REPORT

TEST FACILITY

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CONFIDENTIAL

STUDY TITLE

ISO Closed Patch Sensitization Study in Guinea Pigs

TEST ARTICLE NAME

M110 Pressure Sensitive Adhesive

TEST ARTICLE IDENTIFICATION

M110 Pressure Sensitive Adhesive

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Summary

The test article, M110 Pressure Sensitive Adhesive, was evaluated for the potential to elicit delayed dermal contact sensitization in the guinea pig. This study was conducted based on the requirements of ISO 10993-10, Biological evaluation of medical devices, Part 10: Tests for irritation and skin sensitization.

The test article was occlusively patched to the intact skin of ten animals for 6 hours (±30 minutes), three times a week, over a 3 week period. The control article was similarly patched to five animals. Following a 2-week recovery period, the ten test and five control animals were occlusively patched with the test article and the control article. All sites were observed for evidence of dermal reactions at 24 and 48 hours after patch removal.

The test article showed no evidence of causing delayed dermal contact sensitization in the guinea pig.

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Approved by: Arizona E. Carter, BS, ALAT
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08-24-15
Date

1. Introduction

1.1 Purpose

The purpose of this study was to evaluate the potential of the test article to cause delayed dermal contact sensitization following repeated occlusive patching in the guinea pig.

1.2 Testing Guidelines

This study was conducted based on the International Organization for Standardization 10993-10, Biological evaluation of medical devices, Part 10: Tests for irritation and skin sensitization.

1.3 Dates

Test Article Received:	March 25, 2015 and July 10, 2015
Treatment Started:	July 15, 2015
Observations Concluded:	August 19, 2015

2. Identification of Test and Control Articles

The test article provided by the sponsor was identified and handled as described below:

Table 1: Test Article

Name:	M110 Pressure Sensitive Adhesive
Identification:	M110 Pressure Sensitive Adhesive
Physical Description of the Test Article:	M110 adhesive on supporting substrate
Storage Conditions:	Room Temperature

Table 2: Control Article

Name:	Approximate 25 mm x 25 mm sections of 4-ply gauze
Strength, Purity, Composition or Other Characteristics:	Gauze: Purity: FDA Quality System Requirements (QSR) as stipulated in 21 CFR Part 820. Composition: 20% rayon, 80% polyester blend

3. Test System

3.1 Test System

Species:	Guinea pig (*Cavia porcellus*)
Strain:	Hartley
Source:	Elm Hill Labs
Sex:	Female; nulliparous and nonpregnant
Body Weight Range:	330 grams to 419 grams at study initiation
Age:	Young adult
Acclimation Period:	Minimum 5 days
Number of Animals:	Fifteen
Identification Method:	Ear tag

3.2 Justification of Test System

The Hartley albino guinea pig (animal) has been used historically for sensitization studies. Repeated patching of the test article to fur-clipped intact skin was employed. Topical applications are related to the human exposure route and permit the evaluation of dermal contact and/or absorption of potential sensitizers during induction and challenge phases. Reactions directly under the topical application site can be observed. The susceptibility of the Hartley guinea pig strain to a known sensitizing agent, 1-chloro-2,4-dinitrobenzene (DNCB), has been substantiated at NAMSA with this method under lab number 15T_40358_01 completed on July 23, 2015.

4. Animal Management

4.1 Husbandry, Housing and Environment

Conditions conformed to NAMSA Standard Operating Procedures that are based on the "*Guide for the Care and Use of Laboratory Animals*." Animals were housed in groups in stainless steel or plastic suspended cages identified by a card indicating the lab number, animal numbers, test code, sex, and first treatment date.

The animal housing room temperature and relative humidity were monitored daily. The temperature for the room was set to 68-79°F and the relative humidity was set to 30-70%. There were no significant temperature or relative humidity excursions that adversely affected the health of the animals.

The light cycle was controlled using an automatic timer (12 hours light, 12 hours dark).

4.2 Food, Water and Contaminants

A commercially available guinea pig feed, PROLAB Guinea Pig - 5P18, was provided daily. Potable water was provided *ad libitum* through species appropriate water containers or delivered through an automatic watering system.

No contaminants present in the feed and water impacted the results of this study.

4.3 Accreditation

NAMSA is an AAALAC International accredited facility and is registered with the United States Department of Agriculture. Additionally, NAMSA maintains an approved Animal Welfare Assurance on file with the National Institutes of Health, Office for Laboratory Animal Welfare.

4.4 Personnel

Associates involved in this study were appropriately qualified and trained.

4.5 Veterinary Care

Standard veterinary medical care was provided in this study.

4.6 IACUC

This procedure has been approved by the NAMSA Institutional Animal Care and Use Committee (IACUC), and is reviewed at least annually by the same committee.

4.7 Selection

Only healthy, previously unused animals were selected.

5. Method

5.1 Test and Control Article Preparation

The test article was a white adhesive. The white release liner was removed and excluded from the preparation. One 25 mm x 25 mm section of the test article was prepared per animal.

5.2 Test Procedure

On the day of first induction treatment, each animal was weighed. The hair was removed with an electric clipper from the left flank of ten guinea pigs designated as test animals and five guinea pigs designated as control animals.

5.2.1 Induction

An approximate 25 mm x 25 mm section of the test or control article was applied to the appropriate animals. The patch was then secured with hypoallergenic tape to the intact skin. The trunk of each animal was wrapped with an elastic band to maintain the occluded patch in position.

After 6 hours (±30 minutes), the wraps and patches were removed. The sites were wiped with dry gauze to remove any residue.

The application procedure was repeated three times each week (e.g., Monday-Wednesday-Friday) for 3 consecutive weeks until nine applications were made to the left flank of the animals. The hair was removed with an electric clipper as necessary to provide a clear site.

5.2.2 Challenge

At 14 days (±1 day) after the final induction patch, the hair of each animal was removed with an electric clipper from the right flank area. An approximate 25 mm x 25 mm section of both the control and test article was applied to the intact skin on the dorsal and ventral regions of the right flank of each test and control animal. The trunk of each animal was wrapped with an elastic band to hold the occluded patch in place.

After 6 hours (±30 minutes), the wraps and patches were removed. The sites were wiped with dry gauze to remove any residue. At 24 hours after patch removal, the challenged sites and surrounding area were shaved.

5.2.3 Laboratory Observations

1. Animals were observed daily for general health.
2. Body weights were recorded for each animal at pretreatment.
3. Dermal observations for erythema were recorded at 2-4 hours following shaving of the animals. Scoring was also conducted at 48 hours after challenge patch removal. Sites were wiped with 35% IPA saturated gauze before scoring at each interval. Evaluations for the challenge phase were based on dermal reactions which were scored as outlined below:

Table 3: Test Scoring

Patch Test Reaction	Grading Scale
No visible change	0
Discrete or patchy erythema	1
Moderate and confluent erythema	2
Intense erythema and swelling	3

All times and temperatures reported herein are approximate and are within ranges established by the external standards described in the References section of this report and/or NAMSA standard operating procedures.

6. Evaluation

Grades greater than or equal to 1 in the test group generally indicate sensitization, provided grades of less than 1 are seen in the control animals. If grades greater than or equal to 1 are noted in the control animals, then the reactions of the test animals which exceed the most severe reaction in the control animals are presumed to be due to sensitization.

Occasionally, the test group will have a greater number of animals showing a response than the control group, although the intensity of the reaction is not greater than that exhibited by the control group. In these instances, a rechallenge may be necessary to define the response clearly.

A true sensitization reaction can be confirmed by rechallenge. Absence of dermal responses at rechallenge may nullify earlier findings. Recurring observations in at least one of the same animals verify findings of the primary challenge.

7. Results

7.1 Body Weights and Clinical Observations

Individual body weights and clinical observations are presented in Appendix 1. All animals were clinically normal throughout the study.

7.2 Dermal Observations

Individual results of dermal scoring for the challenge phase are presented in Appendix 2. No evidence of sensitization was observed.

8. Conclusion

The test article showed no evidence of causing delayed dermal contact sensitization in the guinea pig.

Results and conclusions apply only to the test article tested. Any extrapolation of these data to other articles is the sponsor's responsibility.

9. Records

All raw data pertaining to this study and a copy of the final report are retained in designated NAMSA archive files in accordance with NAMSA SOPs.

10. ISO Compliance

All procedures were certified to ISO 13485 and accredited to ISO 17025.

11. References

Code of Federal Regulations (CFR), Title 9, Parts 1-4, Animal Welfare Act.

International Organization for Standardization (ISO) 10993-1, Biological evaluation of medical devices - Part 1: Evaluation and testing within a risk management process (2009/Technical Corrigendum 1 2010).

International Organization for Standardization (ISO) 10993-2, Biological evaluation of medical devices - Part 2: Animal welfare requirements (2006).

International Organization for Standardization (ISO) 10993-10, Biological evaluation of medical devices - Part 10: Tests for irritation and skin sensitization (2010).

International Organization for Standardization (ISO) 10993-12, Biological evaluation of medical devices - Part 12: Sample preparation and reference materials (2012).

International Organization for Standardization (ISO) 13485, Medical devices - Quality management systems - Requirements for regulatory purposes (2003/Technical Corrigendum 1 2009).

International Organization for Standardization/International Electrotechnical Commission (ISO/IEC) 17025, General requirements for the competence of testing and calibration laboratories (2005/Technical Corrigendum 1 2006).

National Research Council, *Guide for the Care and Use of Laboratory Animals*, Washington, DC: National Academy Press, 2011.

Office of Laboratory Animal Welfare (OLAW), Public Health Service Policy on Humane Care and Use of Laboratory Animals.

Appendix 1 - Individual Body Weights and Clinical Observations

Group	Animal Number	Individual Observation	
		Pretreatment Body Weight (g)	Clinical Observations
Test	4293	389	No findings
	4294	330	No findings
	4295	377	No findings
	4296	398	No findings
	4297	419	No findings
	4298	368	No findings
	4299	355	No findings
	4300	334	No findings
	4301	338	No findings
	4302	374	No findings
Control	4303	367	No findings
	4304	363	No findings
	4305	349	No findings
	4306	394	No findings
	4307	390	No findings

Appendix 2 - Dermal Reactions - Challenge

Group	Animal Number	Hours Following Patch Removal			
		24 Hour Score		48 Hour Score	
		Control	Test Article	Control	Test Article
Test	4293	0	0	0	0
	4294	0	0	0	0
	4295	0	0	0	0
	4296	0	0	0	0
	4297	0	0	0	0
	4298	0	0	0	0
	4299	0	0	0	0
	4300	0	0	0	0
	4301	0	0	0	0
	4302	0	0	0	0
Control	4303	0	0	0	0
	4304	0	0	0	0
	4305	0	0	0	0
	4306	0	0	0	0
	4307	0	0	0	0

Appendix 3 - Periodic Positive Control Study for the Closed Patch Sensitization Test

What was tested

1-chloro-2,4-dinitrobenzene (DNCB)

Dates

Treatment Started: June 15, 2015 under NAMSA Lab Number: 15T_40358_01
Observations Concluded: July 15, 2015

Purpose

A periodic positive control study was conducted for the ISO Closed Patch Sensitization Study in Guinea Pigs to meet the following objectives: 1) the methodology in International Organization for Standardization 10993-10, Biological Evaluation of Medical Devices - Part 10: Tests for Irritation and Skin Sensitization was confirmed, 2) the potential of DNCB to cause delayed dermal contact sensitization following repeated occlusive application was substantiated, 3) proper training of the technologists/technicians performing these studies was verified, and 4) the susceptibility of the Hartley guinea pig strain to sensitization was substantiated.

Methods

The test utilized young adult, nulliparous and not pregnant, female Hartley albino guinea pigs supplied by Elm Hill Labs. The weight at study initiation ranged from 332 grams to 500 grams. A 0.1% (w/v) concentration of DNCB in 70% isopropyl alcohol (IPA) was occlusively patched for 6 hours (±30 minutes) to the intact skin of ten guinea pigs, once a week, for a total of three induction treatments over a 3 week period. The 70% isopropyl alcohol was similarly patched to five control guinea pigs. Following a recovery period, the ten test and five control animals received a challenge patch of 0.1% (w/v) concentration of DNCB in acetone and the acetone control article. All sites were scored at 24 and 48 hours after patch removal. The patch sites were graded using the scale:

Patch Test Reaction	Grading Scale
No visible change	0
Discrete or patchy erythema	1
Moderate and confluent erythema	2
Intense erythema and swelling	3

Results

All of the test animals demonstrated a positive sensitization response to the known sensitizer, DNCB. None of the control animals demonstrated a sensitization response. The results are shown below:

Treatment Group	Animal Number	Dermal Reactions				Results (+) or (-)
		24 Hour Score		48 Hour Score		
		Control Site	Test Article Site	Control Site	Test Article Site	
Test	1713	0	3*	0	3*	+
	1714	0	3	0	2	+
	1715	0	2	0	2	+
	1716	0	3	0	3	+
	1717	0	3	0	2	+
	1718	0	2	0	2	+
	1719	0	1	0	1	+
	1720	0	2	0	2	+
	1721	0	2	0	2	+
	1722	0	1	0	2	+
Control	1723	0	0	0	0	-
	1724	0	0	0	0	-
	1725	0	0	0	0	-
	1726	0	0	0	0	-
	1727	0	0	0	0	-

*Two areas of eschar on test site.

Conclusion

The known sensitizer DNCB did produce evidence of causing delayed dermal contact sensitization in the Hartley strain of guinea pig. Therefore, the following objectives were met: 1) the methodology in International Organization for Standardization 10993-10, Biological Evaluation of Medical Devices, Part 10: Tests for Irritation and Skin Sensitization was confirmed, 2) the potential for DNCB to cause delayed contact sensitization was substantiated, 3) proper training of the technologists/technicians performing these studies was verified and 4) the susceptibility of the Hartley guinea pig strain to sensitization was substantiated.