In addition, the date of Study Director signature on the Master Protocol Cover Sheet shall be considered the date of study initiation.

Modification of the protocol may be accomplished during the course of this investigation. However, no changes will be made in the study design without the verbal or written permission of the Sponsor. In the event that the Sponsor verbally requests or approves a change in the protocol, such changes will be made by appropriate documentation in the form of a protocol amendment. All alterations of the protocol and reasons for the modification(s) will be signed by the Study Director and the Sponsor Representative.

XVII. ANIMAL WELFARE ACT COMPLIANCE

This study will comply with all applicable sections of the Final Rules of the Animal Welfare Act regulations (9 CFR). The Sponsor should make particular note of the following:

1. The Sponsor signature on this protocol documents for the Study Director the Sponsor's assurance that the study described in this protocol does not unnecessarily duplicate previous experiments.

2. Whenever possible, procedures used in this study have been designed to avoid or minimize discomfort, distress or pain to animals. All methods are described in this study protocol or in written laboratory Standard Operating Procedures.

3. Animals that experience severe or chronic pain or distress that cannot be relieved will be painlessly euthanized as deemed appropriate by the veterinary staff and Study Director. The Sponsor will be advised by the Study Director of all circumstances which could lead to this action in as timely a manner as possible.

4. Methods of euthanasia used during this study are in conformance with the above-referenced regulation.

XVIII. PROTOCOL APPROVAL

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Carol Finlay

Carol Finlay, B.A.
Sponsor Representative

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Date

Tracy A. Filliben

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Date

WIL Research Laboratories, Inc.
Ashland, OH 44805-9281

Tom G. Kern, B.S.
Study Director

6/4/97
Date

APPENDIX A

SCORING CRITERIA FOR DERMAL REACTIONS*

Evaluation of Dermal Reactions*

Value	Erythema and Eschar Formation
0	No erythema
1	Very slight erythema (barely perceptible, edges or area not well defined)
2	Mild erythema (pale red in color and edges definable)
3	Moderate to severe erythema (definite red in color and area well defined)
4	Severe erythema (beet or crimson red) to slight eschar formation (injuries in depth)

	Edema Formation
0	No edema
1	Very slight edema (barely perceptible, edges of area not well defined)
2	Mild edema (edges of area well defined by definite raising)
3	Moderate edema (raised approximately 1 mm)
4	Severe edema (raised more than 1 mm and extending beyond area of exposure)

*Draize, J.H., 1959. The Appraisal of the Safety of Chemicals in Foods, Drugs and Cosmetics. Dermal Toxicity, pp. 46-59. Assoc. of Food and Drug Officials of the U.S., Topeka, Kansas.