TOXIKON

ADVANCING YOUR INNOVATION

FINAL GLP REPORT: 12-4297-G2

L929 MEM ELUTION TEST – USP

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

21 CFR Part 58 Compliance
GLP for Nonclinical Laboratory Studies

Report Date
October 2, 2012

Study Director
Ryan Ross, B.S.

Sponsor
Sigma Inks Corporation
12113 Kirkham Road
Poway, CA 92064

TABLE OF CONTENTS

STUDY SUMMARY

The potential biological reactivity of a mammalian cell culture (mouse fibroblast L929) in response to exposure to the extract of the test article, 20 square inches of dry ink and 50 grams of liquid ink, was determined. The test article was extracted in Minimum Essential Medium (MEM) with 10% Fetal Bovine Serum (referred to as complete MEM) for 24 ± 2 hours at 37 ± 1 °C. Negative and positive controls were prepared similarly. The maintenance medium of L929 cells grown in 6–well plates was replaced with the neat (100%) extracts in 3 replicates, and the cells were incubated for 48 ± 2 hours at 37 ± 1 °C. The biological reactivity of the cells following the exposure to the extracts was visually observed with a microscope, and graded on a scale of 0 to 4.

There was slight biological reactivity (Grade 1) of the cells exposed to the test article extract. The response obtained from the positive and negative control article extracts confirmed the suitability of the test system.

Based on the criteria of the protocol and the USP 35-NF 30 <87> guidelines, the test article meets the requirements of the test and is not considered to have a cytotoxic effect.

QUALITY ASSURANCE STATEMENT

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

Phase	Inspection Date	Date Reported to Study Director	Date Reported to Management
SAMPLE PREPARATION	09/20/12	09/20/12	09/20/12
RAW DATA	10/02/12	10/02/12	10/02/12
FINAL REPORT	10/02/12	10/02/12	10/02/12

Jirom S. Yibrah, B.S.
Quality Assurance

10/2/12
Date

GLP COMPLIANCE STATEMENT

This study meets the technical requirements of the protocol.

This study was conducted in compliance with the current U.S. Food and Drug Administration 21 CFR, Part 58 Good Laboratory Practices for Nonclinical Laboratory Studies.

The sections of the regulations not performed by or under the direction of Toxikon Corporation, exempt from this Good Laboratory Practice Statement, included characterization and stability of the test article, 21 CFR, Part 58.105, and its mixture with carriers, 21 CFR, Part 58.113.

SIGNATURES

Signature Information	
Protocol Number	P12-1512-00A
Study Director	Ryan Ross, B.S.
Study Supervisor	Stephen Bond, B.S.
Company	Toxikon Corporation

VERIFICATION DATES

The study initiation day is the date the protocol is signed by the Study Director.

Verification Dates	
Test Article Receipt	09/11/12
Project Log	09/12/12
Study Initiation	09/13/12
Study Completion	10/02/12

Ryan Ross, B.S.
Study Director

10/2/12
Date

1.0 PURPOSE

The purpose of the study was to determine the potential biological reactivity of a mammalian cell culture (L929) in response to the test article extract.

2.0 REFERENCES

The study was based upon the following references:

2.1 United States Pharmacopeia 35, National Formulary 30, 2012. <87> Biological Reactivity Tests, *In Vitro*.

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

3.0 COMPLIANCE

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

4.0 IDENTIFICATION OF TEST AND CONTROL ARTICLES

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

4.1 Test Article:

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

4.2 Negative Control Article (Toxikon Supplied):

Name	Negative Control High Density Polyethylene Equivalent to Negative Control USP High Density Polyethylene Reference Standard (Negative Control Plastic)
Toxikon QC Number	CSC-04-05-009-CC

4.3 Positive Control Article (Toxikon Supplied):

Name	Natural Rubber
Toxikon QC Number	CSC-12-03-004-CC

4.4 Untreated Control – Extraction Medium (Toxikon Supplied):

Name	Serum-Supplemented (complete) Minimum Essential Medium (MEM)
Additive	10% of fetal bovine serum, 100 U/mL Penicillin, 0.1 mg/mL Streptomycin, 2 mM L–Glutamine (final concentrations in medium)
Toxikon QC Number	LPR-12-09-008-CC

5.0 IDENTIFICATION OF TEST SYSTEM

The test system was mouse fibroblast CCL–1 (NCTC clone 929) cells, also known as 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 Justification of Test System:
Historically, mouse fibroblast L929 cells have been used for cytotoxicity studies because they demonstrate sensitivity to extractable cytotoxic articles.

6.2 Route of Administration:
The test article was extracted and administered *in vitro* to mouse fibroblast L929 cells through a medium compatible with the test system, as indicated on the GLP Test Requisition Form.

7.0 EXPERIMENTAL DESIGN AND DOSAGE

7.1 Preparation of Test and Control Articles:

7.1.1 Per Sponsor request, a thin layer of ink was put on glass slides and allowed to dry prior to testing. The test article was prepared according to the USP guidelines and Sponsor specifications, as itemized in the table below.

Sample	Amount	Vehicle	Volume	Ratio	Temperature	Time
Test Article	82.5 cm^2	complete MEM	13.8 mL	120 cm^2/20 mL	37 ± 1°C	24 ± 2 hours
Positive Control	30 cm^2	complete MEM	10 mL	60 cm^2/ 20 mL	37 ± 1°C	24 ± 2 hours
Negative Control	30 cm^2	complete MEM	10 mL	60 cm^2/20 mL	37 ± 1°C	24 ± 2 hours
Untreated Control	N/A	complete MEM	10 mL	N/A	37 ± 1°C	24 ± 2 hours

N/A: Not Applicable

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

7.1.3 The test article was placed in an extraction vessel and the appropriate medium was added. The medium completely covered the test article.

7.1.4 The positive (Natural Rubber, 0.23 cm thick) and negative (Negative Control Plastic, 0.06 cm thick) control articles were prepared following USP ratios and extracted with the same medium at the same temperature and for the same duration as the test article, as itemized in the table above.

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

7.1.6 Each extract was agitated vigorously prior to administration.

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

7.2 Pre–Dose Procedure:

7.2.1 Cell Culture Preparation:
Cell cultures were removed from culture flasks by enzymatic digestion (trypsin/EDTA) and the cell suspension was centrifuged. The cells were then re-suspended in culture medium and seeded at 2×10^5 cells per well in 2 mL of complete MEM in a 6–well plate. The cultures were incubated for not less than 24 hours (5 ± 1% carbon dioxide (CO_2), 37 ± 1 °C, > 90% humidity) so that cells formed a sub–confluent monolayer.

7.2.2 pH Measurement:
The color of the test article extract did not indicate an obvious change of pH (yellow or purple) so the pH of the extract was not adjusted.

7.2.3 Sterility:
The test article extract was filter sterilized by passage through a 0.2 μm pore filter prior to being applied to the cell monolayer to prevent false positive results that could be due to bacterial contamination.

7.3 Dose Administration:
A 2 mL volume of extract of the test article and control articles, as well as the untreated control, were used to replace the maintenance medium of the cell culture. All dosing was done in duplicate.

7.4 Post–Dose Procedure:

7.4.1 Incubation:
All cultures were incubated for 48 ± 2 hours at 37 ± 1 °C, in a humidified atmosphere containing 5 ± 1% CO_2.

7.4.2 Grading:
The reactivity of the cells were evaluated at time 24 and 48 hours. The response of the cell monolayer was evaluated under a microscope at a 10 x 10 magnification. A cytochemical stain (Trypan Blue) was used in the evaluation. The biological reactivity (cellular degeneration and malformation) was rated on a scale of 0 to 4 based on the following table.

Grade	Reactivity	Description of Reactivity Zone
0	None	Discrete intracytoplasmic granules; no cell lysis.
1	Slight	Not more than 20% of the cells are round, loosely attached, and without intracytoplasmic granules; occasional lysed cells are present.
2	Mild	Not more than 50% of the cells are round and devoid of intracytoplasmic granules; no extensive cell lysis and empty areas between cells.
3	Moderate	Not more than 70% of the cell layers contain rounded cells or are lysed.
4	Severe	Nearly complete destruction of the cell layers.

8.0 EVALUATION CRITERIA

8.1 Test System Suitability:
The test system is considered suitable if the following conditions are met:

- The negative control article and untreated control show no signs of cellular reactivity (Grade 0).
- The positive control article shows greater than a Mild reactivity (Grade 2).

If the test system is not considered suitable, the test is repeated.

8.2 Determination of Cytotoxic Effect:
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).

8.3 Control of Bias Statement:
The study and its design employed methodology to minimize uncertainty of measurement and control of bias for data collection and analysis, which included but was not limited to: concurrent control data, system suitability assessment, blanks, and replicates.

9.0 RESULTS

The Reactivity grades are summarized in the following table:

Time	Date	Test Article		Controls					
				Untreated		Negative		Positive	
		A	B	A	B	A	B	A	B
24 Hours	09/22/12	0	0	0	0	0	0	4	4
48 Hours	09/23/12	0	0	0	0	0	0	4	4

10.0 CONCLUSION

Based on the criteria of the protocol and the USP 35-NF 30 <87> guidelines, the test article meets the requirements of the test and is not considered to have cytotoxic effect.

11.0 RECORDS

11.1 Original raw data will be archived at Toxikon Corporation.

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

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

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

12.0 CONFIDENTIALITY AGREEMENT

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

13.0 UNFORESEEN CIRCUMSTANCES

Any unforeseen circumstances were documented in the raw data. However, no unforeseen circumstances that affected the integrity of the study were noted.

14.0 PROTOCOL AMENDMENTS/DEVIATIONS

There were no protocol amendments or deviations. No changes to the protocol were required.

APPENDIX I
Software Systems

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

TOXIKON

ADVANCING YOUR INNOVATION

TOXIKON TEST PROTOCOL
FDA GLP REGULATIONS
FILE COPY/CONFIDENTIAL PROPERTY OF TOXIKON

L929 MEM ELUTION TEST – USP

TOXIKON PROTOCOL NUMBER: P12–1512–00A

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

MANAGEMENT OF THE STUDY

Performing Laboratory
Toxikon Corporation
15 Wiggins Avenue
Bedford, MA 01730

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

ORIGINAL

124297 G 2
Toxikon Use Only: 003

PROTOCOL ACCEPTANCE

James Cheng
PRINT NAME

Sponsor's Representative Signature
Sigma Inks Corp.
12113 Kirkham Road
Poway, CA 92064

9-10-12
Date

Allison Lyons-Hook
PRINT NAME

Quality Assurance Signature
Toxikon Corporation
15 Wiggins Avenue
Bedford, MA 01730

9/11/12
Date

Ryan Ross
PRINT NAME

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

9/13/12
Date

Toxikon Use Only: 003 G 2

TABLE OF CONTENTS

1.0 PURPOSE

The purpose of the study is to determine the potential biological reactivity of a mammalian cell culture (L929) in response to the test article extract.

2.0 REFERENCES

The study will be based upon the following references:

2.1 United States Pharmacopeia 35, National Formulary 30, 2012. <87> Biological Reactivity Tests, *In Vitro*.

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

3.0 COMPLIANCE

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

4.0 IDENTIFICATION OF TEST AND CONTROL ARTICLES

The Sponsor will supply the following information on a GLP Test Requisition Form or other correspondence, wherever applicable (excluding confidential or trade secret information). The Sponsor will be responsible for all test article characterization data as specified in the GLP regulations. Test and control articles (exclusive of extracts) that are mixed with carriers require verification of concentration, homogeneity, and stability. Samples of test and control article mixtures will be returned to the Sponsor for characterization and verification, unless this work was specifically contracted to Toxikon by Sponsor under a separate analytical protocol, whichever is applicable.

4.1 Test Article:

Name	To Be Determined (TBD)
CAS/Code Number	TBD
Lot/Batch Number	TBD

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

Name	Negative Control High Density Polyethylene Equivalent to Negative Control USP High Density Polyethylene Reference Standard (Negative Control Plastic)
Toxikon QC Number	To Be Determined (TBD)

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

Name	Natural Rubber
Toxikon QC Number	To Be Determined (TBD)

4.4 Untreated Control (Extraction Medium; Toxikon Supplied, unless specified by the Sponsor):

Name	To Be Determined (TBD)
Toxikon QC Number	TBD

5.0 IDENTIFICATION OF TEST SYSTEM

The test system will be mouse fibroblast CCL–1 (NCTC clone 929) cells, also known as 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 Justification of Test System:
Historically, mouse fibroblast L929 cells have been used for cytotoxicity studies because they demonstrate sensitivity to extractable cytotoxic articles.

6.2 Route of Administration:
The test article will be extracted and administered *in vitro* to mouse fibroblast L929 cells through a medium compatible with the test system, as indicated on the GLP Test Requisition Form.

7.0 EXPERIMENTAL DESIGN AND DOSAGE

7.1 Preparation of Test and Control Articles:

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

1. According to USP (default)
2. Sponsor–Specified

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

1. Minimum Essential Medium (MEM) Supplemented with 10% Fetal Bovine Serum, 100 U/mL Penicillin, 0.1 mg/mL Streptomycin, and 2 mM L–Glutamine (referred to as complete MEM)
2. USP 0.9% Sodium Chloride for Injection (NaCl)
3. Sponsor–Specified Medium (NOTE: Extraction medium not specified by USP guidelines may be required to be justified.)

124297 G 2

7.1.3 The test article will be dynamically extracted at the following condition (please indicate on the GLP 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 (NOTE: Extraction conditions not specified by USP guidelines may be required to be justified.)

7.1.4 Extracts prepared with NaCl, or other medium specified by the Sponsor, will be tested at the highest physiological concentration. NaCl extracts will be diluted to 25% concentration with complete MEM before testing, unless specified otherwise by the Sponsor. Extracts prepared with complete MEM will be tested at 100% (neat) concentration, unless specified by the Sponsor.

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

7.1.6 The positive and negative control articles will be prepared following USP ratios and extracted with the same medium at the same temperature and for the same duration as the test article.

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

7.1.8 Each extract will be agitated vigorously prior to administration.

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

7.2 Pre–Dose Procedure:

7.2.1 Cell Culture Preparation:
Cell cultures will be removed from culture flasks by enzymatic digestion (trypsin/EDTA) and the cell suspension will be centrifuged. The cells will then be re-suspended in culture medium and seeded at 2 x 10^5 cells per well in 2 mL of complete MEM in a 6–well plate. The cultures will be incubated for not less than 24 hours (5 ± 1% carbon dioxide (CO_2), 37 ± 1 °C, > 90% humidity) so that cells form a sub–confluent (> 80%) monolayer. Alternative well sizes may be used with adjustment of the cell number and dosing volumes.

7.2.2 pH Measurement:
If the color of the extract indicates an obvious 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 requested by the Sponsor.

7.2.3 Sterility:
If the test article is not provided sterile, or if the sample preparation compromises its sterility, extracts will be filter sterilized by passage through a 0.2 µm pore filter prior to being applied to the cell monolayer to prevent false positive results that can be due to bacterial contamination.

7.3 Dose Administration:
An appropriate volume of extract of the test article and control articles, as well as the untreated control, will be used to replace the maintenance medium of the cell culture. All dosing will be done in duplicate.

7.4 Post–Dose Procedure:

7.4.1 Incubation:
All cultures will be incubated for 48 ± 2 hours or as specified by the Sponsor on the GLP Test Requisition Form, at 37 ± 1 °C, in a humidified atmosphere containing 5 ± 1% CO_2.

7.4.2 Grading:
The reactivity of the cells will be evaluated at time 24 and 48 hours, unless specified by the Sponsor. The response of the cell monolayer will be evaluated under a microscope at a magnification suitable for the evaluation of cytotoxicity. 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 based on the following table:

Grade	Reactivity	Description of Reactivity Zone
0	None	Discrete intracytoplasmic granules; no cell lysis.
1	Slight	Not more than 20% of the cells are round, loosely attached, and without intracytoplasmic granules; occasional lysed cells are present.
2	Mild	Not more than 50% of the cells are round and devoid of intracytoplasmic granules; no extensive cell lysis and empty areas between cells.
3	Moderate	Not more than 70% of the cell layers contain rounded cells or are lysed.
4	Severe	Nearly complete destruction of the cell layers.

8.0 EVALUATION CRITERIA

8.1 Test System Suitability:
The test system will be considered suitable if the following conditions are met:

- The negative control article and untreated control show no signs of cellular reactivity (Grade 0).
- The positive control article shows greater than a Mild reactivity (Grade 2).

If the test system is not considered suitable, the test will be repeated.

8.2 Determination of Cytotoxic Effect:
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).

1242976 2

8.3 Control of Bias Statement:
The study and its design will employ methodology to minimize uncertainty of measurement and control of bias for data collection and analysis, which may include but is not limited to: control data (retrospective, concurrent, or prospective), system suitability assessment, randomization, method controls such as blanks and replicates, or others as required by the specific study or guideline. Methods employed will be specified in the final report.

9.0 RECORDS

9.1 Original raw data will be archived at Toxikon Corporation.

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

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

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

10.0 CONFIDENTIALITY AGREEMENT

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

11.0 UNFORESEEN CIRCUMSTANCES

All unforeseen circumstances will be documented in the raw data. Any unforeseen circumstances that affect the integrity of the study will be discussed in the final report.

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. A Protocol Amendment/Deviation Report (PADR) will be generated as closely as possible to the time of the change. The document will be created and signed by the Study Director and sent to the Sponsor. Sponsor's signature will be required for amendments to indicate approval of the amendment. Acknowledgement of notification of deviations will either be with a signature or other form of documentation.

APPENDIX I
Software Systems

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

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