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FINAL GLP REPORT: 15-03990-G3

SYSTEMIC INJECTION TEST – ISO

Test Article
THERMOLAST ® M TM9LFT

21 CFR Part 58 Compliance
Good Laboratory Practice for Nonclinical Laboratory Studies

Report Date
12/3/2015

Study Director
Sarah Goulet, M.S.

Sponsor
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STUDY SUMMARY

The USP 0.9% Sodium Chloride for Injection (NaCl) and Cottonseed Oil (CSO) extracts of the test article, THERMOLAST ® M TM9LFT, did not induce a significantly greater biological reaction than the control extracts following a single dose to Albino Swiss mice.

This test passed and is considered to be negative based on standards set by ISO 10993–11.

QUALITY ASSURANCE STATEMENT

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

The final report was reviewed to assure that the report accurately describes the methods and standard operating procedures. The reported results accurately reflect the raw data of the nonclinical study conducted per the protocol.

Phase	Inspection Date	Date Reported to Study Director	Date Reported to Management
BODY WEIGHT	11/18/2015	11/18/2015	11/18/2015
DATA	12/3/2015	12/3/2015	12/3/2015
FINAL REPORT	12/3/2015	12/3/2015	12/3/2015

Melissa M. Metzger, B.S.
Quality Assurance Signature

12/3/2015
Date

GLP COMPLIANCE STATEMENT

This study meets the technical requirements of the protocol.

This study was conducted in compliance with the current U.S. Food and Drug Administration 21 CFR, Part 58 Good Laboratory Practices for Nonclinical Laboratory Studies.

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, 21 CFR, Part 58.105, and its mixture with carriers, 21 CFR, Part 58.113.

SIGNATURES

Signature Information	
Protocol Number	P15-0301-00A
Study Director	Sarah Goulet, M.S.
Study Supervisor	Allan Sleger, A.S., LAT
Company	Toxikon Corporation

VERIFICATION DATES

The study initiation day is the date the protocol is signed by the Study Director.

Verification Dates	
Test Article Receipt	11/11/2015
Project Log	11/11/2015
Study Initiation	11/12/2015
Study Completion	12/3/2015

Sarah Goulet

Sarah Goulet, M.S.
Study Director Signature

12/3/15

Date

1.0 PURPOSE

The purpose of the study was to determine the potential toxic effects of the test article extract as a result of a single-dose systemic injection in mice.

2.0 REFERENCES

The study was based upon the following references:

2.1 ISO 10993–11, 2006, Biological Evaluation of Medical Devices – Part 11: Tests for Systemic Toxicity.

2.2 ISO 10993–12, 2012, Biological Evaluation of Medical Devices – Part 12: Sample Preparation and Reference Materials.

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

3.0 COMPLIANCE

The study conformed to the current FDA 21 CFR, Part 58 – Good Laboratory Practice for Nonclinical Laboratory Studies.

4.0 IDENTIFICATION OF TEST AND CONTROL ARTICLES

The Sponsor supplied the following information on a GLP 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:

Name	THERMOLAST ® M TM9LFT
CAS/Code Number	Not Supplied by Sponsor (N/S)
Lot/Batch Number	Not Supplied by Sponsor (N/S)
Storage Condition	Room Temperature
Physical State	Solid
Color	natural
Density	1,10

4.2 Negative Control Articles (Toxikon Supplied):

4.2.1 Negative Control Article:

Name	USP 0.9% Sodium Chloride for Injection (NaCl)
Toxikon QC Number	CSC-15-08-00085

4.2.2 Negative Control Article:

Name	Cottonseed Oil (CSO)
Toxikon QC Number	CSC-15-10-00165

5.0 IDENTIFICATION OF TEST SYSTEM

5.1 Animals Used in the Study:

Number and Species: 20 Albino Swiss Mice (*Mus musculus*)

Sex: female (females were non–pregnant and nulliparous)

Weight/Age Range: 20.4 – 27.2 grams / at least 34 days old (adult)
weighed to the nearest 0.1 g

Health Status: healthy, not previously used in other experimental procedures

Animal Purchase: Envigo, Indianapolis, IN

Animal Identification: ear punch

Acclimation: minimum 5 days, under same conditions as for the actual test

Animal Selection: selected from larger pool and examined to ensure lack of adverse clinical signs

5.2 Animal Care and Maintenance:

Animal Room Target Temperature: 68 ± 5 °F

Animal Room Target Relative Humidity: 30–70%

Air Exchanges per Hour: a minimum of 10 changes per hour

Lights: 12–hour light/dark cycle, full spectrum fluorescent lights

Housing: group housed (5 per cage of same sex)

Cages: polycarbonate

Bedding: hardwood chips, PJ Murphy, Montville, NJ (contact)

Animal Rations: Teklad 2020X Rodent Diet, Envigo, Madison, WI,
ad libitum

Water: tap water, *ad libitum*

There were no known contaminants present in the feed, water, or bedding expected to interfere with the test data.

The laboratory and animal rooms were maintained as limited–access facilities.

6.0 JUSTIFICATION OF TEST SYSTEM AND ROUTE OF ADMINISTRATION

6.1 Justification of Test System:

Historically, mice have been used in systemic safety evaluation studies because the guidelines have no alternative (non–animal) methods.

6.2 Route of Administration:

All animals were treated by intravenous and intraperitoneal routes. The animal species, number, and route of test article administration are recommended by the ISO 10993–11 guidelines. The test article was extracted and administered *in vivo* through a medium compatible with the test system, as indicated on the GLP Test Requisition Form.

7.0 EXPERIMENTAL DESIGN AND DOSAGE

7.1 Preparation of Test and Control Articles:

7.1.1 The test article (60 cm^2) was combined with 20 mL of vehicle following an ISO 10993–12 ratio of 3 cm^2 per 1 mL. The test article was extracted in NaCl and CSO at 70 ± 2 °C for 24 ± 2 hours.

7.1.2 Properly prepared test articles were placed in separate extraction vessels, and to each vessel the appropriate medium was added. The extraction medium completely covered the test article.

7.1.3 An untreated control (blank) was prepared for parallel treatment and comparison. The untreated control was the extraction medium that was subjected to the same temperature and for the same duration as the test article.

7.1.4 Following extraction, the vessel containing each test or control article was cooled to room temperature.

7.1.5 Each extract was agitated vigorously prior to administration.

7.1.6 After the completion of the extraction, the extracts were kept at room temperature and were used the same day the extraction was completed. The test article appeared unchanged by the extraction procedure. The extracts were clear and free from particulates. No storage of the extracts occurred. The extracts were not filtered, centrifuged, or pH adjusted.

7.1.7 All other test article preparation was as specified by the Sponsor.

7.2 Pre–Dose Procedure:

Acclimated animals were weighed prior to dosing.

7.3 Dose Administration:

7.3.1 Groups of 5 animals were injected with either the test article extract or the corresponding control article extract in the same amounts and by the same routes set forth below:

Extract	Route	Dose/kg	Injection Rate
NaCl	Intravenous	50 mL	2 mL/minute
CSO	Intraperitoneal	50 mL	—

7.3.2 Extracts prepared with NaCl and CSO were tested at 100% (neat) concentration.

7.4 Post–Dose Procedure:

7.4.1 The animals were observed for clinical signs immediately after injection, 4 hours after injection, and at 24 ± 2, 48 ± 2, and 72 ± 2 hours after injection. Observations were conducted per Toxikon SOP # 6.2.6 and in accordance with ISO 10993–11 (Appendix I). Observations conducted included all clinical and toxicologic signs.

7.4.2 Animals were weighed at 24 ± 2, 48 ± 2, and 72 ± 2 hours after injection.

7.4.3 Animals were sacrificed by carbon dioxide (CO_2) inhalation.

8.0 EVALUATION CRITERIA

8.1 Evaluation of Data:

The test passes and is considered negative if none of the animals injected with the test article show a significantly greater biological reaction than the animals treated with the control article. If two or more mice die, or show signs of toxicity such as convulsions or prostration, or if a body weight loss greater than 10% occurs in three or more animals, the test article does not meet the requirements of the test. If any animal treated with a test article shows only slight signs of biological reaction, and not more than one animal shows gross signs of biological reaction or dies, a repeat test should be conducted using groups of 10 mice. On the repeat test, all 10 animals must not show a significantly greater biological reaction than the animals treated with the control article.

8.2 Control of Bias Statement:

The study and its design employed methodology to minimize uncertainty of measurement and control of bias for data collection and analysis, which included but was not limited to: concurrent control data, system suitability assessment, blanks, and replicates.

9.0 RESULTS

9.1 Animal Weights (Table 1):

Three test animals and four control animals lost a biologically insignificant amount of weight (less than 5%). All of the other test and control animals maintained or increased in weight.

9.2 Clinical Observations (Table 1):

None of the test or control animals exhibited overt signs of toxicity at any of the observation points.

9.3 The test is considered negative because none of the animals injected with the extracts of the test article showed a significantly greater biological reaction than the animals treated with the control articles.

10.0 CONCLUSION

The USP 0.9% Sodium Chloride for Injection (NaCl) and Cottonseed Oil (CSO) extracts of the test article, THERMOLAST ® M TM9LFT, did not induce a significantly greater biological reaction than the control extracts following a single dose to Albino Swiss mice.

This test passed and is considered to be negative based on standards set by ISO 10993–11.

11.0 RECORDS

11.1 Original raw data will be archived by Toxikon Corporation.

11.2 A copy of the final report and any report amendments will be archived by Toxikon Corporation.

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

11.4 The test article shall be disposed by Toxikon.

11.5 Test article retention upon study completion is the responsibility of the Sponsor.

12.0 CONFIDENTIALITY AGREEMENT

Per corporate policy, confidentiality shall be maintained in general, and in specific accordance with any relevant agreement specifically executed between Toxikon and the Sponsor.

13.0 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 distress 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 (November 2013 edition), Subchapter A–Animal Welfare.

"Guide for the Care and Use of Laboratory Animals," National Research Council, 2011. (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 2015.

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.

14.0 UNFORESEEN CIRCUMSTANCES

Any unforeseen circumstances were documented in the raw data. However, no unforeseen circumstances that affected the integrity of the study were noted.

15.0 PROTOCOL AMENDMENTS/DEVIATIONS

There were no protocol amendments or deviations. No changes to the protocol were required.

TABLE 1:
Animal Weights and Clinical Observations

Group	Animal #	Sex	Dose (mL)	Body Weight (g)					Signs of Toxicity*
				Day 0 11/18/15	Day 1 11/19/15	Day 2 11/20/15	Day 3 11/21/15	Weight Change	
NaCl Test IV 2 mL/min 50 mL/kg	1	Female	1.2	23.7	23.7	24.0	24.3	0.6	None
	2	Female	1.2	23.4	22.9	23.3	23.1	-0.3	None
	3	Female	1.3	26.5	25.7	25.9	25.4	-1.1	None
	4	Female	1.4	27.2	27.7	28.2	28.2	1.0	None
	5	Female	1.2	23.4	23.3	23.8	22.9	-0.5	None
NaCl Control IV 2 mL/min 50 mL/kg	6	Female	1.0	20.9	19.8	20.3	21.3	0.4	None
	7	Female	1.0	20.8	20.4	20.9	21.2	0.4	None
	8	Female	1.0	20.8	20.8	21.3	20.5	-0.3	None
	9	Female	1.3	25.6	24.5	24.9	25.7	0.1	None
	10	Female	1.1	21.6	21.6	22.0	21.9	0.3	None
CSO Test IP 50 mL/kg	11	Female	1.2	23.9	24.0	24.6	25.0	1.1	None
	12	Female	1.2	23.4	23.0	23.5	23.4	0.0	None
	13	Female	1.3	25.5	25.5	26.0	26.6	1.1	None
	14	Female	1.2	23.7	24.0	24.4	24.3	0.6	None
	15	Female	1.1	22.0	21.0	21.5	22.3	0.3	None
CSO Control IP 50 mL/kg	16	Female	1.3	25.4	23.9	24.4	25.3	-0.1	None
	17	Female	1.2	23.3	23.4	23.7	24.5	1.2	None
	18	Female	1.2	24.6	24.4	25.1	23.5	-1.1	None
	19	Female	1.2	24.9	23.3	23.7	23.7	-1.2	None
	20	Female	1.0	20.4	20.6	21.1	21.4	1.0	None

* Summary of clinical observations, Immediately, 4, 24, 48, and 72 hours after injection.

IV = Intravenous Route
IP = Intraperitoneal Route

APPENDIX I:
Common Clinical Signs and Observations

Clinical Observation	Observed Sign	Involved System(s)
Respiratory	Dyspnea (abdominal breathing, gasping), apnea, cyanosis, tachypnea, nostril discharges	CNS, pulmonary, cardiac
Motor Activities	Decrease/increase somnolence, loss of righting, anaesthesia, catalepsy, ataxia, unusual locomotion, prostration, tremors, fasciculation	CNS, somatomotor, sensory, neuromuscular, autonomic
Convulsion	Clonic, tonic, tonic–clonic, asphyxial, opisthotonos	CNS, neuromuscular, autonomic, respiratory
Reflexes	Corneal, righting, myotact, light, startle reflex	CNS, sensory, autonomic, neuromuscular
Ocular Signs	Lacrimation, miosis, mydriasis, exophthalmos, ptosis, opacity, iritis, conjunctivitis, chromodacryorrhea, relaxation of nictitating membrane	Autonomic, irritation
Cardiovascular Signs	Bradycardia, tachycardia, arrhythmia, vasodilation, vasoconstriction	CNS, autonomic, cardiac, pulmonary
Salivation	Excessive	Autonomic
Piloerection	Rough hair	Autonomic
Analgesia	Decrease reaction	CNS, sensory
Muscle Tone	Hypotonia, hypertonia	Autonomic
Gastrointestinal	Soft stool, diarrhea, emesis, diuresis, erythruria	CNS, autonomic, sensory, GI motility, kidney
Skin	Edema, erythema	Tissue damage, irritation

APPENDIX II:
Software Systems

Software	Use	Publisher/Vendor	Location
Adobe Acrobat 8, 9 and 10 Professional	Document preparation	Adobe Systems, Inc.	San José, CA
Lotus Domino Rel. 5	Client–server application for Sponsor, sample, test codes, and quotation management application databases	IBM Corporation	Armonk, NY
Matrix Gemini 5.3.5	Laboratory Information Management System	Autoscribe Limited	Reading, UK
MS Office 2010 Small Business Suite and MS Office 2013 Professional Suite	Business software (suite includes Word, Excel, PowerPoint, Outlook, Publisher, Office tools)	Microsoft Corporation	Redmond, WA
Rees CentronSQL System 2.0	Environmental monitoring and metrology system	Rees Scientific	Trenton, NJ
TMS Web 7	Document management for SOPs and training records management software system	Quality Systems Integrators	Eagle, PA
Toxikon Protocol Manager 1.0	Protocol requisition application	Custom developed	Toxikon Corporation, Bedford, MA