ISO 10993-5 MEM Elution Test Report

Assessment of Dehydrated Boulders

September 22, 2024

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.

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## 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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| Wile E. Coyote | Date |
| Study Director |  |

## 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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| 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 cm²/1 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 cm²/1 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.