FINAL GLP REPORT: 20-03086-G2

SYSTEMIC 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, did not induce a significantly greater biological reaction than the control extracts following a single dose to Albino Swiss mice.

Based on the criteria of the protocol, the test article meets the requirements of the ISO 10993–11 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	9/1/2020	9/1/2020	9/1/2020
DATA	9/15/2020	9/15/2020	9/15/2020
FINAL REPORT	9/15/2020	9/15/2020	9/15/2020

Ami Martinez, B.S.
Quality Assurance

9-15-20
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	p19-1806-00d
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
Sarah Goulet, M.S.
Study Director

9/15/2020
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:

- ISO 10993–11, 2017, Biological Evaluation of Medical Devices – Part 11: Tests for Systemic Toxicity.
- 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: 0.9% USP 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: 20 Albino Swiss mice (*Mus musculus*)

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

Weight/Age Range: 21.0 – 25.6 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. The animal species, number, and route of test article administration are recommended by the ISO 10993–11 guidelines.

6.2 Route of Administration:

All animals were treated by intravenous or intraperitoneal routes. 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 cm^2 as per Sponsor) was combined with 52.0 mL of vehicle following an ISO 10993-12 ratio of 3 cm^2 per 1 mL. The test article was separately extracted in NaCl and CSO at 50 ± 2 ºC 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:

Acclimated animals were weighed prior to dosing.

7.3 Dose Administration:

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	—

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

7.4 Post–Dose Procedure:

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.

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

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 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 (Table 1):

Three test animals lost a biologically insignificant amount of weight (less than 7%). All of the other test and control animals 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.

10.0 CONCLUSION

The USP 0.9% Sodium Chloride for Injection (NaCl) and Cottonseed Oil (CSO) extracts of the test article, TM9MEP, did not induce a significantly greater biological reaction than the control extracts following a single dose to Albino Swiss mice.

Based on the criteria of the protocol, the test article meets the requirements of the ISO 10993–11 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	Dose (mL)	Body Weight (g)					Signs of Toxicity*
				Day 0 9/1/2020	Day 1 9/2/2020	Day 2 9/3/2020	Day 3 9/4/2020	Weight Change	
NaCl Test IV 2 mL/min 50 mL/kg	1	Female	1.2	23.4	22.8	22.8	23.5	0.1	None
	2	Female	1.1	22.8	20.3	20.5	21.4	-1.4	None
	3	Female	1.1	21.6	22.4	22.7	23.7	2.1	None
	4	Female	1.3	25.6	23.6	24.6	26.1	0.5	None
	5	Female	1.2	24.8	24.3	24.4	25.4	0.6	None
NaCl Control IV 2 mL/min 50 mL/kg	6	Female	1.2	23.3	22.9	23.1	24.0	0.7	None
	7	Female	1.1	21.9	22.3	22.4	23.1	1.2	None
	8	Female	1.1	22.2	21.8	22.4	22.8	0.6	None
	9	Female	1.2	23.9	23.3	23.6	25.4	1.5	None
	10	Female	1.1	22.9	22.1	22.6	23.2	0.3	None
CSO Test IP 50 mL/kg	11	Female	1.1	22.7	23.0	23.6	24.4	1.7	None
	12	Female	1.2	23.6	23.9	24.7	25.9	2.3	None
	13	Female	1.2	24.4	22.7	24.1	24.2	-0.2	None
	14	Female	1.1	22.2	20.2	20.2	21.0	-1.2	None
	15	Female	1.1	22.0	21.0	21.6	22.5	0.5	None
CSO Control IP 50 mL/kg	16	Female	1.2	23.9	22.7	23.1	24.6	0.7	None
	17	Female	1.1	21.2	20.3	20.8	21.8	0.6	None
	18	Female	1.2	24.1	24.2	25.5	25.7	1.6	None
	19	Female	1.2	23.5	23.4	23.9	24.4	0.9	None
	20	Female	1.1	21.0	21.3	21.7	22.0	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, 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, myotactic, 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	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
RIGHT FROM THE START.SM

TOXIKON TEST PROTOCOL
FDA GLP REGULATIONS
CONFIDENTIAL PROPERTY OF TOXIKON

SYSTEMIC INJECTION TEST - ISO

TOXIKON PROTOCOL NUMBER: p19-1806-00d

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

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PROTOCOL SIGNATURES

Jared Forsyth
PRINT NAME

Jared Forsyth
Sponsor's Representative Approval
Nelson Laboratories, NV
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Begium

14 Jan 2020
Date

Colin McFadden
PRINT NAME

Colin McFadden
Quality Assurance Review
Toxikon Corporation
15 Wiggins Avenue
Bedford, MA 01730

1/15/20
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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List of Appendices:

1.0 PURPOSE

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

- ISO 10993-11, 2017, Biological Evaluation of Medical Devices - Part 11: Tests for Systemic Toxicity.
- 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: Albino Swiss mice (*Mus musculus*); 10 animals per extract

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

Weight/Age Range: at least 17 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: registered commercial breeder

Animal Identification: ear punch or ear tag

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: group housed (5 per cage of same sex)

Cages: polycarbonate

Bedding: laboratory grade bedding used as contact bedding

Animal Rations: commercial rodent 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, mice have been used in systemic 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 10993-11 guidelines.

6.2 Route of Administration:

All animals will be treated by the intravenous or intraperitoneal route. 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.)

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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:

Acclimated animals will be weighed prior to dosing.

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7.3 Dose Administration:

7.3.1 Test Article Extract(s):

Groups of 5 animals will be 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	—
EtOH	Intravenous**	50 mL	2 mL/minute
PEG*	Intraperitoneal	10 g	—

+ Alternative polar or non-polar media will be administered in the same amounts and by the same routes.

* Prior to injection, the PEG extract (test and control), if used, will be diluted with NaCl to obtain an approximate concentration of 200 mg of PEG per mL.

**If the polar extract is found to contain particulates, the extract may be dosed via the Intraperitoneal route.

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

7.4 Post-Dose Procedure:

7.4.1 Clinical Observations:

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

7.4.2 Body Weights:

Animals will be weighed at 24 ± 2, 48 ± 2, and 72 ± 2 hours after injection.

7.4.3 Euthanasia:

Animals will be 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 (at additional cost to the Sponsor). On the repeat test, all 10 animals must not show a significantly greater biological reaction than the animals treated with the control article.

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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.

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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:
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, 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, myotactic, 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

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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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