



Health and Environmental
Sciences Department

Chinese Hamster Ovary (CHO)/HGPRT Mutation Assay of Tertiary Amyl Methyl Ether (TAME)

DECEMBER 1996

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Chinese Hamster Ovary (CHO)/HGPRT Mutation Assay of Tertiary Amyl Methyl Ether (TAME)

Health and Environmental Sciences Department

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FINAL REPORT AMENDMENT ISPONSOR: **American Petroleum Institute**TEST ARTICLE I.D.: **Tertiary Amyl Methyl Ether (TAME)**MA STUDY NO: **G95CA89.782**DATE OF FINAL REPORT: **06/19/96**PROTOCOL TITLE: **CHO/HGPRT MUTATION ASSAY**

1. PART OF FINAL REPORT TO BE AMENDED: Entire report.**AMENDMENTS:**

- (a) Change page numbers to accomodate Sponsor's Quality Assurance Report and report format.
- (b) Minor grammatical and formatting changes throughout report.
- (c) Amend the Table of Contents to include section subheadings.
- (d) Add protocol amendment 1 to Appendix B.
- (e) Add memo on cell history record to Appendix B.

REASON FOR THE AMENDMENT: To accomodate changes in report format requested by the Sponsor.

APPROVALS:

STUDY DIRECTOR

11/22/96

DATE



QUALITY ASSURANCE

Nov 22, 1996

DATE

The Quality Assurance Report for the American Petroleum Institute contained on these pages was performed for two laboratory studies. This publication, TR411, corresponds to MBA Study G95CA89.782. The remaining MBA study (G95CA89.330) is published under API publication TR410.



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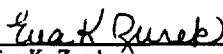
**REPORT OF THE INSPECTION
FOR COMPLIANCE WITH THE
GOOD LABORATORY PRACTICE REGULATIONS
of the
ENVIRONMENTAL PROTECTION AGENCY**

Conducted at

MICROBIOLOGICAL ASSOCIATES, INC.
9630 Medical Drive
Rockville, MD

on

May 1 and 9, 1996


Eva K. Zurek
Senior Associate

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INTRODUCTION

This inspection of Microbiological Associates, Inc. was carried out on behalf of the American Petroleum Institute (API), Washington DC under the Environmental Protection Agency's Testing Consent Order concerning *tert*-amyl methyl ether (TAME). The inspection was to ascertain the facilities' compliance with the Good Laboratory Practice (GLP) regulations issued by the EPA under the Toxic Substances Control Act (TSCA) at 40 CFR 792. The nonclinical laboratory studies audited on this site visit include:

Chromosome Aberrations in Chinese Hamster Ovary (CHO) Cells
(MBA Study Number G95CA89.330)

CHO/HGPRT Mutation Assay (MBA Study Number G95CA89.782)

The inspection/audit was performed on May 1 and May 9, 1996, by Eva Zurek, Senior Associate, Goldman Associates International, Inc.

PROCEDURES

This inspection was conducted by a tour of the new genetic tox facility and review of personnel files, QAU procedures, study protocols, relevant standard operating procedures (SOPs), and raw data generated during the conduct of the studies. The studies were in the final draft report stage at the time of the audit.

Claire Courtemanche, Quality Assurance Manager, Toxicology Group, provided all assistance and documents during the inspection. The inspection was initiated at approximately 10 a.m. on May 1, 1996. The inspector returned at 10 a.m. on May 9, 1996 to complete the inspection.

SUMMARY OF FINDINGS

In general, inspection of the facilities, equipment, personnel files, QAU, test article receipt and handling, and SOPs showed management's firm commitment to compliance with the regulations. Thorough documentation of study conduct is enhanced by the use of pre-printed raw data worksheets. However, the following deficiencies were noted with respect to documentation of test system receipt and evaluation for the Mutation Assay:

- There is no documentation that the CHO-K₁-BH₄ cells used for the Mutation Assay were obtained from A. Hsie, Oak Ridge, TN, as stated in the protocol and raw data workbook.

- The earliest record of this cell line is an ampule freeze date of 2/2/81. It was next reconstituted on 1/11/93. There is no documentation to show characterization or karyotypic stability for this or any subsequent freeze lot that was ultimately used for this study.

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- There was no record of testing the freeze lot of cells for mycoplasma contamination, as required by protocol.

- Results of mycoplasma testing for each new frozen lot of cells were not being maintained in the cell line log book, as specified by SOP OPGTO322.R4

- Preparation of the "cleansing" medium (HAT) should be documented. "HAT" should be defined in the raw data.

The findings were discussed with Ms. Courtemanche at the close of the inspection on May 9, 1996. She stated that the study director will be notified, and that he will provide deviation reports, where necessary. The study director will contact appropriate API personnel to determine the best course of action. It may be advisable to perform characterization and mycoplasma testing on another representative sample from the same freeze lot that was used for this testing.

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REPORT OF THE INSPECTION

PERSONNEL

All study personnel had the appropriate training, education, and experience for their assigned tasks. Training records were reviewed for representative technicians involved in the studies audited, and were found to be complete.

MANAGEMENT

Test facility management is supportive of the GLP program as is evidenced by the personnel, facilities, equipment, and methodology provided for the studies that were reviewed. Quality Assurance submits bimonthly written reports to keep management informed of the compliance status of all studies.

STUDY DIRECTOR

Dr. Richard San is the designated study director for the CHO/HGPRT Mutation Assay, and Dr. Patrick Curry is the study director for the Chromosome Aberration study. An SOP has been developed that addresses the replacement of a study director, or the appointment of an alternate study director.

It was noted that Dr. San will have to issue a deviation report with respect to the lack of documentation for the mycoplasma testing for the freeze lot of cells used in the Mutation Assay. Dr. San should provide documentation to support the statement in the final report that "the CHO-K₁-BH₄ cells were obtained from A. Hsie, Oak Ridge National Laboratory, Oak Ridge, TN."

QUALITY ASSURANCE

The Quality Assurance responsibilities for genetic toxicology studies are managed by Claire Courtemanche. Records show Ms. Courtemanche to be an experienced and well-qualified Quality Assurance professional. GLP training is provided to all personnel by members of the QAU staff. SOPs are in place and are followed for all QAU activities. All studies (including acutes) are monitored at least once during the in-life phase. QAU has issued the required statements for the two studies audited that state when inspections were conducted and when reports were made to study director and management.

FACILITIES

The Genetic Toxicology laboratory was re-located to a new facility on March 18-20, 1996. The new facilities are clean and spacious and afford the room for complete separation of study activities. A tour of the facility provided by the QAU Manager included the following specialized areas: Test Article Repository; Cold Room (251);

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Tissue Processing Lab; Media Preparation Lab; Ames Lab; Bacterial Counting Lab; Gene Mutation Lab; Counting Room; Microscope Room. A routine maintenance form was located next to each piece of equipment that requires any maintenance.

FACILITIES FOR HANDLING TEST AND CONTROL SUBSTANCES

Specific SOPs have been prepared to control the receipt, storage and distribution of test and control articles at MBA. All test articles are delivered to the loading dock, brought upstairs, opened and unpacked under hoods. All test articles are handled and stored in the Test Article Repository (Room 261), which is a limited access area. Only designated personnel may sign in, open and log in test and control articles. All test articles are logged into a computerized tracking system (TATS), and well-controlled records are maintained for use and distribution. All refrigerators and freezers are alarmed and connected to a central control room and security company. If an alarm sounds, the appropriate personnel are notified.

EQUIPMENT

SOPs are available for each piece of equipment in the genetic toxicology area. Detailed equipment maintenance logs are kept for routine and specialized cleaning, testing, calibration or maintenance operations. Appropriate temperature logs are maintained for all freezers, refrigerators, incubators and water baths. A NIST traceable thermometer is used to calibrate all laboratory thermometers.

STANDARD OPERATING PROCEDURES

All standard operating procedures have been established, as required by the regulations. Relevant SOPs are readily available in the laboratory areas. SOPs are current and well-written. SOPs applicable to these genetic toxicology studies were reviewed. One deviation from an SOP was noted (SOP OPGTO322.R4). This SOP states that "each new frozen lot of cells will be tested for mycoplasma contamination. Results will be maintained in the cell line log book". The cell line log book used to trace the CHO cells for the Mutation Assay did not contain any reported results of mycoplasma testing. If it is more convenient to maintain these reports elsewhere, the SOP should be revised.

ANIMAL AND OTHER TEST SYSTEM CARE

There are SOPs for the handling of the test systems used in the studies that were audited. Good study records were maintained for the preparation of cells for testing. Records show that the CHO-K₁ cell line used for the Chromosome Aberration test was obtained from ATCC, Rockville, MD. There are no records to show that the CHO cells used for the Mutation Assay were received from Dr. Hsie, Oak Ridge, TN, as is stated in the protocol. There are no records for the original receipt of these cells. The earliest record documents an ampule freeze date of 2/2/81. These cells were reconstituted on 1/11/93, and subsequently split, passed and frozen again for a number of times before achieving

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the freeze lot used in the current study. It is suggested that the study director provide some documentation regarding the receipt, characterization or karyotypic stability of this cell line. The preparation of the cleansing medium (HAT) that is added to the cell line prior to freezing should be documented.

TEST, CONTROL, AND REFERENCE SUBSTANCE CHARACTERIZATION

The sponsor has assumed responsibility for all test substance characterization, and this is stated in the study protocol signed by the study director and sponsor.

PROTOCOL

The protocols for the two TAME genetic tox studies were signed by the Study Directors on January 23, 1996. The protocols met all GLP requirements. As mentioned above, Dr. San, the study director for the Mutation Assay, should provide documentation for the receipt of the CHO-K1-BH4 cell line, and prepare a deviation report for the mycoplasma testing for this freeze lot.

CONDUCT OF THE STUDY

Recording and maintenance of raw data is facilitated by the use of pre-printed data sheets which form the Raw Data Workbook for each study. In addition to all of the results, the workbooks contain the study protocols, test article receipt, use and disposition records, identification of study personnel, documentation error codes, test article measuring reports, solubility determinations, pH measurements, Full list of materials and supplies with batch or lot numbers, where needed, equipment identification, and target cell history and preparation. Data sheets were properly completed.

ABSTRACT

The test article, Tertiary Amyl Methyl Ether (TAME), was tested in the CHO/HGPRT Mutation Assay in the absence of Aroclor[®]-induced rat liver S9 at doses of 1000 to 5000 µg/ml and in the presence of Aroclor[®]-induced rat liver S9 at doses of 500 to 5000 µg/ml. No positive responses, i.e., treated cultures with mutant frequencies >40 mutants per 10⁶ clonable cells, were observed. All concentrations were soluble in treatment medium. Toxicity, i.e., cloning efficiency ≤50% of the solvent control, was observed at doses of 4000 and 5000 µg/ml with S9 activation. Under the conditions of this study, test article Tertiary Amyl Methyl Ether (TAME) was concluded to be negative in the CHO/HGPRT Mutation Assay.

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AMENDED FINAL REPORT

Study Title

CHO/HGPRT MUTATION ASSAY

Test Article

Tertiary Amyl Methyl Ether (TAME)

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Study Completion Date

06/19/96

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