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220F0265S-X01

STUDY NUMBER

20F0265S-X01

FINAL REPORT

STUDY TITLE

Primary Skin Irritation Test (FHSA/16 CFR 1500.41)

TEST ARTICLE

Eastlon Polyolefin Fiber (mask application)
Lot Number: 202006
Other Identifier: SP-22301EP

ANALYST

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TABLE OF CONTENTS

SIGNATURE PAGE

This report is being submitted by the following personnel:

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16 Jul 2020

X

I approve the content of this document.
Signed by: Zuzana Karjala

RESPONSIBLE PERSONNEL

1. Rick Shin, Ph.D., Director, Toxicology
2. Roger O'Meara, B.S., LATg, Manager, Toxicology
3. F. Michael Yakes, Ph.D., Executive Vice President
4. Tom Spalding, President

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STATEMENT OF COMPLIANCE

Study Number 20F0265S-X01 was conducted according to Pacific BioLabs Standard Operating Procedures (SOPs) and in compliance with the Food and Drug Administration (FDA) Good Manufacturing Practice Regulations (cGMP), Title 21 of the U.S. Code of Federal Regulations, Parts 210 and 211.

Analyst Signature

16 Jul 2020

X

I approve the content of this document.
Signed by: Zuzana Karjala

The Signature below indicates review of the Final Report by Quality Assurance and specifies the date of the review.

16 Jul 2020

X

QA Review
Signed by: Kenneth Tan

STUDY SUMMARY

Purpose: The purpose of this study is to evaluate the degree of skin irritation caused by the test article in rabbits. This study was performed according to Pacific BioLabs Standard Operating Procedure (SOP) 16F-03, which incorporates procedures outlined in 16 CFR Part 1500.41, which complies with the Consumer Product Safety Commission guidelines.

Procedures: Six New Zealand White rabbits were used in this study. Approximately 24 hours prior to the test, the entire dorsal surface of each animal was clipped free of hair. Only animals with healthy, intact skin were used in this study.

The test article "Eastlon Polyolefin Fiber (mask application)" was administered as received from the Sponsor. A portion (1 inch by 1 inch) of the test article was cut and applied directly to the rabbit's skin on each dosing site, one intact skin dose site on one side of the rabbit and one abraded skin dose site on the other side of the rabbit. The test article was secured in place by adhesive surgical tape to create a patch on both sides. The entire trunk of the animal was then wrapped with gauze and with an elastic bandage, for the 24-hour period of exposure.

After 24 hours of exposure, the wrapping and test article were removed. The dose sites were cleaned using lukewarm water. Animals were examined and the resulting reactions (intact and abraded skin) were evaluated according to the scoring system recommended by 16 CFR 1500.41. Readings were again made at the end of a total of 72 hours (48 hours after the first reading). Separate scores were recorded for edema and erythema, for intact and abraded skin. The Primary irritation score was determined.

Interpretation of Results: According to 16 CFR 1500.3 (*Definitions*), a primary irritant is defined as a test material that produces a Primary Irritation Score greater or equal to five.

Results: The Primary Irritation Score was 0.

Conclusion: Under the conditions of the study, based on the group mean comparison, the test article was well tolerated when applied topically to the skin of the New Zealand white rabbits and no severe reactions were observed. The test article was rated as non-irritating to the rabbit skin.

1. GENERAL INFORMATION

1.1. Study Dates

Date Test Article Received:	18 Jun 2020
Experimental Start Date:	29 Jun 2020
Experimental End Date:	02 Jul 2020
Study Completion Date:	14 Jul 2020

1.2. Key Personnel and Laboratories

Study Director:	Zuzana Karjala, Ph.D, RLATg Senior Scientist, Toxicology Pacific BioLabs 551 Linus Pauling Drive Hercules, CA 94547 United States Phone: (510) 964-9000
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2. INTRODUCTION

Purpose: The purpose of this study is to evaluate the degree of skin irritation caused by the test article in rabbits. This study was performed according to Pacific BioLabs SOP 16F-03, which incorporates procedures outlined in 16 CFR Part 1500.41, which complies with the Consumer Product Safety Commission guidelines.

Justification of Test System: Justification for the use of animals in this study is based on the premise that animal testing is an appropriate and ethical prerequisite to testing new medical devices in humans, and that data obtained from nonclinical animal models will have relevance to the behavior of the test material in humans. Because of the complex interactions that occur *in vivo*, an *in vitro* system does not provide sufficient information for evaluation of a compound's *in vivo* activities. The albino rabbit is specified for use in the dermal irritation test by 16 CFR 1500.41.

Justification of Number of Animals: Six animals were used in this study as required by 16 CFR Part 1500.41 guidelines. The minimum number of animals required for this test was used.

Justification of Route of Administration: The topical exposure was selected because it is required by 16 CFR 1500.41 guidelines.

Dose Rationale: The dose was selected based on 16 CFR 1500.41 guidelines and Sponsor's requirements.

3. MATERIALS AND METHODS

3.1. Test Materials

3.1.1. Test Article Identification

Test Article Name:	Eastlon Polyolefin Fiber (mask application)
Physical Description:	Solid
Total Quantity Received for Testing:	1 bag
Quantity Used for This Study:	Representative portions
Lot Number:	202006
Part Number:	Not provided by Sponsor
Other Identifier:	SP-22301EP
Expiration Date:	Not provided by Sponsor
Special Handling and/or Precautions:	None
Sterilization Data:	Non-Sterile
Storage Conditions:	Room Temperature

3.1.2. Reserve Sample and Sample Disposition

After completion of the study, all remaining test articles will be disposed according to Pacific BioLabs SOPs.

3.2. Test System

Species:	Rabbit
Strain:	New Zealand White
Source:	Charles River Laboratories, Wilmington, MA
Number Used:	Six
Initial Weight:	2.5 to 2.6 kg
Age:	Young Adult
Sex:	Female
Identification:	Ear tags and cage cards

Environment: Animals were housed individually in stainless steel cages. Animals were maintained in a controlled environment at a nominal temperature range of 16 to 22°C, a humidity range of 50 ± 20%, and a light/dark cycle of 12 hours. Animals were maintained in rooms with at least 10 room air changes per hour. Room logs documenting temperature and humidity are kept on file at Pacific BioLabs.

Housing: Animals were maintained and monitored for good health in accordance with Pacific BioLabs animal husbandry SOPs. During acclimation, animals were housed individually in metal suspended cages. During study, animals were housed individually in metal suspended cages.

Acclimation Period: Animals placed on study were acclimated to the testing facility for at least six days prior to test. Health observations were performed prior to the study to ensure that the animals were acceptable for study use.

Diet and Feed: Animals received a Certified Laboratory Rabbit Diet (approximately 165 grams per day). The feed was analyzed by the supplier for nutritional components and environmental contaminants. There were no known contaminants in the feed that are reasonably expected to interfere with the conduct of this study.

Water: Fresh, potable drinking water was provided *ad libitum* to all animals via a sipper tube. Water is supplied by the local utility and is analyzed two times per year by Pacific BioLabs for potential contaminants; results of water analyses are archived at Pacific BioLabs. There were no known contaminants in the water at levels expected to interfere with the conduct of this study.

Veterinary Care: Veterinary care was available throughout the study and was supplied when required by changes in clinical signs or other changes. No veterinary medical treatments were administered during the study.

Disposition: Disposition of study animals is documented in the Pacific BioLabs study records. Alternate animals not selected for the study were returned to Pacific BioLabs animal colony for use in subsequent studies or procedures.

3.3. Study Materials

Text Table 1. Supplies

Item	Lot Number	Manufacturer	Expiration Date
6" Roll Gauze	38310	Dynarex	N/A
Surgical Tape (Zonas®)	0340B20	Johnson & Johnson	N/A
Micropore™ Surgical Tape	2022-03BB	3M	Mar 2022
Latex Sheeting (Dental Dam)	31934748	MSC Industrial Supply Co.	N/A
25 Gauge Needle	9193551	Becton Dickinson	30 Sep 2024
Alcohol Wipes	S20180605	Fisher Healthcare	Jun 2023

3.4. Experimental Design

3.4.1. Animal Preparation

During the 24 hours prior to exposure, the entire dorsal surface of each animal was clipped free of hair. All of the animals were weighed prior to dosing administration. Only animals with healthy, intact skin were used in this study. Each animal had two dose sites; one intact skin located on one side of the rabbit and one abraded skin located on the on the other side (Fig. 1). The abraded site was prepared by carefully scraping the skin with the point of a 25-gauge needle. This was performed in a way so to penetrate the stratum corneum, but not the dermis.

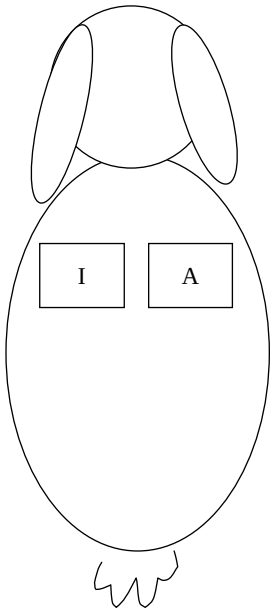

Figure 1: Approximate dose site location. I-Intact skin; A-Abraded skin

3.4.2. Test Article Preparation

The test article was "Eastlon Polyolefin Fiber (mask application)." The test article was administered as received from the Sponsor. A portion of the test article was arranged into (1 inch by 1 inch) squares.

3.4.3. Dosing Procedure

The study design is presented in Text Table 2. The dose sites were marked with indelible ink. One inch by one inch square pieces of the test article were arranged on each dosing site, one intact skin dose site on one side of the rabbit, and one abraded skin dose site on the other side of the rabbit. The test article was secured in place by adhesive surgical tape (Micropore™). The entire trunk of each animal was wrapped with a six-inch wide gauze bandage, and with and elastic bandage, which was held in place with Zonas® porous tape so that complete occlusion was obtained. The test sites were uncovered after 24 hours of exposure.

Text Table 2. Study Design

Number of Animals (n)	Route of Administration	Number of Sites/ Animal	Condition of Site	Dose per Site	Duration of Exposure	Scoring (Hours Post-Dosing)
6	Topical	2	Intact (Left)	1 in^2 piece	24 hours	24 ± 2 hr
			Abraded (Right)	1 in^2 piece	24 hours	72 ± 2 hr

3.5. In Life Observations and Measurements

3.5.1. Mortality/Moribundity Checks

General morbidity and moribundity checks (cage side observations) were performed once daily.

3.5.2. Clinical Observations

Clinical observations were performed daily. All of the animals were observed for adverse reactions immediately after dosing and daily until the end of the study. Animals were observed for changes in their general appearance including, but not limited to, signs of dehydration, loss of weight, and abnormal posture. Other characteristics observed included appearance of skin and fur, appearance of eyes and mucous membranes, urine and fecal output, and changes in locomotor behavior.

3.5.3. Body Weight Measurement

Body weights were measured prior to the start of the study and at the end of the study.

3.5.4. Scoring

All of the animals were scored at 24 and 72 hours after application of the test article. Signs of edema, erythema, and/or eschar formation were scored as indicated in Text Table 3. Separate scores were recorded for the intact and abraded sites.

Text Table 3. Evaluation of Skin Reactions

Erythema and Eschar Formation	Value*
No erythema	0
Very slight erythema (barely perceptible)	1
Well-defined erythema	2
Moderate to severe erythema	3
Severe erythema (beet redness) to slight eschar formation (injuries in depth)	4
Edema Formation	Value
No edema	0
Very slight edema (barely perceptible)	1
Slight 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 area of exposure)	4

Table adopted from 16 CFR Part 1500.41

*The "value" recorded for each reading is the average value of the six or more animals subject to the test.

3.6. Interpretation and Analysis

The Primary Irritation score was calculated as follows:

The values recorded for each reading was the average score of the six animals subject to the test. The Erythema and Eschar formation values were added for intact and abraded skin at 24 hours and 72 hours (four values). Similarly, the Edema formation values were added for intact and abraded skin at 24 hours and 72 hours (four values). The eight values were totaled and then divided by four to give the Primary Irritation Score. As defined in 16 CFR 1500.3 (*Definitions*), a primary irritant is a substance that results in an empirical score of five or greater when tested by this method.

3.7. Statistical Analysis

No statistical analysis was conducted for the evaluation of data.

3.8. Data Acquisition and Analysis

Major computer software systems used on this study included Microsoft Word®, and the Rees Scientific Environmental Monitoring System® for study room environmental control.

3.9. Maintenance of Raw Data, Records, and Specimens

Following issuance of the Final Report, records (including, but not limited to, protocol, protocol amendment(s), in-life records, pathology records, dose preparation records, correspondence related to the study, Final Report, and histopathology records) and materials (including, but not limited to, slides, specimens, wet tissues, and blocks) will be archived at Pacific BioLabs (Hercules, CA) for a period of one year. After one year, the Sponsor will be contacted concerning continued storage or return of materials.

Records and materials associated with activities external to Pacific BioLabs (including, but not limited to, clinical pathology, histopathology, and bioanalysis) and activities conducted by the Sponsor (including, but not limited to, dose solution analysis) will be archived by the individual performing laboratories or the Sponsor in a manner consistent with their individual operating SOPs and regulatory requirements.

4. RESULTS AND DISCUSSION

4.1. In Life Observations and Measurements

4.1.1. Survival

No mortality occurred during the study; all animals survived until scheduled termination. At the end of the study, all animals were returned to Pacific BioLabs animal colony.

4.1.2. Clinical Observations

No test article related abnormalities were noted in any of the tested animals. All animals appeared healthy during the course of the study.

4.1.3. Body Weight Measurement

Body weights are presented in Text Table 4. All animals had acceptable body weight when placed on study. All animals exhibited typical body weight at the end of the study.

Text Table 4. Body Weights

Animal Number	Initial Body Weight (kg)	Final Body Weight (kg)	Body Weight Change* (kg)
75163	2.5	2.6	+0.1
75164	2.5	2.6	+0.1
75165	2.5	2.6	+0.1
75166	2.6	2.6	0
75167	2.6	2.6	0
75168	2.5	2.6	+0.1

*Initial body weight was subtracted from final body weight

4.1.4. Scoring

The individual erythema and edema scores are presented in Summary Table 1. Average scores and Primary Irritation Scores are presented in Summary Table 2.

The Primary Irritation Score was 0. The test article was not a primary irritant in rabbits used in this study.

5. CONCLUSION

This test was performed according to 16 CFR 1500.41, which complies with the Consumer Product Safety Commission guidelines. The test article was considered non-irritating to the skin of the six test animals used in this study.

6. REFERENCES

16 CFR 1500.3, rev. 7/6/2020, *Definitions*

16 CFR 1500.41, rev. 7/6/2020, *Method of Testing Primary Irritant Substances*

Pacific BioLabs SOP 16F-03, rev. 1N.00, *Primary Skin Irritation Test*

7. SUMMARY OF RESULTS

Summary Table 1. Dermal Irritation Scores

Rabbit Number	Sex	Time (hours)	Intact		Abraded	
			Erythema	Edema	Erythema	Edema
75163	F	24	0	0	0	0
75164	F	24	0	0	0	0
75165	F	24	0	0	0	0
75166	F	24	0	0	0	0
75167	F	24	0	0	0	0
75168	F	24	0	0	0	0
75163	F	72	0	0	0	0
75164	F	72	0	0	0	0
75165	F	72	0	0	0	0
75166	F	72	0	0	0	0
75167	F	72	0	0	0	0
75168	F	72	0	0	0	0

Summary Table 2. Averages and Primary Irritation Score

Skin Reaction	Exposure Time (Hours)	Evaluation Value[1]
Erythema and Eschar Formation		
Intact Skin	24	0
Intact Skin	72	0
Abraded Skin	24	0
Abraded Skin	72	0
	Subtotal	
Edema Formation		
Intact Skin	24	0
Intact Skin	72	0
Abraded Skin	24	0
Abraded Skin	72	0
	Subtotal	0
	TOTAL	0
	Primary Irritation Score[2]	0

1. The "value" recorded for each reading is the average value of the six animals subject to the test.

2. The Primary Irritation Score is obtained by dividing the total by four.