

**HAZLETON**

LABORATORIES AMERICA, INC.

Chemical & BioMedical Sciences Division

3301 KINSMAN BLVD. • P.O. BOX 7545 • MADISON, WISCONSIN 53707 • PHONE (608) 241-4471 • TLX 703956 HAZRAL MDS.UD



REPORT OF ANALYSIS

W. C. MCCORMICK
MINNESOTA MINING AND MANUFACTURING
TOXICOLOGY SERVICES
ST. PAUL, MN 55101

SAMPLE NUMBER: 407039

DATE ENTERED: 07/19/

REPORT PRINTED: 10/05/

SAMPLE: T-3609

PURCHASE ORDER NUMBER: T351371

ENCLOSED: GUINEA PIG MAXIMIZATION - METHOD, SUMMARY
RAW DATA APPENDIX

SIGNED:

Steven M. Glaza
.....
STEVEN M. GLAZA
STUDY DIRECTOR
ACUTE TOXICOLOGY

BY AND FOR HAZLETON LABORATORIES AMERICA, INC.

RAW DATA FOR THIS STUDY ARE KEPT ON FILE AT HAZLETON LABORATORIES
AMERICA, INC., MADISON, WISCONSIN.

005219



SAMPLE NUMBER: 40703984

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SAMPLE: T-3609

GUINEA PIG MAXIMIZATION

Test Animal: Young adult male albino guinea pigs of the Hartley strain were procured, maintained individually in stainless-steel cages in temperature- and humidity-controlled rooms, provided continuous access to Purina Guinea Pig Chow and water and held for an acclimation period of at least 7 days. The animals were identified by animal number and corresponding ear tag.

Test System: Fourteen male albino guinea pigs, weighing between 443 g and 549 g, were chosen at random for this study. The animals were divided into two groups consisting of a test group of 10 animals and a naive control group of four animals. A common vehicle control group of ten animals, weighing between 473 g and 553 g, was utilized for this study and Study Nos. 40703985 and 40705145.

Dose Range Study: The purpose of the range-finding study was to find a moderately irritating dose level to use for the topical application and a subirritating concentration to be used for the challenge application. Four additional animals were treated with the test material at concentrations of 25, 50, 75 and 90% w/v in 80% ethanol/distilled water. The test mixtures were applied to 2.0 x 2.0-cm Whatman No. 3 filter papers which were applied to the shaved animals' backs and were then overwrapped with Blenderm and Elastoplast tapes. The dressings remained in place for 24 hours. The test sites were evaluated for dermal irritation 24 and 48 hours after bandage removal. Based upon the results obtained, the test material was administered as a 90% w/v suspension for the topical and challenge applications.

Preparation of Test Material:**Intradermal Injections**

Solution No. 1: The Freund's Complete Adjuvant Solution was prepared by adding 3.0 ml of sterile water for injection in increments to 3.0 ml of Freund's Complete Adjuvant.

Solution No. 2: A 5% w/v mixture was prepared by weighing 0.50 g of T-3609 into a 30-ml beaker and adding 80% ethanol/distilled water to a total volume of 10.0 ml.

Solution No. 3: A 5% w/v mixture of test material in Freund's Adjuvant was prepared by adding 2.0 ml of a 10% w/v suspension of T-3609 in 80% ethanol/distilled water in increments to 2.0 ml of Freund's Complete Adjuvant.

For the topical induction and challenge application, the test material was administered as a 90% w/v suspension in 80% ethanol/distilled water.

All test mixtures were thoroughly stirred during preparation and dosing.

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SAMPLE: T-3609

GUINEA PIG MAXIMIZATION

(CONTINUED)

Preparation of Vehicle Control Mixtures:

Intradermal Injections

Solution No. 1: The Freund's Complete Adjuvant Solution was prepared by adding 3.0 ml of sterile water for injection in increments to 3.0 ml of Freund's Complete Adjuvant.

Solution No. 2: A 5% w/v mixture of 80% ethanol/distilled water in sterile water was prepared by adding 0.5 g of 80% ethanol/distilled water into a 30 ml beaker and adding sterile water for injection to a total volume of 10 ml.

Solution No. 3: A 5% w/v mixture of 80% ethanol/distilled water in Freund's Adjuvant was prepared by adding 1.3 ml of a 10% w/v mixture of 80% ethanol/distilled water in sterile water for injection in increments to 1.3 ml of Freund's Adjuvant.

For the topical induction and challenge application, the vehicle control animals were treated with 80% ethanol in distilled water.

All vehicle control mixtures were thoroughly stirred during preparation and dosing.

Method: A 4.0 x 6.0-cm area was clipped along the midline over the shoulder region. Two rows of three-deep intradermal injections (total of six injection sites) were made within the boundaries of a 2.0 x 4.0-cm area, one row on each side of the midline as follows per site: 0.05 ml of the prepared Freund's Adjuvant Solution, 0.05 ml of the 5% w/v test mixture, and 0.05 ml of the 5% w/v test material in Freund's Complete Adjuvant mixture. The respective vehicle control mixtures were administered to the vehicle control animals in a similar manner.

Because the test and vehicle control materials were non-irritating, the areas of the intradermal injections were pretreated with a 10% w/w sodium lauryl sulfate (SLS) suspension in petrolatum 24 hours prior to topical application. Six days after the intradermal injections the test area was closely shaved and the 10% SLS was massaged into the skin with a glass rod. Twenty-four hours after pretreatment a 2.0 x 4.0-cm patch of Whatman No. 3 filter paper was saturated with a 90% w/v suspension of T-3609 in 80% ethanol/distilled water, placed over the injection sites, then covered with an overlapping of Blenderm tape and secured by an overwrap of Elastoplast tape. The dressing remained in place for 48 hours. The vehicle control animals were dosed in the same manner using 80% ethanol/distilled water only.

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SAMPLE: T-3609

GUINEA PIG MAXIMIZATION

(CONTINUED)

Two weeks after topical induction the test and naive control (previously untreated) animals received a challenge dose. The hair was removed from a 5.0 x 5.0-cm area on the right flank by shaving as before. The 90% w/v suspension of test material in 80% ethanol/distilled water was applied to saturation on a 2.0 x 2.0-cm piece of filter paper. The vehicle control animals were dosed in the same manner using 80% ethanol/distilled water. The patch was sealed to the right flank for 24 hours under strips of Blenderm tape. Complete occlusion was made by an overwrap with Elastoplast tape wound around the trunk.

Observations: Twenty-four hours following patch removal the test sites were examined for erythema and edema. The sites were examined again at 48 hours after patch removal to detect any weak, slowly developing reactions. The test sites were shaved 3 hours prior to the 24-hour reading. The reactions were scored according to the following 4-point scale:

- 0 = no reaction
- 1 = scattered mild redness
- 2 = moderate and diffuse redness
- 3 = intense redness and swelling

The important statistic in maximization testing is the frequency of sensitization, not the intensity.

Pathology: At study termination all animals were euthanatized and discarded.

Maximization Ratings

Sensitization Rate

0%
1% - 9%
10% - 39%
40% - 69%
70% - 100%

Classification

Not a Skin Sensitizer
Weak Sensitizer
Mild Sensitizer
Moderate Sensitizer
Strong Sensitizer

005222

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SAMPLE: T-3609

GUINEA PIG MAXIMIZATION

(CONTINUED)

Test Animal: Albino guinea pigs

Source: Hazleton Dutchland, Inc., Denver PA

Date Animals Received: 07/10 and 07/24/84

Date Test Started: 08/08/84

Date Test Completed: 09/07/84

Test Material: T-3609

Dermal Reactions to Challenge Application

Animal Number	24-Hour Score	48-Hour Score
	Test Group	
EO-2117	1	1
EO-2118	1	1
EO-2119	0	0
EO-2133	0	0
EO-2121	0	0
EO-2122	0	0
EO-2123	0	0
EO-2124	1	0
EO-2125	0	0
EO-2126	1	1
Average Score	0.4	0.3

Naive Control Group

EO-2127	0	0
EO-2128	0	0
EO-2131	0	0
EO-2111	0	0
Average Score	0.0	0.0

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GUINEA PIG MAXIMIZATION

(CONTINUED)

Vehicle Control (80% Ethanol in Distilled Water)

Animal Number	24-Hour Score	48-Hour Score
	Test Group	
EO-2030	0	0
EO-2027	0	0
EO-2051	0	0
EO-2052	0	0
EO-2053	0	0
EO-2135	0	0
EO-2140	1	0
EO-2054	0	0
EO-2055	0	0
EO-2056	0	0
Average Score	0.1	0.0

General Behavior and Appearance: All of the guinea pigs used in this study appeared normal throughout the study period with the exception of one test group animal, EO-2133, and one vehicle control group animal, EO-2140, which had diarrhea on day 3 of the study.

Normal body weight gains were recorded for all animals during the study with the exception of one vehicle control and two test group animals which exhibited body weight losses of 3-24 g during the first week of the study. During the last week of the study, six test, four naive control and five vehicle control animals exhibited body weight losses of 2-31 g.

Skin Reactions to T-3609: Slight dermal reactions were observed in four test group animals 24 hours following the challenge application. None of the naive control animals reacted to the challenge application of the test material. A slight dermal reaction was observed in one vehicle control animal following the challenge application with 80% ethanol/distilled water.

Conclusion: Based on the results obtained, the test material, T-3609, is considered to be a moderate skin sensitizer in guinea pigs.

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GUINEA PIG MAXIMIZATION

(CONTINUED)

References:

1. Magnusson, B., MD, and A. Kligman, PhD, MD, Allergic Contact Dermatitis in the Guinea Pig, Charles C. Thomas Pub., pp. 113-117 (1970).
2. Hitch, R.K., "Dermal Sensitization Study," Pesticide Assessment Guidelines, Subdivision F, Hazard Evaluation: Human and Domestic Animals, U.S. Environmental Protection Agency Office of Pesticide and Toxic Substance Series 81-1, pp. 59-62, November 1982.

005225

Guinea Pigs - Body Weights (grams)

Test Group: T-3609Vehicle 80% Ethanol / Distilled waterPositive Control Group NAVehicle NAVehicle Control Group NANaive Control Group NAVehicle NA

Animal Number <u>EO-</u>							Tech-nician	Date
2117	2118	2119	2133	2121	2122	2123		1984
500	508	515	498	503	461	443	MH	8-14
476	545	543	485	542	523	492	MP	8-21
548	602	583	565	594	545	508	MP	8/28
589	677	666	659	663	598	573	SP	9/4
577	680	655	662	673	591	575	SP	9/7

Animal Number <u>EO-</u>				Tech-nician	Date
2124	2125	2126			1984
515	549	516	Scale Used: KTRON 1348	MH	8-14
556	601	559	Scale Used: KTron 15019	MP	8-21
576	614	566	Scale Used: KTron 15019	MP	8/28
644	693	619	Scale Used: KTron 5228	SP	9/4
635	664	607	Scale Used: KTron 5228	SP	9/7
			Scale Used:		

NA - Not Applicable

Reviewed by JBW Date 9-19-84

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RT No. 4070398
Room No. 4

Guinea Pigs - Daily Observations

Test Group: T-3609
Positive Control Group NA
Vehicle Control Group NA
Naive Control Group NA

Vehicle 80% Ethanol / Distilled water
Vehicle NA
Vehicle NA

Animal Number EO-										Tech- nician	Date
2117	2118	2119	2133	2121	2122	2123	2124	2125	2126		
N	N	N	N	N	N	N	N	N	N	MP	8-14
N	N	N	N	N	N	N	N	N	N	MP	8-15
N	N	N	D	N	N	N	N	N	N	SP	8-16
N	N	N	N	N	N	N	N	N	N	MP	8-17
N	N	N	N	N	N	N	N	N	N	dcb	8/18
N	N	N	N	N	N	N	N	N	N	dcb	8/19
N	N	N	N	N	N	N	N	N	N	MP	8/20
N	N	N	N	N	N	N	N	N	N	MP	8/21
N	N	N	N	N	N	N	N	N	N	MP	8/22
N	N	N	N	N	N	N	N	N	N	MP	8/23
N	N	N	N	N	N	N	N	N	N	MP	8/24
N	N	N	N	N	N	N	N	N	N	MP	8/25
N	N	N	N	N	N	N	N	N	N	slh	8-26
N	N	N	N	N	N	N	N	N	N	MP	8-27
N	N	N	N	N	N	N	N	N	N	MP	8-28
N	N	N	N	N	N	N	N	N	N	MP	8-29
N	N	N	N	N	N	N	N	N	N	MP	8-30
N	N	N	N	N	N	N	N	N	N	SP	8-31

D = Diarrhea SP 8-16-84

N - No Visible Abnormalities
NA - Not Applicable

005227

RT No. 4070398
Room No. 4

Test Group: T-3609

Positive Control Group NA

Vehicle Control Group NA

Naive Control Group NA

Vehicle 80% Ethanol / Distilled
Water

Vehicle NA

Vehicle NA

N - No Visible Abnormalities
NA - Not Applicable

005228

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GUINEA PIG MAXIMIZATION TEST

Test Group

Test Compound T-3609
Date Animals Received 7-24-84
Date of Intradermal Injections 8-14-84
Date of Topical Application 8-21-84
Date of Challenge 9-4-84

RT No. 40703984
Source Hazleton Dutchland
Technician MP MN
Technician MP
Technician SP

Animal No., Sex ♂	24 Hours		48 Hours	
	Right	Left	Right	Left
EO- 2117	1 slight		1 slight	
2118	1 slight		1 slight	
2119	0		0	
2133	0		0	
2121	0		0	
2122	0		0	
2123	0		0	
2124	1 slight		0	
2125	0		0	
2126	1		1 slight	
Technician	SG		SP	
Recorded By	SP		SP	
Date	1984 9/6		9/7	

Scoring Code: 0 = Normal, No Reaction
1 = Scattered Mild Redness
2 = Moderate and Diffuse Redness
3 = Intense Redness and Swelling

REVIEWED BY: SGW

DATE: 9-19-84

SG

9-27-84

005229

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RT No. 40703984

Guinea Pigs - Body Weights (grams)

Test Group: NA

Vehicle NA

Positive Control Group NA

Vehicle NA

Vehicle Control Group NA

Naive Control Group T-3609

Vehicle 80% Ethanol/Distilled Water

Animal Number					Tech-nician	Date
2127	2128	2131	2111	① 2133		1984
500	526	546	528	① 598	MH	8-14
549	590	621	587		MP	8-21
580	645	675	617		MP	8-28
638	681	744	654		SP	9/4
628	650	736	630		SP	9/7

Animal Number				Tech-nician	Date
			Scale Used: KTRON 1348	MH	8-14
			Scale Used: KTRON 15019	MP	8-21
			Scale Used: KTRON 15019	MP	8-28
			Scale Used: KTRON 5228	SP	9/4
			Scale Used: KTRON 5228	SP	9/7
			Scale Used:		

NA - Not Applicable

Reviewed by gaw Date 9-19-84

① Entry Error 8/14/84 MH

005230

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RT No. 40703984
Room No. 4

Guinea Pigs - Daily Observations

Test Group: NA
Positive Control Group NA
Vehicle Control Group NA
Naive Control Group T-3609

Vehicle NA
Vehicle NA
Vehicle 80% Ethanol/Distilled water

Animal Number <u>EO</u>										Tech- nician	Date
2127	2128	2131	2111								1984
N	N	N	N							MP	8-14
N	N	N	N							MP	8-15
N	N	N	N							SP	8-16
N	N	N	N							MP	8-17
N	N	N	N							dcb	8-18
N	N	N	N							dcb	8-19
N	N	N	N							MP	8-20
N	N	N	N							MP	8-21
N	N	N	N							MP	8-22
N	N	N	N							MP	8-23
N	N	N	N							MP	8-24
N	N	N	N							MP	8-25
N	N	N	N							MP	8-26
N	N	N	N							MP	8-27
N	N	N	N							MP	8-28
N	N	N	N							MP	8-29
N	N	N	N							MP	8-30
N	N	N	N							SP	8-31

N - No Visible Abnormalities
NA - Not Applicable

① Animals observed to be normal on 8/23/84 but mistakenly not recorded - MP 9-24-84

005231

RT No. 40703984
Room No. 4

Test Group: NA
Positive Control Group NA
Vehicle Control Group NA
Naive Control Group T-3609

Vehicle NA

Vehicle 80% Ethanol/Distilled
water

N - No Visible Abnormalities
NA - Not Applicable

005232

GUINEA PIG MAXIMIZATION TEST

Naive Group

Test Compound T-3609 RT No. 40703984
 Date Animals Received 7-24-84 Source Hazleton Dutchland
 Date of Intradermal Injections NA Technician NA
 Date of Topical Application NA Technician NA
 Date of Challenge 9-4-84 Technician SP

Animal No., Sex ♂	24 Hours		48 Hours	
	Right	Left	Right	Left
EO- 2127	0		0	
2128	0		0	
2131	0		0	
2111	0		0	
Technician	SG		SP	
Recorded By	SP		SP	
Date 1984	9/6		9/7	

Scoring Code: 0 = Normal, No Reaction
 1 = Scattered Mild Redness
 2 = Moderate and Diffuse Redness
 3 = Intense Redness and Swelling

REVIEWED BY: SP DATE: 9-19-84
 SU 9-27-84

005233

40705145
RT No. 40703984
40703985

Guinea Pigs - Body Weights (grams)

Test Group: NA Vehicle NA
Positive Control Group NA Vehicle NA
Vehicle Control Group 80% Ethanol / Distilled Water
Naive Control Group NA Vehicle NA

Animal Number EO-							Tech-nician	Date
2030	2027	2051	2052	2053	2135	2140		1984
527	511	549	517	553	477	477	MH	8-14
598	560	581	549	593	477 523	477 483	MP	8-21
608	562	606	549	609	522	496	MP	8/28
668	625	694	612	668	582	550	SP Sm	9-4
680	625	684	607	666	587	567	SP	9-7

Animal Number EO-				Tech-nician	Date
2054	2055	2056			1984
473	520	476	Scale Used: KTRON 1349	MH	8-14
506 483	543 506	523 513	Scale Used: KTRON 15019	MP	8-21
538	574	540	Scale Used: KTRON 15019	MP	8/28
596	631	612	Scale Used: KTRON 5228	SP Sm	9-4
594	648	595	Scale Used: KTRON 5228	SP	9-7
			Scale Used:		

NA - Not Applicable

Reviewed by db Date 9/28/84

① Entry errors MP 8-21-84

005234

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40705145
RT No. 4070398
Room No. 7

Guinea Pigs - Daily Observations

Test Group: NA

Vehicle NA

Positive Control Group NA

Vehicle NA

Vehicle Control Group 80% Ethanol / Distilled Water

Naive Control Group NA

Vehicle NA

Animal Number EO-										Tech- nician	Date
2030	2027	2051	2052	2053	2135	2140	2054	2055	2056		
N	N	N	N	N	N	N	N	N	N	MP	8-14
N	N	N	N	N	N	N	N	N	N	MP	8-15
N	N	N	N	N	N	D	N	N	N	SP	8-16
N	N	N	N	N	N	N	N	N	N	MP	8-17
N	N	N	N	N	N	N	N	N	N	ab	8/18
N	N	N	N	N	N	N	N	N	N	ab	8/19
N	N	N	N	N	N	N	N	N	N	SP	8/20
N	N	N	N	N	N	N	N	N	N	MP	8/21
N	N	N	N	N	N	N	N	N	N	MP	8/22
N	N	N	N	N	N	N	N	N	N	MP	8/23
N	N	N	N	N	N	N	N	N	N	MP	8/24
N	N	N	N	N	N	N	N	N	N	jp	8/25
N	N	N	N	N	N	N	N	N	N	slh	8-26
N	N	N	N	N	N	N	N	N	N	MP	8/27
N	N	N	N	N	N	N	N	N	N	MP	8/28
N	N	N	N	N	N	N	N	N	N	MP	8/29
N	N	N	N	N	N	N	N	N	N	MP	8/30
N	N	N	N	N	N	N	N	N	N	SP	8/31

D = Diarrhea SP 8-16-84

N - No Visible Abnormalities
NA - Not Applicable

005235

40705145
40703984
RT No. 40703984
Root No. 4

Vehicle NA

Vehicle NA

Naive Control Group NA

Vehicle NA

N - No Visible Abnormalities
NA - Not Applicable

005236

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GUINEA PIG MAXIMIZATION TEST

Vehicle Group

40705145

40703984

RT No. 40703985

Test Compound 80% Ethanol/Distilled Water

pH Result NA

Sponsor No. NA

Date Animals Received 7-24-84

Source Hazleton Dutchland

Date of Intradermal Injections 8-14-84

Technician MP mmh

Date of Topical Application 8-21-84

Technician MP

Date of Challenge 9-4-84

Technician deb

Animal No., Sex	24 Hours		48 Hours	
	Right	Left	Right	Left
EQ- 2030	0	0	0	
2027	0	0	0	
2051	0	0	0	
2052	0	0	0	
2053	0	0	0	
2135	0	0	0	
2140	1 slight	0	0	
2054	0	0	0	
2055	0	0	0	
2056	0	0	0	
Technician	SL	SP	SP	
Recorded By	SP	SP	SP	
Date 1984	9/6	9/7	9/7	

Scoring Code: 0 = Normal, No Reaction
1 = Scattered Mild Redness
2 = Moderate and Diffuse Redness
3 = Intense Redness and Swelling

① Entry error MP 8-22-84

② Recording Errors SP 9-7-84

REVIEWED BY:

deb

DATE: 9/28/84

005237

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Dose Range

RT No. 40703984

Test Group: T-3609

Vehicle 80% Ethanol / Distilled
water

Positive Control Group NA

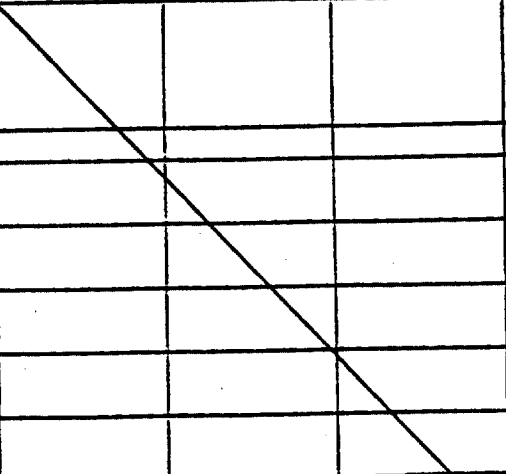
Vehicle NA

Vehicle Control Group NA

Naive Control Group NA

Vehicle NA

[illegible]

Animal Number				Tech- nician	Date
					1984
			Scale Used: KTron 15019	MP SP	8-8-84
			Scale Used:		
			Scale Used:		
			Scale Used:		
			Scale Used:		

NA - Not Applicable

Reviewed by gcu Date 9.19.84

① Recording Error SP 8-8-84

005238

Dose Range

Guinea Pigs - Daily Observations

Vehicle 80% Ethanol / Distilled
water

Vehicle NA

Vehicle NA

N - No Visible Abnormalities

005239

Dose Range
GUINEA PIG MAXIMIZATION TEST

Test Compound T-3609
Date Animals Received 7-10-84
Date of Intradermal Injections NA
Date of Topical Application NA
Date of Challenge 8-8-84

RT No. 40703984
Source Hazleton Dutchland
Technician NA
Technician NA
Technician MP

Animal No., Sex	24 Hours		48 Hours	
	w/v Right	w/v Left	Right	Left
EO- 2008	90% 0	25% 0	0	0
2009	75% 0	50% 0	0	0
2010	25% 0	75% 0	0	0
1992	50% 0	90% 0	0	0
Technician	el	el	slu	slu
Recorded By	lch	lch	slu	slu
Date 1984	8/10	8/10	8-11	8-11

Scoring Code: 0 = Normal, No Reaction
1 = Scattered Mild Redness
2 = Moderate and Diffuse Redness
3 = Intense Redness and Swelling

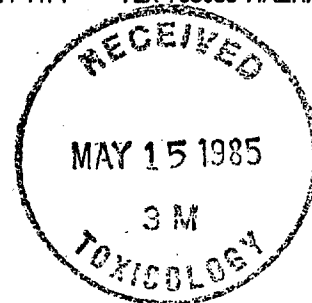
REVIEWED BY: JRW DATE: 9-19-
SG 9-27-84

005240



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Guinea Pig Maximization
Sample: T-3609

Study No. 40703984

Sponsor

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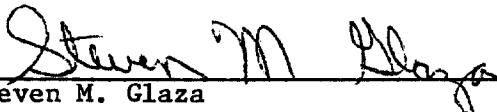
Steven M. Glaza

Amendment No. 1 to the Final Report
Effective May 13, 1985

This amendment modifies the following portion of the report.

Page 6, Conclusion. To correctly identify the degree of skin sensitization, substitute the underlined for "moderate".

Based on the results obtained, the test material, T-3609, is considered to be a mild skin sensitizer in guinea pigs.


Steven M. Glaza

Study Director, Acute Toxicology
Hazleton Laboratories America, Inc.

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Date

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