MPC Series, Polycarbonate (with White Thumb Latch)

Product Validation Guide

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Product Line Overview and Validation Guide Summary

Colder Product's MPC03 Series, polycarbonate couplings are widely used in bioprocessing and medical device applications. MPC03 couplings are manufactured from USP Class VI, animal-free materials. Couplings within this product line include inserts and bodies with hose barbs for 1/8", 1/4" and 3/8" internal diameter tubing as well as pressure sealing caps and plugs.

This guide is intended to document specifications of the MPC03 Series polycarbonate couplings and testing that has been performed on this series. This information is valid for the following part numbers:

Part Number	Description
MPC17002T03	1/8" In-Line Hose Barb Non-valved Coupling Body
MPC17004T03	1/4" In-Line Hose Barb Non-valved Coupling Body
MPC17006T03	3/8" In-Line Hose Barb Non-valved Coupling Body
MPCK17002T03	1/8" In-Line Hose Barb Non-valved Coupling Body with Lock
MPCK17004T03	1/4" In-Line Hose Barb Non-valved Coupling Body with Lock
MPCK17006T03	3/8" In-Line Hose Barb Non-valved Coupling Body with Lock
MPC22002T03M	1/8" In-Line Hose Barb Non-valved Insert with Silicone Seal
MPC22004T03M	1/4" In-Line Hose Barb Non-valved Insert with Silicone Seal
MPC22006T03M	3/8" In-Line Hose Barb Non-valved Insert with Silicone Seal
MPC32003	Sealing Cap
MPCK32003	Sealing Cap with Lock
MPC30003M	Sealing Plug
MPC17C1703	Back-to-Back MPC Body Adapter
MPC17X1703	MPC to MPX Body Reducer

If you desire additional information on the MPC03 Series polycarbonate couplings, please contact your Colder Products Company representative.

Summaries:

Specifications: Listing of materials and appropriate operational and sterilization conditions.

Biocompatibility Tests: The polycarbonate and platinum-cured silicone materials were tested to USP Class VI criteria. Tests performed include: biological reactivity tests *in vivo*, physicochemical studies, cytotoxicity studies, systemic toxicity studies and *In Vitro* hemolysis studies.

Functional Testing: The MPC03 couplings were tested for burst pressure, helium leak test and cycle test. All tested samples passed.

Product Specifications

OPEN FORMAT CONNECTION TECHNOLOGY

MPC
SERIES CONNECTORS

MPC Series Connecters add ease of use and security to critical fluid handling applications. Choose from a full line of connectors and configurations, including pressure sealing caps and plugs, in sizes to fit 1/8" to 3/8" tubing. MPC couplings offer optional locking sleeves to further guard against accidental disconnects. In addition, coupling halves can be rotated when connected to reduce tube kinks.

SPECIFICATIONS

OPERATING PRESSURE
Vacuum to 60 psi, 4.1 bar

OPERATING TEMPERATURE
Polycarbonate:
-40°F to 250°F (-40°C to 121°C)
Polysulfone:
-40°F to 300°F (-40°C to 149°C)

STERILIZATION
Gamma: Up to 50 kGy irradiation
OR
Autoclave:
Polycarbonate: Up to 250°F (121°C), 30 minutes, up to 10 repetitions
Sterilize uncoupled only
Polysulfone: Up to 270°F (132°C), 60 minutes, up to 25 repetitions
Sterilize uncoupled only

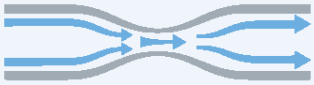
TERMINATIONS
1/8" to 3/8" ID (3.2mm to 9.5mm)

MATERIALS
Main components:
Polycarbonate (purple tint)
Polysulfone (amber tint)
Locking sleeves:
Polysulfone (white)
Thumb Latches:
Polycarbonate (white)
PVDF (white)
O-rings:
Silicone (clear), platinum-cured

WARNING: Pressure, temperature, chemicals, and operating environment can affect the performance of couplings. It is the customer's responsibility to test the suitability of CPC's products in their own application conditions.

FEATURES	BENEFITS
Ergonomic thumb latch	Easy to operate – even with gloved hands
Parting line-free hose barb	Prevent potential leak path
Optional locking sleeve	Prevents accidental disconnection
Various options on termination size and material	Better flexibility to fit more applications

TYPICAL FLOW RATE:
Cv Value Range: 0.1 - 8
for MPC hose barb terminations



Cv values represent the approximate expected flow rate in gallons per minute of water at room temperature for a 1 PSI pressure drop. The flow is generally constrained by the smallest diameter, which in some cases will be the termination diameter and not the Nominal Flow Path.

NOTE
Validation and Extractables data can be requested at cpcworldwide.com/MPC

DID YOU KNOW
The MPC and MPX connectors are perfect for smaller bag systems for aliquoted media or other product stored in bags.

Scan code to visit webpage

SCAN

cpcworldwide.com/MPC

Part Number	Description
Coupling Bodies	
MPC17002T03	1/8" In-Line Hose Barb Non-valved Coupling Body
MPC17004T03	1/4" In-Line Hose Barb Non-valved Coupling Body
MPC17006T03	3/8" In-Line Hose Barb Non-valved Coupling Body

Coupling Bodies with Locks	
MPCK17002T03	1/8" In-Line Hose Barb Non-valved Coupling Body with Lock
MPCK17004T03	1/4" In-Line Hose Barb Non-valved Coupling Body with Lock
MPCK17006T03	3/8" In-Line Hose Barb Non-valved Coupling Body with Lock

Coupling Inserts	
MPC22002T03M	1/8" In-Line Hose Barb Non-valved Insert with Silicone Seal
MPC22004T03M	1/4" In-Line Hose Barb Non-valved Insert with Silicone Seal
MPC22006T03M	3/8" In-Line Hose Barb Non-valved Insert with Silicone Seal

Sealing Caps	
MPC32003	Sealing Cap
MPCK32003	Sealing Cap with Lock

Sealing Plug	
MPC30003M	Sealing Plug

Back-to-Back Body Adapters	
MPC17C1703	MPC Back-to-Back Body Adapter
MPC17X1703	MPC to MPX Body Reducer

Helium Leak Test

Colder Products Company
Engineering Test Lab

1001 Westgate Drive
St. Paul, MN 55114 USA

Telephone (651) 645-0091
Fax (651) 603-2638

HELIUM LEAK TEST:

Test #: 2007-005

Purpose:

The purpose of the Helium Leak Test is to verify the sealing performance of Colder Products Company's polycarbonate MPC couplings with white thumb latches. Testing was completed on both virgin and sterilized parts.

Procedure:

The polycarbonate MPC couplings with white thumb latches were tested at full vacuum. Forty (40) of each part number were tested while coupled. Of the forty (40) parts, twenty (20) will be virgin parts, twenty (20) parts were gamma irradiated to a minimum of 50 kGy then autoclaved sterilized for 10 cycles at 250°F for 30 minutes with a 15 minute dry time.

Each polycarbonate MPC coupling was capped on one end and attached to a Helium Mass Spectrometer Leak Detector via its hose barb. The leak detector was activated, thereby applying a full vacuum to the MPC coupling. The part will then be completely enveloped with helium by means of a cylinder placed directly over of the part. The maximum leak rate was recorded.

Results:

For the helium leak test, the leak rate must be below 1.0×10^{-5} atm-cc/sec. All parts passed the Helium Leak Test.

Mary Wallraff
Mechanical Test Engineer

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Cycle Test

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CYCLE TEST:

Test #: 2007-005

Purpose:

The purpose of the Cycle Test is to verify the functionality of Colder Products Company's polycarbonate MPC couplings with white thumb latches after 1,000 connect/disconnect cycles. Testing was completed on both virgin, gamma irradiated, and autoclave sterilized parts.

Procedure:

The gamma sterilized parts were irradiated to a minimum of 50kGy. The autoclaved sterilized parts completed 10 cycles at 250°F for 30 minutes with a 15 minute dry time.

The MPC couplings shall be disconnected and then connected 1,000 times by an automatic mechanism. One cycle consists of depressing the thumb latch, removing the insert from the body, releasing the thumb latch, and then placing the insert into the body. One connect/disconnect cycle lasts six seconds.

The MPC couplings are cycled in intervals of 200 cycles. After every interval the parts are examined for any deformation. The parts are then returned to the cycle test fixture.

Ten virgin, ten gamma irradiated, and ten autoclave sterilized parts were cycled.

Results:

To pass the Cycle Test, the parts must maintain their functionality after 1,000 cycles. All parts passed the Cycle Test.

Mary Wallraff
Mechanical Test Engineer

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Burst Test

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BURST TEST:

Test #: 2007-005

Purpose:

The purpose of the Burst Test is to demonstrate that Colder Products Company's polycarbonate MPC couplings with white thumb latches have an adequate safety margin above their rated operating pressure at room temperature. Testing was completed on both virgin and sterilized parts.

Procedure:

The sterilized parts were gamma irradiated to a minimum of 50kGy and then autoclaved sterilized for 10 cycles at 250°F for 30 minutes with a 15 minute dry time.

This test is modeled after National Fluid Power Association (NFPA), ANSI/B93.42M-1977. Each coupled set of polycarbonate MPC inserts and bodies shall be attached to a hydraulic burst fixture. Each sample shall be pressurized with water at ambient temperature. The pressure is increased until failure occurs. The rate of pressurization shall not exceed 100 psi per second. A minimum burst pressure of 300 psi is considered passing.

Results:

A test of 10 virgin and 10 sterilized samples all passed with burst values greater than 300 psi.

Mary Wallraff
Mechanical Test Engineer

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Flow Test

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FLOW TEST:

Purpose:

The purpose of the Flow Test is to determine the C_V values of the MPC product line.

Procedure:

An MPC coupling set with each available size of hosebarb shall be tested.

Each coupling set shall be installed in the flow test bench. A minimum of five flow rate and pressure drop measurements shall be recorded: one each at the maximum and minimum practical flow rates and at least three measurements spaced approximately equally between the maximum and minimum flow rates. The coupling set shall be tested with the flow direction from body-to-insert and from insert-to-body.

This procedure shall be repeated without the coupling installed for a tare measurement.

The C_V values shall be calculated by using the following equation:

$$C_V = Q \Big/ \sqrt{P_{In} - P_{Out} - \left(P_{In_{Tare}} - P_{Out_{Tare}}\right)}$$

Where:
Q is the flow rate in gallons-per-minute
P_{In} is a pressure measurement, in pounds-per-square-inch, upstream of the test unit
P_{Out} is a pressure measurement, in pounds-per-square-inch, downstream of the test unit
P_{Tare} is a pressure measurement taken during the measurement of the flow test bench

The minimum C_V value shall be reported.

Results:

The C_V value of the MPC with 1/4" hosebarb terminations is 2.8.
The C_V value of the MPC with 3/8" hosebarb terminations is 5.5.

Erik Long
Engineering Test Lab Manager

Polycarbonate Systemic Toxicity – (USP <88>/ISO 10993-11)

REPORT

TEST FACILITY

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419.666.9455

SPONSOR

Kayla Vangsgard
Colder Products Company
1001 Westgate Drive
St. Paul, MN 55114
United States

CONFIDENTIAL

STUDY TITLE

USP and ISO Acute Systemic Toxicity Study in Mice

TEST ARTICLE NAME

Polycarbonate, Lexan HPS1-1H1124

TEST ARTICLE IDENTIFICATION

24235560

NAMSA

PEOPLE > SCIENCE > SOLUTIONS	P.O. Number 182004705	Lab Number 18T_20417_04 18T_20417_05 18T_20417_06 18T_20417_07	T0625_500/S Report	Page 1 of 15

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Summary

The test article, Polycarbonate, Lexan HPS1-1H1124, was evaluated for acute systemic toxicity in mice. This study was conducted based on ISO 10993-11, Biological evaluation of medical devices - Part 11: Tests for systemic toxicity, and the United States Pharmacopeia, National Formulary, General Chapter <88>, Biological Reactivity Tests, In Vivo. The test article was extracted in 0.9% sodium chloride USP solution (SC), sesame oil, NF (SO), alcohol in saline (AS) and polyethylene glycol (PEG). A single dose of the appropriate test article extract was injected into a group of five animals. Similarly, a separate group of five animals was dosed with each corresponding extract vehicle alone (control). The animals were observed for signs of systemic toxicity immediately after injection and at 4, 24, 48 and 72 hours after injection. Body weights were recorded prior to dosing and on days 1, 2 and 3.

There was no mortality or evidence of systemic toxicity from the extracts injected into mice. Each test article extract met the requirements of the study.

Supervisory Personnel: Mark A. Shumaker, MBA, ILAM, LAT
Manager, In Vivo Biocompatibility

Austin M. Zdawczyk, BS, MBA, ALAT
Manager, Biocompatibility

Approved by: Arizona Eugene Carter 01-23-18
Arizona E. Carter, BS, ALAT — Date
Technical Reviewer

1. Introduction

1.1 Purpose

The purpose of this study was to evaluate acute systemic toxicity of a test article extract following a single intravenous or intraperitoneal injection in mice.

1.2 Testing Guidelines

This study was conducted based on the International Organization for Standardization 10993-11, Biological evaluation of medical devices, Part 11: Tests for systemic toxicity, and the United States Pharmacopeia, National Formulary, General Chapter <88>, Biological Reactivity Tests, In Vivo.

This test was performed under an ISO 13485 certified Quality System, with the test method accredited to the ISO 17025 Standard.

1.3 Dates

Test Article Received:	December 19, 2017
Treatment Started:	January 11, 2018
Observations Concluded:	January 14, 2018

1.4 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. 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:	Polycarbonate, Lexan HPS1-1H1124
Identification:	24235560
Physical Description of the Test Article:	Part # 2471700
Storage Conditions:	Room Temperature

Table 2: Control Articles

Name:	0.9% sodium chloride USP solution (SC) Sesame oil, NF (SO) Alcohol in saline 1:20 solution (AS) Polyethylene glycol 400 (PEG)
Strength, Purity, Composition or Other Characteristics:	SC: Purity: Meets requirements of USP Sodium Chloride for Injection and is certified as USP Grade; Composition: 0.9% NaCl ± 5.0% of label claim, balance is water; sodium chloride CAS No.: 7647-14-5/water CAS No.: 7732-18-5 SO: Purity: Meets the requirements of National Formulary. Composition: CAS No.: 8008-74-0 AS: Composition: ethanol in saline 1:20; ethanol CAS No.: 64-17-5/sodium chloride CAS No.: 7647-14-5/water CAS No.: 7732-18-5 PEG: Identity: Matches infrared spectrum of polyethylene glycol 400 with average molecular weight of 380 to 420; Composition: Neat: CAS No.: 25322-68-3

3. Test System

3.1 Test System

Species:	Mouse (*Mus musculus*)
Strain:	Outbred albino
Source:	Hilltop Lab Animals
Sex:	Male
Body Weight Range:	19 grams to 23 grams at injection
Acclimation Period:	Minimum 1 day
Number of Animals:	Forty
Identification Method:	Ear punch

3.2 Justification of Test System

Mice have historically been used to evaluate biomaterial extracts. The use of albino mice injected with a single intravenous (IV) or intraperitoneal (IP) dose of test article extract or control blank have been suggested in the current USP and ISO standards for evaluation of medical plastics.

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 of five in shoebox cages identified by a card indicating the lab number, animal numbers, test code, sex, animal code and date dosed.

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 rodent feed, PROLAB RMH 1000 - 5P07, 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.

4.1 Sedation, Analgesia or Anesthesia

It has been determined that the use of sedation, analgesia or anesthesia was not necessary during the routine course of this procedure.

4.2 Veterinary Care

Standard veterinary medical care was provided in this study.

4.3 IACUC

The procedures for this study were approved by the NAMSA Institutional Animal Care and Use Committee (IACUC) prior to conduct.

4.4 Selection

Only healthy, previously unused animals were selected.

5. Method

5.1 Test and Control Article Preparation

The subdivided test article and the control blank (extraction vehicle without the test article) were subjected to the extraction conditions as described below. The extracts were continuously agitated during extraction.

Table 3: Extraction

Vehicle	Extraction Ratio	Article Amount	Volume of Vehicle	Extraction Condition
SC	3 cm^2:1 mL	39.2 cm^2	13 mL	50°C for 72 hours
SO	3 cm^2:1 mL	39.2 cm^2	13 mL	50°C for 72 hours
AS	3 cm^2:1 mL	39.2 cm^2	13 mL	50°C for 72 hours
PEG	3 cm^2:1 mL	39.2 cm^2	13 mL	50°C for 72 hours

The following table contains a description of the test and control article extract conditions.

Table 4: Condition of Extracts

Vehicle	Time Observed	Extract	Condition of Extracts		
			Color	Clarity	Particulates
SC	Before Extraction	Test	Colorless	Clear	No
		Control	Colorless	Clear	No
	After Extraction	Test	Colorless	Clear	No
		Control	Colorless	Clear	No
	Prior to Use	Test	Colorless	Clear	No
		Control	Colorless	Clear	No
SO	Before Extraction	Test	Colorless	Clear	No
		Control	Colorless	Clear	No
	After Extraction	Test	Colorless	Clear	No
		Control	Colorless	Clear	No
	Prior to Use	Test	Colorless	Clear	No
		Control	Colorless	Clear	No
AS	Before Extraction	Test	Colorless	Clear	No
		Control	Colorless	Clear	No
	After Extraction	Test	Colorless	Clear	No
		Control	Colorless	Clear	No
	Prior to Use	Test	Colorless	Clear	No
		Control	Colorless	Clear	No
PEG	Before Extraction	Test	Colorless	Clear	No
		Control	Colorless	Clear	No
	After Extraction	Test	Colorless	Clear	No
		Control	Colorless	Clear	No
Diluted PEG	After Dilution	Test	Colorless	Clear	No
		Control	Colorless	Clear	No
	Prior to Use	Test	Colorless	Clear	No
		Control	Colorless	Clear	No

The test article remained visually unchanged following the extraction process. The PEG test article extract and control were diluted with saline to yield a 200 mg PEG/mL concentration before dosing the animals. The extracts were stored at room temperature for less than 3 hours prior to dosing. The extracts were not centrifuged, filtered, or otherwise altered prior to dosing.

5.2 Test Procedure

Prior to dosing, the animals were individually identified, weighed and arbitrarily assigned to a treatment group as shown below:

Table 5: Treatment Group Assignment

Extract	Treatment Group	Number of Animals	Sex	Dose	Route of Administration
AS	Test	5	Male	50 mL/kg	Intravenous
	Control	5	Male	50 mL/kg	Intravenous
PEG	Test	5	Male	10 g/kg	Intraperitoneal
	Control	5	Male	10 g/kg	Intraperitoneal
SC	Test	5	Male	50 mL/kg	Intravenous
	Control	5	Male	50 mL/kg	Intravenous
SO	Test	5	Male	50 mL/kg	Intraperitoneal
	Control	5	Male	50 mL/kg	Intraperitoneal

A single dose of each test article extract was injected into each animal in the test group. Each control blank was similarly injected into each animal in the control group. Dosing occurred on day 0. Animals were observed for any adverse clinical reactions immediately after injection. The animals were then returned to their cages. The animals were observed for signs of systemic reactions at 4, 24, 48 and 72 hours after injection. The animals were weighed daily for three days after dosing. After the test was completed, all animals were euthanized according to an IACUC approved NAMSA procedure.

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

No statistical analysis of the data was performed. If during the observation period none of the animals treated with the test extract exhibited a significantly greater reaction than the corresponding control animals, then the test article met the ISO and USP requirements. If two or more animals died, or if abnormal behavior such as convulsions or prostration occurred in two or more animals, or if body weight loss greater than 2 grams occurred in three or more animals, the test article did not meet the ISO and USP requirements.

7. Results

7.1 Mortality Data

There was no mortality during the study. The mortality data are presented in Table 1 in the appendices.

7.2 Clinical Observations

The test and control animals injected with AS appeared lethargic immediately after the injection; this was considered an expected pharmacological effect due to the alcohol content of the extract. All animals were clinically normal throughout the study. The clinical observations are presented in Table 2 in the appendices.

7.3 Body Weight

Body weight data were acceptable. Body weight data are presented in Table 3 in the appendices.

8. Conclusion

There was no mortality or evidence of systemic toxicity from the extracts injected into mice. Each test article extract met the requirements of the study.

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. 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) 10993-11, Biological evaluation of medical devices - Part 11: Tests for systemic toxicity (2017).

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

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.

United States Pharmacopeia 40, National Formulary 35 (USP), General Chapter <88>, Biological Reactivity Tests, In Vivo (2017).

Appendix 1 - Observations - AS Extract

Table 1: Mortality Data

Extract	Treatment Group	Number Dead/Number Tested
AS	Test Extract	0/5
	Control Blank	0/5

Table 2: Clinical Observations

			Observation				
Extract	Treatment Group	Animal Number	Immediate	4 Hours	24 Hours	48 Hours	72 Hours
AS	Test Extract	101	Lethargic	Normal	Normal	Normal	Normal
		102	Lethargic	Normal	Normal	Normal	Normal
		103	Lethargic	Normal	Normal	Normal	Normal
		104	Lethargic	Normal	Normal	Normal	Normal
		105	Lethargic	Normal	Normal	Normal	Normal
	Control Blank	41	Lethargic	Normal	Normal	Normal	Normal
		42	Lethargic	Normal	Normal	Normal	Normal
		43	Lethargic	Normal	Normal	Normal	Normal
		44	Lethargic	Normal	Normal	Normal	Normal
		45	Lethargic	Normal	Normal	Normal	Normal

Table 3: Body Weight Data

			Weight (g)			
Extract	Treatment Group	Animal Number	Day 0	Day 1	Day 2	Day 3
AS	Test Extract	101	20	21	22	23
		102	23	23	25	26
		103	21	22	24	25
		104	20	21	22	23
		105	20	21	23	23
	Control Blank	41	19	21	22	24
		42	20	21	21	23
		43	22	22	23	25
		44	21	21	23	24
		45	20	21	23	24

Appendix 2 - Observations - SC Extract

Table 1: Mortality Data

Extract	Treatment Group	Number Dead/Number Tested
SC	Test Extract	0/5
	Control Blank	0/5

Table 2: Clinical Observations

			Observation				
Extract	Treatment Group	Animal Number	Immediate	4 Hours	24 Hours	48 Hours	72 Hours
SC	Test Extract	91	Normal	Normal	Normal	Normal	Normal
		92	Normal	Normal	Normal	Normal	Normal
		93	Normal	Normal	Normal	Normal	Normal
		94	Normal	Normal	Normal	Normal	Normal
		95	Normal	Normal	Normal	Normal	Normal
	Control Blank	31	Normal	Normal	Normal	Normal	Normal
		32	Normal	Normal	Normal	Normal	Normal
		33	Normal	Normal	Normal	Normal	Normal
		34	Normal	Normal	Normal	Normal	Normal
		35	Normal	Normal	Normal	Normal	Normal

Table 3: Body Weight Data

			Weight (g)			
Extract	Treatment Group	Animal Number	Day 0	Day 1	Day 2	Day 3
SC	Test Extract	91	20	20	21	22
		92	21	22	23	25
		93	20	21	23	25
		94	21	21	22	23
		95	20	21	22	23
	Control Blank	31	20	21	22	24
		32	21	22	23	24
		33	21	22	24	25
		34	21	23	24	25
		35	22	23	24	25

Appendix 3 - Observations - PEG Extract

Table 1: Mortality Data

Extract	Treatment Group	Number Dead/Number Tested
PEG	Test Extract	0/5
	Control Blank	0/5

Table 2: Clinical Observations

			Observation				
Extract	Treatment Group	Animal Number	Immediate	4 Hours	24 Hours	48 Hours	72 Hours
PEG	Test Extract	106	Normal	Normal	Normal	Normal	Normal
		107	Normal	Normal	Normal	Normal	Normal
		108	Normal	Normal	Normal	Normal	Normal
		109	Normal	Normal	Normal	Normal	Normal
		110	Normal	Normal	Normal	Normal	Normal
	Control Blank	46	Normal	Normal	Normal	Normal	Normal
		47	Normal	Normal	Normal	Normal	Normal
		48	Normal	Normal	Normal	Normal	Normal
		49	Normal	Normal	Normal	Normal	Normal
		50	Normal	Normal	Normal	Normal	Normal

Table 3: Body Weight Data

			Weight (g)			
Extract	Treatment Group	Animal Number	Day 0	Day 1	Day 2	Day 3
PEG	Test Extract	106	22	22	25	26
		107	20	21	22	23
		108	19	21	23	23
		109	21	22	24	26
		110	19	20	21	22
	Control Blank	46	20	22	24	26
		47	21	22	23	25
		48	21	22	24	26
		49	20	22	23	25
		50	20	21	22	23

Appendix 4 - Observations - SO Extract

Table 1: Mortality Data

Extract	Treatment Group	Number Dead/Number Tested
SO	Test Extract	0/5
	Control Blank	0/5

Table 2: Clinical Observations

			Observation				
Extract	Treatment Group	Animal Number	Immediate	4 Hours	24 Hours	48 Hours	72 Hours
SO	Test Extract	96	Normal	Normal	Normal	Normal	Normal
		97	Normal	Normal	Normal	Normal	Normal
		98	Normal	Normal	Normal	Normal	Normal
		99	Normal	Normal	Normal	Normal	Normal
		100	Normal	Normal	Normal	Normal	Normal
	Control Blank	36	Normal	Normal	Normal	Normal	Normal
		37	Normal	Normal	Normal	Normal	Normal
		38	Normal	Normal	Normal	Normal	Normal
		39	Normal	Normal	Normal	Normal	Normal
		40	Normal	Normal	Normal	Normal	Normal

Table 3: Body Weight Data

			Weight (g)			
Extract	Treatment Group	Animal Number	Day 0	Day 1	Day 2	Day 3
SO	Test Extract	96	22	24	25	26
		97	21	23	25	26
		98	21	21	23	25
		99	20	22	23	25
		100	19	20	22	24
	Control Blank	36	20	21	23	25
		37	22	23	24	24
		38	22	23	24	25
		39	22	24	25	27
		40	20	21	22	25

Polycarbonate Muscle Implantation Test (USP <88>)

REPORT

TEST FACILITY

NAMSA
6750 Wales Road
Northwood, OH 43619
419.666.9455

SPONSOR

Kayla Vangsgard
Colder Products Company
1001 Westgate Drive
St. Paul, MN 55114

CONFIDENTIAL

STUDY TITLE

USP Muscle Implantation Study in Rabbits - 7 Day

TEST ARTICLE NAME

Polycarbonate, Lexan HPS1-1H1124

TEST ARTICLE IDENTIFICATION

24235560

NAMSA

PEOPLE > SCIENCE > SOLUTIONS	P.O. Number 182004705	Lab Number 18T_20417_02	TU014_807 Report	Page 1 of 9

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Summary

The test article, Polycarbonate, Lexan HPS1-1H1124, was implanted in muscle tissue of the rabbit to evaluate the local tissue response. This study was conducted in accordance with the USP, General Chapter <88>, Biological Reactivity Tests, In Vivo.

Implant test articles and negative control articles were sterilized by steam. The test article and negative control were intramuscularly implanted and animals were euthanized 7 days later. Muscle tissues were excised and the implant sites examined macroscopically.

The macroscopic reaction was not significant as compared to the negative control article. The implanted test article met the USP requirements.

Supervisory Personnel: Michelle E. Zdawczyk, MS, ALAT
Manager, Preclinical Functional Studies

Approved by: Arizona Eugene Carter 01-28-18
Arizona E. Carter, BS, ALAT — Date
Technical Reviewer

1. Introduction

1.1 Purpose

The purpose of this study was to evaluate the local tissue response to the test article when implanted in muscle tissue in rabbits.

1.2 Testing Guidelines

This study was based on the United States Pharmacopeia, National Formulary, General Chapter <88>, Biological Reactivity Tests, In Vivo.

This test was performed under an ISO 13485 certified Quality System, with the test method accredited to the ISO 17025 Standard.

1.3 Dates

Test Article Received:	December 19, 2017
Implanted:	January 11, 2018
Explanted:	January 18, 2018

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:	Polycarbonate, Lexan HPS1-1H1124
Identification:	24235560
Physical Description of the Test Article:	Part # 2471700
Storage Conditions:	Room Temperature

Table 2: Negative Control Article

Name:	USP high density polyethylene reference standard was purchased from the US Pharmacopeial Convention.
Stability Testing:	Marketed product, stability characterized by its labeling
Strength, Purity, Composition or Other Characteristics:	Purity: USP Certified Standard; Composition: polyethylene

3. Test System

3.1 Test System

Species:	Rabbit (*Oryctolagus cuniculus*)
Breed:	New Zealand White
Source:	Robinson Services, Inc.
Sex:	Male
Body Weight Range:	2.8 kg to 3.6 kg at selection
Age:	Young adult
Acclimation Period:	Minimum 5 days
Number of Animals:	Two
Identification Method:	Ear tag

3.2 Justification of Test System

The rabbit is the animal model identified for USP implant testing. The muscle tissue is evaluated because the response to an implanted test article is easily graded and compared to a known negative control article.

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 individually housed in stainless steel or plastic suspended cages identified by a card indicating the lab number, animal number, test code, sex, and date implanted.

The animal housing room temperature and relative humidity were monitored daily. The temperature for the room was set to 61-72°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 rabbit feed, Laboratory Rabbit Diet – 5326, 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 were appropriately qualified and trained.

4.5 Veterinary Care

Standard veterinary medical care was provided in this study.

4.6 IACUC

The procedures for this study were approved by the NAMSA Institutional Animal Care and Use Committee (IACUC) prior to conduct.

4.7 Selection

Healthy animals were selected. To reduce the number of animals used for testing, and to comply with the directives of the NAMSA IACUC, animals on this study were used previously in an unrelated test model. Any previously evaluated test or control articles did not cause a response in the animals. Complete history of animal usage is traceable in laboratory records. Animals used for previous evaluations are identified in the report.

5. Method

5.1 Test and Control Article Preparation

The test article was an opaque plastic. All rough and/or sharp edges of the test articles and negative control articles were trimmed. A minimum of four sections of the test article were prepared, per animal. Each test article was approximately 10 mm x 1 mm x 1 mm, and were loaded into 16 gauge needles. For each animal, a minimum of two negative control articles, each approximately 10 mm x 1 mm x 1 mm, were loaded into the same size needles as used for the test article. Test and control articles were sterilized by steam.

5.2 Test Procedure

No more than 1 day prior to implantation, rabbits were weighed and clipped free of fur over the paravertebral muscles. For analgesia, on the day of implantation, each rabbit was injected subcutaneously with 0.02 mg/kg buprenorphine. For general anesthesia, each rabbit was injected intramuscularly with a mixture of ketamine hydrochloride and xylazine at a dose volume of 0.6 mL/kg. After the anesthetic had taken effect, a non-medicated ophthalmic ointment was applied to both eyes of each rabbit. The surgical site was scrubbed with povidone iodine scrub, wiped with 70% isopropyl alcohol and painted with povidone iodine solution.

One incision was made on each side of the back through the skin and parallel to the lumbar region of the vertebral column. A sterile stylet was placed in the hub of a loaded needle. Approximately 2.5 to 5.0 cm from the midline and parallel to the spinal column, the needle was inserted into the muscle through the incision at an angle until the bevel disappeared, but not deeper than 2.5 cm. The needle was withdrawn over the stylet, leaving the article in the paravertebral muscle. This was repeated until four test article sections were implanted in the right paravertebral muscle and two negative control sections were implanted in the left paravertebral muscle of each rabbit. The sections were placed at appropriately spaced intervals. The skin was closed with stainless steel wound clips.

Following the procedure, to aid in anesthetic recovery, the rabbits received intramuscular injections of atipamezole dosed at 0.5 mg/kg. The rabbits were monitored for recovery from the anesthetic and returned to their respective cages. Another dose of buprenorphine was administered at the end of the day. On the day following implantation, a third buprenorphine injection was administered.

5.2.1 Laboratory Observations

1. Rabbits were observed daily for general health.
2. Body weights were recorded on the day of implantation and at termination.

5.2.2 Terminal Procedures

After 7 days, the rabbits were weighed and then euthanized by an intravenous injection of a sodium pentobarbital based euthanasia solution. The paravertebral muscles were dissected free and methodically cut to locate four test article sites and two negative control sites in each rabbit. Capsule formation or other evidence of irritation was scored using an auxiliary light source (if needed) and a low magnification instrument. The scores were recorded as follows:

Table 3: Macroscopic Scoring

Score	Encapsulation
0	No capsule, no adverse reaction (other than minimal hemorrhage)
1	Up to 0.5 mm capsule or reaction area
2	0.6 to 1.0 mm capsule or reaction area
3	1.1 to 2.0 mm capsule or reaction area
4	>2.0 mm capsule or reaction area

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 average macroscopic score for test article sites was compared with the average score for control article sites. Calculations were rounded to the nearest 0.1. A difference of scores (test minus control) is regarded as follows:

Table 4: Reaction Index

Average Difference	Reaction Index
0.0 to 0.5	Not significant
0.6 to 1.0	Trace
1.1 to 2.0	Slight
2.1 to 3.0	Moderate
≥3.1	Marked

The requirements of the USP test were met if the difference between test and control score averages was not greater than 1.0. The requirements were not met if the difference between the test and control scores for two (or more) implant sites exceeds 1 for any animal implanted.

7. Results

7.1 Clinical Observations

All animals appeared clinically normal throughout the duration of the study.

7.2 Body Weight Data

Body weight data for individual animals were considered acceptable. Individual body weights are presented in Appendix 1.

7.3 Macroscopic Observations

There was no visible reaction at any test or control site. This resulted in a macroscopic reaction classification of not significant tissue contact irritation. The findings for the macroscopic evaluation are shown in Appendix 1.

8. Conclusion

The implanted test article met the USP requirements.

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. References

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

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

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.

United States Pharmacopeia 40, National Formulary 35 (USP), General Chapter <88>, Biological Reactivity Tests, In Vivo (2017).

Appendix 1 - Body Weights and Macroscopic Observations

Animal Number	Sex	Body Weight (kg)		Test Article	Negative Control
		Day 0	Day 7		
23510*	Male	3.6	3.6	0	0
				0	0
				0	[redacted]
				0	
23752*	Male	2.8	2.9	0	0
				0	0
				0	[redacted]
				0	
			Average:	0.0	0.0

*Previous use history traceable in laboratory records.

Polycarbonate Intracutaneous Injection (ISO 10993-10)

REPORT

TEST FACILITY

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6750 Wales Road
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SPONSOR

Kayla Vangsgard
Colder Products Company
1001 Westgate Drive
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CONFIDENTIAL

STUDY TITLE

USP and ISO Intracutaneous Study in Rabbits

TEST ARTICLE NAME

Polycarbonate, Lexan HPS1-1H1124

TEST ARTICLE IDENTIFICATION

24235560

NAMSA

PEOPLE > SCIENCE > SOLUTIONS	P.O. Number 182004705	Lab Number 18T_20417_08 18T_20417_09 18T_20417_10 18T_20417_11	TI251_800/S Report	Page 1 of 13

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Summary

The test article, Polycarbonate, Lexan HPS1-1H1124, was evaluated for the potential to cause irritation following intracutaneous injection in rabbits. 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, and United States Pharmacopeia, National Formulary, General Chapter <88>, Biological Reactivity Tests, In Vivo. The test article was extracted in 0.9% sodium chloride USP solution (SC), sesame oil, NF (SO), alcohol in saline (AS) and polyethylene glycol (PEG). A 0.2 mL dose of the appropriate test article extract was injected intracutaneously into five separate sites on the right side of the back of each of three animals. Similarly, the extract vehicle alone (control) was injected on the left side of the back of each animal. The injection sites were observed immediately after injection. Observations for erythema and edema were conducted at 24, 48, and 72 hours after injection.

The test article met the requirements of the test since the difference between each test article extract overall mean score and corresponding control extract overall mean score was 0.0, 0.0, 0.0 and 0.0 for the SC, SO, AS and PEG test article extracts, respectively.

Supervisory Personnel: Mark A. Shumaker, MBA, ILAM, LAT
Manager, In Vivo Biocompatibility

Austin M. Zdawczyk, BS, MBA, ALAT
Manager, Biocompatibility

Approved by: Arizona Eugene Carter — 01-25-19

Arizona E. Carter, BS, ALAT
Technical Reviewer

Date

1. Introduction

1.1 Purpose

The purpose of this study was to evaluate the local dermal irritation of a test article extract following intracutaneous injection in rabbits.

1.2 Testing Guidelines

This study will be conducted based on the International Organization for Standardization 10993-10, Biological evaluation of medical devices - Part 10: Tests for irritation and skin sensitization, and United States Pharmacopeia, National Formulary, General Chapter <88>, Biological Reactivity Tests, In Vivo.

This test was performed under an ISO 13485 certified Quality System, with the test method accredited to the ISO 17025 Standard.

1.3 Dates

Test Article Received:	December 19, 2017
Treatment Started:	January 11, 2018
Observations Concluded:	January 14, 2018

1.4 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. 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:	Polycarbonate, Lexan HPS1-1H1124
Identification:	24235560
Physical Description of the Test Article:	Part # 2471700
Storage Conditions:	Room Temperature

Table 2: Control Articles/Extraction Vehicles

Name:	0.9% sodium chloride USP solution (SC) Sesame oil, NF (SO) Alcohol in saline 1:20 solution (AS) Polyethylene glycol 400 (PEG)
Strength, Purity, Composition or Other Characteristics:	SC: Purity: Meets requirements of USP Sodium Chloride for Injection and is certified as USP Grade; Composition: 0.9% NaCl ± 5.0% of label claim, balance is water; sodium chloride CAS No.: 7647-14-5/water CAS No.: 7732-18-5 SO: Purity: Meets the requirements of National Formulary. Composition: CAS No.: 8008-74-0 AS: Composition: ethanol in saline 1:20; ethanol CAS No.: 64-17-5/sodium chloride CAS No.: 7647-14-5/water CAS No.: 7732-18-5 PEG: Identity: Matches infrared spectrum of polyethylene glycol 400 with average molecular weight of 380 to 420; Composition: Neat: CAS No.: 25322-68-3

3. Test System

3.1 Test System

Species:	Rabbit (*Oryctolagus cuniculus*)
Breed:	New Zealand White
Source:	Robinson Services, Inc.
Sex:	Male
Body Weight Range:	2.6 kg to 3.0 kg at selection
Age:	Young adult
Acclimation Period:	Minimum 5 days
Number of Animals:	Six
Identification Method:	Ear tag

3.2 Justification of Test System

The intracutaneous injection test in rabbits is specified in the current USP and ISO testing standards and has been used historically to evaluate biomaterial extracts.

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 individually housed in stainless steel or plastic suspended cages identified by a card indicating the lab number, animal number, test code, sex, and date dosed.

The animal housing room temperature and relative humidity were monitored daily. The temperature for the room was set to 61-72°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 rabbit feed, Laboratory Rabbit Diet – 5326, 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.

4.1 Sedation, Analgesia or Anesthesia

It has been determined that the use of sedation, analgesia or anesthesia was not necessary during the routine course of this procedure.

4.2 Veterinary Care

Standard veterinary medical care was provided in this study.

4.3 IACUC

The procedures for this study were approved by the NAMSA Institutional Animal Care and Use Committee (IACUC) prior to conduct.

4.4 Selection

Only healthy, previously unused, thin-skinned animals free of mechanical irritation or trauma that could interfere with the test were selected.

5. Method

5.1 Test and Control Article Preparation

The subdivided test article and the control blank (extraction vehicle without the test article) were subjected to the extraction conditions as described below. The extracts were continuously agitated during extraction.

Table 3: Extraction

Vehicle	Extraction Ratio	Article Amount	Volume of Vehicle	Extraction Condition
SC	3 cm^2:1 mL	19.6 cm^2	6.5 mL	50°C for 72 hours
SO	3 cm^2:1 mL	19.6 cm^2	6.5 mL	50°C for 72 hours
AS	3 cm^2:1 mL	19.6 cm^2	6.5 mL	50°C for 72 hours
PEG	3 cm^2:1 mL	19.6 cm^2	6.5 mL	50°C for 72 hours

The following table contains a description of the test and control article extract conditions.

Table 4: Condition of Extracts

Vehicle	Time Observed	Extract	Condition of Extracts		
			Color	Clarity	Particulates
SC	Before Extraction	Test	Colorless	Clear	No
		Control	Colorless	Clear	No
	After Extraction	Test	Colorless	Clear	No
		Control	Colorless	Clear	No
	Prior to Use	Test	Colorless	Clear	No
		Control	Colorless	Clear	No
SO	Before Extraction	Test	Colorless	Clear	No
		Control	Colorless	Clear	No
	After Extraction	Test	Colorless	Clear	No
		Control	Colorless	Clear	No
	Prior to Use	Test	Colorless	Clear	No
		Control	Colorless	Clear	No
AS	Before Extraction	Test	Colorless	Clear	No
		Control	Colorless	Clear	No
	After Extraction	Test	Colorless	Clear	No
		Control	Colorless	Clear	No
	Prior to Use	Test	Colorless	Clear	No
		Control	Colorless	Clear	No
PEG	Before Extraction	Test	Colorless	Clear	No
		Control	Colorless	Clear	No
	After Extraction	Test	Colorless	Clear	No
		Control	Colorless	Clear	No
Diluted PEG	After Dilution	Test	Colorless	Clear	No
		Control	Colorless	Clear	No
	Prior to Use	Test	Colorless	Clear	No
		Control	Colorless	Clear	No

The test article remained visually unchanged following the extraction process. The PEG test article extract and control extract were diluted with saline to yield a 120 mg PEG/mL concentration before dosing the animal. The extracts were stored at room temperature for less than 5 hours prior to dosing. The extracts were not centrifuged, filtered, or otherwise altered prior to dosing.

5.2 Test Procedure

Prior to treatment, each animal was identified and weighed. Within a 4 to 18 hour period before treatment, each animal was clipped free of fur from the back and both sides of the spinal column to yield a sufficient injection area. Three animals were prepared per pair of extracts. A 0.2 mL dose of the appropriate test article extract was injected by the intracutaneous route into five separate sites on the right side of the back of each animal. Similarly, the corresponding control was injected on the left side of the back of each animal. Injections were spaced approximately 2 cm apart.

The appearance of each injection site was noted immediately after injection. The animals were returned to their respective cages following the procedure.

Observations for erythema and edema were conducted at 24, 48, and 72 hours after injection. Reactions were scored on a 0 to 4 basis. Any reactions at the injection sites were also noted. The reactions were evaluated according to the following subjective rating scale:

Table 5: Test Scoring

Score	Erythema (ER)	Edema (ED)
0	No erythema	No edema
1	Very slight erythema (barely perceptible)	Very slight edema (barely perceptible)
2	Well-defined erythema	Well-defined edema (edges of area well-defined by definite raising)
3	Moderate erythema	Moderate edema (raised approximately 1 mm)
4	Severe erythema (beet redness) to eschar formation preventing grading of erythema	Severe edema (raised more than 1 mm, and extending beyond exposure area)

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

No statistical analysis of the data was performed. All erythema grades and edema grades (24, 48 and 72 hours) were calculated separately for each test and control for each individual animal. The score of a test article or control on each individual animal was calculated by dividing each of the totals by 15 (3 scoring time points x 5 sites). The overall mean for each test and control was determine by adding the scores for the 3 animals and dividing by 3. The difference between the overall mean score of the test article extracts and corresponding control extracts was calculated by subtracting the overall mean score for the control from the overall mean score for the test article extract. If the overall mean score of the test article extracts was less than the overall mean score of the corresponding control extracts, 0.0 was recorded for the overall mean difference between test and control.

The ISO and USP requirements of the test were met when the difference between the test article extract overall mean score and the corresponding control overall mean score was 1.0 or less. When at any observation period the average reaction to the test article extract was questionably greater than the average reaction to the control, the test was repeated using three additional rabbits.

Ischemia or necrosis present at the majority of the test sites of both animals for any scoring interval was considered as significant regardless of the calculated result. The test article failed when either of these findings were observed at the majority of the test sites of all animals.

7. Results

All animals appeared normal throughout the study. Results of erythema and edema scores for individual animals are presented in Appendix 1. All injection sites appeared normal immediately following injection. The overall mean difference for the extracts is summarized below:

Table 6: Mean Erythema and Edema Scores

Extract	Overall Test Group Mean	Overall Control Group Mean	Overall Mean Difference (Test - Control)
SC	0.0	0.0	0.0
SO	0.7	0.7	0.0
AS	0.0	0.0	0.0
PEG	0.0	0.0	0.0

8. Conclusion

The test article met the requirements of the test since the difference between each test article extract overall mean score and corresponding control extract overall mean score was 0.0, 0.0, 0.0 and 0.0 for the SC, SO, AS and PEG test article extracts, respectively.

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. 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).

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.

United States Pharmacopeia 40, National Formulary 35 (USP), General Chapter, <88> Biological Reactivity Tests, In Vivo (2017).

Appendix 1 - ISO Intracutaneous Observations

Extract	Animal Number	Sex	Body Weight (kg)	Scoring Interval											
				24 Hours				48 Hours				72 Hours			
				Test		Control		Test		Control		Test		Control	
				ER	ED	ER	ED	ER	ED	ER	ED	ER	ED	ER	ED
SC	24141	Male	2.6	0	0	0	0	0	0	0	0	0	0	0	0
				0	0	0	0	0	0	0	0	0	0	0	0
				0	0	0	0	0	0	0	0	0	0	0	0
				0	0	0	0	0	0	0	0	0	0	0	0
				0	0	0	0	0	0	0	0	0	0	0	0
SC	24142	Male	2.7	0	0	0	0	0	0	0	0	0	0	0	0
				0	0	0	0	0	0	0	0	0	0	0	0
				0	0	0	0	0	0	0	0	0	0	0	0
				0	0	0	0	0	0	0	0	0	0	0	0
				0	0	0	0	0	0	0	0	0	0	0	0
SC	24143	Male	2.6	0	0	0	0	0	0	0	0	0	0	0	0
				0	0	0	0	0	0	0	0	0	0	0	0
				0	0	0	0	0	0	0	0	0	0	0	0
				0	0	0	0	0	0	0	0	0	0	0	0
				0	0	0	0	0	0	0	0	0	0	0	0
SO	24141	Male	2.6	1	0	1	0	0	0	1	0	0	0	1	0
				1	0	1	0	0	0	0	0	0	0	0	0
				1	0	1	0	1	0	0	0	1	0	0	0
				1	0	1	0	0	0	0	0	0	0	0	0
				1	0	1	0	0	0	0	0	0	0	1	0
SO	24142	Male	2.7	1	0	1	0	1	0	0	0	1	0	0	0
				1	0	1	0	1	0	0	0	1	0	1	0
				1	0	1	0	1	0	1	0	1	0	1	0
				1	0	1	0	1	0	1	0	1	0	1	0
				1	0	1	0	1	0	1	0	0	0	1	0
SO	24143	Male	2.6	1	0	1	0	0	0	1	0	0	0	1	0
				1	0	1	0	1	0	1	0	1	0	1	0
				1	0	1	0	1	0	1	0	1	0	1	0
				1	0	1	0	0	0	0	0	1	0	1	0
				1	0	1	0	0	0	0	0	1	0	1	0

ER = Erythema
ED = Edema

Appendix 1 (continued) - ISO Intracutaneous Observations

Extract	Animal Number	Sex	Body Weight (kg)	Scoring Interval											
				24 Hours				48 Hours				72 Hours			
				Test		Control		Test		Control		Test		Control	
				ER	ED	ER	ED	ER	ED	ER	ED	ER	ED	ER	ED
AS	24145	Male	2.7	0	0	0	0	0	0	0	0	0	0	0	0
				0	0	0	0	0	0	0	0	0	0	0	0
				0	0	0	0	0	0	0	0	0	0	0	0
				0	0	0	0	0	0	0	0	0	0	0	0
				0	0	0	0	0	0	0	0	0	0	0	0
AS	24146	Male	3.0	0	0	0	0	0	0	0	0	0	0	0	0
				0	0	0	0	0	0	0	0	0	0	0	0
				0	0	0	0	0	0	0	0	0	0	0	0
				0	0	0	0	0	0	0	0	0	0	0	0
				0	0	0	0	0	0	0	0	0	0	0	0
AS	24147	Male	2.6	0	0	0	0	0	0	0	0	0	0	0	0
				0	0	0	0	0	0	0	0	0	0	0	0
				0	0	0	0	0	0	0	0	0	0	0	0
				0	0	0	0	0	0	0	0	0	0	0	0
				0	0	0	0	0	0	0	0	0	0	0	0
PEG	24145	Male	2.7	0	0	0	0	0	0	0	0	0	0	0	0
				0	0	0	0	0	0	0	0	0	0	0	0
				0	0	0	0	0	0	0	0	0	0	0	0
				0	0	0	0	0	0	0	0	0	0	0	0
				0	0	0	0	0	0	0	0	0	0	0	0
PEG	24146	Male	3.0	0	0	0	0	0	0	0	0	0	0	0	0
				0	0	0	0	0	0	0	0	0	0	0	0
				0	0	0	0	0	0	0	0	0	0	0	0
				0	0	0	0	0	0	0	0	0	0	0	0
				0	0	0	0	0	0	0	0	0	0	0	0
PEG	24147	Male	2.6	0	0	0	0	0	0	0	0	0	0	0	0
				0	0	0	0	0	0	0	0	0	0	0	0
				0	0	0	0	0	0	0	0	0	0	0	0
				0	0	0	0	0	0	0	0	0	0	0	0
				0	0	0	0	0	0	0	0	0	0	0	0

ER = Erythema
ED = Edema

Polycarbonate Class VI USP Certificate

NAMSA

CONFIDENTIAL

CERTIFICATE OF COMPLIANCE

PEOPLE > SCIENCE > SOLUTIONS

Test Facility
6750 Wales Road
Northwood, OH 43619
419.666.9455

TEST ARTICLE NAME
Polycarbonate, Lexan HPS1-1H1124

TEST ARTICLE IDENTIFICATION
24235560

TEST ARTICLE PHYSICAL DESCRIPTION
Part # 2471700

TEST ARTICLE RECEIVED
December 19, 2017

SPONSOR
Kayla Vangsgard
Colder Products Company
1001 Westgate Drive
St. Paul, MN 55114

USP Biological Reactivity Tests, *In Vivo*
USP Plastic Class VI

USP & ISO Systemic Toxicity Study in the Mouse
The test article was prepared as indicated below and injected into mice. The saline, alcohol in saline, polyethylene glycol 400 and sesame oil extracts did not produce a significantly greater systemic reaction than the blank extractants.

USP & ISO Intracutaneous Toxicity Study in the Rabbit
The test article was prepared as indicated below and injected intracutaneously into rabbits. The saline, alcohol in saline, polyethylene glycol 400 and sesame oil extracts did not produce a significantly greater tissue reaction than the blank extractants.

USP Muscle Implantation Study in the Rabbit
The macroscopic reaction of the test article, implanted in rabbit muscle for 1 week, was not significant when compared to the USP negative control plastic.

The test article was prepared at a ratio of 3 cm^2:1 mL and extracted at 50°C for 72 hours. The test article extracts met the requirements of a USP Plastic Class VI.

APPROVAL Arizona E. Carter, BS, ALAT — Technical Reviewer — 01-29-18 — Date

P.O. No.: 182004705	Lab Number: 18T_20417_03	TCLAS_VI7/S	Page 1 of 1

Polycarbonate Cytotoxicity Study (USP <87>/ISO 10993-5)

REPORT

TEST FACILITY

NAMSA
6750 Wales Road
Northwood, OH 43619
419.666.9455

SPONSOR

Kayla Vangsgard
Colder Products Company
1001 Westgate Drive
St. Paul, MN 55114

CONFIDENTIAL

STUDY TITLE

Cytotoxicity Study Using a Modified USP and ISO Elution Method

TEST ARTICLE NAME

Polycarbonate, Lexan HPS1-1H1124

TEST ARTICLE IDENTIFICATION

24235560

NAMSA

PEOPLE > SCIENCE > SOLUTIONS	P.O. Number 182004705	Lab Number 18T_20417_13	V0835_001 Report	Page 1 of 8

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Summary

The test article, Polycarbonate, Lexan HPS1-1H1124, was evaluated for potential cytotoxic effects using an *in vitro* mammalian cell culture test. This study was conducted following the guidelines of ISO 10993-5, Biological evaluation of medical devices - Part 5: Tests for *in vitro* cytotoxicity and the USP, General Chapter <87>, Biological Reactivity Tests, In Vitro. A single preparation of the test article was extracted in single strength Minimum Essential Medium (1X MEM) at 37°C for 24 hours. The negative control, reagent control, and positive control were similarly prepared. Triplicate monolayers of L-929 mouse fibroblast cells were dosed with each extract and incubated at 37°C in the presence of 5% CO_2 for 48 hours. Following incubation, the monolayers were examined microscopically for abnormal cell morphology and cellular degeneration.

The test article extract showed no evidence of causing cell lysis or toxicity. The test article extract met the requirements of the test since the grade was less than or equal to a grade 2 (mild reactivity).

Supervisory Personnel: Austin M. Zdawczyk, BS, MBA, ALAT
Manager, Biocompatibility

Approved by:

Jennifer N. Plaskey, BS
Senior Technical Reviewer

1-11-18
Date

1. Introduction

1.1 Purpose

The purpose of this study was to determine the potential of a test article to cause cytotoxicity.

1.2 Testing Guidelines

This study was based on the requirements of the International Organization for Standardization 10993-5, Biological evaluation of medical devices - Part 5: Tests for *in vitro* cytotoxicity and the United States Pharmacopeia, National Formulary, General Chapter <87>, Biological Reactivity Tests, In Vitro.

This test was performed under an ISO 13485 certified Quality System, with the test method accredited to the ISO 17025 Standard.

1.3 Dates

Test Article Received:	December 19, 2017
Cells Dosed:	January 6, 2018
Observations Concluded:	January 8, 2018

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:	Polycarbonate, Lexan HPS1-1H1124
Identification:	24235560
Physical Description of the Test Article:	Part # 2471700
Storage Conditions:	Room Temperature

2.1 Control Article (System Suitability)

Negative Control: The test facility provided USP Reference Standard - high density polyethylene (HDPE) for use as the negative control. The purpose of the negative control was to demonstrate background response of the cells.

Reagent Control: A single aliquot of the extraction vehicle without test article for use as the reagent control. The purpose of the reagent control was to demonstrate background response of the cells.

Positive Control: The test facility provided powder-free latex gloves for use as the positive control. The purpose of the positive control was to demonstrate an appropriate test system response.

3. Test System

3.1 Test System and Justification of Test System

Mammalian cell culture monolayer consisting of L-929 mouse fibroblast cells free from mycoplasma (ECACC Catalog No. 85103115) was used. *In vitro* mammalian cell culture studies have been used historically to evaluate cytotoxicity of biomaterials and medical devices

3.2 Test System Management

L-929 mouse fibroblast cells were propagated and maintained in flasks containing 1X MEM at 37°C with 5% carbon dioxide (CO_2). For this study, cells were seeded in 10 cm^2 cell culture wells, labeled with passage number and date, and incubated at 37°C in the presence of 5% CO_2 to obtain subconfluent monolayers of cells prior to use. Aseptic procedures were used in the handling of the cell cultures following approved NAMSA Standard Operating Procedures.

4. Method

4.1 Test and Control Article Preparation

The test article was prepared based on the sponsor supplied surface area of 19.61 cm^2 per test article. Two test articles were included in the preparation. A single preparation of the test article and each of the controls were subjected to the extraction conditions as described below. The extracts were manually agitated during extraction. All extractions were performed in sterile borosilicate glass containers. The 1X MEM extraction method was conducted in the presence of serum to optimize extraction of both polar and non-polar components.

Table 2: Extraction

Article	Extraction Ratio	Article Amount	Volume of Vehicle	Extraction Condition
Test	60 cm^2:20 mL	39.2 cm^2	13 mL	37°C with 5% CO_2 for 24 hours
Negative Control	60 cm^2:20 mL	30 cm^2	10 mL	37°C with 5% CO_2 for 24 hours
Reagent Control	Not Applicable	Not Applicable	10 mL	37°C with 5% CO_2 for 24 hours
Positive Control	120 cm^2:20 mL	60 cm^2	10 mL	37°C with 5% CO_2 for 24 hours

The following table contains a description of the test and control article extract conditions.

Table 3: Condition of Extracts

Vehicle	Time Observed	Extract	Condition of Extracts		
			Color	Clarity	Particulates
1X MEM	Before Extraction	Test Article	Pink	Clear	No
		Negative Control	Pink	Clear	No
		Reagent Control	Pink	Clear	No
		Positive Control	Pink	Clear	No
	After Extraction	Test Article	Pink	Clear	No
		Negative Control	Pink	Clear	No
		Reagent Control	Pink	Clear	No
		Positive Control	Pink	Clear	No

The test article remained visually unchanged following the extraction process. The extracts were tested immediately following extraction. The extracts were not centrifuged, filtered, or otherwise altered prior to dosing.

4.2 Test Procedure

Triplicate culture wells were selected which contained a subconfluent cell monolayer. The growth medium contained in the triplicate cultures was replaced with 2.0 mL of the test extract in each well. Similarly, the growth medium in triplicate 10 cm^2 wells was replaced with 2.0 mL of the reagent control, the negative control and the positive control extracts. The wells of each plate were labeled with the appropriate lab number or control and the replicate number. Each plate was labeled with the test code and the dosing date. The wells were incubated at 37°C in 5% CO_2 for 48 hours.

Following incubation, the cells were examined microscopically (100X) to evaluate cellular characteristics and percent lysis.

Table 4: Test Scoring

Grade	Reactivity	Conditions of all Cultures
0	None	Discrete intracytoplasmic granules, no cell lysis, no reduction of cell growth.
1	Slight	Not more than (less than or equal to) 20% of the cells are round, loosely attached and without intracytoplasmic granules, or show changes in morphology; occasional lysed cells are present; only slight growth inhibition observable.
2	Mild	Not more than 50% (greater than 20% to less than or equal to 50%) of the cells are round, devoid of intracytoplasmic granules; no extensive cell lysis; not more than 50% growth inhibition observable.
3	Moderate	Not more than 70% (greater than 50% to less than or equal to 70%) of the cell layers contain rounded cells or are lysed; cell layers not completely destroyed, but more than 50% growth inhibition observed.
4	Severe	Nearly complete or complete destruction of the cell layers.

The color of the test medium was observed to determine any change in pH. A color shift toward yellow would have indicated an acidic pH range, and a color shift toward magenta to purple would have indicated an alkaline pH range.

For the test to be valid, the reagent control and the negative control must have had a reactivity of none (grade 0) and the positive control must have been a grade 3 or 4. Percent rounding and percent cells without intracytoplasmic granules are not evaluated in the event of 100% lysis. The test article met the requirements of the test if the biological response was less than or equal to grade 2 (mild). The test would have been repeated if the controls did not perform as anticipated.

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.

5. Results

No cytotoxicity or cell lysis was noted in any of the test wells. No pH shift was observed at 48 hours. The reagent control, negative control and the positive control performed as anticipated. The individual reactivity grades are presented in Appendix 1.

6. Conclusion

The test article extract showed no evidence of causing cell lysis or toxicity. The test article extract met the requirements of the test since the grade was less than or equal to a grade 2 (mild reactivity).

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

7. 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.

8. References

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) 10993-5, Biological evaluation of medical devices - Part 5: Tests for *in vitro* cytotoxicity (2009).

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).

United States Pharmacopeia 40, National Formulary 35 (USP), General Chapter <87>, Biological Reactivity Tests, In Vitro (2017).

Appendix 1 - Reactivity Grades For Elution Testing

Well	Percent Rounding	Percent Cells Without Intracytoplasmic Granules	Percent Lysis	Grade	Reactivity
Test (1)	0	0	0	0	None
Test (2)	0	0	0	0	None
Test (3)	0	0	0	0	None
Negative Control (1)	0	0	0	0	None
Negative Control (2)	0	0	0	0	None
Negative Control (3)	0	0	0	0	None
Reagent Control (1)	0	0	0	0	None
Reagent Control (2)	0	0	0	0	None
Reagent Control (3)	0	0	0	0	None
Positive Control (1)	Not Applicable	Not Applicable	100	4	Severe
Positive Control (2)	Not Applicable	Not Applicable	100	4	Severe
Positive Control (3)	Not Applicable	Not Applicable	100	4	Severe

Note: 1, 2 and 3 denote replicates.
Percent rounding and percent cells without intracytoplasmic granules are not evaluated in the event of 100% lysis.

Polycarbonate Physico-Chemical Test (USP <661>)

NAMSA

CONFIDENTIAL REPORT

PEOPLE > SCIENCE > SOLUTIONS

Test Facility
6750 Wales Road
Northwood, OH 43619
419.666.9455

STUDY TITLE
Physicochemical Testing Using a Purified Water Extract

TEST ARTICLE NAME
Polycarbonate, Lexan HPS1-1H1124

TEST ARTICLE IDENTIFICATION
24235560

TEST ARTICLE PHYSICAL DESCRIPTION
Part # 2471700

TEST ARTICLE RECEIVED
December 19, 2017

SPONSOR
Kayla Vangsgard
Colder Products Company
1001 Westgate Drive
St. Paul, MN 55114

PURPOSE
The purpose of this study was to describe the physicochemical attributes as part of the overall characterization of the test article.

RESULTS

	Assay Results
Non-Volatile Residue	1 mg*
Residue on Ignition	≤1 mg**
Heavy Metals	<1 ppm
Buffering Capacity	<1.0 mL

*Reference Deviation
**Based on non-volatile residue results

	Condition of Extracts
Test Article	Clear and colorless with no particulates
Control Blank	Clear and colorless with no particulates

Date Extract Prepared: January 8, 2018 Date Test Concluded: January 16, 2018

METHOD
A 588.3 cm^2 portion (30 pieces) of the test article was rinsed twice with a sufficient volume of purified water to cover the test article and then extracted at 50°C for 72 hours in 98 mL of purified water. A control of purified water was similarly prepared without the test article. Non-volatile residue, residue on ignition, heavy metals, and buffering capacity were determined on the test article extract. The non-volatile residue testing utilized a 50.0 mL portion of the test article extract.

DEVIATION
Because the non-volatile residue (NVR) of the test extract was significantly lower than the control extract, the control NVR was not subtracted from the test NVR, and the reported NVR result is considered worst case scenario. Based on the value of the test NVR, this did not impact the outcome of the study.

COMMENT
This analysis was performed to the testing requirements of USP <661> "Containers – Plastics" 2015 edition. Since this methodology is no longer current with the USP, the results should be considered investigational or used for comparison purposes to previous USP <661> testing.

P.O. No.: 182004705	Lab Number: 18T_20417_12	C0019_000	Page 1 of 2

NAMSA

CONFIDENTIAL REPORT

PEOPLE > SCIENCE > SOLUTIONS

Test Facility
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REFERENCES

United States Pharmacopeia 38, National Formulary 33 (USP), General Chapter <231>, Heavy Metals (2015).

United States Pharmacopeia 38, National Formulary 33 (USP), General Chapter <281>, Residue on Ignition (2015).

United States Pharmacopeia 38, National Formulary 33 (USP), General Chapter <661>, Containers - Plastics (2015).

APPROVAL Margaret LaPlant 25JAN2018

Margaret K. LaPlante, BS — Date
Technical Reviewer, Analytical Services

Results apply only to the test article tested. Any extrapolation of these data to other articles is the sponsor's responsibility. This test was performed under all applicable GMP regulations and an ISO 13485 certified Quality System, with the test method accredited to the ISO 17025 Standard.

Silicone Systemic Toxicity (USP <88>/ISO 10993-11)

REPORT

TEST FACILITY

NAMSA
6750 Wales Road
Northwood, OH 43619
419.666.9455

SPONSOR

Kayla Vangsgard
Colder Products Company
1001 Westgate Drive
St. Paul, MN 55114
United States

CONFIDENTIAL

STUDY TITLE

USP and ISO Acute Systemic Toxicity Study in Mice

TEST ARTICLE NAME

Silicone Lim 6071

TEST ARTICLE IDENTIFICATION

24240091

NAMSA

PEOPLE > SCIENCE > SOLUTIONS	P.O. Number 182004705	Lab Number 18T_20414_04 18T_20414_05 18T_20414_06 18T_20414_07	T0625_500/S Report	Page 1 of 15

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Summary

The test article, Silicone Lim 6071, was evaluated for acute systemic toxicity in mice. This study was conducted based on ISO 10993-11, Biological evaluation of medical devices - Part 11: Tests for systemic toxicity, and the United States Pharmacopeia, National Formulary, General Chapter <88>, Biological Reactivity Tests, In Vivo. The test article was extracted in 0.9% sodium chloride USP solution (SC), sesame oil, NF (SO), alcohol in saline (AS) and polyethylene glycol (PEG). A single dose of the appropriate test article extract was injected into a group of five animals. Similarly, a separate group of five animals was dosed with each corresponding extract vehicle alone (control). The animals were observed for signs of systemic toxicity immediately after injection and at 4, 24, 48 and 72 hours after injection. Body weights were recorded prior to dosing and on days 1, 2 and 3.

There was no mortality or evidence of systemic toxicity from the extracts injected into mice. Each test article extract met the requirements of the study.

Supervisory Personnel: Mark A. Shumaker, MBA, ILAM, LAT
Manager, In Vivo Biocompatibility

Austin M. Zdawczyk, BS, MBA, ALAT
Manager, Biocompatibility

Approved by: Arizona E. Carter, BS, ALAT
Technical Reviewer

01-23-18
Date

1. Introduction

1.1 Purpose

The purpose of this study was to evaluate acute systemic toxicity of a test article extract following a single intravenous or intraperitoneal injection in mice.

1.2 Testing Guidelines

This study was conducted based on the International Organization for Standardization 10993-11, Biological evaluation of medical devices, Part 11: Tests for systemic toxicity, and the United States Pharmacopeia, National Formulary, General Chapter <88>, Biological Reactivity Tests, In Vivo.

This test was performed under an ISO 13485 certified Quality System, with the test method accredited to the ISO 17025 Standard.

1.3 Dates

Test Article Received:	December 19, 2017
Treatment Started:	January 11, 2018
Observations Concluded:	January 14, 2018

1.4 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. 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:	Silicone Lim 6071
Identification:	24240091
Physical Description of the Test Article:	Part # 1437000
Storage Conditions:	Room Temperature

Table 2: Control Articles

Name:	0.9% sodium chloride USP solution (SC) Sesame oil, NF (SO) Alcohol in saline 1:20 solution (AS) Polyethylene glycol 400 (PEG)
Strength, Purity, Composition or Other Characteristics:	SC: Purity: Meets requirements of USP Sodium Chloride for Injection and is certified as USP Grade; Composition: 0.9% NaCl ± 5.0% of label claim, balance is water; sodium chloride CAS No.: 7647-14-5/water CAS No.: 7732-18-5 SO: Purity: Meets the requirements of National Formulary. Composition: CAS No.: 8008-74-0 AS: Composition: ethanol in saline 1:20; ethanol CAS No.: 64-17-5/sodium chloride CAS No.: 7647-14-5/water CAS No.: 7732-18-5 PEG: Identity: Matches infrared spectrum of polyethylene glycol 400 with average molecular weight of 380 to 420; Composition: Neat: CAS No.: 25322-68-3

3. Test System

3.1 Test System

Species:	Mouse (*Mus musculus*)
Strain:	Outbred albino
Source:	Hilltop Lab Animals
Sex:	Male
Body Weight Range:	19 grams to 23 grams at injection
Acclimation Period:	Minimum 1 day
Number of Animals:	Forty
Identification Method:	Ear punch

3.2 Justification of Test System

Mice have historically been used to evaluate biomaterial extracts. The use of albino mice injected with a single intravenous (IV) or intraperitoneal (IP) dose of test article extract or control blank have been suggested in the current USP and ISO standards for evaluation of medical plastics.

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 of five in shoebox cages identified by a card indicating the lab number, animal numbers, test code, sex, animal code and date dosed.

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 rodent feed, PROLAB RMH 1000 - 5P07, 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.

4.1 Sedation, Analgesia or Anesthesia

It has been determined that the use of sedation, analgesia or anesthesia was not necessary during the routine course of this procedure.

4.2 Veterinary Care

Standard veterinary medical care was provided in this study.

4.3 IACUC

The procedures for this study were approved by the NAMSA Institutional Animal Care and Use Committee (IACUC) prior to conduct.

4.4 Selection

Only healthy, previously unused animals were selected.

5. Method

5.1 Test and Control Article Preparation

The test article and the control blank (extraction vehicle without the test article) were subjected to the extraction conditions as described below. The extracts were continuously agitated during extraction.

Table 3: Extraction

Vehicle	Extraction Ratio	Article Amount	Volume of Vehicle	Extraction Condition
SC	3 cm^2:1 mL	34.8 cm^2	12 mL	50°C for 72 hours
SO	3 cm^2:1 mL	34.8 cm^2	12 mL	50°C for 72 hours
AS	3 cm^2:1 mL	34.8 cm^2	12 mL	50°C for 72 hours
PEG	3 cm^2:1 mL	34.8 cm^2	12 mL	50°C for 72 hours

The following table contains a description of the test and control article extract conditions.

Table 4: Condition of Extracts

Vehicle	Time Observed	Extract	Condition of Extracts		
			Color	Clarity	Particulates
SC	Before Extraction	Test	Colorless	Clear	No
		Control	Colorless	Clear	No
	After Extraction	Test	Colorless	Clear	No
		Control	Colorless	Clear	No
	Prior to Use	Test	Colorless	Clear	No
		Control	Colorless	Clear	No
SO	Before Extraction	Test	Colorless	Clear	No
		Control	Colorless	Clear	No
	After Extraction	Test	Colorless	Clear	No
		Control	Colorless	Clear	No
	Prior to Use	Test	Colorless	Clear	No
		Control	Colorless	Clear	No
AS	Before Extraction	Test	Colorless	Clear	No
		Control	Colorless	Clear	No
	After Extraction	Test	Colorless	Clear	No
		Control	Colorless	Clear	No
	Prior to Use	Test	Colorless	Clear	No
		Control	Colorless	Clear	No
PEG	Before Extraction	Test	Colorless	Clear	No
		Control	Colorless	Clear	No
	After Extraction	Test	Colorless	Clear	No
		Control	Colorless	Clear	No
Diluted PEG	After Dilution	Test	Colorless	Clear	No
		Control	Colorless	Clear	No
	Prior to Use	Test	Colorless	Clear	No
		Control	Colorless	Clear	No

The test article remained visually unchanged following the extraction process. The PEG test article extract and control were diluted with saline to yield a 200 mg PEG/mL concentration before dosing the animals. The extracts were stored at room temperature for less than 3 hours prior to dosing. The extracts were not centrifuged, filtered, or otherwise altered prior to dosing.

5.2 Test Procedure

Prior to dosing, the animals were individually identified, weighed and arbitrarily assigned to a treatment group as shown below:

Table 5: Treatment Group Assignment

Extract	Treatment Group	Number of Animals	Sex	Dose	Route of Administration
AS	Test	5	Male	50 mL/kg	Intravenous
	Control	5	Male	50 mL/kg	Intravenous
PEG	Test	5	Male	10 g/kg	Intraperitoneal
	Control	5	Male	10 g/kg	Intraperitoneal
SC	Test	5	Male	50 mL/kg	Intravenous
	Control	5	Male	50 mL/kg	Intravenous
SO	Test	5	Male	50 mL/kg	Intraperitoneal
	Control	5	Male	50 mL/kg	Intraperitoneal

A single dose of each test article extract was injected into each animal in the test group. Each control blank was similarly injected into each animal in the control group. Dosing occurred on day 0. Animals were observed for any adverse clinical reactions immediately after injection. The animals were then returned to their cages. The animals were observed for signs of systemic reactions at 4, 24, 48 and 72 hours after injection. The animals were weighed daily for three days after dosing. After the test was completed, all animals were euthanized according to an IACUC approved NAMSA procedure.

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

No statistical analysis of the data was performed. If during the observation period none of the animals treated with the test extract exhibited a significantly greater reaction than the corresponding control animals, then the test article met the ISO and USP requirements. If two or more animals died, or if abnormal behavior such as convulsions or prostration occurred in two or more animals, or if body weight loss greater than 2 grams occurred in three or more animals, the test article did not meet the ISO and USP requirements.

7. Results

7.1 Mortality Data

There was no mortality during the study. The mortality data are presented in Table 1 in the appendices.

7.2 Clinical Observations

The test and control animals injected with AS appeared lethargic immediately after the injection; this was considered an expected pharmacological effect due to the alcohol content of the extract. All animals were clinically normal throughout the study. The clinical observations are presented in Table 2 in the appendices.

7.3 Body Weight

Body weight data were acceptable. Body weight data are presented in Table 3 in the appendices.

8. Conclusion

There was no mortality or evidence of systemic toxicity from the extracts injected into mice. Each test article extract met the requirements of the study.

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. 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) 10993-11, Biological evaluation of medical devices - Part 11: Tests for systemic toxicity (2017).

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

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.

United States Pharmacopeia 40, National Formulary 35 (USP), General Chapter <88>, Biological Reactivity Tests, In Vivo (2017).

Appendix 1 - Observations - AS Extract

Table 1: Mortality Data

Extract	Treatment Group	Number Dead/Number Tested
AS	Test Extract	0/5
	Control Blank	0/5

Table 2: Clinical Observations

			Observation				
Extract	Treatment Group	Animal Number	Immediate	4 Hours	24 Hours	48 Hours	72 Hours
AS	Test Extract	81	Lethargic	Normal	Normal	Normal	Normal
		82	Lethargic	Normal	Normal	Normal	Normal
		83	Lethargic	Normal	Normal	Normal	Normal
		84	Lethargic	Normal	Normal	Normal	Normal
		85	Lethargic	Normal	Normal	Normal	Normal
	Control Blank	41	Lethargic	Normal	Normal	Normal	Normal
		42	Lethargic	Normal	Normal	Normal	Normal
		43	Lethargic	Normal	Normal	Normal	Normal
		44	Lethargic	Normal	Normal	Normal	Normal
		45	Lethargic	Normal	Normal	Normal	Normal

Table 3: Body Weight Data

			Weight (g)			
Extract	Treatment Group	Animal Number	Day 0	Day 1	Day 2	Day 3
AS	Test Extract	81	21	22	23	25
		82	20	21	23	24
		83	20	20	21	22
		84	19	19	20	20
		85	20	21	21	22
	Control Blank	41	19	21	22	24
		42	20	21	21	23
		43	22	22	23	25
		44	21	21	23	24
		45	20	21	23	24

Appendix 2 - Observations - SC Extract

Table 1: Mortality Data

Extract	Treatment Group	Number Dead/Number Tested
SC	Test Extract	0/5
	Control Blank	0/5

Table 2: Clinical Observations

			Observation				
Extract	Treatment Group	Animal Number	Immediate	4 Hours	24 Hours	48 Hours	72 Hours
SC	Test Extract	71	Normal	Normal	Normal	Normal	Normal
		72	Normal	Normal	Normal	Normal	Normal
		73	Normal	Normal	Normal	Normal	Normal
		74	Normal	Normal	Normal	Normal	Normal
		75	Normal	Normal	Normal	Normal	Normal
	Control Blank	31	Normal	Normal	Normal	Normal	Normal
		32	Normal	Normal	Normal	Normal	Normal
		33	Normal	Normal	Normal	Normal	Normal
		34	Normal	Normal	Normal	Normal	Normal
		35	Normal	Normal	Normal	Normal	Normal

Table 3: Body Weight Data

			Weight (g)			
Extract	Treatment Group	Animal Number	Day 0	Day 1	Day 2	Day 3
SC	Test Extract	71	20	21	23	25
		72	20	21	22	23
		73	20	22	23	25
		74	21	21	23	25
		75	20	21	23	25
	Control Blank	31	20	21	22	24
		32	21	22	23	24
		33	21	22	24	25
		34	21	23	24	25
		35	22	23	24	25

Appendix 3 - Observations - PEG Extract

Table 1: Mortality Data

Extract	Treatment Group	Number Dead/Number Tested
PEG	Test Extract	0/5
	Control Blank	0/5

Table 2: Clinical Observations

			Observation				
Extract	Treatment Group	Animal Number	Immediate	4 Hours	24 Hours	48 Hours	72 Hours
PEG	Test Extract	86	Normal	Normal	Normal	Normal	Normal
		87	Normal	Normal	Normal	Normal	Normal
		88	Normal	Normal	Normal	Normal	Normal
		89	Normal	Normal	Normal	Normal	Normal
		90	Normal	Normal	Normal	Normal	Normal
	Control Blank	46	Normal	Normal	Normal	Normal	Normal
		47	Normal	Normal	Normal	Normal	Normal
		48	Normal	Normal	Normal	Normal	Normal
		49	Normal	Normal	Normal	Normal	Normal
		50	Normal	Normal	Normal	Normal	Normal

Table 3: Body Weight Data

			Weight (g)			
Extract	Treatment Group	Animal Number	Day 0	Day 1	Day 2	Day 3
PEG	Test Extract	86	20	21	23	24
		87	21	22	23	24
		88	21	22	24	25
		89	21	22	24	25
		90	22	23	25	26
	Control Blank	46	20	22	24	26
		47	21	22	23	25
		48	21	22	24	26
		49	20	22	23	25
		50	20	21	22	23

Appendix 4 - Observations - SO Extract

Table 1: Mortality Data

Extract	Treatment Group	Number Dead/Number Tested
SO	Test Extract	0/5
	Control Blank	0/5

Table 2: Clinical Observations

			Observation				
Extract	Treatment Group	Animal Number	Immediate	4 Hours	24 Hours	48 Hours	72 Hours
SO	Test Extract	76	Normal	Normal	Normal	Normal	Normal
		77	Normal	Normal	Normal	Normal	Normal
		78	Normal	Normal	Normal	Normal	Normal
		79	Normal	Normal	Normal	Normal	Normal
		80	Normal	Normal	Normal	Normal	Normal
	Control Blank	36	Normal	Normal	Normal	Normal	Normal
		37	Normal	Normal	Normal	Normal	Normal
		38	Normal	Normal	Normal	Normal	Normal
		39	Normal	Normal	Normal	Normal	Normal
		40	Normal	Normal	Normal	Normal	Normal

Table 3: Body Weight Data

			Weight (g)			
Extract	Treatment Group	Animal Number	Day 0	Day 1	Day 2	Day 3
SO	Test Extract	76	21	23	23	25
		77	20	21	23	24
		78	20	22	23	24
		79	20	22	23	24
		80	20	21	22	24
	Control Blank	36	20	21	23	25
		37	22	23	24	24
		38	22	23	24	25
		39	22	24	25	27
		40	20	21	22	25

Silicone Intracutaneous Injection (USP <88>/ISO 10993-10)

REPORT

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CONFIDENTIAL

STUDY TITLE

USP and ISO Intracutaneous Study in Rabbits

TEST ARTICLE NAME

Silicone Lim 6071

TEST ARTICLE IDENTIFICATION

24240091

NAMSA

PEOPLE > SCIENCE > SOLUTIONS	P.O. Number 182004705	Lab Number 18T_20414_08 18T_20414_09 18T_20414_10 18T_20414_11	TI251_800/S Report	Page 1 of 13

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Summary

The test article, Silicone Lim 6071, was evaluated for the potential to cause irritation following intracutaneous injection in rabbits. 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, and United States Pharmacopeia, National Formulary, General Chapter <88>, Biological Reactivity Tests, In Vivo. The test article was extracted in 0.9% sodium chloride USP solution (SC), sesame oil, NF (SO), alcohol in saline (AS) and polyethylene glycol (PEG). A 0.2 mL dose of the appropriate test article extract was injected intracutaneously into five separate sites on the right side of the back of each of three animals. Similarly, the extract vehicle alone (control) was injected on the left side of the back of each animal. The injection sites were observed immediately after injection. Observations for erythema and edema were conducted at 24, 48, and 72 hours after injection.

The test article met the requirements of the test since the difference between each test article extract overall mean score and corresponding control extract overall mean score was 0.0, 0.0, 0.0 and 0.0 for the SC, SO, AS and PEG test article extracts, respectively.

Supervisory Personnel: Mark A. Shumaker, MBA, ILAM, LAT
Manager, In Vivo Biocompatibility

Austin M. Zdawczyk, BS, MBA, ALAT
Manager, Biocompatibility

Approved by: Virginia Lynne Carter 01-25-18
Arizona E. Carter, BS, ALAT — Date
Technical Reviewer

1. Introduction

1.1 Purpose

The purpose of this study was to evaluate the local dermal irritation of a test article extract following intracutaneous injection in rabbits.

1.2 Testing Guidelines

This study will be conducted based on the International Organization for Standardization 10993-10, Biological evaluation of medical devices - Part 10: Tests for irritation and skin sensitization, and United States Pharmacopeia, National Formulary, General Chapter <88>, Biological Reactivity Tests, In Vivo.

This test was performed under an ISO 13485 certified Quality System, with the test method accredited to the ISO 17025 Standard.

1.3 Dates

Test Article Received:	December 19, 2017
Treatment Started:	January 11, 2018
Observations Concluded:	January 14, 2018

1.4 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. 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:	Silicone Lim 6071
Identification:	24240091
Physical Description of the Test Article:	Part # 1437000
Storage Conditions:	Room Temperature

Table 2: Control Articles/Extraction Vehicles

Name:	0.9% sodium chloride USP solution (SC) Sesame oil, NF (SO) Alcohol in saline 1:20 solution (AS) Polyethylene glycol 400 (PEG)
Strength, Purity, Composition or Other Characteristics:	SC: Purity: Meets requirements of USP Sodium Chloride for Injection and is certified as USP Grade; Composition: 0.9% NaCl ± 5.0% of label claim, balance is water; sodium chloride CAS No.: 7647-14-5/water CAS No.: 7732-18-5 SO: Purity: Meets the requirements of National Formulary. Composition: CAS No.: 8008-74-0 AS: Composition: ethanol in saline 1:20; ethanol CAS No.: 64-17-5/sodium chloride CAS No.: 7647-14-5/water CAS No.: 7732-18-5 PEG: Identity: Matches infrared spectrum of polyethylene glycol 400 with average molecular weight of 380 to 420; Composition: Neat: CAS No.: 25322-68-3

3. Test System

3.1 Test System

Species:	Rabbit (*Oryctolagus cuniculus*)
Breed:	New Zealand White
Source:	Robinson Services, Inc.
Sex:	Five male, one female; females were nulliparous and nonpregnant
Body Weight Range:	2.6 kg to 3.0 kg at selection
Age:	Young adult
Acclimation Period:	Minimum 5 days
Number of Animals:	Six
Identification Method:	Ear tag

3.2 Justification of Test System

The intracutaneous injection test in rabbits is specified in the current USP and ISO testing standards and has been used historically to evaluate biomaterial extracts.

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 individually housed in stainless steel or plastic suspended cages identified by a card indicating the lab number, animal number, test code, sex, and date dosed.

The animal housing room temperature and relative humidity were monitored daily. The temperature for the room was set to 61-72°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 rabbit feed, Laboratory Rabbit Diet – 5326, 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.

4.1 Sedation, Analgesia or Anesthesia

It has been determined that the use of sedation, analgesia or anesthesia was not necessary during the routine course of this procedure.

4.2 Veterinary Care

Standard veterinary medical care was provided in this study.

4.3 IACUC

The procedures for this study were approved by the NAMSA Institutional Animal Care and Use Committee (IACUC) prior to conduct.

4.4 Selection

Only healthy, previously unused, thin-skinned animals free of mechanical irritation or trauma that could interfere with the test were selected.

5. Method

5.1 Test and Control Article Preparation

The test article and the control blank (extraction vehicle without the test article) were subjected to the extraction conditions as described below. The extracts were continuously agitated during extraction.

Table 3: Extraction

Vehicle	Extraction Ratio	Article Amount	Volume of Vehicle	Extraction Condition
SC	3 cm^2:1 mL	31.3 cm^2	10 mL	50°C for 72 hours
SO	3 cm^2:1 mL	31.3 cm^2	10 mL	50°C for 72 hours
AS	3 cm^2:1 mL	31.3 cm^2	10 mL	50°C for 72 hours
PEG	3 cm^2:1 mL	31.3 cm^2	10 mL	50°C for 72 hours

The following table contains a description of the test and control article extract conditions.

Table 4: Condition of Extracts

Vehicle	Time Observed	Extract	Condition of Extracts		
			Color	Clarity	Particulates
SC	Before Extraction	Test	Colorless	Clear	No
		Control	Colorless	Clear	No
	After Extraction	Test	Colorless	Clear	No
		Control	Colorless	Clear	No
	Prior to Use	Test	Colorless	Clear	No
		Control	Colorless	Clear	No
SO	Before Extraction	Test	Colorless	Clear	No
		Control	Colorless	Clear	No
	After Extraction	Test	Colorless	Clear	No
		Control	Colorless	Clear	No
	Prior to Use	Test	Colorless	Clear	No
		Control	Colorless	Clear	No
AS	Before Extraction	Test	Colorless	Clear	No
		Control	Colorless	Clear	No
	After Extraction	Test	Colorless	Clear	No
		Control	Colorless	Clear	No
	Prior to Use	Test	Colorless	Clear	No
		Control	Colorless	Clear	No
PEG	Before Extraction	Test	Colorless	Clear	No
		Control	Colorless	Clear	No
	After Extraction	Test	Colorless	Clear	No
		Control	Colorless	Clear	No
Diluted PEG	After Dilution	Test	Colorless	Clear	No
		Control	Colorless	Clear	No
	Prior to Use	Test	Colorless	Clear	No
		Control	Colorless	Clear	No

The test article remained visually unchanged following the extraction process. The PEG test article extract and control extract were diluted with saline to yield a 120 mg PEG/mL concentration before dosing the animal. The extracts were stored at room temperature for less than 6 hours prior to dosing. The extracts were not centrifuged, filtered, or otherwise altered prior to dosing.

5.2 Test Procedure

Prior to treatment, each animal was identified and weighed. Within a 4 to 18 hour period before treatment, each animal was clipped free of fur from the back and both sides of the spinal column to yield a sufficient injection area. Three animals were prepared per pair of extracts. A 0.2 mL dose of the appropriate test article extract was injected by the intracutaneous route into five separate sites on the right side of the back of each animal. Similarly, the corresponding control was injected on the left side of the back of each animal. Injections were spaced approximately 2 cm apart.

The appearance of each injection site was noted immediately after injection. The animals were returned to their respective cages following the procedure.

Observations for erythema and edema were conducted at 24, 48, and 72 hours after injection. Reactions were scored on a 0 to 4 basis. Any reactions at the injection sites were also noted. The reactions were evaluated according to the following subjective rating scale:

Table 5: Test Scoring

Score	Erythema (ER)	Edema (ED)
0	No erythema	No edema
1	Very slight erythema (barely perceptible)	Very slight edema (barely perceptible)
2	Well-defined erythema	Well-defined edema (edges of area well-defined by definite raising)
3	Moderate erythema	Moderate edema (raised approximately 1 mm)
4	Severe erythema (beet redness) to eschar formation preventing grading of erythema	Severe edema (raised more than 1 mm, and extending beyond exposure area)

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

No statistical analysis of the data was performed. All erythema grades and edema grades (24, 48 and 72 hours) were calculated separately for each test and control for each individual animal. The score of a test article or control on each individual animal was calculated by dividing each of the totals by 15 (3 scoring time points x 5 sites). The overall mean for each test and control was determine by adding the scores for the 3 animals and dividing by 3. The difference between the overall mean score of the test article extracts and corresponding control extracts was calculated by subtracting the overall mean score for the control from the overall mean score for the test article extract. If the overall mean score of the test article extracts was less than the overall mean score of the corresponding control extracts, 0.0 was recorded for the overall mean difference between test and control.

The ISO and USP requirements of the test were met when the difference between the test article extract overall mean score and the corresponding control overall mean score was 1.0 or less. When at any observation period the average reaction to the test article extract was questionably greater than the average reaction to the control, the test was repeated using three additional rabbits.

Ischemia or necrosis present at the majority of the test sites of both animals for any scoring interval was considered as significant regardless of the calculated result. The test article failed when either of these findings were observed at the majority of the test sites of all animals.

7. Results

All animals appeared normal throughout the study. Results of erythema and edema scores for individual animals are presented in Appendix 1. All injection sites appeared normal immediately following injection. The overall mean difference for the extracts is summarized below:

Table 6: Mean Erythema and Edema Scores

Extract	Overall Test Group Mean	Overall Control Group Mean	Overall Mean Difference (Test - Control)
SC	0.0	0.0	0.0
SO	0.2	0.2	0.0
AS	0.0	0.0	0.0
PEG	0.0	0.0	0.0

8. Conclusion

The test article met the requirements of the test since the difference between each test article extract overall mean score and corresponding control extract overall mean score was 0.0, 0.0, 0.0 and 0.0 for the SC, SO, AS and PEG test article extracts, respectively.

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. 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).

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.

United States Pharmacopeia 40, National Formulary 35 (USP), General Chapter, <88> Biological Reactivity Tests, In Vivo (2017).

Appendix 1 - ISO Intracutaneous Observations

Extract	Animal Number	Sex	Body Weight (kg)	Scoring Interval											
				24 Hours				48 Hours				72 Hours			
				Test		Control		Test		Control		Test		Control	
				ER	ED	ER	ED	ER	ED	ER	ED	ER	ED	ER	ED
SC	24157	Male	2.8	0	0	0	0	0	0	0	0	0	0	0	0
				0	0	0	0	0	0	0	0	0	0	0	0
				0	0	0	0	0	0	0	0	0	0	0	0
				0	0	0	0	0	0	0	0	0	0	0	0
				0	0	0	0	0	0	0	0	0	0	0	0
SC	24158	Male	2.7	0	0	0	0	0	0	0	0	0	0	0	0
				0	0	0	0	0	0	0	0	0	0	0	0
				0	0	0	0	0	0	0	0	0	0	0	0
				0	0	0	0	0	0	0	0	0	0	0	0
				0	0	0	0	0	0	0	0	0	0	0	0
SC	24159	Male	2.8	0	0	0	0	0	0	0	0	0	0	0	0
				0	0	0	0	0	0	0	0	0	0	0	0
				0	0	0	0	0	0	0	0	0	0	0	0
				0	0	0	0	0	0	0	0	0	0	0	0
				0	0	0	0	0	0	0	0	0	0	0	0
SO	24157	Male	2.8	1	0	0	0	0	0	0	0	0	0	0	0
				1	0	0	0	0	0	0	0	0	0	0	0
				0	0	0	0	0	0	0	0	0	0	0	0
				0	0	0	0	0	0	0	0	0	0	0	0
				0	0	0	0	0	0	0	0	0	0	0	0
SO	24158	Male	2.7	1	0	1	0	1	0	0	0	0	0	0	0
				1	0	1	0	0	0	0	0	0	0	0	0
				0	0	1	0	0	0	0	0	0	0	0	0
				1	0	1	0	1	0	0	0	0	0	0	0
				0	0	1	0	0	0	0	0	0	0	0	0
SO	24159	Male	2.8	1	0	1	0	0	0	0	0	0	0	0	0
				1	0	1	0	0	0	0	0	0	0	0	0
				1	0	0	0	0	0	0	0	0	0	0	0
				1	0	1	0	0	0	0	0	0	0	0	0
				1	0	0	0	0	0	0	0	0	0	0	0

ER = Erythema
ED = Edema

Appendix 1 (continued) - ISO Intracutaneous Observations

Extract	Animal Number	Sex	Body Weight (kg)	Scoring Interval											
				24 Hours				48 Hours				72 Hours			
				Test		Control		Test		Control		Test		Control	
				ER	ED	ER	ED	ER	ED	ER	ED	ER	ED	ER	ED
AS	24160	Male	2.8	0	0	0	0	0	0	0	0	0	0	0	0
				0	0	0	0	0	0	0	0	0	0	0	0
				0	0	0	0	0	0	0	0	0	0	0	0
				0	0	0	0	0	0	0	0	0	0	0	0
				0	0	0	0	0	0	0	0	0	0	0	0
AS	24161	Male	3.0	0	0	0	0	0	0	0	0	0	0	0	0
				0	0	0	0	0	0	0	0	0	0	0	0
				0	0	0	0	0	0	0	0	0	0	0	0
				0	0	0	0	0	0	0	0	0	0	0	0
				0	0	0	0	0	0	0	0	0	0	0	0
AS	24164	Female	2.6	0	0	0	0	0	0	0	0	0	0	0	0
				0	0	0	0	0	0	0	0	0	0	0	0
				0	0	0	0	0	0	0	0	0	0	0	0
				0	0	0	0	0	0	0	0	0	0	0	0
				0	0	0	0	0	0	0	0	0	0	0	0
PEG	24160	Male	2.8	0	0	0	0	0	0	0	0	0	0	0	0
				0	0	0	0	0	0	0	0	0	0	0	0
				0	0	0	0	0	0	0	0	0	0	0	0
				0	0	0	0	0	0	0	0	0	0	0	0
				0	0	0	0	0	0	0	0	0	0	0	0
PEG	24161	Male	3.0	0	0	0	0	0	0	0	0	0	0	0	0
				0	0	0	0	0	0	0	0	0	0	0	0
				0	0	0	0	0	0	0	0	0	0	0	0
				0	0	0	0	0	0	0	0	0	0	0	0
				0	0	0	0	0	0	0	0	0	0	0	0
PEG	24164	Female	2.6	0	0	0	0	0	0	0	0	0	0	0	0
				0	0	0	0	0	0	0	0	0	0	0	0
				0	0	0	0	0	0	0	0	0	0	0	0
				0	0	0	0	0	0	0	0	0	0	0	0
				0	0	0	0	0	0	0	0	0	0	0	0

ER = Erythema
ED = Edema

Silicone Muscle Implantation (USP <88>)

REPORT

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STUDY TITLE

USP Muscle Implantation Study in Rabbits - 7 Day

TEST ARTICLE NAME

Silicone Lim 6071

TEST ARTICLE IDENTIFICATION

24240091

NAMSA

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Summary

The test article, Silicone Lim 6071, was implanted in muscle tissue of the rabbit to evaluate the local tissue response. This study was conducted in accordance with the USP, General Chapter <88>, Biological Reactivity Tests, In Vivo.

Implant test articles, location markers and negative control articles were sterilized by steam. The test article along with location markers and negative control were intramuscularly implanted and animals were euthanized 7 days later. Muscle tissues were excised and the implant sites examined macroscopically.

The macroscopic reaction was not significant as compared to the negative control article. The implanted test article met the USP requirements.

Supervisory Personnel: Michelle E. Zdawczyk, MS, ALAT
Manager, Preclinical Functional Studies

Approved by: Arizona Eugene Carter 01-29-18
Arizona E. Carter, BS, ALAT — Date
Technical Reviewer

1. Introduction

1.1 Purpose

The purpose of this study was to evaluate the local tissue response to the test article when implanted in muscle tissue in rabbits.

1.2 Testing Guidelines

This study was based on the United States Pharmacopeia, National Formulary, General Chapter <88>, Biological Reactivity Tests, In Vivo.

This test was performed under an ISO 13485 certified Quality System, with the test method accredited to the ISO 17025 Standard.

1.3 Dates

Test Article Received:	December 19, 2017
Implanted:	January 11, 2018
Explanted:	January 18, 2018

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:	Silicone Lim 6071
Identification:	24240091
Physical Description of the Test Article:	Part # 1437000
Storage Conditions:	Room Temperature

Table 2: Negative Control Article/Location Markers

Name:	USP high density polyethylene reference standard was purchased from the US Pharmacopeial Convention.
Stability Testing:	Marketed product, stability characterized by its labeling
Strength, Purity, Composition or Other Characteristics:	Purity: USP Certified Standard; Composition: polyethylene

3. Test System

3.1 Test System

Species:	Rabbit (*Oryctolagus cuniculus*)
Breed:	New Zealand White
Source:	Robinson Services, Inc.
Sex:	Male
Body Weight Range:	3.0 kg to 3.4 kg at selection
Age:	Young adult
Acclimation Period:	Minimum 5 days
Number of Animals:	Two
Identification Method:	Ear tag

3.2 Justification of Test System

The rabbit is the animal model identified for USP implant testing. The muscle tissue is evaluated because the response to an implanted test article is easily graded and compared to a known negative control article.

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 individually housed in stainless steel or plastic suspended cages identified by a card indicating the lab number, animal number, test code, sex, and date implanted.

The animal housing room temperature and relative humidity were monitored daily. The temperature for the room was set to 61-72°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 rabbit feed, Laboratory Rabbit Diet – 5326, 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 were appropriately qualified and trained.

4.5 Veterinary Care

Standard veterinary medical care was provided in this study.

4.6 IACUC

The procedures for this study were approved by the NAMSA Institutional Animal Care and Use Committee (IACUC) prior to conduct.

4.7 Selection

Healthy animals were selected. To reduce the number of animals used for testing, and to comply with the directives of the NAMSA IACUC, animals on this study may have been used previously in an unrelated test model. Any previously evaluated test or control articles did not cause a response in the animals. Complete history of animal usage is traceable in laboratory records. Animals used for previous evaluations are identified in the report.

5. Method

5.1 Test and Control Article Preparation

The test article was a clear plastic. All rough and/or sharp edges of the test articles, negative control articles, and location markers were trimmed. A minimum of four sections of the test article along with location markers were prepared, per animal. Each test article and location marker was approximately 10 mm x 1 mm x 1 mm, and were loaded into 16 gauge needles. For each animal, a minimum of two negative control articles, each approximately 10 mm x 1 mm x 1 mm, were loaded into the same size needles as used for the test article. Test articles, control articles and location markers were sterilized by steam prior to implantation.

5.2 Test Procedure

No more than 1 day prior to implantation, rabbits were weighed and clipped free of fur over the paravertebral muscles. For analgesia, on the day of implantation, each rabbit was injected subcutaneously with 0.02 mg/kg buprenorphine. For general anesthesia, each rabbit was injected intramuscularly with a mixture of ketamine hydrochloride and xylazine at a dose volume of 0.6 mL/kg. After the anesthetic had taken effect, a non-medicated ophthalmic ointment was applied to both eyes of each rabbit. The surgical site was scrubbed with povidone iodine scrub, wiped with 70% isopropyl alcohol and painted with povidone iodine solution.

One incision was made on each side of the back through the skin and parallel to the lumbar region of the vertebral column. A sterile stylet was placed in the hub of a loaded needle. Approximately 2.5 to 5.0 cm from the midline and parallel to the spinal column, the needle was inserted into the muscle through the incision at an angle until the bevel disappeared, but not deeper than 2.5 cm. The needle was withdrawn over the stylet, leaving the article and location marker in the paravertebral muscle. This was repeated until four test article sections were implanted in the right paravertebral muscle and two negative control sections were implanted in the left paravertebral muscle of each rabbit. The sections were placed at appropriately spaced intervals. The skin was closed with stainless steel wound clips.

Following the procedure, to aid in anesthetic recovery, the rabbits received intramuscular injections of atipamezole dosed at 0.5 mg/kg. The rabbits were monitored for recovery from the anesthetic and returned to their respective cages. Another dose of buprenorphine was administered at the end of the day. On the day following implantation, a third buprenorphine injection was administered.

5.2.1 Laboratory Observations

1. Rabbits were observed daily for general health.
2. Body weights were recorded on the day of implantation and at termination.

5.2.2 Terminal Procedures

After 7 days, the rabbits were weighed and then euthanized by an intravenous injection of a sodium pentobarbital based euthanasia solution. The paravertebral muscles were dissected free and methodically cut to locate four test article sites and two negative control sites in each rabbit. Capsule formation or other evidence of irritation was scored using an auxiliary light source (if needed) and a low magnification instrument. The scores were recorded as follows:

Table 3: Macroscopic Scoring

Score	Encapsulation
0	No capsule, no adverse reaction (other than minimal hemorrhage)
1	Up to 0.5 mm capsule or reaction area
2	0.6 to 1.0 mm capsule or reaction area
3	1.1 to 2.0 mm capsule or reaction area
4	>2.0 mm capsule or reaction area

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 average macroscopic score for test article sites was compared with the average score for control article sites. Calculations were rounded to the nearest 0.1. A difference of scores (test minus control) is regarded as follows:

Table 4: Reaction Index

Average Difference	Reaction Index
0.0 to 0.5	Not significant
0.6 to 1.0	Trace
1.1 to 2.0	Slight
2.1 to 3.0	Moderate
≥3.1	Marked

The requirements of the USP test were met if the difference between test and control score averages was not greater than 1.0. The requirements were not met if the difference between the test and control scores for two (or more) implant sites exceeds 1 for any animal implanted.

7. Results

7.1 Clinical Observations
All animals appeared clinically normal throughout the duration of the study.

7.2 Body Weight Data
Body weight data for individual animals were considered acceptable. Individual body weights are presented in Appendix 1.

7.3 Macroscopic Observations
There was no visible reaction at any test or control site. This resulted in a macroscopic reaction classification of not significant tissue contact irritation. The findings for the macroscopic evaluation are shown in Appendix 1.

8. Conclusion

The implanted test article met the USP requirements.

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. References

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

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

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.

United States Pharmacopeia 40, National Formulary 35 (USP), General Chapter <88>, Biological Reactivity Tests, In Vivo (2017).

Appendix 1 - Body Weights and Macroscopic Observations

Animal Number	Sex	Body Weight (kg)		Test Article	Negative Control
		Day 0	Day 7		
23811	Male	3.0	3.0	0	0
				0	0
				0	[redacted]
				0	
22432*	Male	3.4	3.4	0	0
				0	0
				0	[redacted]
				0	
			Average:	0.0	0

*Previous use history traceable in laboratory records.

Silicone Class VI Certificate

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CERTIFICATE OF COMPLIANCE

PEOPLE > SCIENCE > SOLUTIONS

Test Facility
6750 Wales Road
Northwood, OH 43619
419.666.9455

TEST ARTICLE NAME
Silicone Lim 6071

TEST ARTICLE IDENTIFICATION
24240091

TEST ARTICLE PHYSICAL DESCRIPTION
Part # 1437000

TEST ARTICLE RECEIVED
December 19, 2017

SPONSOR
Kayla Vangsgard
Colder Products Company
1001 Westgate Drive
St. Paul, MN 55114

USP Biological Reactivity Tests, *In Vivo*

USP Plastic Class VI

USP & ISO Systemic Toxicity Study in the Mouse

The test article was prepared as indicated below and injected into mice. The saline, alcohol in saline, polyethylene glycol 400 and sesame oil extracts did not produce a significantly greater systemic reaction than the blank extractants.

USP & ISO Intracutaneous Toxicity Study in the Rabbit

The test article was prepared as indicated below and injected intracutaneously into rabbits. The saline, alcohol in saline, polyethylene glycol 400 and sesame oil extracts did not produce a significantly greater tissue reaction than the blank extractants.

USP Muscle Implantation Study in the Rabbit

The macroscopic reaction of the test article, implanted in rabbit muscle for 1 week, was not significant when compared to the USP negative control plastic.

The test article was prepared at a ratio of 3 cm^2:1 mL and extracted at 50°C for 72 hours. The test article extracts met the requirements of a USP Plastic Class VI.

APPROVAL Arizona Eugene Carter 01-29-18

Arizona E. Carter, BS, ALAT — Date
Technical Reviewer

P.O. No.:	Lab Number:	TCLAS_VI7/S	Page 1 of 1
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Silicone Cytotoxicity Study (USP <87>/ISO 10993-5)

REPORT

TEST FACILITY

NAMSA
6750 Wales Road
Northwood, OH 43619
419.666.9455

SPONSOR

Kayla Vangsgard
Colder Products Company
1001 Westgate Drive
St. Paul, MN 55114

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STUDY TITLE

Cytotoxicity Study Using a Modified USP and ISO Elution Method

TEST ARTICLE NAME

Silicone Lim 6071

TEST ARTICLE IDENTIFICATION

24240091

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Summary

The test article, Silicone Lim 6071, was evaluated for potential cytotoxic effects using an *in vitro* mammalian cell culture test. This study was conducted following the guidelines of ISO 10993-5, Biological evaluation of medical devices - Part 5: Tests for *in vitro* cytotoxicity and the USP, General Chapter <87>, Biological Reactivity Tests, In Vitro. A single preparation of the test article was extracted in single strength Minimum Essential Medium (1X MEM) at 37°C for 24 hours. The negative control, reagent control, and positive control were similarly prepared. Triplicate monolayers of L-929 mouse fibroblast cells were dosed with each extract and incubated at 37°C in the presence of 5% CO_2 for 48 hours. Following incubation, the monolayers were examined microscopically for abnormal cell morphology and cellular degeneration.

The test article extract showed no evidence of causing cell lysis or toxicity. The test article extract met the requirements of the test since the grade was less than or equal to a grade 2 (mild reactivity).

Supervisory Personnel: Austin M. Zdawczyk, BS, MBA, ALAT
Manager, Biocompatibility

Approved by:

Jennifer N. Plaskey, BS
Senior Technical Reviewer

1-11-18
Date

1. Introduction

1.1 Purpose

The purpose of this study was to determine the potential of a test article to cause cytotoxicity.

1.2 Testing Guidelines

This study was based on the requirements of the International Organization for Standardization 10993-5, Biological evaluation of medical devices - Part 5: Tests for *in vitro* cytotoxicity and the United States Pharmacopeia, National Formulary, General Chapter <87>, Biological Reactivity Tests, In Vitro.

This test was performed under an ISO 13485 certified Quality System, with the test method accredited to the ISO 17025 Standard.

1.3 Dates

Test Article Received:	December 19, 2017
Cells Dosed:	January 6, 2018
Observations Concluded:	January 8, 2018

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:	Silicone Lim 6071
Identification:	24240091
Physical Description of the Test Article:	Part # 1437000
Storage Conditions:	Room Temperature

2.1 Control Article (System Suitability)

Negative Control: The test facility provided USP Reference Standard - high density polyethylene (HDPE) for use as the negative control. The purpose of the negative control was to demonstrate background response of the cells.

Reagent Control: A single aliquot of the extraction vehicle without test article for use as the reagent control. The purpose of the reagent control was to demonstrate background response of the cells.

Positive Control: The test facility provided powder-free latex gloves for use as the positive control. The purpose of the positive control was to demonstrate an appropriate test system response.

3. Test System

3.1 Test System and Justification of Test System

Mammalian cell culture monolayer consisting of L-929 mouse fibroblast cells free from mycoplasma (ECACC Catalog No. 85103115) was used. *In vitro* mammalian cell culture studies have been used historically to evaluate cytotoxicity of biomaterials and medical devices

3.2 Test System Management

L-929 mouse fibroblast cells were propagated and maintained in flasks containing 1X MEM at 37°C with 5% carbon dioxide (CO_2). For this study, cells were seeded in 10 cm^2 cell culture wells, labeled with passage number and date, and incubated at 37°C in the presence of 5% CO_2 to obtain subconfluent monolayers of cells prior to use. Aseptic procedures were used in the handling of the cell cultures following approved NAMSA Standard Operating Procedures.

4. Method

4.1 Test and Control Article Preparation

The test article was prepared based on the sponsor supplied surface area of 1.74 cm^2 per test article. Eighteen test articles were included in the preparation. A single preparation of the test article and each of the controls were subjected to the extraction conditions as described below. The extracts were manually agitated during extraction. All extractions were performed in sterile borosilicate glass containers. The 1X MEM extraction method was conducted in the presence of serum to optimize extraction of both polar and non-polar components.

Table 2: Extraction

Article	Extraction Ratio	Article Amount	Volume of Vehicle	Extraction Condition
Test	60 cm^2:20 mL	31.3 cm^2	10 mL	37°C with 5% CO_2 for 24 hours
Negative Control	60 cm^2:20 mL	30 cm^2	10 mL	37°C with 5% CO_2 for 24 hours
Reagent Control	Not Applicable	Not Applicable	10 mL	37°C with 5% CO_2 for 24 hours
Positive Control	120 cm^2:20 mL	60 cm^2	10 mL	37°C with 5% CO_2 for 24 hours

The following table contains a description of the test and control article extract conditions.

Table 3: Condition of Extracts

Vehicle	Time Observed	Extract	Condition of Extracts		
			Color	Clarity	Particulates
1X MEM	Before Extraction	Test Article	Pink	Clear	No
		Negative Control	Pink	Clear	No
		Reagent Control	Pink	Clear	No
		Positive Control	Pink	Clear	No
	After Extraction	Test Article	Pink	Clear	No
		Negative Control	Pink	Clear	No
		Reagent Control	Pink	Clear	No
		Positive Control	Pink	Clear	No

The test article remained visually unchanged following the extraction process. The extracts were tested immediately following extraction. The extracts were not centrifuged, filtered, or otherwise altered prior to dosing.

4.2 Test Procedure

Triplicate culture wells were selected which contained a subconfluent cell monolayer. The growth medium contained in the triplicate cultures was replaced with 2.0 mL of the test extract in each well. Similarly, the growth medium in triplicate 10 cm^2 wells was replaced with 2.0 mL of the reagent control, the negative control and the positive control extracts. The wells of each plate were labeled with the appropriate lab number or control and the replicate number. Each plate was labeled with the test code and the dosing date. The wells were incubated at 37°C in 5% CO_2 for 48 hours.

Following incubation, the cells were examined microscopically (100X) to evaluate cellular characteristics and percent lysis.

Table 4: Test Scoring

Grade	Reactivity	Conditions of all Cultures
0	None	Discrete intracytoplasmic granules, no cell lysis, no reduction of cell growth.
1	Slight	Not more than (less than or equal to) 20% of the cells are round, loosely attached and without intracytoplasmic granules, or show changes in morphology; occasional lysed cells are present; only slight growth inhibition observable.
2	Mild	Not more than 50% (greater than 20% to less than or equal to 50%) of the cells are round, devoid of intracytoplasmic granules; no extensive cell lysis; not more than 50% growth inhibition observable.
3	Moderate	Not more than 70% (greater than 50% to less than or equal to 70%) of the cell layers contain rounded cells or are lysed; cell layers not completely destroyed, but more than 50% growth inhibition observed.
4	Severe	Nearly complete or complete destruction of the cell layers.

The color of the test medium was observed to determine any change in pH. A color shift toward yellow would have indicated an acidic pH range, and a color shift toward magenta to purple would have indicated an alkaline pH range.

For the test to be valid, the reagent control and the negative control must have had a reactivity of none (grade 0) and the positive control must have been a grade 3 or 4. Percent rounding and percent cells without intracytoplasmic granules are not evaluated in the event of 100% lysis. The test article met the requirements of the test if the biological response was less than or equal to grade 2 (mild). The test would have been repeated if the controls did not perform as anticipated.

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.

5. Results

No cytotoxicity or cell lysis was noted in any of the test wells. No pH shift was observed at 48 hours. The reagent control, negative control and the positive control performed as anticipated. The individual reactivity grades are presented in Appendix 1.

6. Conclusion

The test article extract showed no evidence of causing cell lysis or toxicity. The test article extract met the requirements of the test since the grade was less than or equal to a grade 2 (mild reactivity).

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

7. 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.

8. References

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) 10993-5, Biological evaluation of medical devices - Part 5: Tests for *in vitro* cytotoxicity (2009).

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).

United States Pharmacopeia 40, National Formulary 35 (USP), General Chapter <87>, Biological Reactivity Tests, In Vitro (2017).

Appendix 1 - Reactivity Grades For Elution Testing

Well	Percent Rounding	Percent Cells Without Intracytoplasmic Granules	Percent Lysis	Grade	Reactivity
Test (1)	0	0	0	0	None
Test (2)	0	0	0	0	None
Test (3)	0	0	0	0	None
Negative Control (1)	0	0	0	0	None
Negative Control (2)	0	0	0	0	None
Negative Control (3)	0	0	0	0	None
Reagent Control (1)	0	0	0	0	None
Reagent Control (2)	0	0	0	0	None
Reagent Control (3)	0	0	0	0	None
Positive Control (1)	Not Applicable	Not Applicable	100	4	Severe
Positive Control (2)	Not Applicable	Not Applicable	100	4	Severe
Positive Control (3)	Not Applicable	Not Applicable	100	4	Severe

Note: 1, 2 and 3 denote replicates.
Percent rounding and percent cells without intracytoplasmic granules are not evaluated in the event of 100% lysis.

Silicone Elastomeric Closures for Injection (USP <381>)

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REPORT

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Test Facility
6750 Wales Road
Northwood, OH 43619
419.666.9455

STUDY TITLE
USP <381> Elastomeric Closures for Injections

TEST ARTICLE NAME
Silicone Lim 6071

TEST ARTICLE IDENTIFICATION
24240091

TEST ARTICLE PHYSICAL DESCRIPTION
Part # 1437000

TEST ARTICLE RECEIVED
December 19, 2017

SPONSOR
Kayla Vangsgard
Colder Products Company
1001 Westgate Drive
St. Paul, MN 55114

PURPOSE
The purpose of this study was to determine the response of the test article.

RESULTS

Test	Results	Type I / Type II Limits
Appearance of Solution: Determination of Turbidity (Opalescence)	The turbidity of Solution S was less opalescent than Reference Suspension B. The turbidity of Solution S was less opalescent than Reference Suspension C.	Type I: Solution S is no more opalescent than Reference Suspension B. Type II: Solution S is no more opalescent than Reference Suspension C.
Appearance of Solution: Determination of Color	Solution S was less intense in color than the Color Standard.	Solution S is not more intensely colored than the Color Standard.
Acidity or Alkalinity	<0.3 mL of 0.01N NaOH was required to produce a blue color.	≤0.3 mL of 0.01N NaOH is required to produce a blue color, ≤0.8 mL of 0.01N HCl is required to produce a yellow color, or no titration is required.
Absorbance	Maximum absorbance of Solution S is (-) 0.0005494 at AU at 353.9 nm.	Type I: Maximum absorbance of Solution S is ≤0.2 AU between wavelengths 220 nm and 360 nm. Type II: Maximum absorbance of Solution S is ≤4.0 AU between wavelengths 220 nm and 360 nm.
Reducing Substances	The difference between the titration volumes of Solution S and the Blank was 0.15 mL.	Type I: The difference between the titration volumes of Solution S and the Blank is ≤3.0 mL. Type II: The difference between the titration volumes of Solution S and the Blank is ≤7.0 mL.
Heavy Metals	<2 ppm	≤2 ppm
Extractable Zinc*	<0.100 ppm	≤5 ppm
Ammonium	<2 ppm	≤2 ppm

P.O. No.: 182004705 | Lab Number: 18T_20414_12 | C0126_000 | Page 1 of 2

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Test Facility
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Test	Results	Type I / Type II Limits
Volatile Sulfides	The black stain on the paper produced by the Test Solution was less intense than that produced by the Control Solution.	Any black stain on the paper produced by the Test Solution is not more intense than that produced by the Control substance.
Residue on Evaporation**	0.40 mg	No Limit**

*USP indicates to use either an Atomic Absorption (AA) Spectrophotometer or an Inductively Coupled Plasma Optical Emission (ICP-OES) Spectrophotometer/Inductively Coupled Plasma/Mass Spectrophotometer (ICP-MS) for extractable zinc analysis. EP indicates to use an AA for extractable zinc analysis. An ICP with equivalent or greater sensitivity and accuracy was used for the extractable zinc testing. The AA validation activities outlined in EP for the zinc analysis were followed using the ICP and met the EP acceptance criteria, verifying that the use of ICP instead of AA is appropriate for the analysis of extractable zinc per EP.
**There is no applicable USP limit, as Residue on Evaporation is not required per USP <381>.

Date Test Concluded: January 29, 2018

METHOD

The implant portion of the test article was steam sterilized prior to analysis. The standard methodology of USP <381> was followed for this study.

REFERENCE

United States Pharmacopeia 40, National Formulary 35 (USP), General Chapters <381>, Elastomeric Closures for Injections (2017).

APPROVAL Margaret LaPlante 29JAN2018

Margaret K. LaPlante, BS
Technical Reviewer, Analytical Services
Date

Results apply only to the test article tested. Any extrapolation of these data to other articles is the sponsor's responsibility. This test was performed under all applicable GMP regulations and an ISO 13485 certified Quality System.

P.O. No.: 182004705	Lab Number: 18T_20414_12	C0126_000	Page 2 of 2

Other Information

For additional information regarding any of the topics listed below, please refer to the CPC Supplier Qualification Guide:

- Headquarters and Manufacturing Locations
- ISO Certifications
- Shelf Life, Storage, and Shipping Conditions
- Animal Derived Component Free
- Materials of Construction
- Food and Drug Administration (FDA)
- Cleanliness, Bioburden, and Endotoxin
- Biocompatibility and USP Class VI
- Bisphenol A (BPA)
- Residual Solvents (ICH Q3C)
- Residual Metals/Elemental Impurities (ICH Q3D)
- Allergens and Other Chemicals of Concern
- Restriction of Hazardous Substances (RoHS)
- Conflict Minerals
- REACH
- California Proposition 65
- Flow Path Surface Areas and Volumes
- Product Validation Guides
- Sample Certificate of Compliance (CoC)

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Legal Statement

Further Information:

For additional information regarding the MPC Series polycarbonate couplings, please contact Colder Products Company or your bag manufacturer/local distributor.

Colder Warranty Statement: Colder Products Company warrants its products against defects in workmanship and materials a period of 12 months from the date of sale by Colder Products Company to its initial customer (regardless of any subsequent sale of the products). This warranty is void if the product is misused, altered, tampered with or is installed or used in a manner that is inconsistent with Colder Product Company's written recommendations, specifications and/or instructions, or fails to perform due to normal wear and tear. Colder Products Company does not warrant the suitability of the product for any particular application. Determining product application suitability is solely the customer's responsibility. Colder Products Company is not liable for special, indirect, incidental, consequential or other damages including, but not limited to, loss, damage, personal injury, or any other expense directly or indirectly arising from the use of or inability to use its products either separately or in combination with other products. ALL OTHER WARRANTIES EXPRESS OR IMPLIED, WHETHER ORAL, WRITTEN OR IN ANY OTHER FORM, INCLUDING BUT NOT LIMITED TO WARRANTIES OF MERCHANTABILITY OR FITNESS FOR A PARTICULAR PURPOSE, ARE EXPRESSLY EXCLUDED.

Colder Patent Statement: Colder Products Company takes pride in it's innovative quick disconnect coupling solutions, many of which have been awarded United States and International patents. Colder Products Company has a strong tradition of leadership in the quick disconnect market, and aggressively pursues and protects its proprietary information and intellectual property. In cases where it is practical and as a benefit to its customers, Colder Products Company has licensed its proprietary technology. Please contact Colder Products to discuss your unique needs.