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FINAL GLP REPORT: 18-04221-G1

CLASS VI TEST - USP

Test Article
UV Ink

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

Final Report Date
1/3/2019

Study Director
Radhika Devalaraja, Ph.D.

Sponsor
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STUDY SUMMARY

The USP 0.9% Sodium Chloride for Injection (NaCl), Cottonseed Oil (CSO), 1 in 20 Ethanol in NaCl (EtOH), and Polyethylene Glycol 400 (PEG) extracts of the test article, UV Ink, following Intracutaneous Injection in rabbits and Systemic Injection in mice, and the test article, following Implantation in rabbits, did not produce a biological response.

Based on the criteria of the protocol and the USP guidelines for Class VI Plastics - 70 °C, the test article meets the requirements of the test.

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
CONTENT OF DATA PACKAGE	1/3/2019	1/3/2019	1/3/2019
DATA	1/3/2019	1/3/2019	1/3/2019
FINAL REPORT	1/3/2019	1/3/2019	1/3/2019

Priti Patel, B.S.
Quality Assurance

1/3/19
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	P18-1763-00A
Study Director	Radhika Devalaraja, Ph.D.
Study Supervisor	Catherine Maciaszek, B.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	11/7/2018
Project Log	11/27/2018
Study Initiation	11/27/2018
Study Completion	1/3/2019

D. Radhika
Radhika Devalaraja, Ph.D.
Study Director

1/3/2019
Date

1.0 PURPOSE

The purpose of the study was to determine the biological response of animals to direct and indirect contact with the test article or injection of the test article extract.

2.0 REFERENCES

The study was based upon the following references:

- United States Pharmacopeia 41, National Formulary 36, 2018. <88> Biological Reactivity Tests, *In Vivo*.
- 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: UV Ink

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

Lot/Batch Number: N/S

Physical State: N/S

Color: N/S

Expiration Date: N/S

Density: N/S

Stability: N/S

Sterility: N/S

Sterilization Conditions: N/S

Storage Condition: N/S

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-18-11-00079

4.2.2 Negative Control Article 2:

Name: Cottonseed Oil (CSO)

Toxikon QC Number: CSC-18-11-00063

4.2.3 Negative Control Article 3:

Name: 1 in 20 Ethanol in NaCl (EtOH)

Toxikon QC Number: CSC-18-11-0629

4.2.4 Negative Control Article 4:

Name: Polyethylene Glycol 400 (PEG)

Toxikon QC Number: CSC-18-10-00182

4.2.5 Negative Control Article 5:

Name: Negative Control High Density Polyethylene Equivalent to Negative Control USP High Density Polyethylene Reference Standard (Negative Control Plastic)

Toxikon QC Number: CSC-04-05-009-CC

5.0 IDENTIFICATION OF TEST SYSTEM

5.1 Animals Used in the Study:

5.1.1 Systemic Injection Test:

Number and Species: 40 Albino Swiss mice (*Mus musculus*)

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

Weight/Age Range: 17.0 – 21.5 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

5.1.2 Intracutaneous Injection and Intramuscular Implant Tests:

Number and Species: 6 New Zealand White rabbits (*Oryctolagus cuniculus*)

Sex: 2 male and 4 females (females were non-pregnant and nulliparous)

Weight/Age Range: 2.48 – 2.92 kilograms for Intracutaneous Test
2.89 – 3.34 kilograms for Implant Test
at least 10 weeks old (young adult)
weighed to nearest 10 g

Health Status: healthy, Animal #81030 and Animal #81032 were previously used in other experimental procedures. Animal #81152, #81153, #81154 and #81155 were not 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:

5.2.1 Systemic Injection Test:

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.

5.2.2 Intracutaneous Injection and Intramuscular Implant Tests:

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:

6.1.1 Systemic Injection Test:

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

6.1.2 Intramuscular Implant and Intracutaneous Injection Tests:

Historically, New Zealand White rabbits have been used in intracutaneous injection and intramuscular implantation safety evaluation studies because the guidelines have no alternative (non-animal) methods.

6.2 Route of Administration:

6.2.1 Systemic Injection Test:

Animals were treated by intravenous and intraperitioneal routes. The animal species, number, and route of test article administration were recommended by the USP guidelines.

6.2.2 Implant and Intracutaneous Injection Tests:

Animals were treated by intracutaneous injections and intramuscular implantation. The animal species, number, and route of test article administration were recommended by the USP guidelines.

The test article was administered *in vivo* directly and/or 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 Extract Preparation for Injection Tests:

Per Sponsor request, test article was extracted intact. The test article (116 cm^2) was combined with 19.3 mL of vehicle following a USP ratio of 120 cm^2 per 20 mL.

7.1.2 Extraction Medium:

The test article was separately extracted in NaCl, CSO, EtOH, and PEG.

7.1.3 Extraction Conditions:

The test article was extracted at 70 ± 2 °C for 24 ± 2 hours under dynamic conditions for the Systemic Injection and Intracutaneous Injection tests.

7.1.4 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.

7.1.5 Control Conditions:

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

7.1.6 Extract Agitation:

Each extract was agitated vigorously prior to administration.

7.1.7 Extract Examination:

The test article appeared unchanged by the CSO and PEG extraction procedures. Some of the adhesive of the test article peeled off by the NaCl and EtOH extraction procedures. The extracts were clear and free of particulates and the color of the vehicle unchanged.

7.1.8 Extract Manipulation:

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

7.1.9 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.

7.1.10 Preparation for Implant Test:

For the implant test, all apparatus strips were prepared according to the USP guidelines. The test article was cut or shaped to measure approximately 1 mm in width, 1 mm in thickness and 10 mm in length, with a rounded cross section and rounded ends.

The Control strips were Negative Control Plastic cut to measure approximately 1 mm in diameter by 10 mm in length. The test and control strips were sterilized by dipping in 70% alcohol.

7.1.11 Other Test Article Preparation:

The Systemic and Intracutaneous Injection tests were performed using the same extracts. All other test article preparation was as specified by the Sponsor.

7.2 Pre-Dose Procedure:

7.2.1 Systemic Injection Test:

Acclimated animals were weighed prior to dosing.

For the Systemic Injection Test, the PEG test article extract and the corresponding control were diluted with NaCl to obtain PEG concentration of approximately 200 mg/mL.

7.2.2 Intracutaneous Injection Test:

On the day of the test, the animals were weighed and clipped free of fur on the dorsal side.

For the Intracutaneous Injection test, the PEG test article extract and the corresponding control were diluted with NaCl to obtain PEG concentration of approximately 120 mg/mL.

7.2.3 Intramuscular Implantation:

7.2.3.1 Animal Assignment:

Two rabbits were used for the USP Intramuscular Implantation Test.

7.2.3.2 Body Weights:

Each animal was weighed prior to implantation.

7.2.3.3 Fur Clipping:

On the day of the test, the dorsal side of the animals was clipped free of fur and loose hair was removed by means of a vacuum.

7.2.3.4 Anesthesia

Each animal was appropriately anesthetized. Prior to implantation, the area was swabbed with a surgical preparation solution.

7.3 Dose Administration:

7.3.1 Systemic Injection Test:

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	0.1 mL/second
CSO	Intraperitoneal	50 mL	—
EtOH	Intravenous	50 mL	0.1 mL/second
*PEG	Intraperitoneal	10 g	—

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

The extracts were dosed at a neat (100%) concentration.

7.3.2 Intracutaneous Injection Test:

A volume of 0.2 mL per site of each extract was injected intracutaneously at five sites on one side of each of two rabbits.

Similarly, at five sites on the other side of each rabbit, 0.2 mL of the corresponding control was injected.

The maximum injections per rabbit was limited to 2 test articles and 2 corresponding control articles. The extracts were dosed at a neat (100%) concentration.

7.3.3 Intramuscular Implantation Test:

Four samples of the test article were implanted into the paravertebral muscle on one side of the spine of each of two rabbits (2.5 to 5.0 cm from the midline, parallel to the spinal column, and about 2.5 cm from each other). In a similar fashion, two strips of the Negative Control Plastic were implanted in the contralateral muscle of each animal.

7.4 Post-Dose Procedure:

7.4.1 Systemic Injection Test:

7.4.1.1 Clinical Observations:

The animals were observed for clinical signs immediately after injection, 4 hours after injection, and 24 ± 2, 48 ± 2, and 72 ± 2 hours after injection. Observations conducted included all clinical and toxicologic signs.

7.4.1.2 Body Weights:

The animals were weighed at the end of the observation period.

7.4.1.3 Euthanasia:

Animals were sacrificed by carbon dioxide (CO_2) inhalation.

7.4.2 Intracutaneous Injection Test:

7.4.2.1 Clinical Observations:

The injection sites on each animal were observed for signs of erythema and edema immediately after 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 (Appendix I). Observations conducted also included all clinical signs.

7.4.2.2 Body Weights:

Animals were weighed at the end of the observation period.

7.4.2.3 Euthanasia:

The animals were returned to the general colony.

7.4.3 Intramuscular Implant Test:

7.4.3.1 Implant Duration:

The animals were maintained for a period of 7 days.

7.4.3.2 Clinical Observations:

The animals were observed daily for this period to ensure proper healing of the implant sites and for clinical signs of toxicity. Observations included all clinical manifestations.

7.4.3.3 Body Weights:

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

7.4.3.4 Euthanasia:

Each animal was sacrificed by an injectable barbiturate.

7.4.3.5 Necropsy:

Sufficient time was allowed to elapse for the tissue to be cut without bleeding.

7.4.3.6 USP Macroscopic Evaluation (Intramuscular or Subcutaneous Implant):

The area of the tissue surrounding the center portion of each implant strip was examined macroscopically using a magnifying lens. Hemorrhaging, necrosis, discolorations, and infections were scored using the following scale:

0 = Normal
1 = Mild
2 = Moderate
3 = Severe

Encapsulation, if present, was scored by first measuring the width of the capsule (the distance from the periphery of the implant to the periphery of the capsule) rounded to the nearest 0.1 mm. The encapsulation was scored as follows:

Capsule Width	Score
None	0
Up to 0.5 mm	1
0.6 to 1.0 mm	2
1.1 to 2.0 mm	3
Greater than 2.0 mm	4

The differences between the average scores for the test article and control article implant sites were calculated.

8.0 EVALUATION CRITERIA

8.1 Systemic Injection Test:

The test passes and is considered negative if none of the animals injected with the test article shows 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 2 grams in three or more mice, 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 Intracutaneous Injection Test:

All average erythema and edema scores for the test and control sites at 24 ± 2 hours, 48 ± 2 hours, and 72 ± 2 hours will be totaled separately and divided by 12 (2 animals x 3 scoring time points x 2 scoring categories) to determine the overall mean score for the test article versus the corresponding control vehicle. The requirements of the test will be met if the difference between the test article and control article mean reaction scores (erythema/edema) is 1.0 or less.

If at any observation point, the average reaction to the test article sites is questionably greater than the corresponding control article sites, a repeat for the particular test article extract/solution will be conducted using an additional 3 rabbits. On the repeat test, the requirements of the test will be met if the difference between the test article and control article mean reaction scores (erythema/edema) is 1.0 or less.

8.3 Intramuscular Implantation Test:

The test is considered negative if, in each rabbit, the difference between the average scores for each category of biological reaction for the test article and control article implant sites do not exceed 1.0; or if the difference between the mean scores for all categories of biological reaction for each test article and the average score for all categories for all the control implant sites do not exceed 1.0, for not more than one of four test article strips.

8.4 Class VI Requirements:

The test article satisfies the requirements of the USP Class VI test if the requirements described above are met.

8.5 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 Systemic Injection Test:

9.1.1 Animal Weights (Table 1):

One control animal lost a biologically insignificant amount of weight (less than 1%). All of the other test and control animals increased in weight.

9.1.2 Clinical Observations (Table 1):

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

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

9.2 Intracutaneous Injection Test:

9.2.1 Animal Weights (Table 2):

Animal #81154 lost a biologically insignificant amount of weight (less than 1%). All of the other animals increased in weight.

9.2.2 Clinical Observations (Table 2):

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

The difference between the test article and control article mean reaction scores (erythema/edema) was less than 1.0. The test article meets the requirements of the Intracutaneous Test (Table 3).

9.3 Implant Test:

9.3.1 Animal Weights (Table 2):

Both animals increased in weight.

9.3.2 Clinical Observations (Table 2 and Table 4):

Neither of the animals exhibited overt signs of toxicity at any of the observation points. Macroscopic evaluation of the test and control article implant sites showed no significant infection, encapsulation, hemorrhage, necrosis, or discoloration.

The test is considered negative, since in each rabbit the difference between the average scores for all of the categories of biological reaction for the test article and control article implant sites did not exceed 1.0, and the difference between the mean scores for all categories of biological reaction for all of the test article implant sites and the average score for all categories for all the control implant sites did not exceed 1.0. The test article meets the requirements of the Intramuscular Implantation Test (Table 4).

10.0 CONCLUSION

The USP 0.9% Sodium Chloride for Injection (NaCl), Cottonseed Oil (CSO), 1 in 20 Ethanol in NaCl (EtOH), and Polyethylene Glycol 400 (PEG) extracts of the test article, UV Ink, following Intracutaneous Injection in rabbits and Systemic Injection in mice, and the test article, following Intramuscular Implantation in rabbits, did not produce a biological response.

Based on the criteria of the protocol and the USP guidelines for Class VI Plastics - 70 °C, the test article meets the requirements of the test.

11.0 RECORDS

- Original raw data will be archived by Toxikon Corporation.
- A copy of the final report and any report amendments will be archived by Toxikon Corporation.
- The original 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 (January 2017 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:
Systemic Injection Test: Animal Weights and Clinical Observations

Group	Animal #	Sex	Dose (mL)	Body Weight (g)			Signs of Toxicity*
				Day 0 12/10/2018	Day 3 12/13/2018	Weight Change	
	1	Female	0.9	18.0	19.5	1.5	None
NaCl Test	2	Female	0.9	17.0	19.9	2.9	None
IV	3	Female	0.9	17.5	20.1	2.6	None
50 mL/kg	4	Female	1.0	19.5	20.9	1.4	None
	5	Female	0.9	17.4	19.4	2.0	None
	6	Female	0.9	18.2	20.1	1.9	None
NaCl Control	7	Female	0.9	18.4	19.8	1.4	None
IV	8	Female	1.1	21.5	23.2	1.7	None
50 mL/kg	9	Female	0.9	18.2	20.1	1.9	None
	10	Female	0.9	17.0	17.8	0.8	None
	11	Female	0.9	17.6	19.0	1.4	None
CSO Test	12	Female	1.0	20.6	22.5	1.9	None
IP	13	Female	0.9	18.1	20.2	2.1	None
50 mL/kg	14	Female	0.9	18.6	20.4	1.8	None
	15	Female	0.9	17.4	20.0	2.6	None
	16	Female	1.0	20.6	21.0	0.4	None
CSO Control	17	Female	1.0	19.3	19.2	-0.1	None
IP	18	Female	0.9	17.0	18.5	1.5	None
50 mL/kg	19	Female	1.0	20.8	22.7	1.9	None
	20	Female	1.0	20.1	22.4	2.3	None
	21	Female	0.9	18.9	21.0	2.1	None
EtOH Test	22	Female	0.9	17.9	20.6	2.7	None
IV	23	Female	1.0	20.8	21.3	0.5	None
50 mL/kg	24	Female	0.9	17.4	19.5	2.1	None
	25	Female	0.9	17.5	18.4	0.9	None
	26	Female	0.9	18.5	19.8	1.3	None
EtOH Control	27	Female	0.9	18.7	20.8	2.1	None
IV	28	Female	0.9	17.5	20.3	2.8	None
50 mL/kg	29	Female	1.0	19.3	19.4	0.1	None
	30	Female	0.9	18.2	20.9	2.7	None
	31	Female	0.9	17.4	18.8	1.4	None
PEG Test	32	Female	1.0	20.4	21.6	1.2	None
IP	33	Female	1.0	19.2	21.0	1.8	None
10 g/kg	34	Female	0.9	18.4	20.4	2.0	None
	35	Female	1.0	20.4	21.1	0.7	None
	36	Female	1.0	20.5	22.4	1.9	None
PEG Control	37	Female	1.0	20.8	21.8	1.0	None
IP	38	Female	0.9	18.1	20.5	2.4	None
10 g/kg	39	Female	0.9	18.0	20.1	2.1	None
	40	Female	1.0	20.4	21.4	1.0	None

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

IV = Intravenous IP - Intraperitoneal

TABLE 2:
Intracutaneous Injection and Implant Tests:
Animal Weights and Clinical Observations

Group	Animal #	Sex	Body Weight (kg)			Signs of Toxicity*
			Day 0 12/10/2018	Day 3 12/13/2018	Weight Change	
NaCl & CSO	81152	Female	2.70	2.74	0.04	None
	81153	Male	2.92	2.99	0.07	None
EtOH & PEG	81154	Female	2.48	2.47	-0.01	None
	81155	Male	2.85	2.92	0.07	None
Group	**Animal #**	**Sex**	**Body Weight (kg)**			**Signs of Toxicity***
			Day 0 12/20/2018	**Day 7 12/27/2018**	**Weight Change**	
USP Implant (7 Days)	81030	Female	3.34	3.35	0.01	None
	81032	Female	2.89	2.92	0.03	None

* Summary of Clinical Observations, Day 0 through Day 3, excluding skin reactions for the Intracutaneous Injection Test, Day 0 through Day 7 for the Implant Test (USP).

TABLE 3:
Intracutaneous Test Skin Reaction Scores

NaCl Extract

Animal #	Vehicle	Time	Site Numbers Scoring (ER/ED)									
			A-1	A-2	A-3	A-4	A-5	D-1	D-2	D-3	D-4	D-5
81152	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
81153	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
Total/5 (sites)			0.0					0.0				

Overall Mean Score* for Test Article = 0.0/12 = 0.0
Overall Mean Score* for Control Article = 0.0/12 = 0.0
A and B = Test C and D = Control
Difference between Test Article and Control Article Overall Mean Score = 0.0 - 0.0 = 0.0

CSO Extract

Animal #	Vehicle	Time	Site Numbers Scoring (ER/ED)									
			B-6	B-7	B-8	B-9	B-10	C-6	C-6	C-6	C-6	C-10
81152	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	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
81153	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	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
Total/5 (sites)			0.0					0.0				

Overall Mean Score* for Test Article = 0.0/12 = 0.0
Overall Mean Score* for Control Article = 0.0/12 = 0.0
A and B = Test sites
C and D = Control sites
Difference between Test Article and Control Article Overall Mean Score = 0.0 - 0.0 = 0.0

ER = Erythema; ED = Edema
* Overall Mean Score = Total erythema plus edema scores divided by 12
(2 animals × 3 scoring periods × 2 scoring categories)

TABLE 3:
Intracutaneous Test Skin Reaction Scores (Cont.)

EtOH Extract

Animal #	Vehicle	Time	Site Numbers Scoring (ER/ED)									
			E-6	E-7	E-8	E-9	E-10	H-6	H-7	H-8	H-9	H-10
81154	EtOH	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	1/0	1/0	1/0	1/0	1/0	0/0	0/0	0/0	0/0	0/0
81155	EtOH	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
Total/5 (sites)			1.0					0.0				

Overall Mean Score* for Test Article = 1.0/12 = 0.08
Overall Mean Score* for Control Article = 0.0/12 = 0.0
E = Test H = Control
Difference between Test Article and Control Article Overall Mean Score = 0.08 - 0.0 = 0.08

PEG Extract

Animal #	Vehicle	Time	Site Numbers Scoring (ER/ED)									
			F-6	F-7	F-8	F-9	F-10	G-6	G-7	G-8	G-9	G-10
81154	PEG	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
81155	PEG	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
Total/5 (sites)			0.0					0.0				

Overall Mean Score* for Test Article = 0.0/12 = 0.0
Overall Mean Score* for Control Article = 0.0/12 = 0.0
E and F= Test sites
G and H = Control sites
Difference between Test Article and Control Article Overall Mean Score = 0.0 - 0.0 = 0.0

ER = Erythema; ED = Edema
* Overall Mean Score = Total erythema plus edema scores divided by 12
(2 animals × 3 scoring periods × 2 scoring categories)

TABLE 4:
USP Implant Test Macroscopic Observations 7 Days

Animal #: 81030

Tissue Site	T1	T2	T3	T4	Test Average	C1	C2	Control Average
Infection	0	0	0	0	0	0	0	0
Encapsulation	0	0	0	0	0	0	0	0
Hemorrhage	0	0	0	0	0	0	0	0
Necrosis	0	0	0	0	0	0	0	0
Discoloration	0	0	0	0	0	0	0	0
Total	0	0	0	0	0	0	0	0
Mean Score (total/5)	0	0	0	0	N/A	0	0	N/A

Animal #: 81032

Tissue Site	T1	T2	T3	T4	Test Average	C1	C2	Control Average
Infection	0	0	0	0	0	0	0	0
Encapsulation	0	0	0	0	0	0	0	0
Hemorrhage	0	0	0	0	0	0	0	0
Necrosis	0	0	0	0	0	0	0	0
Discoloration	0	0	0	0	0	0	0	0
Total	0	0	0	0	0	0	0	0
Mean Score (total/5)	0	0	0	0	N/A	0	0	N/A

T = Test
C = Control
N/A = Not Applicable

APPENDIX I:
Evaluation of 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 or 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	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

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TOXIKON TEST PROTOCOL
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CLASS VI TEST - USP

TOXIKON PROTOCOL NUMBER: P18-1763-00A

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

MANAGEMENT OF THE STUDY

Test Facility	Sponsor
Toxikon Corporation	Sigma Ink
15 Wiggins Avenue	12800 Brookprinter Place
Bedford, MA 01730	Poway, CA 92064

PROTOCOL SIGNATURES

Andy Bonk
PRINT NAME

Andy Bonk
Sponsor's Representative Approval
Sigma Ink
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Poway, CA 92064

10/10/18
Date

Priti Patel
PRINT NAME

Priti Patel
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10/10/18
Date

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D. Radhika
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11/27/2018
Date

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List of Appendices:

1.0 PURPOSE

The purpose of the study is to determine the potential biological response of animals to direct and indirect contact with the test article or injection of the test article extract.

2.0 REFERENCES

The study will be based upon the following references:

- United States Pharmacopeia 41, National Formulary 36, 2018. <88> Biological Reactivity Tests, *In Vivo*.
- 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

Storage Conditions: TBD

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)

4.2.5 Negative Control Article 5:

Name: Negative Control High Density Polyethylene Equivalent to Negative Control USP High Density Polyethylene Reference Standard (Negative Control Plastic)

Toxikon QC Number: To Be Determined (TBD)

5.0 IDENTIFICATION OF TEST SYSTEM

5.1 Animals Used in the Study:

5.1.1 Systemic Injection Test:

Number and Species: 40 Albino Swiss mice (*Mus musculus*)

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

Weight/Age Range: 17-23 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

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

5.1.2 Intracutaneous Injection and Intramuscular Implant Tests:

Number and Species: at least 6 New Zealand White rabbits (*Oryctolagus cuniculus*)

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

Weight/Age Range: at least 2.0 kilograms (animals will weigh at least 2.5 kilograms for implant test) / at least 10 weeks old (young adult)
weighed to nearest 10 g

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

Animal Purchase: registered commercial breeder

Animal Identification: ear marker or 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

5.1.3 Subcutaneous Implant Test:

Number and Species: 5 Albino rats (*Rattus norvegicus*)

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

Weight/Age Range: 225-350 grams / at least 5 weeks old
weighed to nearest 0.1 g

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

Animal Purchase: registered commercial breeder

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:

5.2.1 Systemic Injection Test:

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: 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.

5.2.2 Intracutaneous Injection and Intramuscular Implant Tests:

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: 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.

5.2.3 Subcutaneous Implant Test:

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: 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:

6.1.1 Systemic Injection Test:

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

6.1.2 Intramuscular Implant and Intracutaneous Injection Tests:

Historically, New Zealand White rabbits have been used in intracutaneous injection and intramuscular implantation safety evaluation studies because the guidelines have no alternative (non-animal) methods.

6.1.3 Subcutaneous Implant Test:

Historically, albino rats have been used in subcutaneous implantation safety evaluation studies because the guidelines have no alternative (non-animal) methods.

6.2 Route of Administration:

6.2.1 Systemic Injection Test:

Animals will be treated by intravenous and intraperitioneal routes. The animal species, number, and route of test article administration are recommended by the USP guidelines.

6.2.2 Implant and Intracutaneous Injection Tests:

Animals will be treated by intracutaneous injections and intramuscular or subcutaneous implantation. The animal species, number, and route of test article administration are recommended by the USP guidelines.

The test article will be administered *in vivo* directly and/or 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 Extract Preparation for Injection Tests:

For the systemic and intracutaneous injection tests, the test article will be prepared at the following ratio (please indicate on the Test Requisition Form):

- According to USP
- 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):

- USP 0.9% Sodium Chloride for Injection (NaCl)
- Cottonseed Oil (CSO)
- 1 in 20 Ethanol in NaCl (EtOH)
- Polyethylene Glycol 400 (PEG)
- Sponsor-Specified Medium (NOTE: Extraction medium not specified by USP 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):

- 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 USP 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 Preparation for Implant Tests:

For the implant tests, all apparatus strips will be prepared according to the USP guidelines.

The test article (Sponsor-supplied) will be cut or shaped to measure approximately 1 mm to 3 mm in diameter, 1 mm in width and 10 mm in length, with a rounded cross section and rounded ends. It is the Sponsor's responsibility to ensure that the test article is manufactured, processed, cleaned of contaminants, and sterilized by the methods intended for the final end use product. Unless supplied sterile from the Sponsor, test articles will be sterilized by either autoclaving at 121 ± 2 °C for at least 15 minutes or sanitized by submerging in alcohol.

The control article will be as similar as possible to the test article in terms of surface area exposed to the host tissue. The Sponsor will take all necessary steps to provide a control article that is similar to the test article. The control article will be cut or shaped to a size similar to the test article, if possible. If a control article is not supplied by the Sponsor, Toxikon Negative Control Plastic may be used. The control article, unless supplied sterile from the Sponsor, will be sterilized by either autoclaving at 121 ± 2 °C for at least 15 minutes or sanitized by submerging in alcohol.

7.1.11 Other Test Article Preparation:

The systemic and intracutaneous injection tests may be performed using the same extracts.

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

7.2 Pre-Dose Procedure:

7.2.1 Systemic Injection Test:

Acclimated animals will be weighed prior to dosing.

For the Systemic Injection Test, the PEG test article extract and the corresponding control will be diluted with NaCl to obtain a PEG concentration of approximately 200 mg/mL.

7.2.2 Intracutaneous Injection Test:

On the day of the test, the animals will be weighed and clipped free of fur on the dorsal side.

For the Intracutaneous Injection Test, the PEG test article extract and the corresponding control will be diluted with NaCl to obtain a PEG concentration of approximately 120 mg/mL.

7.2.3 Intramuscular Implantation Test:

7.2.3.1 Animal Assignment:

At least two rabbits will be used for the Intramuscular Implantation Test.

7.2.3.2 Body Weights:

On the day of the test, the animals will be weighed prior to implantation.

7.2.3.3 Fur Clipping:

On the day of the test, the skin on both sides of the spinal column will be clipped free of fur and loose hair will be removed by means of a vacuum.

7.2.3.4 Anesthesia:

Each animal will be appropriately anesthetized. Prior to implantation, the area will be swabbed with a surgical preparation solution.

7.2.4 Subcutaneous Implantation Test:

For materials with physical characteristics unsuitable for routine intramuscular implantation, the subcutaneous rat implantation model is a viable alternative.

7.2.4.1 Animal Assignment:

Five rats will be used for the Subcutaneous Implantation Test.

7.2.4.2 Body Weights:

Each animal will be weighed prior to implantation.

7.2.4.3 Fur Clipping:

On the day of the test, the skin on both sides of the spinal column will be clipped free of fur and loose hair will be removed by means of a vacuum.

7.2.4.4 Anesthesia:

Each animal will be appropriately anesthetized. Prior to implantation, the area will be swabbed with a surgical preparation solution.

7.3 Dose Administration:

7.3.1 Systemic Injection Test:

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	0.1 mL/second
CSO	Intraperitoneal	50 mL	—
EtOH	Intravenous	50 mL	0.1 mL/second
PEG	Intraperitoneal	10 g	—

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

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

7.3.2 Intracutaneous Injection Test:

A volume of 0.2 mL of each test article extract will be injected intracutaneously at five sites on one side of each of two rabbits. More than one test article extract may be used per rabbit.

Similarly, at five sites on the other side of each rabbit, 0.2 mL of the corresponding control will be injected.

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

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

7.3.3 Intramuscular Implantation Test:

Four samples of the test article will be implanted into the paravertebral muscle on one side of the spine of each of two rabbits (2.5 to 5.0 cm from the midline, parallel to the spinal column, and about 2.5 cm from each other). In a similar fashion, two strips of the Negative Control Plastic will be implanted in the contralateral muscle of each animal. Additional strips may be implanted to assure the recovery of four test article strips and two control article strips.

7.3.4 Subcutaneous Implantation Test:

Two test samples and two Negative Control Plastic samples will be implanted in each of five rats. A small pocket will be created in the subcutaneous tissue and the implant material will be placed in the pocket (base of pocket approximately 20 mm from the incision site).

7.3.5 Test Articles with Multiple Component/Materials (Additional Cost):

This study is designed to evaluate a single material, however, if a test article has multiple components/materials to be implanted, up to two components/materials can be implanted in each animal. In this case, at least four test articles of one component will be implanted on one side of the spine. The second component will be similarly implanted in the contralateral muscle. At least two control articles will be implanted caudal (toward the tail) to the test articles on either side of the spine (total of at least four articles). Test articles with more than two components/materials to be implanted require additional rabbits (at an additional cost) or a separate study. The Sponsor is responsible for identifying test article components / materials for implantation.

7.4 Post-Dose Procedure:

7.4.1 Systemic Injection Test:

7.4.1.1 Clinical Observations:

The animals will be observed for clinical signs immediately after injection, 4 hours after injection, and then at least 24 ± 2, 48 ± 2, and 72 ± 2 hours after injection. Observations conducted will include all clinical and toxicologic signs.

7.4.1.2 Body Weights:

The animals will be weighed at the end of the observation period.

7.4.1.3 Euthanasia:

Animals will be sacrificed by carbon dioxide (CO_2) inhalation.

7.4.2 Intracutaneous Injection Test:

7.4.2.1 Clinical Observations:

The injection sites on each animal will be observed for signs of erythema and edema immediately after injection and at 24 ± 2, 48 ± 2, and 72 ± 2 hours after injection of the test article. Observations will be scored according to the Evaluation of Skin Reactions (see Appendix I). Observations conducted will also include all clinical signs.

7.4.2.2 Body Weights:

Animals will be weighed at the end of the observation period.

7.4.2.3 Euthanasia:

The animals may be euthanized by an injectable barbiturate or returned to the general colony.

7.4.3 Intramuscular or Subcutaneous Implantation Test:

7.4.3.1 Implant Duration:

The animals will be maintained for a period of not less than 120 hours for the Intramuscular Implantation Test.

The animals will be maintained for a period of at least seven days for the Subcutaneous Implantation Test.

7.4.3.2 Clinical Observations:

The animals will be observed daily for this period to ensure proper healing of the implant sites and for clinical signs of toxicity. Observations include all clinical manifestations.

7.4.3.3 Body Weights:

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

7.4.3.4 Euthanasia:

Each animal will be sacrificed by an injectable barbiturate for the intramuscular implant. For the subcutaneous implant, each animal will be sacrificed by CO_2 inhalation.

7.4.3.5 Necropsy:

Sufficient time will be allowed to elapse for the tissue to be cut without bleeding.

7.4.3.6 USP Macroscopic Evaluation (Intramuscular or Subcutaneous Implant):

The area of the tissue surrounding the center portion of each implant strip will be examined macroscopically using a magnifying lens. Hemorrhaging, necrosis, discolorations, and infections will be scored using the following scale:

0 = Normal
1 = Mild
2 = Moderate
3 = Severe

Encapsulation, if present, will be scored by first measuring the width of the capsule (the distance from the periphery of the implant to the periphery of the capsule) rounded to the nearest 0.1 mm. The encapsulation will be scored as follows:

Capsule Width	Score
None	0
Up to 0.5 mm	1
0.6 to 1.0 mm	2
1.1 to 2.0 mm	3
Greater than 2.0 mm	4

The differences between the average scores for the test article and control article implant sites will be calculated.

8.0 EVALUATION CRITERIA

8.1 Systemic Injection Test:

The test passes and will be considered negative if none of the animals injected with the test article shows 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 three or more mice lose more than 2 g of body weight, 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 treated with the test article must not show a significantly greater biological reaction than the animals treated with the control article.

8.2 Intracutaneous Injection Test:

All average erythema and edema scores for the test and control sites at 24 ± 2, 48 ± 2, and 72 ± 2 hours will be totaled separately and divided by 12 (2 animals × 3 scoring periods × 2 scoring categories) to determine the overall mean score for the test article versus the corresponding control article. The requirements of the test will be met if the difference between the test article and control article mean reaction scores (erythema/edema) is 1.0 or less.

If at any observation point, the average reaction to the test article sites is questionably greater than the corresponding control article sites, a repeat for the particular test article extract/solution should be conducted using an additional 3 rabbits. On the repeat test, the requirements of the test will be met if the difference between the test article and control article mean reaction scores (erythema/edema) is 1.0 or less.

8.3 Intramuscular Implantation Test:

The test is considered negative if, in each rabbit, the difference between the average scores for each category of biological reaction for the test article and control article implant sites does not exceed 1.0; or if the difference between the mean scores for all categories of biological reaction for each test article and the average score for all categories for all the control implant sites does not exceed 1.0, for not more than one of four test article strips.

8.4 Subcutaneous Implantation Test:

The test is considered negative if the difference between the average scores for the test article and control article implant sites does not exceed 1.0.

8.5 Class VI Requirements:

The test article will satisfy the requirements of the USP Class VI test if the requirements described above are met.

8.6 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.
- A copy of the final report and any report amendments will be archived by Toxikon Corporation.
- The original 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 consent 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 (January 2017 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.

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:
Evaluation of Skin Reactions

Erythema and Eschar Formation	Score
No erythema	0
Very slight erythema (barely perceptible)	1
Well-defined erythema	2
Moderate to severe erythema	3
Severe erythema (beet redness) to slight eschar formation (injuries in depth)	4

Total possible erythema score = 4

Edema Formation*	Score
No edema	0
Very slight edema (barely perceptible)	1
Slight edema (edges of area 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

* Excludes non-inflammatory (mechanical) edema from the blank or extract fluid.

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