Study Title

Skin Sensitization Test
with [redacted] in Guinea Pigs

Study Completed On

August 14, 1991

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.

[redacted]

Laboratory Project ID

Haskell Laboratory Report No. 433-91

COMPLIANCE STATEMENT

This study was conducted in compliance with the Principles of Good Laboratory Practices (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 792, Thursday August 17, 1989.

U.S. Environmental Protection Agency as stated in the Federal Register, 40 CFR, Part 160, 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 No.: [redacted]

"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 conclusions."

Susan E. Armondi
Study Director

July 23, 1991
Date

GENERAL INFORMATION

	Test Material
Material Tested:	[redacted]
Medical Research No.:	[redacted]
Haskell No.:	18,920
Haskell Test Code No.:	[redacted]
Pharmakon Study No.:	[redacted]
Physical Form:	[redacted]
Purity:	[redacted]
Composition:	[redacted]
Synonyms:	[redacted]
Other Codes:	[redacted]
Contaminants:	[redacted]
CAS Registry Number:	[redacted]
Stability:	In the absence of visible evidence to the contrary, the test material was assumed to be stable under the conditions of administration.

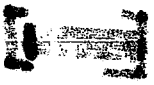
GENERAL INFORMATION (CONT.)

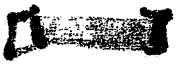
Positive Control Material

Material Tested:	N-Phenyl-1,4-Phenylenediamine
Purity:	98%
Synonyms:	o 1,4-Benzenediamine o p-phenylenediamine o PPD
Other Codes:	o Aldrich Chemical Co., Lot #05012MK o Aldrich Chemical Co., Catalog #24139-3
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
Materials Submitted By:	K. Spencer Prowse Du Pont Chemicals E. I. du Pont de Nemours and Company Jackson Laboratory Deepwater, New Jersey
Pharmakon Notebook No.:	1470, pp. 389, 392, 395, and 399 1489, pp. 13-29
Study Initiated - Completed:	4/11/91 - 8/14/91
In-Life Phase Initiated - Completed:	4/29/91 - 6/14/91

There are 68 pages in this report.

Distribution: [redacted]





Company Sanitized. Does not contain TSCA CBI

PHARMAKON RESEARCH INTERNATIONAL, INC.

WAVERLY, PENNSYLVANIA 18471

PHONE
(717) 586-2411

FAX
(717) 586-3450

Skin Sensitization Test with H-18,920 in Guinea Pigs

[redacted]

Submitted to

E. I. Du Pont De Nemours
Newark, Delaware

Susan E. Armondi

Susan E. Armondi, LAT
Study Director

Date July 23, 1991

Test Facility Management

Date 7/23/91

Skin Sensitization Test with H-18,920 In Guinea Pigs
SUMMARY

Test Article H-18,920 at an induction concentration of 1.0% (w/v) and challenge and rechallenge concentrations of 100% and 10% (w/v) in distilled water was tested on the clipped, intact skin of male and female guinea pigs. p-Phenylenediamine (PPD) as a 35% (w/v) suspension in distilled water was used to demonstrate the ability of the test system to detect a skin sensitizer (positive control group). Distilled water served as a vehicle control.

During the primary irritation phase, no dermal irritation was observed in vehicle control animals. No signs of erythema were observed at 24 or 48 hours in animals receiving the test article at 10% or 100% concentrations. No erythema was observed in positive control animals receiving PPD at a 35% concentration.

During the challenge phase, no to severe erythema was observed in the positive control group treated with p-phenylenediamine at a 35% concentration. Slight erythema was observed in one test article-treated animal at the 100% concentration site at 24 hours. One animal exhibited severe erythema at the 100% site at 24 hours with mild erythema at 48 hours. Slight erythema was observed in the same test article-treated animal at the 10% concentration site. No signs of erythema were observed in vehicle or negative control animals.

Due to the spurious positive response observed in one animal at challenge, all test article-treated animals, along with an additional group of five naive control animals (2 males and 3 females), were rechallenged with test article at 100% and 10% concentrations. Slight erythema was observed at 24 hours in one test article-treated animal at the 100% concentration site. No signs of erythema were observed at 24 or 48 hours after rechallenge in test article-treated animals at 10%. No signs of erythema were observed at 24 or 48 hours in naive control animals at the 100% or 10% concentration sites.

Under the conditions of this study protocol, H-18,920 produced weak delayed contact hypersensitivity in one guinea pig at a 100% concentration at challenge. Delayed contact hypersensitivity was not observed at 10% after challenge. During rechallenge, H-18,920 did not produce sensitization at 100% or 10% concentrations. Rechallenge results did not reinforce the spurious response observed at challenge, and after evaluation of all data, H-18,920 did not produce Delayed Contact Hypersensitivity in Guinea Pigs. The results of the rechallenge support that the reaction observed at challenge was indicative of irritation and not sensitization.

Work by: Charles Boyarsky
Charles Boyarsky
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.: [redacted]

Study Director: Susan E. Armondi

This study was conducted in compliance with the Good Laboratory Practice Regulations. 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	April 29, 1991
Reporting Phase	July 23, 1991

Date QAU Report Issued:

To Study Director	To Management
July 23, 1991	July 23, 1991

Leslie Pinnell
Quality Assurance

July 23, 1991
Date

INTRODUCTION

The purpose of this study was to evaluate the potential of H-18,920 to produce delayed hypersensitivity or allergic reactions 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 as the criterion in this study protocol to identify compounds that are sensitizers. The sensitivity of the test system to detect chemical sensitizers was evaluated with p-phenylenediamine. This study was conducted according to Good Laboratory Practice Regulations as listed on page 2 of this report. Areas of noncompliance are documented in the study records. No deviations existed that significantly affected the validity of the study.

MATERIAL AND METHODS

A. Animal Husbandry

Young adult male and female Duncan Hartley albino guinea pigs 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 a unique identification card on which the 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 in 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 studies were used to select the exposure concentrations for the main study. The main sensitization study consisted of 4 phases: a primary irritation phase, an induction phase, a challenge phase and a rechallenge 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 topical range-finding test was conducted on 2 male and 1 female guinea pigs ranging in weight from 318-332 grams. Aliquots (approximately 0.05 mL) of 1.0%, 10%, 50% and 100% (w/v) suspensions of the test material in distilled water were applied and lightly rubbed onto four separate test sites on the clipped, intact skin of the back of each animal.

The primary irritation phase was conducted in 10 guinea pigs (5 males and 5 females), weighing from 400-440 grams, by applying and lightly rubbing in 1 drop (approximately 0.05 mL) of 100% and 10% (w/v) suspensions of the test material in distilled water onto two separate sites of the clipped, intact skin of each animal. In addition, 10 positive control guinea pigs (5 males and 5 females), weighing from 401-451 grams, were treated by applying and lightly rubbing in 1 drop of a 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 406-441 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 dermal 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 intradermal applications (1 each week) of 0.1 mL of the test material at a 1.0% (w/v) concentration in 0.9% saline. A virgin site on the back of each animal was used for each induction. The same induction procedure was followed for the 10 positive control guinea pigs using 0.1 mL of a 1.0% (w/v) solution of p-phenylenediamine in saline. The vehicle control animals were similarly treated with 0.1 mL saline. Skin responses were evaluated approximately 24 hours after each induction.

Approximately 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% (w/v) suspensions of the test material in distilled water onto two separate sites of clipped, intact shoulder skin. The sites used during challenge were the same used on each animal during the primary dermal irritation phase, respectively. The positive control animals were challenged for sensitization by applying and lightly rubbing in 1 drop of a 35% (w/v) suspension of p-phenylenediamine distilled water onto one site of clipped, intact skin. The vehicle control animals were treated at one site with distilled water, in the same manner. Also, 2 male and 3 female previously untreated guinea pigs, weighing 410-450 grams at study initiation, were treated by applying 1 drop each of 100% and 10% (w/v) suspensions of the test material in distilled water onto two separate sites of clipped, intact skin and served as negative

control animals. In addition, these animals received 1 drop of distilled water and 1 drop of p-phenylenediamine at 35%. These animals also served as negative control animals for the vehicle and positive control materials.

Seven days following the primary challenge, all test article-treated animals, along with an additional group of five negative control animals (2 males and 3 females) were rechallenged with the test article at 100% and 10% concentrations.

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. A copy of the raw data is presented in Appendix C. In the range-finding test, no signs of erythema were observed. Based upon the results of the range-finding study, the test article was dosed as received, and as per protocol, dose concentrations used for the primary-irritation phase were 100% and 10%. The dose concentration used for induction was 1% and as per protocol, dose concentrations chosen for challenge and rechallenge were 100% and 10%.

During the primary irritation phase, no signs of erythema were observed in the test article-treated animals at 100% or 10% concentration sites. No erythema was observed in positive control animals. No erythema was observed in vehicle control animals receiving distilled water. Individual animal data are presented in Table II.

During the induction phase, no to mild erythema, with and without edema, was observed in test article-treated animals. No to mild erythema, with and without edema, was observed in positive control animals. No signs of erythema were observed in vehicle control animals. Individual animal data is presented in Table III.

During the challenge phase, no to severe erythema was observed in the positive control group treated with p-phenylenediamine at a 35% concentration. Slight erythema was observed in one test article-treated animal at the 100% concentration site at 24 hours. One animal exhibited severe erythema at the 100% site at 24 hours with mild erythema at 48 hours. Slight erythema was observed in the same test article-treated animal at the 10% concentration site. No signs of erythema were observed in vehicle or negative control animals.

Due to the spurious positive response observed in one animal at challenge, all test article-treated animals, along with an

additional group of five naive control animals (2 males and 3 females), were rechallenged with test article at 100% and 10% concentrations. Slight erythema was observed at 24 hours in one test article-treated animal at the 100% concentration site. No signs of erythema were observed at 24 or 48 hours after rechallenge in test article-treated animals at 10%. No signs of erythema were observed at 24 or 48 hours in naive control animals at the 100% or 10% concentration sites.

Under the conditions of this study protocol, H-18,920 produced weak delayed contact hypersensitivity in one guinea pig at a 100% concentration at challenge. Delayed contact hypersensitivity was not observed at 10% after challenge. During rechallenge, H-18,920 did not produce sensitization at 100% or 10% concentrations. Rechallenge results did not reinforce the spurious response observed at challenge and after evaluation of all data, H-18,920 did not produce Delayed Contact Hypersensitivity in Guinea Pigs. The results of the rechallenge support that the reaction observed at challenge was indicative of irritation and not sensitization.

Dermal responses observed during the challenge phase are summarized in the following table (page 14). Individual animal data are presented in Table IV.

Summary of Skin Responses:
Challenge Phase

	Positive Control PPD		Test Animals H-18,920			
	35%		100%		10%	
Responses	24 hr	48 hr	24 hr	48 hr	24 hr	48 hr
No erythema	1/10	1/10	8/10	9/10	9/10	10/10
Slight erythema	2/10	4/10	1/10	0/10	1/10	0/10
Mild erythema	5/10	3/10	0/10	1/10	0/10	0/10
Moderate erythema	1/10	1/10	0/10	0/10	0/10	0/10
Severe erythema with necrosis	1/10	1/10	1/10	0/10	0/10	0/10

	Negative Control							
	H-18,920				PPD		Vehicle	
	100%		10%		35%		Distilled Water	
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	
	24 hr	48 hr
No erythema	5/5	5/5

At challenge, H-18,920 produced weak delayed contact hypersensitivity at a 100% concentration but did not produce delayed contact hypersensitivity at a 10% concentration. Due to the responses observed at challenge, a rechallenge was performed. Dermal responses during the rechallenge phase are summarized in the following table (page 15). Individual animal data are presented in Table V.

Summary of Skin Responses:
Rechallenge Phase

Test Animals
H-18,920

Responses	100% 24 hr	100% 48 hr	10% 24 hr	10% 48 hr
No erythema	9/10	10/10	10/10	10/10
Slight erythema	1/10	0/10	0/10	0/10
Mild erythema	0/10	0/10	0/10	0/10

	Naive Control							
	H-18,920				H-18,920			
	100%		10%		100%		10%	
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

The results of the rechallenge did not reinforce the spurious response observed at challenge and after evaluation of all data, H-18,920 did not produce Delayed Contact Hypersensitivity in Guinea Pigs. The results of the rechallenge support that the reaction observed at challenge was indicative of irritation and not sensitization.

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 formations or necrosis)

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

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-18,920

GUINEA PIG NUMBER	LEFT FRONT 100%		RIGHT FRONT 10%	
	24 hr	48 hr	24 hr	48 hr
1131	0	0	0	0
1132	0	0	0	0
1133	0	0	0	0
1134	0	0	0	0
1135	0	0	0	0
1136	0	0	0	0
1137	0	0	0	0
1138	0	0	0	0
1139	0	0	0	0
1140	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
1141	0	0
1188	0	0
1143	0	0
1144	0	0
1197	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
1121	0	0
1122	0	0
1123	0	0
1187	0	0
1125	0	0
1193	0	0
1194	0	0
1128	0	0
1129	0	0
1195	0	0

TABLE III

INDUCTION PHASE

SKIN RESPONSES OBSERVED IN TEST GUINEA PIGS 24 HOURS FOLLOWING INTRADERMAL ADMINISTRATION OF 1.0% H-18,920

GUINEA PIG NUMBER	Week 1 Dose #1 (LEFT)	Week 2 Dose #2 (RIGHT)	Week 3 Dose #3 (LEFT)	Week 4 Dose # 4 (RIGHT)
1131	1ED	1	1ED	1
1132	2ED	1ED	2ED	2ED
1133	1	2ED	1	1
1134	1	1	1	1ED
1135	2ED	2	1	1
1136	1	2	0	2
1137	1	1	1	1
1138	1	1	1	1ED
1139	2ED	1	1	1
1140	1	1	1	1

ED = Edema

TABLE III (continued)

INDUCTION PHASE

SKIN RESPONSES OBSERVED IN TEST GUINEA PIGS 24 HOURS FOLLOWING INTRADERMAL ADMINISTRATION OF DISTILLED WATER

GUINEA PIG NUMBER	Week 1 Dose #1 (LEFT)	Week 2 Dose #2 (RIGHT)	Week3 Dose #3 (LEFT)	Week 4 Dose #4 (RIGHT)
1141	0	0	0	0
1188	0	0	0	0
1143	0	0	0	0
1144	0	0	0	0
1197	0	0	0	0

TABLE III (continued)

INDUCTION PHASE

SKIN RESPONSES OBSERVED IN TEST GUINEA PIGS 24 HOURS FOLLOWING INTRADERMAL ADMINISTRATION OF p-PHENYLENEDIAMINE

GUINEA PIG NUMBER	Week 1 Dose #1 (LEFT)	Week 2 Dose #2 (RIGHT)	Week 3 Dose #3 (LEFT)	Week 4 Dose #4 (RIGHT)
1121	0	2ED	0	1
1122	0	2ED	0	1
1123	0	1	1	1
1187	1	1	1	0
1125	1	1	1ED	1
1193	0	1	0	0
1194	0	1ED	1ED	1
1128	0	1	1	0
1129	1	1	2ED	1
1195	0	1	0	1

ED = edema

TABLE IV

CHALLENGE PHASE

SKIN RESPONSES OBSERVED IN TEST GUINEA PIGS
FOLLOWING TOPICAL APPLICATION OF H-18,920

GUINEA PIG NUMBER	LEFT FRONT 100%		RIGHT FRONT 10%	
	24 hr	48 hr	24 hr	48 hr
1131	0	0	0	0
1132	0	0	0	0
1133	0	0	0	0
1134	0	0	0	0
1135	4	2	1	0
1136	0	0	0	0
1137	0	0	0	0
1138	0	0	0	0
1139	1	0	0	0
1140	0	0	0	0

TABLE IV (cont'd)

CHALLENGE PHASE

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

GUINEA PIG NUMBER	LEFT FRONT Distilled water 24 hr	48 hr
1141	0	0
1188	0	0
1143	0	0
1144	0	0
1197	0	0

TABLE IV (Cont'd)

CHALLENGE PHASE

SKIN RESPONSES OBSERVED IN NEGATIVE CONTROL GUINEA PIGS FOLLOWING TOPICAL APPLICATION OF H-18,920 DISTILLED WATER AND p-PHENYLENEDIAMINE

GUINEA PIG NUMBER	LEFT FRONT H-18,920 100%		RIGHT FRONT H-18,920 10%		LEFT REAR Distilled Water 100%		RIGHT REAR PPD 35%	
	24 hr	48 hr	24 hr	48 hr	24 hr	48 hr	24 hr	48 hr
1146	0	0	0	0	0	0	0	0
1147	0	0	0	0	0	0	0	0
1198	0	0	0	0	0	0	0	0
1149	0	0	0	0	0	0	0	0
1150	0	0	0	0	0	0	0	0

TABLE IV (Cont'd)

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
1121	2	2
1122	2	2
1123	4	3
1187	2	1
1125	2	1
1193	3	4
1194	1	1
1128	2	2
1129	1	1
1195	0	0

TABLE V

RECHALLENGE PHASE

SKIN RESPONSES OBSERVED IN TEST GUINEA PIGS
FOLLOWING TOPICAL APPLICATION OF H-18,920

GUINEA PIG NUMBER	LEFT FRONT H-18,920 100%		RIGHT FRONT H-18,920 10%	
	24 hr	48 hr	24 hr	48 hr
1131	1	0	0	0
1132	0	0	0	0
1133	0	0	0	0
1134	0	0	0	0
1135	0	0	0	0
1138	0	0	0	0
1137	0	0	0	0
1138	0	0	0	0
1139	0	0	0	0
1140	0	0	0	0

TABLE V (Continued)

RECHALLENGE PHASE

SKIN RESPONSES OBSERVED IN NAIVE CONTROL GUINEA PIGS
FOLLOWING TOPICAL APPLICATION OF H-18,920

GUINEA PIG NUMBER	LEFT FRONT H-18,920 100%		RIGHT FRONT H-18,920 10%	
	24 hr	48 hr	24 hr	48 hr
5821	0	0	0	0
5822	0	0	0	0
5823	0	0	0	0
5824	0	0	0	0
5825	0	0	0	0

APPENDIX A

PROTOCOL

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. duPont de Nemours Haskell Laboratory for Toxicology and Industrial Medicine Newark, DE 19714
Testing Facility:	Pharmakon Research International, Inc. Waverly, Pennsylvania 18471
Test Facility S.O.P. No.:	[redacted]
Study No.:	To be assigned upon study initiation. PH424-DU-006-91
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:	Susan E. Loveless ~~Dolores E. Malek~~, Ph.D., E.I. duPont de Nemours
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 following 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:	This protocol has been reviewed by the Institutional Animal Care and Use Committee (IACUC) and complies with acceptable standard animal welfare and humane care.
Tentative Date of Submission of Draft Report:	Within three weeks following the completion of the in-life phase of the study.

080489

Records Maintained:	All raw data, final reports, documentation and protocol will be maintained in the Pharmakon Central Files. 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):	BuckberG 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
Weight at Initiation:	400 - 650 grams

080489

Protocol - 424D[1] 3
Skin Sensitization Test in Guinea Pigs

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:	Treatment 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. 23-107 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 - Maintained at a temperature of 22°C ± 3°C and a humidity of 30 to 70%.
Housing:	Guinea pigs are housed individually 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

080489

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: Availability - 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 finding 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.

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.

080489

Protocol - 424D[1] 5
Skin Sensitization Test in Guinea Pigs

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).
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.

080489

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.

SZ I
4/3/91

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 ~~which is no more than mildly irritating~~ 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 pig will remain untreated until the challenge phase. Observations for signs of dermal irritation will be recorded at 24 and 48 hours after application.

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 appropriate vehicle, e.g., 0.9% saline or dimethyl phthlate, 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 quinea pigs will receive similar treatments using the positive control article.

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-hour evaluations.

080489

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.

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

080489

Ratio of Responsers	Degree of Sensitization
5 to 6/10	Moderate Senitizer
7 and above	Strong Senitizer

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 surive 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 will 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.

Protocol - 424D[1] 10
Skin Sensitization Test in Guinea Pigs

Test Article
(Name or Code):

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:

N/A

Shipping Instructions: No special handling

AMENDMENTS

APPROVAL OF PROTOCOL

Date 4/3/91 Study Monitor Scott E Fowler

Date 4/11/91 Study Director Susan E. Simonds

ATXPL4

APPENDIX A

Test Article Information

I Identification:

Test Article (Name or Code): H-18920
Lot or Sample No:
Physical Description: [redacted]
Purity: [redacted]
Expiration Date:
Density/~~Specific Gravity~~: [redacted]
Solubility (check one): Water [redacted] Acetone [redacted]
It's dispersible in water. Ethanol [redacted] Corn Oil [redacted] DMSO [redacted]
Other (please specify) [redacted]
Chemical Classification: Flammable [redacted] Corrosive [redacted]
Other

II Storage Information:

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

III Handling Information:

Known Hazards: [redacted]

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

Other recommended precaution: [redacted]

In Case of Emergency Related to this substance, contact:

Sue E. Lawler (person) of D-Pont/CR+P (company/division) at 302 451 4806 (phone number)
or call 1-800-441-3637 -DuPont Hot Line

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. N/A

Name:

Address:

VI Signature: Sue E Lawler Date: 4/3/91

ATXPL4

APPENDIX B

WEEKLY BODY WEIGHTS (g)

WEEKLY BODY WEIGHTS (g)

Animal Number	Sex	Initial	Week II	Week III	Week IV	Week V	Week VI	Wt. after Challenge	Final
Test Group									
1131	M	429	496	556	607	632	675	691	724
1132	M	421	470	530	579	683	740	767	821
1133	M	419	494	552	616	689	743	773	800
1134	M	422	479	529	576	624	658	684	728
1135	M	440	514	590	652	717	765	788	835
1136	F	408	462	528	571	628	646	670	691
1137	F	400	421	474	513	558	578	592	611
1138	F	412	465	519	531	603	639	653	685
1139	F	432	486	519	585	599	646	662	671
1140	F	417	454	503	570	603	651	656	708

WEEKLY BODY WEIGHTS (g)

Animal Number	Sex	Initial	Week II	Week III	Week IV	Week V	Week VI	Final
Positive Control Group								
1121	M	451	544	611	668	740	804	830
1122	M	411	465	511	564	623	669	680
1123	M	431	496	545	616	673	737	736
1187	M	434	462	521	543	593	612	604
1125	M	443	502	574	635	705	741	780
1193	F	402	458	540	573	649	676	722
1194	F	407	446	485	534	585	615	638
1128	F	420	467	538	584	648	678	651
1129	F	444	479	529	526	611	629	626
1195	F	401	435	467	517	563	593	613

WEEKLY BODY WEIGHTS (g)

Animal Number	Sex	Initial	Week II	Week III	Week IV	Week V	Week VI	Final
Vehicle Control Group								
1141	M	419	478	539	595	659	705	720
1188	M	441	520	607	683	761	827	857
1143	M	423	498	585	657	743	787	768
1144	F	412	459	508	561	622	644	674
1197	F	406	420	459	502	554	584	591

WEEKLY BODY WEIGHTS (g)

Animal Number	Sex	Initial	Week II	Week III	Week IV	Week V	Week VI	Final
Negative Control								
1146	M	450	512	542	577	623	664	668
1147	M	433	510	594	672	739	793	813
1198	F	421	463	527	574	641	650	666
1149	F	410	482	513	579	639	673	695
1150	F	432	481	543	581	636	661	671

WEEKLY BODY WEIGHTS (g)

Animal Number	Sex	Initial	Final
Naive Control (Rechallenge)			
5821	M	642	638
5822	M	521	525
5823	M	551	556
5824	F	477	473
5825	F	530	542

Notebook # 1470
Page # 389

PHARMAKON RESEARCH INTERNAIONAL, INC.

Skin Sensitization Test in Guinea Pigs

Body Weights (grams)

Sponsor: E.I. DuPont De Nemours
Test Article: H-18790

Study Number: PH424-DU-006-91 and PH424-DU-001-91
Lot Number: Not Applicable

Animal Number	Sex	Initial Week I	Week II	Week III	Week IV	Week V	Week VI	Final
Positive Control								
1121	♂	451	544	611	668	740	804	830
1122	♂	411	465	511	564	623	669	680
1123	♂	431	496	545	616	673	737	736
1187	♂	434	462	521	543	593	612	604
1125	♂	443	502	574	635	705	741	780
1193	♀	462	458	540	573	649	676	722
1194	♀	407	448	486	534	585	615	638
1128	♀	420	467	538	584	648	678	651
1189	♀	444	479	529	526	611	629	626
1195	♀	401	435	467	517	563	593	613
		4-29-91 MHO 8:43 AM	5-6-91 MHO 8:50 AM	5-13-91 MHO 9:35 AM	5-20-91 MHO	5/27/91	6-3-91 MHO	6/7/91 CB

Charles Boyarsky 6/7/91
Investigator Date

Susan E. Ormond 6/7/91
Study Director Date

ATABL3(5)

PHARMAKON RESEARCH ...TERNATIONAL, INC.

Individual Animal Scores During Primary Irritation Phase

Sponsor: E.I. DuPont De Nemours Study Number: PH424-DU-001-91 & PH424-DU-006-91 Notebook # 1470

Test Article: H-18,790 Lot Number: N/A Page # 392

Positive Control

Animal No.	Sex	Dose Conc. Left Shoulder	Dose Conc. Right Shoulder	Time of Dose	24 Hours	Time of Reading	48 Hours	Time of Reading
1121	♂			1:29 pm	0	1:23 pm	0	1:17 pm
1122	♂				0		0	
1123	♂				0		0	
1187	♂				0		0	
1125	♂	0.1 mL 35%			0		0	
1193	♀				0		0	
1194	♀				0		0	
1128	♀				0		0	
1129	♀				0		0	
1195	♀			1:31 pm 4-29-91 MB	0	1:26 pm 4-30-91 MB	0	1:20 pm 5-1-91 MB
Initials								

ATABL3(9)

PHARMAKON RESEARCH INTERNATIONAL, INC.

Individual Animal Scores During Induction

Sponsor: E I DuPont De Nemours Study Number: ~~PH424 DU-001-91~~ PH424-DU-006-91 Notebook # 1470

Test Article: H-18,790 Lot Number: N/A Page # 395

			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														
1121	♂	1.0 90 ~~H-18790~~ PPD (~~0.1ml~~) ED 5/1/91	1:40 PM	0	1:37 pm	9:05	2 ED	8:31 AM	11:00	0	11:23 Am	11:18 AM	1	11:07 Am
1122	♂		↓	0	↓	↓	2 ED	↓	↓	0	↓	↓	1	↓
1123	♂		↓	0	↓	↓	1	↓	↓	1	↓	↓	1	↓
1187	♂		↓	1	↓	↓	1	↓	↓	1	↓	↓	0	↓
1125	♂		↓	1	↓	↓	1	↓	↓	1 Ed	↓	↓	1	↓
1193	♀		↓	0	↓	↓	1	↓	↓	0	↓	↓	0	↓
1194	♀		↓	0	↓	↓	1 ED	↓	↓	1 Ed	↓	↓	1	↓
1138	♀		↓	0	↓	↓	1	↓	↓	1	↓	↓	0	↓
1131	♀		↓	1	↓	↓	1	↓	↓	2 Ed	↓	↓	1	↓
1195	♀		1:46 PM	0	1:30 pm	9:12	1	8:39 Am	11:12	0	11:26 Am	11:27 AM	1	11:11 Am
			5/1/91 CB MAS	5-3-91 MAS		5-8-91 MAS	5/9/91 CB		5/15/91 CB MAS	5-16-91 MAS		5/22/91 CB MAS	5-23-91 MAS	
Initials														

Ed = Edema

ATABL3(10)

PHARMAKON RESEARCH INTERNATIONAL, INC.

Individual Animal Scores after Challenge

Sponsor: E. I. du Pont de Nemours Study Number: PH424-DU-001-91 & PH424DU006-91 Notebook # 1470

Test Article: # -18,990 Lot Number: N/A Page # 399

Positive Control (10% Sodium Lauryl Sulfate)

Animal No.	Sex	Challenge Scores: Dose Conc. Left Shoulder	Dose Conc. Right Shoulder	Time of Dose	24 Hrs.	Time of Reading	48 Hrs.	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
1121	♂			11:40	2	11:10 Am	2	11:01 Am							
1122	♂				2		2								
1123	♂				4		3								
1187	♂				2		1								
1125	♂				2		1								
1193	♀				3		4								
1194	♀				1		1								
1128	♀				2		2								
1129	♀			↓	1	↓	1	↓							
1195	♀			11:47	0	11:17 Am	0	11:09 Am							
				6/5/91		6/6/91		6/7/91							
Initials															

ATABL3(10)

PHARMAKON RESEARCH INTERNATIONAL, INC.

N.B.# 1489
Page # 13

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

Sponsor: E. I. duPont de Nemours

Purpose: To determine the dermal sensitization potential of the test material in guinea pigs

Study No.: PH 424-DU-006-91

Method: refer to protocol 424

Date of Initiation: 4/15/91

Date of Termination: 4/17/91

Test Article: #-18,920 dated received 4/9/91 Lot# N/A

Description: [redacted]

Amount Received: 18.759 (gross)

Date Submitted: 4/9/91

Vehicle: distilled H_2O

Dose Levels: 1.0%, 10%, 50%, 100%

Route of Administration: topical

Animal P.O. #: 5001-032791I

Species: Guinea Pig

Strain: Hartley

Food Lot #: D22891A

Identification: Cage card & tail #

No. of Animals on Study: 3

Sex: Male 2 4 weeks Female 1

Scale#: 30 (Animals); Balance #32

Light Cycle Checked: 4/14/91 JC

Animals Clipped: 4/14/91 JC

Compound Preparation: @ 1.0% = 50mg = 5ml distilled H_2O
@ 10% - 500mg = 5ml distilled H_2O
@ 50% - 2.5g = 5ml distilled H_2O
@ 100% - dosed as received
~6.5g of #A was removed from stock 4/15/91 JC

Charles Rayanker
Investigator

Date 4/17/91

Susan E. Armondi
Study Director

Date 4/17/91

ATABL3 (1)

PHARMAKON RESEARCH INTERNATIONAL, INC.

N.B.# 1489
Page # 14

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

In this test, both sides of the animal are shaved 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

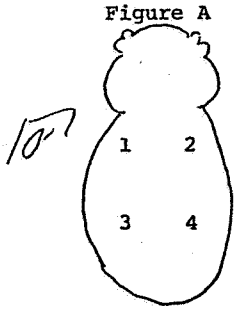
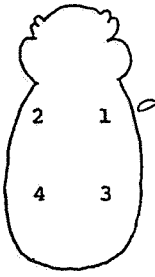


Figure B



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

Figure C

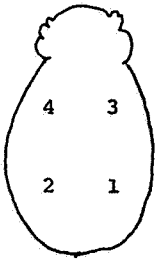
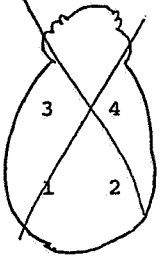


Figure D



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-18,920 @	332	10:52	0	0	0	0	359
2	♂	1.0, 10, 50	319	↓	0	0	0	0	326
3	♀	and 100%	318	10:54	0	0	0	0	330
			4/15/91	4/15/91	4/16/91			CB	4/17/91

48 Hour Scores				
0	0	0	0	
0	0	0	0	
0	0	0	0	
	4/17/91			CB

Charles Boyarsky — Investigator — Date 4/17/91

Susan E. Ormond — Study Director — Date 4/17/91

ATABL3(2)

Company Sanitized. Does not contain TSCA CBI

PHARMAKON RESEARCH INTERNATIONAL, INC.

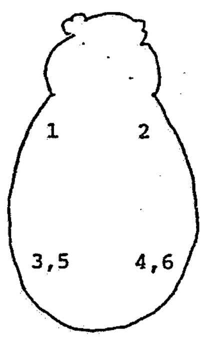
N.B.# 1489
Page # 15

Skin Sensitization Test in Guinea Pigs

Title: Skin Sensitization Test in Guinea Pigs
Sponsor: E.I. du Pont de Nemours
Purpose: Evaluate the dermal sensitization potential of the test material
Study No.: PH 424-DU-006-91
Method: Refer to protocol 424D²

Technical Initiation: 4/29/91	Technical Termination: 6/14/91
Test Article: H-18920	Positive Control: N-Phenyl-4-phenylenediamine
Lot Number: N/A	Lot Number: 05012MK
Description: [redacted]	Description: Black Flakes
Vehicle: Distilled Water	Vehicle: Distilled Water
Amount Submitted: 1.6775 g (gross)	
Receipt Date: 4/23/91 4/30/91	Route of Administration: Topical 4/29/91
Animal P.O. #: 5011-0417918-5011-041791A-10 17 5011-042591B 5011-042591C 10,19 5011-050691B 10,12	Toxicology Lab. No.: 1/1/2
Species: Guinea Pig	Strain: Hartley
Weight Range: 400-650 Grams	Food Lot #: 0228911A 0321911A 0326911A
No. of Animals on Study: 30 35 JDE SCA 6/14/91	Sex: Male 15 17 SEA 6/14/91 Female 15 18
Scale #: Balance #30 Balance #32	Light Cycle Checked: 4/29/91 CB 5/6/91 CB 5/14/91 CB 5/20/91 CB 6/3/91 CB 6/10/91 CB
Animals Clipped: 4/29/91 CB 4/30/91 CB 5/7/91 CB 5/14/91 CB 5/21/91 CB 6/4/91 CB 6/11/91 CB	

Format for Primary Irritation, Induction and Challenge Phases



1-2 Primary Irritation
and Challenge

3-6 Sensitization Phase
(Induction)

Charles Boyarsky
Investigator — Date 6/14/91

Susan E. Armonchi
Study Director — Date 6/14/91

ATABLE3

Notebook # 1489
Page # 116

PHARMAKON RESEARCH INTERNATIONAL, INC.

Skin Sensitization in Guinea Pigs (Modified Buehler Method)

GENERAL OBSERVATIONS

Study No.: PH424-DV-006-91 Sponsor: E.I. du Pont de Nemours
Test Article: IT-1,892D

Date	AN = (appears normal)	Initials
4/29/91	Positive Control animals #'s 1121-1125, 1193, 1194, 1128, 1129, and 1195 will serve as common controls for PH424-DV-001-91 and PH424-DV-006-91.	CB
6/12/91	All test article-treated animals, along with an additional group of five naive guinea pigs rechallenged.	SSA

ATABL3(4)

N.B. # 1489
Page# 17

PHARMAKON RESEARCH INTERNATIONAL, INC.

Skin Sensitization Test in Guines Pigs

Test Article Preparation

Study No.: PH424-DU-006-91

Test Article: H-18,920 Sponsor: E.I. Du Pont de Nemours

Primary Irritation Phase (Week I): @ 100% → dosed as received @ 10% → 500 mgs test article q.s 5 ml. Vehicle → Distilled Water. Total amount of test article used 4.5 gms 4/29/91 CB; Positive Control 35% PPD → 3.5 grams of PPD q.s 10 ml; 4/29/91 CB;

Induction (Week I): @ 1.0% → 50 mgs of test article q.s 5 ml. Vehicle → 0.9% saline; Positive Control PPD @ 1% → 50 mgs PPD q.s 5 ml; Total amount of test article used 50 mgs. 5/1/91 CB

Induction (Week II): @ 1.0% → 100 mgs of test article q.s 10 ml. Vehicle → 0.9% saline; Positive Control PPD @ 1% → 100 mgs PPD q.s 10 ml; Total amount of test article used 100 mgs. 5/9/91 CB

Induction (Week III): @ 1.0% → 100 mgs of test article q.s 10 ml. Vehicle → 0.9% saline; Positive Control 1.0% PPD → 100 mgs PPD q.s 10 ml; Total amount of test article used 100 mgs. 5/15/91 CB

ATABL3(3)

N.B. # 1489
Page # 18

PHARMAKON RESEARCH INTERNATIONAL, INC.

Skin Sensitization Test in Guinea Pigs

Test Article Preparation (Continued)

Study No.: PH424-DV-006-91

Test Article: H-18,920 Sponsor: E.I. du Pont de Nemours

Induction (Week IV): @ 1.0% → 100 mgs of test article q.s 10 ml; Vehicle → 0.9% saline; Positive Control 1.0% PPD → 100 mgs of PPD q.s 10 ml; Total amount of test article used 100 mgs 5/13/91 CB;

Challenge (Week VI): @ 100% → dosed as received (0.05 ml 0.300 mg/site) I of CB 5/91 @ 10% → 300 mgs of test article q.s 5 ml; Vehicle → Distilled water; Positive Control 3.5% PPD → 3.5 grams PPD q.s 10 ml; Total amount of test article used 5.0 grams 6/5/91 CB

Rechallenge (Week VII): @ 100% → test article dosed as received (0.05 ml/site); @ 10% → 300 mgs of test article q.s 5 ml; Total amount of test article used 6/12/91 CB; 2.5 grams.

ATABL3(4)

Notebook # 1489
Page # 79

PHARMAKON RESEARCH INTERNAIONAL, INC.

Skin Sensitization Test in Guinea Pigs

Body Weights (grams)

Sponsor: E.I. DuPont De Nemours
Test Article: 41-15920

Study Number: PH424-Du-006-91
Lot Number: Not Applicable (N/A)

Animal Number	Sex	Initial Week I	Week II	Week III	Week IV	Week V	Week VI	Wt. after Challenge Final	Final
TEST VEHICLE									
1131	♂	429	496	556	607	632	675	691	724
1132	♂	421	470	530	579	683	740	767	821
1133	♂	419	494	552	616	689	743	773	800
1134	♂	422	479	529	576	624	658	684	728
1135	♂	440	514	590	652	717	765	788	835
1136	♀	408	462	528	571	628	646	670	691
1196	♀	400	451	474	513	558	578	592	611
1138	♀	412	465	519	531	603	639	653	685
1139	♀	432	486	519	585	599	646	662	671
1140	♀	417	454	503	570	603	651	656	708
		4-29-91 MLS 8:46 Am	5-6-91 MLS 8:55 Am	5-13-91 MLS 9:35 Am	5-20-91 MLS	5/27/91 SE	6-3-91 MLS	6/7/91 CB	6/14/91 JM

Charles Loyansby 6/14/91
Investigator Date

ATABL3(5)

Susan E. Armondi 6/14/91
Study Director Date

Notebook # 1489
Page # 20

PHARMAKON RESEARCH INTERNAIONAL, INC.

Skin Sensitization Test in Guinea Pigs

Body Weights (grams)

Sponsor: E.I. DuPont De Nemours
Test Article: A-18920

Study Number: PH124-Du-006-91
Lot Number: N/A

Animal Number	Sex	Initial Week I	Week II	Week III	Week IV	Week V	Week VI	Final
Vehicle Control								
1141	♂	419	478	539	595	659	705	720
1188	♂	441	500	607	683	761	807	857
1143	♂	423	498	585	657	743	787	768
1144	♀	412	459	508	561	622	644	674
1197	♀	406	480	459	502	554	584	591
		4-29-91 MLB 8:48 Am	5-6-91 MLB 8:68 Am	5-13-91 MLB 9:37 Am	5-20-91 MLB	5/27/91 SL	6-3-91 MLB	6/7/91 CB
Negative Control								
1146	♂	450	512	542	577	623	664	668
1147	♂	433	510	594	672	739	793	813
1198	♀	421	463	527	574	641	650	666
1149	♀	410	482	513	579	639	673	695
1150	♀	432	481	543	581	636	661	671
		4-29-91 MLB 8:51 Am	5-6-91 MLB 9:00 Am	5-13-91 MLB 9:40 Am	5-20-91 MLB	5/27/91 SL	6-3-91 MLB	6/7/91 CB

Charles Bayarsky 6/14/91
Investigator Date

Susan E. Ormond 6/14/91
Study Director Date

ATABL3(5)

Notebook # 1489
Page # 21

PHARMAKON RESEARCH INTERNAIONAL, INC.

Skin Sensitization Test in Guinea Pigs

Body Weights (grams)

Sponsor: E.I. du Pont de Nemours
Test Article: H-18928

Study Number: PH434-PV-006-91
Lot Number: N/A

Animal Number	Sex	Initial Week I	Week II	Week III	Week IV	Week V	Week VI	Final
Naive Control (Rechallenge)								
5821	♂	642						638
5822	♂	501						525
5823	♀	551						556
5824	♀	477						473
5825	♀	530						542
		6/5/91 DJS						6/14/91 JM

Charles Bryansky 6/14/91
Investigator Date

Susan E. Ormond 6/14/91
Study Director Date

ATABL3(5)

PHARMAKON RESEARCH INTERNATIONAL, INC.

Individual Animal Scores During Primary Irritation Phase

Sponsor: E.I. DuPont De Nemours Study Number: PH454-DU-006-91 Notebook # 1489

Test Article: N-18920 Lot Number: N/A Page # 22

TEST Article

Animal No.	Sex	Dose Conc. Left Shoulder	Dose Conc. Right Shoulder	Time of Dose	a 24 b Hours	Time of Reading	a 48 b Hours	Time of Reading
1131	♂	Saved as Received (0.05 ml/site)	(0.10 ml/site)	1:33 PM	0 0	1:27 pm	0 0	1:20 pm
1132	♂				0 0		0 0	
1133	♂				0 0		0 0	
1134	♂				0 0		0 0	
1135	♂				0 0		0 0	
1136	♀				0 0		0 0	
1196	♀				0 0		0 0	
1138	♀				0 0		0 0	
1139	♀				0 0		0 0	
1140	♀			1:36 PM 4/29/91 RCB	0 0	1:31 pm 4-30-91 MDO	0 0	1:24 pm 5-1-91 mlo
		a = left shoulder						
		b = right shoulder						
Initials								

ATABL3(9)

PHARMAKON RESEARCH INTERNATIONAL, INC.

Individual Animal Scores During Primary Irritation Phase

Sponsor: E.I. DuPONT DE NEMOURS Study Number: PH454-DU-0016-91 Notebook # 1489

Test Article: H-18,920 Lot Number: N/A Page # 23

VEHICLE CONTROL

Animal No.	Sex	Dose Conc. Left Shoulder	Dose Conc. Right Shoulder	Time of Dose	24 Hours	Time of Reading	48 Hours	Time of Reading
1141	♂	Distilled Water		1:36 PM	0	1:31 pm	0	1:24 pm
1188	♂			↓	0	↓	0	↓
1143	♂				0		0	
1144	♀			↓	0	↓	0	↓
1197	♀			1:38 PM 4/29/91 MLG	0	1:33 pm 4-30-91 mlg	0 5-1-91	1:26 pm mlg
NEGATIVE CONTROL								
1146	♂							
1147	♂							
1198	♀							
1149	♀							
1150	♀							
Initials								

ATABL3(9)

PHARMAKON RESEARCH INTERNATIONAL, INC.

Individual Animal Scores During Induction

Sponsor: E I DuPont De Nemours Study Number: PH424-Du-006-91 Notebook # 1489

Test Article: H-1890 Lot Number: N/A Page # 24

			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
TEST ARTICLE														
1131	♂		1:47PM	1 ED	1:30pm	9:12	1	8:40 Am	11:12	1 ED	11:27 Am	11:27AM	1	11:11 Am
1132	♂			2 ED			1 ED			0 ED			2 ED	
1133	♂			1			2 ED			1			1	
1134	♂			1			1			1			1 ED	
1135	♂			2 ED			2			1			1	
1136	♂			1			2			0			0	
1196	♂			1			1			1			1	
1138	♂			1			1			1			1 ED	
1139	♂			2 ED			1			1			1	
1140	♂		1:53PM	1	1:35pm	9:16	1	8:45 Am	11:17	1	11:32 Am	11:32AM	1	11:15 Am
			5/11/91 MLB	5-10-91 MLB		5/18/91 MLB	5/19/91		5/25/91 MLB	5-16-91 MLB		5/30/91 MLB	5-23-91 MLB	
Initials														

ED=Edema

ATABL3(10)

PHARMAKON RESEARCH INTERNATIONAL, INC.

Individual Animal Scores During Induction

Sponsor: E.I. DuPont De Nemours Study Number: 7H424-Du-006-91 Notebook # 1489

Test Article: H-18900 Lot Number: N/A Page # 25

			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
Vehicle Control														
1141	♂	(0.1 ml 0.9% saline)	1:53PM	0	1:35pm	9:16	0	8:45 Am	11:17	0	11:32 Am	11:32	0	11:15Am
1153	♂		↓	0	↓	↓	0	↓	↓	0	↓	↓	0	↓
1143	♀			0			0			0			0	
1144	♀			0			0			0			0	
1197	♀		1:36PM	0	1:37 Am	9:18	0	8:47 Am	11:20	0	11:35 Am	11:35PM	0	11:17 Am
			5/1/91 MLG	5-0-91 MLG		5/8/91 MLG		5/9/91	5/15/91 MLG	5-16-91 MLG		5/22/91 MLG	5-23-91 MLG	
Negative Control														
1146	♂													
1147	♂													
1198	♀													
1149	♀													
1150	♀													
Initials														

ATABL3(10)

PHARMAKON RESEARCH INTERNATIONAL, INC.

Individual Animal Scores after Challenge

Sponsor: E. I. duPont de Nemours Study Number: PH484-DU-006-91 Notebook # 1489

Test Article: H-18920 Lot Number: N/A Page # 26

Test Article

Animal No.	Sex	Challenge Dose Conc. Left Shoulder	Challenge Dose Conc. Right Shoulder	Challenge Time of Dose	Challenge a 24 Hrs.	Challenge b 24 Hrs.	Challenge Time of Reading	Challenge a 48 Hrs.	Challenge b 48 Hrs.	Challenge Time of Reading	Rechallenge Dose Conc. Left Shoulder	Rechallenge Dose Conc. Right Shoulder	Rechallenge Time of Dose	Rechallenge a 24 Hrs.	Rechallenge b 24 Hrs.	Rechallenge Time of Reading	Rechallenge a 48 Hrs.	Rechallenge b 48 Hrs.	Rechallenge Time of Reading
1131	♂	Undiluted received H-18920 (0.05 mL/site)	10 % H-18920 (0.05 mL/site)	11:48	0	0	11:17 Am	0	0	11:10 Am	Diluted as received H-18920 (0.05 mL/site)	10 % H-18920 (0.05 mL/site)	11:10	1	0	11:09 Am	0	0	11:30 Am
1132	♂				0	0		0	0					0	0		0	0	
1133	♂				0	0		0	0					0	0		0	0	
1134	♂				0	0		0	0					0	0		0	0	
1135	♂				4	1		2	0					0	0		0	0	
1136	♀				0	0		0	0					0	0		0	0	
1196	♀				0	0		0	0					0	0		0	0	
1138	♀				0	0		0	0					0	0		0	0	
1139	♀			↓	1	0	↓	0	0	↓			↓	0	0	↓	0	0	↓
1140	♀			11:53	0	0	11:24 Am	0	0	11:18 Am			11:15	0	0	11:16 Am	0	0	11:39 Am
				6/5/91			6/6/91			6/7/91			6/12/91			6/13/91			6/14/91
Initials																			

a = left shoulder
b = right shoulder

ATABL3(10)

PHARMAKON RESEARCH INTERNATIONAL, INC.

Individual Animal Scores after Challenge

Sponsor: E.I. duPont de Nemours Study Number: PH424-DU-006-91 Notebook # 1489

Test Article: H-18,920 Lot Number: N/A Page # 27

Vehicle Control

Animal No.	Sex	Challenge Scores: Dose Conc. Left Shoulder	Dose Conc. Right Shoulder	Time of Dose	24 Hrs.	Time of Reading	48 Hrs.	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
1141	♂	Distilled Water		11:53	0	11:24 Am	0	11:18 Am							
1188	♂			↓	0	↓	0	↓							
1143	♂			↓	0	↓	0	↓							
1144	♀			↓	0	↓	0	↓							
1197	♀			11:56	0	11:27 Am	0	11:21 Am							
				4/5/91	6/6/91		6/7/91								
Initials															

ATABL3(10)

PHARMAKON RESEARCH INTERNATIONAL, INC.

Individual Animal Scores after Challenge

Sponsor: E.I. du Pont de Nemours   Study Number: PH424-DU-006-91   Notebook # 1489

Test Article: H-18920   Lot Number: N/A   Page # 28

Negative Control

Animal No.	Sex	Dose Conc. Left Shoulder	Dose Conc. Right Shoulder	Time of Dose	24 Hrs. (a b)	Time of Reading	48 Hrs.	Time of Reading	Dose Conc. Left ~~Shoulder~~ Flank	Dose Conc. Right ~~Shoulder~~ Flank	Time of Dose	24 Hrs (c d)	Time of Reading	48 Hrs.	Time of Reading
				Challenge Scores								Rechallenge Scores			
1146	♂	Dosed as received H-18920 (0.05ml/site)	10% H-18920 (0.05ml/site)	11:57	0 0	11:27 AM	0 0	11:21 AM	Distilled Water	PPD 35%	/	0 0	11:27 AM	0 0	11:21 AM
1147	♂			↓	0 0	↓	0 0	↓			/	0 0	↓	0 0	↓
1198	♀			↓	0 0	↓	0 0	↓			/	0 0	↓	0 0	↓
1149	♀			↓	0 0	↓	0 0	↓			/	0 0	↓	0 0	↓
1150	♀			10:00	0 0	11:30 AM	0 0	11:28 AM			/	0 0	11:30 AM	0 0	11:28 AM
				6/5/91	6/6/91		6/7/91					6/6/91		6/7/91	
Initials															

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

ATABL3(10)

PHARMAKON RESEARCH INTERNATIONAL, INC.

Individual Animal Scores after Challenge

Sponsor: E.I du Pont de Nemours Study Number: PH424-DV-006-91 Notebook # 1489

Test Article: H-18920 Lot Number: N/A Page # 29

Naive Control (Rechallenge)

		Challenge Scores							Rechallenge Scores						
Animal No.	Sex	Dose Conc. Left Shoulder	Dose Conc. Right Shoulder	Time of Dose	24 Hrs.	Time of Reading	48 Hrs.	Time of Reading	Dose Conc. Left Shoulder	Dose Conc. Right Shoulder	Time of Dose	a 24 b Hrs	Time of Reading	a 48 b Hrs.	Time of Reading
5821	♂								100% H-18920 Dosed as received (0.05ml/site)	10% H-18920 (0.05ml/site)	11:15	0 0	11:16 Am	0 0	11:40 Am
5822	♂											0 0		0 0	
5823	♀											0 0		0 0	
5824	♀											0 0		0 0	
5825	♀										11:18	0 0	11:19 Am	0 0	11:43 Am
											6/12/91		6/13/91		6/14/91
Initials															

a = left shoulder

b = right shoulder

ATABL3(10)