



HAZLETON

LABORATORIES AMERICA, INC.

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Chemical & BioMedical Sciences Division

AR226-0315

REPORT OF ANALYSIS



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

SAMPLE NUMBER: 4070

DATE ENTERED: 07/

REPORT PRINTED: 10/

SAMPLE: T-3610

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.

005541

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PAGE 2

SAMPLE NUMBER: 40703985

SAMPLE: T-3610

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 430 g and 568 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. 40703984 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 paper 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-3610 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-3610 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

thoroughly stirred during preparation and dos

005542

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PAGE

SAMPLE NUMBER: 40703985

SAMPLE: T-3610

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 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, 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 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 piece of Whatman No. 3 filter paper was saturated with a 90% w/v suspension of T-3610 in 80% ethanol/distilled water, placed over the injection site then covered with an overlapping of Blendederm 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.

C05543

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PAGE

SAMPLE NUMBER: 40703985

SAMPLE: T-3610

GUINEA PIG MAXIMIZATION

(CONTINUED)

Two weeks after topical induction the test and naive control (previous 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

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PAGE

SAMPLE NUMBER: 40703985

SAMPLE: T-3610

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-3610

Dermal Reactions to Challenge Application

Animal Number	24-Hour Score		48-Hour Score	
	Test Group			
EO-2132	0		0	
EO-2134	0		0	
EO-2136	0		0	
EO-2137	0		0	
EO-2138	0		0	
EO-2139	0		0	
EO-2141	0		0	
EO-2142	0		0	
EO-2143	0		1	
EO-2144	1			
Average Score	0.1		0.1	

Naive Control Group			
EO-2150	0		0
EO-2147	0		0
EO-2148	0		0
EO-2145	0		
Average Score	0.0		0.0

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PAGE 6

SAMPLE NUMBER: 40703985

SAMPLE: T-3610

(CONTINUED)

GUINEA PIG MAXIMIZATION

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 vehicle control group animal, EO-2140, which had diarrhea on Day 3.

Normal body weight gains were recorded for all animals during the study with the exception of one vehicle control group animal which exhibited a body weight loss of 3 g during the first week of the study and one test animal which exhibited a body weight loss of 67 g during the second week. During the last three days of the study, nine test, three naive control and five vehicle control animals exhibited body weight losses of 2-28 g.

Skin Reactions to T-3610: A slight dermal reaction was observed in one test group animal at 24 and 48 hours following the challenge application. The dermal reaction in this animal (No. EO-2144) was possibly caused by tape irritation. The other nine test group and all of the naive control animals did not react 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-3610, is not considered to be a skin sensitizer in guinea pigs.

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PAGE 7

SAMPLE NUMBER: 40703985

SAMPLE: T-3610

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.

005547

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RT No. 40703985

Guinea Pigs - Body Weights (grams)

Test Group: T-3610
 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
2132	2134	2136	2137	2138	2139	2141		198
485	324	491	502	430	566	489	MH	8-11
534	597	537	536	524	632	513	MP	8-2
534	617	555	578	457	658	550	MP	8-2
600	689	602	634	520	695	589	SP	9/4
591	676	585	610	507	693	581	SP	9/

Animal Number EO-			Tech-nician	Date
2142	2143	2144		19
449	517	503	Scale Used: KTRON 1348	MH 8
491	517	566	Scale Used: KTron 15019	MP 8-
508	553	580	Scale Used: KTRON 15019	MP 8-
573	605	671	Scale Used: KTron 5228	SP 9
574	596	667	Scale Used: KTron 5228	SP 9
			Scale Used:	

NA - Not Applicable

Reviewed by JSW Date 9-19-84

005548

RT No. 40703985
Room No. 4

Guinea Pigs - Daily Observations

Test Group: T-3610
Positive Control Group NA
Vehicle Control Group NA
Naive Control Group NA

Vehicle 80% Ethanol/Distilled water

Vehicle NA

Vehicle NA

Animal Number <u>EO-</u>										Tech- nician	Date 1984
2132	2134	2136	2137	2138	2139	2141	2142	2143	2144		
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	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	deb	8-18
N	N	N	N	N	N	N	N	N	N	ckb	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	jp	8-25
N	N	N	N	N	N	N	N	N	N	Sh	8-26
N	N	N	N	N	N	N	N	N	N	SP	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

005549

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RT No. 40703985
Room No. 4

Guinea Pigs - Daily Observations

Test Group: T-3610
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 1984
2132	2134	2136	2137	2138	2139	2141	2142	2143	2144		
N	N	N	N	N	N	N	N	N	N	dch	9-1
N	N	N	N	N	N	N	N	N	N	slh	9-2
N	N	N	N	N	N	N	N	N	N	slh	9-3
N	N	N	N	N	N	N	N	N	N	SP	9-4
N	N	N	N	N	N	N	N	N	N	SP	9-5
N	N	N	N	N	N	N	N	N	N	SG	9-6
N	N	N	N	N	N	N	N	N	N	SP	9-7

N - No Visible Abnormalities

005550

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GUINEA PIG MAXIMIZATION TEST

Test Compound T-3610
 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

Test Group
 RT No. 40703985
 Source Harleton Dutchland
 Technician MP
 Technician MP
 Technician SP

Animal No., Sex ♂	24 Hours		48 Hours	
	Right	Left	Right	Left
EO- 2132	0		0	
2134	0		0	
2136	0		0	
2137	0		0	
2138	0		0	
2139	0		0	
2141	0		0	
2142	0		0	
2143	0		0	
2144	1 *		1 *	
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

* POSSIBLY
 TAPE IRRITATION

REVIEWED BY: SG DAT

SG 9-6-84
 SP 9-6-84

005551

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RT No. 40703985

Guinea Pigs - Body Weights (grams)

Test Group: NA
Positive Control Group NA
Vehicle Control Group NA
Naive Control Group T-3610

Vehicle NA

Vehicle NA

Vehicle 80% Ethanol / Distilled water

Animal Number EO-				Tech-nician	Date
2150	2147	2148	2145		1984
① 489 489	568	508	460	MH	8-14
555	640	593	529	MP	8-2
608	692	630	621	MP	8-2
664	742	680	693	SP	9/
636	739	685	676	SP	9/

Animal Number EO-				Tech-nician	Date
				MH	8-
				MP	8
				MP	8
				SP	9
				SP	9

Scale Used: KTRON 1348

Scale Used: KTRON 15019

Scale Used: KTRON 15019

Scale Used: KTRON 5228

Scale Used: KTRON 5228

Scale Used:

Reviewed by gaw

Date 9-19-84

NA - Not Applicable

① Recording Error 9/24/84 MH

005552

RT No. 40703985
Room No. 4

Test Group: _____ NA

Positive Control Group _____ NA

Vehicle Control Group _____ NA

Naive Control Group _____ T-3610

Vehicle NA

Vehicle 80% Ethanol / Distilled
Water

N - No Visible Abnormalities
NA - Not Applicable

005553

RT No. 407039
Room No. 4

Test Group: NA
Positive Control Group NA
Vehicle Control Group NA
Naive Control Group T-3610

Vehicle NA
Vehicle NA

Vehicle 80% Ethanol/Dist
water

[illegible]

N - No Visible Abnormalities
NA - Not Applicable

005554

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GUINEA PIG MAXIMIZATION TEST

Test Compound T-3610
Date Animals Received 7-24-84
Date of Intradermal Injections NA
Date of Topical Application NA
Date of Challenge 9-4-84

RT No. 40703985
Source Hazleton Dutckland
Technician NA
Technician NA
Technician SP

Animal No., Sex	24 Hours		48 Hours	
	Right	Left	Right	Left
E0- 2150	0		0	
2147	0		0	
2148	0		0	
2145	0		0	
Technician	SG		SP	
Recorded By	SP		SP	
Date	9/6		9/7	
1984				

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

REVIEWED BY: SGW DATE: SG

005555

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40705145
RT No. 40703984
40703985

Guinea Pigs - Body Weights (grams)

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

Vehicle NA
Vehicle NA
Vehicle NA

Animal Number EO-							Tech- nician	Da 198
2030	2027	2051	2052	2053	2135	2140		
527	511	549	517	553	477	477	MH	8-1
598	560	581	549	593	477 ⁵²² ①	474 ⁴⁸³ ①	MP	8-2
608	562	606	549	609	522	496	MP	8/2
668	625	694	612	668	582	550	SP Sam	9-4
680	625	684	607	666	587	567	SP	9-

Animal Number EO-			Tech- nician	198
2054	2055	2056		
473	520	476	MH	8
506 ⁴⁸³ ①	543 ⁵⁰⁶ ①	523 ⁵¹³ ①	MP	8
538	574	540	MP	8
596	631	612	SP Sam	9
594	648	595	SP	9
Scale Used: KTRON 1349				
Scale Used: KTRON 15019				
Scale Used: KTRON 15019				
Scale Used: KTRON 5223				
Scale Used: KTRON 5228				
Scale Used:				

NA - Not Applicable

Reviewed by dlb Date 9/28/84

① Entry errors MP 8-21-84

005556

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Test Group:

NA

Vehicle

NA

Positive Control Group

NA

Vehicle

NA

Vehicle Control Group

80% Ethanol / Distilled Water

Vehicle

NA

Naive Control Group

NA

D = Diarrhea SP 8-16-84

N - No Visible Abnormalities
NA - Not Applicable

005557

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GUINEA PIG MAXIMIZATION TEST

Test Compound 80% Ethanol/Distilled Water
pH Result NA
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

Vehicle Group

40705145
40703984
RT No. 40703985
Sponsor No. NA
Source Hazleton Dutchland
Technician MP mmh
Technician MP
Technician deb

Animal No., Sex ♂	24 Hours		48 Hours	
	Right	Left	Right	Left
EO- 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

005559

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Dose Range
Guinea Pigs - Body Weights (grams)

RT No. 4070398

Test Group: T-3610
Positive Control Group NA
Vehicle Control Group NA
Naive Control Group NA

Vehicle 80% Ethanol/Distilled water

Vehicle NA

Vehicle NA

Animal Number				Tech- nician	Da te
2022	1932	2015	2016		
509	550	539	560	SP	8-8

Animal Number				Tech- nician	
			Scale Used: KTron 15019	SP	8-
			Scale Used:		
			Scale Used:		
			Scale Used:		
			Scale Used:		
			Scale Used:		

NA - Not Applicable

Reviewed by gbl Date 9-19-84

005560

Dose Range

Guinea Pigs - Daily Observations

Vehicle 80% Ethanol/Distilled
water

Vehicle NA

Vehicle NA

[illegible]

005561

GUINEA PIG MAXIMIZATION TEST

RT No. 40703985
Source Hazleton Dutchland
Technician NA
Technician NA
Technician MP

Technician

Recorded By

Date _____

Scoring Code:

REVIEWED BY:

DATE: 9-19-81

5-

927-8

005562