GLP Final Report

Report No.: T60122018-004(E)
Date: 04/29/2018

Exclusively prepared for:

SPONSOR
Solaplus biotech co.,ltd.
No.75 FengFang Road, Ouhai Economic Development Zone, Wenzhou

STUDY TITLE
ISO Systemic Toxicity Study in Mice

TEST ARTICLE
hemostatic xerogel sponge
Model: XLJ-Ⅰ

ANAB ACCREDITED ISO/IEC 17025 TESTING LABORATORY
AT-2046

ilac-MRA

TESTING FACILITY
Mid-Link Technology Testing Co., Ltd.
B6-05, RongTong Building,
No. 80, Haiyun Street, TEDA
Tianjin, 300457, China

Table of Content

Summary

The test article, hemostatic xerogel sponge , XLJ-Ⅰ , was evaluated for acute systemic toxicity in mice 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 solution (SC) and Cotton Seed Oil (CSO). 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.

Approved by: Xiaojie Bo
Xiaojie Bo, Study Director

04/29/2018
Date

GLP STATEMENT

This nonclinical laboratory study was conducted in accordance with the United States Food and Drug Administration Good Laboratory Practice Regulations, 21 CFR Part 58.

There was no deviation to the protocol or provisions of GLP Regulation noted during the course of the study.

Approved by: Xiaojie Bo 04/29/2018

Xiaojie Bo, Study Director — Date

1. Generals

1.1 Purpose

The purpose of this study was to determine whether acute systemic toxicity occurs following injection into mice.

1.2 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 (2006).

1.3 Dates

Test Article Received:	03/12/2018
Injection:	04/26/2018
Observations Concluded:	04/29/2018

2. Materials

Test Article	hemostatic xerogel sponge
Model	XLJ-I
Status	Sterile Finished Device Gamma Radiation Sterilization
Physical Description	White, Flaky sponge, Solid
Composition	Chitosan, Sodium polyacrylate, Polyethylene glycol
Stability	Stability testing is completed and on file with the sponsor **Expiration Date:** 2 years
Strength	Not applicable, no active ingredient
Purity	Not applicable, no active ingredient
Storage Condition	Room Temperature
Extraction Vehicle (Control) Polar	0.9% sodium chloride
Manufacturer	China Otsuka Pharmaceutical Co.,Ltd.
Lot Number	7K70G3
Physical Description	Clear, Colourless, Liquid
Composition	NaCl
Strength	500ml:4.5g
Purity	Conforms to China Pharmacopeia (2015)
Stability	Marketed product, stability is characterized by its labelling
Storage Condition	Room Temperature
Extraction Vehicle (Control) Non-Polar	Cotton Seed oil

Manufacturer	Acros Organics
Lot Number	A0387833
Physical Description	Clear, Yellow to Green, Liquid
Composition	Cotton Seed Oil
Strength	500mL
Purity	Pure
Stability	Marketed product, stability is characterized by its labelling
Storage Condition	Room Temperature

Extractions Procedure The sample was saturated in the extraction medium before extraction. 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.

Group	**Polar (SC)**		**Non-Polar (SO)**	
	Test	**Control**	**Test**	**Control**
Extraction Ratio	0.1g: 1ml	N.A.	0.1g: 1ml	N.A.
Sample Amount	3.21 g	N.A.	3.34 g	N.A.
Extraction Vehicle Volume	32.1 ml	20.0 ml	33.4 ml	20.0 ml
Extraction Condition	50 ℃ 72 hour	50 ℃ 72 hour	50 ℃ 72 hour	50 ℃ 72 hour
Condition of Extracts	Clear	Clear	Clear	Clear
	No Particulate	No Particulate	No Particulate	No Particulate

Note: All extracts were not centrifuged, filtered or otherwise altered prior to dosing. It was dosed immediately after extraction.

3. Test Systems and Justification

Species:	Mouse (Mus musculus)
Breed:	Kunming
Source:	Tianjin Yuda Laboratory Animal Breeding Co., Ltd.
Sex:	Male and Female femals were nulliparous and non-pregnant
Body Weight Range:	23.2-29.2grams at injection
Acclimation Period:	Minimum 5 days
Number of Animals:	Twenty (20) 5 SC Test Group, 5 SC Control Group 5 CSO Test Group, 5 CSO Control Group
Identification Method:	Ear Punch

Justification: 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 by ISO for evaluation of medical plastics.

4. Animal Management

Husbandry, Housing and Environment	Conditions conform to MID-LINK Standard Operating Procedures. Animals with same sex and in same group were housed in group of five in a box cage with an identification card indicating the animal number, test code.
Food, Water and Contaminants	A commercially available mouse feed was provided daily. Potable water was provided ad libitum through species appropriate water containers. No contaminant present in the feed and water was expected to impact the results of this study.
Personnel	Associates involved in this study were appropriately qualified and trained.
Veterinary Care	Standard veterinary medical care was provided during the study, if applicable.
Selection	Only healthy, previously unused animals will be selected.

5. Methods

Prior to dosing, the mice were identified and weighed. Five animals were injected with test extracts(SC) intravenously via the lateral tail vein at a dose of 50 mL/kg, another five animals were similarly injected with the corresponding SC blank solution (without test article). Five animals were injected with test extracts (CSO) intraperitoneally at a dose of 50 mL/kg and not exceeding 2ml/minute, another five animals were similarly injected with the corresponding CSO blank solution (without test article). Dosing occurred on day 0. Animals were observed for adverse reactions immediately after dosing, and at 4, 24, 48 and 72 hours after injection. The animals were weighed daily for three days after dosing. After the test is completed, all animals were euthanized according to Mid-Link procedure.

6. Evaluation

If during the observation period none of the animals treated with the test extract showed a significantly greater reaction than the corresponding control animals, then the test sample met the test requirements. If two or more animals died or if abnormal behaviour such as convulsions or prostration occurred in to or more animals or if body weight loss greater than 10% occurred in three or more animals; the test sample did not meet the test requirements.

7. Results

Mortality Data

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

Clinical Observations

All animals were clinically normal throughout the study. The clinical observations are presented in Table 2 and Table 3 in the attachment.

Body Weight

No animal has a weight loss greater 10%. Body weight data are presented in Table 4 in the attachment.

8. Conclusion

Under the conditions of this study, there was no mortality or evidence of systemic toxicity from the extracts injected

Under the conditions of this study, 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 final report are retained in designated Mid-Link's archive files in accordance with Mid-Link SOP.

10. References

1. Code of Federal Regulations (CFR), Title 21, Part 58, Good Laboratory Practice for Nonclinical Laboratory Studies.
2. International Organization for Standardization (ISO) 17025 - General requirements for the competence of testing and calibration laboratories (2005)
3. International Organization for Standardization (ISO) 10993-1, Biological evaluation of medical devices - Part 1: Evaluation and testing within a risk management process (2009).
4. International Organization for Standardization (ISO) 10993 11, Biological evaluation of medical devices - Part 11: Tests for systemic toxicity (2006).
5. International Organization for Standardization (ISO) 10993-12, Biological evaluation of medical devices - Part 12: Sample preparation and reference materials (2012).
6. GLP Study Protocol, T60122018-004

STATEMENT OF QUALITY ASSURANCE ACTIVITIES

Phase Inspected	Date Inspected	Date Reported to Study Director	Date Reported to Management
Injection	04/26/2018	04/26/2018	04/26/2018
Study Data Review	04/29/2018	04/29/2018	04/29/2018
Final Report Review	04/29/2018	04/29/2018	04/29/2018

Based on a review of this study, it has been concluded that this report accurately describes the methods and standard operating procedures, and that the reported results accurately reflect the raw data of the study. This study has been reviewed in accordance with the provisions of the FDA Good Laboratory Practice Regulations (21 CFR, part 58).

QA Representative 李师慧
Authorized Signature

2018.04.29
Date

ATTACHMENT: OBSERVATIONS

Table 1 Mortality Data

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

Table 2 Clinical Observations (SC)

			Observation				
Extract	Treatment Group	Animal Number	Immediate	4 Hours	24 Hours	48 Hours	72 Hours
SC	Test Extract	1	Appeared Normal	Appeared Normal	Appeared Normal	Appeared Normal	Appeared Normal
		2	Appeared Normal	Appeared Normal	Appeared Normal	Appeared Normal	Appeared Normal
		3	Appeared Normal	Appeared Normal	Appeared Normal	Appeared Normal	Appeared Normal
		4	Appeared Normal	Appeared Normal	Appeared Normal	Appeared Normal	Appeared Normal
		5	Appeared Normal	Appeared Normal	Appeared Normal	Appeared Normal	Appeared Normal
	Control Blank	6	Appeared Normal	Appeared Normal	Appeared Normal	Appeared Normal	Appeared Normal
		7	Appeared Normal	Appeared Normal	Appeared Normal	Appeared Normal	Appeared Normal
		8	Appeared Normal	Appeared Normal	Appeared Normal	Appeared Normal	Appeared Normal
		9	Appeared Normal	Appeared Normal	Appeared Normal	Appeared Normal	Appeared Normal
		10	Appeared Normal	Appeared Normal	Appeared Normal	Appeared Normal	Appeared Normal

Table 3 Clinical Observations (CSO)

			Observation				
Extract	Treatment Group	Animal Number	Immediate	4 Hours	24 Hours	48 Hours	72 Hours
CSO	Test Extract	11	Appeared Normal	Appeared Normal	Appeared Normal	Appeared Normal	Appeared Normal
		12	Appeared Normal	Appeared Normal	Appeared Normal	Appeared Normal	Appeared Normal
		13	Appeared Normal	Appeared Normal	Appeared Normal	Appeared Normal	Appeared Normal
		14	Appeared Normal	Appeared Normal	Appeared Normal	Appeared Normal	Appeared Normal
		15	Appeared Normal	Appeared Normal	Appeared Normal	Appeared Normal	Appeared Normal
	Control Blank	16	Appeared Normal	Appeared Normal	Appeared Normal	Appeared Normal	Appeared Normal
		17	Appeared Normal	Appeared Normal	Appeared Normal	Appeared Normal	Appeared Normal
		18	Appeared Normal	Appeared Normal	Appeared Normal	Appeared Normal	Appeared Normal
		19	Appeared Normal	Appeared Normal	Appeared Normal	Appeared Normal	Appeared Normal
		20	Appeared Normal	Appeared Normal	Appeared Normal	Appeared Normal	Appeared Normal

Table 4 Weight (SC Group)

			Weight (g)			
Extract	Treatment Group	Animal Number	Day 0	Day 1	Day 2	Day 3
SC	Test Extract	1	28.9	28.9	29.1	29.0
		2	28.3	28.5	29.5	29.3
		3	27.1	28.0	28.5	28.2
		4	29.2	29.3	29.3	29.4
		5	27.2	28.2	28.2	28.2
	Control Blank	6	27.3	27.4	28.1	28.0
		7	26.5	26.9	25.9	26.5
		8	26.3	26.8	26.9	26.7
		9	28.9	28.9	29.1	29.8
		10	26.2	27.2	28.2	28.1

Table 5 Weight (CSO Group)

			Weight (g)			
Extract	Treatment Group	Animal Number	Day 0	Day 1	Day 2	Day 3
CSO	Test Extract	11	26.6	25.9	26.1	26.2
		12	24.5	25.2	25.3	25.5
		13	24.6	24.9	25.1	25.0
		14	24.3	25.7	26.4	26.2
		15	23.6	24.7	25.1	24.9
	Control Blank	16	24.5	25.6	26.1	26.2
		17	23.5	24.9	25.2	25.3
		18	23.2	24.5	25.1	25.0
		19	24.6	25.9	26.2	26.4
		20	23.9	25.0	26.1	26.6

Table 4 Weight (SC Group)

			Weight (g)			
Extract	Treatment Group	Animal Number	Day 0	Day 1	Day 2	Day 3
SC	Test Extract	1	28.9	28.9	29.1	29.0
		2	28.3	28.5	29.5	29.3
		3	27.1	28.0	28.5	28.2
		4	29.2	29.3	29.3	29.4
		5	27.2	28.2	28.2	28.2
	Control Blank	6	27.3	27.4	28.1	28.0
		7	26.5	26.9	25.9	26.5
		8	26.3	26.8	26.9	26.7
		9	28.9	28.9	29.1	29.8
		10	26.2	27.2	28.2	28.1

Table 5 Weight (CSO Group)

			Weight (g)			
Extract	Treatment Group	Animal Number	Day 0	Day 1	Day 2	Day 3
CSO	Test Extract	11	26.6	25.9	26.1	26.2
		12	24.5	25.2	25.3	25.5
		13	24.6	24.9	25.1	25.0
		14	24.3	25.7	26.4	26.2
		15	23.6	24.7	25.1	24.9
	Control Blank	16	24.5	25.6	26.1	26.2
		17	23.5	24.9	25.2	25.3
		18	23.2	24.5	25.1	25.0
		19	24.6	25.9	26.2	26.4
		20	23.9	25.0	26.1	26.6