REVISED REPORT

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CONFIDENTIAL

STUDY TITLE

Cytotoxicity Study Using the ISO Elution Method

TEST ARTICLE NAME

M109 Pressure Sensitive Adhesive

TEST ARTICLE IDENTIFICATION

2601-84B

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Summary

The test article, M109 Pressure Sensitive Adhesive, was evaluated for potential cytotoxic effects using an *in vitro* mammalian cell culture test. This study was conducted following the guidelines of ISO 10993-5, Biological evaluation of medical devices - Part 5: Tests for *in vitro* cytotoxicity. A single preparation of the test article was extracted in single strength Minimum Essential Medium (1X MEM) at 37°C for 24 hours. The negative control, reagent control, and positive control were similarly prepared. Triplicate monolayers of L-929 mouse fibroblast cells were dosed with each extract and incubated at 37°C in the presence of 5% CO_2 for 48 hours. Following incubation, the monolayers were examined microscopically for abnormal cell morphology and cellular degeneration.

The test article extract showed no evidence of causing cell lysis or toxicity. The test article extract met the requirements of the test since the grade was less than or equal to a grade 2 (mild reactivity).

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This report has been revised to change the test article name and physical description. The conclusions were not affected. The original report was signed on December 1, 2014 by Jennifer N. Plaskey.

Approved by: Jennifer N. Plaskey, BS
Technical Reviewer

2-18-15
Date

1. Introduction

1.1 Purpose

The purpose of this study was to determine the potential of a test article to cause cytotoxicity.

1.2 Testing Guidelines

This study was based on the requirements of the International Organization for Standardization 10993-5, Biological evaluation of medical devices - Part 5: Tests for *in vitro* cytotoxicity.

1.3 Dates

Test Article Received:	November 19, 2014
Cells Dosed:	November 25, 2014
Observations Concluded:	November 27, 2014

2. Identification of Test and Control Articles

The test article provided by the sponsor was identified and handled as described below:

Table 1: Test Article

Name:	M109 Pressure Sensitive Adhesive
Identification:	2601-84B
Physical Description of the Test Article:	M109 Pressure Sensitive adhesive on polyethylene film, covered with white paper release liner
Storage Conditions:	Room Temperature

Table 2: Negative Control Article

Name:	High density polyethylene (HDPE)
Purity, Composition, and Other Characteristics:	Purity: Meets USP <661> Polyethylene Containers, Multiple Internal Reflectance, Thermal Analysis, Heavy Metals, and Non-Volatile Residue; Composition: polyethylene

Table 3: Reagent Control Article

Name:	Single strength Minimum Essential Medium supplemented with 5% fetal bovine serum, 2% antibiotics (100 units/mL penicillin, 100 µg/mL streptomycin and 2.5 µg/mL amphotericin B) and 1% (2 mM) L-glutamine (1X MEM)
Purity, Composition, and Other Characteristics:	Composition: 92% Gibco MEM with Earle's salts, 5% fetal bovine serum, 2% antibiotics (100 units/mL penicillin, 100 µg/mL streptomycin and 2.5 µg/mL amphotericin B), and 1% (2 mM) L-glutamine

Table 4: Positive Control Article

Name:	Powder-Free Latex Gloves
Purity, Composition, and Other Characteristics:	Composition: natural rubber latex, zinc carbamate accelerators, zinc oxide, and titanium dioxide

Table 5: Extraction Vehicle

Name:	1X MEM

3. Test System

3.1 Test System and Justification of Test System

Mammalian cell culture monolayer consisting of L-929 mouse fibroblast cells (ECACC Cat #85103115, or equivalent source) was used. *In vitro* mammalian cell culture studies have been used historically to evaluate cytotoxicity of biomaterials and medical devices.

3.2 Test System Management

L-929 mouse fibroblast cells were propagated and maintained in flasks containing 1X MEM at 37°C with 5% carbon dioxide (CO_2). For this study, cells were seeded in 10 cm^2 cell culture wells, labeled with passage number and date, and incubated at 37°C in the presence of 5% CO_2 to obtain subconfluent monolayers of cells prior to use. Aseptic procedures were used in the handling of the cell cultures following approved NAMSA Standard Operating Procedures.

4. Method

4.1 Test and Control Article Preparation

The release liner was removed and excluded from the preparation. A single preparation of the test article and each of the controls were subjected to the extraction conditions as described below. The extracts were continuously agitated during extraction. The 1X MEM extraction method was conducted in the presence of serum to optimize extraction of both polar and non-polar components.

Table 6: Extraction

Article	Extraction Ratio	Article Amount	Volume of Vehicle	Extraction Condition
Test	6 cm^2:1 mL	100 cm^2	17 mL	37°C for 24 hours
Negative Control	3 cm^2:1 mL	32.1 cm^2	11 mL	37°C for 24 hours
Reagent Control	Not Applicable	Not Applicable	10 mL	37°C for 24 hours
Positive Control	6 cm^2:1 mL	60 cm^2	10 mL	37°C for 24 hours

The following table contains a description of the test and control article extracts before and after extraction.

Table 7: Condition of Extracts

Vehicle	Time Observed	Extract	Condition of Extracts		
			Color	Clarity	Particulates
1X MEM	Before	Test Article	Pink	Clear	No
		Negative Control	Pink	Clear	No
		Reagent Control	Pink	Clear	No
		Positive Control	Pink	Clear	No
	After	Test Article	Pink	Clear	No
		Negative Control	Pink	Clear	No
		Reagent Control	Pink	Clear	No
		Positive Control	Pink	Clear	No

The condition of the extracts was unchanged during the extraction process.

The test article remained unchanged during the extraction process.

The extracts were tested immediately following extraction. The extracts were not centrifuged, filtered, or otherwise altered prior to dosing.

4.2 Test Procedure

Triplicate culture wells were selected which contained a subconfluent cell monolayer. The growth medium contained in the triplicate cultures was replaced with 2.0 mL of the test extract in each well. Similarly, the growth medium in triplicate 10 cm^2 wells was replaced with 2.0 mL of the reagent control, the negative control and the positive control extracts. The wells of each plate were labeled with the appropriate lab number or control and the replicate number. Each plate was labeled with the test code and the dosing date. The wells were incubated at 37°C in 5% CO_2 for 48 hours.

Following incubation, the cells were examined microscopically (100X) to evaluate cellular characteristics and percent lysis.

Table 8: Test Scoring

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 observed.
4	Severe	Nearly complete or complete destruction of the cell layers.

The color of the test medium was observed to determine any change in pH. A color shift toward yellow would have indicated an acidic pH range, and a color shift toward magenta to purple would have indicated an alkaline pH range.

For the test to be valid, the reagent control and the negative control must have had a reactivity of none (grade 0) and the positive control must have been a grade 3 or 4. Percent rounding and percent cells without intracytoplasmic granules are not evaluated in the event of 100% lysis. The test article met the requirements of the test if the biological response was less than or equal to grade 2 (mild). The test would have been repeated if the controls did not perform as anticipated.

All times and temperatures reported herein are approximate and are within ranges established by the external standards described in the References section of this report and/or NAMSA standard operating procedures.

5. Results

No cytotoxicity or cell lysis was noted in any of the test wells. No pH shift was observed at 48 hours. The reagent control, negative control and the positive control performed as anticipated. The individual reactivity grades are presented in Appendix 1.

6. Conclusion

The test article extract showed no evidence of causing cell lysis or toxicity. The test article extract met the requirements of the test since the grade was less than or equal to a grade 2 (mild reactivity).

Results and conclusions apply only to the test article tested. Any extrapolation of these data to other articles is the sponsor's responsibility.

7. Records

All raw data pertaining to this study and a copy of the final report are retained in designated NAMSA archive files in accordance with NAMSA SOPs.

8. ISO Compliance

All procedures were certified to ISO 13485 and accredited to ISO 17025.

9. References

International Organization for Standardization (ISO) 10993-1, Biological evaluation of medical devices - Part 1: Evaluation and testing within a risk management process (2009/Technical Corrigendum 1 2010).

International Organization for Standardization (ISO) 10993-5, Biological evaluation of medical devices – Part 5: Tests for *in vitro* cytotoxicity (2009).

International Organization for Standardization (ISO) 10993-12, Biological evaluation of medical devices - Part 12: Sample preparation and reference materials (2012).

International Organization for Standardization (ISO) 13485, Medical devices - Quality management systems - Requirements for regulatory purposes (2003/Technical Corrigendum 1 2009).

International Organization for Standardization/International Electrotechnical Commission (ISO/IEC) 17025, General requirements for the competence of testing and calibration laboratories (2005/Technical Corrigendum 1 2006).

United States Pharmacopeia 37, National Formulary 32 (USP), General Chapter <87>, Biological Reactivity Tests, In Vitro (2014).

Appendix 1 - Reactivity Grades For Elution Testing

Well	Percent Rounding	Percent Cells Without Intracytoplasmic Granules	Percent Lysis	Grade	Reactivity
Test (1)	0	0	0	0	None
Test (2)	0	0	0	0	None
Test (3)	0	0	0	0	None
Negative Control (1)	0	0	0	0	None
Negative Control (2)	0	0	0	0	None
Negative Control (3)	0	0	0	0	None
Reagent Control (1)	0	0	0	0	None
Reagent Control (2)	0	0	0	0	None
Reagent Control (3)	0	0	0	0	None
Positive Control (1)	Not Applicable	Not Applicable	100	4	Severe
Positive Control (2)	Not Applicable	Not Applicable	100	4	Severe
Positive Control (3)	Not Applicable	Not Applicable	100	4	Severe

Note: 1, 2 and 3 denote replicates.

Percent rounding and percent cells without intracytoplasmic granules are not evaluated in the event of 100% lysis.