

elf atochem

ATO

PDCN: 88450000

Elf Atochem North America, Inc.

2000 Market Street

Philadelphia, Pa. 19103-3222

RECEIVED

~~CONFIDENTIAL~~

95 JAN 24 11:53

January 19, 1995

ORIGINAL

VIA CERTIFIED MAIL
RETURN RECEIPT REQUESTED

(B)



8ENQ-95-13305
SP001 01/24/95

Document Processing Center (TS-790)
Office of Toxic Substances
Environmental Protection Agency
401 M St. S.W.
Washington, D.C. 20460
Attn: Section 8(e) Coordinator

ORIGINAL

Contains No CBI

Subject: TSCA Section 8(e) Submission

8ENQ-0195-13305

Dear Sir/Madam:

Elf Atochem North America Inc. has received the final report of a skin sensitization study in guinea pigs and is submitting it to the Environmental Protection Agency (EPA) pursuant to the Toxic Substances Control Act (TSCA) Section 8(e). Preliminary results from this study were submitted to the Agency by Elf Atochem on January 4, 1995. This study provides information on t-Butyl mercaptan (CAS No. 75-66-1) and does not involve effects in humans. The title of the study is A Dermal Sensitization Study in Guinea Pigs with t-Butyl Mercaptan - Modified Buehler Design.

Nothing in this letter or the enclosed study report is considered confidential business information of Elf Atochem.

In light of the strong disagreeable odor and the very low odor threshold of t-butyl mercaptan (0.08 ppb), there is a low potential for skin contact with this material. It is the opinion of Elf Atochem that the effects noted in this study do not, therefore, necessarily support a conclusion of substantial health risk, but are being submitted in response to the EPA 8(e) reporting standards.

Sincerely,

C.H. Farr, PhD, DABT
Manager, Product Safety
and Toxicology



89950000163

Enclosure

4/4/95

Springborn Laboratories, Inc.

Life Sciences Division

640 N. Elizabeth Street • Spencerville, Ohio 45887 • (419) 647-4196 • Telex 4436041 • Facsimile 419-647-6560

**A DERMAL SENSITIZATION STUDY IN GUINEA
PIGS WITH t-BUTYL MERCAPTAN
• MODIFIED BUEHLER DESIGN •**

FINAL REPORT

Contains No CBI

Author

Deborah A. Douds, M.S.

Study Completed on

January 12, 1995

Performing Laboratory

Springborn Laboratories, Inc. (SLS)
Life Sciences Division
640 North Elizabeth Street
Spencerville, OH 45887

SLS Study No.

3255.21

Submitted to

Elf Atochem North America, Inc.
900 First Avenue
P.O. Box 1536
King of Prussia, PA 19406-0018

Page 1 of 60

RECEIVED
CPI/PHS
95 JAN 21 AM 11:51

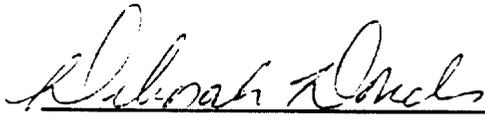
SLS Study No. 3255.21

(2)

COMPLIANCE STATEMENT

This study was conducted in compliance with the Good Laboratory Practice Regulations as described by the FDA (21 CFR Part 58) and the EPA (40 CFR Parts 160 and 792) with the following exception:

The dose preparations were not analyzed to confirm test article concentration, stability or homogeneity.



Deborah A. Douds, M.S.
Study Director/Author
Springborn Laboratories, Inc.

Date 1/12/95

QUALITY ASSURANCE STATEMENT

This study was inspected by the Quality Assurance Unit and reports were submitted to management and the study director in accordance with SLS's Standard Operating Procedures as follows:

<u>Phase</u>	<u>Date</u>
Dermal Observations	11/22/94
Data Audit	12/20/94
Draft Report Review	12/22/94
Final Report Review	01/12/95
Reports to Study Director and Management	12/22/94, 01/12/95

This study was conducted in compliance with the Good Laboratory Practice Regulations as described by the FDA (21 CFR Part 58) and the EPA (40 CFR Parts 160 and 792) except as noted on the Compliance Statement.



Anita M. Bosau, Director
Quality Assurance

Date

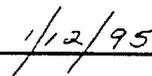


TABLE OF CONTENTS

	<u>Page No.</u>
COMPLIANCE STATEMENT	2
QUALITY ASSURANCE STATEMENT	3
TABLE OF CONTENTS	4
SUMMARY	6
 <u>Section</u>	
I. INTRODUCTION	7
II. MATERIALS AND METHODS	7
III. EXPERIMENTAL PROCEDURES	9
IV. ANALYSIS OF DATA	11
V. MAINTENANCE OF RAW DATA AND RECORDS	12
VI. RESULTS	12
VII. CONCLUSION	13
VIII. REPORT REVIEW	14
IX. REFERENCES	15
 <u>Tables</u>	
1. Individual Induction Data	16
2. Individual Challenge Data	17

TABLE OF CONTENTS--(Continued)

Page No.

Appendices

A. Protocol and Amendment	19
B. Dermal Grading System	43
C. Topical Range-Finding Data	47
D. Individual Body Weight Data	49
E. Hexylcinnamaldehyde Historical Control Data	52
F. SLS Personnel Responsibilities	59

SUMMARY

The dermal sensitization potential of t-Butyl Mercaptan was evaluated in Hartley-derived albino guinea pigs. Ten male and ten female guinea pigs were topically treated with 100% t-Butyl Mercaptan, once per week, for three consecutive weeks. Following a two week rest period, a challenge was performed whereby the twenty test and ten previously untreated (naive) challenge control guinea pigs were topically treated with 75% w/v t-Butyl Mercaptan in mineral oil. Challenge responses in the test animals were compared to those of the challenge control animals.

t-Butyl Mercaptan: Following induction 1 with 100% t-Butyl Mercaptan, dermal scores of 1 (some with edema) were noted in 18/20 test animals at the 24 hour scoring interval and in 17/20 test animals at the 48 hour scoring interval. Following induction 2 with the same concentration, the responses were much more severe. Dermal scores of 1 to 3 (some with blanching, edema and/or eschar) were noted in 20/20 test animals at both the 24 and 48 hour scoring intervals. This increased irritation may have, in part, been a result of cumulative irritation since inductions 1 and 2 were performed on the same test site. Following induction 3, increased dermal responses were again noted in the test animals, although they were not as severe as those noted at induction 2. Dermal scores of 1 to 2 (some with edema and/or pinpoint blanching) were noted in 20/20 test animals at the 24 hour scoring interval and in 18/20 test animals at the 48 hour scoring interval. The increase in dermal scores seen at induction 3 (performed on a naive test site) over those seen during induction 1 may be suggestive of dermal sensitization. Following challenge with 75% w/v t-Butyl Mercaptan in mineral oil, dermal scores of 1 to 3 (with edema and/or blanching) were noted in 20/20 test animals at both the 24 and 48 hour scoring intervals. Dermal reactions in the challenge control animals were limited to scores of 1 (three with slight edema) in 10/10 animals at the 24 hour scoring interval and in 9/10 animals at the 48 hour scoring interval. Group mean dermal scores were noted to be higher in the test animals as compared to the challenge control animals.

Hexylcinnamaldehyde Historical Control: Using Hexylcinnamaldehyde as a mild to moderate positive control, Springborn Laboratories, Inc., Spencerville, Ohio, has compiled historical control data for contact sensitization to this agent utilizing the test system described herein (Modified Buehler Design). As indicated, 16.7% of animals induced with Hexylcinnamaldehyde elicited a contact sensitization response following challenge with Hexylcinnamaldehyde and 50% of animals induced with Hexylcinnamaldehyde elicited a contact sensitization response following rechallenge with Hexylcinnamaldehyde, thereby demonstrating the susceptibility of the test system to this sensitizing agent.

Based on the results of this study, t-Butyl Mercaptan is considered to be a contact sensitizer in guinea pigs. The results of the Hexylcinnamaldehyde Historical Control demonstrates that the test design utilized by Springborn Laboratories could detect mild to moderate potential contact sensitizers.

I. INTRODUCTION

This study was performed to assess the dermal sensitization potential of t-Butyl Mercaptan in Hartley-derived albino guinea pigs when administered by multiple topical applications. This study is intended to provide information on the potential health hazards of the test article with respect to dermal exposure. Data from this study may serve as a basis for classification and/or labeling of the test article. This study was performed at Springborn Laboratories, Inc., 553 North Broadway, Spencerville, Ohio.

II. MATERIALS AND METHODS

Study Dates

Study Initiation: July 22, 1994
Experimental Initiation: November 4, 1994
Experimental Completion: December 14, 1994

Protocol

The study protocol and Protocol Amendment No. 1 are presented in Appendix A.

Test Article

Sponsor I.D.: t-Butyl Mercaptan
Lot No.: 3300TBM94
Springborn I.D.: S94.002.3255
Receipt Date: July 7, 1994
Physical Description: Clear colorless liquid
Storage Conditions: Room temperature (cool dark area)
Expiration Date: June 24, 1995

The Sponsor is responsible for any necessary evaluations related to chemical composition, purity, strength, stability and other data required by 21 CFR Part 58.105, 40 CFR Parts 160.105 and 792.105.

Test Article Preparations

The test article was utilized at 100% and at 25%, 50% and 75% w/v in mineral oil for the range-finding study. For the sensitization study, the test article was utilized at 100% (induction) and 75% w/v in mineral oil (challenge).

Animals and Animal Husbandry

Description: Young adult, Hartley-derived albino guinea pigs were received at SLS from Harlan Sprague Dawley, Inc., Haslett, MI.

Method of Identification: Upon receipt, plastic ear tags displaying unique identification numbers were used to individually identify the animals. Cage cards displaying at least the study number, animal number and sex were affixed to each cage.

Housing: The animals were housed individually in suspended stainless steel cages. All housing and care were based on the standards recommended by the Guide for the Care and Use of Laboratory Animals [1].

Environment: The animal room temperature and relative humidity ranges were 65-71 °F and 35-59%, respectively. Environmental control equipment was monitored and adjusted as necessary to minimize fluctuations in the animal room environment. Light timers were set to maintain a 12-hour light/12-hour dark cycle. There were ten to twelve air changes in the animal room per hour. The animal room temperature and relative humidity were recorded a minimum of once daily.

Food: Purina Certified Guinea Pig Chow #5026 was provided ad libitum to the animals throughout the study. The lot number and expiration date of each batch of diet used during the study were recorded. The feed was analyzed by the supplier for nutritional components and environmental contaminants. Dietary limitations for various environmental contaminants, including heavy metals, pesticides, polychlorinated biphenyls and total aflatoxin are set by the manufacturer. Within these limits, contaminants which may have been present were not expected to compromise the purpose of this study. Results of the dietary analyses (Certificates of Analysis) are provided by the manufacturer for each lot of diet. These are maintained by SLS.

Water: Municipal tap water treated by reverse osmosis or deionization (back-up system) was available to the animals ad libitum throughout the study. The purified water was supplied by an automatic watering system. Monitoring of the drinking water for contaminants was conducted by SLS and the records are available for inspection. Within generally accepted limits, contaminants which may have been present were not expected to compromise the purpose of this study.

Quarantine: Upon receipt, animals were examined, identified with plastic ear tags and then quarantined for a minimum of five days.

Animal Selection: The animals chosen for study use were arbitrarily selected from healthy stock animals to avoid potential bias. All animals received a detailed pretest observation prior to dosing. Only healthy animals were chosen for study use. Females were nulliparous and nonpregnant.

III. EXPERIMENTAL PROCEDURES [2]

Topical Range-Finding Study

Dosing: On the day prior to dose administration, four topical range-finding guinea pigs were weighed and the hair removed from the right and left side of the animals with a small animal clipper. Care was taken to avoid abrading the skin during clipping procedures. On the following day, four concentrations of the test article were prepared and each concentration was applied to each topical range-finding animal as indicated below:

Group	Material	Concentration (%)	Test Site No.	Amount Applied	Patch Design	No. of Animals	
						Male	Female
Topical Range-Finding	t-Butyl Mercaptan	25	4	0.4 ml	2 x 2 cm Webril Patch	2	2
		50	3	0.4 ml	2 x 2 cm Webril Patch		
		75	2	0.4 ml	2 x 2 cm Webril Patch		
		100	1	0.4 ml	2 x 2 cm Webril Patch		

Following patch application, the trunk of the animal was wrapped with elastic wrap which was secured with adhesive tape to prevent removal of the patches and the animal was returned to its cage. Approximately six hours after patch application, the elastic wrap, tape and patches were removed. The test sites were then wiped with gauze moistened in distilled water to remove test article residue. The animals were then returned to their cages.

Dermal Observations: The test sites of the topical range-finding animals were graded for irritation at approximately 24 and 48 hours following patch application using the Dermal Grading System presented in Appendix B.

Clinical Observations: Any unusual observations and/or mortality were recorded. The topical range-finding animals were observed for mortality twice daily, once in the morning and once in the afternoon.

Body Weights: Individual body weights were obtained for the topical range-finding animals on the day prior to dosing.

Gross Necropsy: Following the 48 hour scoring interval, all topical range-finding animals were euthanized (carbon dioxide inhalation). Gross necropsy examinations were not required for these animals.

Induction

Dosing: On the day prior to first induction dose administration, the hair was removed from the left side of the twenty test animals with a small animal clipper. Care was taken to avoid abrading the skin during clipping procedures. On the following day (day 0), the appropriate concentration of the test article was prepared and applied to the animals as indicated below:

Group	Material	Induction No.	Concentration (%)	Test Site No.	Amount Applied	Patch Design	No. of Animals Male Female	
Test	t-Butyl Mercaptan	1	100	1	0.4 ml	2 x 2 cm Webril Patch	10	10
		2	100	1	0.4 ml	2 x 2 cm Webril Patch		
		3	100	3	0.4 ml	2 x 2 cm Webril Patch		

Following patch application, the trunk of the animal was wrapped with elastic wrap which was secured with adhesive tape to prevent removal of the patch and the animal was returned to its cage. Approximately six hours after patch application, the elastic wrap, tape and patches were removed. The test sites were then wiped with gauze moistened in distilled water to remove test article residue. The animals were then returned to their cages.

Dermal Observations: The test sites were graded for irritation at approximately 24 and 48 hours following patch application using the Dermal Grading System presented in Appendix B.

The induction procedure was repeated on study day 7 and on study day 14 so that a total of three consecutive induction exposures were made to the twenty test animals.

Challenge

Dosing: On the day prior to challenge dose administration, the hair was removed from the right side of the twenty test and ten challenge control animals with a small animal clipper. Care was taken to avoid abrading the skin during clipping procedures. On the following day (day 28), the appropriate concentration of the test article was prepared and applied to the animals as indicated below:

Group	Material	Concentration (%)	Test Site No.	Amount Applied	Patch Design	No. of Animals Male Female	
Test	t-Butyl Mercaptan	75	2	0.4 ml	2 x 2 cm Webril Patch	10	10
Challenge Control	t-Butyl Mercaptan	75	2	0.4 ml	2 x 2 cm Webril Patch	5	5

Following patch application, the trunk of the animal was wrapped with elastic wrap which was secured with adhesive tape to prevent removal of the patch and the animal was returned to its cage. Approximately six hours after patch application, the elastic wrap, tape and patches were removed. The test sites were then wiped with gauze moistened in distilled water to remove test article residue. The animals were then returned to their cages.

Dermal Observations: The test sites were graded for irritation at approximately 24 and 48 hours following patch removal using the Dermal Grading System presented in Appendix B.

Clinical Observations

Any unusual observations and/or mortality were recorded. The sensitization study animals were observed for mortality twice daily, once in the morning and once in the afternoon.

Body Weights

Individual body weights were obtained for all sensitization study animals on the day prior to the first induction (day -1) and for the test and challenge control animals on the day prior to challenge dosing.

Gross Necropsy

All sensitization study animals were euthanized (carbon dioxide inhalation) following each animal's final scoring interval. Gross necropsy examinations were not required for these animals.

Protocol Deviations

The relative humidity of the animal room (35-59%) exceeded the range specified in the protocol (40-70%) during this study. The supplies utilized on 11/21/94 for Induction 2 were inadvertently not documented. The incorrect dermal scoring system was inadvertently used for the range-finding study. Rechallenge control animals were placed on study, although not required by protocol. These occurrences are considered to have had no adverse effect on the outcome of this study.

IV. ANALYSIS OF DATA

The sensitization potential of the test article was based on the dermal responses observed on the test and control animals at challenge. Generally, dermal scores of ≥ 1 in the test

animals with scores of 0 to \pm noted in the controls is considered indicative of sensitization. Dermal scores of 1 in the controls is generally considered equivocal unless a higher dermal response (\geq grade 2) is noted in the test animals. Group mean dermal scores were calculated for challenge.

V. MAINTENANCE OF RAW DATA AND RECORDS

The remaining test article was returned to the Sponsor following completion of the in-life phase of the study. An approximate 1 ml retention sample of the test article was placed in an amber vial, sealed and archived at the test facility. All original paper data, the final report and magnetically encoded records were transferred to the SLS archives for a period of 10 years. The Sponsor will be contacted prior to final disposition of these items.

VI. RESULTS

Topical Range-Finding Study

Individual Range-Finding Data: Appendix C

The results of the range-finding study indicated that a test article concentration of 100% produced only minimal irritation and was considered appropriate for induction. The range-finding study also indicated that the test article concentration of 75% was the maximum non-irritating concentration; therefore, this concentration was considered appropriate for challenge.

Main Sensitization Study

Dermal Sensitization Observations

Individual Induction Data: Table 1

Individual Challenge Data: Table 2

t-Butyl Mercaptan: Following induction 1 with 100% t-Butyl Mercaptan, dermal scores of 1 (some with edema) were noted in 18/20 test animals at the 24 hour scoring interval and in 17/20 test animals at the 48 hour scoring interval. Following induction 2 with the same concentration, the responses were much more severe. Dermal scores of 1 to 3 (some with blanching, edema and/or eschar) were noted in 20/20 test animals at both the 24 and 48 hour scoring intervals. This increased irritation may have, in part, been a result of cumulative irritation since inductions 1 and 2 were performed on the same test site.

Following induction 3, increased dermal responses were again noted in the test animals, although they were not as severe as those noted at induction 2. Dermal scores of 1 to 2 (some with edema and/or pinpoint blanching) were noted in 20/20 test animals at the 24 hour scoring interval and in 18/20 test animals at the 48 hour scoring interval. The increase in dermal scores seen at induction 3 (performed on a naive test site) over those seen during induction 1 may be suggestive of dermal sensitization. Following challenge with 75% w/v t-Butyl Mercaptan in mineral oil, dermal scores of 1-3 (with edema and/or blanching) were noted in 20/20 test animals at both the 24 and 48 hour scoring intervals. Dermal reactions in the challenge control animals were limited to scores of 1 (three with slight edema) in 10/10 animals at the 24 hour scoring interval and in 9/10 animals at the 48 hour scoring interval. Group mean dermal scores were noted to be higher in the test animals as compared to the challenge control animals.

Hexylcinnamaldehyde Historical Control: Using Hexylcinnamaldehyde as a mild to moderate positive control, Springborn Laboratories, Inc., Spencerville, Ohio, has compiled historical control data for contact sensitization to this agent utilizing the test system described herein (Modified Buehler Design). As indicated, 16.7% of animals induced with Hexylcinnamaldehyde elicited a contact sensitization response following challenge with Hexylcinnamaldehyde and 50% of animals induced with Hexylcinnamaldehyde elicited a contact sensitization response following rechallenge with Hexylcinnamaldehyde, thereby demonstrating the susceptibility of the test system to this sensitizing agent.

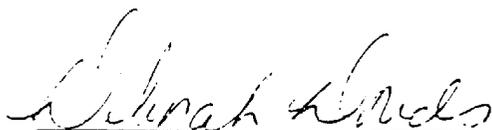
Clinical Observations/Body Weights

Individual Body Weight Data: Appendix D

All sensitization study animals gained weight during the test period and generally appeared in good health.

VII. CONCLUSION

Based on the results of this study, t-Butyl Mercaptan is considered to be a contact sensitizer in guinea pigs. The results of the Hexylcinnamaldehyde Historical Control demonstrates that the test design utilized by Springborn Laboratories could detect potential mild to moderate contact sensitizers.



Deborah A. Douds, M.S.
Study Director

Date

1/13/95

VIII. REPORT REVIEW



Kimberly L. Bonnette, M.S., LATG
Manager of Acute Toxicology

Date 1/12/95



Rusty E. Rush, M.S., LAT, DABT
Manager of Special Studies

Date 1-12-95

IX. REFERENCES

1. Guide for the Care and Use of Laboratory Animals, DHHS Publication No. (NIH) 86-23, 1985.
2. E. V. Buehler, Delayed Contact Hypersensitivity in the Guinea Pig, Arch. Dermat., 91:171-177, 1965.

SLS STUDY NO.: 3255.21
 CLIENT: ELF ATOCHEM

TABLE 1
 A DERMAL SENSITIZATION STUDY IN GUINEA PIGS
 INDIVIDUAL INDUCTION DATA

PAGE 1

Group	Sex	Induction 1 Dermal Scores		Induction 2 Dermal Scores		Induction 3 Dermal Scores	
		24 Hr	48 Hr	24 Hr	48 Hr	24 Hr	48 Hr
Test	4931/M	1	1	1 ED-1,BLA-1	3 ED-2,BLA-2	2 ED-2	2 ED-1
	4932/M	0	0	1	1	1 ED-1	1
	4933/M	1	1	3 ED-2,BLA-2,ES-2	3 ED-2,BLA-2,ES-2	2 ED-2,BLA-1	2 ED-2,BLA-1
	4934/M	1 ED-1	1	3 ED-2,BLA-3	3 ED-2,BLA-3,ES-1	2 ED-2	2 ED-2
	4935/M	1 ED-1	1	3 ED-2,BLA-2	3 ED-2,BLA-2,ES-1	2 ED-1	1 ED-1
	4936/M	1 ED-1	1	3 ED-2,BLA-2	3 ED-2,BLA-2,ES-2	2 ED-2	2 ED-2
	4937/M	1 ED-1	1 ED-1	3 ED-2,BLA-4	3 ED-2,BLA-4,ES-1	2 ED-2	2 ED-2
	4938/M	1 ED-1	1	3 ED-2,BLA-3,ES-2	3 ED-2,BLA-3,ES-2,EXF	2 ED-2	1 ED-1
	4939/M	1 ED-1	1 ED-1	3 ED-2,BLA-2,ES-3	3 ED-2,BLA-1,ES-4,EXF	2 ED-2	1 ED-1
	4940/M	1 ED-1	1	3 ED-2,BLA-2,ES-1	3 ED-2,BLA-2,ES-2	2 ED-2	1 ED-1
	4950/F	1	1	3 ED-2,BLA-3,ES-2	3 ED-1,BLA-2,ES-2	2 ED-2,BLA-1	1 ED-1
	4951/F	1	1	3 ED-2,BLA-4	3 ED-1,BLA-2,ES-1	2 ED-2,BLA-1	1 ED-2
	4952/F	1	1	3 ED-2,BLA-4	3 ED-2,BLA-3,ES-1	2 ED-2	2 ED-2
	4953/F*	1	1	3 ED-2,BLA-3,ES-2	3 ED-2,BLA-3,ES-3	1	0
	4954/F	0	0	3 ED-2,BLA-3,ES-2	3 ED-1,BLA-2,ES-2	2 ED-2	1 ED-1
	4955/F	1	1	3 ED-2,BLA-2,ES-2	3 ED-2,BLA-2,ES-3	2 ED-2	1 ED-1
	4956/F*	1 ED-1	1	3 ED-2,BLA-4,ES-1	3 ED-1,BLA-4,ES-1	1 ED-1	1
	4957/F	1	1	3 ED-2,BLA-3,ES-1	3 ED-2,BLA-3,ES-2	2 ED-2,BLA-1	2 ED-2
	4958/F*	1	0	3 ED-2,BLA-1,ES-4	3 ED-2,BLA-1,ES-4,EXF	1	0
	4959/F	1	1	3 ED-2,BLA-2	3 ED-2,BLA-2,ES-1	2 ED-2	2 ED-2

*Animal found with wrap and patch removed at time of unwrapping for Induction 3.

See Appendix B for definition of codes.

TABLE 2
 A DERMAL SENSITIZATION STUDY IN GUINEA PIGS
 INDIVIDUAL CHALLENGE DATA

Group	Animal No./ Sex	Dermal Scores	
		24 Hr	48 Hr
Test			
	4931/M	2 ED-1	2 ED-1
	4932/M	1 ED-1	1 ED-1
	4933/M	1 ED-1,BLA-1	1 ED-1
	4934/M	1 ED-1,BLA-1	1 ED-1
	4935/M	1 ED-1	1 ED-1
	4936/M	3 ED-2,BLA-2	2 ED-1,BLA-1
	4937/M	1 ED-1	1
	4938/M	2 ED-2	2 ED-1,BLA-1
	4939/M	2 ED-1	2 ED-1
	4940/M	2 ED-1	1
	4950/F	2 ED-1	1 ED-1
	4951/F	1 ED-1	1
	4952/F	1 ED-1	2 ED-1
	4953/F	2 ED-1,BLA-1	2 ED-1,BLA-2
	4954/F	2 ED-1	1
	4955/F	3 ED-2,BLA-2	2 ED-1,BLA-1
	4956/F	2 ED-1,BLA-2	2 ED-2,BLA-1
	4957/F	3 ED-2,BLA-2	3 ED-1,BLA-2
	4958/F	3 ED-2,BLA-3	3 ED-2,BLA-3
	4959/F	2 ED-2	2 ED-1,BLA-1

Mean 1.9

1.7

See Appendix B for definition of codes.

SLS STUDY NO.: 3255.21
 CLIENT: ELF ATOCHEM

TABLE 2
 A DERMAL SENSITIZATION STUDY IN GUINEA PIGS
 INDIVIDUAL CHALLENGE DATA

PAGE 2

Group	Animal No./ Sex	Dermal Scores	
		24 Hr	48 Hr
Challenge Control	4941/M	1 ED-1	1
	4942/M	1	1
	4943/M	1 ED-1	1 ED-1
	4944/M	1	1 ED-1
	4945/M	1	1
	4960/F	1	0
	4961/F	1	1
	4962/F	1 ED-1	1
	4963/F	1	1
	4964/F	1	1
	Mean	1.0	0.9

(18)

For the purpose of calculation, $\pm = 0.5$.

See Appendix B for definition of codes.

APPENDIX A

Protocol and Amendment

A DERMAL SENSITIZATION STUDY IN GUINEA PIGS WITH t-BUTYL MERCAPTAN
● MODIFIED BUEHLER DESIGN ●

Springborn Study No. 3255.21

Springborn Laboratories, Inc. (SLS)
Life Sciences Division
640 North Elizabeth Street
Spencerville, Ohio 45887

Deborah A. Douds, M.S.
Study Director

For

Elf Atochem North America, Inc.
900 First Avenue
P.O. Box 1536
King of Prussia, PA 19406-0018

TABLE OF CONTENTS

<u>Section</u>	<u>Page No.</u>
I. PURPOSE	1
II. SPONSOR	1
III. SPONSOR'S REPRESENTATIVE	1
IV. TESTING LOCATION	1
V. SPRINGBORN PERSONNEL RESPONSIBILITIES	1
VI. PROPOSED STUDY SCHEDULE	2
VII. TEST ARTICLE IDENTIFICATION	3
VIII. TEST SYSTEM	4
IX. ANIMAL HUSBANDRY AND EXPERIMENTAL DESIGN	6
X. EXPERIMENTAL PROCEDURES	7
XI. DATA REPORTING	14
XII. ANALYSIS OF DATA	14
XIII. MAINTENANCE OF RAW DATA, RECORDS AND SPECIMENS	14
XIV. REGULATORY COMPLIANCE	15
XV. QUALITY ASSURANCE	15
XVI. USDA ANIMAL WELFARE COMPLIANCE STATEMENT	15
XVII. PROTOCOL APPROVAL	16
XVIII. REFERENCES	17
<u>APPENDIX</u>	
A. DERMAL GRADING SYSTEM	18

I. PURPOSE

To assess the dermal sensitization potential of a test article in guinea pigs when administered by multiple topical applications. This study is intended to provide information on the potential health hazards of the test article with respect to dermal exposure. Data from this study may serve as a basis for classification and/or labeling of the test article.

II. SPONSOR

Elf Atochem North America, Inc.
900 First Avenue
P.O. Box 1536
King of Prussia, PA 19406-0018

III. SPONSOR'S REPRESENTATIVE

Roy M. Bannister, Ph.D., DABT
Phone: (610) 337-6875
Fax: (610) 337-8660

IV. TESTING LOCATION

Springborn Laboratories, Inc.
Life Sciences Division
553 North Broadway
Spencerville, OH 45887
Phone: (419) 647-4196
FAX: (419) 647-6458

V. SPRINGBORN PERSONNEL RESPONSIBILITIES

Deborah A. Douds, M.S.
Study Director/Toxicologist

Rusty E. Rush, M.S., LAT
Alternate Contact/Manager of Acute Toxicology and Special Studies

Malcolm Blair, Ph.D.
Vice President/Director of Research

Joseph C. Siglin, M.S., DABT
Associate Director of Toxicology

Kimberly L. Bonnette, M.S., LATG
Assistant Manager of Acute Toxicology

Todd N. Merriman, A.S., LATG
Associate Toxicologist

Patricia K. Jenkins, AAS, LATG, RLAM
Acute Toxicology Supervisor

Pamela S. Smith, ALAT
Unit Leader

Delores P. Knippen
Pharmacy Supervisor

Steven H. Magness, B.S., LATG
Supervisor of Gross & Fetal Pathology

Anita M. Bosau
Director of Quality Assurance

Raymond V. Karcher, B.A., LAT
Quality Assurance Supervisor

VI. PROPOSED STUDY SCHEDULE

A. Experimental Initiation: July 1994

B. Experimental Completion: August 1994

C. Audited Report Date: Eight weeks following experimental completion

VII. TEST ARTICLE IDENTIFICATION

A. Sponsor's Identification

t-Butyl Mercaptan

B. SLS Test Article Identification Number

S94.002.3255

C. Characteristics

The Sponsor is responsible for any necessary evaluations related to chemical composition, purity, strength, stability and other data required by 40 CFR Part 792.105. Any special storage conditions for the test article will be supplied by the Sponsor.

D. Handling Precautions

Safety data regarding the test article should be provided by the Sponsor (Material Safety Data Sheet or equivalent, if available). Technical personnel should review this information prior to handling the test article. In addition, any special handling precautions will be provided by the Sponsor/Study Director.

E. Method of Dosage Preparation

1. Test Article

The test article will be administered as received and/or diluted with an appropriate vehicle. Selected dosages will be achieved by adjustment of test article concentration in the vehicle. Solids and powders may need to be ground and/or sieved prior to preparation in order to enhance test article contact with the skin or improve homogeneity of the dose preparation. The test article will be prepared and/or dispensed fresh on the day of dosing. The method of preparation will be documented in the raw data and presented in the final report. An approximate 1 g or 1 ml retention sample of the test article will be placed in an amber vial, sealed and archived at the test facility.

VIII. TEST SYSTEM

A. Justification of Test System

1. The guinea pig is the preferred species for dermal sensitization testing by various U.S. and International regulatory agencies.
2. The Hartley-derived albino guinea pig has been shown to be sensitive to the hyperallergenic effects of a variety of drugs and chemicals. Therefore, this species and strain is a reasonable alternative to larger mammals for dermal sensitization testing of drugs and chemicals for human safety assessment.
3. The Hartley-derived albino guinea pig has been used extensively for dermal sensitization testing. Thus, data from this study may be compared and contrasted to other studies performed in Hartley-derived albino guinea pigs.
4. Historical information concerning Hartley-derived albino guinea pigs is available at SLS and in the published literature.
5. Healthy, outbred Hartley-derived albino guinea pigs may be obtained from reliable, USDA approved and regulated suppliers.
6. The laboratory guinea pig may be safely handled and manipulated by trained technical personnel.

B. Justification of Route of Exposure and Number of Animals

1. Dermal administration of the test substance was selected since this is a potential route of human exposure. The dermal tissue also plays a significant role in the hypersensitization process.
2. Since Hartley-derived albino guinea pigs have no pigment, dermal responses may be easily observed.
3. The number of animals used on this study will be consistent with the guidelines published by a number of U.S. and International regulatory agencies including EPA-FIFRA, EPA-TSCA, FDA, CPSC-FHSA, DOT, IMO, EEC, OECD, MAFF and MOHW.

4. If a range-finding study is required, only two animals/sex will be utilized in order to produce scientifically meaningful results.

C. Description

1. Species

Guinea Pig

2. Strain

Hartley-derived albino

3. Source

Harlan Sprague Dawley or another USDA approved supplier

4. Age and Body Weight Range

Young adult, approximately 300 to 500 g (Topical Range-Finding Study: the day prior to dosing, Sensitization Study: the day prior to first induction)

5. Number and Sex

Topical Range-finding: 4 (2 males and 2 females)

Test: 20 (10 males and 10 females)

Challenge Control: 10 (5 males and 5 females)

Rechallenge Control: 10 (5 males and 5 females)

Additional range-finding animals may be necessary depending on the results obtained at each level.

D. Method of Identification

Plastic ear tags displaying unique identification numbers will be used to individually identify the animals. Cage cards displaying at least the study number, animal number, and sex will be affixed to each cage.

IX. ANIMAL HUSBANDRY AND EXPERIMENTAL DESIGN

A. Animal Housing

1. Housing

The animals will be housed individually in suspended stainless steel cages. All housing and care will conform to the standards recommended by the Guide for the Care and Use of Laboratory Animals [1].

2. Environment

The environmental conditions in the animal room will be controlled. The desired animal room temperature and relative humidity ranges are 65-79°F and 40-70%, respectively. Environmental control equipment will be monitored and adjusted as necessary to minimize fluctuations in the animal room environment. Light timers will be set to maintain a 12-hour light/12-hour dark cycle. There will be ten to twelve air changes in the animal room per hour. The animal room temperature and relative humidity will be recorded a minimum of once daily.

3. Food

Purina Certified Guinea Pig Chow #5026 will be provided ad libitum to the animals throughout the study. The lot number and expiration date of each batch of diet used during the study will be recorded. The feed is analyzed by the supplier for nutritional components and environmental contaminants. Dietary limitations for various environmental contaminants, including heavy metals, pesticides, polychlorinated biphenyls and total aflatoxin are set by the manufacturer. Within these limits, contaminants which may be present are not expected to compromise the purpose of this study. Results of the dietary analyses (Certificates of Analysis) are provided by the manufacturer for each lot of diet. These will be maintained by the testing laboratory.

4. Water

Municipal tap water treated by reverse osmosis or deionization (back-up system) will be available ad libitum throughout the study. The purified water will be supplied by an automatic watering system. Monitoring of

the drinking water for contaminants will be conducted by the testing laboratory and the records will be available for inspection. Within generally accepted limits, contaminants which may be present are not expected to compromise the purpose of this study.

B. Quarantine

Upon receipt, the animals will be examined, identified with plastic ear tags, and then quarantined for a minimum of 5 days.

C. Animal Selection

The animals chosen for study use will be arbitrarily selected from healthy stock animals to avoid potential bias. All animals will receive a detailed pretest observation prior to dosing. Only healthy animals will be chosen for study use. Females will be nulliparous and nonpregnant.

D. Experimental Design [2]

This study will consist of at least a topical range-finding group, a test group and a challenge control group. The Sponsor may select one or more of the following options:

Rechallenge Control group

Historical Positive Control data (mild/moderate sensitizer)

X. EXPERIMENTAL PROCEDURES

A. Topical Range-Finding Study

1. Methodology

Four graded levels (generally 25% w/v, 50% w/v, 75% w/v and 100%) are utilized for this procedure. Optimally, the topical range-finding study should produce no systemic toxicity and a spectrum of dermal responses that include grades 0, 1 and 2 unless the test article is not dermally irritating at 100%. All dose levels utilized will be documented in the raw data and presented in the final report.

2. Dosing

On the day prior to dose administration, four topical range-finding guinea pigs will be weighed and the hair removed from the right and left side of the animals with a small animal clipper. Care will be taken to avoid abrading the skin during clipping procedures. On the following day, up to four closed patches/chambers at four different concentrations of test article can be applied to the clipped area of each animal (one patch/chamber for each level of test article). For liquids, gels and pastes, a dose of 0.4 ml will be placed on a Webril patch (approximately 2 cm x 2 cm) backed by adhesive tape (occlusive patch). For solids and powders, the maximum volume of solid/powder that can be contained in a 25 mm Hilltop Chamber (with cotton pad removed) will be utilized (occlusive patch). The weight of the solid/powder placed in the chamber will be recorded. Prior to chamber application, the test sites will be moistened to enhance solid/powder contact with the skin by wiping the site with gauze moistened in distilled water. The patches/chambers will then be applied to the clipped surface as quickly as possible. The trunk of the animal will be wrapped with elastic wrap which is secured with adhesive tape (if necessary) to prevent removal of the patch/chamber and the animal returned to its cage. Approximately six hours after patch/chamber application, the elastic wrap, tape and patches/chambers will be removed. The test sites will then be wiped with gauze moistened in distilled water to remove test article residue and the animals returned to their cages. If the distilled water does not sufficiently remove the test article residue, the Study Director/Sponsor may choose to use another solvent.

3. Body Weights

Individual body weights will be obtained for the topical range-finding animals on the day prior to dosing.

4. Dermal Observations

The test sites of the topical range-finding animals will be graded for irritation at approximately 24 and 48 hours following patch/chamber application using the Dermal Grading System in Protocol Appendix A.

5. Clinical Observations

Any unusual observation and mortality will be recorded. The topical range-finding animals will be observed for mortality twice daily, once in the morning and once in the afternoon.

6. Unscheduled Deaths

Any topical range-finding animals dying or euthanized (due to a possible accidental injury) during the study will be necropsied. Body cavities (cranial, thoracic, abdominal and pelvic) will be opened and examined. No tissues will be retained.

7. Scheduled Euthanasia

Following the 48 hour scoring interval, all surviving topical range-finding animals will be euthanized by carbon dioxide inhalation. A gross necropsy examination will not be required for surviving topical range-finding animals.

B. Sensitization Study

1. Induction

a. Methodology

Optimally, the test article concentration used for induction should produce no systemic toxicity and a mild dermal response unless the test article is not dermally irritating at 100%. The test article concentration may be varied during the induction period depending on the dermal responses produced. All dose levels utilized will be documented in the raw data and presented in the final report.

b. Dosing

On the day prior to the first induction dose administration (day -1), all sensitization study animals will be weighed. The hair will then be removed from the left side of the twenty test animals with a small animal clipper. Care will be taken to avoid abrading the skin during clipping procedures. On the following day (day 0), patches/chambers containing the appropriate material will be

applied to the clipped area of the twenty test animals. For liquids, gels and pastes, a dose of 0.4 ml will be placed on a Webril patch (approximately 2 cm x 2 cm) that is backed by adhesive tape (occlusive patch). For solids and powders, the maximum volume of solid/powder that can be contained in a 25 mm Hilltop Chamber (with cotton pad removed) that is backed by adhesive tape will be utilized (occlusive patch). The weight of the solid/powder placed in the chamber will be recorded. Prior to chamber application, the test sites will be moistened to enhance solid/powder contact with the skin by wiping the site with gauze moistened in distilled water. The patch/chamber will then be applied to the clipped surface as quickly as possible. The trunk of each animal will be wrapped with elastic wrap which is secured with adhesive tape (if necessary) to prevent removal of the patch/chamber and the animal returned to its cage. Approximately six hours after dosing, the elastic wrap, tape, and patch/chamber will be removed. The test sites will be wiped with gauze moistened in distilled water to remove test article residue and the animals returned to their cages. If the distilled water does not sufficiently remove the test article residue, the Study Director/Sponsor may choose to use another solvent. The induction clipping, patch application and grading procedure will be repeated on study day 7 (± 1 day) and study day 14 (± 1 day) so that a total of three consecutive induction exposures will be made to the twenty test animals.

c. Dermal Observations

Test sites will be graded for dermal irritation at approximately 24 and 48 hours following patch application using the Dermal Grading System presented in Protocol Appendix A. The application site may be moved if irritation persists from a previous induction exposure but will remain on the left side of the animal.

2. Challenge

a. Methodology

Optimally, the test article concentration(s) used for challenge should produce no systemic toxicity and be the highest non-irritating dose. All dose levels will be documented in the raw data and presented in the final report.

b. Dosing

On the day prior to challenge dose administration, all test and challenge control animals will be weighed. The hair will then be removed from the right side of the twenty test and ten challenge control animals with a small animal clipper. Care will be taken to avoid abrading of the skin during clipping procedures. On the following day (day 28 \pm 1 day), patches/chambers containing the appropriate material will be applied to a naive site within the clipped area of the twenty test and ten challenge control animals. For liquids, gels and pastes, a dose of 0.4 ml will be placed on a Webril patch (approximately 2 cm x 2 cm) that is backed by adhesive tape (occlusive patch). For solids and powders, the maximum volume of solid/powder that can be contained in a 25 mm Hilltop Chamber (with cotton pad removed) that is backed by adhesive tape will be utilized (occlusive patch). The weight of the solid/powder placed in the chamber will be recorded. Prior to chamber application, the test sites will be moistened to enhance solid/powder contact with the skin by wiping the site with gauze moistened in distilled water. The patch/chamber will then be applied to the clipped surface as quickly as possible. The trunk of each animal will be wrapped with elastic wrap which is secured with adhesive tape (if necessary) to prevent removal of the patch/chamber and the animal returned to its cage. Approximately six hours after dosing, the elastic wrap, tape, and patch/chamber will be removed. The test sites will be wiped with gauze moistened in distilled water to remove test article residue and the animals returned to their cages. If the distilled water does not sufficiently remove the test article residue, the Study Director/Sponsor may choose to use another solvent.

c. Dermal Observations

Test sites will be graded for dermal irritation at approximately 24 and 48 hours following patch removal using the Dermal Grading System presented in Protocol Appendix A. A 72 hour grading interval may be conducted as deemed necessary by the Study Director/Sponsor to allow further evaluation of challenge responses.

3. Rechallenge

a. Methodology

If the results of the challenge procedure are not conclusive, then a rechallenge may need to be performed to help clarify the challenge responses. Optimally, the test article concentration(s) used for rechallenge should produce no systemic toxicity and dermal responses generally consisting of grade 0. All dose levels will be documented in the raw data and presented in the final report.

b. Dosing

On the day prior to rechallenge dose administration, all test and rechallenge control animals used on study will be weighed. The hair will then be removed from the right side of the twenty test and ten rechallenge control animals with a small animal clipper. On the following day (day 35 \pm 1 day), patches/chambers containing the test article will be applied to a naive site within the clipped area of the twenty test and ten rechallenge control animals. For liquids, gels and pastes, a dose of 0.4 ml will be placed on a Webril patch (approximately 2 cm x 2 cm) that is backed by adhesive tape (occlusive patch). For solids and powders, the maximum volume of solid/powder that can be contained in a 25 mm Hilltop Chamber (with cotton pad removed) that is backed by adhesive tape will be utilized (occlusive patch). The weight of the solid/powder placed in the chamber will be recorded. Prior to chamber application, the test sites will be moistened to enhance solid/powder contact with the skin by wiping the site with distilled water prior to application of the chamber. The patch/chamber will then be applied to the clipped surface as quickly as possible. The trunk of each animal will then be wrapped with elastic wrap which is secured with adhesive tape (if necessary) to prevent removal of the patch/chamber and the animal returned to its cage. Approximately six hours after dosing, the elastic wrap, tape, and patch chamber will be removed. The test sites will be wiped with gauze moistened in distilled water to remove test article residue and the animals returned to their cages. If the distilled water does not sufficiently remove the test article residue, the Study Director Sponsor may choose to use another solvent.

BEST COPY AVAILABLE

c. Dermal Observations

Test sites will be graded for dermal irritation at approximately 24 and 48 hours following patch removal using the Dermal Grading System presented in Protocol Appendix A. A 72 hour grading interval may be conducted as deemed necessary by the Study Director/Sponsor to allow further evaluation of rechallenge responses.

4. Body Weights

Individual body weights will be obtained for all sensitization study animals on the day prior to the first induction (day -1) and for the test and challenge control animals on the day prior to challenge dosing. The test and rechallenge control animals will be weighed on the day prior to rechallenge dosing if a rechallenge is performed. Individual body weights will also be obtained for all sensitization study animals prior to scheduled euthanasia.

5. Clinical Observations

Any unusual observations and mortality will be recorded. The sensitization study animals will be observed for mortality twice daily, once in the morning and once in the afternoon.

6. Unscheduled Deaths

Any sensitization study animals dying or euthanized (due to a possible accidental injury) during the study will be necropsied. Body cavities (cranial, thoracic, abdominal and pelvic) will be opened and examined. No tissues will be retained.

7. Scheduled Euthanasia

All surviving sensitization study animals will be euthanized by carbon dioxide asphyxiation after their final scoring interval. A gross necropsy examination will not be required for surviving sensitization study animals.

XI. DATA REPORTING

One unbound copy of the draft report will be submitted to the Sponsor. Two copies of the final report (one bound and one unbound) will be submitted to the Sponsor. The final report will include all information necessary to provide a complete and accurate description and evaluation of the experimental procedures and results.

The report will include at least the following information and data:

- Table of Contents
- Regulatory Compliance
- Summary
- Introduction
- Experimental Design and Test Procedures
- Presentation and Discussion of Results
- Conclusion
- References
- Data Tables
- Protocol and Amendments
- SLS Personnel Responsibilities

XII. ANALYSIS OF DATA

The sensitization potential of the test article will be based on the dermal responses observed on the test and control animals at challenge and rechallenge (if conducted). Generally, dermal scores of ≥ 1 in the test animals with scores of 0 noted in the controls is considered indicative of sensitization. Dermal scores of 1 in the controls is generally considered equivocal unless a higher dermal response (\geq grade 2) is noted in the test animals. Group mean dermal scores will be calculated for challenge and rechallenge (if conducted).

XIII. MAINTENANCE OF RAW DATA, RECORDS AND SPECIMENS

All original data, magnetically encoded records, specimens and reports from this study are the property of the Sponsor. These materials shall be available at SLS to facilitate auditing of the study during its progress and prior to acceptance of the final report. The remaining test article(s) will be returned to the Sponsor following completion of the in-life phase of the study. All original paper data, the final report, magnetically encoded records, and any specimens will be transferred to the SLS archives for a period of 10 years. The Sponsor will be contacted prior to the final disposition of these items.

XIV. REGULATORY COMPLIANCE

This study may be submitted to and will be performed in general compliance with the OECD Guidelines for Testing of Chemicals, Section 4: Health Effects, Subsection 406, July 1992, EPA TSCA guidelines (40 CFR 798.4100) and the EEC Council Directive 67/548/EEC, September 19, 1984; the principles of the Good Laboratory Practice regulations as described by the EPA (40 CFR Part 792) and the OECD Annex 2 C(81)30. Changes may be made in this protocol prior to, during, and/or following study completion. A protocol amendment will be prepared for such changes and will be signed by the Study Director, SLS Quality Assurance Unit and the Sponsor. The Sponsor shall be notified as soon as practical whenever an event occurs that is unexpected and may have an effect on the study.

XV. QUALITY ASSURANCE

The study will be inspected at least once during the in-life phase by the Springborn Laboratories, Inc., Life Sciences Division's Quality Assurance Unit to assure compliance with Good Laboratory Practice regulations, SLS's Standard Operating Procedures and for conformance with the protocol and protocol amendments. The final report will be audited prior to submission to the Sponsor to ensure that it completely and accurately describes the test procedures and results of the study.

XVI. USDA ANIMAL WELFARE COMPLIANCE STATEMENT

This study will comply with all applicable sections of the Final Rules of the Animal Welfare Act regulations (9 CFR) and the Public Health Service Policy on Humane Care and Use of Laboratory Animals (OPRR, NIH, 1986). Wherever possible, procedures used in this study have been designed to avoid or minimize discomfort, distress and pain to animals. All methods are described in this study protocol or in written laboratory standard operating procedures. These procedures are based on the most currently available technologies concerning proper laboratory animal use and management. This protocol has been reviewed and approved by Springborn Laboratories, Inc. Institutional Animal Care and Use Committee (IACUC) for a maximum of 70 animals.

This study is being conducted to evaluate potential dermal sensitization effects of the test article. Following dosing, the Study Director will be notified by the technician if severe local reactions occur or if the animals exhibit overt clinical indications of pain/distress immediately postdose. If such is noted, the Sponsor

will be contacted to see if the animals should be humanely euthanized. In the event that the Sponsor cannot be contacted, the Study Director and/or Facility Veterinarian may decide to humanely euthanize the animals. Methods of euthanasia used during this study are in conformance with the above referenced regulations and the American Veterinary Medical Association Panel on Euthanasia (JAVMA, 1993).

XVII. PROTOCOL APPROVAL

The Sponsor's signature below documents for the Study Director that there are no acceptable non-animal alternatives for this study, the study does not unnecessarily duplicate previous studies and that the study is needed for regulatory purposes and/or human safety assessment.

Deborah A. Douds Date 7/22/94
Deborah A. Douds, M.S.
Study Director (SLS)

Raymond V. Karcher Date 7-22-94
Raymond V. Karcher, B.A., LAT
Quality Assurance Unit (SLS)

Patricia K. Jenkins Date 7-25-94
Patricia K. Jenkins, A.A.S., LATG, RLAM
IACUC Representative (SLS)

Roy M. Bannister Date 6/27/94
Roy M. Bannister, Ph.D., DABT
Sponsor's Representative
(Principal Investigator)

XVIII. REFERENCES

1. Guide for the Care and Use of Laboratory Animals, DHHS Publication No. (NIH) 86-23, 1985.
2. E. V. Buehler, Delayed Contact Hypersensitivity in the Guinea Pig, Arch. Dermat., 91:171-177, 1965.

PROTOCOL APPENDIX A
DERMAL GRADING SYSTEM

ERYTHEMA	
OBSERVATION	CODE
No visible change	0
Discrete or patchy erythema	1
Moderate, confluent erythema	2
Severe erythema with or without edema	3

EDEMA	
OBSERVATION	CODE
Very slight edema (barely perceptible)	ED-1
Slight edema (edges of area well defined by definite raising)	ED-2
Moderate edema (raised approximately 1 millimeter)	ED-3
Severe edema (raised more than 1 millimeter and extends beyond the area of exposure)	ED-4

An erythema code will be assigned to each test site. An edema code will be assigned only if edema is present at the test site.

PROTOCOL APPENDIX A--(Continued)
DERMAL GRADING SYSTEM

NOTABLE DERMAL LESIONS		
OBSERVATION	CODE	DEFINITION
Eschar - Focal/pinpoint	ES-1	Focal and/or pinpoint areas in test site.
Eschar - Mild	ES-2	>focal/pinpoint < 25% of test site.
Eschar - Moderate	ES-3	>25% < 50% of test site.
Eschar - Severe	ES-4	>50% of test site.
Blanching - Focal/pinpoint	BLA-1	Focal and/or pinpoint areas in test site.
Blanching - Mild	BLA-2	>focal/pinpoint < 25% of test site.
Blanching - Moderate	BLA-3	>25% < 50% of test site.
Blanching - Severe	BLA-4	>50% of test site.
Ulceration - Focal/pinpoint	U-1	Focal and/or pinpoint areas in test site.
Ulceration - Mild	U-2	>focal/pinpoint < 25% of test site.
Ulceration - Moderate	U-3	>25% < 50% of test site.
Ulceration - Severe	U-4	>50% of test site.

If the eschar, blanching and/or ulceration is focal and/or pinpoint, the remaining portion of the test site will be assigned the appropriate erythema and edema score (ie: 0, ±, 1, 2 or 3) and the score footnoted with the appropriate code (ie: BLA-1, ES-1 and/or U-1).

If the eschar, blanching and/or ulceration is greater than the focal and/or pinpoint, the maximum score of "3" will be assigned to the site for erythema and the score footnoted with the appropriate code (ex: BLA-2, ES-3 and/or U-4). The appropriate edema score will then be assigned to the site, if present.

PROTOCOL APPENDIX A--(Continued)
DERMAL GRADING SYSTEM

SECONDARY DERMAL FINDINGS		
OBSERVATION	CODE	DEFINITION
Desquamation	DES	Characterized by scaling or flaking of dermal tissue with or without denuded areas. Scab-like areas of eschar are not scored for desquamation.
Fissuring	FIS	Characterized by cracking of the skin with or without moist exudate. Fissuring should be checked prior to manipulating the test site. Scab-like areas of eschar are not scored for fissuring
Eschar Exfoliation	EXF	The process by which a scab-like or slough-like formation flakes off the test site.
Test Article Staining Test Site	TAS	Skin located at test site appears to be discolored due to the test article (note color of staining).
Ancillary Irritation on the Trunk Outside the Test Site	IT	Dermal irritation or lesions that are located outside the test site on the animals' trunk. These findings are commonly mechanically induced (ex: animal movement following wrapping, compression of the skin by the tape/binder and/or removal of the adhesive tape).

Any additional dermal findings will be noted in the raw data and in the final report.

A DERMAL SENSITIZATION STUDY IN GUINEA PIGS
WITH t-BUTYL MERCAPTAN
• MODIFIED BUEHLER DESIGN •

PROTOCOL AMENDMENT
NO. 1

JAN 04 1995

SLS STUDY NO. 3255.21

PAGE 1 OF 1

TEST ARTICLE t-Butyl Mercaptan

1) PART TO BE CHANGED/REVISED: VI. PROPOSED STUDY SCHEDULE

CHANGE/REVISION: Replace this section with the following:

- A. Experimental Initiation: November 1994
- B. Experimental Completion: December 1994
- C. Audited Report Date: January 6, 1995

REASON FOR CHANGE/REVISION: Initiation of the study was delayed.

2) PART TO BE CHANGED/REVISED: IX.D. ANIMAL HUSBANDRY AND EXPERIMENTAL DESIGN

CHANGE/REVISION: Hexylcinnamaldehyde will be utilized as the mild to moderate sensitizer for the Historical Positive Control Data.

REASON FOR CHANGE/REVISION: To specify the mild to moderate sensitizer used for the Historical Positive Control Data.



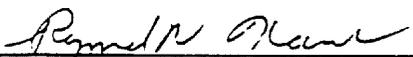
Deborah A. Douds, M.S.
Study Director (SLS)

Date 12/22/94



Roy M. Bannister, Ph.D., DABT
Sponsor's Representative

Date 12/29/94



Raymond V. Karcher, B.A., LAT
Quality Assurance Supervisor (SLS)

Date 12-22-94

APPENDIX B

Dermal Grading System

DERMAL GRADING SYSTEM

ERYTHEMA	
OBSERVATION	CODE
No visible change	0
Discrete or patchy erythema	1
Moderate, confluent erythema	2
Severe erythema with or without edema	3

EDEMA	
OBSERVATION	CODE
Very slight edema (barely perceptible)	ED-1
Slight edema (edges of area well defined by definite raising)	ED-2
Moderate edema (raised approximately 1 millimeter)	ED-3
Severe edema (raised more than 1 millimeter and extends beyond the area of exposure)	ED-4

An erythema code was assigned to each test site. An edema code was assigned only if edema was present at the test site.

DERMAL GRADING SYSTEM

NOTABLE DERMAL LESIONS		
OBSERVATION	CODE	DEFINITION
Eschar - Focal/pinpoint	ES-1	Focal and/or pinpoint areas in test site.
Eschar - Mild	ES-2	> focal/pinpoint < 25% of test site.
Eschar - Moderate	ES-3	> 25% < 50% of test site.
Eschar - Severe	ES-4	> 50% of test site.
Blanching - Focal/pinpoint	BLA-1	Focal and/or pinpoint areas in test site.
Blanching - Mild	BLA-2	> focal/pinpoint < 25% of test site.
Blanching - Moderate	BLA-3	> 25% < 50% of test site.
Blanching - Severe	BLA-4	> 50% of test site.
Ulceration - Focal/pinpoint	U-1	Focal and/or pinpoint areas in test site.
Ulceration - Mild	U-2	> focal/pinpoint < 25% of test site.
Ulceration - Moderate	U-3	> 25% < 50% of test site.
Ulceration - Severe	U-4	> 50% of test site.

If the eschar, blanching and/or ulceration was focal and/or pinpoint, the remaining portion of the test site was assigned the appropriate erythema and edema score (i.e., 0, 1, 2 or 3) and the score footnoted with the appropriate code (i.e., BLA-1, ES-1 and/or U-1).

If the eschar, blanching and/or ulceration was greater than the focal and/or pinpoint, the maximum score of "3" was assigned to the site for erythema and the score footnoted with the appropriate code (ex: BLA-2, ES-3 and/or U-4). The appropriate edema score was then assigned to the site, if present.

DERMAL GRADING SYSTEM

SECONDARY DERMAL FINDINGS		
OBSERVATION	CODE	DEFINITION
Desquamation	DES	Characterized by scaling or flaking of dermal tissue with or without denuded areas. Scab-like areas of eschar are not scored for desquamation.
Fissuring	FIS	Characterized by cracking of the skin with or without moist exudate. Fissuring should be checked prior to manipulating the test site. Scab-like areas of eschar are not scored for fissuring.
Eschar Exfoliation	EXF	The process by which a scab-like or slough-like formation flakes off the test site.
Test Article Staining Test Site	TAS	Skin located at test site appears to be discolored due to the test article (note color of staining).
Ancillary Irritation on the Trunk Outside the Test Site	IT	Dermal irritation or lesions that are located outside the test site on the animals' trunk. These findings are commonly mechanically induced (ex: animal movement following wrapping, compression of the skin by the tape/binder and/or removal of the adhesive tape).

APPENDIX C

Topical Range-Finding Data

SLS STUDY NO.: 3255.21
 CLIENT: ELF ATOCHEM

A DERMAL SENSITIZATION STUDY IN GUINEA PIGS
 TOPICAL RANGE-FINDING DATA

PAGE 1

Group	Animal No./Sex	Range-Finding Dermal Scores*													
		24 Hr	48 Hr	24 Hr	48 Hr	24 Hr	48 Hr	24 Hr	48 Hr	24 Hr	48 Hr				
Range-finding	4071/M 477	0	0	0	0	±	0	±	0	±	0	±	0	±	0
	4073/M 459	0	0	0	0	0	0	0	0	±	0	±	0	±	0
	4261/F 462	0	0	0	0	0	0	±	0	±	0	±	0	±	0
	4262/F 429	0	0	0	0	0	0	0	0	0	0	±	0	±	0

*An incorrect dermal grading system was utilized. A score of ± was utilized to indicate slight, patchy erythema. See Appendix B for definition of codes.

APPENDIX D

Individual Body Weight Data

SLS STUDY NO.: 3255.21
 CLIENT: ELF ATOCHEM

A DERMAL SENSITIZATION STUDY IN GUINEA PIGS
 INDIVIDUAL BODY WEIGHT DATA

PAGE 1

(50)

Group	Animal No./Sex	Body Weight (g)		
		Day -1	Day 27	Terminal
Test	4931/M	400	536	538
	4932/M	349	492	508
	4933/M	417	567	586
	4934/M	395	556	573
	4935/M	389	576	605
	4936/M	389	501	496
	4937/M	378	501	524
	4938/M	360	543	548
	4939/M	406	565	591
	4940/M	357	542	553
	4950/F	310	450	440
	4951/F	354	499	522
	4952/F	306	399	416
	4953/F	326	394	399
	4954/F	333	417	429
	4955/F	336	453	464
	4956/F	334	402	396
	4957/F	316	447	435
	4958/F	328	502	527
4959/F	314	432	437	
Challenge Control	4941/M	368	622	634
	4942/M	357	484	479
	4943/M	386	549	555
	4944/M	412	621	639
	4945/M	422	695	696
	4960/F	301	403	411
	4961/F	341	537	542
	4962/F	312	385	381
	4963/F	351	533	531
	4964/F	363	482	487

SLS STUDY NO.: 3255.21
CLIENT: ELF ATOCHEM

A DERMAL SENSITIZATION STUDY IN GUINEA PIGS
INDIVIDUAL BODY WEIGHT DATA

PAGE 2

Group	Animal No./Sex	Body Weight (g)		
		Day -1	Day 27	Terminal
Rechallenge Control*	4946/M	382	-	585
	4947/M	349	-	541
	4948/M	367	-	567
	4949/M	395	-	552
	4855/M	373	-	627
	4965/F	327	-	425
	4966/F	339	-	470
	4967/F	319	-	469
	4968/F	347	-	502
	4930/F	347	-	478

*A rechallenge irritation control group was maintained on the study; however, the rechallenge procedure was not required.

APPENDIX E

Hexylcinnamaldehyde Historical Control Data

**SPRINGBORN LABORATORIES, INC.
MODIFIED BUEHLER HISTORICAL CONTROL DATA
USING HEXYLCINNAMALDEHYDE
(A KNOWN MILD TO MODERATE SENSITIZER)**

Purpose

To assess the dermal sensitization potential of Hexylcinnamaldehyde when administered by multiple topical applications.

Study Dates

Experimental Initiation: September 16, 1994
Experimental Completion: October 23, 1994

Test Article

Supplier's I.D.: Hexylcinnamaldehyde
Lot Number: 10021HF
Springborn I.D.: S94.004.N
Receipt Date: May 31, 1994
Storage Conditions: Room temperature
Expiration Date: December 31, 2004

The test article was utilized at 100% (induction, challenge and rechallenge).

Experimental Procedures

Young adult Hartley-derived albino guinea pigs were obtained from Harlan Sprague Dawley, Inc., Haslett, Michigan. The guinea pigs were uniquely identified by ear tag, individually housed in suspended stainless steel cages and received Purina Certified Guinea Pig Chow #5026 and water purified by reverse osmosis ad libitum. The animals were quarantined for a minimum of 5 days prior to study initiation.

On the day prior to the first induction dose administration (day -1), the hair was removed from the left side of the six test animals. On the following day, 0.3 ml of 100% Hexylcinnamaldehyde was placed in a 25 mm Hilltop Chamber and applied to the animals' backs. The trunk of each animal was wrapped with elastic wrap which was secured with adhesive tape to prevent removal of the chamber. Approximately six hours after chamber application, the elastic wrap, tape and chambers were removed. The test sites were wiped with gauze moistened with distilled water to removed test article residue. The test sites were graded for irritation at approximately 24 and 48 hours following chamber application using the Dermal Grading System. The induction procedure was repeated on study day 7 and on study day 14 so that a total of three induction exposures were made to the animals.

On the day prior to challenge dose administration, the hair was removed from the right side of the six test and four challenge control animals. On the following day (day 27), 0.3 ml of 100% Hexylcinnamaldehyde was placed in a 25 mm Hilltop Chamber and applied to the animal's backs. Wrapping, unwrapping and rinsing procedures were the same as those utilized for the induction phase. The test sites were graded for irritation at approximately 24 and 48 hours following chamber removal.

On the day prior to rechallenge dose administration, the hair was removed from the right side of the six test and four rechallenge control animals. On the following day (day 35), 0.3 ml of 100% Hexylcinnamaldehyde was placed in a 25 mm Hilltop Chamber and applied to the animals' backs. Wrapping, unwrapping and rinsing procedures were the same as those utilized for the challenge phase. The test sites were graded for irritation at approximately 24 and 48 hours following chamber removal.

Any unusual observations and/or mortality were recorded. Body weights were recorded for the test and challenge control animals on the day prior to first induction (day -1) and on the day prior to challenge dosing. Body weights were recorded for the test and rechallenge control animals on the day prior to rechallenge dosing. All sensitization study animals were euthanized (carbon dioxide inhalation) following each animal's final scoring interval. Gross necropsy examinations were not required for these animals.

NOTE: The relative humidity of the animal room (34-65%) exceeded the range specified in the protocol (40-70%) during this study. This occurrence is considered to have had no apparent impact on the outcome of this study.

Results

Individual Induction Data: Table 1

Individual Challenge Data: Table 2

Individual Rechallenge Data: Table 3

Hexylcinnamaldehyde: Following challenge with 100% Hexylcinnamaldehyde, dermal scores of 1 were noted in 1/6 test animals. Dermal reactions in the remaining test animals and in the challenge control animals were limited to scores of 0 to ±. Group mean dermal scores were noted to be lightly higher in the test animals as compared to the challenge control animals. Following rechallenge with 100% Hexylcinnamaldehyde, dermal scores of 1 were noted in 3/6 test animals. Dermal reactions in the remaining test animals and in the rechallenge control animals were limited to scores of 0 to ±. Group mean dermal scores were again noted to be slightly higher in the test animals as compared to the rechallenge control animals.

Conclusion

Based on the results of this study, Hexylcinnamaldehyde is considered to be a mild to moderate contact sensitizer in guinea pigs.

TABLE 1
 A DERMAL SENSITIZATION STUDY IN GUINEA PIGS
 INDIVIDUAL INDUCTION DATA

Group	Sex	Animal No./	Induction 1 Dermal Scores			Induction 2 Dermal Scores			Induction 3 Dermal Scores		
			24 Hr	48 Hr	100%	24 Hr	48 Hr	100%	24 Hr	48 Hr	100%
Test	3304/M	0	0	0	2 ED-2	2 ED-1	2 ED-1, DES	2 ED-1	2 ED-1, DES	2 ED-1, DES	
	3305/M	0	0	0	1 ED-2	1 ED-1	2 ED-1	2 ED-1	2 ED-1	2 ED-1	
	3306/M	0	0	0	± ED-1	±	± ED-1	± ED-1	±	±	
	3366/F	0	0	0	2 ED-2	1 ED-1	2 ED-1	2 ED-1	1 ED-1	1 ED-1	
	3368/F	0	0	0	1 ED-1	±	±	1 ED-1	1 ED-1	1 ED-1	
	3369/F	0	0	0	± ED-1	± ED-1	± ED-1	1 ED-1	1 ED-1	± ED-1	

TABLE 2
 A DERMAL SENSITIZATION STUDY IN GUINEA PIGS
 INDIVIDUAL CHALLENGE DATA

Group	Animal No./ Sex	Dermal Scores	
		24 Hr	48 Hr
Test	3304/M	±	±
	3305/M	1 ^{ED-1}	±
	3306/M	±	±
	3366/F	±	±
	3368/F	±	0
	3369/F	±	±
	Mean	0.6	0.4
Challenge Control	3307/M	±	±
	3308/M	±	0
	3370/F	±	±
	3371/F	±	±
	Mean	0.5	0.4

(56)

For the purpose of calculation, ± = 0.5.

TABLE 3
 A DERMAL SENSITIZATION STUDY IN GUINEA PIGS
 INDIVIDUAL RECHALLENGE DATA

Group	Animal No./ Sex	Dermal Scores		
		24 Hr	48 Hr	100%
Test	3304/M	1 ^{ED-1}	1	
	3305/M	1 ^{ED-1}	1	
	3306/M	±	±	
	3366/F	±	±	
	3368/F	1	±	
	3369/F	±	±	
	Mean	0.8	0.7	
Rechallenge Control	4047/M	±	±	
	4048/M	±	0	
	4089/F	±	±	
	4112/F	±	±	
	Mean	0.5	0.4	

(57)

For the purpose of calculation, ± = 0.5.

TABLE 4
 A DERMAL SENSITIZATION STUDY IN GUINEA PIGS
 INDIVIDUAL BODY WEIGHT DATA

Group	Animal No./Sex	Body Weight (g)		
		Day -1	Day 26	Day 34
Test	3304/M	414	617	666
	3305/M	418	632	688
	3306/M	356	514	556
	3366/F	380	540	555
	3368/F	345	473	477
	3369/F	403	561	585
Challenge Control	3307/M	418	630	--
	3308/M	350	511	--
	3370/F	331	465	--
	3371/F	341	513	--
Rechallenge Control	4047/M	--	--	422
	4048/M	--	--	387
	4089/F	--	--	314
	4112/F	--	--	362

APPENDIX F

SLS Personnel Responsibilities

SLS PERSONNEL RESPONSIBILITIES

Deborah A. Douds, M.S. -	Study Director/Toxicologist
Rusty E. Rush, M.S., LAT, DABT	Alternate Contact/Manager of Special Studies
Malcolm Blair, Ph.D.	Vice President/Director of Research
Joseph C. Siglin, M.S., DABT	Associate Director of Toxicology
Kimberly L. Bonnette, M.S., LATG	Manager of Acute Toxicology
Todd N. Merriman, A.S., LATG	Toxicologist
Patricia K. Jenkins, AAS, LATG, RILAM	Acute Toxicology Supervisor
Pamela S. Smith, ALAT	Unit Leader
Delores P. Knippen	Pharmacy Supervisor
Steven H. Magness, B.S., LATG	Gross and Fetal Pathology Supervisor
Anita M. Bosau	Director of Quality Assurance
Raymond V. Karcher, B.A., LAT	Quality Assurance Supervisor