

REPORT

TEST FACILITY

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CONFIDENTIAL

STUDY TITLE

ISO Skin Irritation Study in Rabbits

TEST ARTICLE NAME

100% COTTON WITH SB SERIES PAD PRINTING
INK

NAMSA

TABLE OF CONTENTS

Page

Summary 3

1. Introduction..... 4

2. Materials..... 4

3. Test System 4

4. Animal Management 5

5. Method 5

6. Evaluation and Statistical Analysis..... 6

7. Results 6

8. Conclusion..... 6

9. Records 6

10. References 6

Appendix 1 - Classification System For Skin Reaction 7

Appendix 2 - Dermal Observations..... 8

Summary

The test article, 100% COTTON WITH SB SERIES PAD PRINTING INK, was evaluated for primary skin irritation in accordance with the guidelines of ISO 10993, Biological Evaluation of Medical Devices - Part 10: Tests for Irritation and Delayed-Type Hypersensitivity. Two approximate 25 mm x 25 mm sections of the test article and control article were topically applied to the skin of each of three rabbits and left in place for 24 hours. The sites were graded for erythema and edema at 1, 24, 48 and 72 hours after removal of the single sample application.

There was no erythema and no edema observed on the skin of the animals. The Primary Irritation Index for the test article was calculated to be 0.0. The response of the test article was categorized as negligible.

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1. Introduction

Purpose

The test article identified below was evaluated the potential of a single topical application to irritate skin of the rabbit.

Testing Guidelines

This study was conducted based on the International Organization for Standardization 10993, Biological Evaluation of Medical Devices, Part 10: Tests for Irritation and .Delayed-Type Hypersensitivity.

Dates

Test Article Receipt: March 5, 2010

Treatment Started: March 12, 2010 and March 16, 2010

Observations Concluded: March 16, 2010 and March 20, 2010

2. Materials

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

Test Article Name:	100% COTTON WITH SB SERIES PAD PRINTING INK
Test Article Identification:	Not Supplied
Physical Description of the Test Article:	Fun Pal Brief
Storage Conditions:	Room Temperature
Control Article:	Four-ply gauze supplied by the test facility, was cut into 25 mm x 25 mm sections, moistened with 0.5 mL of saline per section and backed with polyethylene plastic.
Control Article Stability Testing:	Marketed product, stability characterized by its labeling.
Control Article Strength, Purity and Composition:	Gauze: Strength: Not applicable, no active components in the formulation; 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 ink side of each section of the test article was moistened with 0.5 mL of saline and applied to the skin of the rabbit.
Sample Disposition:	Any remaining used and unused sample will be returned to the sponsor.

3. Test System

Test System

Species:	Rabbit (<i>Oryctolagus cuniculus</i>)
Breed:	New Zealand White
Source:	Myrtle's Rabbitry, Inc.
Sex:	One male, three female
Body Weight Range:	2.3 kg to 2.6 kg at selection
Age:	Young adult
Acclimation Period:	Minimum 5 days
Number of Animals:	Three
Identification Method:	Ear tag

Justification of Test System

The rabbit (animal) is specified as an appropriate animal model for evaluating potential skin irritants by the current ANSI/AAMI/ISO testing standards. The rabbit is widely used for this purpose and relative ranking of irritant scores can be determined.

4. Animal Management

Husbandry:	Conditions conformed to NAMSA Standard Operating Procedures that are based on the " <i>Guide for the Care and Use of Laboratory Animals</i> ."
Food:	A commercially available rabbit feed, PROLAB Hi-Fiber Rabbit - 5P25, was provided daily.
Water:	Potable water was provided <i>ad libitum</i> 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 individually housed in stainless steel suspended cages identified by a card indicating the lab number, animal number, test code, sex, and date dosed.
Environment:	The animal housing room temperature and relative humidity were monitored daily. The temperature for the room was 61-72°F and the relative humidity was 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).
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 were appropriately qualified and trained.
Selection:	Only healthy, previously unused, animals free from irritation or other dermatological lesions that could interfere with the test 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 Committees (IACUC), and is reviewed at least annually by the same committees.

5. Method

The animals were weighed and the fur on the back of each animal was clipped with an electric clipper 4 to 24 hours prior to treatment. On the day of treatment, four sites, two on each side of the back and positioned cranially and caudally, were designated on each animal. The sites were free of blemishes that could interfere with the interpretation of results.

An approximate 25 mm x 25 mm section of the test article was moistened with 0.5 mL of saline, and applied to one cranial site and one caudal site (two sites per animal) by introduction under a 4 ply gauze layer to an area of skin approximately 25 mm x 25 mm square. The patches were backed with plastic and covered with a nonreactive tape. The control was similarly applied to the opposite cranial and caudal sites. The trunk of each animal was wrapped with an elastic binder to maintain the test patches in . Animals were returned to their cages after treatment.

After the 24 hour exposure, the binders, tape, and patches were removed. The sites were gently wiped with a gauze sponge dampened with deionized water in an attempt to remove any remaining residue.

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 and edema were recorded at 1, 24, 48 and 72 hours after patch removal in accordance with the criteria in Appendix 1.

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 and Statistical Analysis

The Primary Irritation Index of the test was calculated following test completion for each animal. The erythema and edema scores obtained at the 24, 48 and 72 hour intervals were added together and divided by the total number of observations. This calculation was conducted separately for the test and control article for each animal. The score for the control was subtracted from the score for the test article to obtain the Primary Irritation Score. The Primary Irritation Score for each animal was added together and divided by the number of animals to obtain the Primary Irritation Index. The Primary Irritation Index was characterized based on the definitions outlined in Appendix 1.

7. Results

All animals were clinically normal throughout the study. Individual results of dermal scoring appear in Appendix 2. No irritation was observed on the skin of the animals. The Maximum Irritation Response was not applicable. The Primary Irritation Index of the test article was calculated to be 0.0. The irritation calculations are shown below:

Animal Number	Test Score Average	-	Control Score Average	Individual Primary Irritation Score	Combined Primary Irritation Score (CPIS)	Primary Irritation Index (CPIS ÷ 3)	Response Category
66666	0.0	-	0.0	0.0	0.0	0.0	Negligible
66667	0.0	-	0.0	0.0			
66754	0.0	-	0.0	0.0			

8. Conclusion

There was no erythema and no edema observed on the skin of the animals. The Primary Irritation Index for the test article was calculated to be 0.0. The response of the test article was categorized as negligible.

Results and conclusions apply only to the test article tested. Any extrapolation of these data to other articles is the sponsor's responsibility. All procedures were conducted in conformance with good manufacturing practices and certified to ISO 13485:2003.

9. Records

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

10. References

Code of Federal Regulations (CFR), Title 9, Parts 1-199, Animal Welfare Act (2008).

National Research Council, *Guide for the Care and Use of Laboratory Animals*, Washington, DC: National Academy Press, 1996.

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

Code of Federal Regulations (CFR), Title 16, Part 1500, Federal Hazardous Substances Act (FHSA) Regulations (2008).

International Organization for Standardization (ISO) 10993, Biological Evaluation of Medical Devices - Part 2: Animal Welfare Requirements (2006).

International Organization for Standardization (ISO) 10993, Biological Evaluation of Medical Devices - Part 10: Tests for Irritation and Delayed-Type Hypersensitivity (2002).

Appendix 1 - Classification System For Skin Reaction

Reaction	Numerical Grading
Erythema and Eschar Formation	
No erythema	0
Very slight erythema (barely perceptible)	1
Well-defined erythema	2
Moderate erythema	3
Severe erythema (beet redness) to eschar formation preventing grading of erythema	4
Edema Formation	
No edema	0
Very slight edema (barely perceptible)	1
Well-defined edema (edges of area well-defined by definite raising)	2
Moderate edema (raised approximately 1 mm)	3
Severe edema (raised more than 1 mm and extending beyond exposure area)	4
Total possible score for irritation	8

NOTE: Other adverse changes at the skin sites shall be recorded and reported

Irritation Response Categories in the Rabbit

Response Category	Mean Score
Negligible	0.0 to 0.4
Slight	0.5 to 1.9
Moderate	2.0 to 4.9
Severe	5.0 to 8.0

Appendix 2 - Dermal Observations

Animal Number/ Sex	Weight (kg)	Group	Observation	Interval (hours)							
				1		24		48		72	
				Left	Right	Left	Right	Left	Right	Left	Right
66666 Female	2.6	Test	Erythema	0	0	0	0	0	0	0	0
			Edema	0	0	0	0	0	0	0	0
		Control	Erythema	0	0	0	0	0	0	0	0
			Edema	0	0	0	0	0	0	0	0
66667 Female	2.6	Test	Erythema	0	0	0	0	0	0	0	0
			Edema	0	0	0	0	0	0	0	0
		Control	Erythema	0	0	0	0	0	0	0	0
			Edema	0	0	0	0	0	0	0	0
66668 Male	2.6	Test	Erythema	NA	0	NA	0	NA	0	NA	0
			Edema	NA	0	NA	0	NA	0	NA	0
		Control	Erythema	NA	NA	NA	NA	NA	NA	NA	NA
			Edema	NA	NA	NA	NA	NA	NA	NA	NA
66754 Male	2.3	Test	Erythema	0	0	0	0	0	0	0	0
			Edema	0	0	0	0	0	0	0	0
		Control	Erythema	0	0	0	0	0	0	0	0
			Edema	0	0	0	0	0	0	0	0

NA = Not Applicable as the test article on the right side was the only patch found. Replaced with Animal Number 66754.