REPORT

TEST FACILITY

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CONFIDENTIAL

STUDY TITLE

ISO Closed Patch Sensitization Study in Guinea Pigs

TEST ARTICLE NAME

M102 Pressure Sensitive Adhesive

TEST ARTICLE IDENTIFICATION

M102 Batch 0001884115 11-16-15

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Summary

The test article, M102 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 Eugene Carter 02-02-16
Arizona E. Carter, BS, ALAT — Date
Technical Reviewer

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:	November 17, 2015
Treatment Started:	December 18, 2015
Observations Concluded:	January 22, 2016

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:	M102 Pressure Sensitive Adhesive
Identification:	M102 Batch 0001884115 11-16-15
Physical Description of the Test Article:	M102 unsupported adhesive between two white paper release liners. Test adhesive only. Remove both white paper release liners before the test.
Storage Conditions:	Ambient 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:	303 grams to 464 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_54251_01 completed on October 27, 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 pressure sensitive adhesive. One paper release liner was removed during preparation. The other white release liner was removed prior to patching. Two approximate 25 mm x 25 mm sections were prepared per animal. Either adhesive side of the test article was applied to the skin of the guinea pig.

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

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.

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	6616	303	No findings
	5184	373	No findings
	5735	405	No findings
	5736	345	No findings
	5737	431	No findings
	5738	424	No findings
	5739	384	No findings
	5740	453	No findings
	5741	464	No findings
	5742	411	No findings
Control	5743	399	No findings
	5744	395	No findings
	5745	389	No findings
	5746	430	No findings
	5747	431	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	6616	0	0	0	0
	5184	0	0	0	0
	5735	0	0	0	0
	5736	0	0	0	0
	5737	0	0	0	0
	5738	0	0	0	0
	5739	0	0	0	0
	5740	0	0	0	0
	5741	0	0	0	0
	5742	0	0	0	0
Control	5743	0	0	0	0
	5744	0	0	0	0
	5745	0	0	0	0
	5746	0	0	0	0
	5747	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: September 16, 2015 under NAMSA Lab Number: 15T_54251_01
Observations Concluded: October 16, 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 the 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 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, female, nulliparous and not pregnant, Hartley albino guinea pigs supplied by Elm Hill Labs. The weight at study initiation ranged from 326 grams to 393 grams. A 0.1% (w/v) concentration of DNCB in 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% IPA was similarly patched to five control guinea pigs. Following a recovery period, the ten test and five control animals received a challenge patch of a 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 ten 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				Sensitization Assessment
		24 Hour Score		48 Hour Score		
		Control	Test Article	Control	Test Article	
Test	9126	0	2*	0	1*	+
	9127	0	2*	0	1*	+
	9128	0	2*	0	1*	+
	9129	0	2*	0	2*	+
	9130	0	2*	0	2*	+
	9131	0	2*	0	2*	+
	9132	0	1*	0	1*	+
	9133	0	2*	0	1*	+
	9134	0	3*	0	2*	+
	9135	0	2*	0	2*	+
Control	9136	0	0*	0	0*	-
	9137	0	0*	0	0*	-
	9138	0	0*	0	0*	-
	9139	0	0*	0	0*	-
	9140	0	0*	0	0*	-

*Yellow residue at test site; did not interfere with scoring.

Conclusion

The known sensitizer DNCB did produce significant evidence of causing delayed dermal contact sensitization in the Hartley strain of guinea pig. Therefore, the following objectives were met: 1) the methodology in the 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 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.