MPC39 Series, Polysulfone (White Thumb Latch)

Product Validation Guide

Revision Date:
October 3, 2022

COLDER PRODUCTS COMPANY
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Validation Guide Summary

Colder Product's MPC39 Series, Polysulfone couplings are widely used in bioprocessing and medical device applications. MPC39 couplings are manufactured from USP Class VI animal-free materials. Couplings within this product line include inserts and bodies with hose barbs for ⅛", ¼" and ⅜" internal diameter tubing as well as pressure sealing caps and plugs.

This guide is intended to document specifications of the MPC39 Series, Polysulfone couplings and testing that has been performed on this series. This information is valid for the following part numbers:

Part Number	Description
MPC17002T39	⅛" In-Line Hose Barb Non-valved Coupling Body
MPC17004T39	¼" In-Line Hose Barb Non-valved Coupling Body
MPC17006T39	⅜" In-Line Hose Barb Non-valved Coupling Body
MPCK17002T39	⅛" In-Line Hose Barb Non-valved Coupling Body with Lock
MPCK17004T39	¼" In-Line Hose Barb Non-valved Coupling Body with Lock
MPCK17006T39	⅜" In-Line Hose Barb Non-valved Coupling Body with Lock
MPC22002T39M	⅛" In-Line Hose Barb Non-valved Insert with Silicone Seal
MPC22004T39M	¼" In-Line Hose Barb Non-valved Insert with Silicone Seal
MPC22006T39M	⅜" In-Line Hose Barb Non-valved Insert with Silicone Seal
MPC32039	Sealing Cap
MPCK32039	Sealing Cap with Lock
MPC30039M	Sealing Plug
MPC3301239	¾" Sanitary Non-valved Coupling Body
MPC3301639	1" Sanitary Non-valved Coupling Body
MPC44012T39	¾" Sanitary Non-valved Coupling Insert with Silicone Seal
MPC44024T39	1-1/2" Sanitary Non-valved Coupling Insert with Silicone Seal
MPC17C1739	MPC Back-to-Back Body Adapter
MPC17X1739	MPC to MPX Body Reducer

If you desire additional information on the MPC39 Series, Polysulfone couplings, please contact your Colder Products Company representative.

Summaries:

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

Barrier Challenge: The couplings were tested to ensure that bacteria could not ingress into the flow path of a connected insert and body. The couplings were sterilized at the maximum rating of gamma and autoclave prior to testing.

Biocompatibility Tests: The polysulfone and platinum-cured silicone materials were tested post sterilization to USP Class VI criteria. Tests performed include physicochemical, hemolysis, systemic injection, intracutaneous injection, intramuscular implantation, and MEM elution. The couplings were sterilized by gamma and autoclave prior to testing.

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
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

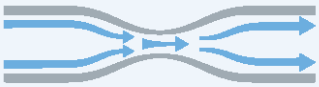
TERMINATIONS
1/8" to 3/8" ID (3.2mm to 9.5mm)

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

Coupling Bodies

MPC17002T39	⅛” In-Line Hose Barb Non-Valved Coupling Body
MPC17004T39	¼" In-Line Hose Barb Non-Valved Coupling Body
MPC17006T39	⅜" In-Line Hose Barb Non-Valved Coupling Body

Coupling Bodies with Locks

MPCK17002T39	⅛" In-Line Hose Barb Non-Valved Coupling Body with Lock
MPCK17004T39	¼" In-Line Hose Barb Non-Valved Coupling Body with Lock
MPCK17006T39	⅜" In-Line Hose Barb Non-Valved Coupling Body with Lock

Coupling Bodies with Sanitary Fittings

MPC3301239	¾” Mini Sanitary Non-Valved Coupling Body
MPC3301639	1” Maxi Sanitary Non-Valved Coupling Body

Coupling Inserts

MPC22002T39M	⅛" In-Line Hose Barb Non-Valved Insert with Silicone Seal
MPC22004T39M	¼" In-Line Hose Barb Non-Valved Insert with Silicone Seal
MPC22006T39M	⅜" In-Line Hose Barb Non-Valved Insert with Silicone Seal

Coupling Inserts with Sanitary Fittings

MPC44012T39M	¾” Sanitary Non-Valved Coupling Insert w/ Silicone Seal
MPC44024T39M	1-1/2” Sanitary Non-Valved Coupling Insert w/ Silicone Seal

Sealing Caps

MPC32039	Sealing Cap
MPCK32039	Sealing Cap with Lock

Sealing Plug

MPC30039M	Sealing Plug

Barrier Challenge – Closure Method

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MG049-000

Lab No. 00C 11708 01
P.O. No. 53787
XP1688S
Revised Page

REVISED REPORT

STUDY TITLE:

BARRIER CHALLENGE - CLOSURE METHOD

TEST ARTICLE:

QUICK-DISCONNECT COUPLERS

IDENTIFICATION NO.:

MPC17006T39 AND MPC22004T39M

(SMALL AMBER COUPLER)

TEST FACILITY:

NAMSA
California Division

SPONSOR:

ERIK LONG
COLDER PRODUCTS COMPANY
1001 WESTGATE DRIVE
ST PAUL, MN 55114

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Ensuring Medical Device Safety and Compliance™

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SUMMARY

The purpose of the study was to determine the integrity of the Quick-Disconnect Coupler, MPC17006T39 And MPC22004T39M (Small Amber Coupler). The Microbial Challenge Test using *Brevundimonas diminuta* (ATCC #19146) was performed to demonstrate that indicator organism cannot ingress through the assembly and into the container from an external source. A Microbial Challenge was conducted on the test article in accordance with the NAMSA Protocol No. 00C 11708 00. Twenty (20) couplers were immersed into the Saline Lactose Broth (SLB) containing *B. diminuta* indicator organisms for 24 hours. Three (3) couplers were not exposed to the challenge suspension and served as negative controls. Three (3) couplers containing teflon tape between the O-ring and bore served as positive controls and the couplers were challenged in the same manner. Under the conditions of this study, all the challenge couplers, negative controls, and positive controls, passed the Microbial Challenge Test and demonstrated the container closure resists the bacterial ingress through the assembly.

Study and Supervisory Personnel: John J. Broad, B.S.
Esther R. Cantu, B.S.
Johnathan Randall, B.S.

Reviewed by: Esther R. Cantu, B.S. — Date: 1-15-01
Study Director, Microbiology

Approved by: John J. Broad, B.S. — Date: 1-15-01
Senior Scientist
NAMSA California Division

Comment: This report has been revised to correct the identification number, clarify positive control information within the body of the report and clarify the method.

cj

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METHOD

The challenge organism *Brevundomonas diminuta* ATCC #19146 from stock culture (maintained on a TSA slant) was used to inoculate 10 ml of Trypticase Soy Broth (TSB) and the TSB cultures were incubated at 30-35°C for 18-24 hours. Three (3) ml aliquots of the 24 hour broth culture from the organism were used to inoculate each aspirator bottle containing 3000 ml of Soybean Casein Digest Broth (SCDB) which were then incubated at 30-35°C for 18-24 hours. This 24 hour broth culture was used as the challenge medium.

Sterile trays were filled with the challenge medium and twenty (20) couplers were submerged in the inoculated SLB ensuring the whole coupler is submersed. The couplers remained in the challenge medium for 24 hours at room temperature.

After 24 hours, the couplers were removed, disinfected, and incubated at 30-35°C for 7 days. After 7 days the cultures were removed and checked for sterility.

Three (3) test articles, not exposed to the challenge suspension, but treated in the same manner as above, served as negative controls. Three (3) test articles were prepared by inserting teflon tape between the O-ring and bore (recommended by NAMSA) prior to exposing them to the challenge suspension to serve as positive controls. The tape remained in the device for the 24 hour exposure time. The positive controls were treated in the same manner as the challenged test articles. The test article was received on August 15, 2000. The test was initiated on October 18, 2000 and terminated on October 26, 2000.

RESULTS

All challenged couplers and negative control couplers remained negative. The positive control couplers showed growth for the indicator organism (See Table I).

Results and conclusions apply only to the test article tested. No further evaluation of these results is made by NAMSA. Any extrapolation of these data to other samples is the responsibility of the sponsor. All procedures were conducted in conformance with good laboratory practice and EN45001 Quality Standards (TÜV Product Services 1/96).

INTERPRETATION

The ability of the Quick-Disconnect Couplers, MPC17006T39 and MPC22004T39M (Small Amber Coupler), to maintain a sterile barrier will be demonstrated if the following criteria are met:

A. The sterility for all challenged couplers is maintained.

B. All negative controls are verified sterile.

C. All positive controls demonstrate growth for the indicator organism.

CONCLUSION

Under the conditions of this study, the couplers supplied by Colder Products Company demonstrated the ability to maintain a sterile barrier. The test articles met the criteria described in the Interpretation above.

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RECORD STORAGE

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

REFERENCES

A. NAMSA Standard Operating Procedures.

B. NAMSA Protocol No.: 00C 11708 00

C. United States Pharmacopoeia (USP), current edition.

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TABLE I

MICROBIAL CHALLENGE RESULTS FOR

QUICK-DISCONNECT COUPLERS, MPC17006T39 AND MPC22004T39M (SMALL AMBER COUPLER)

Sample #	Results
1	(-)
2	(-)
3	(-)
4	(-)
5	(-)
6	(-)
7	(-)
8	(-)
9	(-)
10	(-)
11	(-)
12	(-)
13	(-)
14	(-)
15	(-)
16	(-)
17	(-)
18	(-)
19	(-)
20	(-)
Negative Controls	
1	(-)
2	(-)
3	(-)
Positive Controls	
1	(+)
2	(+)
3	(+)

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MPC PVDF Thumb Latch Technical Requirement Checklist Testing

CPC Technology Development

Test Report

MPC PVDF Thumb Latch Technical Requirement Checklist Testing

File Number: 2017-116

Date Revised: 9/19/17 (Rev.2)

Date: 9/19/17

Kayla Vangsgard Digitally signed by Kayla Vangsgard Date: 2017.09.19 11:56:43 -05'00'

Kayla Vangsgard
Test Engineer

Greg Zeien Digitally signed by Greg Zeien Date: 2017.09.19 12:43:42 -05'00'

Read by: Greg Zeien
Test Engineer

This report is prepared for the exclusive benefit of the Requesting Party and may not be relied upon by any other party for any reason whatsoever. The information contained in this report relates only to the materials and/or products tested under the test conditions specified.

Colder Products Company
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Telephone (651) 645-0091

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CPC TDL Report

Summary

MPC39 product made with the white PVDF thumb latch were tested to ensure proper function. The Technical Requirement Checklist (TRC) consisted of samples being exposed to autoclave and gamma irradiation, and tested to ensure the thumb latches performed as designed. All samples passed.

Methods

Parts were either kept virgin, autoclaved at 132°C for 60min 25 times or exposed to a minimum of 50kGy gamma irradiation.

Cycle Test

20 sets of the three terminations (MPC17002T39, MPC17004T39 and MPC3301239) post gamma irradiation, autoclave and virgin were cycle tested. 10 of each termination to 500 cycles, the remaining 10 to 1,000. Cycle testing was completed to ensure proper thumb latch functionality up to 1,000 cycles with no change in performance. CPC's standard work instruction WI-0995[1] details the test protocol.

Verify Thumb Latch Function

After 500 and 1,000 cycles were complete, thumb latches were manually pushed in to ensure thumb latch was functional.

Bubble Leak

The bubble leak test was conducted using ASTM E515-11, Standard Practice for Leaks Using Bubble Emission Techniques [2]. After 1,000 cycles MPC17002T39, MPC17004T39 and MPC3301239 parts were connected, submerged into a tank, pressurized at 60psi and held for 2min. A pass would entail no bubbles forming or coming off of the part. 15 non-cycled MPC17002T39, MPC17004T39 and MPC3301239 samples of virgin, autoclave and gamma irradiation were also bubble leaked. CPC's standard work instruction WI-1102[3] details the test protocol.

Water Burst

After 1,000 cycles MPC17002T39, MPC17004T39 and MPC3301239 parts were tested according to CPC standard WI-0998[4]. Parts were connected and ramped at 200 psi/sec until structural failure. The resulting failure pressure is recorded. 15 new MPC17002T39, MPC17004T39 and MPC3301239 samples of virgin, autoclave and gamma irradiation were also burst.

Tensile to Leak

15 of each of the three terminations (MPC17002T39, MPC17004T39 and MPC3301239) post gamma irradiation, autoclave and virgin were fixtured to the load cell in an Ametek LS1SH-115V tensile tester. The samples were connected and pressurized to 60psi. Parts were pulled apart in

a tension setting at a speed of 2in/min per CPC standard protocol WI-0983[5]. Force was recorded when pressure in the connected set began to drop.

Tensile to Break

15 of each of the three terminations (MPC17002T39, MPC17004T39 and MPC3301239) post gamma irradiation, autoclave and virgin were fixtured under the load cell in an Ametek LS1SH-115V tensile tester. The samples were connected then pulled apart in a tension setting at a speed of 2in/min per CPC standard protocol WI-0983[5]. Results were recorded at peak strength/force.

Verify PVDF Thumb Latch is Functional with Lock Sleeve

After 30 MPCK17004T39 parts were autoclaved and 30 parts were gamma irradiated (60 total), they were checked for functionality with the lock sleeve. The lock sleeve was rotated by hand.

Results

Cycle, Verify Thumb Latch Function, Bubble and Burst

All 180 samples passed the thumb latch function test post 500 and 1,000 cycles. All 90 bubble leaked samples passed at 60psi for two minutes. All 180 samples passed the burst test.

Bubble Leak

30 samples of gamma irradiated and 30 samples of autoclaved parts from three different terminations were bubble leak tested. All 180 samples passed the bubble leak test at 60psi for two minutes.

Water Burst

15 samples of gamma irradiated and 15 samples of autoclaved parts from three different terminations were burst tested. All parts passed the burst test.

Tensile to Leak

15 samples of gamma irradiation and 15 samples of autoclaved parts from three different terminations were tested for force to leak. Failure modes consisted of the hose barb breaking, the insert or the body giving away. All parts passed.

Tensile to Break

15 samples of gamma irradiation and 15 samples of autoclaved parts from three different terminations were tested for force to break. Failure modes consisted of the hose barb breaking, the insert or the body giving away. All parts passed.

Verify PVDF Thumb Latch Works with Lock Sleeve

30 MPCK17004T39 parts were smoothly rotated by hand to check the lock sleeve, all rotated with no issues.

Flow Test

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Engineering Test Lab

1001 Westgate Drive
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Fax (651) 603-2638

FLOW TEST:

Test #: 2003-007

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

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Helium Leak Test

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HELIUM LEAK TEST:

Test #: 1998-005

Purpose:

The purpose of the Helium Leak Test is to verify the sealing performance of Colder Products Company's polysulfone MPX39 and MPC39 couplings. Testing was completed on autoclave sterilized parts.

Procedure:

A total of fifty-five couplings were tested. Of the fifty -five couplings, twenty were MPX39 inserts and bodies and twenty-five were MPC39 inserts and bodies. All were autoclave sterilized at 270°F (132°C) for 60 minutes with a 15 minute dry time for a total of 25 cycles.

The polysulfone MPX39 and MPC39 couplings with were tested at full vacuum. Each polysulfone MPX39 and MPC39 coupling were capped on one end and attached to a helium source and pressurized to 20 psi. A "sniffer" probe attached to a Helium Mass Spectrometer Leak Detector was used to inspect the seals. 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 – ACCELERATED AGING:

Test #: 2006-017

Purpose:

The purpose of this Cycle Test is to verify the sealing performance on artificially aged MPC couplings after 1000 connect/disconnect cycles.

Procedure:

The samples have been placed in an elevated temperature oven, twelve (12) coupled and twelve (12) uncoupled parts, for 69 days, to simulate a shelf life of three years. The formula used to arrive at the oven temperature and the time of exposure is:

$$\text{Days} = 365/2^{\Delta T/10}$$

where "Days" is the time in the oven and ΔT is the temperature difference, in Celsius, between the oven and room temperature. This equated to one year on the shelf was 22.8 days in a 60°C oven.
Ten (10) gamma irradiated samples were sterilized prior to aging. The samples were subjected to a minimum of 50 kilograys of gamma radiation.
Ten (10) autoclave samples were sterilized post aging. The samples were subjected to twenty- five (25) autoclave sterilization cycles of 270°F for 60 minutes with 15 minutes of drying time. The MPC parts were removed from the autoclave and a minimum of one hour cool down time was allowed between each cycle.
Four (4) virgin parts were not sterilized in any fashion.

The MPC couplings shall be disconnected and then connected 1000 times by an automatic mechanism. One cycle consists of depressing the clip, removing the insert from the body, releasing the clip, and then placing the insert into the body. One connect/disconnect cycle lasts six seconds.

After the MPC couplings have been cycled 1000 times, the parts are subjected to a Helium Leak Test.

Pass/Fail Criteria:

To pass the Cycle Test, the parts must maintain their sealing capabilities. For the vacuum test, the leak rate must be below 1.0×10^{-5} atm-cc/sec.

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Results:

Accelerated Aging 3 Years - Gamma Irradiated

Part Number	Aged	Sample	Pass/Fail
MPC17004T39 & MPC22004T39M	Uncoupled	1	Pass
MPC17004T39 & MPC22004T39M	Uncoupled	2	Pass
MPC17004T39 & MPC22004T39M	Uncoupled	3	Pass
MPC17004T39 & MPC22004T39M	Uncoupled	4	Pass
MPC17004T39 & MPC22004T39M	Uncoupled	5	Pass
MPC17004T39 & MPC22004T39M	Coupled	6	Pass
MPC17004T39 & MPC22004T39M	Coupled	7	Pass
MPC17004T39 & MPC22004T39M	Coupled	8	Pass
MPC17004T39 & MPC22004T39M	Coupled	9	Pass
MPC17004T39 & MPC22004T39M	Coupled	10	Pass

Accelerated Aging 3 Years - Autoclave Sterilized

Part Number	Aged	Sample	Pass/Fail
MPC17004T39 & MPC22004T39M	Uncoupled	11	Pass
MPC17004T39 & MPC22004T39M	Uncoupled	12	Pass
MPC17004T39 & MPC22004T39M	Uncoupled	13	Pass
MPC17004T39 & MPC22004T39M	Uncoupled	14	Pass
MPC17004T39 & MPC22004T39M	Uncoupled	15	Pass
MPC17004T39 & MPC22004T39M	Coupled	16	Pass
MPC17004T39 & MPC22004T39M	Coupled	17	Pass
MPC17004T39 & MPC22004T39M	Coupled	18	Pass
MPC17004T39 & MPC22004T39M	Coupled	19	Pass
MPC17004T39 & MPC22004T39M	Coupled	20	Pass

Accelerated Aging 3 Years - Virgin

Part Number	Aged	Sample	Pass/Fail
MPC17004T39 & MPC22004T39M	Uncoupled	37	Pass
MPC17004T39 & MPC22004T39M	Uncoupled	38	Pass
MPC17004T39 & MPC22004T39M	Coupled	39	Pass
MPC17004T39 & MPC22004T39M	Coupled	40	Pass

Mary Wallraff
Mechanical Test Engineer

MPC39 Series Technical Requirement Checklist Testing – Accelerated Aging

Technology Development **Test Report**

MPC39 Series, (White Thumb Latch) Technical Requirement Checklist Testing, Accelerated Aging

File Number: 2017-116

Date Revised: 9/20/17 (Rev.2)

Date: 9/20/17

Kayla Vangsgard — Digitally signed by Kayla Vangsgard Date: 2017.09.20 09:13:35 -05'00'

Kayla Vangsgard
Test Engineer

Greg Zeien — Digitally signed by Greg Zeien Date: 2017.09.20 09:29:37 -05'00'

Read by: Greg Zeien
Test Engineer

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Summary

MPC39 PVDF clips (white thumb latch) were tested to ensure proper function post accelerated aging. Samples were accelerated aged to three years and tested to complete the Technical Requirement Checklist (TRC). All samples passed.

Methods

30 virgin MPC17004T39 parts were placed in an oven at 60°C for 84.25 days to simulate three (3) year accelerated aging, this is in reference to Arrhenius' equation.

Bubble Leak

The bubble leak test was conducted using ASTM E515-11, Standard Practice for Leaks Using Bubble Emission Techniques [1]. After samples were taken out of the accelerated aging oven, samples were coupled and submerged into a tank, pressurized at 60 psi and held for 2 min. A pass would entail no bubbles forming on or coming off of the part. CPCs standard work instruction WI-1102[2] details the test protocol.

Cycle Test

After samples were bubble leak tested, parts were then cycle tested 500 times. One cycle is the process of depressing and releasing the thumb latch one time. Parts were then bubble leak tested again and samples were placed back in the cycle tester for another 500 cycles. Once complete, parts were pulled off and bubble leak tested again. The cycle testing was completed to ensure proper thumb latch functionality after being cycled 1,000 times. CPCs standard work instruction WI-0995[3] details the test protocol.

Water Burst

After sample parts were cycled 1,000 times, parts were tested according to CPC standard WI-0998[4]. Parts were coupled and ramped at 200 psi/sec until structural failure. The resulting failure pressure is recorded.

Results

All 30 samples passed the initial bubble leak test post three (3) year accelerated aging. All samples passed bubble leak test post 500 and 1,000 cycles. Table 1 shows the average burst pressures of 30 samples after cycle and bubble leak testing.

Table 1: Average Water Burst Pressure

	Burst Pressure (psi)
	Virgin
MPC17004T39	707.7

Conclusion

The Technical Requirement Checklist (TRC) consisted of samples being accelerated aged for three (3) years and tested to ensure proper functionality of the thumb latch. All samples passed.

References

1. ASTM E515-11, "Standard Test Method for Leaks Using Bubble Emission Techniques", ASTM International, Conshohocken, PA, 2011.
2. WI-1102, "Bubble Emission Test-Work Instructions"
3. WI-0995, "Cycle Test Work Instructions"
4. WI-0998, "Burst Test Work Instruction"

Conclusion

The Technical Requirement Checklist (TRC) consisted of samples being gamma irradiated and autoclaved, and tested to ensure no issues with functionality, leaking or low breaking force of the thumb latch. All samples passed.

References

1. WI-0995, "Cycle Test Work Instructions"
2. ASTM E515-11, "Standard Test Method for Leaks Using Bubble Emission Techniques", ASTM International, Conshohocken, PA, 2011.
3. WI-1102, "Bubble Emission Test-Work Instructions"
4. WI-0998, "Burst Test Work Instruction"
5. WI-0983, "Tensile Leak or Breakage Test Work instructions"

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

REPORT

TEST FACILITY

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SPONSOR

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CONFIDENTIAL

STUDY TITLE

USP and ISO Acute Systemic Toxicity Study in Mice

TEST ARTICLE NAME

Polysulfone, Udel P-1700 NT11 (with Veggie Lube)

TEST ARTICLE IDENTIFICATION

24223232

NAMSA

PEOPLE > SCIENCE > SOLUTIONS

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Summary

The test article, Polysulfone, Udel P-1700 NT11 (with Veggie Lube), 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

01-22-18
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 12, 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:	Polysulfone, Udel P-1700 NT11 (with Veggie Lube)
Identification:	24223232
Physical Description of the Test Article:	Part # 782600M
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:	20 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 was prepared based on the sponsor supplied surface area of 24.32 cm^2 per test article. Two test articles were included in each 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	48.6 cm^2	16 mL	50°C for 72 hours
SO	3 cm^2:1 mL	48.6 cm^2	16 mL	50°C for 72 hours
AS	3 cm^2:1 mL	48.6 cm^2	16 mL	50°C for 72 hours
PEG	3 cm^2:1 mL	48.6 cm^2	16 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	51	Lethargic	Normal	Normal	Normal	Normal
		52	Lethargic	Normal	Normal	Normal	Normal
		53	Lethargic	Normal	Normal	Normal	Normal
		54	Lethargic	Normal	Normal	Normal	Normal
		55	Lethargic	Normal	Normal	Normal	Normal
	Control Blank	31	Lethargic	Normal	Normal	Normal	Normal
		32	Lethargic	Normal	Normal	Normal	Normal
		33	Lethargic	Normal	Normal	Normal	Normal
		34	Lethargic	Normal	Normal	Normal	Normal
		35	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	51	22	22	24	24
		52	21	23	24	25
		53	21	21	22	22
		54	22	24	25	26
		55	22	24	25	26
	Control Blank	31	21	23	24	25
		32	22	24	25	25
		33	21	22	23	24
		34	21	23	25	26
		35	23	24	25	25

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	41	Normal	Normal	Normal	Normal	Normal
		42	Normal	Normal	Normal	Normal	Normal
		43	Normal	Normal	Normal	Normal	Normal
		44	Normal	Normal	Normal	Normal	Normal
		45	Normal	Normal	Normal	Normal	Normal
	Control Blank	1	Normal	Normal	Normal	Normal	Normal
		2	Normal	Normal	Normal	Normal	Normal
		3	Normal	Normal	Normal	Normal	Normal
		4	Normal	Normal	Normal	Normal	Normal
		5	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	41	22	23	24	25
		42	22	22	23	23
		43	21	23	24	24
		44	23	24	26	26
		45	22	24	25	26
	Control Blank	1	20	21	22	24
		2	20	22	23	25
		3	22	23	24	25
		4	21	22	23	24
		5	20	21	23	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	56	Normal	Normal	Normal	Normal	Normal
		57	Normal	Normal	Normal	Normal	Normal
		58	Normal	Normal	Normal	Normal	Normal
		59	Normal	Normal	Normal	Normal	Normal
		60	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
PEG	Test Extract	56	22	23	25	26
		57	22	23	25	25
		58	22	23	24	25
		59	22	24	26	27
		60	22	23	25	27
	Control Blank	36	20	21	23	23
		37	22	23	24	24
		38	22	23	25	25
		39	21	22	23	23
		40	20	22	23	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	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
	Control Blank	16	Normal	Normal	Normal	Normal	Normal
		17	Normal	Normal	Normal	Normal	Normal
		18	Normal	Normal	Normal	Normal	Normal
		19	Normal	Normal	Normal	Normal	Normal
		20	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	46	21	22	23	24
		47	22	24	25	26
		48	23	25	27	28
		49	22	23	25	26
		50	23	24	26	27
	Control Blank	16	20	22	23	24
		17	21	23	24	25
		18	21	22	24	25
		19	19	21	22	23
		20	20	22	23	24

Polysulfone Intracuteneous Injection Test

REPORT

TEST FACILITY

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STUDY TITLE

USP and ISO Intracutaneous Study in Rabbits

TEST ARTICLE NAME

Polysulfone, Udel P-1700 NT11 (with Veggie Lube)

TEST ARTICLE IDENTIFICATION

24223232

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Summary

The test article, Polysulfone, Udel P-1700 NT11 (with Veggie Lube), 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 01-24-18
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 12, 2018
Observations Concluded:	January 15, 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:	Polysulfone, Udel P-1700 NT11 (with Veggie Lube)
Identification:	24223232
Physical Description of the Test Article:	Part # 782600M
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:	2.3 kg to 2.8 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	48.6 cm^2	16 mL	50°C for 72 hours
SO	3 cm^2:1 mL	48.6 cm^2	16 mL	50°C for 72 hours
AS	3 cm^2:1 mL	48.6 cm^2	16 mL	50°C for 72 hours
PEG	3 cm^2:1 mL	48.6 cm^2	16 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	0.8	0.8	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	24035	Male	2.4	0	0	0	0	0	0	0	0	0	0	0	0
				0	0	0	0	0	0	0	0	0	0	0	0
				0	0	0	0	0	0	0	0	0	0	0	0
				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	24036	Male	2.3	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
SC	24037	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	24035	Male	2.4	1	0	1	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
				0	0	0	0	1	0	1	0	0	0	1	0
				1	0	0	0	1	0	1	0	0	0	1	0
SO	24036	Male	2.3	1	0	1	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	0	0	1	0
				1	0	0	0	1	0	0	0	0	0	0	0
				1	0	0	0	1	0	0	0	0	0	1	0
SO	24037	Male	2.6	1	0	1	0	0	0	1	0	0	0	1	0
				1	0	1	0	1	0	1	0	0	0	1	0
				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

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	24038	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	24039	Male	2.4	0	0	0	0	0	0	0	0	0	0	0	0
				0	0	0	0	0	0	0	0	0	0	0	0
				0	0	0	0	0	0	0	0	0	0	0	0
				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	24040	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	24038	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	24039	Male	2.4	0	0	0	0	0	0	0	0	0	0	0	0
				0	0	0	0	0	0	0	0	0	0	0	0
				0	0	0	0	0	0	0	0	0	0	0	0
				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	24040	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

ER = Erythema
ED = Edema

Polysulfone 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

Modified USP Muscle Implantation Study in Rabbits - 7 Day

TEST ARTICLE NAME

Polysulfone, Udel P-1700 NT11 (with Veggie Lube)

TEST ARTICLE IDENTIFICATION

24223232

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Summary

The test article, Polysulfone, Udel P-1700 NT11 (with Veggie Lube), was implanted in muscle tissue of the rabbit to evaluate the local tissue response. This study was conducted based on the USP, General Chapter <88>, Biological Reactivity Tests, In Vivo. The study was modified to implant the test article surgically due to the nature of the test article and the constraints of the trocar method.

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 E. Carter, BS, ALAT — 01-28-18
Technical Reviewer — Date

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. The study was modified to implant the test article surgically due to the nature of the test article and the constraints of the trocar method.

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 10, 2018
Explanted:	January 17, 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:	Polysulfone, Udel P-1700 NT11 (with Veggie Lube)
Identification:	24223232
Physical Description of the Test Article:	Part # 782600M
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:	3.2 kg to 3.5 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, previously unused animals were selected.

5. Method

5.1 Test and Control Article Preparation
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 5 mm x 5 mm x 1 mm. For each animal, a minimum of two negative control articles, each approximately 5 mm x 5 mm x 1 mm, were prepared. 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. The rabbits were placed on inhalant anesthetic for continued general anesthesia during the procedure.

The operative site was aseptically draped. One incision was made through the skin over the lumbar region of the vertebral column. The fascia was cut to expose the paravertebral muscle. A pocket was formed with a hemostat between the muscle fibers into which the article was introduced. The fascia was closed with nonabsorbable 4-0 prolene suture. 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. Test article sections were placed at appropriately spaced intervals. The skin incision 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 1 day prior to 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

On day 5, the wound clip was missing and the mid incision of animal 24217 was open; a wound clip was placed to close. Otherwise, both animals appeared clinically normal throughout the duration of the study.

7.2 Body Weight Data

Body weight data for individual animals were considered acceptable. Weight loss was noted for animal 24217. This weight loss was considered to be acceptable following this type of procedure. Since this study was conducted to evaluate local tissue response, the weight loss had no impact to the outcome of this study. 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 presented 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 -1	Day 7		
24217	Male	3.5	3.4*	0	0
				0	0
				0	[redacted]
				0	
24214	Male	3.2	3.2	0	0
				0	0
				0	[redacted]
				0	
			Average:	0.0	0.0

*Weight loss noted.

Polysulfone USP 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
Polysulfone, Udel P-1700 NT11

TEST ARTICLE IDENTIFICATION
24134999

TEST ARTICLE PHYSICAL DESCRIPTION
Part # 2541200M

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
182004705	18T_20370_12		06

Polysulfone Physico-Chemical Test (USP <661>)

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REPORT

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Test Facility
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STUDY TITLE
Physicochemical Testing Using a Purified Water Extract

TEST ARTICLE NAME
Polysulfone, Udel P-1700 NT11 (with Veggie Lube)

TEST ARTICLE IDENTIFICATION
24223232

TEST ARTICLE PHYSICAL DESCRIPTION
Part # 782600M

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 608 cm^2 portion (25 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 101 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_20384_10	C0019_000	Page 1 of 2

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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 K. LaPlante 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.

Polysulfone Cytotoxicity Test (USP <87>/ISO 10993-5)

REPORT

TEST FACILITY

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SPONSOR

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St. Paul, MN 55114

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STUDY TITLE

Cytotoxicity Study Using a Modified USP and ISO Elution Method

TEST ARTICLE NAME

Polysulfone, Udel P-1700 NT11 (with Veggie Lube)

TEST ARTICLE IDENTIFICATION

24223232

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Summary

The test article, Polysulfone, Udel P-1700 NT11 (with Veggie Lube), 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, 2017
Observations Concluded:	January 8, 2017

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:	Polysulfone, Udel P-1700 NT11 (with Veggie Lube)
Identification:	24223232
Physical Description of the Test Article:	Part # 782600M
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 24.32 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	48.6 cm^2	16 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 USP Class VI Certificate

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9 Morgan, Irvine, CA 92618-2078 / 949.951.3110 / Fax 949.951.3280
Affiliates: France • Germany • Taiwan

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Lab No. 99T 04489 00
P.O. No. 52950

ERIK LONG
COLDER PRODUCTS COMPANY
1001 WESTGATE DRIVE
ST PAUL, MN 55114

ID No. Test #99008

CERTIFICATE OF COMPLIANCE
USP BIOLOGICAL REACTIVITY TESTS, *IN VIVO*

CLASSIFICATION VI

Test Article: Silicone O-Ring (P/N 12917-00): GE LIM 6071

USP 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 cottonseed oil extracts did not produce a significantly greater systemic reaction than the blank extractants.

USP 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 cottonseed 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 5 days, was not significant when compared to the USP negative control plastic.

The test article was prepared at an elastomeric ratio of 25 cm^2:20 ml and extracted at 70°C for 24 hours. The test article extracts met the requirements of a USP Plastic Class VI.

lb

Date Completed 5 May 1994

Approved By

J. Matthew Buchanan, BS, MS

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Lab No. 99T 04489 00
P.O. No. 52950

ERIK LONG
COLDER PRODUCTS COMPANY
1001 WESTGATE DRIVE

ID No. Test #99008

ST PAUL, MN 55114

PHYSICO-CHEMICAL - ELASTOMERIC CLOSURES

Test Article: Silicone O-Ring (P/N 12917-00): GE LIM 6071

The test article was received on 4-12-99.

Experimental Procedure: A 118.3 cm^2 portion of the test material was washed by autoclaving at 121°C for 30 minutes in 355 ml of Purified Water, then rinsed with two 100 ml portions of Purified Water. The test material was extracted at 121°C in an autoclave for 2 hours in 237 ml of Purified Water. Turbidity, reducing agents, heavy metals, pH change and total extractables were determined in the eluate as outlined in the current USP.

Results:

Turbidity:	<0.1 NTU
Reducing Agents:	<0.1 ml 0.01 N Iodine per 50 ml extract
Heavy Metals:	≤1 ppm
pH Change:	0.2 pH units
Total Extractables:	8 mg solids per 200 ml extract

Results and conclusions apply only to the test article tested. No further evaluation of these results is made by NAMSA. Any extrapolation of these data to other samples is the responsibility of the sponsor. All procedures were conducted in conformance with good laboratory practice and EN45001 Quality Standards (TÜV Product Services 1/96).

Comments: The test extract and control solution were clear.

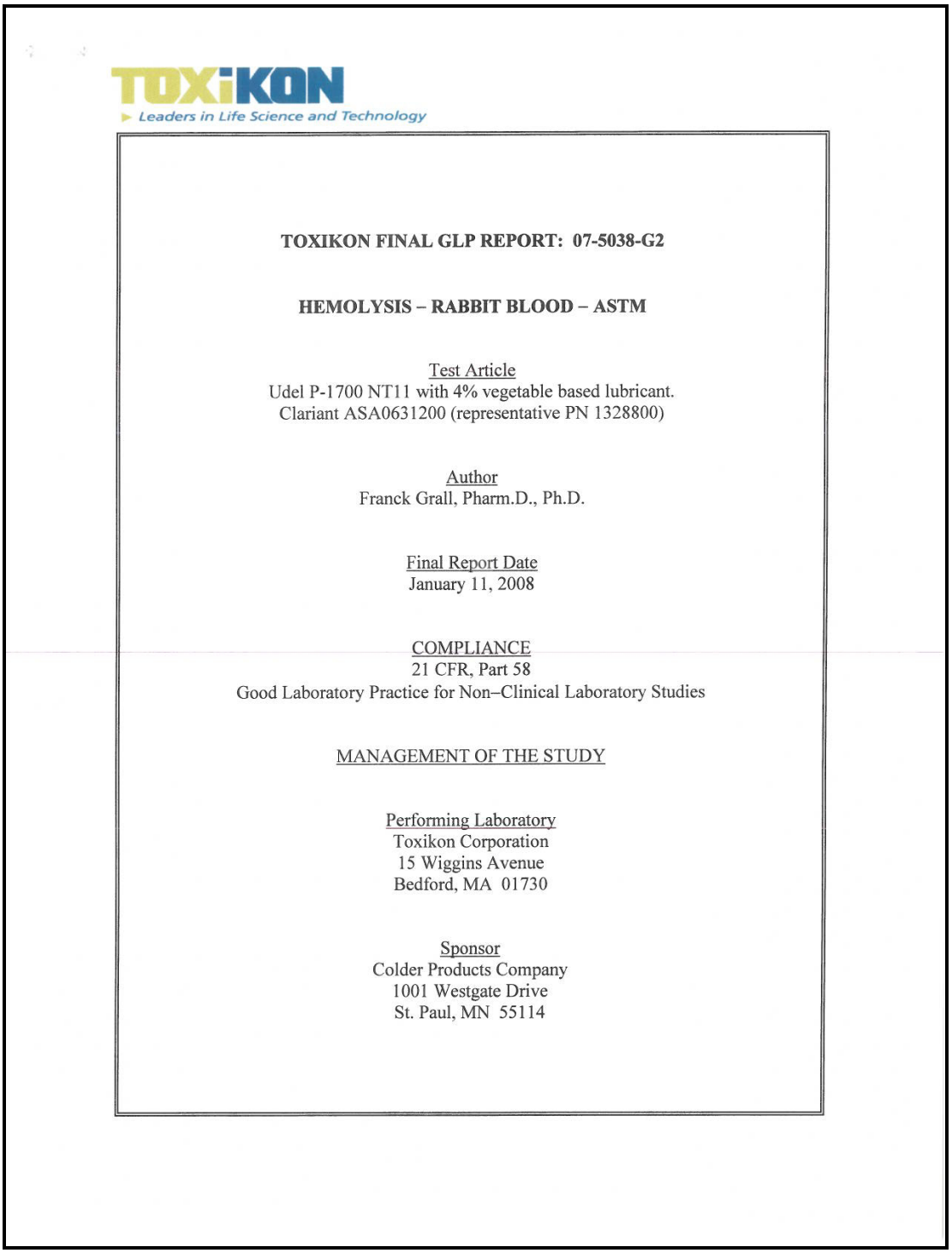
Record Storage: All raw data pertaining to this study and a copy of the final report are to be retained in designated NAMSA archive files.

Test Facility: NAMSA, 2261 Tracy Road, Northwood, OH 43619-1397.

las Date Completed 4-19-99 Approved By Marisol Halligan
Marisol M. Halligan, BS, MS

Page 1 of 1

Polysulfone Hemolysis Study



TOXIKON
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TOXIKON FINAL GLP REPORT: 07-5038-G2

HEMOLYSIS – RABBIT BLOOD – ASTM

Test Article
Udel P-1700 NT11 with 4% vegetable based lubricant.
Clariant ASA0631200 (representative PN 1328800)

Author
Franck Grall, Pharm.D., Ph.D.

Final Report Date
January 11, 2008

COMPLIANCE
21 CFR, Part 58
Good Laboratory Practice for Non–Clinical Laboratory Studies

MANAGEMENT OF THE STUDY

Performing Laboratory
Toxikon Corporation
15 Wiggins Avenue
Bedford, MA 01730

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

TABLE OF CONTENTS

TOXIKON

Hemolysis – Rabbit Blood – ASTM
Project Number: 07-5038-G2
Test Article: Udel P-1700 NT11 with 4% vegetable based lubricant. Clariant ASA0631200 (representative PN 1328800)

STUDY SUMMARY

The hemolytic activity of the test article, Udel P-1700 NT11 with 4% vegetable based lubricant. Clariant ASA0631200 (representative PN 1328800), was evaluated by direct and indirect contact using ASTM method F756–00. The test article exhibited 0.0% hemolysis above the level of hemolysis exhibited by the negative control via the direct method. The test article exhibited 0.0% hemolysis above the level of hemolysis exhibited by the negative control via the indirect method. The test article is considered non–hemolytic under the experimental conditions employed.

TOXIKON

Hemolysis – Rabbit Blood – ASTM
Project Number: 07-5038-G2
Test Article: Udel P-1700 NT11 with 4% vegetable based lubricant. Clariant ASA0631200 (representative PN 1328800)

QUALITY ASSURANCE STATEMENT

This study was conducted in compliance with U.S. Food and Drug Administration regulations set forth in 21 CFR, Part 58.

The sections of the regulations not performed by or under the direction of Toxikon Corporation, exempt from this Good Laboratory Practice Statement, included characterization and stability of the test article and its mixture with carriers, 21 CFR, Parts 58.105 and 58.113.

The Quality Assurance Unit conducted inspections on the following dates. The findings were reported to the Study Director and to Toxikon's Management.

INSPECTIONS	DATE OF INSPECTION	DATE REPORTED STUDY DIRECTOR	DATE REPORTED MANAGEMENT
SCORING (INDIRECT)	11/19/07	11/20/07	11/20/07
SCORING (DIRECT)	11/20/07	11/20/07	11/20/07
RAW DATA	01/11/08	01/11/08	01/11/08
FINAL REPORT	01/11/08	01/11/08	01/11/08

Allison Lyons-Hook, B.A.
Quality Assurance

1/11/08
Date

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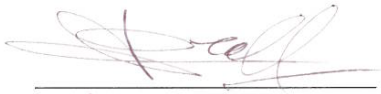
TOXIKON

Hemolysis – Rabbit Blood – ASTM
Project Number: 07-5038-G2
Test Article: Udel P-1700 NT11 with 4% vegetable based lubricant. Clariant ASA0631200 (representative PN 1328800)

STUDY DIRECTOR SIGNATURE AND VERIFICATION DATES

This study meets the technical requirements of the protocol. The study also meets with the requirements of the Good Laboratory Practice Regulations, 21 CFR, Part 58, with the exemptions as stated in the Quality Assurance Statement.

Protocol Name and Number:	CLD/VIVO/001–07/001
Study Director:	Franck Grall, Pharm.D., Ph.D.
Company:	Toxikon Corporation
Signature:	
Date:	01/11/08
Study Supervisor:	Franck Grall, Pharm.D., Ph.D.

VERIFICATION DATES:
The Study Initiation Date is the date the protocol is signed by the Study Director.

Test Article Receipt:	11/13/07
Project Log Date:	11/14/07
Study Initiation Date:	10/25/07
Extraction Dates:	11/19/07 – 11/20/07
Technical Initiation:	11/19/07
Technical Completion:	11/20/07

1.0 PURPOSE

The purpose of this study was to assess the hemolytic activity of the test article in both direct and indirect contact with rabbit blood.

2.0 REFERENCES

The study was conducted based upon the following references:

2.1 ASTM F756–00, Standard Practice for Assessment of Hemolytic Properties of Materials, 2000.

2.2 Hemolysis – Rabbit Blood, Evaluation of Hemodialyzers and Dialysis Membranes, DHEW Publication # (NIH) 77–1294, pg. 213, 1977.

2.3 Autian Method, ATTP–I, Material Sciences Toxicology Laboratories, University of Tennessee Center for the Health Sciences, Memphis, TN, April 18, 1977.

2.4 Feldman, Bernard F., Joseph G. Zinkl, and Nemi C. Jain, eds. Schalm's Veterinary Hematology. 5th edition. Baltimore: Lippincott Williams & Wilkins, 200. 858 – 859.

2.5 ISO 10993–12, 2007, Biological Evaluation of Medical Devices – Part 12: Sample Preparation and Reference Materials.

2.6 ISO/IEC 17025, 2005, General Requirements for the Competence of Testing and Calibration Laboratories.

3.0 COMPLIANCE

The study will conform to the current FDA 21 CFR, Part 58 – Good Laboratory Practice for Non–Clinical Laboratory Studies.

4.0 IDENTIFICATION OF TEST AND CONTROL ARTICLES

The Sponsor supplied the following information on a test requisition form or other correspondence, wherever applicable (excluding confidential or trade secret information). The Sponsor was responsible for all test article characterization data as specified in the GLP regulations.

4.1 Test Article:

Test Article Name: Udel P-1700 NT11 with 4% vegetable based lubricant. Clariant ASA0631200 (representative PN 1328800)
CAS/Code #: Not Supplied by Sponsor (N/S)
Lot/Batch #: N/S
Physical State: N/S
Color: N/S
Expiration Date: N/S

Density: N/S
Stability: N/S
Solubility: N/S
pH: N/S
Storage Conditions: Room Temperature
Safety Precautions: Standard Toxikon Laboratory Safety Precautions

4.2 Vehicle and Control Articles (Toxikon Supplied):

4.2.1 Negative Control Article Name: Negative Control High Density Polyethylene (Negative Control Plastic)
Toxikon QC #: CSC-04-05-009-CC
Physical State: Solid
Color: White
Storage Conditions: Room Temperature
Safety Precautions: Standard Laboratory Safety Precautions

4.2.2 Positive Control Article Name: Buna–N–Rubber
Toxikon QC #: CSC-03-07-005-CC
Physical State: Solid
Color: Black
Stability: Stable at Room Temperature
Storage Conditions: Room Temperature
Safety Precautions: Standard Laboratory Safety Precautions

4.2.3 Vehicle Control Name: Phosphate Buffered Saline (PBS)
Toxikon QC #: CSC-07-07-038-CC
Physical State: Liquid
Color: Colorless
Stability: Stable at Room Temperature
Storage: Room Temperature
Safety Precautions: Standard Laboratory Safety Precautions

5.0 IDENTIFICATION OF TEST SYSTEM

Six adult, healthy New Zealand White rabbits (*Oryctolagus cuniculus*) were used in this study. The animals were purchased from Millbrook Breeding Laboratories, Amherst, MA. The rabbits, 1 female and 2 males, (2.63, 2.55 and 2.61 kilograms and ≥ 7 weeks of age, respectively) for the indirect test and 1 male and 2 females (2.97, 2.75 and 3.11 kilograms and ≥ 7 weeks of age, respectively) for the direct contact were acclimated for a minimum of five days and weighed prior to the removal of a blood sample. In an attempt to reduce the number of animals used in testing, only three donors were used per test to provide the minimum requirement for blood.

6.0 JUSTIFICATION OF TEST SYSTEM AND ROUTE OF ADMINISTRATION

6.1 The system for the determination of hemolytic activity of a test article, when in direct or indirect contact with rabbit blood, was recommended in the ASTM Designation: F756 – 00, Standard Assessment of Hemolytic Properties of Materials. The guidelines have no alternative (non–animal) methods.

6.2 The test article was administered *in vitro*, directly and through a solvent compatible with the test system. This is the optimal route of administration available in this test system.

7.0 EXPERIMENTAL DESIGN AND DOSAGE

7.1 Preparation of Test and Control Articles – Indirect Contact:

7.1.1 The test article (6 g) was combined with 30 mL of vehicle at a ratio of 0.2 g per 1.0 mL per ISO 10993–12 and ASTM F56-00 guidelines. The test article was extracted in PBS at 70 ± 2 °C for 24 ± 2 hours.

7.1.2 The negative control (Negative Control Plastic) and positive control (Buna–N–Rubber) were extracted at a ratio of 3 cm^2 per 1 mL at 70 ± 2 °C for 24 ± 2 hours.

7.1.3 Properly prepared test articles were placed in separate extraction bottles, and to each bottle the appropriate medium was added. The extraction medium completely covered the test article.

7.1.4 Each extracting medium (control article) was prepared for parallel treatments and comparisons. Each control article was prepared in the same manner as the test article.

7.1.5 The test article appeared unchanged by the extraction procedure. It was not degraded or deformed. The extract was clear and free from particulates. Each extract was agitated vigorously prior to administration. All other test article preparation was as specified by the Sponsor.

7.2 Preparation of Test and Control Articles – Direct Contact:

7.2.1 An amount of 3.5 g of the test article was placed in capped test tubes in triplicate. The tube size was chosen in such a way that the test article could be covered by 7 mL of PBS.

7.2.2 An amount of 21 cm^2 of the positive and negative controls was placed in capped test tubes in triplicate.

7.3 Pre–Dose Procedure:

7.3.1 Blood Sample:
Fresh, whole rabbit blood was collected from three donors on the test day and sodium citrate was added as anticoagulant. Approximately 5 mL blood was drawn from each animal and pooled.

7.3.2 Hemoglobin Determination (Direct Method):
For hemoglobin standardization, reference standards reference standards consistent with the specifications of International Committee for Standardization in Hematology (ICSH) were used. A standard curve was prepared from the stock hemoglobin 8 dilutions accommodating a range of 0.03 to 1.4 mg/mL. The Drabkin's reagent was used as zero blank in the spectrophotometer and the absorbance was measured at 540 nm. The calibration curve was plotted from the values of the dilutions using mg/mL of the hemoglobin (Hb) on the y–axis and absorbances (A_{540}) on the x–axis. The calibration coefficient (F) is the slope of this plot. The y–intercept should be approximately zero.

7.3.3 Determination of Plasma Free Hemoglobin (PFH):
3 mL of sample of pooled blood was centrifuged at 800 *g* for 15 minutes. A volume of 0.5 mL of plasma was added to 0.5 mL of Drabkin's reagent. Absorbance was measured at 540 nm and the concentration was obtained from the standard curve. The amount of PFH was calculated using the formula:

$PFH = A^{PFH} * F * 2$ where F is the calibration coefficient.
PFH = Plasma Free Hemoglobin
A^{PFH} = Absorbance at 540 nm sample (0.5 mL plasma + 0.5 mL of Drabkin's reagent)
F = Calibration coefficient, slope of the hemoglobin curve

7.3.4 Determination of Total Blood Hemoglobin Concentration:
20 µL of well–mixed pooled whole blood was added to 5.0 mL of Drabkin's reagent, allowed to stand for 15 minutes and the absorbance of the solution was measured at 540 nm. The total hemoglobin content of the blood is measured using the formula $C = A^{C} * F * 251$. The total hemologbin content was adjusted to 10 ± 1 mg/mL by diluting with an appropriate amount of calcium and magnesium free PBS. The hemoglobin content was once again verified by using triplicate samples of 400 µL of diluted blood to 5 mL of reagent (dilution factor = 13.5). The hemoglobin content was measured using the formula:

$C = A^{C} * F * 13.5$
C = Concentration of total blood hemoglobin
A^{C} = Absorbance at 540 nm of whole blood (400 µL whole blood + 5.0 mL of Drabkin's reagent)
F = Calibration coefficient

7.4 Dose Administration:

7.4.1 A volume of 7 mL of the test article extract, negative control extract, and positive control extract were placed in capped test tubes.

7.4.2 A volume of 7 mL of PBS was added to all tubes prepared in Section 7.2.

7.4.3 Addition of Blood:
One mL of blood prepared as described in Section 7.3.4 was added to each tube.

7.4.4 The resulting solution after addition of blood was maintained at 37 ± 2 °C for 3 ± 0.1 hours in a water bath. The tubes were gently inverted twice every 30 minutes to maintain contact of the blood and material.

7.4.5 Replication:
The test article was tested in triplicate. The positive and negative controls were also tested in triplicate.

7.5 Post–Dose Procedure:

7.5.1 At the end of the incubation, the fluid was transferred into an appropriate tube and centrifuged at 800 *g* for 15 minutes. The supernatant was carefully collected into a screw cap vial and any coloration was recorded.

7.5.2 Determination of Supernatant Hemoglobin:

7.5.2.1 A volume of 1 mL of supernatant was added to 1 mL of Drabkin's reagent and the sample was allowed to stand for 15 minutes. The absorbance of the solution was then measured at 540 nm and hemoglobin concentration was determined using calibration curve.

7.5.2.2 The hemoglobin concentration in the supernatant (S) is given by:

$$S = A^S * F * 2$$

Where A^S = absorbance of the supernatant, F = a calibration coefficient (the value is negligible), and 2 = the dilution factor (1 mL supernatant to 1 mL reagent).

7.5.2.3 The percentage of hemolysis or hemolysis index is calculated as follows:

$$\% \text{ hemolysis} = \frac{\text{Concentration of Hemoglobin in the supernatant}}{\text{Total Hemoglobin in Tube}} * 100$$

The dilution corrected hemolysis is calculated as follows:

$$\text{Dilution Corrected \% hemolysis} = \frac{\text{Concentration of Hemoglobin in the supernatant (S)} * 8 * 100}{\text{Total Hemoglobin in Tube}}$$

Where 8 is the dilution factor based upon the addition of 1 mL total blood to 7 mL extract.

8.0 EVALUATION CRITERIA

8.1 The average absorbance values are used to determine the percent (%) Hemolysis of the test article.

8.2 Negative Control hemolytic index is subtracted from the test article hemolytic index.

8.3 Negative control should exhibit hemolytic index value of less than 2% and the positive control should exhibit greater than 8% hemolysis. If these conditions are not met, the test is repeated.

8.4 If the percent (%) Hemolysis of the test article is 0 – 2 or less, the test article is considered non–hemolytic under the experimental conditions employed. If the percent hemolysis is between 2 – 5, the test article is considered slightly hemolytic and a value higher than 5 would be concluded as hemolytic.

8.5 If deemed necessary by the Study Director, a retest is performed using fresh blood from the same donor and a new sample of test article.

8.6 The study and its design employ methodology to minimize uncertainty of measurement and control of bias for data collection and analysis.

9.0 RESULTS – INDIRECT CONTACT

9.1 Hemoglobin Standard

The hemoglobin concentration (C) has a coefficient of correlation with the absorbance (A) of 0.9922, and a residual error of 0.0347. The Calibration Coefficient F is 1.82.

$C = A \times F = A \times 1.82$

9.2 Plasma Free Hemoglobin PFH

Plasma Free Hemoglobin		
	Absorbance	Concentration (C = A × F)
Replicate 1	0.0970	0.177 mg/mL
Replicate 2	0.0972	0.177 mg/mL
Replicate 3	0.0967	0.176 mg/mL
Average	0.0970	0.177 mg/mL

Reagent dilution correction: $C^{PFH} = 2 \times \text{Average} = 2 \times 0.177 = 0.354$ mg/mL

The plasma free hemoglobin concentration is 0.354 mg/mL, which is less than the 2 mg/mL allowed by the ASTM guidelines.

9.3 Concentration of total hemoglobin = C^T

$C^T = A^C * F * 13.5.$
$A^C = 0.4299$
F = Calibration coefficient = 1.82
$C^T = 10.56$ mg/mL

9.4 Concentration of hemoglobin in Blank Control

Blank Control		
	Absorbance	Concentration (C = A × F)
Replicate 1	0.0030	0.005 mg/mL
Replicate 2	0.0000	0.000 mg/mL
Replicate 3	0.0006	0.001 mg/mL
Average	0.0012	0.002 mg/mL

Reagent dilution correction: C = 2 × Average = 2 × 0.002 = 0.004 mg/mL

9.5 Concentration of hemoglobin in Negative Control

Negative Control Article		
	Absorbance	Concentration (C = A × F)
Replicate 1	0.0020	0.004 mg/mL
Replicate 2	0.0000	0.000 mg/mL
Replicate 3	0.0037	0.007 mg/mL
Average	0.0019	0.003 mg/mL

Reagent dilution correction: C = 2 × Average = 2 × 0.003 = 0.006 mg/mL

Blank subtracted concentration = 0.006 – 0.004 = 0.002 mg/mL

% hemolysis in Negative Control = 0.002 / 10.56 × 100 = 0.02%

Dilution corrected % hemolysis in Negative Control = ((0.002 / 10.56) × 8) × 100 = 0.15%.

9.6 Concentration of hemoglobin in Positive Control

Positive Control Article		
	Absorbance	Concentration (C = A × F)
Replicate 1	0.0815	0.148 mg/mL
Replicate 2	0.0658	0.120 mg/mL
Replicate 3	0.0750	0.137 mg/mL
Average	0.0741	0.135 mg/mL

Reagent dilution correction: C = 2 × Average = 2 × 0.135 = 0.270 mg/mL

Blank subtracted concentration = 0.270 – 0.04 = 0.266 mg/mL

% hemolysis in Positive Control = 0.266 / 10.56 × 100 = 2.52%

Dilution corrected % hemolysis in Positive Control = ((0.266 / 10.56) × 8) × 100 = 20.15%.

9.7 Concentration of hemoglobin in Test Article

Test Article		
	Absorbance	Concentration (C = A × F)
Replicate 1	0.0044	0.008 mg/mL
Replicate 2	0.0006	0.001 mg/mL
Replicate 3	0.0000	0.000 mg/mL
Average	0.0017	0.003 mg/mL

Reagent dilution correction: C = 2 × Average = 2 × 0.003 = 0.006 mg/mL

Blank subtracted concentration = 0.006 – 0.004 = 0.0002 mg/mL

% hemolysis in Test Article = 0.002 / 10.56 × 100 = 0.02%

Dilution corrected % hemolysis in Test Article = ((0.002 / 10.56) × 8) × 100 = 0.15%.

9.8 Hemolysis Summary

Sample	Blank Subtracted and Dilution Corrected Hemolysis	Hemolyis above Negative Control	Hemolytic Grade
Negative Control	0.15%	0.00 %	Non–hemolytic
Positive Control	20.15%	20.0 %	Hemolytic
Test Article	0.15%	0.00 %	Non–hemolytic

The results indicate that the test article is non–hemolytic, under indirect conditions.

10.0 RESULTS – DIRECT CONTACT

10.1 Hemoglobin Standard

The hemoglobin concentration (C) has a coefficient of correlation with the absorbance (A) of 1.0000, and a residual error of 0.0026. The Calibration Coefficient F is 1.87.

C = A × F = A × 1.87

10.2 Plasma Free Hemoglobin PFH

Plasma Free Hemoglobin		
	Absorbance	Concentration (C = A × F)
Replicate 1	0.0769	0.144 mg/mL
Replicate 2	0.0771	0.144 mg/mL
Replicate 3	0.0771	0.144 mg/mL
Average	0.0770	0.144 mg/mL

Reagent dilution correction: C^{PFH} = 2 × Average = 2 × 0.144 = 0.288 mg/mL

The plasma free hemoglobin concentration is 0.288 mg/mL, which is less than the 2 mg/mL allowed by the ASTM guidelines.

10.3 Concentration of total hemoglobin = C^T

$C^T = A^C * F * 13.5.$
$A^C = 0.4282$
F = Calibration coefficient = 1.87
$C^T = 10.81$ mg/mL

10.4 Concentration of hemoglobin in Blank Control

Blank Control		
	Absorbance	Concentration (C = A × F)
Replicate 1	0.0000	0.000 mg/mL
Replicate 2	0.0000	0.000 mg/mL
Replicate 3	0.0000	0.000 mg/mL
Average	0.0000	0.000 mg/mL

Reagent dilution correction: C = 2 × Average = 2 × 0.000 = 0.000 mg/mL

10.5 Concentration of hemoglobin in Negative Control

Negative Control Article		
	Absorbance	Concentration (C = A × F)
Replicate 1	0.0029	0.005 mg/mL
Replicate 2	0.0007	0.001 mg/mL
Replicate 3	0.0006	0.001 mg/mL
Average	0.0014	0.002 mg/mL

Reagent dilution correction: C = 2 × Average = 2 × 0.002 = 0.004 mg/mL

Blank subtracted concentration = 0.004 – 0.000 = 0.004 mg/mL

% hemolysis in Negative Control = 0.004 / 10.81 × 100 = 0.04%

Dilution corrected % hemolysis in Negative Control = ((0.004 / 10.81) × 8) × 100 = 0.30%.

10.6 Concentration of hemoglobin in Positive Control

Positive Control Article		
	Absorbance	Concentration (C = A × F)
Replicate 1	0.0900	0.168 mg/mL
Replicate 2	0.0831	0.155 mg/mL
Replicate 3	0.0912	0.170 mg/mL
Average	0.0881	0.165 mg/mL

Reagent dilution correction: C = 2 × Average = 2 × 0.165 = 0.330 mg/mL

Blank subtracted concentration = 0.330 – 0.000 = 0.330 mg/mL

% hemolysis in Positive Control = 0.330 / 10.81 × 100 = 3.05%

Dilution corrected % hemolysis in Positive Control = ((0.330 / 10.81) × 8) × 100 = 24.4%.

10.7 Concentration of hemoglobin in Test Article

Test Article		
	Absorbance	Concentration (C = A × F)
Replicate 1	0.0021	0.004 mg/mL
Replicate 2	0.0000	0.000 mg/mL
Replicate 3	0.0006	0.001 mg/mL
Average	0.0009	0.002 mg/mL

Reagent dilution correction: C = 2 × Average = 2 × 0.002 = 0.004 mg/mL

Blank subtracted concentration = 0.004 - 0.000 = 0.004 mg/mL

% hemolysis in Test Article = 0.004 / 10.81 × 100 = 0.04%

Dilution corrected % hemolysis in Test Article = ((0.004 / 10.81) × 8) × 100 = 0.30%.

10.8 Hemolysis Summary

Sample	Blank Subtracted and Dilution Corrected Hemolysis	Hemolyis above Negative Control	Hemolytic Grade
Negative Control	0.30%	0.00 %	Non–hemolytic
Positive Control	24.4%	24.1 %	Hemolytic
Test Article	0.30%	0.00 %	Non–hemolytic

The results indicate that the test article is non–hemolytic, under direct contact conditions.

11.0 CONCLUSION

The hemolytic activity of the test article, Udel P-1700 NT11 with 4% vegetable based lubricant. Clariant ASA0631200 (representative PN 1328800), was evaluated by direct and indirect contact using ASTM method F756–00. The test article exhibited 0.0% hemolysis above the level of hemolysis exhibited by the negative control via the direct method. The test article exhibited 0.00% hemolysis above the level of hemolysis exhibited by the negative control via the indirect method. The test article is considered non–hemolytic under the experimental conditions employed.

12.0 RECORDS

11.1 Original raw data are archived at Toxikon Corporation.

11.2 A copy of the final report and any report amendments is archived at Toxikon Corporation.

11.3 The original final report, and a copy of any protocol amendments or deviations, is forwarded to the Sponsor.

11.4 All used and unused test article shall be disposed of by Toxikon, per Sponsor's request.

13.0 CONFIDENTIALITY AGREEMENT

Statements of confidentiality were as agreed upon prior to study initiation.

14.0 ANIMAL WELFARE STATEMENT

The Sponsor assured that, to the best of their knowledge, this study did not unnecessarily duplicate previous testing and that there were no non–animal alternatives acceptable for the evaluation of this test article as defined by the protocol.

No evidence of pain and suffering was reported to the Veterinarian and/or Study Director.

Toxikon strictly adhered to the following standards in maintaining the animal care and use program:

United States Department of Agriculture (USDA), Animal and Plant Health Inspection Service, 9 CFR Ch. 1 (1/1/95 edition), Subchapter A–Animal Welfare.

"Guide for the Care and Use of Laboratory Animals," National Research Council, 1996. (NIH).

Office for Laboratory Animal Welfare (OLAW), "Public Health Service Policy on Humane Care and Use of Laboratory Animals," Health Research Extension Act of 1985 (Public Law 99–158 November 20, 1985), Reprinted 1996.

ISO 10993–2, 2006, Biological Evaluation of Medical Devices – Part 2: Animal Welfare Requirements.

Association for the Assessment and Accreditation of Laboratory Animal Care (AAALAC) International.

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

REPORT

TEST FACILITY

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SPONSOR

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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

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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 Test (USP <88>/ISO 10993-10)

REPORT

TEST FACILITY

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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: Arizona Eugene 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 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

Silicone Lim 6071

TEST ARTICLE IDENTIFICATION

24240091

TABLE OF CONTENTS Page

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 USP Class VI Certificate

NAMSA

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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 Eugenee Carter 01-29-18

Arizona E. Carter, BS, ALAT
Technical Reviewer

Date

P.O. No.: 182004705	Lab Number: 18T_20414_03	TCLAS_VI7/S	Page 1 of 1

Silicone Elastomeric Closures for Injection Test (USP <381>)

NAMSA

CONFIDENTIAL
REPORT

PEOPLE > SCIENCE > SOLUTIONS

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

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Test Facility
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Northwood, OH 43619
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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 29.JAN2018

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.

Silicone Cytotoxicity Test (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 Hemolysis Study

NAMSA
Ensuring Medical Device Safety and Compliance™

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Lab No. 99T 04489 00
P.O. No. 52950

ERIK LONG
COLDER PRODUCTS COMPANY
1001 WESTGATE DRIVE

ID No. Test #99008

ST PAUL, MN 55114

IN VITRO HEMOLYSIS STUDY (EXTRACTION METHOD)

Test Article: Silicone O-Ring (P/N 12917-00): GE LIM 6071

The test article was received on 4-12-99.

Experimental Procedure: A 39.4 cm^2 portion of the test article was placed in 32 ml of 0.9% sodium chloride solution and extracted at 70°C for 24 hours. The extract was divided into individual tubes of 10 ml each and allowed to cool to room temperature.

To duplicate aliquots of the extract and to a similarly treated set of positive and negative control tubes was added 0.2 ml of rabbit blood previously collected in a vacuum tube containing EDTA. The tubes were inverted gently to mix the contents, then placed in a constant temperature water bath at 37°C for 1 hour. The blood-saline mixture, positive and negative controls were then centrifuged for 10 minutes at a speed of not less than 1000 Xg.

The absorbance of each test article solution was determined spectrophotometrically at 545 nm. Similarly, absorbances were recorded for the positive control (10 ml water and 0.2 ml blood) and the negative control (10 ml 0.9% sodium chloride solution and 0.2 ml blood). Absorbance values for controls were used to calculate percent (%) hemolysis of the test article.

Results:
Test #1 = 0.0% hemolysis
Test #2 = 0.0% hemolysis
Mean Hemolysis = 0%

Results and conclusions apply only to the test article tested. No further evaluation of these results is made by NAMSA. Any extrapolation of these data to other samples is the responsibility of the sponsor. All procedures were conducted in conformance with good laboratory practice and EN45001 Quality Standards (TÜV Product Services 1/96).

Under the conditions of this test, the test article would be considered nonhemolytic.
Both the negative and positive control values were as expected.

Date Prepared: 4-15-99 Date Completed: 4-16-99

Comments: The test extract and controls were clear.

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

Test Facility: NAMSA, 2261 Tracy Road, Northwood, OH 43619-1397.

las Date Completed 4-16-99 Approved By Angela Watson
Angela M. Watson, PhD

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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)
- Extractables
- 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
- Sample Certificate of Compliance (CoC)

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Legal Statements

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

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