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

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OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Methamidophos (Monitor): 84-2 Mutagenicity Study - Gene Mutation in Cultured Chinese Hamster Ovary Cells (CHO/HGPRT)

DP Barcode No.: D194341
EPA ID No.: 101201
Rereg. Case No.: 0043
CAS No.: 10265-92-6

Submission No.: S446546
P.C. Code No.: 101201
Case No.: 819351
Tox. Chem. No.: 378 A

FROM: Krystyna K. Locke, Toxicologist
Section I, Toxicology Branch I
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TO: Larry Schnaubelt/Robert Richards, PM Team No. 72
Reregistration Branch
Special Review and Reregistration Division (7508W)

THRU: Roger Gardner, Section Head
Section I, Toxicology Branch I
Health Effects Division (7509C) Roger Gardner 2/8/94 2/22/94

Clement International Corporation (the contractor) and Toxicology Branch I/HED have completed an evaluation of the following study:

CHO/HGPRT Mutation Assay - Monitor Technical (Batch No. 0-06-7009); C. Anita H. Bigger and Cynthia I. Sigler; Microbiological Associates Inc., Rockville, MD; Laboratory Study No.: TC865.332; Sponsor Study Numbers: 93-C500-SO and 105076 Study Completion Date: May 27, 1993. MRID No.: 42854701

The above study was submitted to replace another study (CHO/HGPRT Mutation Assay; MRID No.: 42013701) which was evaluated by the contractor and Toxicology Branch I/HED in February, 1992 and classified as Unacceptable. In that study, performed also by Microbiological Associates, Inc., Rockville, MD, no definitive conclusion could be reached regarding the mutagenic potential of Methamidophos.

The currently submitted study was classified by Toxicology Branch I/HED as Acceptable. In this study, doses up to 5000 ug/mL (limit dosing) were evaluated. Methamidophos was negative for inducing forward mutation in nonactivated cultures, but was

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weakly positive at the highest activated doses (4000 and 5000 ug/mL). Dr. Irving Mauer, Geneticist, Toxicology Branch I/HED, who secondarily evaluated this study, commented as follows:

> " Although an apparent dose-response increase in mutant frequency was recorded at high activated concentrations (Table 2 in DER), it did not achieve background (lab. and expert literature) level (40×10^6), but came close. Hence, one may consider Monitor "weakly positive" in acceptable assays for gene mutation. " This comment, which is in Tox. Branch file, has been dated January 26, 1994.

NOTE: This memorandum supersedes all comments, both typed and hand-written, that have been made in the review regarding the acceptability of this study (MRID No. 42854701). In other words, this study is ACCEPTABLE and does not have to be repeated, and additional data on the test material do not have to be submitted.

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DATA EVALUATION REPORT

METHAMIDOPHOS

Study Type: Mutagenicity: Gene Mutation in Cultured Chinese Hamster Ovary Cells (CHO/HGPRT)

Prepared for:

Health Effects Division
Office of Pesticide Programs
Environmental Protection Agency
1921 Jefferson Davis Highway
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Prepared by

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Independent Reviewer	Nancy E. McCarroll, B.S.	Date 12/8/93
QA/QC Manager	Sharon Segal, Ph.D.	Date 12/8/93

Contract Number: 68D10075
Work Assignment Number: 3-24
Clement Number: 83
Project Officer: Caroline Gordon

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EPA Reviewer: Irving Mauer, Ph.D.
Immediate Office, Toxicology Branch I
Health Effects Division (7509C)

Signature: Irving Mauer
Date: 01-03-94

EPA Section Head: Marion Copley, D.V.M., D.A.B.T.
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Health Effects Division (7509C)

Signature: Marion Copley
Date: 1/8/94

DATA EVALUATION REPORT

STUDY TYPE: Gene mutation in cultured Chinese hamster ovary cells (CHO/HGPRT)

CHEMICAL: Methamidophos

TOX. CHEM. NUMBER: 378A

P.C. CODE: 101201

MRID Number: 428547-01

SYNONYMS/CAS No.: Monitor Technical/10265-92-6

SPONSOR: Miles Inc., Agriculture Division, Kansas City, MO

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

TITLE OF REPORT: CHO/HGPRT Mutation Assay -- Monitor Technical (Batch No. 0-06-7009)

AUTHORS: Bigger, C.A.H. and Sigler, C.I.

STUDY NUMBERS: TC865.332 (Testing Facility); 93-C500-SO (Sponsor)

REPORT ISSUED: May 27, 1993

CONCLUSION/EXECUTIVE SUMMARY: Reported to be negative for the induction of forward gene mutations at the HGPRT locus in Chinese hamster ovary (CHO) cells. However, dose-related increases in the mutation frequency (MF) were observed at the two highest S9-activated levels (4000 and 5000 μg/mL). The suggestive evidence of mutagenic activity at high S9-activated doses was in agreement with the observation of similar responses in a previously-submitted CHO/HGPRT study conducted with Monitor technical (see Data Evaluation Record 1-40/91-139; MRID No. 420137-01). We assess, however, that the results from both studies are equivocal and that definitive conclusions regarding the mutagenic potential of Monitor technical can not be reached. There are also concerns regarding the failure to characterize ≈25% of the test material (purity was listed as 75.6%). It is, therefore, possible that the equivocal positive results, which only occurred at high S9-activated levels, may have been associated with the uncharacterized portion of the test material (see Section D., Reviewers'

Discussion/Conclusions for a detailed discussion). Based on these considerations, Monitor technical is classified as presumptively positive in this in vitro mammalian cell assay and further comprehensive (repeat) testing across a comparable range of test material doses is required. In addition, detailed information on the test material should be submitted.

CLASSIFICATION: Unacceptable. The study does not satisfy the requirements of FIFRA Test Guideline Series 84.2 for genetic effects Category I, Gene Mutations, and is not acceptable for regulatory purposes but is upgradable, see conclusions/discussion

A. MATERIALS:

1. Test Material: Monitor technical

Description: Crystalline white solid (upon receipt); clear colorless liquid (sponsor characterization)[1]
Identification numbers: Batch number 0-06-7009; code number C865
Purity: 75.6% Active ingredient
Contaminants: None listed
Receipt date: January 22, 1993 (sample I); March 16, 1993 (sample II)
Expiration date: January 8, 1994 (estimated)
Storage conditions: -20°±5°C; protected from light
Vehicle used: Sterile, distilled water (dH_2O)
Other provided information: Dosing solutions were prepared immediately prior to use. Aliquots of representative solutions used in the preliminary cytotoxicity test and in the mutation assay were shipped frozen to the sponsor for analysis. In addition, the osmolality and pH of the tissue culture medium containing 5000 µg/mL were determined. The report did not indicate which phase(s) of the study was conducted with sample I and/or sample II; the shipment of two samples of the test material was not explained.

2. Control Materials:

Negative: Culture medium (Ham's F-12 medium containing 5% dialyzed fetal bovine serum, 2 mM L-glutamine/mL, and antibiotics)

Solvent/final concentration: DH_2O/1%

[1] Note: The difference in the physical descriptions of the test material was assumed by our reviewers to be related to shipment conditions (shipped or arrived at the performing laboratory frozen) At the time of use, the test material was melted at 37°C and became a clear colorless liquid as described by the sponsor. The effect, if any, on test material stability, was not addressed.

Positive: Nonactivation (concentration, solvent): Ethyl methanesulfonate (EMS) was prepared in dimethyl sulfoxide (DMSO) to yield a final concentration of 0.2 µL/mL.

Activation (concentrations, solvent): Benzo(a)pyrene (BaP) was prepared in DMSO to yield final concentrations of 4 and 5 µg/mL.

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 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(α)anthracene to mutagenic forms using Salmonella typhimurium strain TA100. The S9 mix was prepared as follows:

Component	Final Concentration
Sodium phosphate buffer (pH 8.0)	50 mM
Glucose-6-phosphate	5 mM
NADP	4 mM
KCl	30 mM
$MgCl_2$	10 mM
$CaCl_2$	10 mM
S9 fraction	100 µL/mL

4. Test Cells:

___ mouse lymphoma L5178Y cells
x Chinese hamster ovary (CHO-K_1-BH_4) cells
___ V79 cells (Chinese hamster lung fibroblasts)
___ other (list):

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

5. Locus Examined:

___ thymidine kinase (TK)
selection agent: _____ bromodeoxyuridine (BrdU)
(give concentration) _____ fluorodeoxyuridine (FdU)

_x_ hypoxanthine guanine phosphoribosyl transferase (HGPRT)
selection agent: _____ 8-azaguanine (8-AG)
(give concentration) 10 µM 6-thioguanine (6-TG)

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

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

6. Test Compound:

(a) Preliminary cytotoxicity test: Nine doses (0.5, 1.5, 5, 15, 50, 150, 500, 1500, and 5000 µg/mL) were evaluated with and without S9 activation.

(b) Mutation assay:

- Nonactivated conditions: 1000, 2000, 3000, 4000, and 5000 µg/mL
- S9-activated conditions: As above for the nonactivated phase of testing

B. TEST PERFORMANCE:

1. Cell Treatment:

(a) Cells were exposed to the test compound, solvent or positive control for 5 hours (nonactivated) and 5 hours (S9-activated).

(b) After washing, cells were cultured for 7-9 days (expression period) before cell selection.

(c) After expression, $2x10^5$ cells/dish (5 dishes/culture) were cultured for 7-10 days in selection medium to determine numbers of mutants; 100 cells/dish (3 dishes/culture) were cultured for 7-10 days in nonselection medium to determine cloning efficiency (CE).

2. Statistical Methods: The data were not analyzed for statistical significance.

3. Evaluation Criteria:

(a) Assay validity: The assay was considered valid if the following criteria were met: (1) the CEs of the negative and solvent controls were greater than 50%; (2) the spontaneous MFs in the negative and solvent controls were $\leq 25 \times 10^{-6}$ clonable cells; and (3) the MFs of the positive controls were at least three times that of the solvent control and/or at least 40 mutants/10^6 surviving cells.

(b) Positive response: The test material was considered positive if a reproducible, dose-dependent increase in the MFs occurred with at least two consecutive dose levels having MFs >40 mutants per 10^6 clonable cells.

C. REPORTED RESULTS

1. Analytical Determinations: Solutions containing nominal concentrations of 0.05 and 500 mg/mL (preliminary cytotoxicity test) and 100 and 500 mg/mL (mutation assay) were analyzed for actual concentrations. Results indicated that with the exception of the high-dose solution used for the preliminary study, which was 82% of the nominal dose, all other samples were within 10% of the intended concentrations. Osmolality and pH measurements indicated that Monitor technical at 5000 µg/mL had no adverse effects on the treatment medium.

2. Preliminary Cytotoxicity Test: The highest assayed dose (5000 µg/mL) was moderately cytotoxic in both the nonactivated and S9-activated phases of testing, with relative percent CEs ≥53%. Lower levels (≤1500 µg/mL +/-S9) had no adverse effects on cell survival. Based on these findings, 1000, 2000, 3000, 4000, and 5000 µg/mL were selected for the nonactivated and S9-activated mutation assays.

3. Mutation Assay:

Nonactivated conditions: Representative results presented in Table 1 indicated that the nonactivated test material was neither cytotoxic nor mutagenic at any dose level. The nonactivated positive control (0.2 µL/mL EMS), however, caused a marked increase in total mutant colonies and in the MF.

S9-activated conditions: Approximately 39% of the cells survived exposure to 5000 µg/mL; below 5000 µg/mL, relative survival was ≥78%. As shown in Table 2, the generally dose-related increase in the MF, which ranged from 5.3 $\times 10^{-6}$ at 2000 µg/mL to 31.9 $\times 10^{-6}$ at 5000 µg/mL, was accompanied by a similar dose-related increase in total mutant colonies. The study authors dismissed these findings because the MFs of the test material did not exceed 40 mutants/10^6 surviving cells and concluded that Monitor technical was negative in this mammalian cell forward gene mutation assay.

TABLE 1. Representative Results of the Nonactivated Chinese Hamster Ovary (CHO) Cell Forward Gene Mutation Assay with Monitor Technical

Substance	Dose	Average Relative Percent Survival[a] (posttreatment)	Total Mutant Colonies	Average Cloning Efficiency[a] (at selection)	Average Mutation Frequency[b] ($x10^{-6}$)
Negative Control					
Culture medium	-	113	27	1.08	12.5
Solvent Control					
Distilled water	1%	100	4	1.05	1.9
Positive Control					
Ethyl methane-sulfonate	0.2 µL/mL	84	400	0.98	203.7
Test Material					
Monitor technical	5000 µg/mL[c]	86	10[d]	1.04	5.4

[a] Values were based on results of three dishes/flask from duplicate cultures in all groups.

[b] Mutation Frequency (MF) = $\frac{\text{Total Mutant Colonies}}{\text{No. of Selection Dishes (5/culture) x No. Cells Plated } (2x10^5) \text{ x Cloning Efficiency}} \times 10^6$

[c] Results for lower doses (1000, 2000, 3000, and 4000 µg/mL) did not suggest a mutagenic response.

[d] One of the ten selection dishes was contaminated.

Note: Data were extracted from the study report, pp. 15-16.

TABLE 2. Results of the S9-Activated Chinese Hamster Ovary (CHO) Cell Forward Gene Mutation Assays with Monitor Technical

Substance	Dose	Average Relative Percent Survival[a] (posttreatment)	Total Mutant Colonies	Average Cloning Efficiency[a] (at selection)	Average Mutation Frequency ($x10^{-6}$)[b]
Negative Control					
Culture medium	-	112	6	1.14	2.6
Solvent Control					
Distilled water	1%	100	11	0.86	6.4
Positive Control[c]					
Benzo(a)pyrene	4 µL/mL	43	233	0.89	131.4
Test Material					
Monitor technical	1000 µg/mL	103	27	1.11	12.1
	2000 µg/mL	103	12	1.12	5.3
	3000 µg/mL	91	13	0.99	6.5
	4000 µg/mL	78	28	0.86	16.2
	5000 µg/mL	39	60	0.94	31.9

[a] Values were based on results of three dishes/flask from duplicate cultures in all groups.

[b] Mutation Frequency (MF) = $\frac{\text{Total Mutant Colonies}}{\text{No. of Selection Dishes (5/culture) x No. Cells Plated } (2x10^5) \text{ x Cloning Efficiency}} \times 10^6$

[c] Two levels of the positive control were assayed; data from the lowest dose were selected as representative.

Note: Data were extracted from the study report, pp. 15 and 17.

D. REVIEWERS' DISCUSSION/CONCLUSIONS: While we agree that the nonactivated test material was negative, we disagree with the study authors' conclusions regarding S9-activated Monitor technical. Dose-related increases in the MF occurring at S9-activated doses that were not severely cytotoxic suggested a positive response. Also, the MF at the highest dose (5000 μg/mL +S9) fell well outside of the generally accepted spontaneous MF range for CHO cells (0-20 mutants/10^6 cells)[2] Overall, the results were not sufficient to classify the S9-activated test material as a mutagen; however, the findings do not fully support a negative conclusion. At best, the results of the study are equivocal.

We also noted that definitive conclusions could not be reached for a previously submitted CHO/HGPRT assay of Monitor technical conducted by the same performing laboratory (see Data Evaluation Record 1-40/91-139; MRID No. 420137-01). In this study, Monitor technical, prepared in DMSO, induced significant ($p<0.05$) increases in the MF at S9-activated doses of 2.0 and 3.5 μL/mL but only in two of four trials. The study was rejected primarily because suboptimal conditions were considered by EPA reviewers to have contributed to the conflicting results. In addition, similar problems (i.e., the unexplained use of two separate test material samples; no indication as to which phase(s) of the study were performed with sample I and/or sample II; and a failure to characterize approximately 25% of the test material) confounded the evaluation of both studies.

Based on these considerations, we conclude that even when the data from both studies were combined the results, suggesting that S9-activated Monitor technical induced a mutagenic response, are still equivocal. Further, it is conceivable that components of the uncharacterized portion of the test material may have been responsible for the equivocal positive results. However, without a full description of the test material components, we are unable to verify this assumption.

E. QUALITY ASSURANCE MEASURES: Was the test performed under GLP? Yes (A quality assurance statement was signed and dated May 27, 1993.)

F. CLASSIFICATION: Unacceptable. The study does not satisfy the requirements of FIFRA Test Guideline Series 84.2 for genetic effects Category I., Gene Mutations, and is not acceptable for regulatory purposes but is upgradable

[2] Li, A.P., Gupta, R.S., Heflich, R.H., and Wassom, J.S. (1988). A review and analysis of the Chinese hamster ovary/hypoxanthine guanine phosphoribosyl transferase assay to determine the mutagenicity of chemical agents. Mutat. Res. 196:17-36.