UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

009369

MAR 23 1992

OFFICE OF
PESTICIDES AND TOXIC
SUBSTANCES

MEMORANDUM

SUBJECT: Review of Mutagenicity Studies Using Technical Methanearsonic Acid (Guidelines 84-1 and 84-2)

TO: Barbara Briscoe/Betty Crompton, PM-51
Registration Division (H-7508W)

FROM: David S. Liem, Ph.D. David Liem 3/12/92
Toxicology Branch II/Sect. II (H-7509C)

THROUGH: K. Clark Swentzel, Section Head K. Clark Swentzel 3/13/92
Toxicology Branch II/Sect. II (H-7509C)
and
Marcia van Gemert, Ph.D., Branch Chief Marcia van Gemert 3/17/92
Toxicology Branch II/HED (H-7509C)

DP BARCODE NO.: D166936 CASWELL NO.: 549A (not 582)

HED PROJECT NO: 1-2070 MRID NO.: 416519-02 to 05

ACTION REQUESTED: To review four mutagenicity studies, Salmonella typhimurium/mammalian microsome mutagenicity assay, Gene mutation in cultures Mouse lymphoma cells, In Vitro Chromosome aberrations in Chinese hamster ovary cells, and In Vitro unscheduled DNA synthesis assay in primary rat hepatocytes with technical methanearsonic acid. It should be noted that the test compound used in the submitted studies was methanearsonic acid (Caswell# 549A) instead of monosodium acid methanarsonate (CASWELL# 582) as indicated in the Data Package Record (Bean Sheet) of 7/26/91 received from the Registration Division (DP Barcode D166936 Rereg Case# 2395).

CONCLUSIONS AND RECOMMENDATIONS:

A. Salmonella/Mammalian-Microsome Plate Incorporation Mutagenicity Assay (Ames Test) With and Without Metabolic Activation With Methanearsonic Acid (MAA). MRID# 416519-02

Under the conditions of two independently performed Salmonella typhimurium/mammalian microsome plate incorporation assays, dose levels ranging from 667 to 10,000 μg/plate of methanearsonic acid did not induce a cytotoxic or mutagenic response in Salmonella typhimurium strains TA1535, TA1537, TA1538, TA98, or TA100, either in the presence or absence of microsome derived from Aroclor 1254-induced rat livers (S). Based on these findings, there is no evidence that methanearsonic acid is mutagenic at this dose range. This study is acceptable. A copy of the DER is attached.

B. In Vitro Chromosomal Aberration Assay in Chinese Hamster Ovary (CHO) cells with Methanearsonic Acid (MAA). MRID# 416519-03.1

Dose levels of methanearsonic acid from 625 to 5000 μg/mL +/-S9 in the initial trial and from 1250 to 10,000 μg/mL in the confirmatory assay did not induce a clastogenic effect in Chinese hamster ovary (CHO) cells harvested 10 hours post-treatment. In the confirmatory assay the percentage of cells with aberrations and aberrations/cell were slightly increased (not statistically significant) relative to negative and solvent controls at 10,000 μg/mL without S9-activation and at 5,000 and 10,000 μg/mL with S9 activation. Evidence of cytotoxicity on monolayer cultures was noted for non-activated doses of more than 5,000 μg/mL and S9-activated dose at 10,000 μg/mL. Thus, methanearsonic acid was adequately tested and it was negative under the conditions of this test system. This study is acceptable. A copy of the DER is attached.

C. L5178Y $TK^{+/-}$ Mouse Lymphoma Mutagenesis Assay with Methanearsonic Acid (MAA). MRID# 416519-04.

Methanearsonic acid was evaluated for the potential to induce forward mutations at the $TK^{+/-}$ locus in L5178Y mouse lymphoma cells in two different trials. Without S9 activation, dose levels from 300 to 4000 μg/mL were not mutagenic, but at levels of more than 5000 μg/mL they were severely cytotoxic. In the S9-activated trials, significant increase ($P \leq 0.05$) in the mutation frequency were obtained at 712 an 949 μg/mL (initial trial) and at 750 μg/mL (confirmatory trial). While the mutation frequencies were significantly increased, they ranged from 0.6 to 1.0 mutants per 10^4 survivors. Since these values fall within the generally accepted spontaneous mutation frequency range for mouse lymphoma cells (0.2-1.0 mutants/10^4 survivors), these results are not indicative of a mutagenic response. From the results of this study, methanearsonic acid was tested to cytotoxic levels but failed to produce convincing evidence of a mutagenic response. This study is acceptable. A copy of the DER is attached.

D. Unscheduled DNA Synthesis Assay in Rat Primary Hepatocytes with Methanearsonic Acid (MAA). MRID# 416519-05.

Methanearsonic acid did not induce unscheduled DNA synthesis (UDS) in primary rat hepatocytes at concentrations ranging from 10 to 750 µg/mL. At 1000 µg/mL (highest dose tested) it was cytotoxic. Methanearsonic acid was tested over an appropriate dose range, with appropriate control. Under the conditions of this assay the exposure to methanearsonic acid did not induce unscheduled DNA synthesis in primary rat hepatocytes. This study is acceptable. A copy of the DER is attached.

Attachments

DOC 920137

FINAL

009369

DATA EVALUATION REPORT

Methanearsonic Acid (MAA)

Study Type: Mutagenicity: Salmonella typhimurium/Mammalian Microsome Mutagenicity Assay

Prepared for:

Health Effects Division
Office of Pesticide Programs
Environmental Protection Agency
1921 Jefferson Davis Highway
Arlington, VA 22202

Prepared by:

Clement International Corporation
9300 Lee Highway
Fairfax, VA 22031-1207

Principal Reviewer Lynne T. Haber, Ph.D. Date 2/6/92

Independent Reviewer Nancy E. McCarroll, B.S. Date 2/6/92

QA/QC Manager Sharon Segal, Ph.D. Date 2/6/92

Contract Number: 68D10075
Work Assignment Number: 1-32
Clement Number: 91-120
Project Officer: James Scott

4

1

MUTAGENICITY STUDIES

EPA Reviewer: David Liem, Ph.D.
Review Section II
Toxicology Branch (II)/HED
Signature: David Liem
Date: 3/10/92

EPA Secondary Reviewer:
Byron T. Backus, Ph.D.
Review Section II
Toxicology Branch (II)/HED
Signature: Byron T. Backus
Date: 3/11/92

EPA Section Head: Clark Swentzel
Review Section II
Toxicology Branch (II)/HED
Signature: K. Clark Swentzel
Date: 3/12/92

DATA EVALUATION REPORT

STUDY TYPE: Mutagenicity: Salmonella typhimurium/mammalian microsome mutagenicity assay

EPA IDENTIFICATION Numbers:

Tox Chem. Number: 549A

MRID Number: 416519-02

TEST MATERIAL: Methanearsonic Acid (MAA)

SYNONYMS: None listed.

SPONSOR: Fermenta ASC Corporation, Mentor, OH

SPONSOR'S REPRESENTATIVE: Ricerca, Inc., Painesville, OH

STUDY NUMBER: T8471.501014

TESTING FACILITY: Microbiological Associates, Inc., Rockville, MD

TITLE OF REPORT: Salmonella/Mammalian-Microsome Plate Incorporation Mutagenicity Assay (Ames Test) With and Without Metabolic Activation With Methanearsonic Acid (MAA).

AUTHORS: Curren, R. D. and Schadly, M. B. (Microbiological Associates, Inc.); Chun, J. S. and Killeen, J. C. (Ricerca, Inc.)

REPORT ISSUED: August 24, 1989 (Microbiological Associates, Inc.); October 30, 1989 (Ricerca, Inc.)

NOTE: Report prepared by the sponsor's representative, Ricerca, Inc., is a summary of the laboratory report prepared by Microbiological Associates, Inc. The following review focuses on the data provided by Microbiological Associates, Inc.

CONCLUSIONS--EXECUTIVE SUMMARY: Under the conditions of two independently performed Salmonella typhimurium/mammalian microsome plate incorporation assays, doses ranging from 667 to 10,000 µg/plate of methanearsonic acid did not induce a cytotoxic or mutagenic response in S. typhimurium strains TA1535, TA1537, TA1538, TA98, or TA100, either in the absence or the presence of microsomes derived from Aroclor 1254-induced rat livers (S9). Based on these findings, it was concluded that methanearsonic acid was tested over an appropriate range of concentrations with no evidence of a mutagenic effect. The study, therefore, satisfies Guideline requirements for genetic effects Category I, Gene Mutations.

STUDY CLASSIFICATION: The study is acceptable.

A. MATERIALS:

1. Test Material: Methanearsonic Acid (MAA)

Description: White crystalline solid
Lot number: 107/84
Purity: 99.8%
Receipt date: March 13, 1989
Stability: Not reported
Contaminants: None listed
Solvent used: Deionized distilled water (DH_2O)
Other provided information: The test material was stored at room temperature and was protected from light. The frequency of dosing solution preparation was not reported. A frozen sample of the test material stock solution was shipped to Ricerca, Inc., for analytical determinations.

2. Control Materials:

Solvent/final concentration: Water/50µL per plate

Positive:

Nonactivation:

Sodium azide	1	µg/plate TA100, TA1535
2-Nitrofluorene	1	µg/plate TA98, TA1538
ICR 191	2	µg/plate TA1537

Activation:

2-Aminoanthracene (2-AA)	0.5	µg/plate all strains

3. Activation: S9 derived from male Sprague-Dawley

x Aroclor 1254	x induced	x rat	x liver
_____ phenobarbital	_____ noninduced	_____ mouse	_____ lung
_____ none		_____ hamster	_____ other
_____ other		_____ other	

The rat liver S9 was prepared by Microbiological Associates, Inc. Prior to use the S9 fraction was characterized for its ability to convert 7,12-dimethylbenzanthracene and 2-AA to mutagenic forms.

S9 mix composition:

Component	Volume/mL
DH_2O	0.56 mL
1.00 M Sodium phosphate buffer (pH 7.4)	0.10 mL
0.05 M Glucose 6-phosphate	0.10 mL
0.04 M NADP	0.10 mL
0.2 M $MgCl_2$/0.825 M KCl	0.04 mL
S9	0.10 mL (10% final)

4. Test Organism Used: S. typhimurium strains
_____ TA97 __x__ TA98 __x__ TA100 _____ TA102 _____ TA104
__x__ TA1535 __x__ TA1537 __x__ TA1538
list any others:

Test organisms were properly maintained: Yes.
Checked for appropriate genetic markers (rfa mutation, R factor): Yes.

5. Test Compound Concentrations Used:

(a) Preliminary cytotoxicity assay: Ten doses (10, 33, 67, 100, 333, 667, 1000, 3333, 6667, and 10,000 µg/plate) were evaluated with and without S9 activation in S. typhimurium strain TA100. A single plate was used per dose per condition.

(b) Mutation assays:

(1) Initial: Five doses (667, 1000, 3333, 6667, and 10,000 µg/plate) were evaluated in the presence and absence of S9 activation; all tester strains were used.

(2) Confirmatory: As above.

B. TEST PERFORMANCE:

1. Type of Salmonella assay:
__x__ Standard plate test
_____ Pre-incubation (_____) minutes
_____ "Prival" modification
_____ Spot test
_____ Other (describe)

2. Protocol:

(a) Preliminary cytotoxicity/mutation assays: Similar procedures were used for the preliminary cytotoxicity and the mutation assays.

47

Approximately 1-2 x 10^8 cells (0.1 mL of a 1-2 x 10^9 cells/mL late log phase culture) of the appropriate tester strain and 50 µL of the appropriate test material dose, solvent, or positive controls were added to tubes containing 2.5-mL volumes of molten top agar. Sufficient DH_2O was added to the top agar in the nonactivated tests to ensure that equivalent concentrations of amino acid supplements were available in the nonactivated and S9-activated tests. For the S9-activated tests, 0.5 mL of the appropriate S9-cofactor mix was added to 2 mL of the top agar. Tester strains and test and control solutions were added as described. The contents of the tubes were mixed, poured over Vogel-Bonner minimal medium E, and incubated at 37°C for ≈48 hours. At the end of incubation, plates were either scored immediately for revertant colonies or were refrigerated and subsequently counted. Means and standard deviations for the mutation test were determined from the counts of triplicate plates per strain, per dose, per condition.

(b) Sterility controls: The sterility of the highest test dose and the S9 mix were determined.

3. Evaluation Criteria:

(a) Assay validity: The assay was considered valid if it met the following criteria: (1) the presence of the appropriate genetic markers must be verified; (2) tester strain culture titers must be ≥0.6 x 10^9; (3) positive control values must show at least a tripling in the mean number of revertants for each strain; and (4) the test compound should be tested to 10 mg/plate, or at least one dose showing cytotoxicity. In addition, the spontaneous revertants for each strain should fall into the following range: TA98, 10-50; TA100, 80-240; TA1535, 5-45; TA1537, 3-21; and TA1538, 5-35.

(b) Positive response: The test material was considered positive if it caused a ≥2-fold increase in the mean number of revertants per plate of at least one strain, and the increase was dose-related. If the increase observed with strains TA1537 or TA1538 was less than three-fold, it must be confirmed in a repeat assay.

4. Protocol: See Appendix C for the Microbiological Associates, Inc. protocol. The Ricerca, Inc. protocol is not included in the appendix. The raw data was also included in the report.

C. REPORTED RESULTS

1. Analytical Determination: Ricerca, Inc. analyzed the test compound stock solution (200 mg/mL) and found it to be 98% ± 2% of the nominal concentration.

2. Preliminary Cytotoxicity Assay: Ten doses of the test material ranging from 10 to 10,000 µg/plate were evaluated with and without S9 activation using strain TA100. No compound precipitation was

was apparent at any of the nonactivated or S9-activated doses. The only evidence of cytotoxicity was a slight thinning of the background lawn of growth at the highest dose (10,000 µg/plate +/- S9). Based on these findings, the dose range selected for the nonactivated and S9-activated mutation assays was 667-10,000 µg/plate.

2. Mutation Assays: The study authors stated that due to unacceptable vehicle control values, unacceptable positive control values, or contamination, the initial assays with S. typhimurium TA98 (+/- S9) and TA1537 (+ S9), and the confirmatory assays with all five strains (+/- S9), were repeated. Data from the assays that were judged unacceptable were not presented. Representative results from the successfully completed phases of the nonactivated and S9-activated initial and confirmatory mutation assays with methanearsonic acid are presented in Tables 1 and 2, respectively. As shown in Table 1, the mean revertant colony counts for the majority of strains was lower than the corresponding solvent control at 10,000 µg/plate +/- S9. Similar slight reductions were seen at this level with and without S9 activation in the confirmatory assay (Table 2). Overall, the results suggest that 10,000 µg/plate +/- S9 may represent the highest subcytotoxic dose of methanearsonic acid. The slight variations in the number of revertant colonies per plate observed at lower levels were not dose-related, and probably resulted from normal plating variability.

   Exposure to graded doses of methanearsonic acid did not induce a mutagenic effect in any tester strain in either the presence or absence of S9 activation. In contrast, all strains responded to the appropriate nonactivated and S9-activated positive controls. From the overall findings, the study authors concluded that methanearsonic acid was not mutagenic in this test system.

D. REVIEWERS' DISCUSSION/CONCLUSIONS: We assess that the study authors' interpretation of the data was correct. Both in the absence and the presence of exogenous metabolic activation derived from rat liver microsomes, methanearsonic acid was assayed to 10,000 µg/plate but failed to induce a cytotoxic or mutagenic effect in a well-controlled study. In addition, the response of all tester strains to the appropriate direct-acting or promutagenic positive controls indicated that the assay had an adequate level of sensitivity to detect a mutagenesis response. It was concluded, therefore, that methanearsonic acid was not mutagenic in this microbial test system.

E. QUALITY ASSURANCE MEASURES: Was the test performed under GLP? Yes. A quality assurance statement was signed and dated August 28, 1989 (Microbiological Associates, Inc.).

TABLE 1: Representative Results of the Initial Salmonella typhimurium/Mammalian Microsome Mutation Assay with Methanearsonic Acid (MAA)

Substance	Dose/Plate	S9 Activation	Revertants per Plate of Bacterial Tester Strain[a] TA1535	TA1537	TA1538	TA98	TA100
Solvent Control							
Water	50 µL	-	14±5	7±2	8±1	14±4	169±7
	50 µL	+	19±2	7±2	8±1	24±2	191±17
Positive Controls							
Sodium azide	1 µg	-	735±41	--	--	--	953±71
ICR-191	2 µg	-	--	68±5	--	--	--
2-Nitrofluorene	1 µg	-	--	--	765±84	204±16	--
2-Aminoanthracene	0.5 µg	+	132±4	127±34	1069±4	1328±102	1203±44
Test Material							
Methanearsonic acid	6667 µg[b]	-	13±4	5±1	7±5	19±4	168±13
	10,000 µg	-	8±4	5±1	9±4	5±1	166±11
	6667 µg[b]	+	16±7	10±3	9±3	16±3	182±16
	10,000 µg	+	14±3	9±2	8±2	18±3	181±10

[a]Means and standard deviations of the counts from triplicate plates.

[b]Results for lower doses (667, 1000, and 3333 µg/plate) -/+ S9 did not suggest a mutagenic effect.

TABLE 2: Representative Results of the Confirmatory Salmonella typhimurium/Mammalian Microsome Mutation Assay with Methanearsonic Acid (MAA)

Substance	Dose/Plate	S9 Activation	Revertants per Plate of Bacterial Tester Strain[a] TA1535	TA1537	TA1538	TA98	TA100
Solvent Control							
Water	50 µL	-	16±3	7±2	7±3	46±11	157±22
	50 µL	+	14±5	6±1	7±3	19±2	169±11
Positive Controls							
Sodium azide	1 µg	-	713±15	--	--	--	907±24
ICR-191	2 µg	-	--	233±34	--	--	--
2-Nitrofluorene	1 µg	-	--	--	796±22	522±103	--
2-Aminoanthracene	0.5 µg	+	113±26	58±9	442±11	1000±171	713±48
Test Material							
Methanearsonic acid	6667 µg[b]	-	14±5	5±2	9±1	31±5	147±8
	10,000 µg	-	11±4	4±4	9±2	29±2	138±4
	6667 µg[b]	+	11±3	4±1	9±4	20±2	119±12
	10,000 µg	+	13±2	6±2	7±2	11±3	157±17

[a]Means and standard deviations of the counts from triplicate plates.

[b]Results for lower doses (667, 1000, and 3333 µg/plate) -/+ S9 did not suggest a mutagenic effect.

P 11

F. CBI APPENDICES: Appendix A, Materials and Methods, (Ricerca, Inc.) CBI pp. 9-13; Appendix B, Materials and Methods, (Microbiological Associates, Inc.) CBI pp. 60-67; and Appendix C, Protocol (Microbiological Associates, Inc.) CBI pp. 112-124.

APPENDIX A

MATERIALS AND METHODS
RICERCA, INC.
CBI pp. 9-13

Page_____ is not included in this copy.

Pages 11 through 15 are not included.
14 thru 18

The material not included contains the following type of information:

_____ Identity of product inert ingredients.

_____ Identity of product impurities.

_____ Description of the product manufacturing process.

_____ Description of quality control procedures.

_____ Identity of the source of product ingredients.

_____ Sales or other commercial/financial information.

_____ A draft product label.

_____ The product confidential statement of formula.

_____ Information about a pending registration action.

__✓__ FIFRA registration data.

_____ The document is a duplicate of page(s) _____.

_____ The document is not responsive to the request.

The information not included is generally considered confidential by product registrants. If you have any questions, please contact the individual who prepared the response to your request.

APPENDIX B

MATERIALS AND METHODS
MICROBIOLOGICAL ASSOCIATES, INC.
CBI pp. 60-67

Page_____ is not included in this copy.

Pages 17 through 24 are not included.

20 THRU 27

The material not included contains the following type of information:

_____ Identity of product inert ingredients.

_____ Identity of product impurities.

_____ Description of the product manufacturing process.

_____ Description of quality control procedures.

_____ Identity of the source of product ingredients.

_____ Sales or other commercial/financial information.

_____ A draft product label.

_____ The product confidential statement of formula.

_____ Information about a pending registration action.

✓ FIFRA registration data.

_____ The document is a duplicate of page(s) _____.

_____ The document is not responsive to the request.

The information not included is generally considered confidential by product registrants. If you have any questions, please contact the individual who prepared the response to your request.

APPENDIX C

PROTOCOL
MICROBIOLOGICAL ASSOCIATES, INC.
CBI pp. 112-124

Page_____ is not included in this copy.

Pages 26 through 37 are not included.
29 thru 40

The material not included contains the following type of information:

_____ Identity of product inert ingredients.

_____ Identity of product impurities.

_____ Description of the product manufacturing process.

_____ Description of quality control procedures.

_____ Identity of the source of product ingredients.

_____ Sales or other commercial/financial information.

_____ A draft product label.

_____ The product confidential statement of formula.

_____ Information about a pending registration action.

✓ FIFRA registration data.

_____ The document is a duplicate of page(s) _____.

_____ The document is not responsive to the request.

The information not included is generally considered confidential by product registrants. If you have any questions, please contact the individual who prepared the response to your request.

DOC920134

FINAL

003369

DATA EVALUATION REPORT

METHANEARSONIC ACID

Study Type: Mutagenicity: In Vitro Chromosome Aberrations in Chinese Hamster Ovary (CHO) Cells

Prepared for:

Health Effects Division
Office of Pesticide Programs
Environmental Protection Agency
1921 Jefferson Davis Highway
Arlington, VA 22202

Prepared by:

Clement International Corporation
9300 Lee Highway
Fairfax, VA 22031-1207

Principal Reviewer Nancy L. McCarroll Date 2-10-92
Nancy E. McCarroll, B.S.

Independent Reviewer Lynne T. Haber Date 2/10/92
Lynne T. Haber Ph.D.

QA/QC Manager Sharon Q. Segal Date 2/10/92
Sharon Segal, Ph.D.

Contract Number: 68D10075
Work Assignment Number: 1-32
Clement Number: 91-119
Project Officer: James Scott

41

1

MUTAGENICITY STUDIES

EPA Reviewer: David Liem, Ph.D. Signature: David Liem
Review Section II Date: 3/10/92
Toxicology Branch (II)/HED
EPA Secondary Reviewer: Byron T. Backus, Ph.D. Signature: Byron T. Backus
Review Section II Date: 3/11/92
Toxicology Branch (II)/HED
EPA Section Head: Clark Swentzel Signature: K. Clark Swentzel
Review Section II Date: 3/12/92
Toxicology Branch (II)/HED

DATA EVALUATION REPORT

STUDY TYPE: Mutagenicity: In vitro Chromosome aberrations in Chinese hamster ovary (CHO) cells

EPA IDENTIFICATION Numbers:

Tox Chem. Number: 549 A

MRID Number: 416519-03

TEST MATERIAL: Methanearsonic acid (MAA)

SYNONYMS: None provided

SPONSOR: Fermenta ASC Corporation, Mentor, OH

SPONSOR'S REPRESENTATIVE: Ricerca, Inc., Painesville, OH

STUDY NUMBER: T8471.337001

TESTING FACILITY: Microbiological Associates, Inc., Rockville, MD

TITLE OF REPORT: In Vitro Chromosomal Aberration Assay in Chinese Hamster Ovary (CHO) cells with Methanearsonic Acid (MAA).

AUTHORS: Putman, D. L. and Morris, M.J. (Microbiological Associates, Inc.); Chun, J. S., and Killeen, J. C. (Ricerca, Inc.)

REPORT ISSUED: July 20, 1989 (Microbiological Associates, Inc.); October 31, 1989 (Ricerca, Inc.)

NOTE: Report prepared by the sponsor's representative, Ricerca, Inc., is a summary of the laboratory report prepared by Microbiological Associates, Inc. The following review focuses on the data provided by Microbiological Associates, Inc.

CONCLUSIONS-EXECUTIVE SUMMARY: Doses of methanearsonic acid ranging from 625 to 5000 µg/mL +/-S9 in the initial trial and from 1250 to 10,000 µg/mL +/-S9 in the confirmatory assay did not induce a clastogenic effect in Chinese hamster ovary (CHO) cells harvested 10 hours posttreatment. Evidence of cytotoxicity on monolayer cultures was reported for nonactivated doses ≥5000 µg/mL and S9-activated 10,000 µg/mL. We conclude, therefore, that methanearsonic was adequately tested and found to be negative in this test system. The study satisfies Guideline requirements for genetic effects, Category II, Structural Chromosome Aberrations.

STUDY CLASSIFICATION: The study is acceptable.

A. MATERIALS:

1. Test Material: Methanearsonic acid (T-168-2)

Description: White crystalline solid
Identification No: SDS-37161; Lot number: 107/84
Purity: 99.8%
Receipt date: March 13, 1989 (sample I) and April 3, 1989 (sample II)
Stability: Reported to be stable for the duration of the study.
Contaminants: None listed
Solvent used: Distilled water (DH_2O)
Other provided information: Stored in the dark at room temperature in a tightly closed container. Samples of the stock dosing solution were frozen and shipped to Ricerca, Inc. for concentration analysis.

2. Control Materials:

Negative: Untreated cells grown in McCoy's 5A medium supplemented with 10% fetal bovine serum (FBS), 2 mM L-glutamine, and antibiotics.

Solvent/final concentration: DH_2O/50 µL

Positive:

Nonactivation: Triethylenemelamine (TEM) was prepared in DH_2O to yield a final concentration of 0.5 µg/mL.

Activation: Cyclophosphamide (CP) was prepared in DH_2O to yield a final concentration of 50 µg/mL.

3. Activation: S9 derived from adult male Sprague Dawley

x Aroclor 1254	x induced	x rat	x liver
_____ phenobarbital	_____ noninduced	_____ mouse	_____ lung
_____ none		_____ hamster	_____ other
_____ other		_____ other	

The S9 homogenate was prepared by the performing laboratory. Prior to use, the S9 fraction was characterized for its ability to metabolize 2-aminoanthracene and 7,12-dimethylbenz(a)anthracene to mutagenic forms in Salmonella typhimurium TA100.

S9 mix composition:

Component	Concentration/mL of Growth Medium
NADP	1.4 mg
Isocitric acid	2.7 mg
S9	15.0 µL

4. Test Compound Concentrations Used:

(a) Preliminary cytotoxicity assay: The nine concentrations of the test material used were 0.5, 1.5, 5, 15, 50, 150, 500, 1510, and 5000 µg/mL (with and without S9 activation).

(b) Cytogenetic assay:

(1) Nonactivated conditions:

- Initial trial: 625, 1250, 2500, and 5000 µg/mL (10-hour cell harvest).
- Confirmatory trial: 1250, 2500, 5000, and 10,000 µg/mL (10-hour cell harvest).

(2) S9-activated conditions: As above.

5. Test Cells: The Chinese hamster ovary cells (CHO-K_1) used in this assay were obtained from the American Type Culture Collection, Rockville, MD. Prior to use, the CHO cells were grown for 16 to 24 hours in McCoy's 5A complete medium.

Properly maintained? Yes.

Cell line or strain periodically checked for mycoplasma contamination? Not reported.

Cell line or strain periodically checked for karyotype stability? Not reported.

B. TEST PERFORMANCE:

1. Cell Treatments:

(a) Cells exposed to test compound for:
8 hours (nonactivated) 2 hours (activated)

(b) Cells exposed to positive controls for:
8 hours (nonactivated) 2 hours (activated)

(c) Cells exposed to negative and/or solvent controls for:
8 hours (nonactivated) 2 hours (activated)

2. Protocol:

(a) Preliminary cytotoxicity assay: Prepared cultures, seeded at $5x10^5$ cells/flask, were exposed with or without S9 activation to nine concentrations of the test material (0.5 to 5000 µg/mL) and the solvent control (DH_2O).

In the nonactivated system, cells were exposed for 6 hours to the test material; 50 µL of 1 mM BrdU were added to the cultures 2 hours after initiation. After exposure, cells were washed, refed with complete medium containing BrdU (0.01 mM), and reincubated for a total of 24 hours.

In the S9-activated system, cultures were exposed for 2 hours. Following treatment, cells were washed, refed, and reincubated as described for the nonactivated cultures.

Two hours prior to the end of incubation, colcemid, at a final concentration of 0.1 µg/mL, was added to each culture. Metaphases were harvested, fixed, and stained with the modified fluorescent-plus Giemsa technique of Perry and Wolff.[1] One hundred cells from each dose group were examined for the percentage of first (M_1), second (M_2), and third (M_3) division metaphases. Mitotic indices (MIs) were determined by counting the number of mitotic cells in a population of 500 scored cells. Based on these results, the doses and harvest time for the cytogenetic assay were established.

(b) Cytogenetic assay:

(1) Treatment: Prepared cultures (in duplicate), seeded at $5x10^5$ cells, were exposed to the selected test material doses, the solvent control (DH_2O), or positive controls (0.5 µg/mL TEM -S9 and 50 µg/mL CP +S9).

In the nonactivated system, cells were dosed for 8 hours. Cultures were washed, refed medium containing 0.1 µg/mL colcemid, and reincubated for 2 hours. Under S9-activated conditions, cells were exposed for 2 hours, washed, refed culture medium, and incubated for an additional 6 hours. Colcemid was added 2 hours before the cultures were harvested.

Metaphase cells were collected and fixed. Slides were stained with 5% Giemsa and coded.

[1]Perry, P. and Wolff, S. (1974). New Giemsa method for the differential staining of sister chromatids. Nature 251:156-158.

(2) Metaphase analysis: One hundred metaphase cells per group (50/culture) were scored for chromosome aberrations. Chromatid and chromosome gaps were counted but not included in the final analysis. MIs were calculated.

(3) Statistical methods: The data were evaluated for statistical significance at p values of 0.05 and 0.01 by Fisher's exact test.

4. Evaluation Criteria:

(a) Assay validity: The assay was considered valid if the percent of cells with aberrations in the untreated and solvent control did not exceed 6% and the number of cells with aberrations in the positive control was significantly higher ($p \leq 0.05$) than in the solvent control.

(b) Positive response: The test material was considered positive if it caused a significant and dose-related increase in the percentage of cells with aberrations relative to the solvent control.

C. REPORTED RESULTS:

1. Preliminary Cytotoxicity Assay: The study authors stated that the test material was soluble at all levels and that the osmolality of the highest assayed concentration (5000 µg/mL) was 335 mOsm/kg. As shown in Table 1, the MIs for the highest dose with and without S9 activation were reduced compared to the solvent control values (~58% reduction -S9; ~36% reduction +S9). MIs for the remaining nonactivated and S9-activated levels were generally comparable to the control. The data further show that methanearsonic acid had no effect on cell cycle kinetics. Based on these findings, a 10-hour cell harvest was selected to evaluate 625, 1250, 2500, and 5000 µg/mL of the test material in the initial nonactivated and S9-activated cytogenetic assays.

2. Cytogenetic Assays: The study authors stated that the first cytogenetic assay was terminated because of difficulties in adjusting the pH of the test material. Prior to performance of the repeat initial test, the stock concentration (500 mg/mL) was adjusted to pH 6.5. Microscopic examination of the monolayer cultures at harvest revealed slight cytotoxic effects in the two highest nonactivated groups (2500 and 5000 µg/mL) and the highest S9-activated group (5000 µg/mL). As shown in Table 2, the microscopic evidence of cytotoxicity was not clearly reflected as reduced MIs at 5000 µg/mL +/-S9. Similarly, neither the nonactivated nor the S9-activated test levels caused an appreciable increase in the percentage of cells with aberrations or the number of aberrations per cell. For the confirmatory assay, the maximum concentration was increased to 10,000 µg/mL +/-S9 to ensure that a full range of doses up to an insoluble level were tested. The pH of the stock solution (1000 mg/mL) was adjusted to pH 6. The test material was partially insoluble at this concentration but all doses

TABLE 1. Representative Results from the Preliminary Cytotoxicity Assay with Methanearsonic Acid

Substance	Dose/mL	S9-activation	% Cells[a]			Mitotic[b] Index (%)
			M_1	M_2	M_3	
Solvent Control						
Distilled water	50 µL	-	2	98	0	6.6
	50 µL	+	2	98	0	5.6
Test Material						
Methanearsonic acid	1510 µg/mL	-	3	97	0	5.4
	5000 µg/mL	-	5	95	0	2.8
	1510 µg/mL	+	5	95	0	5.8
	5000 µg/mL	+	8	92	0	3.6

[a]Percent cells in first (M_1), second (M_2), or third (M_3) division.
[b]Percent of metaphase cells in a population of 500 scored cells.
[c]Results for lower doses (0.5, 1.5, 5, 15, 50, 150, and 500 µg/mL +/-S9) did not suggest a cytotoxic effect.

TABLE 2. Representative Results of the Initial CHO Cell *in vitro* Cytogenetic Assays with Methanearsonic Acid Following a 10-Hour Nonactivated and S9-Activated Cell Harvest

Substance	Dose/mL	S9-Activation	No. of Cells Scored	Mitotic Index[a] (%)	Cells with Aberrations[a,b] (%)	Aberrations per Cell ± Standard Deviations[a,b]	Biologically Significant Aberrations (No/Type)[c]
Negative Control							
Untreated cells	--	-	100	3.7	0	0.00±0.000	--
	--	+	100	5.2	1	0.01±0.100	1TB
Solvent Control							
Distilled water	50 µL	-	100	2.3	0	0.00±0.000	--
	50 µL	+	100	4.7	1	0.01±0.100	1TB
Positive Control							
Triethylenemelamine	0.5 µg/mL	-	100	1.1	11*	0.13±0.393	12TB; 1TE
Cyclophosphamide	50 µg/mL	+	100	2.2	11*	0.13±0.393	8TB; 2TE; 2D; 1R
Test Material							
Methanearsonic acid	5000 µg/mL[c]	-	100	2.5	2	0.02±0.141	2TB
	5000 µg/mL[c]	+	100	6.0	0	0.00±0.000	--

[a]Results from duplicate cultures, 50 cells/culture were scored.
[b]Gaps excluded
[c]Abbreviations used:

TB = Chromatid break D = Dicentric chromosome
TE = Chromatid exchange R = Ring

[c]Slight cytotoxic effects on monolayer cells reported at this dose. Results for lower treatment groups (625, 1250, and 2500 µg/mL +/-S9) were not significantly higher than the appropriate solvent control group.

*Significantly higher than the solvent control ($p \leq 0.01$) by Fisher's exact test.

48
8

used in the confirmatory assay were reported to be soluble in culture medium. Microscopic examinations indicated that 5000 and 10,000 µg/mL -S9 and 10,000 µg/mL +S9 induced slight cytotoxic effects on the monolayer cultures. Representative results from the confirmatory trial are presented in Table 3. The highest nonactivated dose caused a moderate reduction in the MIs while 10,000 µg/mL +S9 had no effect on the percentage of mitotic cells. The percentage of cells with aberrations and the aberrations per cell were slightly increased compared to the solvent and negative control at the majority of nonactivated levels; the increases were, however, not significant. Although complex aberrations (i.e., 1 chromatid exchange at 10,000 µg/mL, 1 ring at 5000 µg/mL, 1 dicentric at 2500 µg/mL, and 2 dicentrics at 1250 µg/mL) were scored at the nonactivated doses, the relevance, if any, of these findings was diminished by the presence of similar aberrations at comparable frequencies in the nonactivated control cultures.

In the presence of S9 activation, nonsignificant increases in the percentage of cells with aberrations as well as slight elevations in aberrations per cell were seen at 5000 and 10,000 µg/mL. As noted for the nonactivated confirmatory assay, the occurrence of rare aberrations (i.e., 1 dicentric at 10,000 µg/mL and 3 dicentrics at 5,000 µg/mL) in the test groups should not be considered an effect of treatment, since dicentrics were also scored in the S9-activated negative and solvent control groups. In both trials, the positive controls (TEM at 0.5 µg/mL -S9) and 50 µg/mL CP +S9) induced significant ($p<0.01$) increases in the percentage of cells with aberrations and marked increases in the frequency of aberrations per cell.

From the overall results, the study authors concluded that methanearsonic acid was not clastogenic in CHO cells.

3. Analytical Determinations: Results from the analysis of the 500 mg/mL stock solution indicated that the submitted samples contained 98% of the nominal concentration.

D. REVIEWERS' DISCUSSION/CONCLUSIONS: We assess that the study authors' interpretation of the data was correct. We discounted the presence of rare chromatid and chromosome aberrations in the nonactivated and S9-activated treatment groups of the confirmatory test as indicative of a clastogenic response. The types and frequency of these aberrations were comparable in the control groups and also the findings from the first trial were clearly negative.

We conclude, therefore, that methanearsonic acid was assayed to an insoluble level (10,000 µg/mL) induced a slight cytotoxic effect on the cells at high concentrations (5000 and 10,000 µg/mL -S9 and 10,000 µg/mL +S9), but failed to elicit a clastogenic response. Additionally, the sensitivity of the test system to detect clastogenesis was adequately demonstrated under nonactivated and S9-activated conditions in both the initial and confirmatory trials.

TABLE 3. Representative Results of the Confirmatory CHO Cell *in vitro* Cytogenetic Assays with Methanearsonic Acid Following a 10-Hour Nonactivated and S9-Activated Cell Harvest

Substance	Dose/mL	S9-Activation	No. of Cells Scored	Mitotic Index[a] (%)	Cells with Aberrations[a,b] (%)	Aberrations per Cell ± Standard Deviations[a,b]	Biologically Significant Aberrations (No/Type)[c]
Negative Control							
Untreated cells	--	-	100	3.9	4	0.050±0.261	1TE; 2SB; 2D
	--	+	100	6.0	5	0.050±0.219	2SB; 2D; 1R
Solvent Control							
Distilled water	50 µL	-	100	4.3	3	0.040±0.243	2TB; 1SB; 1D
	50 µL	+	100	6.5	3	0.030±0.171	2SB; 1D
Positive Control							
Triethylenemelamine	0.5 µg/mL	-	100	1.6	28*	0.400±0.725	28TB; 7TE; 5SB
Cyclophosphamide	50 µg/mL	+	100	3.4	18*	0.210±0.478	8TB; 4TE; 8SB; 1R
Test Material							
Methanearsonic acid	5000 µg/mL[c,d]	-	100	4.5	5	0.050±0.219	4TB; 1R
	10,000 µg/mL[d]	-	100	2.1	7	0.120±0.518	7TB; 1TE; 4SB
	5000 µg/mL[c]	+	100	6.2	6	0.060±0.239	1TB; 2SB; 3D
	10,000 µg/mL[d]	+	100	6.4	7	0.080±0.307	2TB; 5SB; 1D

[a]Results from duplicate cultures, 50 cells/culture were scored.
[b]Gaps excluded.
[c]Abbreviations used:

TE = Chromatid exchange R = Ring
SB = Chromosome break TB = Chromatid break
D = Dicentric chromosome

[c]Results for lower doses (1250 and 2500 µg/mL +/-S9) were not significantly higher than the appropriate solvent control group.
[d]Slight cytotoxic effects on the monolayers were reported at this level.

*Significantly higher than the control ($p \leq 0.02$) by Fisher's exact test.

50
10

E. QUALITY ASSURANCE MEASURES: Was the test performed under GLPs? Yes. (A quality assurance statement was signed and dated July 21, 1989).

F. CBI APPENDICES: Appendix A, Materials and Methods, (Ricerca, Inc.) CBI pp. 9-13; Appendix B, Materials and Methods, (Microbiological Associates, Inc.) CBI pp. 52-55; and Appendix C, Protocol (Microbiological Associates, Inc.) CBI pp. 73-83.

APPENDIX A

MATERIALS AND METHODS
RICERCA, INC.
CBI pp. 9-13

009369

Page_____ is not included in this copy.

Pages 13 through 17 are not included.

53 thru 57

The material not included contains the following type of information:

_____ Identity of product inert ingredients.

_____ Identity of product impurities.

_____ Description of the product manufacturing process.

_____ Description of quality control procedures.

_____ Identity of the source of product ingredients.

_____ Sales or other commercial/financial information.

_____ A draft product label.

_____ The product confidential statement of formula.

_____ Information about a pending registration action.

✓ FIFRA registration data.

_____ The document is a duplicate of page(s) _____.

_____ The document is not responsive to the request.

The information not included is generally considered confidential by product registrants. If you have any questions, please contact the individual who prepared the response to your request.

APPENDIX B

MATERIALS AND METHODS
MICROBIOLOGICAL ASSOCIATES, INC.
CBI pp. 52-55

009369

Page_____ is not included in this copy.

Pages 19 through 22 are not included.
59 Thru 62

The material not included contains the following type of information:

_____ Identity of product inert ingredients.

_____ Identity of product impurities.

_____ Description of the product manufacturing process.

_____ Description of quality control procedures.

_____ Identity of the source of product ingredients.

_____ Sales or other commercial/financial information.

_____ A draft product label.

_____ The product confidential statement of formula.

_____ Information about a pending registration action.

✓ FIFRA registration data.

_____ The document is a duplicate of page(s) _____.

_____ The document is not responsive to the request.

The information not included is generally considered confidential by product registrants. If you have any questions, please contact the individual who prepared the response to your request.

APPENDIX C

PROTOCOL
MICROBIOLOGICAL ASSOCIATES, INC.
CBI pp. 73-83

TOXR 009369

Page ____ is not included in this copy.

Pages 24 through 34 are not included.

64 thru 74

The material not included contains the following type of information:

____ Identity of product inert ingredients.

____ Identity of product impurities.

____ Description of the product manufacturing process.

____ Description of quality control procedures.

____ Identity of the source of product ingredients.

____ Sales or other commercial/financial information.

____ A draft product label.

____ The product confidential statement of formula.

____ Information about a pending registration action.

✓ ____ FIFRA registration data.

____ The document is a duplicate of page(s) _____.

____ The document is not responsive to the request.

The information not included is generally considered confidential by product registrants. If you have any questions, please contact the individual who prepared the response to your request.

DOC 920135

FINAL

009369

DATA EVALUATION REPORT

METHANEARSONIC ACID

Study Type: Mutagenicity: Gene Mutation in Cultured Mammalian Cells (Mouse Lymphoma Cells)

Prepared for:

Health Effects Division
Office of Pesticide Programs
Environmental Protection Agency
1921 Jefferson Davis Highway
Arlington, VA 22202

Prepared by

Clement International Corporation
9300 Lee Highway
Fairfax, VA 22031-1207

Principal Reviewer Nancy E. McCarroll, B.S Date 2-10-92

Independent Reviewer Lynne T. Haber, Ph.D. Date 2/10/92

QA/QC Manager Sharon Segal, Ph.D. Date 2/10/92

Contract Number: 68D10075
Work Assignment Number: 1-32
Clement Number: 91-118
Project Officer: James Scott

75

1

GUIDELINE SERIES 84: MUTAGENICITY
MAMMALIAN CELLS IN CULTURE GENE MUTATIONS

EPA Reviewer: David Liem, Ph.D. Signature: David Liem
Review Section II, Toxicology Date: 3/10/92
Branch (II)/HED
EPA Secondary Reviewer: Byron T. Backus, Ph.D. Signature: Byron T. Backus
Review Section II, Toxicology Date: 3/11/92
Branch (II)/HED
EPA Section Head: Clark Swentzel Signature: K. Clark Swentzel
Review Section II, Toxicology Date: 3/12/92
Branch (II)/HED

DATA EVALUATION REPORT

STUDY TYPE: Mutagenicity: Gene mutation in cultured mammalian cells (mouse lymphoma cells)

EPA IDENTIFICATION Numbers:

Tox Chem. Number: 549A

MRID Number: 416519-04

TEST MATERIAL: Methanearsonic acid (MAA)

SYNONYMS: None provided

SPONSOR: Fermenta ASC Corporation, Mentor, OH

SPONSOR'S REPRESENTATIVE: Ricerca, Inc., Painesville, OH

STUDY NUMBER: T8471.701020

TESTING FACILITY: Microbiological Associates, Inc., Rockville, MD

TITLE OF REPORT: L5178Y $TK^{+/-}$ Mouse Lymphoma Mutagenesis Assay with Methanearsonic Acid

AUTHORS: J. W. Harbell (Microbiological Associates, Inc.); Chun, J.S., and Killeen, J.C. (Ricerca, Inc.)

REPORT ISSUED: August 28, 1989 (Microbiological Associates, Inc.); December 7, 1989 (Ricerca, Inc.)

NOTE: Report prepared by the sponsor's representative, Ricerca, Inc., is a summary of the laboratory report prepared by Microbiological Associates, Inc. The following review focuses on the data provided by Microbiological Associates, Inc.

76
2

CONCLUSIONS-EXECUTIVE SUMMARY: Methanearsonic acid was evaluated for the potential to induce forward mutations at the $TK^{+/-}$ locus in L5178Y mouse lymphoma cells in two independently performed trials. Without S9 activation, doses ranging from 300 to 4000 µg/mL were not mutagenic; levels ≥5000 µg/mL were severely cytotoxic. In the S9-activated trials, significant increases ($p<0.05$) in the mutation frequency (MF) were obtained at 712 and 949 µg/mL (trial 1) and 750 µg/mL (trial 2). However, while the MFs were significantly increased, compared to concurrent solvent control values, they ranged from 0.6 to 1.0 mutants per 10^4 survivors. We assess that since these values fell within the generally accepted spontaneous MF range for mouse lymphoma cells (i.e., 15-110 mutants/10^6 surviving cells)[1], the results are not indicative of a mutagenic response. Based on the overall results, it was concluded that methanearsonic acid was tested to cytotoxic levels but failed to produce convincing evidence of a mutagenic response. The study satisfies Guideline requirements for genetic effects Category I, Gene Mutations.

STUDY CLASSIFICATION: The study is acceptable.

A. MATERIALS:

1. Test Material: Methanearsonic acid (T-168-2)

Description: White, crystalline solid
Identification No.: SDS-37161; Lot no. 107/84
Purity: 99.8%
Receipt date: March 13, 1989
Stability: Reported to be stable for the duration of the study. (See Data Evaluation Record 91-119).
Contaminants: None listed
Solvent used: Deionized water (DH_2O).
Other provided information: The test material was stored at room temperature, protected from light. Samples of the stock dosing solution were frozen and shipped to Ricerca, Inc. for concentration analysis.

2. Control Materials:

Negative: None

Solvent/final concentration:

Test material: DH_2O

Positive controls: Dimethyl sulfoxide (DMSO); final concentration reported to be a noncytotoxic level.

[1]Caspary, W.J., Lee, Y.J., Poulton, S., Myhr, B.C., Mitchell, A.D., Rudd, C.J. (1988). Evaluation of the L5178Y mouse lymphoma cell mutagenesis assay: Quality-control guidelines and response categories. Environ. Mol. Mutagen. 12:19-36.

Positive: Nonactivation (concentrations, solvent): Ethyl methanesulfonate (EMS) was prepared in DMSO to yield final concentrations of 0.25 and 0.50 µL/mL.

Activation (concentrations, solvent): 7,12-Dimethylbenz(a)anthracene (DMBA) was prepared in DMSO to yield final concentrations of 2.5 and 5.0 µg/mL.

3. Activation: S9 derived from adult male Sprague-Dawley

x Aroclor 1254	x induced	x rat	x liver
_____ phenobarbital	_____ noninduced	_____ mouse	_____ lung
_____ none		_____ hamster	_____ other
_____ other		_____ other	

The S9 liver homogenate was prepared by the performing laboratory. Prior to use, the S9 fraction was characterized for its ability to convert 2-aminoanthracene and DMBA to mutagenic forms.

S9 mix composition:

Component	Concentration/mL of S9 mix
NADP	6.0 mg
Isocitric acid	11.25 mg
S9 homogenate	0.25 mL

4. Test Cells: Mammalian cells in culture

x mouse lymphoma L5178Y cells
_____ Chinese hamster ovary (CHO) cells
_____ V79 cells (Chinese hamster lung fibroblasts)
_____ other (list):

Properly maintained? Yes.
Periodically checked for mycoplasma contamination? Not reported.
Periodically checked for karyotype stability? Not reported.
Periodically "cleansed" against high spontaneous background? Yes.

5. Locus Examined:

x thymidine kinase (TK)
selection agent: (give concentration)
_____ bromodeoxyuridine (BrdU)
_____ fluorodeoxyuridine (FdU)
4 µg/mL trifluorothymidine (TFT)

_____ hypoxanthine-guanine-phosphoribosyl transferase (HGPRT)
Selection agent: (give concentration)
_____ 8-azaguanine (8-AG)
_____ 6-thioguanine (6-TG)

_____ Na^+/K^+ATPase
Selection agent: (give concentration)
_____ ouabain

_____ other (locus and/or selection agent; give details):

78
4

6. Test Compound Concentrations Used:

(a) Cytotoxicity assay: Six doses (0.1, 1, 10, 100, 1000, and 4000 µg/mL) were evaluated in the presence and absence of S9 activation.

(b) Mutation assay:

(1) Nonactivated conditions:

Initial assay: 300, 400, 534, 712, 949, 1266, 1688, 2250, 3000, and 4000 µg/mL (all levels cloned).

Confirmatory assay: 2000, 3000, 4000, 5000, and 6000 µg/mL (2000, 3000, and 4000 µg/mL were cloned).

(2) S9-activated conditions:

Initial assay: 71, 95, 127, 169, 225, 300, 400, 534, 712, 949, 1266, and 1688 µg/mL (doses ≤949 µg/mL were cloned).

Confirmatory assay: 200, 350, 500, 650, 750, 850, and 950 µg/mL (doses ≤750 µg/mL were cloned).

B. TEST PERFORMANCE:

1. Cell Treatments:

(a) Cells exposed to test compound for:
__4__ hours (nonactivated) __4__ hours (activated)

(b) Cells exposed to positive controls for:
__4__ hours (nonactivated) __4__ hours (activated)

(c) Cells exposed to negative and/or solvent controls for:
__4__ hours (nonactivated) __4__ hours (activated)

(d) After washing, cells cultured for __2__ days (expression period) before cell selection

(e) After expression, cells cultured for __10 to 12__ days in selection medium to determine numbers of mutants and for __10 to 12__ days without selection medium to determine cloning efficiency.

2. Statistical Methods: The data were evaluated for statistical significance at $p \leq 0.05$ by the Kastenbaum and Bowman tables.

3. Evaluation Criteria:

(a) Assay validity: For the assay to be considered valid, the following criteria must be satisfied: (1) the cloning efficiency (CE) of the solvent control must exceed 50%; (2) the mutation

frequency (MF) of the solvent control must be between 0.2 and 1.0 mutant colonies/10^4 survivors; and (3) the MF of the positive controls must be ≥2-fold higher than the corresponding solvent control value.

b. Positive response: The test material was considered positive if it induced a reproducible dose-related increase in the MF that exceeded 2 times the MF of the solvent control at one or more doses with ≥10% total survival.

4. Protocol: See Appendix C.

C. REPORTED RESULTS:

1. Cytotoxicity Assays: Dose solution for the cytotoxicity assay was governed by the solubility properties of the test material; the highest soluble concentration that could be prepared in DH_2O was reported to be 400 mg/mL. The study authors further stated that the pH of the 1000- and 4000-µg/mL doses was adjusted to neutrality with NaOH.

In the nonactivated phase of testing, relative suspension growth (RSG) at 1000 and 4000 µg/mL of the test material was 79% and 35%, respectively. No cytotoxicity was seen at nonactivated doses of 0.1, 1, 10, or 100 µg/mL. In the presence of S9 activation, cytotoxicity was more severe at the two highest levels; RSG was 26% at 1000 µg/mL and no cells survived exposure to 4000 µg/mL +S9. For the remaining levels, cytotoxicity was not apparent. Based on these results, doses ranging from 300 to 4000 µg/mL were evaluated in the nonactivated mutation assay; under S9-activated conditions, cells were exposed to a concentration range of 71 to 1688 µg/mL.

2. Mutation Assays:

(a) Nonactivated conditions: The first nonactivated assay was aborted because of a technical error. Representative results presented in Table 1 are from the successfully completed initial and confirmatory assays. In the initial trial, RSG at the two highest doses (3000 and 4000 µg/mL) was 48 and 45%, respectively. For the remaining levels (300 to 2250 µg/mL), RSG was ≥64%. Over the entire range of test material concentrations, the MFs were either comparable or slightly lower than the solvent control value. Similarly, mutant colony counts at all doses were lower than the solvent control. The assay was repeated with 2000, 3000, 4000, 5000, and 6000 µg/mL of the test material. To achieve the 5000- and 6000-µg/mL concentrations, the volume of test material added to the treatment medium was increased to 250 µL. These levels proved to be severely cytotoxic and were not cloned. Results from the plated doses (2000, 3000, and 4000 µg/mL) were in agreement with the earlier findings and indicated that methanearsonic was not mutagenic in the absence of S9 activation.

TABLE 1. Representative Results of the Nonactivated Mouse Lymphoma Forward Mutation Assays with Methanearsonic Acid

Substance	Dose/mL	Percent Relative Suspension Growth	Mutant Colonies[a] ±S.D.	Viable Colonies[a] ±S.D.	Percent Relative Cloning Efficiency[a]	Percent Relative Total Growth[a]	Mutation Frequency per 10^4 survivors[b]
Solvent Control							
Deionized water	--	100[c]	76±9	153±12	100	100	1.0
(Test Material)		100[d]	48±7	168±5	100	100	0.6
Dimethyl sulfoxide	--	100[c]	61	162	100	100	0.8
(Positive Controls)		100[d]	51	167	100	100	0.6
Positive Control[e]							
Ethylmethane sulfonate	0.25 µL	73[c]	225±12	132±6	82	60	3.4
	0.25 µL	72[d]	260±31	144±5[f]	86	62	3.6
Test Material							
Methanearsonic acid	4000 µg[g]	45[c]	51±5	162±12	106	48	0.6
	4000 µg[g,h]	25[d]	52	161	96	23	0.7

[a]Means and standard deviations from the counts of triplicate plates from single cultures. Values without standard deviations were from duplicate cultures (3 plates/culture) that were reported separately by the study authors. Presented average values were calculated by our reviewers.

[b]Mutation Frequency (MF) = $\frac{\text{Mutant Colonies}}{\text{Viable Colonies}} \times 2$.

[c]Results from the initial trial.

[d]Results from the confirmatory trial.

[e]Two levels of the positive control were assayed; results from the lower dose were selected as representative.

[f]Mean and standard deviation from the counts of duplicate plates.

[g]Findings for lower doses (300, 400, 534, 712, 949, 1266, 1688, 2250, and 3000 µg/mL--initial trial and 2000 and 3000 µg/mL--confirmatory trial) did not suggest a mutagenic effect.

[h]Higher doses (5000 and 6000 µg/mL) were too cytotoxic to clone.

(b) S9-Activated conditions: Doses evaluated in the initial S9-activated assay ranged from 71 to 1688 µg/mL. RSG for the two highest concentrations (1266 and 1688 µg/mL) was ≤6%; the cells were, therefore, not plated. RSG immediately after treatment with 71 to 949 µg/mL was dose-related and ranged from 18% at the highest plated level (949 µg/mL) to 98% at 95 µg/mL; at the lowest plated level (71 µg/mL), RSG was 94%. As shown in Table 2, the MFs at 712 and 949 µg/mL were significantly increased ($p<0.05$) compared to concurrent solvent control MF. However, the significantly increased MFs (0.8 mutants/10^4 survivors at 949 µg/mL and 0.6 mutants/10^4 survivors at 712 µg/mL)were well within the generally accepted spontaneous MF range for mouse lymphoma cells (i.e., 15-110 mutants/10^6 survivors)[2] as well as the acceptable background range of the performing laboratory for solvent control cultures (0.2 to 1.0 mutants/ 10^4 survivors). Results for the remaining treatment levels did not suggest a mutagenic response. Mutant colony size distribution analysis performed on mutant colonies from the 949-µg/mL dose group showed an increased proportion of small mutant colonies compared to the mutant colony sizes in the solvent control group. The study authors stated, however, that the relatively low number of mutant colonies on control and treatment plates limited the interpretation of this finding. Based on the significant MFs observed at two levels, the study authors concluded that methanearsonic acid induced an equivocal response and, therefore, repeated the assay with a narrower range of test doses (200, 350, 500, 650, 750, 850, and 950 µg/mL). An insufficient number of cells (RSG≤7%) survived exposure to 850 and 950 µg/mL; these cultures were discarded. Data from the highest plated concentration (750 µg/mL) showed that while the number of mutant colonies was only slightly higher than the concurrent solvent control, the MF was significantly increased ($p<0.05$). As previously observed for the initial trial, the significantly increased MF (1.0 mutant colony/10^4 survivors) was also within the spontaneous MF range for this cell line. Results for the remaining concentrations did not suggest a mutagenic response.

3. Analytical Determinations: Results from the analysis of the 400 mg/mL stock solution indicated that the submitted samples contained 107±1% of the nominal concentration.

The study authors from the performing laboratory made the following statements:

"The results indicate that, under the condition of these mutagenesis tests, test article T-168-2 was negative in the absence of exogenous metabolic activation in both the initial and the confirmatory assays and was equivocal in the initial assay and negative in the confirmatory assay in the presence of metabolic activation."

---

[2]Caspary, W.J., et al. (1988). Environ. Mol. Mutagen 12:19-36.

82

8

TABLE 2. Representative Results of the S9-activated Mouse Lymphoma Forward Mutation Assays with Methanearsonic Acid

Substance	Dose/mL	Percent Relative Suspension Growth	Mutant Colonies[a] ±S.D.	Viable Colonies[a] ±S.D.	Percent Relative Cloning Efficiency[a]	Percent Relative Total Growth[a]	Mutation Frequency Per 10^4 Survivors[b]
Solvent Control							
Deionized water	--	100[c]	35	203	100	100	0.4
(Test material)		100[d]	63	184	100	100	0.7
Dimethyl sulfoxide	--	100[c]	38	191	100	100	0.4
(Positive controls)		100[d]	59	192	100	100	0.6
Positive Control[e]							
7,12-Dimethylbenz(a) anthracene	2.5 µg	82[c]	144±5	138±18	72	59	2.1
	2.5 µg	75[d]	116±4	153±16	80	60	1.5
Test Material							
Methanearsonic acid	534 µg[f]	59[c]	37±1	153±5	76	45	0.5
	712 µg	39	44±8	154±7	76	30	0.6*
	949 µg[g]	18	61±2	155±8	77	14	0.8*
	650 µg[f]	21[d]	65	155	85	18	0.9
	750 µg[g]	15[h]	66±6	127±9	69	10	1.0*

[a]Means and standard deviations from the counts of triplicate plates from single cultures. Values without standard deviations were from duplicate cultures (3 plates/culture) that were reported separately by the study authors. Presented average values were calculated by our reviewers.

[b]Mutation Frequency (MF) = $\frac{\text{Mutant Colonies}}{\text{Viable Colonies}} \times 2$.

[c]Results from the initial trial.

[d]Results from the confirmatory trial.

[e]Two levels of the positive control were assayed; results from the lower dose were selected as representative.

[f]Findings for lower doses (71, 95, 127, 169, 225, 300, and 400 µg/mL--initial trial and 200, 350, and 500 µg/mL--confirmatory trial) did not suggest a mutagenic effect.

[g]Higher levels (1266 and 1688 µg/mL--initial trial and 850 and 950--confirmatory trial) were too cytotoxic to clone.

[h]One of the two replicate cultures was lost; no further explanation was provided.

*Significantly higher than the corresponding solvent control ($p<0.05$) by Kastenbaum and Bowman tables.

From the overall results, the sponsor's representative (Ricerca, Inc.) drew the following conclusions:

It was concluded that methanearsonic acid did not cause positive mutagenic response in the mouse lymphoma mutagenesis assay with or without metabolic activation.

D. REVIEWERS' DISCUSSION AND INTERPRETATION OF RESULTS: We assess that the mutation assays were properly conducted, and we agree with the conclusion that methanearsonic acid was not mutagenic in this test system. Under nonactivated conditions, the test material was evaluated to levels that were severely cytotoxic (≥5000 µg/mL) and failed to induce a mutagenic response at the highest level (4000 µg/mL) that could be evaluated. In the presence of S9 activation; severe cytotoxicity was achieved at doses >949 µg/mL. Although statistically significant increases in the MF were obtained at 712 and 949 µg/mL in the initial trial and at 750 µg/mL in the confirmatory trial, the MFs for these treatment groups ranged from a low of 0.6 mutants per 10^4 survivors to a high of 1.0 mutants per 10^4 survivors. Since all of these values fell within accepted background ranges for mouse lymphoma cells, they should not be interpreted as a positive response. We further agree with the study authors that no meaningful conclusions can be drawn from the increased incidence of small mutant colonies in the 949 µg/mL +S9 dose group. Although small mutant colonies are considered to be indicative of chromosomal damage[3], the mammalian cell cytogenetic assay performed with methanearsonic acid was negative (see Data Evaluation Record 91-119).

We assess, therefore, that there was no compelling evidence to suggest that methanearsonic induced a genotoxic response in this study. In addition, the sensitivity of the test system to detect mutagenesis was adequately demonstrated in both the initial and confirmatory trials by the marked increases in mutation at the $TK^{+/-}$ locus in cells exposed to the nonactivated positive control (0.25 and 0.50 µL/mL EMS) and the S9-activated positive control (2.5 and 5.0 µg/mL DMBA).

E. QUALITY ASSURANCE MEASURES: Was test performed under GLPs? Yes. (A quality assurance statement from the reporting laboratory was signed and dated August 29, 1989.)

F. CBI APPENDICES: Appendix A, Materials and Methods, (Ricerca, Inc.) CBI pp. 9-13; Appendix B, Materials and Methods, (Microbiological Associates, Inc.) CBI pp. 62-68; Appendix C, Protocol (Microbiological Associates, Inc.) CBI pp. 98-111.

---

[3]Moore, M.M. and Clive, D. (1982). The quantitation of $TK^{-/-}$ and $HGPRT^{-}$ mutants of L5178Y/$TK^{+/-}$ mouse lymphoma cells at varying times posttreatment. Environ. Mutagen. 4:499-519.

APPENDIX A

MATERIALS AND METHODS (RICERCA, INC.)
CBI pp. 9-13

TOXR 009369

Page_____ is not included in this copy.

Pages 12 through 16 are not included.

86 THRU 90

The material not included contains the following type of information:

_____ Identity of product inert ingredients.

_____ Identity of product impurities.

_____ Description of the product manufacturing process.

_____ Description of quality control procedures.

_____ Identity of the source of product ingredients.

_____ Sales or other commercial/financial information.

_____ A draft product label.

_____ The product confidential statement of formula.

_____ Information about a pending registration action.

✓ FIFRA registration data.

_____ The document is a duplicate of page(s) _____.

_____ The document is not responsive to the request.

The information not included is generally considered confidential by product registrants. If you have any questions, please contact the individual who prepared the response to your request.

APPENDIX B

MATERIALS AND METHODS (MICROBIOLOGICAL ASSOCIATES, INC.)
CBI pp. 62-68

TOXR 009369

Page_____ is not included in this copy.

Pages 18 through 24 are not included.

92 THRU 98

The material not included contains the following type of information:

_____ Identity of product inert ingredients.

_____ Identity of product impurities.

_____ Description of the product manufacturing process.

_____ Description of quality control procedures.

_____ Identity of the source of product ingredients.

_____ Sales or other commercial/financial information.

_____ A draft product label.

_____ The product confidential statement of formula.

_____ Information about a pending registration action.

✓ FIFRA registration data.

_____ The document is a duplicate of page(s) _____.

_____ The document is not responsive to the request.

The information not included is generally considered confidential by product registrants. If you have any questions, please contact the individual who prepared the response to your request.

APPENDIX C

PROTOCOL (MICROBIOLOGICAL ASSOCIATES, INC.)
CBI pp. 98-111

TOXR 009369

Page_____ is not included in this copy.

Pages 26 through 39 are not included.

100 THRU 113

The material not included contains the following type of information:

____ Identity of product inert ingredients.

____ Identity of product impurities.

____ Description of the product manufacturing process.

____ Description of quality control procedures.

____ Identity of the source of product ingredients.

____ Sales or other commercial/financial information.

____ A draft product label.

____ The product confidential statement of formula.

____ Information about a pending registration action.

✓ FIFRA registration data.

____ The document is a duplicate of page(s) _____.

____ The document is not responsive to the request.

The information not included is generally considered confidential by product registrants. If you have any questions, please contact the individual who prepared the response to your request.

DOC920136

FINAL

009369

DATA EVALUATION REPORT

METHANEARSONIC ACID

Study Type: Mutagenicity: Unscheduled DNA Synthesis Assay in Primary Rat Hepatocytes

Prepared for:

Health Effects Division
Office of Pesticide Programs
Environmental Protection Agency
1921 Jefferson Davis Highway
Arlington, VA 22202

Prepared by:

Clement International Corporation
9300 Lee Highway
Fairfax, VA 22031-1207

Principal Reviewer Lynne T. Haber Ph.D. Date 2/6/92

Independent Reviewer Nancy E. McCarroll, B.S. Date 2/6/92

QA/QC Manager Sharon Segal, Ph.D. Date 2/6/92

Contract Number: 68D10075
Work Assignment Number: 1-32
Clement Number: 91-117
Project Officer: James Scott

114

MUTAGENICITY STUDIES

EPA Reviewer: David Liem, Ph.D.
Review Section II
Toxicology Branch (II)/HED
Signature: David Liem
Date: 3/10/92

EPA Secondary Reviewer:
Byron T. Backus, Ph.D.
Review Section II
Toxicology Branch (II)/HED
Signature: Byron T Backus
Date: 3/11/92

EPA Section Head: Clark Swentzel
Review Section II
Toxicology Branch (II)/HED
Signature: K. Clark Swentzel
Date: 3/12/92

DATA EVALUATION REPORT

STUDY TYPE: Mutagenicity: In vitro unscheduled DNA synthesis assay in primary rat hepatocytes.

EPA IDENTIFICATION Numbers:

Tox Chem. Number: 549A

MRID Number: 416519-05

TEST MATERIAL: Methanearsonic acid (MAA)

SYNONYMS: None provided

SPONSOR: Fermenta ASC Corporation, Mentor, OH

SPONSOR'S REPRESENTATIVE: Ricerca, Inc., Painesville, OH

STUDY NUMBER: T8471.380009

TESTING FACILITY: Microbiological Associates, Inc., Rockville, MD

TITLE OF REPORT: Unscheduled DNA Synthesis Assay in Rat Primary Hepatocytes with Methanearsonic Acid (MAA).

AUTHORS: Curren, R. D. (Microbiological Associates, Inc.); Chun, J. S., and Killeen, J. C. (Ricerca, Inc.)

REPORT ISSUED: July 31, 1989 (Microbiological Associates, Inc.); October 31, 1989 (Ricerca, Inc.)

NOTE: Report prepared by the sponsor's representative, Ricerca, Inc., is a summary of the laboratory report prepared by Microbiological Associates, Inc. The following review focuses on the data provided by Microbiological Associates, Inc.

115
2

CONCLUSIONS-EXECUTIVE SUMMARY: At concentrations ranging from 10 to 750 µg/mL, methanearsonic acid did not induce unscheduled DNA synthesis (UDS) in primary rat hepatocytes. The highest assayed dose (1000 µg/mL) was cytotoxic. Based on the findings of two independently performed trials, it was concluded that methanearsonic acid was tested over an appropriate range of concentrations with appropriate controls and showed no evidence of UDS. Thus, the study satisfies Guideline requirements for genetic effects Category III, Other Mutagenic Mechanisms.

STUDY CLASSIFICATION: The study is acceptable.

A. MATERIALS:

1. Test Material: Methanearsonic acid

   Description: White crystalline solid
   Identification No: SDS-37161; lot No. 107/84
   Purity: 99.8%
   Receipt date: March 13, 1989 (sample I) and April 3, 1989 (sample II)
   Stability: Not reported
   Contaminants: None listed
   Solvent used: Deionized distilled water (DH_2O)
   Other provided information: Stored in the dark at room temperature in a tightly closed container. Test material was dissolved and diluted immediately prior to use. Frozen samples of the most concentrated dosing solution (100 mg/mL) and the solvent control were shipped to Ricerca, Inc., for analytical determinations.

2. Indicator Cells: Primary rat hepatocytes were obtained by the in situ perfusion of the livers of male Sprague-Dawley rats purchased from Harlan Sprague Dawley, Inc., Frederick, Maryland.

3. Control Substances: DH_2O at 10 µL/mL was used as the solvent control for the test compound; dimethyl sulfoxide (DMSO) at 10 µL/mL was used as the solvent control for the positive control. Untreated cells served as the negative control, and 7,12-dimethylbenz(a)anthracene (DMBA) at 3 and 5 µg/mL (Trial 1), and 3 and 10 µg/mL (Trial 2) was used as the positive control.

4. Medium: WME: Williams' Medium E with 2 mM L-glutamine and gentamycin; WME+: WME with 10% fetal bovine serum.

5. Test Compound Concentrations Used:

   (a) Preliminary cytotoxicity assay: 0.06, 0.2, 0.6, 2.0, 6.0, 20, 60, 200, 600, and 2000 µg/mL.

   (b) UDS assay: Two trials were performed with seven doses of the test material (5.0, 10, 50, 100, 500, 750, and 1000 µg/mL).

B. STUDY DESIGN:

1. Cell Preparation:

(a) Perfusion techniques: Rats were anesthetized with metofane (methoxyflurane) and the livers were perfused with Hank's balanced salt solution containing 0.5 mM EGTA, Hepes buffer, pH 7.3, 2 mM L-glutamine, and gentamycin, followed by WME containing 80-100 units/mL collagenase and Hepes buffer pH 7.3. Livers were excised, shaken in the collagenase perfusion solution, and either combed to release the hepatocytes or passed through a stainless-steel sieve.

(b) Hepatocyte harvest/culture preparation: Recovered cells were collected, counted, and seeded at a density of $\approx 5 \times 10^5$ cells, either into preconditioned 35-mm tissue culture dishes for the cytotoxicity assay, or onto coverslips in 35-mm tissue culture plates for the UDS assay. Cultures were placed in an incubator for 90 to 180 minutes, washed in WME+, fed serum-free WME, and assayed as described below.

2. Preliminary Cytotoxicity Assay: Duplicate hepatocyte cultures were exposed to ten doses of the test compound, ranging from 0.06 to 2000 µg/mL, the negative control (WME), or the solvent control (DH_2O) for 18-20 hours. Following exposure, an aliquot of culture fluid was removed and centrifuged, and the level of lactic acid dehydrogenase (LDH) activity was measured. Relative cytotoxicity was assessed by subtracting the LDH activity of the solvent control from the LDH activity in the treated cultures and comparing the values to the amount of LDH released by exposure of the solvent controls to 1% Triton.

3. UDS Assay:

(a) Treatment: Triplicate monolayer cultures on coverslips were fed WME containing 10 µCi/mL [^{3}H] thymidine and were exposed for 18 to 20 hours to the selected test material doses, the positive control (DMBA), the negative control (WME), or the solvent control (DH_2O for the test material, and DMSO for the positive control). Treated hepatocytes attached to coverslips were washed, swollen in 1% sodium citrate, fixed in ethanol-acetic acid, dried, and mounted. In parallel with the test plates, three cultures per dose were assayed for cytotoxicity as described above.

(b) Preparation of autoradiographs/grain development: Slides were coated with Kodak NTB-2 emulsion, dried for 7 days at 4°C in light-tight desiccated boxes, developed in Kodak D-19, fixed, stained with hematoxylin-sodium acetate-eosin, coded, and counted.

(c) Grain counting: The nuclear grains of 50 randomly chosen cells from each of three coverslips per treatment were counted using an automated colony counter. Cytoplasmic background counts were determined by counting three nuclear-sized areas adjacent to the

nucleus. Net nuclear grain counts were determined by subtracting the mean cytoplasmic background count from the nuclear grain count. Nuclei exhibiting toxic effects, such as uneven staining, disrupted membranes, or irregular shape, were not counted.

4. Evaluation Criteria:

   (a) Assay validity: The assay was considered valid if (1) the positive control induced a significant increase (i.e. ≥5 net nuclear grains) in the net nuclear grain count; (2) less than 15% of the cells in the negative control were in repair; and (3) the net nuclear grain count for the vehicle control was less than one.

   (b) Positive response: The test material was considered positive if the mean net nuclear grain count was increased by at least five counts over the control and the response was dose-related.

5. Protocol: See Appendix C for the Microbiological Associates, Inc. protocol. The Ricerca, Inc. protocol is not included in the appendix.

C. REPORTED RESULTS:

1. Analytical Determination: Ricerca, Inc. analyzed the most concentrated dosing solution used in this study (100 mg/mL) and found it to be 95% ± 2% of the nominal concentration.

2. Preliminary Cytotoxicity Study: Ten doses, ranging from 0.06 to 2000 µg/mL, were assayed for cytotoxicity, as measured by release of LDH. The 2000 µg/mL dose was cytotoxic, with a relative survival of 6% (Table 1); relative survival at all lower doses was ≥97%. Microscopic examination of the cultures indicated moderate to slight cytotoxic effects at 2000 and 600 µg/mL, respectively. Cultures exposed to lower doses had normal cellular morphologies. Based on these results, 1000 µg/mL was selected as the highest concentration for the UDS assay.

3. UDS Assay: Seven levels of methanearsonic acid, ranging from 5 to 1000 µg/mL, were tested in two independently performed assays. The study authors reported that examination of the fixed and stained cells showed that the 1000 µg/mL dose was cytotoxic in both trials (Tables 2 and 3). Toxic effects in cells exposed to 1000 µg/mL in the repeat trial included irregularly shaped and darkly stained nuclei. There was, however, no evidence of a genotoxic response at any of the non-cytotoxic concentrations of methanearsonic acid that were scored for UDS (10, 50, 100, 500, or 750 µg/mL). By contrast, the selected doses of the positive control, DMBA at 3 and 5 µg/mL (trial 1), and 3 and 10 µg/mL (trial 2), induced marked increases in UDS. Based on these findings, the study authors concluded that methanearsonic acid was negative in the primary rat hepatocyte UDS assay.

TABLE 1. Representative Results of the Preliminary Cytotoxicity Assay with Methanearsonic Acid (MAA): Lactate Dehydrogenase (LDH) Activity

Treatment	Dose	Average LDH Activity (units/L)[a]	Corrected LDH Activity (units/L)[b]	Relative Survival[c]
Negative Control				
Culture medium	--	44.5	7.0	97%
Solvent Control				
Water	10 µL/mL	37.5	0.0	100
Water +1% Triton	10 µL/mL	289.0	251.5	0
Test Compound Control				
Methanearsonic acid +1% Triton	2000 µg/mL	306.0	268.5	-7
Test Compound				
Methanearsonic acid	600 µg/mL[d]	43.0	5.5	98
	2000 µg/mL	274.0	236.5	6

[a]Average of two samples.

[b]Corrected LDH = Average LDH - Solvent control LDH

[c]$$\text{Relative survival} = 100\% - 100\% \times \frac{\text{Corrected LDH}}{\text{Corrected LDH of solvent control +1\% Triton}}$$

[d]Lower doses (0.06, 0.2, 0.6, 2.0, 6.0, 20, 60, and 200 µg/mL) were not cytotoxic.

TABLE 2. Representative Results of the Initial Unscheduled DNA Synthesis Rat Hepatocyte Assay with Methanearsonic Acid (MAA)

Treatment	Dose	Cytotoxicity: Average LDH Activity (units/L)[a]	Cytotoxicity: Corrected LDH Activity (units/L)[b]	Cytotoxicity: Relative Survival[c]	UDS Activity: Number of Cells Scored[d]	UDS Activity: Mean Net Nuclear Grain Count ± S.D.	UDS Activity: Percent Cells with ≥5 Grains
Negative Control							
Culture medium	--	37.7	9.7	96	150	-0.8±2.2	0
Solvent Controls							
Water (solvent for test compound)	10 µL/mL	28.0	0.0	100	150	-1.1±2.4	0
Water +1% Triton	10 µL/mL	279.0	251.0	0	--	--	--
Dimethyl sulfoxide (solvent for positive compound)	10 µL/mL	35.0	0.0	100	150	-1.4±2.3	0
Positive Control							
7,12-Dimethylbenz(a)anthracene[e]	3 µg/mL	65.5	30.5	88	150	18.6±4.8[f]	100
Test Compound							
Methanearsonic acid	750 µg/mL[g]	75.0	47.0	81	150	-1.2±2.1	0
	1000 µg/mL[h]	85.0	57.0	77	--	Cytotoxic	0

[a]Average of three samples.
[b]Corrected LDH = Average LDH - Solvent control LDH.
[c]Relative survival = $100\% - 100\% \times \frac{\text{Corrected LDH}}{\text{Corrected LDH of solvent control +1\% Triton}}$
[d]Fifty cells were counted for each of three cultures.
[e]Comparable results were obtained with a 5.0 µg/mL dose.
[f]Fulfills the reporting laboratory's criterion for a positive response (i.e., ≥5 net nuclear grains).
[g]Results for lower doses (10, 50, 100, and 500 µg/mL) did not suggest a genotoxic effect.
[h]Highest assayed level; too cytotoxic to be evaluated for unscheduled DNA synthesis.

TABLE 3. Representative Results of the Confirmatory Unscheduled DNA Synthesis Rat Hepatocyte Assay with Methanearsonic Acid (MAA)

Treatment	Dose	Average Lactate Dehydrogenase Activity (units/L)[a]	Corrected Lactate Dehydrogenase Activity (units/L)[b]	Percent Relative Survival[c]	Number Cells Scored[d]	Mean Net Nuclear Grain Count ± S.D.	Percent Cells with ≥5 Grains
Negative Control							
Culture medium	--	40.3	-11.0	104	N.D.[e]	--	--
Solvent Controls							
Water (solvent for test compound)	10 µL/mL	51.3	0.0	100	150	0.3±2.2	0
Water +1% Triton	10 µL/mL	358.3	307.0	0	--	--	--
Dimethyl sulfoxide (solvent for positive compound)	10 µL/mL	72.0	0.0	100	150	0.2±2.2	0
Positive Control							
7,12-Dimethylbenz(a)anthracene[f]	3 µg/mL	87.5	15.5	95	150	18.4±5.5[g]	100
Test Compound							
Methanearsonic acid	750 µg/mL[h]	92.7	41.3	87	150	-1.5±2.5	0
	1000 µg/mL[i]	111.7	60.3	80	--	Cytotoxic	--

[a]Average of three samples.
[b]Corrected LDH = Average LDH - Solvent control LDH

[c]Relative survival = $100\% - 100\% \times \frac{\text{Corrected LDH}}{\text{Corrected LDH of solvent control +1\% Triton}}$

[d]Fifty cells were counted for each of three cultures.
[e]N.D. = not done.
[f]Comparable results were obtained with a 10 µg/mL dose.
[g]Fulfills the reporting laboratory's criterion for a positive response (i.e., ≥5 net nuclear grains).
[h]Results for lower doses (10, 50, 100, and 500 µg/mL) did not suggest a genotoxic effect.
[i]Highest assayed level; too cytotoxic to be evaluated for unscheduled DNA synthesis.

9 12

D. REVIEWERS' DISCUSSION/CONCLUSIONS: We assess that the study was well-conducted and the authors correctly interpreted the data. Cytotoxicity was determined by a highly sensitive method, measuring LDH activity. In two independently performed assays, methanearsonic acid was evaluated to cytotoxic doses but failed to induce UDS. The response of the test system to the positive controls indicated that the assay was sufficiently sensitive to detect a mutagenic response. We, therefore, conclude that methanearsonic acid did not induce UDS in this test system.

E. QUALITY ASSURANCE MEASURES: Was the test performed under GLPs? Yes. Quality assurance statements were signed and dated August 13, 1989 (Microbiological Associates, Inc.).

F. CBI APPENDICES: Appendix A, Materials and Methods, (Ricerca, Inc.) CBI pp. 9-13; Appendix B, Materials and Methods, (Microbiological Associates, Inc.) CBI pp. 53-56; and Appendix C, Protocol (Microbiological Associates, Inc.) CBI pp. 72-82.

APPENDIX A

MATERIALS AND METHODS
RICERCA, INC.
CBI pp. 9-13

Page ___ is not included in this copy.

Pages 11 through 15 are not included in this copy.

124 THRU 128

The material not included contains the following type of information:

_____ Identity of product inert ingredients.

_____ Identity of product inert impurities.

_____ Description of the product manufacturing process.

_____ Description of quality control procedures.

_____ Identity of the source of product ingredients.

_____ Sales or other commercial/financial information.

_____ A draft product label.

_____ The product confidential statement of formula.

_____ Information about a pending registration action.

J FIFRA registration data.

_____ The document is a duplicate of page(s) _____.

_____ The document is not responsive to the request.

The information not included is generally considered confidential by product registrants. If you have any questions, please contact the individual who prepared the response to your request.

APPENDIX B

MATERIALS AND METHODS
MICROBIOLOGICAL ASSOCIATES, INC.
CBI pp. 53-56

009369

Page_____ is not included in this copy.

Pages 17 through 20 are not included.

130 THRU 133

The material not included contains the following type of information:

_____ Identity of product inert ingredients.

_____ Identity of product impurities.

_____ Description of the product manufacturing process.

_____ Description of quality control procedures.

_____ Identity of the source of product ingredients.

_____ Sales or other commercial/financial information.

_____ A draft product label.

_____ The product confidential statement of formula.

_____ Information about a pending registration action.

✓ FIFRA registration data.

_____ The document is a duplicate of page(s) _____.

_____ The document is not responsive to the request.

The information not included is generally considered confidential by product registrants. If you have any questions, please contact the individual who prepared the response to your request.

APPENDIX C

PROTOCOL
MICROBIOLOGICAL ASSOCIATES, INC.
CBI pp. 72-82

009369

Page ___ is not included in this copy.

Pages 22 through 32 are not included in this copy.

135 THRU 145

The material not included contains the following type of information:

_____ Identity of product inert ingredients.

_____ Identity of product inert impurities.

_____ Description of the product manufacturing process.

_____ Description of quality control procedures.

_____ Identity of the source of product ingredients.

_____ Sales or other commercial/financial information.

_____ A draft product label.

_____ The product confidential statement of formula.

___✓___ Information about a pending registration action.

_____ FIFRA registration data.

_____ The document is a duplicate of page(s) _____.

_____ The document is not responsive to the request.

The information not included is generally considered confidential by product registrants. If you have any questions, please contact the individual who prepared the response to your request.