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TOXIKON FINAL GLP REPORT: 11–0059–G2

AGAR DIFFUSION TEST – ISO

Test Article
LSR2650-1

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. The observed cellular response obtained from the positive control article (Grade 3) and negative control article (Grade 0) confirmed the suitability of the test system. Based on the criteria of the protocol, the test article, LSR2650-1, is considered non–cytotoxic and meets the requirements of the Agar Diffusion 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:	P10–1875–00A
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
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 monolayer cell culture (L929) in response to the test article. The study design was suitable for solid test articles in a variety of shapes (e.g., elastomeric closures, etc.), liquid test articles or extracts. The agar layer protects the cells from mechanical damage while allowing the diffusion of leachable chemicals from the test article.

2.0 REFERENCES

The study was conducted 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: LSR2650-1
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 Toxikon Laboratory Safety Precautions
Sponsor Note: Cure Conditions: Press Cured 10 minutes @ 175 C and Post Cured 4 hrs/200 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: Buna–N–Rubber
Toxikon QC #: CSC–03–07–005–CC
Physical State: Solid
Color: Black
Stability: Stable at Room Temperature
Storage Conditions: Room Temperature
Safety Precautions: Standard Laboratory Safety Precautions

5.0 IDENTIFICATION OF TEST SYSTEM

Mouse fibroblast L929 cells are classically used for cytotoxicity studies. The cell line was obtained from the American Type Tissue 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 leachable cytotoxic articles.

6.2 The test article was administered *in vitro*, directly to the test system. This was the optimal route of administration available in the test system as recommended by 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 was cut into pieces measuring approximately 100 mm^2.

7.1.2 The positive control article (Buna–N–Rubber) and negative control article (Negative Control Plastic) were similarly prepared.

7.2 Pre–Dose Procedure:

Cell Culture Preparation:
Cultures of L929 cells were initiated in Minimal Essential Medium supplemented with 10% fetal bovine serum (complete MEM) and incubated at 37 ± 1 °C in a humidified atmosphere containing 5 ± 1% carbon dioxide. Cultures that had grown to approximate confluence at the

end of the log phase of the growth curve were used in the assay. The liquid medium was replaced with a serum–supplemented medium/agar mixture that was stained with a vital dye, neutral red, prior to testing (final concentration of agar = 1%). The culture was protected from light for the duration of the assay to prevent cell damage elicited by photo–activation of the stain.

7.3 Dose Administration:

7.3.1 The test and control articles were applied directly to the surface of the agar, so that approximately one–tenth of the cell layer surface was covered.

7.3.2 The test was performed in triplicate.

7.4 Post–Dose Procedure:

7.4.1 Incubation:
All plates were incubated for 48 ± 2 hours at 37 ± 1 °C, in a humidified atmosphere containing 5 ± 1% carbon dioxide.

7.4.2 The extent of decolorization of the cells stained with neutral red was evaluated at 0, 24, and 48 hours.

8.0 EVALUATION CRITERIA

8.1 The response of the cell monolayer is evaluated under a microscope for cytotoxicity. The zone of biological reactivity (cellular degeneration and malformation) under and around the test and control articles is measured and 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 the negative control article and the positive control article shows 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 shows greater than a Mild reactivity (Grade 2). If the suitability of the system had not been confirmed, the test would have been repeated.

Grade	Reactivity	Description of Reactivity Zone
0	None	No detectable zone around or under specimen
1	Slight	Some malformed or degenerated cells under specimen
2	Mild	Zone limited to the area under the specimen
3	Moderate	Zone extends up to 1.0 cm beyond specimen
4	Severe	Zone extends greater than 1.0 cm beyond the specimen

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

REACTIVITY GRADES

Date	Time	Dish	Test Article		Controls			
					Positive		Negative	
			Zone Size (cm)	Grade	Zone Size (cm)	Grade	Zone Size (cm)	Grade
01/11/11	0 Hours*	A	0.0	0	0.0	0	0.0	0
		B	0.0	0	0.0	0	0.0	0
		C	0.0	0	0.0	0	0.0	0
01/12/11	24 Hours	A	0.0	0	0.5	3	0.0	0
		B	0.0	0	0.5	3	0.0	0
		C	0.0	0	0.5	3	0.0	0
01/13/11	48 Hours	A	0.0	0	0.7	3	0.0	0
		B	0.0	0	0.7	3	0.0	0
		C	0.0	0	0.7	3	0.0	0

* 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. The observed cellular response obtained from the positive control article (Grade 3) and negative control article (Grade 0) confirmed the suitability of the test system. Based on the criteria of the protocol, the test article, LSR2650-1, is considered non–cytotoxic and meets the requirements of the Agar Diffusion 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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AGAR DIFFUSION TEST – ISO

TOXIKON PROTOCOL NUMBER: P10–1875–00A

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

ORIGINAL

11-0059-62 Rec 11/14/10
11005062

PROTOCOL ACCEPTANCE

SHAHZAD ARSHAD
PRINT NAME

Sponsor's Representative Signature
Momentive Performance Materials
260 Hudson River Road
Waterford, NY 12188

8-24-10
Date

Corrie E. Bergen
PRINT NAME

Corrie E. Bergen
Quality Assurance Signature
Toxikon Corporation
15 Wiggins Avenue
Bedford, MA 01730

8/25/10
Date

Ryan Ross
PRINT NAME

Study Director Signature
Toxikon Corporation
15 Wiggins Avenue
Bedford, MA 01730

1/10/14
Date

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1.0 PURPOSE

The purpose of the study is to determine the biological reactivity of a mammalian monolayer cell culture (L929) in response to the test article. The study design is suitable for solid test articles in a variety of shapes (e.g., elastomeric closures, etc.), liquid test articles or extracts. The agar layer protects the cells from mechanical damage while allowing the diffusion of leachable chemicals from the test article.

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

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Solubility: TBD
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: Buna–N–Rubber
Toxikon QC #: To Be Determined (TBD)
Physical State: Solid
Color: Black
Stability: Stable at Room Temperature
Storage Conditions: Room Temperature
Safety Precautions: Standard Laboratory Safety Precautions

5.0 IDENTIFICATION OF TEST SYSTEM

Mouse fibroblast L929 cells are classically used for cytotoxicity studies. The cell line will be obtained from the American Type Tissue 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 leachable cytotoxic articles.

6.2 The test article will be administered *in vitro*, directly or through a solvent compatible with the test system. These are the optimal routes of administration available in this test system as recommended by the ISO 10993–5 guidelines.

7.0 EXPERIMENTAL DESIGN AND DOSAGE

7.1 Preparation of Test and Control Articles:

7.1.1 Test Without Extraction:
The test and control articles will be cut into pieces of appropriate size.

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7.1.2 Test With Extraction:

7.1.2.1 The test and control articles will be prepared at the following ratios (please indicate on the test requisition form):

1. According to ISO 10993–12
2. Specified by the Sponsor

7.1.2.2 The test article extracts will be prepared (if applicable) 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

7.1.2.3 The test article extract will be prepared under one of the following conditions to be specified by the Sponsor on the test requisition form:

1. 37 ± 1 °C for 24 ± 2 hours (Compatible with Complete MEM)
2. 37 ± 1 °C for 72 ± 2 hours (Compatible with Complete MEM)
3. 50 ± 2 °C for 72 ± 2 hours (Not compatible with Complete MEM)
4. 70 ± 2 °C for 24 ± 2 hours (Not compatible with Complete MEM)
5. 121 ± 2 °C for 1 ± 0.1 hour (Not compatible with Complete MEM)
6. Sponsor–specified

7.1.2.4 Properly prepared test articles will be placed in separate extraction bottles, and to each bottle the appropriate medium will be added.

7.1.2.5 The negative control article (Negative Control Plastic) will be similarly prepared and extracted at 3 cm^2/mL.

7.1.2.6 The positive control will be Buna–N–Rubber tested without extraction.

7.1.2.7 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:

Cell Culture Preparation:
Cultures of L929 cells will be initiated in Complete MEM and incubated at 37 ± 1 °C in a humidified atmosphere containing 5 ± 1% carbon dioxide. Cultures that have grown to approximate confluence at the end of the log phase of the growth curve will be used in the assay. The liquid medium will be replaced with a serum–supplemented medium/agar mixture which is stained with a vital dye, neutral red, prior to testing (final concentration of agar = 1%). The culture will be protected from light for the duration of the assay to prevent cell damage elicited by photo–activation of the stain.

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7.3 Dose Administration:

7.3.1 Solid test articles with a minimum surface area of 100 mm^2 will be applied directly to the surface of the agar, so that approximately one–tenth of the cell layer surface is covered.

7.3.2 Test article extracts and liquid test articles will be applied directly to a filter paper disc (surface area ≥ 100 mm^2) at a volume of 100 µL and placed on the surface of the agar.

7.3.3 Absorbent test articles will be pre–wet with the culture medium to prevent dehydration of the agar medium. The pre–wet test article will be placed on the agar.

7.3.4 The test will be performed in triplicate.

7.4 Post–Dose Procedure:

7.4.1 Incubation:
All plates will be incubated for 48 ± 2 hours unless otherwise specified by Sponsor, at 37 ± 1 °C, in a humidified atmosphere containing 5 ± 1% carbon dioxide.

7.4.2 The extent of de–colorization will be evaluated at time 0, 24, and 48 hours, unless otherwise specified by the Sponsor.

8.0 EVALUATION CRITERIA

8.1 The response of the cell monolayer will be evaluated under a microscope for cytotoxicity. The zone of biological reactivity (cellular degeneration and malformation) under and around the test and control articles will be measured and 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 the negative control article and the positive control article shows 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 shows greater than a Mild reactivity (Grade 2). If the suitability of the system is not confirmed, the test will be repeated.

Grade	Reactivity	Description of Reactivity Zone
0	None	No detectable zone around or under specimen
1	Slight	Some malformed or degenerated cells under specimen
2	Mild	Zone limited to the area under the specimen
3	Moderate	Zone extends up to 1.0 cm beyond specimen
4	Severe	Zone extends greater than 1.0 cm beyond the specimen

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