



Health and Environmental
Sciences Department

Chromosome Aberrations in Chinese Hamster Ovary (CHO) Cells Exposed to Tertiary Amyl Methyl Ether (TAME)

DECEMBER 1996

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Chromosome Aberrations in Chinese Hamster Ovary (CHO) Cells Exposed to Tertiary Amyl Methyl Ether (TAME)

Health and Environmental Sciences Department

API PUBLICATION NUMBER TR410

PREPARED UNDER CONTRACT BY:

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DECEMBER 1996



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THE FOLLOWING PEOPLE ARE RECOGNIZED FOR THEIR CONTRIBUTIONS OF TIME AND EXPERTISE DURING THIS STUDY AND IN THE PREPARATION OF THIS REPORT:

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FINAL REPORT AMENDMENT ISPONSOR: **American Petroleum Institute**TEST ARTICLE I.D.: **Tertiary Amyl Methyl Ether (TAME)**MA STUDY NO: **G95CA89.330**DATE OF FINAL REPORT: **06/18/96**PROTOCOL TITLE: **Chromosome Aberrations in Chinese Hamster Ovary (CHO) Cells**

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 2 to Appendix B.

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

Ramadevi Gudi
STUDY DIRECTOR

11/20/96
DATE

Chaire L. Coustemauche
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, TR410, corresponds to MBA Study G95CA89.330. The remaining MBA study (G95CA89.782) is published under API publication TR411.



GOLDMAN ASSOCIATES INTERNATIONAL, INC.

**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

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. Hsieh, 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 Chromosome Aberration Assay using Chinese hamster ovary (CHO) cells, in both the absence and presence of Aroclor®-induced rat liver S9 (fraction of extracted biotransformation enzymes capable of metabolizing putative promutagens into the ultimate mutagenic agent) at doses of 313 to 5000 µg/ml. All concentrations tested in both the non-activated and the S9-activated test systems were soluble in treatment medium. Toxicity, i.e., cell growth inhibition, was 43% at the 5000 µg/ml dose level in the non-activated test system and 72% at the 5000 µg/ml dose level in the S9-activated test system. No positive response was seen in the non-activated test system. In the S9-activated test system there was a positive response with an increasing concentration of the test article. Under the conditions of this study, the test article, Tertiary Amyl Methyl Ether (TAME), was concluded to be positive in the Chromosome Aberration Assay using CHO cells.

TABLE OF CONTENTS

	<u>Page</u>
Laboratory Title Page	12
Laboratory Signature Page	13
Laboratory QA Statement	14
Laboratory Compliance Page	15
SUMMARY	16
INTRODUCTION	18
PURPOSE	18
CHARACTERIZATION OF TEST AND CONTROL ARTICLES	18
MATERIALS AND METHODS	19
TEST SYSTEM	19
METABOLIC ACTIVATION SYSTEM	19
SOLUBILITY TEST	19
PRELIMINARY TOXICITY ASSAY	20
CHROMOSOME ABERRATION ASSAY	20
COLLECTION OF METAPHASE CELLS	21
SLIDE PREPARATION	22
EVALUATION OF METAPHASE CELLS	22
EVALUATION OF TEST RESULTS	23
CRITERIA FOR A VALID TEST	24
ARCHIVES	24
RESULTS AND DISCUSSION	25
SOLUBILITY TEST	25
PRELIMINARY TOXICITY ASSAY	25
CHROMOSOME ABERRATION ASSAY	26
CONCLUSION	28
REFERENCES	29

LIST OF TABLES

1. Preliminary Toxicity Test in the Absence of Exogenous Metabolic Activation	30
2. Preliminary Toxicity Test in the Presence of Exogenous Metabolic Activation	31
3. Preliminary Toxicity Test in the Absence of Exogenous Metabolic Activation	32
4. Preliminary Toxicity Test in the Presence of Exogenous Metabolic Activation	33
5. Concurrent Toxicity Test in the Absence of Exogenous Metabolic Activation	34
6. Cytogenetic Analysis of Cells Treated (Absence of Exogenous Metabolic Activation)	35
7. Concurrent Toxicity Test in the Presence of Exogenous Metabolic Activation	36
8. Cytogenetic Analysis of Cells Treated (Presence of Exogenous Metabolic Activation)	37
9. Summary	38
Appendix A: Historical Control Data	A-1
Appendix B: Study Protocol	B-1

AMENDED FINAL REPORT

Study Title

**CHROMOSOME ABERRATIONS
IN CHINESE HAMSTER OVARY (CHO) CELLS**

Test Article

Tertiary Amyl Methyl Ether (TAME)

Author

Patrick T. Curry, Ph.D.

Study Completion Date

June 18, 1996

Performing Laboratory

Microbiological Associates, Inc.
9900 Blackwell Road
Rockville, Maryland 20850

Laboratory Study Number

G95CA89.330

Sponsor Project Number

HES1621-L-00860-MUTAGEN

Sponsor

American Petroleum Institute
1220 L Street, Northwest
Washington, D.C. 20005

**CHROMOSOME ABERRATIONS
IN CHINESE HAMSTER OVARY (CHO) CELLS**

AMENDED FINAL REPORT

**Sponsor: American Petroleum Institute
1220 L Street, Northwest
Washington, D.C. 20005**

Authorized Representative: Richard Rhoden, Ph.D.

**Performing Laboratory: Microbiological Associates, Inc. (MA)
9900 Blackwell Road
Rockville, Maryland 20850**

Test Article I.D.: Tertiary Amyl Methyl Ether (TAME)

Sponsor Project Number: HES1621-L-00860-MUTAGEN

MA Study No.: G95CA89.330

Test Article Description: Clear liquid

Storage Conditions: Room temperature, protected from exposure to light

Test Article Receipt: December 26, 1995

Study Initiation: January 23, 1996

Laboratory Supervisor:

Elizabeth H. Schady
Elizabeth H. Schady, B.S.

11/20/96

Date

Study Director:

Ramadevi Gudi
Ramadevi Gudi, Ph.D.

11/20/96

Date

QUALITY ASSURANCE STATEMENT

Study Title: CHROMOSOME ABERRATIONS IN CHINESE HAMSTER OVARY
(CHO) CELLS

Study Number: G95CA89.330

Study Director: Ramadevi Gudi, Ph.D.

This study has been divided into a series of in-process phases. Using a random sampling approach, Quality Assurance monitors each of these phases over a series of studies. Procedures, documentation, equipment records, etc., are examined in order to assure that the study is performed in accordance with the U.S. FDA Good Laboratory Practice Regulations (21 CFR 58), the U.S. EPA GLPs (40 CFR 792 and 40 CFR 160), the UK GLP Compliance Programme, the Japanese GLP Standard, and the OECD Principles of Good Laboratory Practice and to assure that the study is conducted according to the protocol and relevant Standard Operating Procedures.

The following are the inspection dates, phases inspected, and report dates of QA inspections of this study.

INSPECT ON 23 JAN 96, TO STUDY DIR 23 JAN 96, TO MGMT 23 JAN 96
PHASE: Protocol Review

INSPECT ON 06 FEB 96, TO STUDY DIR 13 FEB 96, TO MGMT 14 FEB 96
PHASE: Test and/or control material administration

INSPECT ON 04 APR 96-05 APR 96, TO STUDY DIR 05 APR 96, TO MGMT 08 APR 96
PHASE: Draft Report

INSPECT ON 09 MAY 96, TO STUDY DIR 09 MAY 96, TO MGMT 09 MAY 96
PHASE: Draft to Revised Draft Report

INSPECT ON 19 JUN 96, TO STUDY DIR 19 JUN 96, TO MGMT 19 JUN 96
PHASE: Revised Draft to Final Report

INSPECT ON 27 AUG 96, TO STUDY DIR 11 NOV 96, TO MGMT 22 NOV 96
PHASE: Amended Final Report

This report describes the methods and procedures used in the study and the reported results accurately reflect the raw data of the study.

Claire L. Courtemanche
Claire L. Courtemanche
QUALITY ASSURANCE

Nov 22, 1996
DATE

STATEMENT OF COMPLIANCE

Study G95CA89.330 was conducted in compliance with the US FDA Good Laboratory Practice Regulations as published in 21 CFR 58, the US EPA GLP Standards 40 CFR 160 and 40 CFR 792, the UK GLP Compliance Programme, the Japanese GLP Standard and the OECD Principles of Good Laboratory Practice in all material aspects with the following exceptions:

The identity, strength, purity and composition or other characteristics to define the test or control article were not determined by the testing facility.

Analyses to determine the uniformity, concentration, or stability of the test or control mixtures were not performed by the testing facility.

The stability of the test or control article under the test conditions was not determined by the testing facility.



Ramadevi Gudi, Ph.D.
Study Director



Date

SUMMARY

The test article, Tertiary Amyl Methyl Ether (TAME), was tested in the chromosome aberration assay using Chinese hamster ovary (CHO) cells. The assay was performed in two phases. The first phase, a preliminary toxicity test, was performed to establish the dose range and cell collection times for the chromosome aberration assay. The second phase, the chromosome aberration assay, was used to evaluate the clastogenic potential of the test article. In both phases of the assay the test article was assessed in both the absence and presence of an Aroclor®-induced S9-activation system (fraction of extracted biotransformation enzymes capable of metabolizing putative promutagens into the ultimate mutagenic agent).

Ethanol was determined to be the solvent of choice based on solubility of the test article and compatibility with the target cells. The test article was soluble in ethanol at a maximum concentration of approximately 500 mg/ml and was soluble in the treatment medium at a concentration of 5000 µg/ml.

In the preliminary toxicity assay, the maximum dose tested was 5000 µg/ml. This dose was achieved using a stock concentration of 500 mg/ml and a 50 µl dosing aliquot. The test article was soluble in solvent at the stock concentration of 500 mg/ml and was soluble in treatment medium at all concentrations tested. Selection of dose levels for the chromosome aberration assay was based on cell growth inhibition relative to the solvent control. Cell harvest times were determined after evaluating the test article effect on the cell cycle kinetics by measuring the average generation time (AGT). Substantial toxicity, i.e., at least 50% cell growth inhibition, was observed at a dose level of 5000 µg/ml in both the non-activated and S9-activated test systems. Based on these findings, the doses chosen for the chromosome aberration assay ranged from 313 to 5000 µg/ml for both the non-activated and the S9-activated systems. The average generation time was delayed from approximately 12 hours to 23.8 hours at the highest dose tested (5000 µg/ml) in the S9-activated study. No cell cycle delay was seen at any of the doses tested in the non-activated study. Based on the cell cycle