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TEST RESULT CERTIFICATE

Sponsor	Momentive Performance Materials	**Technical Initiation**	5/14/2010
Address	260 Hudson River Road Waterford , NY 12188	**Technical Completion**	6/14/2010
Contact	Shahzad Arshad	**Report Date**	7/2/2010
P.O. Number	4500631255	**Project Number**	10-2223-G1

Test Article	LSR2650	**Ratio**	60 cm^2/20 mL
Lot/Batch #	Pt. ZM 6120 (B-Stufe)	**Vehicles**	USP 0.9% Sodium Chloride for Injection (NaCl), Cottonseed Oil (CSO), 1 in 20 Ethanol in NaCl (EtOH), and Polyethylene Glycol 400 (PEG)
Study	Class VI Test – USP Systemic Toxicity, Intracutaneous Reactivity, 2 Week Muscle Implant – ISO	**Extraction Conditions**	70 ± 2 °C for 24 ± 2 hours
Comments	Test Article Description: Press Cure: 10 min / 170 C; Post Cure: 4hrs/ 200 C		

REFERENCES: The study was conducted based upon the following references: United States Pharmacopeia 32, National Formulary 27, 2009. <88> Biological Reactivity Tests, *In Vivo*. ISO 10993–10, 2002, Biological Evaluation of Medical Devices – Part 10: Tests for Irritation and Delayed–Type Hypersensitivity, as amended 2006. ISO 10993–11, 2006, Biological Evaluation of Medical Devices – Part 11: Tests for Systemic Toxicity. ISO 10993–6, 2007, Biological Evaluation of Medical Devices – Part 6: Tests for Local Effects After Implantation. ASTM F981–04, Standard Practice for Assessment of Compatibility of Biomaterials for Surgical Implants with Respect to Effect of Materials on Muscle and Bone, 2004. ASTM F763–04, Standard Practice for Short Term Screening of Implant Materials, 2004. ISO 10993–12, 2007, Biological Evaluation of Medical Devices – Part 12: Sample Preparation and Reference Materials.

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

GENERAL PROCEDURE: The extraction conditions were performed as stated above. The test article extracts and corresponding blanks were injected systemically and intracutaneously in mice and rabbits, respectively. The injections were in the amounts and routes set forth by USP and ISO 10993–10 and ISO 10993–11 guidelines; including the further dilution of the extracts prepared with PEG. The animals were observed for signs of toxicity and skin reactivity for up to 72 hours post treatment. In addition, the test article was implanted into the paravertebral muscles of rabbits for 7 days (USP) and 2 weeks (ISO) and observed for signs of infection, encapsulation, hemorrhage, necrosis, discoloration, and inflammation. The implant sites from the animals maintained 2 weeks were removed from the muscle tissue by carefully slicing around the implant site with a scalpel and lifting out the tissue with forceps and fixed in 10% Neutral Buffered Formalin (NBF).

USP Macroscopic Evaluation:

The area of the tissue surrounding the center portion of each implant strip was examined macroscopically using a magnifying lens. Hemorrahaging, 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.

ISO Histopathology:

Following fixation in formalin, each of the implant sites was excised from the larger mass of tissue. The implant site, containing the implanted material, was examined macroscopically, aided by a magnifying glass if needed. Each site was examined for signs of inflammation, encapsulation, hemorrhaging, necrosis, and discoloration using the following scale:

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

The presence, form, and location of the implant material was recorded. Photographs of the sites were taken and retained as part of the raw data and macroscopic results were reported.

ISO Pathological Assessment:

The following categories of biological reaction were assessed by microscopic observation and the responses graded according to the following table for each implant site:

TABLE 1
Inflammatory Responses

Cell Type/Response	Score				
	0	1	2	3	4
Polymorphonuclear Cells	0	Rare, 1–5/phf[a]	5–10/phf	Heavy Infiltrate	Packed
Lymphocytes	0	Rare, 1–5/phf	5–10/phf	Heavy Infiltrate	Packed
Plasma Cells	0	Rare, 1–5/phf	5–10/phf	Heavy Infiltrate	Packed
Macrophages	0	Rare, 1–5/phf	5–10/phf	Heavy Infiltrate	Packed
Giant Cells	0	Rare, 1–2/phf	3–5/phf	Heavy Infiltrate	Sheets
Necrosis	0	Minimal	Mild	Moderate	Severe

[a] phf = per high powered (400 ×) field.

TABLE 2
Healing Responses

Cell Type/Response	Score				
	0	1	2	3	4
Neovascularisation	0	Minimal capillary, proliferation, focal, 1–3 buds	Groups of 4–7 capillaries with supporting fibroblastic structures	Broad band of capillaries with supporting structures	Extensive band of capillaries with supporting fibroblastic structures
Fibrosis	0	Narrow band	Moderately thick band	Thick band	Extensive band
Fatty Infiltrate	0	Minimal amount of fat associated with fibrosis	Several layers of fat and fibrosis	Elongated and broad accumulation of fat cells about the implant site	Extensive fat completely surrounding the implant

The relative size of the involved area was scored by assessing the width of the area from the implant/tissue interface to unaffected areas which have the characteristics of normal tissue and normal vascularity. Relative size of the involved area was scored using the following scale:

0 = 0 mm, No site
0.5 = up to 0.5 mm, Very slight
1 = 0.6 – 1.0 mm, Mild
2 = 1.1 – 2.0 mm, Moderate
3 = > 2.0 mm, Severe

RESULTS: None of the mice injected with the test article extracts exhibited any signs of toxicity in the Systemic Injection Test. In addition, none of the rabbits injected intracutaneously with the test article extracts exhibited any signs of erythema, edema, or clinical toxicity. In both the Systemic and Intracutaneous Tests the controls were normal through 72 hours. The implant sites (USP and ISO) exhibited no significant macroscopic signs of infection, encapsulation, hemorrhage, necrosis, discoloration, or inflammation when compared with the control sites. For the ISO Implant Test, microscopic evaluation of the test article implant sites did not show any increase in biological reaction as compared to the control article sites at the 2 week time period. The Bioreactivity Rating for the 2 week time period (average of three animals) was 1.2, indicating no reaction.

CONCLUSION: The test article meets the requirements of the USP guidelines for Class VI Plastics –70 °C, ISO 10993–11 guidelines for the Systemic Injection Test, ISO 10993–10 guidelines for the Intracutaneous Reactivity Test, and is classified as no reaction according to the ISO 10993–6 guidelines for the Implantation Test.

AUTHORIZED PERSONNEL:

Claudine Kos, Ph.D.
Study Director

Jamie Lynne Metzinger, B.A., B.S.
Quality Assurance

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TOXIKON FINAL GLP REPORT: 10–2223–G1

CLASS VI TEST – USP SYSTEMIC TOXICITY, INTRACUTANEOUS REACTIVITY, 2 WEEK MUSCLE IMPLANT – ISO

Test Article
LSR2650

Author
Claudine Kos, Ph.D.

Final Report Date
July 2, 2010

COMPLIANCE
21 CFR, Part 58
Good Laboratory Practice for Non–Clinical Laboratory Studies

MANAGEMENT OF THE STUDY

Performing Laboratory
Toxikon Corporation
15 Wiggins Avenue
Bedford, MA 01730

Sponsor
Momentive Performance Materials
260 Hudson River Road
Waterford, NY 12188

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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. Therefore, the test article, LSR2650, meets the requirements of the USP guidelines for Class VI Plastics – 70 °C, ISO 10993–11 guidelines for the Systemic Injection Test, ISO 10993–10 guidelines for the Intracutaneous Reactivity Test, and is classified as no reaction according to the ISO 10993–6 guidelines for the Implantation Test.

QUALITY ASSURANCE STATEMENT

This study was conducted in compliance with U.S. Food and Drug Administration regulations set forth in 21 CFR, Part 58.

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 and its mixture with carriers, 21 CFR, Parts 58.105 and 58.113.

The Quality Assurance Unit conducted inspections on the following dates. The findings were reported to the Study Director and to Toxikon's Management.

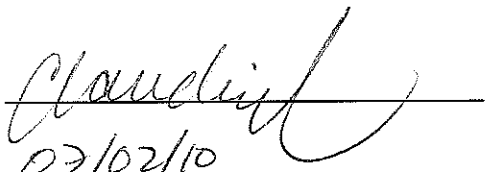
INSPECTIONS	DATE OF INSPECTION	DATE REPORTED STUDY DIRECTOR	DATE REPORTED MANAGEMENT
EXPLANT	06/02/10	06/02/10	06/02/10
RAW DATA	07/02/10	07/02/10	07/02/10
FINAL REPORT	07/02/10	07/02/10	07/02/10

Jamie Lynne Metzinger, B.A., B.S.
Quality Assurance

7/2/10
Date

STUDY DIRECTOR SIGNATURE AND VERIFICATION DATES

This study meets the technical requirements of the protocol. The study also meets the requirements of the Good Laboratory Practice Regulations, 21 CFR, Part 58, with the exemptions as stated in the Quality Assurance Statement.

Protocol Number:	PSW/VIVO/001–09/000
Study Director:	Claudine Kos, Ph.D.
Company:	Toxikon Corporation
Signature:	
Date:	07/02/10
Study Supervisor:	Natalia Tovar, B.S.
Pathology Reviewer:	Ying Ping Yu, B.M.
Pathologist:	Alexander G. Richter, M.S., DVM, DACVP

VERIFICATION DATES:
The Study Initiation Date is the date the protocol is signed by the Study Director.

Test Article Receipt:	05/10/10
Project Log Date:	05/11/10
Study Initiation Date:	05/11/10
Extraction Dates:	05/18/10–05/19/10
Technical Initiation:	05/14/10
Technical Completion:	06/14/10
Histopathology Report:	06/22/10

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 conducted based upon the following references:

2.1 United States Pharmacopeia 32, National Formulary 27, 2009. <88> Biological Reactivity Tests, *In Vivo*.

2.2 ISO 10993–10, 2002, Biological Evaluation of Medical Devices – Part 10: Tests for Irritation and Delayed–Type Hypersensitivity, as amended 2006.

2.3 ISO 10993–11, 2006, Biological Evaluation of Medical Devices – Part 11: Tests for Systemic Toxicity.

2.4 ISO 10993–6, 2007, Biological Evaluation of Medical Devices – Part 6: Tests for Local Effects After Implantation.

2.5 ASTM F981–04, Standard Practice for Assessment of Compatibility of Biomaterials for Surgical Implants with Respect to Effect of Materials on Muscle and Bone, 2004.

2.6 ASTM F763–04, Standard Practice for Short Term Screening of Implant Materials, 2004.

2.7 ISO 10993–12, 2007, Biological Evaluation of Medical Devices – Part 12: Sample Preparation and Reference Materials.

2.8 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 Non–Clinical 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:

Test Article Name: LSR2650
CAS/Code #: Not Supplied by Sponsor (N/S)
Lot/Batch #: Pt. ZM 6120 (B-Stufe)
Physical State: N/S
Color: N/S
Expiration Date: N/S
Density: N/S
Stability: N/S
Solubility: N/S
pH: N/S
Storage Conditions: Room Temperature (N/S)
Safety Precautions: Standard Laboratory Safety Precautions
Sponsor Note: Test Article Description: Press Cure: 10 min / 170 C; Post Cure: 4hrs/ 200 C

4.2 Control Articles (Toxikon Supplied):

4.2.1 Negative Control Article Name: USP 0.9% Sodium Chloride for Injection (NaCl)
Toxikon QC #: CSC-10-04-008-VV
Physical State: Liquid
Color: Colorless
Stability: Stable at Room Temperature
Storage Conditions: Room Temperature
Safety Precautions: Standard Laboratory Safety Precautions

4.2.2 Negative Control Article Name: Cottonseed Oil (CSO)
Toxikon QC #: CSC-10-04-001-VV
Physical State: Liquid
Color: Yellow
Stability: Stable at Room Temperature
Storage Conditions: Room Temperature
Safety Precautions: Standard Laboratory Safety Precautions

4.2.3 Negative Control Article Name: 1 in 20 Ethanol in NaCl (EtOH)
Toxikon QC #: CSC-08-12-008-VV; CSC-10-04-008-VV
Physical State: Liquid
Color: Colorless
Stability: Stable at Room Temperature
Storage Conditions: Room Temperature
Safety Precautions: Standard Laboratory Safety Precautions

4.2.4 Negative Control Article Name: Polyethylene Glycol 400 (PEG)
Toxikon QC #: CSC-09-06-015-VV
Physical State: Liquid
Color: Colorless
Stability: Stable at Room Temperature
Storage Conditions: Room Temperature
Safety Precautions: Standard Laboratory Safety Precautions

4.2.5 Negative Control Article Name: Negative Control High Density Polyethylene (Negative Control Plastic)
Toxikon QC #: CSC-04-05-009-CC
Physical State: Solid
Color: White
Storage Conditions: Room Temperature
Safety Precautions: Standard Laboratory Safety Precautions

4.3 Reagent (Toxikon Supplied):

Reagent Name: Sterile Water for Injection (SWFI)
Toxikon QC #: CSC-09-09-004-VV
Physical State: Liquid
Color: Colorless
Stability: Stable at Room Temperature
Storage Conditions: Room Temperature
Safety Precautions: Standard Laboratory Safety Precautions

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 – 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 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 Implant Tests:

Number and Species: 9 New Zealand White rabbits (*Oryctolagus cuniculus*)

Sex: 4 males and 5 females (females were non-pregnant and nulliparous)

Weight/Age Range: 2.26 – 2.66 kilograms for Intracutaneous
2.66 – 3.32 kilograms for Implant Test
at least 10 weeks old (young adult)
weighed to nearest 10 g

Health Status: healthy, Intracutaneous animals not previously used in other experimental procedures, Implant animals were previously used in other experimental procedures

Animal Purchase: Covance Laboratories, Madison, WI

Animal Identification: ear marker

Acclimation: minimum 5 days, under same conditions as for the actual test

Animal Selection: selected from larger pool and examined to ensure lack of adverse clinical signs

5.2 Animal Care and Maintenance:

5.2.1 Systemic Injection Test:

Animal Room Temperature: 68 ± 5 °F

Animal Room 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: TEK 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 Temperature: 68 ± 5 °F

Animal Room 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: hardwood chips, P.W.I. Industries, St–Hyacinthe, Quebec, Canada (non–contact)

Animal Rations: TEK 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 Mice were used in this study because they have historically been used in systemic safety evaluation studies and the guidelines have no alternative (non–animal) methods. Animals were treated by intravenous and intraperitioneal routes. The animal species, number, and route of test article administration were recommended by both the USP and the ISO 10993–11 guidelines.

6.2 New Zealand White rabbits were used in this study because they have been historically used in intracutaneous and implantation safety evaluation studies and the guidelines have no alternative (non–animal) methods. Animals were treated by Intracutaneous Injections and Intramuscular Implantation. The animal species, number, and route were recommended by the USP, ISO 10993–6 and ISO 10993–10 guidelines.

6.3 The test article was exposed to the test system directly and through solvents compatible with the test system.

7.0 EXPERIMENTAL DESIGN AND DOSAGE

7.1 Preparation of Test and Control Articles:

7.1.1 Test and Control Extracts:

7.1.1.1 The test article (60.0 cm^2) was combined with 20.0 mL of vehicle at a ratio of 60 cm^2 per 20 mL per ISO 10993–12 and 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.

7.1.1.2 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.

7.1.1.3 Properly prepared test articles were placed in separate extraction bottles, and to each bottle the appropriate medium was added. The extraction medium completely covered the test article.

7.1.1.4 Each extracting medium (control article) was prepared for parallel treatments and comparisons. Each control article was prepared in the same manner as the test article.

7.1.1.5 The Systemic Injection and Intracutaneous tests were performed using the same extracts. The test article appeared unchanged by the extraction procedure. It was not degraded or deformed. The extract was clear and free from particulates. Each extract was agitated vigorously prior to administration. All other test article preparation was as specified by the Sponsor.

7.1.2 Test and Control Implants:

7.1.2.1 All apparatus strips were prepared according to the ISO 10993–6 and USP guidelines. The test article (Sponsor–supplied) was cut to measure approximately 1 mm in diameter and 10 mm in length with rounded cross section and rounded ends. It was the Sponsor's responsibility to ensure that the test article was manufactured, processed, cleaned of contaminants, and sterilized by the methods intended for the final end use product. The test article was sterilized by dipping in 70% ethanol.

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

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:

7.2.3.1 Two rabbits were used for the USP Implant Test and three rabbits were used for the ISO Implant Test.

7.2.3.2 Each animal was weighed prior to implantation.

7.2.3.3 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 vacuum.

7.2.3.4 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	2 mL/minute
CSO	Intraperitoneal	50 mL	—
EtOH	Intravenous	50 mL	2 mL/minute
*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 other sites on the other side of each rabbit, 0.2 mL of the corresponding control article was injected.

7.3.3 USP Implant Test:
Four strips 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.

7.3.4 ISO Implant Test:
Six strips of the test article were implanted into each of the paravertebral muscles of each rabbit, approximately 2.5 cm from the midline and parallel to the spinal column and approximately 2.5 cm from each other. The test article strips were implanted on one side of the spine. In a similar fashion, negative control strips were implanted in the contralateral muscle of each animal. A total of at least ten test article strips and ten control strips are required for evaluation.

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 ± 2 hours after injection. Observations conducted included all clinical and toxicologic signs.

7.4.1.2 The animals were weighed at 24, 48, and 72 ± 2 hours after injection.

7.4.1.3 Animals were sacrificed by carbon dioxide 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 immediately after injection and at 24, 48, and 72 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 All average erythema and edema scores for the test and control sites at 24, 48, and 72 hours were 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.

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

7.4.2.4 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 for the USP implant test and a period of 2 weeks for the ISO implant test.

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 Gross Observations:
The paravertebral muscles in which the test or control articles were implanted were excised *in toto* from each animal. The muscle tissue was removed by carefully slicing around the implant sites with a scalpel and lifting out the tissue. The excised implant tissues were examined grossly. For the ISO Implant Test, excessively invasive procedures that may have disrupted the integrity of the tissue for histopathological evaluation were not used. The tissues for histopathological assessment were placed in properly labeled containers containing 10% neutral buffered formalin. The axillary lymph nodes were examined with no findings and were not collected.

7.4.3.6 USP Macroscopic Evaluation:
The area of the tissue surrounding the center portion of each implant strip was examined macroscopically using a magnifying lens. Hemorrahaging, 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.

7.4.3.7 ISO Histopathology:

7.4.3.7.1 Following fixation in formalin, each of the implant sites was excised from the larger mass of tissue. The implant site, containing the implanted material, was examined macroscopically, aided by a magnifying glass if needed. Each site was examined for signs of inflammation, encapsulation, hemorrhaging, necrosis, and discoloration using the following scale:

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

7.4.3.7.2 The presence, form, and location of the implant material were recorded. The implant material was noted to appear unchanged. Photographs of the sites were taken and retained as part of the raw data and microscopic results were reported.

7.4.3.7.3 After macroscopic observation, the implant material was removed and a slice of tissue containing the implant site was processed. Histologic slides of hematoxylin and eosin stained sections were prepared by Toxikon.

7.4.3.7.4 The slides were evaluated and graded by light microscopic examination.

7.4.3.8 ISO Pathological Assessment:

7.4.3.8.1 The following categories of biological reaction were assessed by microscopic observation and the responses graded according to the following tables for each implant site:

TABLE 1
Inflammatory Responses

Cell Type/Response	Score				
	0	1	2	3	4
Polymorphonuclear Cells	0	Rare, 1–5/phf[a]	5–10/phf	Heavy Infiltrate	Packed
Lymphocytes	0	Rare, 1–5/phf	5–10/phf	Heavy Infiltrate	Packed
Plasma Cells	0	Rare, 1–5/phf	5–10/phf	Heavy Infiltrate	Packed
Macrophages	0	Rare, 1–5/phf	5–10/phf	Heavy Infiltrate	Packed
Giant Cells	0	Rare, 1–2/phf	3–5/phf	Heavy Infiltrate	Sheets
Necrosis	0	Minimal	Mild	Moderate	Severe

[a] phf = per high powered (400 ×) field.

TABLE 2
Healing Responses

Cell Type/Response	Score				
	0	1	2	3	4
Neovascularisation	0	Minimal capillary, proliferation, focal, 1–3 buds	Groups of 4–7 capillaries with supporting fibroblastic structures	Broad band of capillaries with supporting structures	Extensive band of capillaries with supporting fibroblastic structures
Fibrosis	0	Narrow band	Moderately thick band	Thick band	Extensive band
Fatty Infiltrate	0	Minimal amount of fat associated with fibrosis	Several layers of fat and fibrosis	Elongated and broad accumulation of fat cells about the implant site	Extensive fat completely surrounding the implant

7.4.3.8.2 The relative size of the involved area was scored by assessing the width of the area from the implant/tissue interface to unaffected areas which have the characteristics of normal tissue and normal vascularity. Relative size of the involved area was scored using the following scale:

0 = 0 mm, No site
1 = up to 0.5 mm, Very slight
2 = 0.6–1.0 mm, Mild
3 = 1.1–2.0 mm, Moderate
4 = > 2.0 mm, Severe

8.0 EVALUATION CRITERIA

8.1 Systemic Injection Test:
The test 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 three or more mice lose more than 10% body weight loss, 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:
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 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 Implant Test:

8.3.1 USP 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 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.

8.3.2 ISO Bioreactivity Rating:

8.3.2.1 For each implanted site, a total score is determined. The inflammatory responses are totaled for each site and weighted by a factor or two (2). The healing responses are totaled separately. Inflammatory and healing responses are then added together resulting in a total score for each site. The average score of the test sites for each animal is compared to the average score of the control sites for that animal. The average difference between test and controls for all animals is calculated and the initial Bioreactivity Rating is assigned as follows:

0 – 2.9	No Reaction*
3.0 – 8.9	Slight Reaction
9.0 – 15.0	Moderate Reaction
> 15	Severe Reaction

* A negative calculation is reported as zero (0).

8.3.2.2 Modification of the Rating:
The pathology observer reviews the calculated level of bioreactivity. Based on the observation of all factors (e.g. relative size, pattern of response, inflammatory vs. resolution), the pathology observer may revise the Bioreactivity Rating. Justification for the modification to the rating is presented in the narrative report (Appendix II).

8.3.2.3 A descriptive narrative report regarding the biocompatibility of the test material is provided by the pathology observer (Appendix II).

8.4 Class VI Requirements:
The test article satisfies the requirements of the USP guidelines for the Class VI test if the requirements described above are met.

8.5 The study and its design employ methodology to minimize uncertainty of measurement and control of bias for data collection and analysis.

9.0 RESULTS

9.1 Systemic Injection Test (Table 3):

9.1.1 Animal Weights:
All test and control animals increased in weight.

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

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

9.2 Intracutaneous Test (Tables 4 and 5):

9.2.1 Animal Weights:
All of the animals increased in weight.

9.2.2 Clinical Observations:
There were no significant signs of erythema or edema observed at any of the test or control article sites.

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

9.3 USP Implant Test (Tables 4 and 6):

9.3.1 Animal Weights:
Both animals increased in weight.

9.3.2 Clinical Observations:
There were no overt signs of toxicity noted in either animal. Macroscopic evaluation of the test and control article implant sites showed no significant infection, encapsulation, hemorrhage, necrosis, or discoloration.

9.3.3 Per USP guidelines, 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.

9.4 ISO Implant Test (Tables 4, 7, and 8)

9.4.1 Animal Weights:
All of the animals increased in weight.

9.4.2 Clinical Observations:
There were no overt signs of toxicity noted in any animal.

9.4.3 Implantation Site Observations (Macroscopic) (ISO 10993–6):
Macroscopic evaluation of the test article implant sites indicated no significant signs of inflammation, encapsulation, hemorrhage, necrosis, or discoloration at the 2 week time period.

9.4.3 Implantation Site Observations (Microscopic):
Microscopic evaluation of the test article implant sites indicated no significant signs of inflammation, fibrosis, hemorrhage, or necrosis as compared to the control article sites. The Bioreactivity Rating for the 2 week time period (average of three animals) was 1.2, indicating no reaction.

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. Therefore, the test article, LSR2650, meets the requirements of the USP guidelines for Class VI Plastics – 70 °C, ISO 10993–11 guidelines for the Systemic Injection Test, ISO 10993–10 guidelines for the Intracutaneous Reactivity Test, and is classified as no reaction according to the ISO 10993–6 guidelines for the Implantation Test.

11.0 RECORDS

11.1 Original raw data are archived at Toxikon Corporation.

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

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

11.4 All used and unused test article shall be disposed of by Toxikon.

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 suffering 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, 1996. (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.

TABLE 3
Systemic Injection Test:
Animal Weights and Clinical Observations

Test Article: LSR2650

Lot/Batch #: Pt. ZM 6120 (B-Stufe)

Group	Animal #	Sex	Dose (mL)	Body Weight (g) Day 0 05/19/10	Day 1 05/20/10	Day 2 05/21/10	Day 3 05/22/10	Weight Change	Signs of Toxicity*
NaCl Test 50 mL/kg	1	Female	0.9	18.1	19.3	19.6	20.3	2.2	None
	2	Female	0.9	18.0	18.7	20.0	20.6	2.6	None
	3	Female	0.9	17.4	18.0	18.6	19.3	1.9	None
	4	Female	1.2	23.0	23.6	25.0	25.7	2.7	None
	5	Female	0.9	17.5	18.1	19.0	20.4	2.9	None
NaCl Control 50 mL/kg	6	Female	1.1	21.0	21.6	22.6	23.2	2.2	None
	7	Female	1.1	22.3	23.3	23.7	24.5	2.2	None
	8	Female	1.0	19.2	20.6	21.7	22.6	3.4	None
	9	Female	0.9	18.9	19.2	19.9	20.8	1.9	None
	10	Female	1.1	22.0	23.4	24.8	25.6	3.6	None
CSO Test 50 mL/kg	11	Female	0.9	17.4	18.2	18.5	19.2	1.8	None
	12	Female	1.0	19.0	19.4	19.9	21.1	2.1	None
	13	Female	1.2	23.0	24.4	24.8	26.0	3.0	None
	14	Female	0.9	18.5	19.8	20.3	21.3	2.8	None
	15	Female	1.0	20.9	22.3	23.2	24.5	3.6	None
CSO Control 50 mL/kg	16	Female	0.9	17.9	18.5	19.5	20.5	2.6	None
	17	Female	1.1	21.6	22.9	23.2	24.4	2.8	None
	18	Female	0.9	17.5	18.1	19.3	20.1	2.6	None
	19	Female	1.1	21.5	22.6	23.8	24.8	3.3	None
	20	Female	0.9	18.9	19.7	20.4	20.8	1.9	None
EtOH Test 50 mL/kg	21	Female	0.9	18.4	19.7	21.0	21.8	3.4	None
	22	Female	1.1	22.1	22.6	24.0	24.4	2.3	None
	23	Female	1.0	20.6	22.0	23.0	24.1	3.5	None
	24	Female	0.9	17.0	18.0	19.4	20.2	3.2	None
	25	Female	0.9	17.3	18.5	18.8	20.0	2.7	None
EtOH Control 50 mL/kg	26	Female	1.0	19.1	20.3	21.4	21.8	2.7	None
	27	Female	0.9	18.7	19.6	20.3	20.6	1.9	None
	28	Female	1.0	20.5	21.6	22.1	23.1	2.6	None
	29	Female	0.9	18.2	19.3	20.6	21.4	3.2	None
	30	Female	1.1	22.7	24.1	24.5	25.5	2.8	None
PEG Test 10 g/kg	31	Female	1.0	19.2	20.5	21.6	22.1	2.9	None
	32	Female	0.9	18.3	19.7	21.1	22.2	3.9	None
	33	Female	1.1	21.1	22.3	23.6	24.0	2.9	None
	34	Female	0.9	17.2	18.0	19.0	19.3	2.1	None
	35	Female	1.0	20.6	21.9	22.3	23.6	3.0	None
PEG Control 10 g/kg	36	Female	1.0	20.1	21.3	21.6	22.1	2.0	None
	37	Female	1.0	20.8	22.1	22.5	22.9	2.1	None
	38	Female	1.0	20.7	21.4	22.1	23.0	2.3	None
	39	Female	1.1	21.5	21.9	22.5	22.9	1.4	None
	40	Female	0.9	18.0	18.3	19.5	20.6	2.6	None

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

TABLE 4
Intracutaneous Injection and Implant Tests: Animal Weights and Clinical Observations

Test Article: LSR2650

Lot/Batch #: Pt. ZM 6120 (B-Stufe)

Group	Animal #	Sex	Body Weight (kg)			Signs of Toxicity*
			Day 0 05/19/10	Day 3 05/22/10	Weight Change	
NaCl & CSO	00609	Male	2.66	2.75	0.09	None
	00610	Female	2.34	2.39	0.05	None
EtOH & PEG	00611	Male	2.34	2.44	0.10	None
	00612	Female	2.26	2.29	0.03	None

Group	Animal #	Sex	Body Weight (kg)			Signs of Toxicity*
			Day 0 05/19/10	Day 7 05/26/10	Weight Change	
USP Implant (7 Days)	00466	Female	3.24	3.36	0.12	None
	00468	Female	3.32	3.37	0.05	None

Group	Animal #	Sex	Body Weight (kg)			Signs of Toxicity*
			Day 0 05/19/10	Day 14 06/02/10	Weight Change	
ISO Implant (2 Weeks)	00470	Female	3.00	3.22	0.22	None
	00471	Male	2.93	3.04	0.11	None
	00475	Male	2.66	2.93	0.27	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), and Day 0 through Day 14 for the Implant Test (ISO).

TABLE 5
Intracutaneous Test:
Skin Reaction Scores

Test Article: LSR2650

Lot/Batch #: Pt. ZM 6120 (B-Stufe)

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
00609	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
00610	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			0.0					0.0				

† = Immediately after injection, not used for the evaluation criteria.
Overall Mean Score* for Test Article = 0.0
Overall Mean Score* for Control Article = 0.0
Difference between Test Article and Control Article Overall Mean Score = 0.0–0.0 = 0.0

CSO Extract

Animal #	Vehicle	Time	Site Numbers Scoring (ER/ED)									
			T–6	T–7	T–8	T–9	T–10	C–6	C–7	C–8	C–9	C–10
00609	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
00610	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			0.0					0.0				

† = Immediately after injection, not used for the evaluation criteria.
Overall Mean Score* for Test Article = 0.0
Overall Mean Score* for Control Article = 0.0
Difference between Test Article and Control Article Overall Mean Score = 0.0–0.0 = 0.0

ER = Erythema; 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 5
Intracutaneous Test:
Skin Reaction Scores (Cont.)

Test Article: LSR2650

Lot/Batch #: Pt. ZM 6120 (B-Stufe)

EtOH Extract

Animal #	Vehicle	Time	Site Numbers Scoring (ER/ED)									
			T–11	T–12	T–13	T–14	T–15	C–11	C–12	C–13	C–14	C–15
00611	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
00612	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			0.0					0.0				

† = Immediately after injection, not used for the evaluation criteria.
Overall Mean Score* for Test Article = 0.0
Overall Mean Score* for Control Article = 0.0
Difference between Test Article and Control Article Overall Mean Score = 0.0–0.0 = 0.0

PEG Extract

Animal #	Vehicle	Time	Site Numbers Scoring (ER/ED)									
			T–16	T–17	T–18	T–19	T–20	C–16	C–17	C–18	C–19	C–20
00611	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
00612	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			0.0					0.0				

† = Immediately after injection, not used for the evaluation criteria.
Overall Mean Score* for Test Article = 0.0
Overall Mean Score* for Control Article = 0.0
Difference between Test Article and Control Article Overall Mean Score = 0.0–0.0 = 0.0

ER = Erythema; 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 6
USP Implant Test:
Macroscopic Observations
7 Days

Test Article: LSR2650

Lot/Batch #: Pt. ZM 6120 (B-Stufe)

Animal #: 00466

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	
Mean Score (total/5)	0	0	0	0		0	0	

Animal #: 00468

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	
Mean Score (total/5)	0	0	0	0		0	0	

T = Test
C = Control

TABLE 7
ISO Implant Test:
Macroscopic Observations
2 Weeks

Test Article: LSR2650

Lot/Batch #: Pt. ZM 6120 (B-Stufe)

Animal #: 00470

Tissue Site	T1	T2	T3	T4	T5	T6	Test Average	C1	C2	C3	C4	C5	C6	Control Average
Inflammation	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Encapsulation	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Hemorrhage	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Necrosis	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Discoloration	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Total	0	0	0	0	0	0		0	0	0	0	0	0	

Animal #: 00471

Tissue Site	T1	T2	T3	T4	T5	T6	Test Average	C1	C2	C3	C4	C5	C6	Control Average
Inflammation	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Encapsulation	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Hemorrhage	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Necrosis	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Discoloration	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Total	0	0	0	0	0	0		0	0	0	0	0	0	

Animal #: 00475

Tissue Site	T1	T2	T3	T4	T5	T6	Test Average	C1	C2	C3	C4	C5	C6	Control Average
Inflammation	NSF	NSF	0	0	0	0	0	0	0	0	NSF	NSF	NSF	0
Encapsulation	NSF	NSF	0	0	0	0	0	0	0	0	NSF	NSF	NSF	0
Hemorrhage	NSF	NSF	0	0	0	0	0	0	0	0	NSF	NSF	NSF	0
Necrosis	NSF	NSF	0	0	0	0	0	0	0	0	NSF	NSF	NSF	0
Discoloration	NSF	NSF	0	0	0	0	0	0	0	0	NSF	NSF	NSF	0
Total	N/A	N/A	0	0	0	0		0	0	0	N/A	N/A	N/A	

T = Test
C = Control

Grading Scale

0 = no reaction
1 = mild reaction
2 = moderate reaction
3 = severe reaction
NSF = No Site Found (Representative Section Subtracted)
N/A = Not Applicable

TABLE 8
Microscopic Observations
2Week Implantation

Test Article: LSR2650

Lot/Batch #: Pt. ZM 6120 (B-Stufe)

Animal #: 00470

Categories of Reaction	Test Sites						Control Sites					
	T1	T2	T3	T4	T5	T6	C1	C2	C3	C4	C5	C6
Foreign Debris	0	0	0	0	0	0	0	0	0	0	0	0
Rel. Size	1	1	1	1	1	1	1	1	1	1	1	1
*Polymorphs	1	2	1	1	2	2	1	1	1	1	1	1
*Lymphocytes	0	0	0	0	0	0	0	0	0	0	0	0
*Plasma Cells	0	0	0	0	0	0	0	0	0	0	0	0
*Macrophages	1	1	1	1	1	1	1	1	1	1	1	1
*Giant Cells	0	0	0	0	0	1	0	1	0	0	0	0
*Necrosis	0	0	0	0	0	0	0	0	0	0	0	0
Subtotal (x2)	**4**	**6**	**4**	**4**	**6**	**8**	**4**	**6**	**4**	**4**	**4**	**4**
*Neovascularisation	1	1	1	1	1	1	1	1	1	1	1	1
*Fibrosis	1	1	1	1	1	1	1	1	1	1	1	1
*Fatty Infiltrate	0	0	0	0	0	0	0	0	0	0	0	0
Subtotal (x1)	**2**	**2**	**2**	**2**	**2**	**2**	**2**	**2**	**2**	**2**	**2**	**2**
TOTAL	**6**	**8**	**6**	**6**	**8**	**10**	**6**	**8**	**6**	**6**	**6**	**6**

Animal Test Score (Average of Totals) = 7.3
Animal Control Score (Average of Totals) = 6.3

Animal Score (Average Test Score - Average Control Score) = 1.0

* Used in calculation of Bioreactivity Rating.

TABLE 8
Microscopic Observations (Cont.)
2 Week Implantation

Test Article: LSR2650

Lot/Batch #: Pt. ZM 6120 (B-Stufe)

Animal #: 00471

Categories of Reaction	Test Sites						Control Sites					
	T1	T2	T3	T4	T5	T6	C1	C2	C3	C4	C5	C6
Foreign Debris	0	0	0	0	0	0	0	0	0	0	0	0
Rel. Size	1	1	1	1	1	1	1	1	1	1	1	1
*Polymorphs	1	2	1	1	2	2	1	1	1	1	1	1
*Lymphocytes	0	0	0	0	0	0	0	0	0	0	0	0
*Plasma Cells	0	0	0	0	0	0	0	0	0	0	0	0
*Macrophages	1	1	1	1	1	1	1	1	1	1	1	1
*Giant Cells	1	0	0	0	1	1	0	1	1	0	1	1
*Necrosis	0	1	0	0	1	0	0	0	0	0	0	0
Subtotal (x2)	**6**	**8**	**4**	**4**	**10**	**8**	**4**	**6**	**6**	**4**	**6**	**6**
*Neovascularisation	1	1	1	1	1	1	1	1	1	1	1	1
*Fibrosis	1	1	1	1	1	1	1	1	1	1	1	1
*Fatty Infiltrate	0	0	0	0	0	0	0	0	0	0	0	0
Subtotal (x1)	**2**	**2**	**2**	**2**	**2**	**2**	**2**	**2**	**2**	**2**	**2**	**2**
TOTAL	**8**	**10**	**6**	**6**	**12**	**10**	**6**	**8**	**8**	**6**	**8**	**8**

Animal Test Score (Average of Totals) = 8.7
Animal Control Score (Average of Totals) = 7.3

Animal Score (Average Test Score - Average Control Score) = 1.4

* Used in calculation of Bioreactivity Rating.

TABLE 8
Microscopic Observations (Cont.)
2 Week Implantation

Test Article: LSR2650

Lot/Batch #: Pt. ZM 6120 (B-Stufe)

Animal #: 00475

Categories of Reaction	Test Sites**				Control Sites**		
	T3	T4	T5	T6	C1	C2	C3
Foreign Debris	0	0	0	0	0	0	0
Rel. Size	1	1	1	1	1	1	1
*Polymorphs	1	1	2	2	1	1	1
*Lymphocytes	0	0	0	0	0	0	0
*Plasma Cells	0	0	0	0	0	0	0
*Macrophages	1	1	1	1	1	1	1
*Giant Cells	0	0	1	1	0	1	1
*Necrosis	0	0	1	0	0	0	0
Subtotal (x2)	**4**	**4**	**10**	**8**	**4**	**6**	**6**
*Neovascularisation	1	1	1	1	1	1	1
*Fibrosis	1	1	1	1	1	1	1
*Fatty Infiltrate	0	0	0	0	0	0	0
Subtotal (x1)	**2**	**2**	**2**	**2**	**2**	**2**	**2**
TOTAL	**6**	**6**	**12**	**10**	**6**	**8**	**8**

Animal Test Score (Average of Totals) = 8.5
Animal Control Score (Average of Totals) = 7.3

Animal Score (Average Test Score - Average Control Score) = 1.2

* Used in calculation of Bioreactivity Rating.
** No site found by pathologist at T1, T2, C4, C5, & C6

Animal Number 00470 = 1.0
Animal Number 00471 = 1.4
Animal Number 00475 = 1.2

Bioreactivity Rating = 1.2 = No Reaction

APPENDIX I
Classification System for Scoring Skin Reactions

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

Total possible erythema score = 4

Edema Formation	
No edema	0
Very slight edema (barely perceptible)	1
Slight 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
Histopathology Report

Project #: 10-2223-G1
Sponsor: Momentive Performance Materials
Calculated Bioreactivity Rating: 1.2
Bioreactivity Rating: No Reaction

Summary
Based on the microscopic and bioreactivity rating, there were no significant differences between the control and test sites. Considering the macroscopic and microscopic observation, the test material was found to be non-reactive in rabbit muscle after two weeks.

Respectfully submitted,

Ying Ping Yu, B.M., Pathology Associate 6/22/10
Pathology Reviewer

Alexander G. Richter, MS, DVM, DACVP 6-22-10
Pathology Approver

APPENDIX III
Software Systems

Software	Use
Adobe Acrobat 8 Professional	Document preparation
DocuKnowledge 3.0	Lotus Domino–based document management system used for SOPs
Lotus Domino Rel. 5	Client–server application for sponsor, sample, test codes, and quotation management application databases
MS Office 2007 Small Business Suite	Business software (suite includes Word, Excel, PowerPoint, Outlook, Publisher, Office tools)
Rees CentronSQL System 2.0	Environmental monitoring and metrology system

TOXIKON

Leaders in Life Science and Technology

TOXIKON TEST PROTOCOL
FDA GLP GUIDELINES
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CLASS VI TEST – USP
SYSTEMIC TOXICITY, INTRACUTANEOUS REACTIVITY, 2 WEEK MUSCLE IMPLANT – ISO

TOXIKON PROTOCOL NUMBER: PSW/VIVO/001–09/000

COMPLIANCE
21 CFR, Part 58
Good Laboratory Practice for Non–Clinical Laboratory Studies

MANAGEMENT OF THE STUDY

Performing Laboratory
Toxikon Corporation
15 Wiggins Avenue
Bedford, MA 01730

Sponsor
Momentive Performance Materials
260 Hudson River Road
Waterford, NY 12188

ORIGINAL

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PROTOCOL ACCEPTANCE

SHAHZAD ARSHAD
PRINT NAME

Sponsor's Representative Signature — 6/5/09 Date
Momentive Performance Materials
260 Hudson River Road
Waterford, NY 12188

Felice Randi LaMadeleine
PRINT NAME

Felice Randi LaMadeleine — 06/05/09 Date
Quality Assurance Signature
Toxikon Corporation
15 Wiggins Avenue
Bedford, MA 01730

Claudine Kos
PRINT NAME

Claudine Kos — 05/11/10 Date
Study Director Signature
Toxikon Corporation
15 Wiggins Avenue
Bedford, MA 01730

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1.0 PURPOSE

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

2.1 United States Pharmacopeia 32, National Formulary 27, 2009. <88> Biological Reactivity Tests, *In Vivo*.

2.2 ISO 10993–10, 2002, Biological Evaluation of Medical Devices – Part 10: Tests for Irritation and Delayed–Type Hypersensitivity, as amended 2006.

2.3 ISO 10993–11, 2006, Biological Evaluation of Medical Devices – Part 11: Tests for Systemic Toxicity.

2.4 ISO 10993–6, 2007, Biological Evaluation of Medical Devices – Part 6: Tests for Local Effects After Implantation.

2.5 ASTM F981–04, Standard Practice for Assessment of Compatibility of Biomaterials for Surgical Implants with Respect to Effect of Materials on Muscle and Bone, 2004.

2.6 ASTM F763–04, Standard Practice for Short Term Screening of Implant Materials, 2004.

2.7 ISO 10993–12, 2007, Biological Evaluation of Medical Devices – Part 12: Sample Preparation and Reference Materials.

2.8 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 Non–Clinical 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 was specifically contracted to Toxikon by Sponsor under a separate analytical protocol, whichever is applicable.

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4.1 Test Article:

Test Article Name: To Be Determined (TBD)
CAS/Code #: TBD
Lot/Batch #: TBD
Physical State: TBD
Color: TBD
Expiration Date: TBD
Density: TBD
Stability: TBD
Solubility: TBD
pH: TBD
Storage Conditions: TBD
Safety Precautions: TBD

4.2 Control Articles (Toxikon Supplied, unless specified by the Sponsor):

4.2.1 Negative Control Article Name: USP 0.9% Sodium Chloride for Injection (NaCl)
Toxikon QC #: To Be Determined (TBD)
Physical State: Liquid
Color: Colorless
Stability: Stable at Room Temperature
Storage Conditions: Room Temperature
Safety Precautions: Standard Laboratory Safety Precautions

4.2.2 Negative Control Article Name: Cottonseed Oil (CSO)
Toxikon QC #: To Be Determined (TBD)
Physical State: Liquid
Color: Yellow
Stability: Stable at Room Temperature
Storage Conditions: Room Temperature
Safety Precautions: Standard Laboratory Safety Precautions

4.2.3 Negative Control Article Name: 1 in 20 Ethanol in NaCl (EtOH)
Toxikon QC #: To Be Determined (TBD)
Physical State: Liquid
Color: Colorless
Stability: Stable at Room Temperature
Storage Conditions: Room Temperature
Safety Precautions: Standard Laboratory Safety Precautions

4.2.4 Negative Control Article Name: Polyethylene Glycol 400 (PEG)
Toxikon QC #: To Be Determined (TBD)
Physical State: Liquid
Color: Colorless

Stability: Stable at Room Temperature
Storage Conditions: Room Temperature
Safety Precautions: Standard Laboratory Safety Precautions

4.2.5 Negative Control Article Name: Negative Control High Density Polyethylene (Negative Control Plastic)

Toxikon QC #: To Be Determined (TBD)
Physical State: Solid
Color: White
Storage Conditions: Room Temperature
Safety Precautions: Standard Laboratory Safety Precautions

4.3 Reagent (Toxikon Supplied):

Reagent Name: Sterile Water for Injection (SWFI)
Toxikon QC #: To Be Determined (TBD)
Physical State: Liquid
Color: Colorless
Storage Conditions: Room Temperature
Safety Precautions: Standard Laboratory Safety Precautions

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 Implant Tests:

Number and Species: 9 New Zealand White rabbits (*Oryctolagus cuniculus*)

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

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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

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 Temperature: 68 ± 5 °F

Animal Room 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 Implant Tests:

Animal Room Temperature: 68 ± 5 °F

Animal Room 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

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 Mice will be used in this study because they have historically been used in systemic safety evaluation studies and the guidelines have no alternative (non–animal) methods. Animals will be treated by intravenous and intraperitioneal routes. The animal species, number and route of test article administration is recommended by both the USP and the ISO 10993–11 guidelines.

6.2 New Zealand White rabbits will be used in this study because they have been historically used in intracutaneous and implantation safety evaluation studies and the guidelines have no alternative (non–animal) methods. Animals will be treated by intracutaneous injections and intramuscular implantation. The animal species, number, and route is recommended by the USP, ISO 10993–6, and ISO 10993–10 guidelines.

6.3 The test article will be exposed to the test system directly and through solvents compatible with the test system.

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 ratios (please indicate on the test requisition form):

1. Specified by the Sponsor
2. No preparation required
3. According to ISO 10993–12
4. According to USP

7.1.2 The test article extracts will be prepared with the following media (please indicate on the 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

7.1.3 Extraction conditions will be determined by the Sponsor from one of the following choices (please indicate on the 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

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7.1.4 For the implant test, all apparatus strips will be prepared according to the ISO 10993–6 and USP guidelines. The test article (Sponsor–supplied) will be cut or shaped to measure approximately 1 mm to 3 mm in diameter, 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 as sterile, the test articles will be sterilized by autoclaving at 121 ± 2 °C for one hour or by dipping in alcohol.

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

7.1.6 Properly prepared test articles will be placed in separate extraction bottles, and to each bottle the appropriate medium will be added. The extraction medium should completely cover the test article.

7.1.7 Each extracting medium (control article) will be prepared for parallel treatments and comparisons. Each control article will be prepared in the same manner as the test article.

7.1.8 The Systemic Injection and Intracutaneous tests may be performed using the same extracts. Each extract will be agitated vigorously prior to administration. 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, extracts of the test article prepared with PEG, and the corresponding control, will be diluted with NaCl to obtain PEG Solution of 200 mg per 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 Test, PEG will be diluted with NaCl to obtain a concentration of about 120 mg PEG per mL.

7.2.3 Implant Test:

7.2.3.1 Two rabbits will be used for the USP Implantation Test and three rabbits will be used for the ISO Implantation Test.

7.2.3.2 Each animal will be weighed prior to implantation.

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

7.2.3.4 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	2 mL/minute
CSO	Intraperitoneal	50 mL	—
EtOH	Intravenous	50 mL	2 mL/minute
PEG	Intraperitoneal	10 g	—

7.3.2 Intracutaneous Injection Test:

7.3.2.1 A volume of 0.2 mL per site of each extract will be injected intracutaneously on one side of each of two rabbits.

7.3.2.2 Similarly, at the other side of each rabbit, the other corresponding control will be injected.

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

7.3.3 USP Implant 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 ISO Implant Test:

Up to six strips will be implanted into each of the paravertebral muscles of each rabbit, approximately 2.5 cm from the midline and parallel to the spinal column and approximately 2.5 cm from each other. The test article strips will be implanted on one side of the spine. In a similar fashion, negative control and/or test article strips will be implanted in the contralateral muscle of each animal. A total of at least ten test article strips and ten control article strips are required for evaluation.

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 at 24, 48, and 72 ± 2 hours after injection. Observations conducted will include all clinical and toxicologic signs.

7.4.1.2 The animals will be weighed 24, 48, and 72 ± 2 hours after injection.

7.4.1.3 Animals will be sacrificed by carbon dioxide inhalation.

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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 immediately after injection and at 24, 48, and 72 hours after injection of the test article. Observations will be scored according to the Classification System for Scoring Skin Reactions (Appendix I). Observations conducted will also include all clinical signs.

7.4.2.2 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.

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

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

7.4.3 Implant Test:

7.4.3.1 The animals will be maintained for a period of not less than 120 hours for the USP implant and a period of 2 weeks for the ISO implant.

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

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

7.4.3.5 Gross Observations:
The paravertebral muscles in which the test or control articles will be implanted (see Section 7.3) will be excised *in toto* from each animal. The muscle tissue will be left *in situ* if possible, or removed by carefully slicing around the implant sites with a scalpel and lifting out the tissue. The excised implant tissues will be examined grossly. For the ISO Implant Test, excessively invasive procedures that might disrupt the integrity of this tissue for histopathological evaluation will not be used. Additionally, axillary lymph nodes will be examined. If any abnormalities are noted grossly, the lymph nodes will be collected and submitted for histopathological evaluation. The tissues for histopathological assessment will be placed in properly labeled containers containing 10% neutral buffered formalin.

7.4.3.6 USP Macroscopic Evaluation:
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

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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.

7.4.3.7 ISO Histopathology:

7.4.3.7.1 Following fixation in formalin, each of the implant sites will be excised from the larger mass of tissue. The implant site, containing the implanted material, will be examined macroscopically, aided by a magnifying glass if needed. Each site will be examined for signs of inflammation, encapsulation, hemorrhaging, necrosis, and discoloration using the following scale:

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

7.4.3.7.2 The presence, form, and location of the implant material will be recorded. Photographs of the sites will be taken and retained as part of the raw data and macroscopic results will be reported.

7.4.3.7.3 After macroscopic observation, the implant material will be removed if possible, and a slice of tissue containing the implant site will be processed. Histologic slides of hematoxylin and eosin stained sections will be prepared by Toxikon.

7.4.3.7.4 The slides will be evaluated and graded by light microscopic examination.

7.4.3.7.5 Histological responses categorized as reactive, according to Section 8.0, will be documented by photomicrograph.

7.4.3.8 ISO Pathological Assessment:

7.4.3.8.1 The following categories of biological reaction will be assessed by microscopic observation and the responses graded according to the following table for each implant site:

TABLE 1
Inflammatory Responses

Cell Type/Response	Score				
	0	1	2	3	4
Polymorphonuclear Cells	0	Rare, 1–5/phf[a]	5–10/phf	Heavy Infiltrate	Packed
Lymphocytes	0	Rare, 1–5/phf	5–10/phf	Heavy Infiltrate	Packed
Plasma Cells	0	Rare, 1–5/phf	5–10/phf	Heavy Infiltrate	Packed
Macrophages	0	Rare, 1–5/phf	5–10/phf	Heavy Infiltrate	Packed
Giant Cells	0	Rare, 1–2/phf	3–5/phf	Heavy Infiltrate	Sheets
Necrosis	0	Minimal	Mild	Moderate	Severe

[a] phf = per high powered (400 ×) field.

TABLE 2
Healing Responses

Cell Type/Response	Score				
	0	1	2	3	4
Neovascularisation	0	Minimal capillary, proliferation, focal, 1–3 buds	Groups of 4–7 capillaries with supporting fibroblastic structures	Broad band of capillaries with supporting structures	Extensive band of capillaries with supporting fibroblastic structures
Fibrosis	0	Narrow band	Moderately thick band	Thick band	Extensive band
Fatty Infiltrate	0	Minimal amount of fat associated with fibrosis	Several layers of fat and fibrosis	Elongated and broad accumulation of fat cells about the implant site	Extensive fat completely surrounding the implant

7.4.3.8.2 The relative size of the involved area will be scored by assessing the width of the area from the implant/tissue interface to unaffected areas which have the characteristics of normal tissue and normal vascularity. Relative size of the involved area will be scored using the following scale:

0 = 0 mm, No site
1 = up to 0.5 mm, Very slight
2 = 0.6–1.0 mm, Mild
3 = 1.1–2.0 mm, Moderate
4 = > 2.0 mm, Severe

8.0 EVALUATION CRITERIA

8.1 Systemic Injection Test:
The test 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.

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If two or more mice die, or show signs of toxicity such as convulsions or prostration, or if a body weight loss greater than 10% (in mice at a pre–dose weight of ≥ 20 g) or 2 g (in mice at a pre–dose weight of < 20 g) occurs in three or more animals, the test article does not meet the requirements of the test. If any animal treated with a test article shows only slight signs of biological reaction, and not more than one animal shows gross signs of biological reaction or dies, a repeat test should be conducted using groups of 10 mice. On the repeat test, all 10 animals must not show a significantly greater biological reaction than the animals treated with the control article.

8.2 Intracutaneous Injection 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.

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 Implant Test:

8.3.1 USP 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.

8.3.2 ISO Bioreactivity Rating:

8.3.2.1 Calculated Rating:
For each implanted site, a total score will be determined. The inflammatory responses will be totaled for each site and weighted by a factor of two (2). The healing responses will be totaled separately. Inflammatory and healing responses will then be added together resulting in a total score for each site. The average score of the test sites for each animal will be compared to the average score of the control sites for that animal. The average difference between test and controls for all animals will be calculated and the Bioreactivity Rating will be assigned as follows:

0.0–2.9	No Reaction*
3.0–8.9	Slight Reaction
9.0–15.0	Moderate Reaction
> 15	Severe Reaction

* A negative calculation will be reported as zero (0).

8.3.2.2 Modification of the Rating:
The pathology observer will review the calculated level of reactivity. Based on the observation of all factors (e.g. relative size, pattern of response, inflammatory vs. resolution), the pathology observer may revise the Bioreactivity Rating. Justification for the modification to the rating will be presented in the narrative report (Section 8.2).

8.3.2.3 The pathology observer will provide a descriptive narrative report regarding the biocompatibility of the test material. This report may also include an assessment of other findings [e.g. hemorrhage, inflammatory pattern, tissue in–growth (for porous material), fibrovascular response, biodegradation (if applicable)], and justification for modification of the calculated Bioreactivity Rating.

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

8.5 The study and its design will employ methodology to minimize uncertainty of measurement and control of bias for data collection and analysis.

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 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 discarded.

10.0 CONFIDENTIALITY AGREEMENT

Statements of confidentiality may be agreed upon prior to study initiation.

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 suffering 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 suffering. 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.

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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, 1996. (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 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. No protocol amendments or deviations will be made without written approval in the form of a Protocol Amendment/Deviation Report (PADR) between the Sponsor and the Study Director, which will be generated as closely as possible to the time of the change.

APPENDIX I
Classification System for Scoring Skin Reactions

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

Total possible erythema score = 4

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

Total possible edema score = 4

Total possible score for irritation = 8

APPENDIX II
Software Systems

The following are the proposed software systems to be used during the conduct of this study. The actual systems used will be documented in the final report.

Software	Use
Adobe Acrobat 8 Professional	Document preparation
DocuKnowledge 3.0	Lotus Domino–based document management system used for SOPs
Lotus Domino Rel. 5	Client–server application for sponsor, sample, test codes, and quotation management application databases
MS Office 2007 Small Business Suite	Business software (suite includes Word, Excel, PowerPoint, Outlook, Publisher, Office tools)
Rees CentronSQL System 2.0	Environmental monitoring and metrology system