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FINAL GLP REPORT: 15-03990-G2

INTRACUTANEOUS 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
KRAIBURG TPE GmbH & Co. KG
Teplitzer Strasse 20
Waldkraiburg 84478
Germany

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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, were evaluated for their potential to produce irritation after intracutaneous injection in New Zealand White rabbits. The test article sites did not show a significantly greater biological reaction than the sites injected with the control article.

Based on the criteria of the protocol, the test article meets the requirements of the ISO 10993–10 guidelines.

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
DOSE ADMINISTRATION	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	P15-0300-00A
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 irritation effects of the test article extract as a result of an intracutaneous injection in New Zealand White rabbits.

2.0 REFERENCES

The study was based upon the following references:

2.1 ISO 10993–10, 2010, Biological Evaluation of Medical Devices – Part 10: Tests for Irritation and Skin Sensitization.

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: 3 New Zealand White rabbits (*Oryctolagus cuniculus*)

Sex: 1 male and 2 females (females were non–pregnant and nulliparous)

Weight/Age Range: 3.00 – 3.78 kilograms / at least 10 weeks old (adult) weighed to the nearest 10 g

Health Status: healthy, previously used in other experimental procedures

Animal Purchase: Covance Laboratories, Denver, PA

Animal Identification: ear tattoo

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: individually housed

Cages: suspended stainless steel

Bedding: Alfa Cobs, Scotts Distributing Inc., Hudson, NH (non-contact)

Animal Rations: Teklad Global High Fiber Rabbit Diet 2031, 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, New Zealand White rabbits have been used in intracutaneous safety evaluation studies because the guidelines have no alternative (non–animal) methods.

6.2 Route of Administration:

Animals were treated by intracutaneous injections. The animal species, number, and route of test article administration are recommended by the ISO 10993–10 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:

7.2.1 Pre–Treatment Screening Procedure:

Animals selected for the study were examined to ensure that their skin was free from irritation, trauma, and disease.

7.2.2 Each animal was weighed on the day of the test prior to injection.

7.2.3 Each animal was clipped free of fur on the dorsal side within 4 to 18 hours prior to injection.

7.3 Dose Administration:

7.3.1 A volume of 0.2 mL per site of one extract was injected intracutaneously at one side of each of three rabbits, five sites for the test article extract and five posterior sites for the control.

7.3.2 Similarly, at the other side of each rabbit, the other extract was injected.

7.3.3 The maximum injections per rabbit was limited to 2 test articles and 2 corresponding control articles.

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

7.4 Post–Dose Procedure:

7.4.1 The injection sites on each animal were observed for signs of erythema and edema immediately following injection and at 24 ± 2 hours, 48 ± 2 hours, and 72 ± 2 hours after injection of the test article. Observations were scored according to the Classification System for Scoring Skin Reactions (see Appendix I).

7.4.2 Observations conducted also included all clinical and toxicologic signs.

7.4.3 At the end of the observation period, the animals were weighed.

7.4.4 At the end of the study, the animals were returned to the general colony.

8.0 EVALUATION CRITERIA

8.1 Evaluation of Data:

After the 72 ± 2 hours grading, all erythema grades plus edema grades 24 ± 2 hours, 48 ± 2 hours, and 72 ± 2 hours were totaled separately for each test article or vehicle control for each individual animal. To calculate the score of a test article or vehicle control on each individual animal, divide each of the totals by 15 (3 scoring time points × 5 test or vehicle control injection sites). To determine the overall mean score for each test article and each corresponding vehicle control, add the scores for the three animals and divide by three. The final test article score was obtained by subtracting the score of the vehicle control from the test article score. The requirements of the test will be met if the difference between the test article mean score and the vehicle control mean score is 1.0 or less. If at any observation period the average reaction to the test article is questionably greater than the average reaction to the vehicle control, the test will be repeated using three additional rabbits.

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:

Animal #50835 lost a biologically insignificant amount of weight (less than 1%). All of the other test animals increased in weight (Table 1).

9.2 Clinical Observations:

None of the animals exhibited overt signs of toxicity at any of the observation points (Table 1).

9.3 The sites injected with the test article did not show a significantly greater biological reaction than the sites treated with the control article (Table 2). The difference of the overall mean score between the test article in NaCl and the control article was 0.0. The difference of the overall mean score between the test article in CSO and the control article was 0.7.

10.0 CONCLUSION

The USP 0.9% Sodium Chloride for Injection (NaCl) and Cottonseed Oil (CSO) extracts of the test article, THERMOLAST ® M TM9LFT, were evaluated for their potential to produce irritation after intracutaneous injection in New Zealand White rabbits. The test article sites did not show a significantly greater biological reaction than the sites injected with the control article.

Based on the criteria of the protocol, the test article meets the requirements of the ISO 10993–10 guidelines.

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	Body Weight (kg)			Signs of Toxicity*
			Day 0 11/18/15	Day 3 11/21/15	Weight Change	
NaCl & CSO	50824	Female	3.78	3.81	0.03	None
	50834	Female	3.72	3.73	0.01	None
	50835	Male	3.00	2.99	-0.01	None

* Summary of Clinical Observations at 24, 48, and 72 hours excluding skin reactions.

TABLE 2:
Intracutaneous Test Skin Reaction Scores

NaCl Extract

Animal #	Vehicle	Time	Site Numbers Scoring (ER/ED)									
			T–1	T–2	T–3	T–4	T–5	C–1	C–2	C–3	C–4	C–5
50824	NaCl	0 hours†	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
		24 hours	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
		48 hours	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
		72 hours	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
50834	NaCl	0 hours†	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
		24 hours	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
		48 hours	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
		72 hours	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
50835	NaCl	0 hours†	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
		24 hours	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
		48 hours	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
		72 hours	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0

† = Immediately after injection, not used for the evaluation criteria.

Animal #	Vehicle	Total Scores (ER + ED)		*Individual Score	
		Test	Control	Test	Control
50824	NaCl	0	0	0.0	0.0
50834	NaCl	0	0	0.0	0.0
50835	NaCl	0	0	0.0	0.0
			**Overall Mean Score	0.0	0.0

*Individual Score = Total (ER + ED) divided by 15 (3 grading periods × 5 test or control sites)

** Overall Mean Score = Total Individual Scores divided by 3 animals
Overall Mean Score for Test Article = 0.0
Overall Mean Score for Control Article = 0.0
Difference between Test Article and Control Article Overall Mean Score = 0.0 – 0.0 = 0.0

ER = Erythema T = Test Site
ED = Edema C = Control Site

TABLE 2:
Intracutaneous Test Skin Reaction Scores (Cont.)

CSO Extract

Animal #	Vehicle	Time	Site Numbers Scoring (ER/ED)									
			T–6	T–7	T–8	T–9	T–10	C–6	C–7	C–8	C–9	C–10
50824	CSO	0 hours†	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
		24 hours	1/1	1/1	1/1	1/1	1/1	0/0	0/0	0/0	0/0	0/0
		48 hours	1/1	1/1	1/1	1/1	1/1	0/0	0/0	0/0	0/0	0/0
		72 hours	1/1	1/1	1/1	1/1	1/1	0/0	0/0	0/0	0/0	0/0
50834	CSO	0 hours†	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
		24 hours	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1
		48 hours	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1
		72 hours	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1
50835	CSO	0 hours†	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
		24 hours	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1
		48 hours	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1
		72 hours	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1

† = Immediately after injection, not used for the evaluation criteria.

Animal #	Vehicle	Total Scores (ER + ED)		*Individual Score	
		Test	Control	Test	Control
50824	CSO	30	0	2.0	0.0
50834	CSO	30	30	2.0	2.0
50835	CSO	30	30	2.0	2.0
			**Overall Mean Score	2.0	1.3

*Individual Score = Total (ER + ED) divided by 15 (3 grading periods × 5 test or control sites)

** Overall Mean Score = Total Individual Scores divided by 3 animals
Overall Mean Score for Test Article = 2.0
Overall Mean Score for Control Article = 1.3
Difference between Test Article and Control Article Overall Mean Score = 2.0 – 1.3 = 0.7

ER = Erythema T = Test Site
ED = Edema C = Control Site

APPENDIX I:
Classification System for Scoring Skin Reactions

Erythema and Eschar Formation	Value
No erythema	0
Very slight erythema (barely perceptible)	1
Well–defined erythema	2
Moderate erythema	3
Severe erythema (beet redness) to eschar formation (preventing grading of erythema)	4

Total possible erythema score = 4

Edema Formation	Value
No edema	0
Very slight edema (barely perceptible)	1
Well–defined edema (edges are well–defined by definite raising)	2
Moderate edema (raised approximately 1 mm)	3
Severe edema (raised more than 1 mm and extending beyond area of exposure)	4

Total possible edema score = 4

Total possible score for irritation = 8

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