ISO 10993-5 MEM Elution Test Report

Assessment of Dehydrated Boulders

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Abstract

This report summarizes the results of the MEM Elution assay for the cytotoxicity testing of Dehydrated Boulders. The purpose of this test is to assess the cytotoxic potential of the test article to determine if the material exhibits any toxic effects on mammalian cells.

Contents

Quality Assurance Statement

The Quality Assurance Unit inspected/audited this study in compliance with the following regulations:

Good Laboratory Practice for Nonclinical Laboratory Studies, Title 21 of the Code of Federal Regulations, Part 58 (21 CFR Part 58)

Inspections Overview

The Quality Assurance Unit conducted inspections on the following dates. The findings were reported to the Study Director. The final report was reviewed by the Quality Assurance Unit. The final report accurately describes the test methods in accordance with standard operating procedures, and the results are consistent with raw data from non-clinical studies conducted according to the study protocol.

Inspection	Date of Inspection	Performed by	Date Reported to Study Director
Study Protocol	2024-09-07	Mr. Bugs Bunny	2024-09-07
Study Procedure	2024-09-11	Mr. Bugs Bunny	2024-09-07
Raw Data	2024-09-14	Mr. Bugs Bunny	2024-09-14
Final Report	2024-09-15	Mr. Bugs Bunny	2024-09-15

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Mr. Bugs Bunny
Quality Manager

Date

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Wile E. Coyote
Study Director

Date

GLP Compliance Statement

This study was fully in accordance with the technical requirements of the study protocol.

This study was conducted in compliance with Good Laboratory Practice (GLP) Regulation 21 CFR Part 58, U.S. Food and Drug Administration (FDA).

Test Article Receipt	2024-09-01
Protocol Effective Date	2024-09-07
Technical Initiation Date	2024-09-08
Technical Completion Date	2024-09-13
Final Report Completion Date	2024-09-15

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Mr. Bugs Bunny Date
Quality Manager

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Wile E. Coyote Date
Study Director

Summary

Test and Control Articles

Article Type	Name	Lot Number	Expiration	Storage
Test Article	Dehydrated Boulders	BB1940WSC	2025-12-31	Room temperature
Negative Control	Polyethylene Pellets	POLY-LOT-5678	2025-03-15	Room temperature
Positive Control	Latex	LATEX-LOT-1234	2025-06-30	Room temperature
Blank Control	N/A	—	N/A	Room temperature

Test Method

Potential toxicity of test article was evaluated using the MEM Elution method in accordance with ISO 10993-5: 2009 Biological evaluation of Medical Devices—Part 5: Tests for in vitro Cytotoxicity. Study protocol number: ACME-CYTO

Conclusion

Under the conditions of this study, the test article extract did not show cytotoxic potential when testing on L929 cells.

Purpose

The purpose of this test was to determine the biological reactivity of mouse fibroblast L929 cells in response to the test article—Dehydrated Boulders. This study evaluated whether the test article exhibited any toxic effects on mammalian cells under in vitro conditions. Specifically, the test assessed the cytotoxic potential to determine the safety and biocompatibility of the material when used according to its intended purpose.

Regulatory Standards

This study was conducted in accordance with the following regulatory standards:

1. **ISO 10993-1**: *Evaluation and Testing within a Risk Management Process*—General requirements for evaluating the biological impact of medical devices.

2. **ISO 10993-5:2009**: *Biological Evaluation of Medical Devices—Part 5: Tests for in vitro Cytotoxicity*—Provides guidelines for evaluating the potential cytotoxic effects of a medical device material on cells.

3. **ISO 10993-12**: *Sample Preparation and Reference Materials*—Guidelines for preparing samples and reference materials for testing to ensure consistent and reliable results.

These standards ensure that the study was conducted with the highest level of scientific rigor and consistency, providing a reliable assessment of the biological impact of the test article.

Compliance

This study complies with the following regulations:

1. **Good Laboratory Practice (GLP) Regulations (21 CFR Part 58)**: GLP standards ensure that the study is conducted with transparency, accountability, and rigorous scientific practices, guaranteeing the integrity of the study data.

2. **ISO/IEC 17025:2017**: *General Requirements for the Competence of Testing and Calibration Laboratories*—ISO 17025 accreditation ensures that the testing facility is competent to perform laboratory testing with a high degree of accuracy and reliability.

Compliance with these regulations guarantees that the study data meets both legal and quality assurance requirements, ensuring the validity and credibility of the test results.

Test and Control Articles

Test Article

Name	Dehydrated Boulders
Manufacturer	ACME Corporation
Batch/Lot Number	BB1940WSC
Expiration Date	2025-12-31
Storage Conditions	Room temperature

*Note** The information about the test article was supplied by the sponsor wherever applicable. The Sponsor is responsible for all test article characterization data as specified in the GLP.

Negative Control Article

Name	High-density Polyethylene (HDPE)
Extraction Ratio	3 cm^2/1 mL
Physical State	Solid
Color	White
Storage Conditions	Room temperature
Extraction Solvent	MEM medium with 10% fetal bovine serum (FBS)

Positive Control Article

Name	Latex
Extraction Ratio	3 cm^2/1 mL
Physical State	Solid
Color	Tan
Storage Conditions	Room temperature
Extraction Solvent	MEM medium with 10% FBS

Media Control

MEM medium with 10% fetal bovine serum (FBS)

Equipment and Reagents

Equipment

Equipment Name	Equipment Number	Calibration Expire
Autoclave	ACME-001	2025-03-09
CO2 Incubator	BUGS-006	2025-03-09
Inverted microscope	DAFFY-007	2025-06-15

Reagents

Reagent Name	Manufacturer	Part Number	Lot Number	Expiration Date
Fetal Bovine Serum (FBS)	Wile E. Biotech Industries	FBS-002	FBS-LOT-2024	2025-01-31
MEM Medium	Road Runner Labs	MEM-003	MEM-LOT-5678	2025-06-30
Trypsin	Bugs Bunny Pharmaceuticals	TRYPSIN-004	TRYPSIN-LOT-9012	2025-08-15
Penicillin/Streptomycin	Daffy Duck Labs	PENSTREP-005	PENSTREP-LOT-3456	2025-11-01
PBS (Phosphate-buffered saline)	Tweety Solutions	PBS-006	PBS-LOT-7890	2026-01-20
Isopropanol (99.9%)	ACME Industrial Reagents	ISO-007	ISO-LOT-1122	2026-03-15

Identification of Test System

L929 mouse fibroblast cells obtained from ATCC (American Type Culture Collection), USA.

Justification of Test System and Route of Administration

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

Experimental Design

Extraction Process

The extraction process was carried out at an extraction ratio of **3 cm³:1 mL** for all samples. Under aseptic conditions, samples were extracted with continuous agitation in closed, inert containers, using **DMEM medium with 10% fetal bovine serum (FBS)** as the extraction solvent.

Sample	Extract Volume (mL)	Appearance of Extract
Test Article	64.9 mL	Clear
Negative Control	64.9 mL	Clear
Positive Control	64.9 mL	Cloudy
Blank Control	64.9 mL	Clear

Experimental Procedure

Once the extracts were prepared, they were tested on **L929 mouse fibroblast cells** using a standard cytotoxicity assay. The cells were cultured in a 96-well plate and exposed to the test article extract and controls for approximately 48 hours.

Evaluation Criteria

Morphological assessment of cytotoxicity was based on the following grading system:

Grade	Criteria
0 - None	No cell lysis observed; cells remained attached, with intracytoplasmic granules.
1 - Slight	20% rounding of cells, with occasional lysed cells observed.
2 - Mild	$>$ 20% to 50% rounding, with no extensive cell lysis.
3 - Moderate	$>$ 50% rounding, with lysed cells present.
4 - Severe	Nearly complete destruction of the cell layer, with extensive lysis.

Statistical Method

The mode of three replicates will be calculated to evaluate cytotoxic potential.

Results

Pre-Extraction Observations

Observation	Details
Pre-Extraction	All extracts were clear and free of particulates prior to the extraction process.

Post-Extraction Observations

Sample	Post-Extraction Observation
Test Article	No significant color changes were observed, and the extract remained clear.
Negative Control	No significant color changes were observed, and the extract remained clear.
Positive Control	The extract became cloudy, and filtration was required before use.

General Observations

No pH adjustments, centrifugation, or dilution processes were performed on the test and control samples.

Post-Extraction Observation

- **Test Article**: No significant color changes were observed, and the extract remained clear.
- **Negative Control**: No significant color changes were observed, and the extract remained clear.
- **Positive Control**: The extract became cloudy, and filtration was required before use.

No pH adjustments, centrifugation, or dilution processes were performed on the test and control samples.

Morphological Scoring

Each sample was tested in triplicate. The table below shows the results of the qualitative assessment of cytotoxicity:

Sample	Replicate 1	Replicate 2	Replicate 3	Modal Score
Test Article	0 (None)	0 (None)	0 (None)	0 (None)
Positive Control	4 (Severe)	4 (Severe)	4 (Severe)	4 (Severe)
Negative Control	0 (None)	0 (None)	0 (None)	0 (None)
Blank Control	0 (None)	0 (None)	0 (None)	0 (None)

Conclusion

The **test article extract**, when tested in triplicate, did not exhibit any cytotoxic effects at the tested concentration, as indicated by the **modal reactivity score** of **0 (None)**. The positive control demonstrated expected **severe cytotoxicity**, while the negative and blank controls showed **no cytotoxicity**.

Record Storage

All raw data generated during this study, along with a copy of the final report, have been securely archived at **ACME Labs**' designated storage facility in accordance with internal protocols and regulatory requirements.

Confidentiality Agreement

The confidentiality of all data and study-related materials has been maintained as per the confidentiality agreement established prior to the initiation of the study.

Deviation Statement

There were no deviations from the approved study protocol that would impact the validity or integrity of the data.