

FINAL GLP REPORT: 20-03086-G1

INTRACUTANEOUS INJECTION TEST – ISO

Test Article
TM9MEP

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

Final Report Date
9/15/2020

Study Director
Sarah Goulet, M.S.

Sponsor
Nelson Laboratories, NV
Romeinsestraat 12
Leuven, Belgium B-3001

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STUDY SUMMARY

The USP 0.9% Sodium Chloride for Injection (NaCl) and Cottonseed Oil (CSO) extracts of the test article, TM9MEP, 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
BODY WEIGHT	9/4/2020	9/4/2020	9/4/2020
DATA	9/15/2020	9/15/2020	9/15/2020
FINAL REPORT	9/15/2020	9/15/2020	9/15/2020



Gaurav Chavan, M.S.
Quality Assurance

9/15/2020

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	p19-1800-00d
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	8/24/2020
Project Log	8/24/2020
Study Initiation	8/25/2020
Study Completion	9/15/2020



Sarah Goulet, M.S.
Study Director

9/15/2020
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:

- ISO 10993–10, 2010, Biological Evaluation of Medical Devices – Part 10: Tests for Irritation and Skin Sensitization.
- ISO 10993–12, 2012, Biological Evaluation of Medical Devices – Part 12: Sample Preparation and Reference Materials.
- ISO/IEC 17025, 2017, 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 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: TM9MEP

CAS/Code Number: Not Supplied by Sponsor (N/S)

Lot/Batch Number: 1175200

Physical State: Solid

Color: N/S

Expiration Date: N/S

Density: N/S

Stability: N/S

Sterility: Not Sterile

Sterilization Conditions: N/S

Storage Condition: Room Temperature

Safety Precautions: N/S

Intended Use: N/S

4.2 Negative Control Articles (Toxikon Supplied):

4.2.1 Negative Control Article 1:

Name: USP 0.9% Sodium Chloride for Injection (NaCl)

Toxikon QC Number: CSC-20-07-00116

4.2.2 Negative Control Article 2:

Name: Cottonseed Oil (CSO)

Toxikon QC Number: CSC-20-07-00115

5.0 IDENTIFICATION OF TEST SYSTEM

5.1 Animals Used in the Study:

Number and Species: 3 New Zealand White rabbits (*Oryctolagus cuniculus*)

Sex: 2 males and 1 female (female was non-pregnant and nulliparous)

Weight/Age Range: 3.28 – 3.84 kilograms / at least 10 weeks old (adult)
 weighed to the nearest 10 g

Health Status: healthy, previously used in other experimental procedures

Animal Purchase: Envigo Global Services, 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, ScottPharma Solutions, Marlborough, MA (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. The animal species, number, and route of test article administration are recommended by the ISO 10993-10 guidelines.

6.2 Route of Administration:

Animals were treated by intracutaneous injections. The test article was extracted and administered *in vivo* through a medium compatible with the test system, as indicated on the Test Requisition Form.

7.0 EXPERIMENTAL DESIGN AND DOSAGE

7.1 Preparation of Test and Control Articles:

7.1.1 Preparation, Extraction Medium, and Extraction Conditions:

The test article (156 cm² as per Sponsor) was combined with 52.0 mL of vehicle following an ISO 10993-12 ratio of 3 cm² per 1 mL. The test article was separately extracted in NaCl and CSO at 50 ± 2 °C for 72 ± 2 hours under dynamic conditions. A total of 2 units were used for testing.

7.1.2 Addition of Extraction Medium:

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 Control Conditions:

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 Extract Agitation:

Each extract was agitated vigorously prior to administration.

7.1.5 Extract Examination:

The test article appeared unchanged by the extraction procedure. The extracts were clear and free of particulates and the color of the vehicle unchanged.

7.1.6 Extract Manipulation:

The extracts were not filtered, centrifuged, or pH adjusted.

7.1.7 Extract Storage:

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

After the completion of the extraction, the extracts were kept at room temperature and were used the same day the extraction was completed. No storage of the extracts occurred.

7.1.8 Other Test Article Preparation:

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 Body Weights:

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

7.2.3 Fur Clipping:

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

7.3 Dose Administration:

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.

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

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

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

7.4 Post-Dose Procedure:

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](#)).

7.4.1 Clinical Observations:

Observations conducted also included all clinical and toxicologic signs.

7.4.2 Body Weights:

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

7.4.3 Euthanasia:

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 from 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 \times 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 as designed employed methodology to minimize uncertainty of measurement and to control bias for data collection and analysis, which included but was not limited to: concurrent control data, system suitability assessment, randomization, and method controls such as blanks and replicates.

9.0 RESULTS

9.1 Animal Weights:

All of the 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](#)).

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 and the control article was 0.0.

10.0 CONCLUSION

The USP 0.9% Sodium Chloride for Injection (NaCl) and Cottonseed Oil (CSO) extracts of the test article, TM9MEP, 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

- Original raw data will be archived by Toxikon Corporation.
- The original final report and any report amendments will be archived by Toxikon Corporation.

- A copy of the final report and a copy of any protocol amendments or deviations will be forwarded to the Sponsor.
- The test article will be disposed by Toxikon.
- 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 during the course of this study.

Toxikon strictly adheres 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, Subchapter A–Animal Welfare.

“Guide for the Care and Use of Laboratory Animals,” National Research Council, 2011.

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), revised 2015.

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

AAALAC International accreditation.

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 9/1/2020	Day 3 9/4/2020	Weight Change	
NaCl & CSO	B0246	Male	3.84	3.90	0.06	None
	B0247	Male	3.65	3.66	0.01	None
	B0255	Female	3.28	3.31	0.03	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	
B0246	NaCl	0 hours†	0/0	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	0/0
		48 hours	0/0	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	0/0
B0247	NaCl	0 hours†	0/0	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	0/0
		48 hours	0/0	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	0/0
B0255	NaCl	0 hours†	0/0	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	0/0
		48 hours	0/0	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	0/0

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

Animal #	Vehicle	Total Scores (ER + ED)		*Individual Score		
		Test	Control	Test	Control	
B0246	NaCl	0	0	0.0	0.0	
B0247	NaCl	0	0	0.0	0.0	
B0255	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 x 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	
B0246	CSO	0 hours†	0/0	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	1/1
		48 hours	1/1	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	1/1
B0247	CSO	0 hours†	0/0	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	0/0
		48 hours	0/0	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	0/0
B0255	CSO	0 hours†	0/0	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	0/0
		48 hours	0/0	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	0/0

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

Animal #	Vehicle	Total Scores (ER + ED)		*Individual Score	
		Test	Control	Test	Control
B0246	CSO	30	30	2.0	2.0
B0247	CSO	0	0	0.0	0.0
B0255	CSO	0	0	0.0	0.0
		Overall Mean Score		* 0.7	*** 0.7

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

** Overall Mean Score = Total Individual Scores divided by 3 animals

Overall Mean Score for Test Article = 0.7

Overall Mean Score for Control Article = 0.7

Difference between Test Article and Control Article Overall Mean Score = 0.7 – 0.7 = 0.0

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

***CSO sensitivity is commonly seen in laboratory rabbits. As scores were observed at the test and control sites it is unlikely this is related to the test article.

**APPENDIX I:
 Classification System for Scoring Skin Reactions**

<u>Erythema and Eschar Formation</u>	<u>Value</u>
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

<u>Edema Formation</u>	<u>Value</u>
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	21 CFR Part 11 Status	Publisher/ Vendor	Location
Adobe Acrobat 8, 9, and 10 Professional	Document preparation	Not Applicable	Adobe Systems, Inc.	San José, CA
Matrix Gemini 5.3.19	Laboratory Information Management System	Compliant	Autoscribe Limited	Reading, UK
MS Office 2010 Small Business Suite and MS Office 2013 Professional Suite and higher	Business software (suite includes Word, Excel, PowerPoint, Outlook, Publisher, Office tools)	Not Applicable	Microsoft Corporation	Redmond, WA
Rees Scientific Centron Presidio 3.0	Automated Environmental Monitoring	Compliant	Rees Scientific	Trenton, NJ
TMS Web 7	Document management for SOPs and training records management software system	Compliant	Quality Systems Integrators	Eagle, PA
Toxikon Protocol Manager 1.0	Protocol requisition application	Not Applicable	Toxikon Corporation	Bedford, MA



TOXIKON TEST PROTOCOL
FDA GLP REGULATIONS
CONFIDENTIAL PROPERTY OF TOXIKON

INTRACUTANEOUS INJECTION TEST - ISO

TOXIKON PROTOCOL NUMBER: p19-1800-00d

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

MANAGEMENT OF THE STUDY

 ORIGINAL

Test Facility
Toxikon Corporation
15 Wiggins Avenue
Bedford, MA 01730

Sponsor
Nelson Laboratories, NV
Romeinsestraat 12
Leuven, B-3001
Belgium

20-03086-G1

PROTOCOL SIGNATURES

Jared Forsyth
PRINT NAME

Jared Forsyth
Sponsor's Representative Approval
Nelson Laboratories, NV
Romeinsestraat 12
Leuven, B-3001
Belgium

17 Jan 2020
Date

Romel Patel
PRINT NAME

R Patel
Quality Assurance Review
Toxikon Corporation
15 Wiggins Avenue
Bedford, MA 01730

1/15/2020
Date

Sarah Goulet
PRINT NAME

Sarah Goulet
Study Director Signature
Toxikon Corporation
15 Wiggins Avenue
Bedford, MA 01730

8/25/2020
Date

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20-03086-G1

1.0 PURPOSE

The purpose of the study is 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 will be based upon the following references:

- ISO 10993-10, 2010, Biological Evaluation of Medical Devices - Part 10: Tests for Irritation and Skin Sensitization.
- ISO 10993-12, 2012, Biological Evaluation of Medical Devices - Part 12: Sample Preparation and Reference Materials.
- ISO/IEC 17025, 2017, 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 Nonclinical Laboratory Studies.

4.0 IDENTIFICATION OF TEST AND CONTROL ARTICLES

The Sponsor will supply the following information on a Test Requisition Form or other correspondence, wherever applicable (excluding confidential or trade secret information). The Sponsor will be responsible for all test article characterization data as specified in the GLP regulations. Test and control articles (exclusive of extracts) that are mixed with carriers require verification of concentration, homogeneity, and stability. Samples of test and control article mixtures will be returned to the Sponsor for characterization and verification, unless this work is specifically contracted to Toxikon by Sponsor under a separate analytical protocol, whichever is applicable.

4.1 Test Article:

Name: To Be Determined (TBD)

CAS/Code Number: TBD

Lot/Batch Number: TBD

Physical State: TBD

Color: TBD

Expiration Date: TBD

Density: TBD

Stability: TBD

Sterility: TBD

Sterilization Conditions: TBD

Storage Condition: TBD

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Safety Precautions: TBD

Intended Use: TBD

4.2 Negative Control Article(s) * (Toxikon Supplied, unless specified by the Sponsor):

4.2.1 Negative Control Article 1:

Name: Physiological Saline (NaCl)

Toxikon QC Number: To Be Determined (TBD)

4.2.2 Negative Control Article 2:

Name: Cottonseed Oil (CSO)

Toxikon QC Number: To Be Determined (TBD)

4.2.3 Negative Control Article 3:

Name: 1 in 20 Ethanol in NaCl (EtOH)

Toxikon QC Number: To Be Determined (TBD)

4.2.4 Negative Control Article 4:

Name: Polyethylene Glycol 400 (PEG)

Toxikon QC Number: To Be Determined (TBD)

* Negative control article(s) will be the vehicle(s) used for extraction, as selected by the Sponsor.

5.0 IDENTIFICATION OF TEST SYSTEM

5.1 Animals Used in the Study:

Number and Species: 3 New Zealand White rabbits (*Oryctolagus cuniculus*) per extract

Sex: male and/or female (females will be non-pregnant and nulliparous)

Weight/Age Range: at least 2.0 kilograms / at least 10 weeks old (adult)
weighed to the nearest 10 g

Health Status: healthy, may be previously used in other experimental procedures

Animal Purchase: registered commercial breeder

Animal Identification: ear tattoo or ear marker

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%

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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: laboratory grade bedding used as non-contact bedding

Animal Rations: commercial rabbit ration, *ad libitum*

Water: tap water, *ad libitum*

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

The laboratory and animal rooms are 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. The animal species, number, and route of test article administration are recommended by the ISO 10993-10 guidelines.

6.2 Route of Administration:

Animals will be treated by intracutaneous injections. The test article will be extracted and administered *in vivo* through a medium compatible with the test system, as indicated on the Test Requisition Form.

7.0 EXPERIMENTAL DESIGN AND DOSAGE

7.1 Preparation of Test and Control Articles:

7.1.1 Preparation:

The test article will be prepared at the following ratio (please indicate on the Test Requisition Form):

- According to ISO 10993-12
- No preparation required
- Sponsor-Specified

7.1.2 Extraction Medium:

The test article extracts will be prepared with the following medium (please indicate on the Test Requisition Form):

- Physiological Saline (NaCl)
- Cottonseed Oil (CSO)
- 1 in 20 Ethanol in NaCl (EtOH)
- Polyethylene Glycol 400 (PEG)
- Sponsor-Specified Medium (NOTE: Extraction medium not specified by ISO 10993-12 may be required to be justified.)

7.1.3 Extraction Conditions:

The test article will be dynamically extracted (except for 121 ± 2 °C) at one of the following conditions (please indicate on the Test Requisition Form):

- 37 ± 1 °C for 72 ± 2 hours
- 50 ± 2 °C for 72 ± 2 hours
- 70 ± 2 °C for 24 ± 2 hours
- 121 ± 2 °C for 60 ± 4 minutes
- Sponsor-Specified (NOTE: Extraction conditions not specified by ISO 10993-12 may be required to be justified.)

7.1.4 Addition of Extraction Medium:

Properly prepared test article will be placed in an extraction vessel and the appropriate medium will be added, unless specified otherwise by the Sponsor. The medium should completely cover the test article, unless specified otherwise by the Sponsor.

7.1.5 Control Conditions:

Each extraction medium (control article) will be prepared for parallel treatments and comparisons. Each control article will be prepared at the same temperature and for the same duration as the test article.

7.1.6 Extract Agitation:

Each extract will be agitated vigorously prior to administration.

7.1.7 Extract Examination:

Each extract will be examined for particulates and changes, which may have occurred during the extraction process.

7.1.8 Extract Manipulation:

The extracts will not be pH adjusted, filtered, centrifuged, or manipulated in any way, unless requested by the Sponsor. Any post extraction manipulations will be reported and justified.

7.1.9 Extract Storage:

No storage of the extracts will occur. The extracts may be cooled to ambient conditions and will be used within 24 hours of the extraction process being completed.

7.1.10 Other Test Article Preparation:

All other test article preparation will be as specified by the Sponsor.

7.2 Pre-Dose Procedure:

7.2.1 Pre-Treatment Screening Procedure:

Animals selected for the study will be examined to ensure that their skin is free from irritation, trauma, and disease.

7.2.2 Body Weights:

Each animal will be weighed on the day of the test prior to injection.

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7.2.3 Fur Clipping:

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

7.2.4 PEG Extract:

Prior to injection, the PEG extracts (test and control), if utilized, will be diluted with NaCl to obtain 120 mg of PEG per mL.

7.3 Dose Administration:

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

Similarly, at the other side of each rabbit, the other test article and control extracts will be injected (if applicable.)

The maximum injections per rabbit will be limited to 2 test article extracts and 2 corresponding control article extracts.

The extracts will be dosed at a neat (100%) concentration unless requested otherwise by the Sponsor.

7.4 Post-Dose Procedure:

The injection sites on each animal will be 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 will be scored according to the Classification System for Scoring Skin Reactions (see Appendix I).

7.4.1 Clinical Observations:

Daily observations conducted will also include all clinical and toxicologic signs.

7.4.2 Body Weights:

At the end of the observation period, the animals will be weighed.

7.4.3 Euthanasia:

At the end of the study, the animals may be euthanized using an injectable barbiturate or 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 from 24 ± 2 hours, 48 ± 2 hours, and 72 ± 2 hours will be 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 \times 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 can be 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

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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 as designed employs methodology to minimize uncertainty of measurement and to control bias for data collection and analysis, which includes but is not limited to: control data (retrospective, concurrent, or prospective), system suitability assessment, randomization, method controls such as blanks and replicates, or others as required by the specific study or guideline. Methods employed will be specified in the final report.

9.0 RECORDS

- Original raw data will be archived by Toxikon Corporation.
- The original final report and any report amendments will be archived by Toxikon Corporation.
- A copy of the final report and a copy of any protocol amendments or deviations will be forwarded to the Sponsor.
- All used and unused test article will be handled as specified on the Test Requisition Form. If not indicated on the Test Requisition Form, all remaining test article will be disposed.
- Test article retention upon study completion is the responsibility of the Sponsor.

10.0 CONFIDENTIALITY AGREEMENT

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

11.0 ANIMAL WELFARE STATEMENT

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

Evidence of pain and distress will be immediately reported to the Veterinarian and/or Study Director, who will make a decision, independently or in concert with the Sponsor, to terminate the study or to continue with or without appropriate analgesics. In toxicity studies, animals cannot be administered analgesics since they would interfere with the toxicity determination. Animals may be immediately euthanized. In other studies, one or more analgesics may be administered to reduce pain and distress. The Institutional Official (IO) and the Institutional Animal Care and Use Committee (IACUC) bases this policy upon Toxikon's Standard Operating Procedures and animal care and welfare standards as governed.

Toxikon strictly adheres to the following standards, where applicable, in maintaining the animal care and use program:

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

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

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), revised 2015.

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

AAALAC International accreditation.

12.0 UNFORESEEN CIRCUMSTANCES

All unforeseen circumstances will be documented in the raw data. Any unforeseen circumstances that affect the integrity of the study will be discussed in the final report.

13.0 PROTOCOL AMENDMENTS/DEVIATIONS

All changes to the approved protocol and the reason for the changes will be documented in writing, signed by the Study Director, dated, and maintained with the protocol. A Protocol Amendment (PA) or a Protocol Deviation (PD) will be generated as closely as possible to the time of the change. The document will be created and signed by the Study Director and sent to the Sponsor. Sponsor's signature will be required for amendments (PA) to indicate approval of the amendment. Acknowledgement of notification of deviations is preferred and may be with a signature or other form of documentation.

APPENDIX I: Classification System for Scoring Skin Reactions

<u>Erythema and Eschar Formation</u>	<u>Value</u>
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

<u>Edema Formation</u>	<u>Value</u>
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

The following are the proposed software systems to be used during the conduct of this study. The actual systems used, as well as 21 CFR Part 11 compliance if applicable, will be documented in the final report.

Software	Use	Publisher/ Vendor	Location
Adobe Acrobat 8, 9, and 10 Professional	Document preparation	Adobe Systems, Inc.	San José, CA
Matrix Gemini 5.3.19	Laboratory Information Management System	Autoscribe Limited	Reading, UK
MS Office 2010 Small Business Suite and MS Office 2013 Professional Suite and higher	Business software (suite includes Word, Excel, PowerPoint, Outlook, Publisher, Office tools)	Microsoft Corporation	Redmond, WA
Rees Scientific Centron Presidio 3.0	Automated Environmental Monitoring	Rees Scientific	Trenton, NJ
Report Automation 1.0	Custom software (add-in) for final report generation, review, approval, distribution to sponsors, and storage	Court Square Group	Springfield, MA
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	Toxikon Corporation	Bedford, MA

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