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FINAL GLP REPORT: 12-4297-G1

CLASS VI TEST – USP

Test Article
20 square inches of dry ink
and 50 grams of liquid
ink

21 CFR Part 58 Compliance
GLP for Nonclinical Laboratory Studies

Report Date
November 2, 2012

Study Director
Kirsten Russell, B.S., RLAT

Sponsor
Sigma Inks Corporation
12113 Kirkham Road
Poway, CA 92064

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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, 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, 20 square inches of dry ink and 50 grams of liquid ink, 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.

Phase	Inspection Date	Date Reported to Study Director	Date Reported to Management
CLINICAL OBSERVATION	10/03/12	10/03/12	10/03/12
RAW DATA	11/02/12	11/02/12	11/02/12
FINAL REPORT	11/02/12	11/02/12	11/02/12

Chrystal A Lucia

Chrystal Lucia, B.S.
Quality Assurance Signature

11/2/12
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	P12-1511-00A
Study Director	Kirsten Russell, B.S., RLAT
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	09/12/12
Additional Sample Receipt	09/18/12
Project Log	09/12/12
Study Initiation	09/13/12
Study Completion	11/02/12

Kirsten Russell

Kirsten Russell, B.S., RLAT
Study Director Signature

11·2·12
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:

2.1 United States Pharmacopeia 35, National Formulary 30, 2012. <88> Biological Reactivity Tests, *In Vivo*.

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

3.0 COMPLIANCE

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

4.0 IDENTIFICATION OF TEST AND CONTROL ARTICLES

The Sponsor supplied the following information on a GLP Test Requisition Form or other correspondence, wherever applicable (excluding confidential or trade secret information). The Sponsor was responsible for all test article characterization data as specified in the GLP regulations.

4.1 Test Article:

Name	20 square inches of dry ink and 50 grams of liquid ink
CAS/Code Number	Not Supplied by Sponsor (N/S)
Lot/Batch Number	Not Supplied by Sponsor (N/S)

4.2 Negative Control Articles (Toxikon Supplied):

4.2.1

Name	USP 0.9% Sodium Chloride for Injection (NaCl)
Toxikon QC Number	CSC–12–07–007–VV

4.2.2

Name	Cottonseed Oil (CSO)
Toxikon QC Number	CSC–12–08–009–VV

4.2.3

Name	1 in 20 Ethanol in NaCl (EtOH)
Toxikon QC Number	CSC–12–02–001–VV; CSC–12–07–007–VV

4.2.4

Name	Polyethylene Glycol 400 (PEG)
Toxikon QC Number	CSC–12–09–001–VV

4.2.5

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

4.3 Reagent (Toxikon Supplied):

Name	Sterile Water for Injection (SWFI)
Toxikon QC Number	CSC–12–08–011–VV

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: 18.3–23.0 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: Harlan Laboratories, Indianapolis, IN

Animal Identification: ear punch

Acclimation: minimum 3 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 Implant Tests:

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

Sex: 4 males and 2 females (females were non–pregnant and nulliparous)

Weight/Age Range: 3.06–3.36 kilograms for Intracutaneous
2.84–2.92 kilograms for Implant Test
at least 10 weeks old (young adult)
weighed to nearest 10 g

Health Status: healthy, previously used in other experimental procedures

Animal Purchase: Millbrook Breeding Labs, Amherst, MA

Animal Identification: ear tattoo

Acclimation: minimum 3 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, P.W.I. Industries, St–Hyacinthe, Quebec, Canada (contact)

Animal Rations: Teklad 7012 Rodent Diet, Harlan Laboratories, 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 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, Scotts Distributing Inc., Hudson, NH (non–contact)

Animal Rations: Teklad Hi–Fiber Rabbit Diet 2031, Harlan Laboratories, 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 Implant and Intracutaneous Injection Test:
Historically, New Zealand White rabbits have been used in intracutaneous and implantation safety evaluation studies because the guidelines have no alternative (non–animal) methods.

6.2 Route of Administration:
Animals were treated by intravenous and intraperitioneal routes for the Systemic Injection Test. Animals were treated by intracutaneous injections and intramuscular implantation. The animal species, number, and route of test article administration were recommended by both the USP guidelines. The test article was extracted and administered *in vivo* through a medium compatible with the test system, as indicated on the GLP Test Requisition Form.

7.0 EXPERIMENTAL DESIGN AND DOSAGE

7.1 Preparation of Test and Control Articles:

7.1.1 Per Sponsor request, a thin layer of ink was put on glass slides and allowed to dry prior to testing. The test article (110 cm^2) was combined with 18.3 mL of vehicle at a ratio of 120 cm^2 per 20 mL per USP guidelines. The test article was separately extracted in NaCl, CSO, EtOH, and PEG at 70 ± 2 °C for 24 ± 2 hours for the Systemic Injection and Intracutaneous Injection tests.

7.1.2 Prior to extraction, the test article was washed two times with 70 mL of SWFI. The test article sample prepared for extraction with CSO was dried at 50 ± 2 °C for 1 ± 0.1 hour.

7.1.3 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.

7.1.4 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.

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

7.1.6 Each extract was agitated vigorously prior to administration.

7.1.7 After the completion of the extraction, the extracts were kept at room temperature and were used the same day the extraction was completed. The Systemic Injection and Intracutaneous tests were performed using the same extracts. The test article appeared unchanged by the extraction procedure. The extracts were clear and free from particulates. No storage of the extracts occurred.

7.1.8 Implant Testing Preparation:
The test and control articles were cut into strips measuring 1 mm × 10 mm. The test and control article strips were sterilized by dipping in 70% ethanol prior to implantation. Per Sponsor request, the negative control plastic was dipped in the test article and allowed to dry before implanting.

7.2 Pre–Dose Procedure:

7.2.1 Systemic Injection Test:

7.2.1.1 Acclimated animals were weighed prior to dosing.

7.2.1.2 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:

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

7.2.2.2 For the Intracutaneous 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 Implant Test:
Two rabbits were used for the Implantation Test. On the day of the test, the animals were weighed and the skin on both sides of the spinal column was clipped free of fur. Each animal was anesthetized to prevent muscular movement.

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.

7.3.2 Intracutaneous Injection Test:

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

7.3.2.2 At five sites on the other side of each rabbit, 0.2 mL of the corresponding control article was injected.

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

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

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

7.4.2 Intracutaneous Injection Test:

7.4.2.1 The injection sites on each animal were observed for signs of erythema and edema 24, 48, and 72 hours after injection of the test article. Observations were scored according to the Evaluation of Skin Reactions (Appendix I). Observations conducted also included all clinical signs.

7.4.2.2 Animals were weighed at the end of the observation period.

7.4.2.3 The animals were returned to the general colony.

7.4.3 Implant Test:

7.4.3.1 The animals were maintained for a period of 7 days.

7.4.3.2 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 At the end of the observation period, the animals were weighed. Each animal was sacrificed by an injectable barbiturate.

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

7.4.3.5 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 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 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 is 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, 48, and 72 hours are 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 are 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 is conducted using an additional 3 rabbits. On the repeat test, the requirements of the test is met if the difference between the test article and control article mean reaction scores (erythema/edema) is 1.0 or less.

8.3 Implant 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 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 employ methodology to minimize uncertainty of measurement and control of bias for data collection and analysis, which includes but is 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):
Three test and one control animal lost an insignificant amount of weight (less than 2 g). All other test and control animals increased in weight.

9.1.2 Clinical Observations (Table 1):
Upon dosing the test NaCl extract, animals displayed signs of dyspnea and lethargy, but returned to normal clinical observation within 60 seconds. None of the other test or control animals exhibited overt signs of toxicity at any of the observation points.

9.1.3 Although clinical signs were noted immediately upon dosing the NaCl test extract, animals returned to normal clinical observation within sixty seconds of dosing. Thus, it can be observed that it was not an overt sign of toxicity. Additionally, the test is considered negative because none of the other 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):
All of the 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.

9.2.3 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 (Tables 2 and 4):

9.3.2 Clinical Observations (Tables 2 and 4):
None 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.

9.3.3 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 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, 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, 20 square inches of dry ink and 50 grams of liquid ink, meets the requirements of the test.

11.0 RECORDS

11.1 Original raw data will be archived at Toxikon Corporation.

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

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

11.4 All used and unused test article shall be disposed of by Toxikon, per Sponsor's request.

12.0 CONFIDENTIALITY AGREEMENT

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

13.0 ANIMAL WELFARE STATEMENT

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

No evidence of pain and distress was reported to the Veterinarian and/or Study Director.

Toxikon strictly adhered to the following standards in maintaining the animal care and use program:

United States Department of Agriculture (USDA), Animal and Plant Health Inspection Service, 9 CFR Ch. 1 (1/1/95 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 1996.

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

Test Article: 20 square inches of dry ink and 50 grams of liquid ink

Lot/Batch #: Not Supplied by Sponsor (N/S)

Group	Animal #	Sex	Dose (mL)	Body Weight (g)			Signs of Toxicity*
				Day 0 10/02/12	Day 3 10/05/12	Weight Change	
NaCl Test IV 50 mL/kg	1	Female	1.0	20.6	19.9	-0.7	1A, 2A, O*
	2	Female	1.1	22.3	22.5	0.2	1A, 2A, O*
	3	Female	1.0	19.7	20.6	0.9	1A, 2A, O*
	4	Female	1.1	22.4	22.1	-0.3	1A, 2A, O*
	5	Female	1.0	20.5	20.5	0.0	1A, 2A, O*
NaCl Control IV 50 mL/kg	6	Female	1.1	21.7	22.6	0.9	None
	7	Female	1.1	21.6	22.0	0.4	None
	8	Female	1.1	22.7	24.5	1.8	None
	9	Female	1.1	22.4	22.5	0.1	None
	10	Female	1.0	20.1	20.6	0.5	None
CSO Test IP 50 mL/kg	11	Female	1.1	21.6	22.7	1.1	None
	12	Female	1.0	20.0	21.9	1.9	None
	13	Female	1.1	21.7	22.3	0.6	None
	14	Female	1.1	21.7	22.2	0.5	None
	15	Female	1.0	20.5	22.8	2.3	None
CSO Control IP 50 mL/kg	16	Female	1.0	20.3	21.0	0.7	None
	17	Female	1.1	22.0	23.8	1.8	None
	18	Female	1.1	21.8	22.1	0.3	None
	19	Female	0.9	18.3	18.4	0.1	None
	20	Female	1.1	21.1	21.8	0.7	None
EtOH Test IV 50 mL/kg	21	Female	1.1	22.6	22.3	-0.3	None
	22	Female	1.0	20.1	20.7	0.6	None
	23	Female	1.1	22.8	24.5	1.7	None
	24	Female	1.0	20.3	20.5	0.2	None
	25	Female	1.1	22.1	22.1	0.0	None
EtOH Control IV 50 mL/kg	26	Female	1.0	20.8	21.7	0.9	None
	27	Female	1.0	19.2	19.1	-0.1	None
	28	Female	1.0	20.4	20.5	0.1	None
	29	Female	1.1	21.8	22.2	0.4	None
	30	Female	1.1	21.6	22.2	0.6	None
PEG Test IP 10 g/kg	31	Female	1.1	22.0	23.6	1.6	None
	32	Female	1.1	21.0	21.4	0.4	None
	33	Female	1.2	23.0	23.3	0.3	None
	34	Female	1.1	22.0	22.3	0.3	None
	35	Female	1.0	20.8	21.3	0.5	None
PEG Control IP 10 g/kg	36	Female	1.1	21.2	21.4	0.2	None
	37	Female	1.0	20.5	21.4	0.9	None
	38	Female	1.0	20.9	21.8	0.9	None
	39	Female	1.1	22.6	23.4	0.8	None
	40	Female	1.1	21.7	22.3	0.6	None

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

1A = Dyspnea

2A = Lethargy

O* = Immediately after dosing, all NaCl test mice displayed dyspnea and lethargy, but within 60 seconds of dosing, mice returned to a normal clinical observation

IV = Intravenous Route

IP = Intraperitoneal Route

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

Test Article: 20 square inches of dry ink and 50 grams of liquid ink

Lot/Batch #: Not Supplied by Sponsor (N/S)

Group	Animal #	Sex	Body Weight (kg)			Signs of Toxicity*
			Day 0 10/02/12	Day 3 10/05/12	Weight Change	
NaCl & CSO	21199	Male	3.06	3.14	0.08	None
	21204	Female	3.31	3.44	0.13	None
EtOH & PEG	21206	Female	3.36	3.43	0.07	None
	21207	Male	3.07	3.17	0.10	None
Group	**Animal #**	**Sex**	**Body Weight (kg)**			**Signs of Toxicity***
			Day 0 09/27/12	**Day 7 10/04/12**	**Weight Change**	
USP Implant (7 Days)	21201	Male	2.84	2.96	0.12	None
	21205	Male	2.92	3.13	0.21	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.

TABLE 3
Intracutaneous Test Skin Reaction Scores

Test Article: 20 square inches of dry ink and 50 grams of liquid ink

Lot/Batch #: Not Supplied by Sponsor (N/S)

NaCl Extract

Animal #	Vehicle	Time	Site Numbers Scoring (ER/ED)									
			T–1	T–2	T–3	T–4	T–5	C–1	C–2	C–3	C–4	C–5
21199	NaCl	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
21204	NaCl	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
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)									
			T–1	T–2	T–3	T–4	T–5	C–1	C–2	C–3	C–4	C–5
21199	CSO	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
21204	CSO	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
Difference between Test Article and Control Article Overall Mean Score = 0.0–0.0 = 0.0

ER = Erythema; ED = Edema; T = Test Sites; C = Control Sites
* 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.)

Test Article: 20 square inches of dry ink and 50 grams of liquid ink

Lot/Batch #: Not Supplied by Sponsor (N/S)

EtOH Extract

Animal #	Vehicle	Time	Site Numbers Scoring (ER/ED)									
			T–1	T–2	T–3	T–4	T–5	C–1	C–2	C–3	C–4	C–5
21206	EtOH	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
21207	EtOH	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
Difference between Test Article and Control Article Overall Mean Score = 0.0–0.0 = 0.0

PEG Extract

Animal #	Vehicle	Time	Site Numbers Scoring (ER/ED)									
			T–1	T–2	T–3	T–4	T–5	C–1	C–2	C–3	C–4	C–5
21206	PEG	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
21207	PEG	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
Difference between Test Article and Control Article Overall Mean Score = 0.0–0.0 = 0.0

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

TABLE 4
Implant Test Macroscopic Observations

Test Article: 20 square inches of dry ink and 50 grams of liquid ink

Lot/Batch #: Not Supplied by Sponsor (N/S)

Animal #: 21201

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		0	0	

Animal #: 21205

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		0	0	

T = Test
C = Control

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

Software	Use	Publisher/Vendor	Location
Adobe Acrobat 8 Professional	Document preparation	Adobe Systems, Inc.	San José, CA
DocuKnowledge 3.0	Lotus Domino–based document management system used for SOPs	Prelude Computer Solutions	Parsippany, NJ
Lotus Domino Rel. 5	Client–server application for Sponsor, sample, test codes, and quotation management application databases	IBM Corporation	Armonk, NY
MS Office 2007 and/or 2010 Small Business Suite	Business software (suite includes Word, Excel, PowerPoint, Outlook, Publisher, Office tools)	Microsoft Corporation	Redmond, WA
Rees CentronSQL System 2.0	Environmental monitoring and metrology system	Rees Scientific	Trenton, NJ

TOXIKON

ADVANCING YOUR INNOVATION

TOXIKON TEST PROTOCOL
FDA GLP REGULATIONS
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CLASS VI TEST – USP

TOXIKON PROTOCOL NUMBER: P12-1511-00A

COMPLIANCE
21 CFR, Part 58
Good Laboratory Practice for Nonclinical Laboratory Studies

MANAGEMENT OF THE STUDY

Performing Laboratory
Toxikon Corporation
15 Wiggins Avenue
Bedford, MA 01730

Sponsor
Sigma Inks Corp.
12113 Kirkham Road
Poway, CA 92064

ORIGINAL

124297 G 1

Toxikon Use Only: 003

PROTOCOL ACCEPTANCE

James Cheng
PRINT NAME

James Cheng
Sponsor's Representative Signature

9-10-12
Date

Sigma Inks Corp.
12113 Kirkham Road
Poway, CA 92064

Melissa Rodrigues
PRINT NAME

Rodrigues
Quality Assurance Signature

9/11/12
Date

Toxikon Corporation
15 Wiggins Avenue
Bedford, MA 01730

Kirsten Russell
PRINT NAME

Kirsten Russell
Study Director Signature

9-13-12
Date

Toxikon Corporation
15 Wiggins Avenue
Bedford, MA 01730

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Toxikon Use Only: 003

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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:

2.1 United States Pharmacopeia 35, National Formulary 30, 2012. <88> Biological Reactivity Tests, *In Vivo*.

2.2 ISO/IEC 17025, 2005, 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 GLP 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 was 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

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

4.2.1 Negative Control Article:

Name	USP 0.9% Sodium Chloride for Injection (NaCl)
Toxikon QC Number	To Be Determined (TBD)

4.2.2 Negative Control Article:

Name	Cottonseed Oil (CSO)
Toxikon QC Number	To Be Determined (TBD)

4.2.3 Negative Control Article:

Name	1 in 20 Ethanol in NaCl (EtOH)
Toxikon QC Number	To Be Determined (TBD)

4.2.4 Negative Control Article:

Name	Polyethylene Glycol 400 (PEG)
Toxikon QC Number	To Be Determined (TBD)

4.2.5 Negative Control Article:

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)

4.3 Reagent(s) (Toxikon Supplied, unless specified by the Sponsor):

Name	Sterile Water for Injection (SWFI)
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 3 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: 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 3 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 3 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 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:
Animals will be treated by intravenous and intraperitioneal routes for the Systemic Injection Test. 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 both 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 GLP Test Requisition Form.

7.0 EXPERIMENTAL DESIGN AND DOSAGE

7.1 Preparation of Test and Control Articles:

7.1.1 The test and control articles will be prepared at the following ratio (please indicate on the GLP Test Requisition Form):

1. According to USP
2. No preparation required
3. Sponsor–Specified

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

1. USP 0.9% Sodium Chloride for Injection (NaCl)
2. Cottonseed Oil (CSO)
3. 1 in 20 Ethanol in NaCl (EtOH)
4. Polyethylene Glycol 400 (PEG)
5. Sponsor–Specified Medium (NOTE: Extraction vehicles not specified by USP may be required to be justified.)

7.1.3 The test article will be dynamically extracted at the following condition (please indicate on the GLP Test Requisition Form):

1. 50 ± 2 °C for 72 ± 2 hours
2. 70 ± 2 °C for 24 ± 2 hours
3. 121 ± 2 °C for 1 ± 0.1 hour
4. Sponsor–Specified (NOTE: Extraction conditions not specified by USP may be required to be justified.)

7.1.4 Prior to extraction, the test article will be washed two times with 70 mL of SWFI. The test article sample prepared for extraction with CSO will be dried at 50 ± 2 °C for 1 ± 0.1 hour.

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

7.1.6 Each extracting 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.

7.1.7 The Systemic Injection and Intracutaneous Injection tests may be performed using the same extracts.

7.1.8 Each extract will be agitated vigorously prior to administration.

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

7.2 Pre-Dose Procedure:

7.2.1 Systemic Injection Test:

7.2.1.1 Acclimated animals will be weighed prior to dosing.

7.2.1.2 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:

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

7.2.2.2 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:
Two rabbits will be used for the Intramuscular Implantation Test. On the day of the test, the animals will be weighed and the skin on both sides of the spinal column will be clipped free of fur. Each animal will be anesthetized to prevent muscular movement.

7.2.4 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.

7.2.5 Subcutaneous Implantation Test:
For materials with physical characteristics unsuitable for routine intramuscular implantation, the subcutaneous rat implantation model is a viable alternative. Five rats will be used for the Subcutaneous Implantation Test. On the day of the test, the animals will be weighed and the skin on both sides of the spinal column will be clipped free of fur. Each animal will be anesthetized to prevent muscular movement.

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	—

7.3.2 Intracutaneous Injection Test:

7.3.2.1 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.

7.3.2.2 At five other sites on the other side of each rabbit, 0.2 mL of the corresponding control article will be injected.

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 line of the implant).

7.4 Post–Dose Procedure:

7.4.1 Systemic Injection Test:

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

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

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

7.4.2 Intracutaneous Injection Test:

7.4.2.1 The injection sites on each animal will be observed for signs of erythema and edema 24, 48, and 72 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 Animals will be weighed at the end of the observation period.

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

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

7.4.3.3 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.

7.4.3.4 At the end of the observation period, the animals will be weighed. Each animal will be sacrificed by an injectable barbiturate for the Intramuscular Implantation Test. For the Subcutaneous Implantation Test, each animal will be sacrificed by CO_2 inhalation.

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

7.4.3.6 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 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, 48 and 72 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 will be 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 and its design will employ methodology to minimize uncertainty of measurement and control of bias for data collection and analysis, which may include 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

9.1 Original raw data will be archived at Toxikon Corporation.

9.2 A copy of the final report and any report amendments will be archived at Toxikon Corporation.

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

9.4 All used and unused test article will be handled as specified on the GLP Test Requisition Form. If not indicated on the GLP Test Requisition Form, all remaining test article will be discarded.

10.0 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.

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 and the Animal Care and Use Committee (IACUC) base 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 (1/1/95 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 1996.

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/Deviation Report (PADR) 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 to indicate approval of the amendment. Acknowledgement of notification of deviations will either 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 will be documented in the final report.

Software	Use	Publisher/Vendor	Location
Adobe Acrobat 8 Professional	Document preparation	Adobe Systems, Inc.	San José, CA
DocuKnowledge 3.0	Lotus Domino–based document management system used for SOPs	Prelude Computer Solutions	Parsippany, NJ
Lotus Domino Rel. 5	Client–server application for Sponsor, sample, test codes, and quotation management application databases	IBM Corporation	Armonk, NY
MS Office 2007 and/or 2010 Small Business Suite	Business software (suite includes Word, Excel, PowerPoint, Outlook, Publisher, Office tools)	Microsoft Corporation	Redmond, WA
Rees CentronSQL System 2.0	Environmental monitoring and metrology system	Rees Scientific	Trenton, NJ

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