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FINAL GLP REPORT: 18-01483-G1

L929 MEM ELUTION TEST – ISO

Test Article
EP30MED

21 CFR Part 58 Compliance
Good Laboratory Practice for Nonclinical Laboratory Studies

Report Date
6/5/2018

Study Director
Sindhura Ramasahayam Ph.D.

Sponsor
Master Bond
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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, EP30MED, 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 100% (neat) 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 no biological reactivity (Grade 0) 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 ISO 10993–5 guidelines, the test article meets the requirements of the test and is not considered to have a cytotoxic potential.

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.

The final report was reviewed to assure that the report accurately describes the methods and standard operating procedures. The reported results accurately reflect the raw data of the nonclinical study conducted per the protocol.

Phase	Inspection Date	Date Reported to Study Director	Date Reported to Management
Dose Administration	4/27/2018	5/11/2018	5/11/2018
Extraction	5/29/2018	5/31/2018	5/31/2018
Data	6/4/2018	6/4/2018	6/4/2018

6/5/2018

X

John Lugo-Toro, B.S.

Signed by: John Lugo-Toro

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	P18-0021-00A
Study Director	Sindhura Ramasahayam Ph.D.
Study Supervisor	Sindhura Ramasahayam Ph.D.
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	4/13/2018
Project Log	4/23/2018
Study Initiation	4/24/2018
Study Completion	6/5/2018

6/5/2018

X 

Sindhura Ramasahayam Ph.D.

Signed by: Sindhura Ramasahayam

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

2.2 ISO 10993–12, 2012, Biological Evaluation of Medical Devices – Part 12: Sample Preparation and Reference Materials.

2.3 ISO/IEC 17025, 2017, 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: EP30MED

CAS/Code Number: Not Supplied by Sponsor

Lot/Batch Number: Not Supplied by Sponsor

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-17-05-00125

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, 0.1mg/mL Streptomycin, 2 mM L-Glutamine (final concentrations in medium)

Toxikon QC Number: LPR-18-05-0374

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* 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 article was prepared following an ISO 10993-12 ratio, as itemized in the table below.

Sample	Amount	Vehicle	Volume	Ratio	Time/Temperature
Test Article	79 cm^2	complete MEM	26.3 mL	3 cm^2/mL	24 ± 2 hours at 37 ± 1 °C
Positive Control	30 cm^2	complete MEM	10.0 mL	3 cm^2/mL	24 ± 2 hours at 37 ± 1 °C
Negative Control	30 cm^2	complete MEM	10.0 mL	3 cm^2/mL	24 ± 2 hours at 37 ± 1 °C
Untreated Control	N/A	complete MEM	10.0 mL	N/A	24 ± 2 hours at 37 ± 1 °C

N/A: Not Applicable

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

7.1.3 The positive (Natural Rubber, 0.23 cm thick) and negative (Negative Control Plastic, 0.06 cm thick) control articles were prepared following ISO 10993–12 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.

7.1.4 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.

7.1.5 Each extract was agitated vigorously prior to administration.

7.1.6 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 of particulates. The test article extract was not centrifuged. 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). The cells were then 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 16 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 not filter sterilized prior to being applied to the cell monolayer.

7.3 Dose Administration:

7.3.1 A 2 mL volume of extract of the test article and a 2 mL volume of extract of the control articles, as well as the untreated control, were used to replace the maintenance medium of the cell culture. All dosing was done in triplicate.

7.3.2 Dosing Concentrations:

The test and control articles were tested at 100% (neat) concentration.

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 was evaluated at time 24 and 48 hours. The response of the cell monolayer was evaluated under a microscope at a 10 × 10 magnification. Trypan Blue was not used in the final scoring of the cell monolayer. The biological reactivity (cellular degeneration and malformation) was rated on a scale of 0 to 4 based on the following table.

Grade	Reactivity	Conditions of all cultures
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 observable.
2	Mild	Not more than 50% of the cells are round, devoid of intracytoplasmic granules, no extensive cell lysis; not more than 50% growth inhibition observable.
3	Moderate	Not more than 70% of the cell layers contain rounded cells or are lysed; cell layers not completely destroyed, but more than 50% growth inhibition observable.
4	Severe	Nearly complete or 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 as designed 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 and method controls such as 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	C	A	B	C	A	B	C	A	B	C
24 Hours	5/31/2018	0	0	0	0	0	0	0	0	0	3	3	3
48 Hours	6/1/2018	0	0	0	0	0	0	0	0	0	3	3	3

10.0 CONCLUSION

Based on the criteria of the protocol and the ISO 10993–5 guidelines, the test article meets the requirements of the test and is not considered to have a cytotoxic potential.

11.0 RECORDS

11.1 Original raw data will be archived by Toxikon Corporation.

11.2 A copy of the final report and any report amendments will be archived by 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 The test article will be disposed by Toxikon.

11.5 Test article retention upon study completion is the responsibility of the Sponsor.

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	21 CFR Part 11 Status	Publisher/Vendor	Location
Adobe Acrobat 8, 9 and 10 Professional	Document preparation	Not Applicable	Adobe Systems, Inc.	San José, CA
Matrix Gemini 5.3.19	Laboratory Information Management System	Compliant	Autoscribe Limited	Reading, UK
MS Office 2010 Small Business Suite and MS Office 2013 Professional Suite	Business software (suite includes Word, Excel, PowerPoint, Outlook, Publisher, Office tools)	Not Applicable	Microsoft Corporation	Redmond, WA
Rees Scientific Centron Presidio 3.0	Automated Environmental Monitoring	Compliant	Rees Scientific	Trenton, NJ
Report Automation 1.0	Custom software (add-in) for final report generation, review, approval, distribution to sponsors, and storage	Compliant	Court Square Group	Springfield, MA
TMS Web 7	Document management for SOPs and training records management software system	Compliant	Quality Systems Integrators	Eagle, PA
Toxikon Protocol Manager 1.0	Protocol requisition application	Not Applicable	Custom Developed	Toxikon Corporation, Bedford, MA