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TOXIKON FINAL GLP REPORT: 11–0060–G1

L929 MEM ELUTION TEST – ISO

Test Article
LSR2650

Author
Ryan Ross, B.S.

Final Report Date
January 14, 2011

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

No biological reactivity (Grade 0) was observed in the L929 mammalian cells at 48 hours post exposure to the test article extract. The observed cellular response obtained from the positive control article extract (Grade 4) and negative control article extract (Grade 0) confirmed the suitability of the test system. Based on the criteria of the protocol, the test article, LSR2650, is considered non–cytotoxic and meets the requirements of the Elution Test defined in ISO 10993–5 guidelines.

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
DOSE ADMINISTRATION	01/11/11	01/11/11	01/11/11
RAW DATA	01/14/11	01/14/11	01/14/11
FINAL REPORT	01/14/11	01/14/11	01/14/11

Allison Lyons-Hook

Allison Lyons-Hook, B.A.
Quality Assurance

1/14/11
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:	P10–2202–00A
Study Director:	Ryan Ross, B.S.
Company:	Toxikon Corporation
Signature:	Ryan Ross
Date:	1/14/11
Study Supervisor:	Ryan Ross, B.S.

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

Test Article Receipt:	11/30/10
Project Log Date:	01/07/11
Study Initiation Date:	01/10/11
Extraction Dates:	01/10/11–01/11/11
Technical Initiation:	01/10/11
Technical Completion:	01/13/11

1.0 PURPOSE

The purpose of the study was to determine the biological reactivity of a mammalian cell culture (L929) in response to the test article extract. The study was designed for the evaluation of solid test articles (e.g., polymeric materials, high–density materials, etc.). This test was appropriate for screening purposes.

2.0 REFERENCES

The study was based upon the following references:

2.1 ISO 10993–5, 2009, Biological Evaluation of Medical Devices – Part 5: Tests for *In Vitro* Cytotoxicity.

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

2.3 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 #: ZM6120
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
Safety Precautions: Standard Laboratory Safety Precautions

Sponsor Note: Cure Conditions: Press Cured 10 minutes @ 175 C

4.2 Control Articles (Toxikon Supplied):

4.2.1 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

4.2.2 Positive Control Article Name: Natural Rubber
Toxikon QC #: CSC–08–02–019–CC
Physical State: Solid
Color: Amber
Stability: Stable at Room Temperature
Storage Conditions: Room Temperature
Safety Precautions: Standard Laboratory Safety Precautions

4.2.3 Untreated Control (Extraction Medium) Name: Serum–Supplemented (Complete) Minimum Essential Medium (MEM)
Toxikon QC #: LPR–11–01–004–CC
Physical State: Liquid
Color: Red/Pink
Stability: Stable
Storage Conditions: 4 ± 2 °C
Safety Precautions: Standard Laboratory Safety Precautions

5.0 IDENTIFICATION OF TEST SYSTEM

The test system was mouse fibroblast L929 cells. The cell line was obtained from the American Type Culture Collection (ATCC), Manassas, Virginia.

6.0 JUSTIFICATION OF TEST SYSTEM AND ROUTE OF ADMINISTRATION

6.1 Historically, mouse fibroblast L929 cells have been used for cytotoxicity studies because they demonstrate sensitivity to extractable cytotoxic articles.

6.2 The test article was extracted and administered *in vitro* to mouse fibroblast L929 cells through a solvent compatible with the test system. This was the optimal route of administration available in this test system as recommended in the ISO 10993–5 guidelines.

7.0 EXPERIMENTAL DESIGN AND DOSAGE

7.1 Preparation of Test and Control Articles:

7.1.1 The test article (30 cm^2) was combined with 10 mL of vehicle at a ratio of 3 cm^2 per 1 mL per ISO 10993–12 guidelines. The test article was extracted in complete MEM supplemented with 10% fetal bovine serum at 37 ± 1 °C for 24 ± 2 hours.

7.1.2 Extracts prepared with complete MEM were tested at 100% (neat) concentration.

7.1.3 The positive control article (Natural Rubber, 0.23 cm thick) and the negative control (Negative Control Plastic, 0.06 cm thick) were extracted at a ratio of 3 cm^2 per mL in complete MEM supplemented with 10% fetal bovine serum at 37 ± 1 °C for 24 ± 2 hours.

7.1.4 A medium control was also prepared. The medium control is the extraction vehicle without the test material that is subjected to the extraction conditions and test procedures.

7.2 Pre–Dose Procedure:

7.2.1 Cell Culture Preparation:
Cultures of L929 cells were initiated in complete MEM not less than 24 hours prior to testing and incubated at 37 ± 1 °C, in a humidified atmosphere containing 5 ± 1% carbon dioxide (CO_2), to form a cell monolayer >80% confluent.

7.2.2 At the completion of the extraction period the test article appeared unchanged by the extraction procedure. The extract was clear and free from particulates. The extracts were filter sterilized by passage through a 0.2 µm pore filter to prevent interference from potential microbial contamination from the test article, prior to being applied to the cell monolayer.

7.3 Dose Administration:
A volume of 2 mL of the test article or control article extract at neat concentration was used to replace the maintenance medium of the cell culture. All extracts were tested in triplicate.

7.4 Post–Dose Procedure:
All cultures were incubated for 48 ± 2 hours, at 37 ± 1 °C, in a humidified atmosphere containing 5 ± 1% CO_2.

8.0 EVALUATION CRITERIA

8.1 The response of the cell monolayer is evaluated under a microscope. The biological reactivity (cellular degeneration and malformation) is rated on a scale of 0 to 4. The test system is considered suitable if no signs of cellular reactivity (Grade 0) are noted for both the negative control article and the medium control; and the positive control article showed greater than a Mild reactivity (Grade 2). The test article meets the requirements of the test if none of the cultures treated with the test article show greater than a Mild reactivity (Grade 2).

Grade	Reactivity	Description of Reactivity Zone
0	None	Discrete intracytoplasmic granules; no cell lysis, no reduction of cell growth
1	Slight	Not more than 20% of the cells are round, loosely attached, and without intracytoplasmic granules or show changes in morphology; occasional lysed cells are present, only slight growth inhibition
2	Mild	Not more than 50% of the cells are round and devoid of intracytoplasmic granules; not more than 50% growth inhibition observable
3	Moderate	Not more than 70% of the cell layers contain rounded cells or are lysed; cells layer not completely destroyed, but more than 50% growth inhibition
4	Severe	Nearly complete or complete destruction of the cell layers

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

9.0 RESULTS

At the 48 hour observations, no signs of cellular reactivity (Grade 0) were observed for the negative control article and the test article extracts. Severe signs of reactivity (Grade 4) were noted for the positive control article extract at the 48 hour observation.

Reactivity Grades

Time	Date	Test Article			Controls								
					Medium			Negative			Positive		
		A	B	C	A	B	C	A	B	C	A	B	C
0 Hours*	01/11/11	0	0	0	0	0	0	0	0	0	0	0	0
24 Hours	01/12/11	0	0	0	0	0	0	0	0	0	4	4	4
48 Hours	01/13/11	0	0	0	0	0	0	0	0	0	4	4	4

* 0 Hours = Pre–dose

10.0 CONCLUSION

No biological reactivity (Grade 0) was observed in the L929 mammalian cells at 48 hours post exposure to the test article extract. The observed cellular response obtained from the positive control article extract (Grade 4) and negative control article extract (Grade 0) confirmed the suitability of the test system. Based on the criteria of the protocol, the test article, LSR2650, is considered non–cytotoxic and meets the requirements of the Elution Test defined in ISO 10993–5 guidelines.

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.

APPENDIX I
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

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TOXIKON TEST PROTOCOL
FDA GLP REGULATIONS
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L929 MEM ELUTION TEST – ISO

TOXIKON PROTOCOL NUMBER: P10–2202–00A

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

MANAGEMENT OF THE STUDY

Performing Laboratory	Sponsor
Toxikon Corporation	Momentive Performance Materials
15 Wiggins Avenue	260 Hudson River Road
Bedford, MA 01730	Waterford, NY 12188

PROTOCOL ACCEPTANCE

SHAHZAD ARSHAD
PRINT NAME

Sponsor's Representative Signature
Momentive Performance Materials
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Waterford, NY 12188

11-8-10
Date

Felice Randi LaMadeleine
PRINT NAME

Felice Randi LaMadeleine
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11/09/2010
Date

PRINT NAME

Study Director Signature
Toxikon Corporation
15 Wiggins Avenue
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1/10/11
Date

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1.0 PURPOSE

The purpose of the study is to determine the biological reactivity of a mammalian cell culture (L929) in response to the test article extract. The study is designed for the evaluation of solid test articles (e.g., polymeric materials, high–density materials, etc.). This test is appropriate for screening purposes.

2.0 REFERENCES

The study will be based upon the following references:

2.1 ISO 10993–5, 2009, Biological Evaluation of Medical Devices – Part 5: Tests for *In Vitro* Cytotoxicity.

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

2.3 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.

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

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pH: TBD
Storage Conditions: TBD
Safety Precautions: TBD

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

4.2.1 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

4.2.2 Positive Control Article Name: Natural Rubber
Toxikon QC #: To Be Determined (TBD)
Physical State: Solid
Color: Amber
Stability: Stable at Room Temperature
Storage Conditions: Room Temperature
Safety Precautions: Standard Laboratory Safety Precautions

4.2.3 Untreated Control (Extraction Medium) Name: To Be Determined (TBD)
Toxikon QC #: TBD
Physical State: Liquid
Color: TBD
Stability: TBD
Storage Conditions: TBD
Safety Precautions: Standard Laboratory Safety Precautions

5.0 IDENTIFICATION OF TEST SYSTEM

The test system will be mouse fibroblast L929 cells. The cell line will be obtained from the American Type Culture Collection (ATCC), Manassas, Virginia.

6.0 JUSTIFICATION OF TEST SYSTEM AND ROUTE OF ADMINISTRATION

6.1 Historically, mouse fibroblast L929 cells have been used for cytotoxicity studies because they demonstrate sensitivity to extractable cytotoxic articles.

6.2 The test article will be extracted and administered *in vitro* to mouse fibroblast L929 cells through a solvent compatible with the test system. This is the optimal route of administration available in this test system as recommended in the ISO 10993–5 guidelines.

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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

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

1. Serum–Supplemented (Complete) Minimum Essential Medium (MEM)
2. USP 0.9% Sodium Chloride for Injection (NaCl)
3. 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. 37 ± 1 °C for 24 ± 2 hours (Compatible with Complete MEM extract)
2. 50 ± 2 °C for 72 ± 2 hours (Not compatible with Complete MEM extract)
3. 70 ± 2 °C for 24 ± 2 hours (Not compatible with Complete MEM extract)
4. 121 ± 2 °C for 1 ± 0.1 hour (Not compatible with Complete MEM extract)
5. Sponsor–specified

7.1.4 Extracts prepared with NaCl will be diluted with complete MEM and tested at 25% extract concentration unless specified otherwise by the Sponsor. Extracts prepared with complete MEM will be tested at 100% (neat) concentration unless specified otherwise by the Sponsor.

7.1.5 The positive control article (Natural Rubber) and negative control article (Negative Control Plastic) will be similarly prepared and extracted.

7.1.6 A medium control will also be prepared. The medium control is the extraction vehicle without the test material that is subjected to the extraction conditions and procedures. All other test article preparation will be as specified by the Sponsor.

7.2 Pre–Dose Procedure:

7.2.1 Cell Culture Preparation:
Cultures of L929 cells will be initiated in complete MEM not less than 24 hours prior to testing and incubated at 37 ± 1 °C, in a humidified atmosphere containing 5 ± 1% carbon dioxide.

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7.2.2 Extracts may be evaluated for a change in pH. If the color of the extract indicates an important change of pH (yellow or purple) the pH of the extract may be adjusted with Hydrochloric Acid (HCl), Sodium Bicarbonate ($NaHCO_3$), or Sodium Hydroxide (NaOH). If the test article is not provided sterile, extracts will be filter sterilized by passage through a 0.2 µm pore filter prior to being applied to the cell monolayer.

7.3 Dose Administration:
An appropriate volume of the test article or control article extract will be used to replace the maintenance medium of the cell culture. All extracts will be tested in triplicate.

7.4 Post–Dose Procedure:
All cultures will be incubated for 48 ± 2 hours or as specified by the Sponsor on the Test Requisition Form, at 37 ± 1 °C, in a humidified atmosphere containing 5 ± 1% carbon dioxide.

8.0 EVALUATION CRITERIA

8.1 The response of the cell monolayer will be evaluated under a microscope. Cytochemical stains may be used in the evaluation. The biological reactivity (cellular degeneration and malformation) will be rated on a scale of 0 to 4. The test system will be considered suitable if no signs of cellular reactivity (Grade 0) are noted for both the negative control article and the media control; and the positive control article showed greater than a Mild reactivity (Grade 2). The test article will meet the requirements of the test if none of the cultures treated with the test article show greater than a Mild reactivity (Grade 2). If the suitability of the system is not confirmed, the test should be repeated.

Grade	Reactivity	Description of Reactivity Zone
0	None	Discrete intracytoplasmic granules; no cell lysis, no reduction of cell growth
1	Slight	Not more than 20% of the cells are round, loosely attached, and without intracytoplasmic granules or show changes in morphology; occasional lysed cells are present, only slight growth inhibition
2	Mild	Not more than 50% of the cells are round and devoid of intracytoplasmic granules; not more than 50% growth inhibition observable
3	Moderate	Not more than 70% of the cell layers contain rounded cells or are lysed; cells layer not completely destroyed, but more than 50% growth inhibition
4	Severe	Nearly complete or complete destruction of the cell layers

8.2 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.

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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

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 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.

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APPENDIX I
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

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