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Study Title

Delayed Contact Hypersensitivity Test
in Guinea Pigs with H-19173

Study Completed On

July 17, 1992

Contracting Laboratory

Pharmakon Research International, Inc.
P.O. Box 313
Waverly, Pennsylvania 18471

Author

Susan Armondi

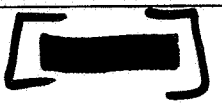
for

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

Scott E. Loveless

Medical Research No.



Laboratory Project ID

Haskell Laboratory Report No. 161-92

COMPLIANCE STATEMENT

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

U.S. Food and Drug Administration, as stated in the Federal Register, 21 CFR, Part 58, Friday, September 4, 1987.

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

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

Organization for Economic Co-operation and Development Guidelines for Testing Chemicals (OECD), ISBN 92-64-12221-4, adopted by the council at its 535th meeting on 12th May, 1981.

Study Number: PH 424-DU-012-91

"To the best of my knowledge, this study was conducted in accordance with applicable Good Laboratory Practice regulations; there were no deviations from these regulations that impacted on study conclusion."

Susan E. Armondi
Study Director

July 9, 1992
Date

GENERAL INFORMATION

Test Material

Material Tested: [redacted]

Medical Research No.: [redacted]

Synonym: H-19173

Haskell Test Code No.: [redacted]

Pharmakon Study No.: PH 424-DU-012-91

Physical Form: Slightly yellow liquid dispersion

Purity: 100%

Composition: H-19173 contains:

[redacted]

Synonym: [redacted]

Submitter's Notebook No.: [redacted]

CAS Registry No.: None availble

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

Positive Control Material

Material Tested: N-Phenyl-1,4-Phenylenediamine

Purity: 98%

Synonyms:
- 1,4-Benzenediamine
- p-phenylenediamine (PPD)

Other Codes:
- Aldrich Chemical Co., Lot #05012MK
- Aldrich Chemical Co., Catalog #24139-3

GENERAL INFORMATION (CONT.)

CAS Registry No.:	101-54-2
Stability:	In the absence of visible evidence to the contrary, the test material was assumed to be stable under the conditions of administration.
Sponsor:	Du Pont Chemicals E. I. du Pont de Nemours and Company Wilmington, Delaware
Study Initiated - Completed:	10-28-91 - 07-17-92
In-Life Phase Initiated - Completed:	01-28-92 - 03-07-92
Pharmakon Notebook No.:	[redacted]

DU PONT CENTRAL RESEARCH AND DEVELOPMENT
HASKELL LABORATORY FOR TOXICOLOGY
AND INDUSTRIAL MEDICINE

K. D. Dastur
Du Pont Chemicals
Brandywine Building

July 17, 1992

Delayed Contact Hypersensitivity Test
in Guinea Pigs with H-19173

Attached is a report from Pharmakon Research International, Inc. (PH 424-DU-012-91). Included in the report are test methods, results, and conclusions.

H-19173 at topical doses of 100% and 10% (v/v) and an intradermal dose of 1.0% (v/v), was tested on the clipped, intact skin of male and female guinea pigs. p-Phenylenediamine at a topical dose of 35% and an intradermal dose of 1.0% (w/v) was used to demonstrate the ability of the test system to detect a skin sensitizer (positive control group). In addition, a group of male and female guinea pigs was treated with 0.9% saline at induction and distilled water at challenge (vehicle control group). At challenge, a group of previously untreated guinea pigs was treated with H-19173 at topical doses of 100% and 10% (v/v), distilled water, and 35% p-phenylenediamine on separate, intact skin sites (negative control group).

One animal died during the course of the study. Necropsy of this animal revealed no visible lesions. The death of this animal was considered spurious in nature and not related to treatment.

During the induction phase, no to slight erythema was observed in the majority of the test article-treated animals. Following the third intradermal injection, a single guinea pig presented with a mild erythema and after the fourth intradermal injection, moderate erythema was noted in another guinea pig. Slight erythema was observed in one vehicle control animal following the third induction. No to severe erythema with/without blanching was observed in the positive control animals.

During the challenge phase, no signs of erythema were observed in the test, vehicle, or negative control animals. No to moderate erythema was observed in the positive control group treated with p-phenylenediamine at a 35% concentration.

Under the conditions of this study, H-19173 did not produce delayed contact hypersensitivity in guinea pigs.

Scott E. Loveless

Scott E. Loveless, Ph.D.
Senior Research Toxicologist

Skin Sensitization Test with H-19,173 in Guinea Pigs

PH 424-DU-012-91

Submitted to

E.I. du Pont de Nemours & Company
Newark, Delaware

Reason for Amendment: In order to include page number on compliance statement, footnote on page 23, and correct weight range of animals on page 47.

Susan E. Armondi

Susan E. Armondi, LAT
Study Director

July 9, 1992
Date

Test Facility Management

July 10, 1992
Date

Skin Sensitization Test with H-19,173 in Guinea Pigs

SUMMARY

Test Article H-19,173 at topical doses of 100% and 10% (v/v) and an intradermal dose of 1.0% (v/v), was tested on the clipped, intact skin of male and female guinea pigs. p-Phenylenediamine (PPD) at a topical dose of 35% and an intradermal dose of 1.0% (w/v) was used to demonstrate the ability of the test system to detect a skin sensitizer (positive control group).

During the primary irritation phase, no signs of erythema were observed in the test, vehicle, or positive control animals.

One animal died during the course of the study. Necropsy of this animal revealed no visible lesions. The death of this animal was considered spurious in nature and not related to treatment. During the induction phase, no to moderate erythema was observed in the test article-treated animals. Slight erythema was observed in one vehicle control animal at 24 hours following the third induction. No to severe erythema with/without blanching was observed in the positive control animals.

During the challenge phase, no signs of erythema were observed in the test, vehicle, or negative control animals. No to moderate erythema was observed in the positive control group treated with p-phenylenediamine at a 35% concentration.

Under the conditions of this study, H-19,173 did not cause delayed contact hypersensitivity in guinea pigs.

Work by: Charles Boyarsky
Charles Boyarsky, LAT
Technician

Study Director: Susan E. Armondi
Susan E. Armondi, LAT

Reviewed and
Approved for Issue: Susan E. Armondi
Susan E. Armondi, LAT
Study Director

QUALITY ASSURANCE UNIT STATEMENT

Study No.: PH 424-DU-012-91

Study Director: Susan E. Armondi

The Quality Assurance Unit conducted the inspections listed below and reported the results to the study director and to management on the dates indicated.

The following inspections were performed:

Interval	Date
In Life Phase	January 30, 1992
Challenge Phase	March 5, 1992
Reporting Phase	March 30, 1992 June 12, 1992
Amended Final	July 8, 1992

Date QAU Report Issued

To Study Director	To Management
March 31, 1992	March 31, 1992
June 12, 1992	June 12, 1992
July 8, 1992	July 8, 1992

Debbi Donnell
Quality Assurance

July 9, 1992
Date

INTRODUCTION

The purpose of this study was to evaluate the potential of H-19,173 to produce delayed hypersensitivity when applied to the skin of guinea pigs. Sensitization was defined as a significant score increase at challenge over the response observed after the primary application of the test material to the test guinea pigs, or the response observed in the negative controls. A significant score increase defined as a 2-or-more step increase (e.g., from 0 to 2, from 1 to 3, etc.) has been used at Haskell Laboratory for its ability to identify compounds that are sensitizers. The sensitivity of the test system and procedures to detect chemical sensitizers was evaluated with p-phenylenediamine.

MATERIAL AND METHODS

A. Animal Husbandry

Young adult male and female Duncan Hartley albino guinea pigs (generally 8 to 10 weeks of age) were received from BuckberG Lab Animals, Tomkins Cove, New York. The guinea pigs were housed singly in suspended, stainless steel, ½" wire-mesh cages. Each guinea pig was assigned an identification card on which a unique animal number, study number, dose level, and sex were recorded and affixed to the cage. Guinea pigs were ear tagged. Purina Certified Guinea Pig Chow® #5026 and water were available ad libitum. Guinea pigs were acclimated and observed for general health during a period of approximately five days. Animal rooms were maintained on a timer-controlled, 12-hour light/12-hour dark cycle. Environmental conditions of the rooms were targeted for a temperature of 22° ± 3°C (66°- 77°F) and relative humidity of 30 - 70%. Any excursions outside these ranges were of small magnitude and/or brief duration and did not adversely affect the validity of the study.

B. Protocol

A copy of the signed original protocol is attached as Appendix A.

A preliminary range-finding test was conducted to estimate the primary irritation potential of the test material. The results of the range-finding study were used to select the exposure concentrations for the main study. The main sensitization study consisted of 3 phases: a primary irritation phase, an induction phase, and a challenge phase. During each phase, skin responses were scored according to the system presented in Table I. During the study, body weights were recorded weekly.

The range-finding test was conducted on 1 male and 2 female guinea pigs ranging in weight from 375 to 417 grams. Aliquots (approximately 0.05 mL) of 1.0%, 10%, 50%, and 100% (v/v) solutions of the test material in distilled water were applied and lightly rubbed onto four separate test sites on the clipped, intact skin on the back of each animal. Irritation responses were scored approximately 24 and 48 hours after treatment.

The primary irritation phase was conducted in 10 guinea pigs (5 males and 5 females), weighing from 428 to 512 grams, by applying and lightly rubbing in 1 drop (approximately 0.05 mL) of 100% and 10% (v/v) solutions of the test material in distilled water onto two separate sites of clipped, intact shoulder skin of each animal. In addition, 10 positive control guinea pigs (5 males and 5 females), weighing from 429 to 506 grams, were treated by applying and lightly rubbing in 1 drop of 35% (w/v) suspension of p-phenylenediamine in distilled water onto the clipped, intact left shoulder skin of each animal. Five vehicle control guinea pigs (3 males and 2 females), weighing from 424 to 522 grams, were treated with distilled water in the same manner at one site only. Dermal responses were scored approximately 24 and 48 hours after application of the test material.

Two days after the primary irritation phase, the induction phase of the study was initiated using the same 10 test guinea pigs in which primary irritation had been evaluated. Induction consisted of a series of 4 sacral intradermal injections (1 each week) of 0.1 mL of 1.0% (v/v) solution of H-19,173 in 0.9% saline. A virgin site on the back of each animal was used for each injection. The same injection procedure was followed for the positive control guinea pigs using 0.1 mL of a 1.0% (w/v) suspension of p-phenylenediamine in 0.9% saline. The vehicle control animals were treated with 0.1 mL 0.9% saline. Skin responses were evaluated approximately 24 hours after each induction.

One animal died during the course of the study. Necropsy of this animal revealed no visible lesions. The death of this animal was considered spurious in nature and not related to treatment. Two weeks after the last induction treatment, the test guinea pigs were challenged for sensitization by applying and lightly rubbing in 1 drop (approximately 0.05 mL) of 100% and 10% (v/v) solutions of the test material in distilled water onto two separate sites of clipped, intact shoulder skin. The positive control animals were challenged for sensitization by applying and lightly rubbing in 1 drop (approximately 0.05 mL) of 35% (w/v) solutions of p-phenylenediamine in distilled water onto the clipped intact left shoulder skin. The sites used during challenge were the same used on each animal during the primary irritation phase, respectively. The vehicle control animals were treated with distilled water in the same manner. Also, 2 male and 3 female previously untreated guinea pigs, weighing 415 to 509 grams at study initiation, were treated by applying 1 drop each (approximately 0.05 mL) of 100% and

10% (v/v) suspensions of the test material in distilled water onto two separate sites of clipped, intact skin and served as negative control animals. These animals also received 0.05 mL of distilled water and 0.05 mL of p-phenylenediamine (35%) in distilled water on separate, intact skin sites. These animals also served as negative control animals for the vehicle and positive control materials.

C. Records Retention

All raw data and the final report will be stored in the archives of Pharmakon Research International, Inc., Waverly, PA.

RESULTS AND CONCLUSIONS

Weekly body weights are presented in Appendix B. There were no statistically or biologically significant differences observed in the group mean body weight data among the four dose groups. A copy of the original raw data is presented in Appendix C. One test article-treated animal died during the study. Necropsy of this animal revealed no visible lesions. The death of this animal was considered spurious in nature and not related to treatment.

In the range-finding test, no signs of erythema were observed at any test site. Based on the results of the range-finding study, 100% and 10% concentrations were used for the main study.

During the primary irritation phase, no signs of erythema were observed in any of the test, vehicle, or positive control animals. Individual animal data are presented in Table II.

During the induction phase, no to moderate erythema was observed in the test article-treated animals. Slight erythema was observed in one vehicle control animal at 24 hours following the third induction. No to severe erythema with/without blanching was osberved in the positive control animals. Individual animal data are presented in Table III.

Individual animal data are presented in Table IV. Dermal responses observed during the challenge phase are summarized in Table V.

During the challenge phase, no signs of erythema were observed in the test, vehicle, or negative control animals. No to moderate erythema was observed in the positive control group treated with p-phenylenediamine at a 35% concentration.

Under the conditions of this study, H-19,173 did not cause delayed contact hypersensitivity in guinea pigs.

TABLE I

SCORING SYSTEM USED TO EVALUATE SKIN RESPONSES

Score	Skin Reaction
0	No erythema or edema
1	Slight erythema (barely perceptible, usually nonconfluent)
2	Mild erythema (well defined, usually confluent)
3	Moderate erythema
4	Severe erythema (beet redness, with or without edema, eschar formation or necrosis)

If edema, blanching, necrosis or eschar formation occurred, they were indicated using the following codes:

ED = Edema

N = Necrosis

E = Eschar

B = Blanching

SN = Superficial Necrosis

TABLE II

PRIMARY IRRITATION PHASE

SKIN RESPONSES OBSERVED IN TEST GUINEA PIGS FOLLOWING TOPICAL EXPOSURE TO H-19,173

GUINEA PIG NUMBER	LEFT FRONT 100%		RIGHT FRONT 10%	
	24 hr.	48 hr.	24 hr.	48 hr.
1531	0	0	0	0
1532	0	0	0	0
1533	0	0	0	0
1534	0	0	0	0
1535	0	0	0	0
1536	0	0	0	0
1537	0	0	0	0
1538	0	0	0	0
1539	0	0	0	0
1540	0	0	0	0

TABLE II (Cont'd)

PRIMARY IRRITATION PHASE

SKIN RESPONSES OBSERVED IN VEHICLE CONTROL GUINEA PIGS FOLLOWING TOPICAL EXPOSURE TO DISTILLED WATER

GUINEA PIG NUMBER	LEFT FRONT Distilled water 24 hr.	48 hr.
1551	0	0
1552	0	0
1553	0	0
1554	0	0
1555	0	0

TABLE II (Cont'd)

PRIMARY IRRITATION PHASE

SKIN RESPONSES OBSERVED IN POSITIVE CONTROL GUINEA PIGS FOLLOWING TOPICAL EXPOSURE TO p-PHENYLENEDIAMINE

GUINEA PIG NUMBER	LEFT FRONT 35%	
	24 hr.	48 hr.
1541	0	0
1542	0	0
1543	0	0
1544	0	0
1545	0	0
1546	0	0
1547	0	0
1548	0	0
1549	0	0
1550	0	0

TABLE III

INDUCTION PHASE

SKIN RESPONSES OBSERVED IN TEST GUINEA PIGS 24 HOURS FOLLOWING INTRADERMAL (ID) INJECTIONS OF 1.0% H-19,173

GUINEA PIG NUMBER	ID1 (LEFT)	ID2 (RIGHT)	ID3 (LEFT)	ID4 (RIGHT)
1531	0	0	1	1
1532	0	0	1	1
1533	0	0	1	1
1534	0	0	2	1
1535	0	0	1	1
1536	1	0	1	1
1537	0	0	1	1
1538	0	0	1	1
1539	0	0	0	3
1540	0	0	1	1

TABLE III (continued)

INDUCTION PHASE

SKIN RESPONSES OBSERVED IN VEHICLE CONTROL GUINEA PIGS 24 HOURS FOLLOWING INTRADERMAL (ID) INJECTIONS OF 0.9% SALINE

GUINEA PIG NUMBER	ID1 (LEFT)	ID2 (RIGHT)	ID3 (LEFT)	ID4 (RIGHT)
1551	0	0	0	0
1552	0	0	0	0
1553	0	0	1	0
1554	0	0	0	0
1555	0	0	0	0

TABLE III

INDUCTION PHASE

SKIN RESPONSES OBSERVED IN POSITIVE CONTROL GUINEA PIGS 24 HOURS FOLLOWING INTRADERMAL (ID) INJECTIONS OF 1.0% p-PHENYLENEDIAMINE

GUINEA PIG NUMBER	ID1 (LEFT)	ID2 (RIGHT)	ID3 (LEFT)	ID4 (RIGHT)
1541	0	1	3	3
1542	0	1	1	3
1543	1	0	1	3^B
1544	0	0	2	4^B
1545	0	0	3	3
1546	0	1	1	2
1547	0	0	4B	3^B
1548	0	0	2	2
1549	0	0	2B	4
1550	0	0	3	3^B

B = Blanching

TABLE IV

CHALLENGE PHASE

SKIN RESPONSES OBSERVED IN TEST GUINEA PIGS FOLLOWING TOPICAL APPLICATION OF H-19,173

GUINEA PIG NUMBER	LEFT FRONT 100%		RIGHT FRONT 10%	
	24 hr.	48 hr.	24 hr.	48 hr.
1531	0	0	0	0
1532	d	d	d	d
1533	0	0	0	0
1534	0	0	0	0
1535	0	0	0	0
1536	0	0	0	0
1537	0	0	0	0
1538	0	0	0	0
1539	0	0	0	0
1540	0	0	0	0

d = denotes animal dying on study.

TABLE IV (continued)

CHALLENGE PHASE

SKIN RESPONSES OBSERVED IN POSITIVE CONTROL GUINEA PIGS FOLLOWING TOPICAL APPLICATION OF p-PHENYLENEDIAMINE

GUINEA PIG NUMBER	LEFT FRONT 35%	
	24 hr.	48 hr.
1541	2	3
1542	2	2
1543	1	2
1544	0	0
1545	0	1
1546	0	0
1547	1	1
1548	1	1
1549	1	2
1550	0	1

TABLE IV (continued)

CHALLENGE PHASE

SKIN RESPONSES OBSERVED IN VEHICLE CONTROL GUINEA PIGS FOLLOWING TOPICAL APPLICATION OF DISTILLED WATER

VEHICLE GUINEA PIG NUMBER	LEFT FRONT Distilled Water 24 hr.	48 hr.
1551	0	0
1552	0	0
1553	0	0
1554	0	0
1555	0	0

TABLE IV (continued)

CHALLENGE PHASE

SKIN RESPONSES OBSERVED IN NEGATIVE CONTROL GUINEA PIGS FOLLOWING TOPICAL APPLICATION OF H-19,173, p-PHENYLENEDIAMINE AND DISTILLED WATER

GUINEA PIG NUMBER	LEFT FRONT H-19,173 100%		RIGHT FRONT H-19,173 10%		LEFT REAR PPD 35%		RIGHT REAR Distilled Water	
	24 hr.	48 hr.	24 hr.	48 hr.	24 hr.	48 hr.	24 hr.	48 hr.
1556	0	0	0	0	0	0	0	0
1557	0	0	0	0	0	0	0	0
1558	0	0	0	0	0	0	0	0
1559	0	0	0	0	0	0	0	0
1560	0	0	0	0	0	0	0	0

Table V

Summary of Skin Responses
Challenge Phases

	Positive Control		Test Animals			
	PPD		H-19,173			
	35%[a]		100%[a]		10%[b]	
Responses	24 hr	48 hr	24 hr	48 hr	24 hr	48 hr
No erythema	4/10	2/10	9/9	9/9	9/9	9/9
Slight erythema	4/10	4/10	0/9	0/9	0/9	0/9
Mild erythema	2/10	3/10	0/9	0/9	0/9	0/9
Moderate erythema	0/10	1/10	0/9	0/9	0/9	0/9

	Negative Control							
	H-19,173				PPD		Vehicle Control	
	100%[a]		10%[b]		35%[c]		Distilled Water[d]	
Responses	24 hr	48 hr	24 hr	48 hr	24 hr	48 hr	24 hr	48 hr
No erythema	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5

	Vehicle Control	
	Distilled Water	
	24 hr	48 hr
No erythema	5/5	5/5

a = left shoulder
b = right shoulder
c = left flank
d = right flank

APPENDIX A

PROTOCOL AND PROTOCOL AMENDMENTS

PHARMAKON RESEARCH INTERNATIONAL, INC.
WAVERLY, PENNSYLVANIA 18471
PHONE (717) 586-2411
FAX (717) 586-3450

Protocol - 424D[1]

Skin Sensitization Test in Guinea Pigs

Sponsor:	E.I. du Pont de Nemours & Company P.O. Box 50 Elkton Road Newark, DE 19714
Testing Facility:	Pharmakon Research International, Inc. Waverly, Pennsylvania 18471
Test Facility S.O.P. No.:	PH-424
Study No.:	To be assigned upon study initiation. PH424-DU-012-91
Purpose of the Study:	This study is designed to evaluate the dermal sensitization potential of the test material in guinea pigs.
Ownership of the Study:	The sponsor owns the study. All raw data, analysis, and reports are the property of the sponsor.
Study Monitor:	Scott E. Loveless, Ph.D., E.I. du Pont de Nemours & Company
Study Director:	Susan Armondi, LAT, Pharmakon Research International, Inc.
Q.A.U. Responsible Personnel:	Leslie J. Pinnell, M.S.
Dates of Performance:	Study will begin within one month of the receipt of the test chemical and authorized protocol.
Good Laboratory Practices Statement:	This study will be conducted in compliance with the U.S. Environmental Protection Agency Toxic Substances Control Act Good Laboratory Practice Standards (40 CFR Part 160) or Good Laboratory Practice Standards (40 CFR Part 792).
IACUC Statement:	Protocol-424D[1] has been reviewed by the Institutional Animal Care and Use Committee (IACUC) and complies with acceptable standard animal welfare and humane care.

Protocol-424D[1]
Delayed Contact Hypersensitivity in Guinea Pigs (Buehler)

Tentative Date of Submission of Final Report:	Within three weeks following the completing of the in-life phase of the study.
Records Maintained:	All raw data, final reports, documentation and protocol will be maintained in the Pharmakon Archives. Amendments to protocol Feed Lot Number Body weights, initial weekly and final Compound preparation Volume administration Observed signs Observed skin reactivity
Raw Data:	Maintained in a Standard Pharmakon Notebook
Record Retention:	All raw data and completed notebooks.
Analytical Chemistry:	Analysis and stability of the test article and test article/carrier mixture is the responsibility of the sponsor. If requested by the sponsor, Pharmakon Research International, Inc. will through its subcontractor, conduct appropriate analytical analysis and will indicate the additional cost involved following receipt and evaluation of the appropriate analytical method. In the case where a satisfactory method is not provided, Pharmakon Research International, Inc. or its subcontractor, at additional cost to the sponsor, will develop appropriate methods. If the sponsor elects to analyze the test article/carrier mixtures, Pharmakon Research International, Inc. will collect the sample at appropriate designated intervals during the study and transmit the samples to the sponsor for analysis.

TEST SYSTEM

Species:	Guinea pig
Strain:	Hartley
Supplier (Source):	BuckerG Lab Animals, Tomkins Cove, New York or from any other U.S.D.A acceptable source.
Sex:	Male and Female (the sex used will be documented in the study records and final report).
Age:	Young adult

053191

Protocol-424D[1]
Delayed Contact Hypersensitivity in Guinea Pigs (Buehler)

Weight at Initiation:	400 - 650 grams
No. on Study:	Dose-Range-Finding: 3 Vehicle control: 5 (at the discretion of the Study Monitor, this group may be excluded from the study.) Negative control: 5 (if re-challenge is necessary, a second set of 5 naive, age-matched negative control animals will be used.) Test article: 10 Positive control: 10 (at the discretion of the Study Director after consultation with the Study Monitor, this group may be excluded from the study.)
Method and Justification for Randomization:	Treat groups will be housed by vertical cage positioning. Any animals showing evidence of clinical signs during acclimation will be eliminated from randomization. Randomization is carried out by use of a computer generated random number table. Before treatment commences, all guinea pigs will be weighed, ranked according to body weight and assigned to treatment groups using a table of random numbers so that each treatment group will have a similar distribution according to body weight. Guinea pigs beyond the extremes of the body weight range will not be assigned to treatment groups. If there is a statistically significant difference in mean body weight between any two groups of the same sex after allocation to the treatment groups, substitution from spare animals will be used to correct the situation.
Acclimation Period:	Minimum: five (5) days
System of Identification:	Cages marked with an animal group number, sex and treatment group. Guinea pigs are ear tagged.

HUSBANDRY

Research Facility Registration:	U.S.D.A. Registration No. 23-R-107 under the Animal Welfare Act 74: SC 2131 et seq.
Animal Rooms:	Separate isolation by test system. Light cycle - 12 hours light, 12 hours dark. Temperature/Humidity - Every attempt will be made to maintain a temperature of 22°C ± 3°C and a humidity of 30 to 70%.

053191

Protocol-424D[1]
Delayed Contact Hypersensitivity in Guinea Pigs (Buehler)

Housing: Guinea pigs are housed individually or two per cage in stainless steel ½" wire mesh cages. Size in accordance with the "Guide for the Care and Use of Laboratory Animals" of the Institute of Laboratory Animal Resources, National Research Council.

Sanitization: Waste material will be removed daily. Cages and feeders will be sanitized every two weeks.

Food: Purina Certified Guinea Pig Chow[R] #5026 or acceptable substitute will be provided ad libitum. Feeders are designed to reduce soiling, bridging, and scattering. The potential effects of dietary contaminants have been considered, and based on the manufacturer's data, contaminant levels are believed to be within acceptable limits.

Food Analysis: There are no contaminants expected to be present in the dietary material known to be capable of interfering with the purpose or conduct of the study.

Water: Fresh tap water, ad libitum.

Water Analysis: Water is monitored for contaminants at periodic intervals according to Standard Operating Procedure PH-018.

METHODS

Rationale for Test System: This study is intended to provide information on the health hazards likely to arise from exposure to the test material by the dermal route; skin contact is a possible worker and consumer exposure route.

Rationale for Dose Selection: Based upon the results of the dose-range study and/or at the sponsor's request.

Justification for Test System: According to EPA Health Effect Test Guidelines EPA 560/6-82-001. The guinea pig has historically been used in the evaluation of chemicals for dermal sensitization.

Compound Preparation: Liquids
Liquids will be applied as received, except when specific instructions request dilution or mixing. The use of a vehicle for dilution will be described in the study records and final report.

053191

Protocol-424D[1]
Delayed Contact Hypersensitivity in Guinea Pigs (Buehler)

Solids
The test material will be pulverized if appropriate. The test material may be moistened with distilled water or a suitable vehicle to ensure good contact with the skin. When a vehicle is used, the influence of the vehicle on penetration of the skin by the test substance will be considered.

Semi-Solids
Extremely viscous liquids may be heated to approximately 50°C to dosing, with the approval of the Study Monitor. The procedure used will be documented in the study records and included in the final report.

Absorption of the Test Material:	For the purpose of this study, all of the test material applied to the test sites is assumed to be absorbed by the test system. All calculations and the evaluation of effects will be based on the applied dose.
Vehicle:	The choice of vehicle varies, depending on the nature of the test substance. Commonly used vehicles are distilled water, saline, ethanol:distilled water (80:20, v/v), dimethyl phthalate, acetone:dimethyl phthalate (1:9, v/v) or propylene glycol. The vehicle used will be documented in the study records and the final report.
Volume Administration:	Approximately 0.05 ml/dose
Route of Administration:	The test and control materials will be applied topically and/or injected intradermally, to the clipped, intact shoulder skin.
Rationale for Route of Administration:	Skin contact is a possible worker and consumer exposure route.
No. and Description of Animals per Dose Group:	Dose-Range-Finding: 3 Vehicle control: 5 (at the discretion of the Study Director, this group may be excluded from the study). Negative control: 5 (if re-challenge is necessary, a second set of 5 naive, age-matched negative control animals will be used.) Test article: 10 Positive control: 10 (at the discretion of the Study Director after consultation with the Study Monitor, this group may be excluded from the study.)

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Protocol-424D[1]
Delayed Contact Hypersensitivity in Guinea Pigs (Buehler)

Method of Study Performance:

Dose-Range-Finding Study

Prior to initiation of the main study, a dose-range-finding study will be performed. In order to evaluate the irritation potential of the test material, 3 animals will be subjected to preliminary studies as follows:

Animals will be closely clipped over the back and sides. The test material concentrations will be based on the solubility of the test compound (solids will be tested at the maximum soluble concentration and at 2 or 3 lower concentrations). Liquids will be tested at 100% (as received or as prepared per the Sponsor's instructions) and at 2 or 3 lower concentrations. Each guinea pig may be dosed with up to 4 different concentrations at different sites (1 concentration/site). In some cases where the solid is not soluble in a common solvent, it will be tested as received by topical application.

One drop (approximately 0.05 ml) of each concentration will be applied directly on the appropriate test site and rubbed gently with the end of the dosing syringe to cover an area of approximately 25 mm in diameter. Observations for signs of dermal irritation, will be recorded at 24 and 48 hours after application. At each observation, all treated sites will be scored according to Table I.

Primary Irritation Phase

For the test guinea pigs there will be 2 sites per animal in the area between the shoulder and the middle of the back. One site will receive the highest slight to non-irritating concentration as determined from the dose-range-finding study. The second site will receive one-tenth of that concentration. Each site will receive 1 drop (approximately 0.05 ml) of the appropriate concentration, rubbed gently into the clipped skin with the end of the dosing syringe to cover an area approximately 25 mm in diameter. The vehicle control guinea pigs, if used, will be treated at 1 site as above using the vehicle only. The positive control guinea pigs will be treated at 1 site with the positive control. The negative control guinea pigs will remain untreated until the challenge phase. Observations for signs of dermal irritation will be recorded at 24 and 48 hours after application.

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Protocol-424D[1]
Delayed Contact Hypersensitivity in Guinea Pigs (Buehler)

Sensitization Phase (Induction)
The first induction application will be made after the 48-hour dermal observation is made for the primary irritation phase. If the test material is soluble in an appropriate vehicle, e.g., 0.9% saline or dimethyl phthalate, induction will be performed by intradermal injection. If the test material is insoluble in the commonly used vehicles a topical induction procedure will be used.

The hair at the appropriate application site will be clipped on the day prior to each application or, if a topical induction procedure is employed, at least weekly during induction. After clipping, the area to be treated may be delineated for the convenience of and at the discretion of the study director or the experimenter. The hair will be clipped as needed during the study for evaluation of the dermal irritation.

A total of 4 intradermal injections will be made, 1 per week at 1 week intervals. A virgin site will be used for each injection. A 0.1 ml aliquot of a 1% solution of the test material in an acceptable solvent (e.g., 0.9% saline, dimethyl phthalate, etc.) will be administered by intradermal injection to the clipped sacral area of each test guinea pig. If distilled water is used as a vehicle in the primary irritation phase, 0.9% saline will be used for the intradermal injections. The vehicle control guinea pigs will be treated as above using the vehicle only. The positive control guinea pigs will be treated as above using the positive control material.

Topical Administration
A total of 9 applications will be made to the sacral area, 3 applications per week for 3 weeks. One drop (approximately 0.05 ml) of a minimally irritating concentration of the test material in the acceptable solvent (e.g., dimethyl phthalate, 0.9% saline, etc. will be applied directly to the skin and then gently rubbed in with the end of the syringe, to cover an area approximately 25 mm in diameter on each test guinea pig. The vehicle control guinea pigs will receive similar treatments using the vehicle only. The positive control guinea pigs will receive similar treatments using the positive control article.

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Challenge Phase
Approximately 14 days after the last induction treatment, the challenge treatment will be administered. The test material will be administered in the same manner as in the primary irritation phase, treating with the same concentrations at the same sites. Following the same procedure the negative control animals will be treated with the same concentrations of the test material, and may be treated with the vehicle and positive control material (up to 4 separate test sites). The vehicle control animals will be treated with the vehicle only. The positive control animals will receive the positive control article. If the test results are conclusive and the test material has either clearly caused allergic sensitization or is not a sensitizer, the test is terminated and the guinea pigs are euthanized. If the reactions are less than conclusive, a second challenge (rechallenge) may be performed approximately 1 week after the initial challenge using the same test guinea pigs and additional naive control guinea pigs. The vehicle and positive control animals may not be rechallenged.

Method Validation/ Positive Control: The procedures and test system will be evaluated with a known dermal sensitizer to confirm the validity of the test. Evaluation of the test system with a known dermal sensitizer may not be conducted concurrently with every study, but testing with a known dermal sensitizer will be performed periodically.

Evaluation of Dermal Response: Dermal evaluations will be made between 23-25 hours and/or 47-49 hours following treatment, but the data will be reported as the 24- or 48-our evaluations.

Intervals:

a. Primary Irritation Test - Dermal evaluations will be made approximately 24 and 48 hours after the test material application.

b. Induction - Dermal evaluations will be made approximately 24 hours after each induction treatment.

c. Challenge and Rechallenge (if required) - Dermal evaluations will be made approximately 24 and 48 hours after the test material applications.

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Protocol-424D[1]
Delayed Contact Hypersensitivity in Guinea Pigs (Buehler)

Interpretation of Results: All test sites will be graded on a scale of 0 to 4 as follows:

TABLE I

Score	Skin Reaction
0	No erythema or edema
1	Slight erythema (barely perceptible, usually nonconfluent)
2	Mild erythema (well defined, usually confluent)
3	Moderate erythema
4	Severe erythema (beet redness, with or without edema, eschar formation or necrosis)

If edema, blanching, necrosis, superficial necrosis or eschar formation occur, they will also be indicated using the following code:

ED = Edema
N = Necrosis
E = Eschar
B = Blanching
SN = Superficial Necrosis

Sensitization Rating: Sensitization is defined as a 2-or-more step increase (e.g., from 0 to 2, from 1 to 3, etc.) in dermal irritation scores in the test animals over those observed in the negative controls at challenge. When the test and negative control animals have similar dermal irritation scores, the test material will not be considered a sensitizer. The rating of a sensitizer is as follows:

Ratio of Responsers	Degree of Sensitization
1 to 2/10	Weak Sensitizer
3 to 4/10	Mild Sensitizer
5 to 6/10	Moderate Sensitizer
7 and above	Strong Sensitizer

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Protocol-424D[1]
Delayed Contact Hypersensitivity in Guinea Pigs (Buehler)

	This rating scale is not fixed rigidly. Scientific evaluation of the data and judgement will be used to decide whether a material will be classified as a weak, mild, etc. sensitizer.
Necropsy:	Pathological evaluations of test animals will not ordinarily be performed. Exceptions will be noted in the study records and final report.
Euthanasia:	Animals that survive the test period will be euthanized according to the Testing Facility's Euthanasia SOP and incinerated.
Statistical Analysis:	Statistical analyses of data will not be performed, unless requested by the sponsor.
Rechallenge:	The sponsor will be notified of animals that are considered sensitized. Verbal instructions for rechallenge may be given by the sponsor, followed by written confirmation. All test article animals, along with an additional group of four naive guinea pigs, can be rechallenged six (6) days after primary challenge, but not before. The negative and positive control groups will not be rechallenged.

POSITIVE CONTROL ARTICLE

Positive Control Article:	N-Phenyl-1,4 phenylenediamine (PPD)
Supplier (Source):	Aldrich Chemical Company, Milwaukee, Wisconsin
Special Handling Instructions:	Standard precautions
Analysis of Purity:	The purity is the responsibility of the manufacturer.
How Supplied:	100 gm bottle
Rationale for Positive Control Article:	As per sponsor's request.

ACT/PT3

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Protocol-424D[1]
Delayed Contact Hypersensitivity in Guinea Pigs (Buehler)

Test Article (Name or Code): H 19173

Chemical Abstract No. or Code No.:

Analysis of Purity/Stability: Analysis of the purity and stability of the test article is the responsibility of the sponsor.

Carrier Mixtures: Analysis for stability, uniformity and correctness of concentration of the test article in the carrier is the responsibility of the sponsor.

___ Return Test Article Carrier Mixtures to the Sponsor

X Dispose of Test Article Carrier Mixtures

Person to whom carrier mixtures should be sent:

Shipping Instructions:

Government Agency Submission: ___FDA X TSCA ___FIFRA
___EEC ___OECD ___MHW
___Other

AMENDMENTS

APPROVAL OF PROTOCOL

Date 10/22/91 Study Monitor Scott E. Lovely

Date 10/28/91 Study Director Susan E. Armondi

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Protocol-424D[1]
Delayed Contact Hypersensitivity in Guinea Pigs (Buehler)

APPENDIX A

Test Article Information

I Identification:

Test Article (Name or Code): H19173
Lot or Sample No.:
Physical Description: Slightly yellow liquid dispersion
Purity: 100%
Expiration Date:
~~Density~~/Specific Gravity: 1.07
Solubility (check one): Water Dispersible Acetone
Ethanol Corn Oil DMSO
Other (please specify)
Chemical Classification: Flammable Corrosive
Other

II Storage Information:

Material Storage (check one):
Room Temperature X ; Refrigerator
Freezer ; Other (specify)

III Handling Information:

Known Hazards: Moderate Eye Irritant; May cause skin irritancy

Precautions: Routine use of protective clothing includes laboratory coats, latex gloves, dust masks, and safety glasses.

Other recommended precautions

In Case of Emergency Related to this substance, contact:

Scott Lovelin (person) of DuPont CR&D (company/division) at 302 451-4806 (phone number)

IV Disposition:

All materials will be returned to the sponsor three months following submission of the final report to the sponsor. Person and address to whom test articles are to be returned.

Name:

Address:

V Signature: Scott Lovelin Date: 10/22/91

053191

Protocol Amendments
Skin Sensitization Test in Guinea Pigs
PH 424-DU-012-91
Test Article: H-19,173

Records Maintained: (page 2 of protocol, line 8)	Original Statement Body weights, initial weekly and final Corrected Statement Body weights, initial, weekly and final (including means and standard deviations)
Reason for Amendment:	As per sponsor's request.
Evaluation of Dermal Response: (page 8 of protocol)	Original Statement Dermal evaluations will be made between 23-35 hours and/or 47-49 hours following treatment, but the data will be reported as the 24- or 48- our evaluations. Corrected Statement Dermal evaluations will be made between 23-25 hours and/or 47-49 hours following treatment, but the data will be reported as the 24- or 48-hour evaluation.
Reason for Amendment:	Typographical error.
Compound Preparation: (page 5 of protocol)	Original Statement Semi-Solids Extremely viscous liquids may be heated to approximately 50°C to dosing, with the approval of the Study Monitor. The procedure used will be documented in the study records and included in the final report.

Protocol Amendments
Skin Sensitization Test in Guinea Pigs
PH 424-DU-012-91
Test Article: H-19,173

Corrected Statement
Semi-Solids
Extremely viscous liquids may be heated to approximately 50°C to facilitate dosing, with the approval of the Study Monitor. The procedure used will be documented in the study records and included in the final report.

Reason for Amendment: Typographical error.

Method and Justification for Randomization: (page 3 of protocol)

Original Statement
Treat groups will be housed by vertical cage positioning.

Corrected Statement
Treatment groups will be housed by vertical cage positioning.

Reason for Amendment: Typographical error

Susan E. Armondi
Susan E. Armondi, LAT
Study Director
Pharmakon Research International, Inc.

June 12, 1992
Date

APPENDIX B

WEEKLY BODY WEIGHTS (g)

WEEKLY BODY WEIGHTS (G)

Animal Number	Sex	Initial	Week II	Week III	Week IV	Week V	Week VI	Final
Test Group								
1531	M	452	481	539	579	612	670	680
1532	M	512	572	648	719	783	d	748*
1533	M	482	573	638	621	717	788	797
1534	M	483	558	612	675	719	692	732
1535	M	507	555	613	658	702	757	766
Mean		487	548	610	650	707	727	744
S.D.		24	38	43	53	61	55	50
N		5	5	5	5	5	4	4
1536	F	446	506	537	572	625	654	683
1537	F	466	509	558	574	634	648	668
1538	F	428	448	492	534	558	583	579
1539	F	496	548	593	610	669	710	713
1540	F	452	489	509	549	588	620	641
Mean		458	500	538	568	615	643	657
S.D.		25	36	40	29	43	47	51
N		5	5	5	5	5	5	5

d = denotes animal dying on study
* = weight upon death, data not included in statistical analysis

WEEKLY BODY WEIGHTS (G)

Animal Number	Sex	Initial	Week II	Week III	Week IV	Week V	Week VI	Final
Vehicle Control Group								
1551	M	519	576	642	710	772	825	840
1552	M	461	518	581	613	668	712	739
1553	M	522	593	668	713	780	830	845
Mean		501	562	630	679	740	789	808
S.D.		34	39	45	57	62	67	60
N		3	3	3	3	3	3	3
1554	F	424	457	506	524	568	605	608
1555	F	465	525	557	597	624	666	694
Mean		445	491	532	561	596	636	651
S.D.		29	48	36	52	40	43	61
N		2	2	2	2	2	2	2

WEEKLY BODY WEIGHTS (G)

Animal Number	Sex	Initial	Week II	Week III	Week IV	Week V	Week VI	Final
Positive Control Group								
1541	M	506	557	616	666	714	746	776
1542	M	503	532	584	617	668	691	710
1543	M	494	544	588	637	677	721	746
1544	M	464	511	571	611	674	710	732
1545	M	499	548	602	647	653	683	697
Mean		493	538	592	636	677	710	732
S.D.		17	18	17	22	23	25	31
N		5	5	5	5	5	5	5
1546	F	429	460	522	526	591	594	633
1547	F	462	533	558	592	627	657	679
1548	F	471	534	590	618	694	744	753
1549	F	457	488	534	536	585	608	599
1550	F	499	542	589	642	683	723	744
Mean		464	511	559	583	636	665	682
S.D.		25	36	31	51	51	67	67
N		5	5	5	5	5	5	5

WEEKLY BODY WEIGHTS (G)

Animal Number	Sex	Initial	Week II	Week III	Week IV	Week V	Week VI	Final
Negative Control Group								
1556	M	482	546	619	663	742	801	812
1557	M	509	575	636	679	761	826	839
Mean		496	561	628	671	752	814	826
S.D.		19	21	12	11	13	18	19
N		2	2	2	2	2	2	2
1558	F	415	454	485	497	530	556	571
1559	F	486	548	592	650	664	725	754
1560	F	447	468	525	526	587	563	608
Mean		449	490	534	558	594	615	644
S.D.		36	51	54	81	67	96	97
N		3	3	3	3	3	3	3

APPENDIX C

COPY OF RAW DATA

PHARMAKON RESEARCH INTERNATIONAL, INC.

N.B.# [redacted]
Page

Skin Sensitization Test in Guinea Pigs
Dose-Range-Finding Study

Sponsor: E.I. du Pont de Nemours & Co.

Purpose: To determine the topical irritation potential of the test article in Guinea Pigs

Study No.: PH424-DU-012-91

Method: Refer to Protocol #2440

Date of Initiation: 1/14/92 Date of Termination: 1/16/92

Test Article: H-19,173 Description: Slightly yellow liquid Dispersion JDC Jm 1/14/92

Amount Received: 182.12g (Gross) Date Submitted: 10/28/91

Vehicle: Distilled water Dose Levels: 1.0, 10, 50 and 100%

Route of Administration: Dermal

Animal P.O. #: 5011-010392B

Species: Guinea Pig Strain: Hartley

Food Lot #: 102391 1A Identification: Cage card & Animal No.

No. of Animals on Study: 3 Sex: Male 1 Female 2

Scale#: 31 (Animal) Balance #32 (Test Article) Light Cycle Checked: 1/14/92 Jm

Animals Clipped: 1/13/92 Jm

Compound Preparation: @ 100% → Dosed as received (0.05mL/site);
@ 50% → 0.5mL test article + 0.5mL vehicle;
@ 10% → 0.1mL test article + 0.9mL vehicle;
@ 1.0% → 0.1mL of the 10% concentration + 0.9mL vehicle;
Vehicle → distilled water. Total amount of test article extracted from containers 0.935 grams, residual disposed of properly. The above concentrations, 1/14/92 Jm were considered good and ~~suitable~~ soluble in the vehicle 1/14/92 JDC, 1/14/92 CR Jm

Marina Biesgaard
Investigator

1-16-92
Date

Susan E. Armondi
Study Director

1/16/92
Date

ATABL3(1)

Amended Final Report

Du Pont HLO 161-92

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N.B.# [redacted]
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PH424-DU-012-91

Skin Sensitization Test in Guinea Pigs

Dose-Range-Finding Study

1/14/92

In this test, both sides of the animal are ~~shaved~~ clipped and exposed to four concentrations of the test material. The different concentrations are applied concurrently to four different test sites. A given concentration is applied to a different test site on each guinea pig so as to minimize variation in response due to dfferent skin locations. The format for application of the test article is described below:

Figure A

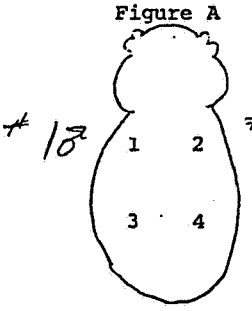
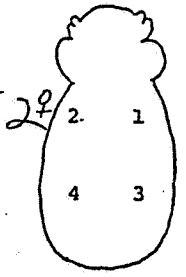


Figure B



Site 1 = 1.0%
Site 2 = 10%
Site 3 = 50%
Site 4 = 100%

Figure C

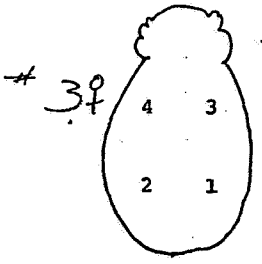
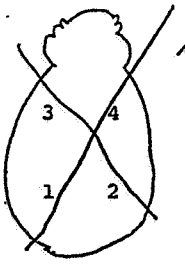


Figure D



Not applicable
1/16/92
SEA

1-4: Test Sites

Animal No.	Sex	Test article and dose	Initial Wt. (gms)	Time of Dose	24 Hour Scores Site 1	Site 2	Site 3	Site 4	Final Wt. (gms)
1	♂	H-19,173	375	2:01Pm	0	0	0	0	
2	♀	1.0, 10, 50	417	↓	0	0	0	0	
3	♀	And 100%	392	2:06Pm	0	0	0	0	
			11/14/92 JM	11/14/92 JM CB	1/15/92			CLH	

48 Hour Scores				
0	0	0	0	384
0	0	0	0	436
0	0	0	0	412
1/16/92			MLS	1-16-92 MLS

Maura Buszczak
Investigator

1-16-92
Date

Susan E. Armondi
Study Director

1/16/92
Date

ATABL3(2)

PHARMAKON RESEARCH INTERNATIONAL, INC.

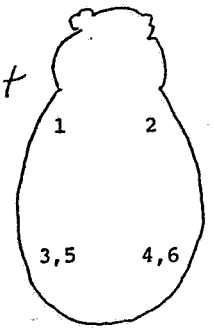
N.B.#
Page

Skin Sensitization Test in Guinea Pigs

Title: Skin Sensitization Test in Guinea Pigs
Sponsor: E.I. du Pont de Nemours + Company
Purpose: To evaluate the dermal sensitization potential of the test article in Guinea Pigs
Study No.: PH424-DU-012-91
Method: Refer to Protocol 424D'
Technical Initiation: 1/28/92 Technical Termination: 3/7/92
Test Article: H-19,173 Positive Control: (PPD) N-phenyl-1, 4-phenylene diamine
Lot Number: Not Applicable Lot Number: 05012MK Aldrich chemical Co.
Description: Slightly yellow liquid dispersion IDE Jm 1/14/92 Description: Black flakes
Vehicle: Distilled water Vehicle: Distilled water
Amount Submitted: 182.12 g
Receipt Date: 10/28/91 Route of Administration: dermal, intradermal
Animal P.O. #: 5011-010392 C Toxicology Lab. No.: VI
Species: Guinea Pig Strain: Ha:Hsy
Weight Range: IDE Jm 1/28/92 LATE entry 7/18/92 Jm 424 - 622 grams 415
Food Lot #: 010792 1B, 010792 1A
No. of Animals on Study: 30 Sex: Male 15♂ Female 15♀
Scale #: 31 (Animal) Balance #32 (Test Article) Light Cycle Checked: 1/28/92 Jm 2/5/92 CLH 2/12/92 CLH, 2/19/92 CLH, 2/26/92 CLH
Animals Clipped: 1/27/92 Jm, 1/29/92 Jm, 2/5/92 Jm, 2/12/92 CLH 2/19/92 CLH

Format for Primary Irritation, Induction and Challenge Phases

6/1/92 - late entry for animal clip on 3/4/92 - Inadvertent omission of data. SEA



1-2 Primary Irritation and Challenge

3-6 Sensitization Phase (Induction)

Investigator — Date 3/7/92

Study Director — Date 3/7/92

ATTACHED

Amended Final Report

Notebook [redacted]
Page #

PHARMAKON RESEARCH INTERNATIONAL, INC.

Skin Sensitization Test in Guinea Pigs

GENERAL OBSERVATIONS

Study No.: PH424-DU-012-91 Sponsor: E.I. du Pont de Nemours & Company
Test Article: H-19173

Date	AN = (appears normal)	Initials
1/28/92	Distilled water - Lot # 22012, Ephrata Diamond Spring Co. Ephrata, PA Exp 11/21/92	JM
1/30/92	0.9% Sodium Chloride Baxter Healthcare Corporation, Deerfield, IL Lot # C 165621 Exp 12/92	JM

ATABL3(7)

PHARMAKON RESEARCH INTERNATIONAL, INC.

Skin Sensitization Test in Guines Pigs

Test Article Preparation

Study No.: PH424-Du-012-91

Test Article: H-19,173 Sponsor: E.I. du Pont de Nemours & Co.

Primary Irritation Phase (Week I): @100% → Test article dosed as received (0.05ml/site). IDE Jm 11/26/92
@10% → 0.5 mL test article ~~q.s. to 5.0mL~~ PLUS 4.5 mL. PPD @ 35% → 1.75 grams PPD q.s. to 5.0mL; Vehicle → distilled water. PPD suspension homogenized using a polytron prior to dosing and forcibly mixed prior to each dose and considered good. Total amount of test article extracted from containers 1.20 grams, residual disposed of properly. 1/28/92 Jm CH

Induction (Week I): @1.0% → 0.1mL test article + 9.9mL vehicle;
Vehicle → 0.9% saline;
Positive control PPD @ 1.0% → 100mgs PPD q.s. to 10mL.
Total amount of test article extracted from container 96 mgs. Polytron used to homogenize PPD. 1/30/92 Jm CH

Induction (Week II): @1.0% → 0.1mL test article + 9.9mL vehicle;
Vehicle → 0.9% saline;
Positive control PPD @1.0% → 100mgs PPD q.s. to 10mL. Polytron used to homogenize PPD. Total amount of test article extracted from container 110 mgs. 2/6/92 Jm CB

Induction (Week III): @ 1.0% → 0.1ml of test article + 9.9 ml of vehicle; Vehicle → 0.9% saline. Positive Control PPD @ 1.0% → 100 mgs of PPD q.s. to 10ml. PPD was homogenized using polytron. Total amount of test article extracted from container: 100 mg. 2/13/93 CB Jm

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Notebook [redacted]
Page # [redacted]

PHARMAKON RESEARCH INTERNATIONAL, INC.

Skin Sensitization Test in Guinea Pigs

GENERAL OBSERVATIONS

Study No.: PH424-DU-012-91 Sponsor: E.I. du Pont de Nemours & Company
Test Article: H-19,173

Date	AN = (appears normal)	Initials
1/28/92	Distilled water - Lot # 22012, Ephrata Diamond Spring Co. Ephrata, PA Exp 11/21/92	JM
1/30/92	0.9% Sodium Chloride Baxter Healthcare Corpration, Deerfield, IL Lot # C 165621 Exp 12/92	JM
		JM

ATABL3(7)

PHARMAKON RESEARCH INTERNATIONAL, INC.

Skin Sensitization Test in Guinea Pigs

Test Article Preparation (Continued)

Study No.: PH424-DU-012-91

Test Article: H-19173 Sponsor: E.I. du Pont de Nemours + Co.

Induction (Week IV): @ 1.0% → 0.1mL test article + 9.9mL vehicle; Positive control PPD @ 1.0% → 100mgs PPD q.s. to 10mL; Vehicle → 0.9% saline. Polytron used to homogenize PPD. Total amount of test article extracted from container 109 mgs. 2/20/92 IDC JM 2/20/92 JM, CB 2/20/92

Challenge (Week VI): Test article @ 100% → test article dosed as received (0.05mL/site); @ 10% → 0.5mL test article + 4.5mL; Positive control PPD @ 35% → 3.5grams PPD q.s. to 10mL; Vehicle → distilled water. Polytron used to homogenize PPD. Total amount of test article extracted from containers 2.76grams, residual disposed of properly. 3/5/92 JM CB

Rechallenge (Week VII):

ATABL3(4)

Notebook [redacted]
Page # [redacted]

PHARMAKON RESEARCH INTERNAIONAL, INC.

Skin Sensitization Test in Guinea Pigs

Body Weights (grams)

Sponsor: E.I. du Pont de Nemours + Co. Study Number: PH424-Du-012-91
Test Article: H-19,173 Lot Number: Not Applicable

Animal Number	Sex	Initial Week I	Week II	Week III	Week IV	Week V	Week VI	Final
1531	♂	452	481	539	579	612	670	680
1532	♂	512	572	648	719	783	d	748*
1533	♂	482	573	638	621	717	788	797
1534	♂	483	558	612	675	719	692	732
1535	♂	507	555	613	658	702	757	766
1536	♀	446	506	537	572	625	654	683
1537	♀	466	509	558	574	634	648	668
1538	♀	428	448	462	534	558	583	579
1539	♀	496	548	593	610	669	710	713
1540	♀	452	489	509	549	588	620	641
		11-28-92 JM	2-4-92 MLB	2-11-92 MLB	2-18-92 MLB	2/25/92 CLH	3-3-92 MLB	3/7/92 CLH
Vehicle Control								
1551	♂	519	576	642	710	772	825	840
1552	♂	461	518	581	613	668	712	739
1553	♂	522	593	668	713	780	830	845
1554	♀	424	457	506	524	568	605	608
1555	♀	465	525	557	597	624	666	694
		11-28-92 JM	2-4-92 MLB	2-11-92 MLB	2-18-92 MLB	2/25/92 CLH	3-3-92 MLB	3/7/92 CLH

d = denotes animal dying on study 3/4/92 CLH
* = weight upon death 3/2/92 CLH

Charles Laurenzo 3/7/92
Investigator Date

Susan E. Armond 3/7/92
Study Director Date

ATABL3(5)

Notebook [redacted]
Page # [redacted]

PHARMAKON RESEARCH INTERNAIONAL, INC.

Skin Sensitization Test in Guinea Pigs

Body Weights (grams)

Sponsor: E.I du Pont de Nemours Co. Study Number: PH424-DU-012-91
Test Article: H-19,173 Lot Number: Not Applicable

Animal Number	Sex	Initial Week I	Week II	Week III	Week IV	Week V	Week VI	Final
Positive Control								
1541	♂	506	557	616	666	714	746	776
1542	♂	503	532	584	617	668	691	710
1543	♂	494	544	588	637	677	721	746
1544	♂	464	511	571	611	674	710	732
1545	♂	499	548	602	647	653	683	697
1546	♀	429	460	522	526	591	594	633
1547	♀	462	533	558	592	627	657	679
1548	♀	471	534	590	618	694	744	753
1549	♀	457	488	534	536	585	608	599
1550	♀	499	542	589	642	683	723	744
		1/23/92 Jm	2-4-92 mlb	2-11-92 mlb	2/18/92 mlb	2/25/92 CHH	3-3-92 mlb	3/7/92 CB
Negative Controls								
1556	♂	482	546	619	663	742	801	812
1557	♂	509	575	636	679	761	926	839
1558	♀	415	451	485	497	530	556	571
1559	♀	486	548	592	650	664	725	754
1560	♀	447	468	525	526	587	563	608
		1/23/92 Jm	2-4-92 mlb	2-11-92 mlb	2-18-92 mlb	2/25/92 CHH	3-3-92 mlb	3/7/92 CB

Investigator 3/7/92 Date

Study Director 3/7/92 Date

ATABL3(5)

PHARMAKON RESEARCH INTERNATIONAL, INC.

Individual Animal Scores During Primary Irritation Phase

Sponsor: E.I. duPont DeNemours & CO Study Number: PH424-DU-012-91 Notebook [redacted]

Test Article: A-18,173 Lot Number: Not Applicable Page # [redacted]

Animal No.	Sex	Dose Conc. Left Shoulder	Dose Conc. Right Shoulder	Time of Dose	24 Hours a	24 Hours b	Time of Reading	48 Hours a	48 Hours b	Time of Reading
1531	♂	Test Article @ 10% (0.05 ml/site)	Test Article @ 10% (0.05 ml/site)	11:35 AM	0	0	11:30 AM	0	0	11:35 AM
1532	♂				0	0		0	0	
1533	♂				0	0		0	0	
1534	♂				0	0		0	0	
1535	♂				0	0		0	0	
1536	♀				0	0		0	0	
1537	♀				0	0		0	0	
1538	♀				0	0		0	0	
1539	♀				0	0		0	0	
1540	♀			11:45 AM	0	0	11:35 AM	0	0	11:41 AM
				11/28/92 JM MAB		1/2	9/92 CLH		11/30/92 JM	
Vehicle Control										
1551	♂	Distilled Water (0.05 ml/site)		11:50 AM	0		11:41 AM	0		11:50 AM
1552	♂				0			0		
1553	♂				0			0		
1554	♀				0			0		
1555	♀			11:52 AM	0		11:42 AM	0		11:53 AM
Initials				11/28/92 JM MAB		1/2	9/92 CLH		11/30/92 JM	

a = Left ~~Flank~~ Shoulder b = Right ~~Flank~~ Shoulder DESCA 11/28/92

ATABL3(9)

PHARMAKON RESEARCH INTERNATIONAL, INC.

Individual Animal Scores During Primary Irritation Phase

Sponsor: E.I. du Pont de Nemours & Co Study Number: PHRM-DU-012-91 Notebook # [redacted]

Test Article: H-19173 Lot Number: Not Applicable Page # [redacted]

Animal No.	Sex	Dose Conc. Left Shoulder	Dose Conc. Right Shoulder	Time of Dose	24 Hours	Time of Reading	48 Hours	Time of Reading
Positive Control		PPD @ 35% (0.05 mL i.d.)						
1541	♂			11:45 AM	0	11:36 AM	0	11:42 AM
1542	♂			↓	0	↓	0	↓
1543	♂				0		0	
1544	♂				0		0	
1545	♂				0		0	
1546	♀				0		0	
1547	♀				0		0	
1548	♀				0		0	
1549	♀				0		0	
1550	♀			11:50 AM	0	11:40 AM IDE CLH 11/29/92	0	11:50 AM
				11/28/92 JM	1/2	9/92 CLH	11/30/92 JM	
Negative Control								
1556	♂							
1557	♂							
1558	♀							
1559	♀							
1560	♀							
Initials								

ATABL3(9)

PHARMAKON RESEARCH INTERNATIONAL, INC.

Individual Animal Scores During Induction

Sponsor: E.I du Pont de Nemours & Co Study Number: PH424-DU-012-91 Notebook #: [redacted]

Test Article: H-19173 Lot Number: Not Applicable Page #: [redacted]

			Week I			Week II			Week III				Week IV		
Animal No.	Sex	Dose Conc.	Time of Dose	24 Hrs.	Time of Reading	Time of Dose	24 Hrs.	Time of Reading	Time of Dose	24 Hrs.	Time of Reading	Time of Dose	24 Hrs.	Time of Reading
1531	♂	Test article (0.1 mL/kg bw)	10:50 Am	0	10:55 Am	11:15 Am	0	10:35 Am	11:10	1	11:20	1:20	1	1:30 Pm
1532	♂			0			0			1			1	
1533	♂			0			0			1			1	
1534	♂			0			0			2			1	
1535	♂			0			0			1			1	
1536	♂			1			0			1			1	
1537	♂			0			0			1			1	
1538	♂			0			0			1			1	
1539	♂			0			0			0			3	
1540	♂		10:57 Am 1/30/92 Jm CLH	0	10:59 Am 1/31/92 JM	11:22 Am 2/6/92 Jm CB	0	10:42 Am 2/7/92 CLH	11:16 2/13/92 1223 m	1 2/14/92	11:27 CB	1:31 2/20/92 CB	1 2-21-92 MLG	1:37 Pm
Vehicle Control														
1551	♂	0.92 mL/kg bw	11:04 Am	0	11:04 Am	11:34 Am	0	10:48 Am	11:21	0	11:35	1:42	0	1:47 Pm
1552	♂			0			0			0			0	
1553	♂			0			0			1			0	
1554	♀			0			0			0			0	
1555	♀		11:06 Am 1/30/92 Jm CLH	0	11:05 Am 1/31/92 JM	11:36 Am 2/6/92 Jm CB	0	10:50 Am 2/7/92 CLH	11:23 2/13/92 1223 m	0 2/14/92	11:37 CB	1:45 2/20/92 CB	0 2-21-92 MLG	1:47 Pm
Initials														

ATABL3(10)

PHARMAKON RESEARCH INTERNATIONAL, INC.

Individual Animal Scores During Induction

Sponsor: E.I du Pont de Nemours Co Study Number: PH424-Du-012-91 Notebook #

Test Article: H-9173 Lot Number: Not Applicable Page #

			Week I			Week II			Week III			Week IV		
Animal No.	Sex	Dose Conc.	Time of Dose	24 Hrs.	Time of Reading	Time of Dose	24 Hrs.	Time of Reading	Time of Dose	24 Hrs.	Time of Reading	Time of Dose	24 Hrs.	Time of Reading
Positive Control														
1541	♂	TPTD 1.0% (a.i./kg/day)	10:57 Am	0	11:00 AM	11:22 Am	1	10:43 pm	11:16	3	11:28 AM	1:32	3	1:39 pm
1542	♂			0			1			1			3	
1543	♂			1			0			1			3B	
1544	♂			0			0			2			4B	
1545	♂			0			0			3			3	
1546	♀			0			1			1			2	
1547	♀			0			0			4B			3B	
1548	♀			0			0			2			2	
1549	♀			0			0			2B			4	
1550	♀		11:04 Am 11/30/92 Jm CLH	0	11:04 Am 11/31/92 Jm	11:34 Am 2/6/92 Jm CB	0	10:48 pm 2/7/92 CLH	11:21 2/12/93 pm	3	11:34 AM	1:41 2/20/93	3B	1:44 pm 2-21-93 pm CB
Negative Control														
1556	♂													
1557	♂													
1558	♀													
1559	♀													
1560	♀													
Initials														

B = Bruising around mouth

ATABL3(10)

PHARMAKON RESE...H INTERNATIONAL, INC.

Individual Animal Scores after Challenge

Sponsor: E.I. du Pont de Nemours + Co. Study Number: PH424-DU-012-91 Notebook [redacted]

Test Article: H-19173 Lot Number: Not Applicable Page #

CLH 3/5/92

a = left shoulder
b = right shoulder

Animal No.	Sex	Dose Conc. Left Shoulder	Dose Conc. Right Shoulder	Challenge Scores: Time of Dose	24 Hrs a	24 Hrs b	Time of Reading	48 Hrs. a	48 Hrs. b	Time of Reading	Rechallenge Scores: Dose Conc. Left Shoulder	Dose Conc. Right Shoulder	Time of Dose	24 Hrs	Time of Reading	48 Hrs.	Time of Reading
1531	♂	Test article @ 10% Dosed w/ vaseline (0.05 mL/site)	Test article @ 10% (0.05 mL/site)	11:00 AM	0	0	10:58 AM	0	0	10:25 AM							
1532	♂				d			d									
1533	♂				0	0		0	0								
1534	♂				0	0		0	0								
1535	♂				0	0		0	0								
1536	♀				0	0		0	0								
1537	♀				0	0		0	0								
1538	♀				0	0		0	0								
1539	♀				0	0		0	0								
1540	♀			11:04 AM 3/5/92 JM CB	0	0	11:03 AM 3/6/92 CB	0	0	10:29 AM 3/7/92 CB							
Vehicle Control																	
1551	♂	Petrolatum (0.05 mL/site)		11:09 AM	0		11:08 AM	0		10:36 AM							
1552	♂				0			0									
1553	♂				0			0									
1554	♀				0			0									
1555	♀			11:11 AM 3/5/92 JM CB	0		11:10 AM 3/6/92 CB	0		10:38 AM 3/7/92 CB							
Initials																	

ATABL3(10) d = denotes Animal dying on Study 3/6/92 JM

Company Sanitized. Does not contain TSCA CBI
Amended Final Report

Du Pont HLO 161-92

PHARMAKON RESE...CH INTERNATIONAL, INC.

Individual Animal Scores after Challenge

Sponsor: E.I. duPont de Nemours & Co. Study Number: PH424-DU-012-91 Notebook # [redacted]

Test Article: H-19173 Lot Number: Not Applicable Page # [redacted]

a = left shoulder b = right shoulder c = left flank d = right flank (init. 3/5/92)

Animal No.	Sex	Challenge: Dose Conc. Left Shoulder	Challenge: Dose Conc. Right Shoulder	Time of Dose	24 Hrs.	Time of Reading	48 Hrs.	Time of Reading	Rechallenge (IDE 3/5/92): Dose Conc. Left ~~Shoulder~~ Flank	Dose Conc. Right ~~Shoulder~~ Flank	Time of Dose	24 Hrs	Time of Reading	48 Hrs.	Time of Reading
Positive Controls									IDE JM	3/5/92					
1541	♂	PPD @ 35% (0.05 mL/site)		11:04 AM	2	11:03 AM	3	10:29							
1542	♂				2		2								
1543	♂				1		2								
1544	♂				0		0								
1545	♂				0		1								
1546	♀				0		0								
1547	♀				1		1								
1548	♀				1		1								
1549	♀				1		2								
1550	♀			11:09 AM	0	11:08 AM	1	10:35 AM							
				3/5/92 JM CB	3/6/92 CB		3/7/92 CB								
Negative Controls					a b		a b				IDE JM 3/5/92	c d		c d	
1556	♂	Test article @ 100% - dosed as received (0.05 mL/site)	Test article @ 10% (0.05 mL/site)	11:11 AM	0 0	11:10 AM	0 0	10:38 AM	PPD @ 35% (0.05 mL/site)	Distilled water (0.05 mL/site)		0 0	11:10 AM	0 0	10:38 AM
1557	♂				0 0		0 0					0 0		0 0	
1558	♀				0 0		0 0					0 0		0 0	
1559	♀				0 0		0 0					0 0		0 0	
1560	♀			11:15 AM	0 0	11:16 AM	0 0	10:43 AM				0 0	11:16 AM	0 0	10:43 AM
Initials				3/5/92 JM CB	3/6/92 CB		3/7/92 CB					3/6/92 CB		3/7/92 CB	

ATABL3(10)

PHARMAKON RESEARCH INTERNATIONAL, INC.
WAVERLY, PENNSYLVANIA 18471

PHONE
(717) 586-2411

Individual Necropsy Sheet

Study No. PH424-DU-012-91	Species: Guinea Pig	Sex: ♂	Weight: 748g	Date: 3/2/92
Animal #: 1532	Ear Tag #: 1532	Reason*: (1) 2 3 4 (circle one)		
Compound: H-19,173	Dose: As received	Study Director: D. Dumond		

Tissue	E	Wt.	Tissue	E	Wt.	Tissue	E	Wt.	Other
Brain	✓		Heart	✓		Cecum	✓		
Sp.Cord	✓		Liver	✓		Colon	✓		
Pituitary	✓		Cholecyst	✓		Mesen.l.node	✓		
Eyes	✓		Kidneys	✓		Testes	✓		
Sal.glands	✓		Spleen	✓		Epididymis	✓		
Submn.l.node	✓		Pancreas	✓		Prostate	✓		
Thyroid	✓		Urocyst	✓		Seminal ves.	✓		
Parathyroid	✓		Adrenals	✓		Ovary	N/A		
Thymus	✓		Stomach	✓		Uterus	N/A		
Esophagus	✓		Duodenum	✓		Sternebrae	✓		
Trachea	✓		Jejunum	✓		Sciatic n.	✓		
Lung	✓		Ileum	✓		Skel.muscle	✓		

E= examine Wt.= weight (gm) *1 = dead 2 = term sac 3 = sac morib 4 = sac weak

Necropsy Observation (NVL = no visible lesions):

NVL

Prosector: Christine L. Stelle STUDY DIRECTOR: D Dumond

ATABLE

Notebook 1554
Page 35A