REPORT

TEST FACILITY

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SPONSOR

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CONFIDENTIAL

STUDY TITLE

ISO Systemic Toxicity Study in Mice

TEST ARTICLE NAME

Poly-ond (R) Plating

TEST ARTICLE IDENTIFICATION

Test Panels

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Summary

The test article, Poly-ond (R) Plating, 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. The test article was extracted in 0.9% sodium chloride USP solution and sesame oil, NF. 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 extraction 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.

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1. Introduction

1.1 Purpose

The purpose of this study was to evaluate the acute systemic toxicity of a test article extract following 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.

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:	April 28, 2017
Treatment Started:	May 16, 2017
Observations Concluded:	May 19, 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:	Poly-ond (R) Plating
Identification:	Test Panels
Physical Description of the Test Article:	1x1x.032 Steel test panels and 1/16 diameter X 3/8 test rods. Both test samples were plated with our Poly-ond Nickel Teflon coating.
Storage Conditions:	Room Temperature

Table 2: Control Articles

Name:	0.9% sodium chloride USP solution (SC) sesame oil, NF (SO)
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

3. Test System

3.1 Test System

Species:	Mouse (*Mus musculus*)
Strain:	Hla®: (ICR) CVF®
Source:	Hilltop Lab Animals, Inc.
Sex:	Male
Body Weight Range:	19 grams to 22 grams at injection
Acclimation Period:	Minimum 1 day
Number of Animals:	Twenty
Identification Method:	Ear Punch

3.2 Justification of Test System

Mice have historically been used to evaluate potential toxicity of test articles. The use of mice injected with a single intravenous (IV) or intraperitoneal (IP) dose of test article extract or control blank is specified in ISO 10993-11.

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.

4.5 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

Only healthy, previously unused animals were selected.

5. Method

5.1 Test and Control Article Preparation

Only the plates were included in the preparation. The test article and the control blank (extraction vehicle without the test article) were subjected to the extraction conditions as described below.

Table 3: Extraction

Vehicle	Extraction Ratio	Article Amount	Volume of Vehicle	Extraction Condition
SC	6 cm^2:1 mL	94.6 cm^2	16 mL	121°C for 1 hour
SO	6 cm^2:1 mL	94.6 cm^2	16 mL	121°C for 1 hour

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	Yellow	Clear	Yes*
		Control	Colorless	Clear	No
	Prior to Use	Test	Yellow	Clear	Yes*†
		Control	Colorless	Clear	No
SO	Before Extraction	Test	Colorless	Clear	No
		Control	Colorless	Clear	No
	After Extraction	Test	Colorless	Opaque	No
		Control	Colorless	Clear	No
	Prior to Use	Test	Colorless	Opaque	No
		Control	Colorless	Clear	No

*The particulates were orange, fine and few in number.
†The test extract was allowed to settle and an aliquot was drawn off the top.

The test extracts changed following the extraction process. There was a slight orange discoloration on the edge of the SC test article following the extraction process. The SO test article remained visually unchanged following the extraction process. The extracts were stored at room temperature for less than 1 hour 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
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

If during the observation period, none of the animals treated with the individual test extract exhibited a significantly greater reaction than the control animals, the test article met the requirements of the standard. 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 test 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

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 (2006).

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).

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.

Appendix 1 - 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	11	Normal	Normal	Normal	Normal	Normal
		12	Normal	Normal	Normal	Normal	Normal
		13	Normal	Normal	Normal	Normal	Normal
		14	Normal	Normal	Normal	Normal	Normal
		15	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	11	19	21	21	22
		12	20	21	22	24
		13	19	21	22	25
		14	20	22	23	24
		15	21	22	24	25
	Control Blank	1	20	22	23	25
		2	20	22	23	24
		3	20	21	22	23
		4	22	23	24	25
		5	20	21	22	24

Appendix 2 - 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	26	Normal	Normal	Normal	Normal	Normal
		27	Normal	Normal	Normal	Normal	Normal
		28	Normal	Normal	Normal	Normal	Normal
		29	Normal	Normal	Normal	Normal	Normal
		30	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	26	19	21	22	23
		27	19	21	22	24
		28	19	21	22	23
		29	20	21	22	24
		30	21	22	22	23
	Control Blank	16	20	22	24	25
		17	20	21	22	23
		18	20	20	22	23
		19	20	20	22	23
		20	21	22	23	24