

GLP REPORT

TEST FACILITY

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CONFIDENTIAL

STUDY TITLE

ISO Closed Patch Sensitization Study in Guinea Pigs

TEST ARTICLE NAME

Viberect Pad Overmold

TEST ARTICLE IDENTIFICATION

Pad Overmold

NAMSA

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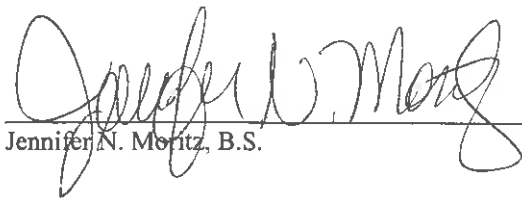
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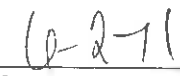
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Statement of GLP Compliance

This study was conducted in accordance with the provisions of the FDA Good Laboratory Practice (GLP) Regulations (21 CFR, Part 58).

Study Director:


Jennifer N. Moritz, B.S.


Date

1. Introduction

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.

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.

Dates

Test Article Received: March 23, 2011
Treatment Started: April 27, 2011
Observations Concluded: June 1, 2011

GLP Compliance

The study initiated by protocol signature on April 13, 2011 was conducted in accordance with the provisions of the FDA Good Laboratory Practice (GLP) Regulations, 21 CFR 58. A Statement of Quality Assurance Activities was issued with this report.

Duplication of Experimental Work

By signature on the protocol, the sponsor confirmed that the conduct of this study did not unnecessarily duplicate previous experiments.

2. Materials

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

Test Article Name:	Vibrect Pad Overmold
Identification:	Pad Overmold
Stability Testing:	In progress (per sponsor)
Expiration Date:	Stable for duration of intended testing (per sponsor)
Strength, Purity and Composition:	Strength: not applicable because no active ingredients are used to formulate a concentration Purity: not applicable because the test article is a multi-component device Composition: Irogran A85P4394 thermoplastic polyurethane elastomer
Physical Description of the Test Article:	Pad overmold (blue) thermoplastic polyurethane for skin contact (penis) for vibratory nerve stimulation. Black is to be removed and discarded.
Storage Conditions:	Room Temperature

Pre-Preparation



Post-Preparation (representative image)



Control Article: Approximate 25 mm x 25 mm sections of 4-ply gauze

Stability Testing: Gauze: Marketed product; stability characterized by labeling

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

Preparation: The test article was cut into approximate 25 mm x 25 mm sections. The black plastic component was removed and the smooth side of the blue pad overmold was applied to the skin of the guinea pig.

3. Test System

Test System

Species: Guinea pig (*Cavia porcellus*)

Strain: Hartley

Source: Elm Hill Labs

Sex: Female (nulliparous and not pregnant)

Body Weight Range: 373 grams to 430 grams at study initiation

Age: Young adult

Acclimation Period: Minimum 5 days

Number of Animals: Fifteen

Identification Method: Ear tag

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 11T_25786_01 completed on May 5, 2011.

4. Animal Management

Husbandry: Conditions conformed to NAMSA Standard Operating Procedures that are based on the "*Guide for the Care and Use of Laboratory Animals*."

Food: A commercially available guinea pig feed, PROLAB Guinea Pig - 5P18, was provided daily.

Water: Potable water was provided *ad libitum* through species appropriate water containers or delivered through an automatic watering system.

Contaminants: Contaminants reasonably expected in feed or water supplies were not believed to have influenced the outcome of this test.

Housing: 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.

Environment: The animal housing room temperature and relative humidity were monitored daily. The temperature for the room was 64-79°F and the recommended relative humidity was 30-70%. There were no significant 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).

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.

Personnel: Associates involved in this study were appropriately qualified and trained.

Selection: Only healthy, previously unused animals were selected.

Veterinary Care: Standard veterinary medical care was provided in this study.

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

5. Method

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.

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.

At 6 to 8 hours, 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.

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.

At 6 to 8 hours, 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.

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% isopropyl alcohol saturated gauze before scoring at each interval. Evaluations for the challenge phase were based on dermal reactions which were scored as outlined below:

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

Body Weights and Clinical Observations

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

Dermal Observations

Individual results of dermal scoring for the challenge phase appear 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. Quality Assurance

Inspections were conducted at intervals adequate to assure the integrity of the study in conformance with 21 CFR 58.35(b)(3). The final report was reviewed for conformance to Section 58.185, Subpart J, of the GLP Regulations. A Statement of Quality Assurance Activities was issued with the report.

10. Records

All raw data, test and control articles pertaining to this study, and a copy of the final report are retained in designated NAMSA archive files.

11. ISO Compliance

All procedures were certified to ISO 13485:2003 and accredited to ISO 17025:2005.

12. References

Code of Federal Regulations (CFR), Title 21, Part 58, Good Laboratory Practice for Nonclinical Laboratory Studies.

Code of Federal Regulations (CFR), Title 9, Parts 1-199, Animal Welfare Act.

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.

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) 13485, Medical Devices - Quality Management Systems - Requirements for Regulatory Purposes (2003).

International Organization for Standardization (ISO) 17025, General Requirements for the Competence of Testing and Calibration Laboratories (2005).

Appendix 1 - Individual Body Weights and Clinical Observations

Group	Animal Number	Individual Observation	
		Pretreatment Body Weight (g)	Clinical Observations
Test	9692	389	Animal was clinically normal throughout the study.
	9693	413	Animal was clinically normal throughout the study.
	9694	414	Animal was clinically normal throughout the study.
	9695	422	Animal was clinically normal throughout the study.
	9696	380	Animal was clinically normal throughout the study.
	9771	420	Animal was clinically normal throughout the study.
	9772	374	Animal was clinically normal throughout the study.
	9773	390	Animal was clinically normal throughout the study.
	9774	394	Animal was clinically normal throughout the study.
	9775	384	Animal was clinically normal throughout the study.
Control	9821	395	Animal was clinically normal throughout the study.
	9822	414	Animal was clinically normal throughout the study.
	9823	425	Animal was clinically normal throughout the study.
	9824	373	Animal was clinically normal throughout the study.
	9825	430	Animal was clinically normal throughout the study.

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	9692	0	0	0	0
	9693	0	0	0	0
	9694	0	0	0	0
	9695	0	0	0	0
	9696	0	0	0	0
	9771	0	0	0	0
	9772	0	0	0	0
	9773	0	0	0	0
	9774	0	0	0	0
	9775	0	0	0	0
Control	9821	0	0	0	0
	9822	0	0	0	0
	9823	0	0	0	0
	9824	0	0	0	0
	9825	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: March 16, 2011 under NAMSA Lab Number: 11T_25786_01

Observations Concluded: April 15, 2011

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, nulliparous and not pregnant, female Hartley albino guinea pigs supplied by Elm Hill Labs. The weight at study initiation ranged from 371 grams to 490 grams. A 0.1% (w/v) concentration of DNCB in isopropyl alcohol was occlusively patched for 6 to 8 hours 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 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: 0 = no visible change, 1 = discrete or patchy erythema, 2 = moderate and confluent erythema, and 3 = intense erythema and swelling.

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				Results (+) or (-)
		24 Hour Score		48 Hour Score		
		Control	Test Article	Control	Test Article	
Test	6414	0	2	0	1	+
	6415	0	1	0	1	+
	6416	0	3	0	2	+
	6417	0	2	0	1	+
	6418	0	2	0	2	+
	6399	0	2	0	2	+
	6400	0	1	0	1	+
	6401	0	2	0	2	+
	6402	0	2	0	2	+
	6403	0	2	0	2	+
Control	6394	0	0	0	0	-
	6395	0	0	0	0	-
	6396	0	0	0	0	-
	6397	0	0	0	0	-
	6398	0	0	0	0	-

(+) = Positive sensitization response

(-) = Negative sensitization response

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.

Statement of Quality Assurance Activities

Phase Inspected	Date Inspected	Date Reported to Study Director	Date Reported to Management
Induction Application	May 6, 2011	May 6, 2011	May 6, 2011
Score	May 31, 2011	May 31, 2011	May 31, 2011
Study Data Review	June 1, 2011	June 1, 2011	June 1, 2011
Final Report Review	June 2, 2011	June 2, 2011	June 2, 2011

Based on a review of this study, it has been concluded that this report accurately describes the methods and standard operating procedures, and that the reported results accurately reflect the raw data of the study. This study has been reviewed in accordance with the provisions of the FDA Good Laboratory Practice Regulations (21 CFR, Part 58).

QA Representative: *Susan Pellitieri* 6-2-11
Susan Pellitieri, B.A. Date
Reviewer II, Quality Assurance