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

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JUL - 9 1993

MEMORANDUM

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

SUBJECT: **ACETOCHLOR:** Review of two mutagenicity studies; one with acetochlor and the other with the PJ2 metabolite of acetochlor.
EPA DP Barcode: D190316, D190318, D190321; EPA Submission No. S438926-8; MRID # 427131-06, 427131-18; EPA Pesticide Chemical Code 121601, Caswell No. 003B.

TO: Robert Taylor/Vickie Walters, PM 25
Herbicide-Fungicide Branch
Registration Division (H7505C)

FROM: Stephen C. Dapson, Ph.D. Stephen C. Dapson 6/30/93
Senior Pharmacologist
Review Section I, TB II/HED (H7509C)

THRU: Yiannakis M. Ioannou, Ph.D., D.A.B.T. Y. M. Ioannou 7/1/93
Section Head, Review Section I
and
Marcia van Gemert, Ph.D. Marcia van Gemert 7/2/93
Chief, Toxicology Branch II
Health Effects Division (H7509C)

Registrant: Acetochlor Registration Partnership - ZENECA

Action Requested: Review two mutagenicity studies; one with acetochlor and the other with the PJ2 metabolite of acetochlor.

Recommendations: TB II reviewed two mutagenicity studies; one with acetochlor and the other with the PJ2 metabolite of acetochlor. The following are the conclusions from the reviews:

MRID# 427131-06: *CHO/HGPRT Gene Mutation Assay with MON 097,* Monsanto Company for Acetochlor Registration Partnership, Monsanto Report No. ML-88-314, 9/6/89.

Negative for inducing forward gene mutations at the HGPRT locus in Chinese hamster ovary (CHO) cells at doses ranging from 50 to 300 µg/mL in the absence and presence of varying levels of S9 (1, 2, 5, or 10%) in the S9--cofactor mix. The findings were confirmed in an independently performed assay. Cytotoxicity was clearly demonstrated at ≥200 µg/mL +/- 10% S9.

The study is classified as Acceptable and satisfies the 1984 Pesticide Assessment Guideline (40 CFR 158.340, §84-2) for a Category 1, in vitro mutagenicity (mammalian cell) gene mutation assay.

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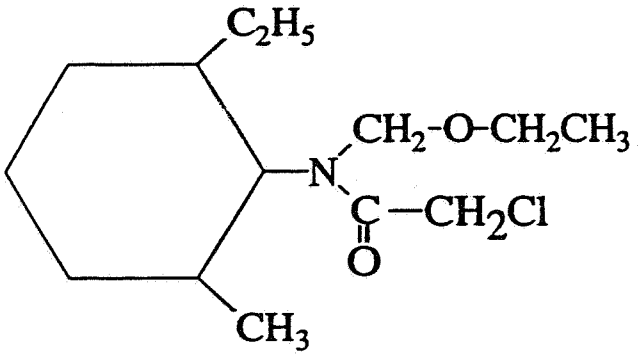
MRID# 427131-18: *PJ2 Acetochlor Metabolite - An Evaluation of Mutagenic Potential Using S.Typhimurium and E.Coli*, ICI Central Toxicology Laboratory for Acetochlor Registration Partnership, Report No. CTL/P/3358 and Study No. YV2989, 7/2/91.

Negative for inducing reverse gene mutation in *Salmonella typhimurium* TA1535, TA1537, TA98, and TA100 and *Escherichia coli* WP2P *uvrA* exposed to a concentration range of 100 to 5000 µg/plate +/-S9. Also negative for *S. typhimurium* TA1538 and *E. coli* WP2 in the nonactivated phase of testing but equivocal for these strains in the presence of S9 activation. Significant and dose-related increases in revertant colonies, which were, <2-fold over control, were seen at 2500 and 5000 µg/plate +S9 in both trials. Two independent trials that were conducted utilized a plate incorporation (Trial 1 and nonactivated phase of Trial 2) and a pre-incubation (S9-activated phase of Trial 2) protocol.

The study is classified as Acceptable and satisfies the 1984 Pesticide Assessment Guideline (40 CFR 158.340, §84-2) for a Category 1, in vitro mutagenicity (bacterial reverse) gene mutation assay.

NOTE: This chemical is classified as a Group B2 carcinogen (see page 4).



ACETOCHLOR

I. Toxicology Profile for Acetochlor (40 CFR 158.340)

Technical: Acetochlor
Use Pattern: food, terrestrial nonfood
Action Type: review of studies for registration

This compound is a registered active ingredient. The following data are required for technical acetochlor.

	Required	Satisfied
§81-1 Acute oral toxicity in rats	Yes	Yes
§81-2 Acute dermal toxicity in rabbits	Yes	Yes
§81-3 Acute inhalation toxicity in rats	Yes	Yes
§81-4 Primary eye irritation in rabbits	Yes	Yes
§81-5 Primary dermal irritation in rabbits	Yes	Yes
§81-6 Dermal sensitization - guinea pig	Yes	Yes
§82-1(a)90 day feeding study - rat	Yes	Yes
§82-1(b)90 day feeding study - nonrodent	Yes	No[1]
§82-2 21 day dermal - rabbit	Yes	Yes
§82-3 90 day dermal	No	
§82-4 Subchronic inhalation	No	
§82-4 Subchronic neurotoxicity	No	
§83-1(a)2-year feeding - rodent	Yes	Yes
§83-1(b)1-year feeding - nonrodent	Yes	Yes
§83-2(a)Carcinogenicity - rat	Yes	Yes
§83-2(b)Carcinogenicity - mouse	Yes	Yes
§83-3(a)Teratology - rat	Yes	Yes
§83-3(b)Teratology - rabbit	Yes	Yes
§83-4 Multigeneration reproduction-rat	Yes	Yes
§83-5 Carcinogenicity/Chronic Toxicity - rat	Yes	Yes
§84-2(a)Mutagenicity - Gene Mutation	Yes	Yes
§84-2(b)Muta - Struct. Chromosome Aberr.	Yes	Yes
§84-4 Muta - Other Genotoxic Effects	Yes	Yes
§85-1 General metabolism - rat	Yes	Yes
§85-2 Domestic Animal Safety	No	
§85-2 Dermal Penetration	Yes	Yes

[1] = This requirement is satisfied by a chronic feeding study in dogs.

II. Data Gaps

There are no data gaps at this time in the technical database.

III. Actions Being Taken to Obtain Additional Information or Clarification

None at this time.

IV. Reference Dose

The RfD is 0.02 mg/kg/day based on the chronic feeding study in the dog with a NOEL of 2.0 mg/kg/day and an uncertainty factor (UF) of 100.

V. Pending Regulatory Actions

None at this time.

VI. Additional Toxicological Information

This chemical has been classified as a Group B2 Carcinogen (Probable Human Carcinogen) by the HED Peer Review Committee (PRC), CRAVE and the Science Advisory Panel (SAP). This is based on the evidence that administration of acetochlor causes increased incidence of benign and malignant tumors at multiple sites in Sprague-Dawley rats (papillary adenomas of the nose/turbinates in both sexes at doses below the MTD; hepatocellular carcinomas in both sexes and thyroid follicular cell adenomas in males at levels exceeding the MTD). Also, increased incidence of benign and malignant tumors at multiple sites in Swiss albino CD-1 mice (hepatocellular carcinoma in both sexes; lung carcinomas in females; uterine histiocytic sarcoma and benign ovarian tumors in females; kidney adenomas in females). There is positive mutagenicity data and structural analogues to Acetochlor that have positive carcinogenicity data.

The unit risk, Q_1^* of acetochlor is $10^{-2}(mg/kg/day)^{-1}$ in human equivalents. This estimate of the Q_1^* is based upon papillary adenomas (of the nose/turbinates) in both male and female Sprague-Dawley rats fed 0, 40, 200, or 1000 ppm in the diet.

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FINAL

DATA EVALUATION REPORT

MON 097

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
Arlington, VA 22202

Prepared by

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

Principal Reviewer Nancy E. McCarroll, B.S Date 6/11/93

Independent Reviewer Lynne Haber, Ph.D. Date 6/11/93

QA/QC Manager Sharon Segal, Ph.D. Date 6/11/93

Contract Number: 68D10075
Work Assignment Number: 2-110
Clement Number: 333
Project Officer: Caroline Gordon

GUIDELINE § 84: MUTAGENICITY
MAMMALIAN CELLS IN CULTURE GENE MUTATION

EPA Reviewer: Stephen C. Dapson, Ph.D.
Review Section I, Toxicology
Branch II/HED (H-7509C)

Signature: Stephen C. Dapson
Date: 6/22/93

EPA Mutagenicity Secondary
Reviewer: Byron T. Backus, Ph.D.
Review Section II, Toxicology
Branch II/HED (H-7509C)

Signature: Byron T. Backus
Date: 6/22/93

DATA EVALUATION REPORT

CHEMICAL: MON 097

Tox Chem. Number: 003B

PC Code: 121601

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

MRID Number: ~~421396-31~~ 427131-06 840

SYNONYM(S)/CAS NO.: Acetochlor

SPONSOR: Monsanto Agricultural Company, St. Louis, MO/Acetochlor Registration Partnership c/o Zeneca Ag Products, Wilmington, DE

TESTING FACILITY: Monsanto Environmental Health Laboratory, Monsanto Agricultural Company, St. Louis, MO

TITLE OF REPORT: CHO/HGPRT Gene Mutation Assay with MON 097

AUTHORS: Li, A.P. and Myers, C.A.

STUDY NUMBER(S): ML-88-314; EHL 88183

REPORT ISSUED: September 6, 1989

CONCLUSIONS--EXECUTIVE SUMMARY: Negative for inducing forward gene mutations at the HGPRT locus in Chinese hamster ovary (CHO) cells at doses ranging from 50 to 300 µg/mL in the absence and presence of varying levels of S9 (1, 2, 5, or 10%) in the S9-cofactor mix. The findings were confirmed in an independently performed assay. Cytotoxicity was clearly demonstrated at ≥200 µg/mL +/- 10% S9.

STUDY CLASSIFICATION: Acceptable. The study satisfies the requirements for FIFRA Test Guideline 84-2 for *in vitro* mutagenicity (mammalian cell gene mutation) data.

MAMMALIAN CELLS IN CULTURE GENE MUTATION

A. MATERIALS:

1. Test Material: MON 097

Description: Dark liquid
Identification No.: nbp 3982351; EHL Cod T880095
Purity: 91.4%
Receipt date: Not listed
Stability: Not provided
Contaminants: None listed
Solvent used: Ethanol (ETOH)
Other provided information: The test material was stored at room temperature, and solutions of the test material were prepared on the day of use. Stock solutions were not evaluated for actual concentration.

2. Control Materials:

Negative: None

Solvent/final concentration: ETOH/final concentration was not reported but assumed to be 0.5%.

Positive: Nonactivation (concentrations, solvent): Ethyl methanesulfonate (EMS) was presumably prepared in ETOH to yield a final concentration of 200 µg/mL.

Activation (concentrations, solvent): Benzo(a)pyrene (BaP), which was presumably prepared in ETOH to yield a final concentration of 1 µg/mL, was included in the initial mutation assay conducted with S9 liver homogenate concentrations in the cofactor mix ranging from 1 to 10%. Dimethylnitrosamine (DMN) at 100 µg/mL was used as the positive control for the confirmatory S9-activated trial conducted with 10% S9.

3. Activation: S9 derived from

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

[a]Neither the strain nor the sex of the rats used to prepare the S9 fraction were reported.

The S9 homogenate (lot number 5C016) was purchased from Organon Teknika Corp.

S9 mix composition:

Component	Concentration/mL of Culture Medium
NADP	4 mM
Glucose 6-phosphate	5 mM
Potassium chloride	30 mM
Magnesium chloride	10 mM
Sodium phosphate buffer, pH 7.5	50 mM
Calcium chloride	10 mM
S9 homogenate	1%, 2%, 5%, and 10% were used in both the cytotoxicity and initial mutagenicity assay. 10% was used in the confirmatory assay.

4. Test Cells: Mammalian cells in culture

_____ mouse lymphoma L5178Y cells
_x_ 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? Not reported.

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 Concentrations Used:

(a) Preliminary cytotoxicity assay: Eight doses (25, 50, 75, 100, 125, 150, 175, and 200 µg/mL) were evaluated in the presence and absence of 1, 2, 5, or 10% S9 in the S9 cofactor mix.

(b) Mutation assay: Two nonactivated and two S9-activated assays were performed; doses tested were as follows:

(1) Nonactivated conditions:

Initial trial: 50, 100, and 200 µg/mL

Confirmatory trial: 50, 75, 100, 150, 200, and 300 µg/mL

(2) S9-activated conditions:

Initial trial: 50, 100, and 200 µg/mL plus 1%, 2%, 5%, or 10% S9

Confirmatory trial: 50, 75, 100, 150, 200, and 300 µg/mL plus 10% S9

B. TEST PERFORMANCE:

1. Cell Treatments:

(a) Cells were exposed to test compound, solvent, or positive controls for: 3 hours (nonactivated) 3 hours (activated)

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

(c) After expression, cells ($2x10^5$ cells/plate, 5 plates) were cultured for 6-10 days in selection medium to determine numbers of mutants and 200 cells/plate, 3 plates were incubated for 6-10 days without selection medium to determine cloning efficiency.

2. Statistical Methods: Data were evaluated according to the method of Snee and Irr[1] (i.e., mutation frequencies were transformed and analyzed by Student's t-test).

3. Evaluation Criteria: No criteria were provided to evaluate assay validity, a positive response, or the biological significance of the findings.

C. REPORTED RESULTS:

1. Preliminary Cytotoxicity Assay: Dose selection for the preliminary cytotoxicity test was based on the findings of an earlier study (Report No. MSL-3021, dated June 9, 1983). Accordingly, the eight evaluated concentrations of MON 097 ranged from 25 to 200 µg/mL in the

[1]Snee, R.D. and Irr J.D. (1981). Design of a statistical method for the analysis of mutagenesis at the hypoxanthine guanine phosphoribosyl transferase locus of cultured Chinese hamster ovary cells. Mutat. Res. 85:77-93.

absence of S9 activation and with S9 levels of 1 to 10%. Results indicated that ≈≤50% of the cells survived treatment with ≥100 µg/mL -S9 or ≥150 µg/mL +1, 2, or 5% S9. Approximately 50% of the cells were recovered following exposure to 125 µg/mL +10% S9. Based on these data, the initial mutation assay was conducted with three doses (50, 100, and 200 µg/mL) under nonactivated conditions and in the presence of graded concentrations of S9.

2. Mutation Assays:

(a) Nonactivated conditions: Representative findings from the initial and confirmatory nonactivated trials conducted with MON 097 are presented in Table 1. Data from both trials were in good agreement and indicated that ≤10% of the cells survived exposure to doses ≥200 µg/mL. There was also no indication of a mutagenic response in either trial. Although increased mutation frequencies (MF) compared to the concurrent controls were noted in both trials, the data did not suggest a dose response and none of the elevated MFs exceeded the generally accepted background range $(0\text{-}20\times10^{-6})$[2] for CHO cells.

(b) S9-activated conditions: Selected results from the initial and confirmatory trials performed with MON 097 in the presence of 10% S9, which were considered by our reviewers to be representative of the overall findings, are shown in Table 2. In the initial trial, cell survival at 200 µg/mL (≈40%) was higher than expected from the preliminary cytotoxicity assessment, which indicated that <10% of the cells were recovered following treatment with 200 µg/mL +10% S9. Additionally, MFs were increased at all doses. Although the increases were not dose related, at 100 µg/mL the calculated MF (30.3×10^{-6}) was ≈11-fold higher than the solvent controls. There was no convincing evidence of mutagenesis in cultures exposed to the selected test material levels (50, 100, or 200 µg/mL) in the presence of 1, 2, or 5% S9. Based on the overall findings, the doses and percentage S9 selected for the confirmatory assay were 50, 75, 100, 150, 200, and 300 µg/mL +10% S9. As the data presented in Table 2 further show, the highest dose (300 µg/mL) was completely cytotoxic. Relative cell survival at the remaining levels ranged from ≈100% at 50 µg/mL to 19% at 200 µg/mL. MFs over this concentration range did not suggest a mutagenic effect. We, therefore, consider the increased MF noted in the initial trial to be an anomalous finding.

Based on the overall results, the study authors concluded that MON 097 was not mutagenic in the CHO/HGPRT gene mutation assay.

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[2]Li, A.P., Carver, J.H., Choy, W.N., Hsie, A.W., Gupta, R.S., Loveday, K.S., O'Neill, J.P., Riddle, J.C., Stankowski, Jr, L.F., and Yang, L.L. (1987). A guide for the performance of the Chinese hamster ovary cell/hypoxanthine-guanine phosphoribosyl transferase gene mutation assay. Mutat. Res. 189:135-141.

TABLE 1. Representative Results of the Nonactivated Chinese Hamster Ovary (CHO) Cell Forward Gene Mutation Assays with MON 097

Substance	Dose/mL	Relative % Cloning Efficiency (Relative survival after treatment)[a,b]	Average Mutant Colonies/ 5 Dishes[a]	Cloning Efficiency (at selection)[a]	Mutation Frequency x 10^{-6}[a,c]
Solvent Control					
Ethanol	0.5%	100[d] (72)[e]	3[f]	0.80[f]	3.8
	0.5%	100[g] (93)	3	0.94	3.2
Positive Control					
Ethylmethane sulfonate	200 µg	94[d]	160	0.62	258.1
	200 µg	77[g]	116	0.78	148.7
Test Material					
MON 097	100 µg[h]	75[d]	5	0.76	6.6
	200 µg	10[h]	0[h]	0.53[f]	0.0
	150 µg[g]	22[g]	2	0.82	2.4
	200 µg[i]	6	10	0.73	13.7

[a]Average results from duplicate cultures in the initial trial and triplicate cultures in the confirmatory trial; calculated by our reviewers. Minor differences between reported and recalculated values presumably resulted from the rounding-off of fewer significant digits by our reviewers.

[b]Cloning efficiency (CE) = $\frac{\text{No. of Cells Recovered}}{\text{No. of Cells Plated (200/Dish)}}$; Relative CE = $\frac{\text{CE of Test Group}}{\text{CE of Solvent Control}}$ x 100.

[c]Mutation Frequency (MF) = $\frac{\text{Average Mutant Colonies/5 Dishes}}{\text{No. of Cells Plated } (1\text{x}10^6) \text{ x Cloning Efficiency (at selection)}}$.

[d]Results from the initial assay

[e]Value in () = absolute CE of the solvent control cultures.

[f]One of the duplicate cultures was contaminated.

[g]Results from the confirmatory assay

[h]Results for lower doses (50 µg/mL--initial trial; 50, 75, or 100 µg/mL--confirmatory trial) did not suggest a mutagenic response.

[i]No cells survived exposure to the highest assayed dose (300 µg/mL).

Note: Data were extracted from the study report, pp. 16, 18, 19, 23, and 25.

TABLE 2. Representative Results of the 10% S9-Activated Chinese Hamster Ovary (CHO) Cell Forward Gene Mutation Assays with MON 097

Substance	Dose/mL	% S9	Relative % Cloning Efficiency (Relative survival after treatment)[a,b]	Average Mutant Colonies/ 5 Dishes[a]	Cloning Efficiency (at selection)[a]	Mutation Frequency x 10^{-6}[a,c]
Solvent Control						
Ethanol	0.5%	10	100[d] (88)[e]	2	0.73	2.7
	0.5%	10	100[f] (68)	10	0.80	12.5
Positive Control						
Benzo(a)pyrene	1 µg	10	92[d]	19	0.68	27.9
Dimethylnitrosamine	100 µg	10	50[f]	34	0.69	49.3
Test Material						
MON 097	50 µg[g]	10	90[d]	7	0.73	9.6
	100 µg	10	73	20[h]	0.66[h]	30.3
	200 µg	10	40	8	0.66	12.1
	100 µg[i]	10	68[f]	~11[j]	0.76	14.5
	150 µg	10	53	8	0.69	11.6
	200 µg[k]	10	19	1	0.59	1.7

[a]Average results from duplicate cultures in the initial trial and triplicate cultures in the confirmatory trial; calculated by our reviewers. Minor differences between reported and recalculated values presumably resulted from the rounding-off of fewer significant digits by our reviewers.

[b]Cloning efficiency (CE) = $\frac{\text{No. of Cells Recovered}}{\text{No. of Cells Plated (200/Dish)}}$; Relative CE = $\frac{\text{CE of Test Group}}{\text{CE of Solvent Control}} \times 100$.

[c]Mutation Frequency (MF) = $\frac{\text{Average Mutant Colonies/5 Dishes}}{\text{No. of Cells Plated } (1\times10^{6}) \times \text{Cloning Efficiency (at selection)}}$

[d]Results from the initial trial

[e]Value in () = absolute CE of the solvent control cultures.

[f]Results from the confirmatory trial

[g]Findings with comparable doses +1, 2, or 5% S9 did not suggest a mutagenic effect.

[h]One of the duplicate cultures was contaminated and not plated for mutant selection or survival.

[i]Lower levels (50 or 75 µg/mL +10% S9) did not suggest a mutagenic effect.

[j]Two of 15 selection plates were contaminated and not scored.

[k]No cells survived exposure to the highest assayed dose (300 µg/mL).

Note: Data were extracted from the study report, pp. 17-19 and 24-26.

MAMMALIAN CELLS IN CULTURE GENE MUTATION

D. REVIEWERS' DISCUSSION AND INTERPRETATION OF RESULTS: We assess that MON 097 (50-300 µg/mL) was comprehensively evaluated and failed to induce a mutagenic response at the HGPRT locus in CHO cell in both the absence and presence of varying levels of S9 (1-10%) in the S9-cofactor mix. Cytotoxicity was clearly demonstrated at ≥200 µg/mL -/+10% S9 in the confirmatory trial. Additionally, the sensitivity of the test system to detect mutagenesis was adequately demonstrated by the results obtained with the nonactivated (200 µg/mL EMS) and S9-activated (1 µg/mL B(a)P +1-10% S9 or 100 µg/mL DMN +10% S9) positive controls. We conclude, therefore, that the study provided valid evidence that MON 097 is not mutagenic in this mammalian gene mutation assay.

E. QUALITY ASSURANCE MEASURES: Was the test performed under GLP? Yes. (A quality assurance statement was signed and dated May 23, 1990).

CORE CLASSIFICATION: Acceptable. The study satisfies the requirements for FIFRA Test Guideline 84-2 for *in vitro* mutagenicity (mammalian cell gene mutation) data.

FINAL

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DATA EVALUATION REPORT

PJ2 ACETOCHLOR METABOLITE
~~MON 097 (PJ2)~~ 6/29/93

Study Type: Mutagenicity: Microbial/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	Nancy E. McCarroll, B.S.	Date 6/23/93
Independent Reviewer	Lynne T. Haber, Ph.D.	Date 6/23/93
QA/QC Manager	Sharon Segal, Ph.D	Date 6/23/93

Contract Number: 68D10075
Work Assignment Number: 2-110
Clement Number: 334
Project Officer: Caroline C. Gordon

039870

MUTAGENICITY STUDIES

EPA Reviewer: Stephen C. Dapson, Ph.D.
Review Section I, Toxicology
Branch II/HED (H7509C)

Signature: Stephen C. Dapson
Date: 6/29/93

EPA Mutagenicity Secondary
Reviewer: Byron T Backus, Ph.D.
Review Section II, Toxicology
Branch II/HEAD (H7509C)

Signature: Byron T. Backus
Date: 6/29/93

DATA EVALUATION REPORT

CHEMICAL: PJ2 Acetochlor metabolite

Tox Chem. Number: 003B (Acetochlor)

PC Code: 121601 (Acetochlor)

STUDY TYPE: Mutagenicity: Microbial/mammalian microsome plate incorporation assay

MRID NUMBER: 427131-18

SYNONYMS/CAS NO: ~~MON-097~~ NONE BLM 6/29/93

SPONSOR: ICI Americas, Inc., Wilmington, DE/Monsanto Agricultural Co., St. Louis, MO/Acetochlor Registration Partnership c/o Zeneca Ag Products, Wilmington, DE

TESTING FACILITY: ICI Central Toxicology Laboratory, Cheshire, UK

TITLE OF REPORT: PJ2 Acetochlor Metabolite - An Evaluation of Mutagenic Potential Using *S. typhimurium* and *E. coli*.

AUTHOR(S): R.D. Callander

STUDY NUMBER: Report No. CTL/P/3358; Study No. YV 2989

REPORT ISSUED: July 2, 1991

CONCLUSIONS--EXECUTIVE SUMMARY: Negative for inducing reverse gene mutation in *Salmonella typhimurium* TA1535, TA1537, TA98, and TA100 and *Escherichia coli* WP2 *uvrA* exposed to a concentration range of 100 to 5000 µg/plate +/-S9. Also negative for *S. typhimurium* TA1538 and *E. coli* WP2 in the nonactivated phase of testing but equivocal for these strains in the presence of S9 activation. Significant and dose-related increases in revertant colonies, which were, <2-fold over control, were seen at 2500 and 5000 µg/plate +S9 in both trials. The two independent trials that were conducted utilized a plate incorporation (Trial 1 and nonactivated phase of Trial 2) and a pre-incubation (S9-activated phase of Trial 2) protocol.

STUDY CLASSIFICATION: Acceptable. The study satisfies the requirements of FIFRA Test Guideline 84-2 for in vitro mutagenicity (bacterial reverse gene mutation) data.

A. MATERIALS:

1. Test Material: PJ2 Acetochlor Metabolite

Description: Off-white powder
Identification number: Batch reference number: ASW-1351-R
Purity: 96%
Receipt date: Not listed
Stability: Reported by the sponsor to be stable at 4°C, and under the conditions of use.
Contaminants: None listed
Solvent used: Dimethyl sulfoxide (DMSO)
Other provided information: It was assumed that the test material was stored at 4°C. The frequency of stock solution preparation was not specified; however, the study author stated that "fresh stock solutions and dilutions were prepared as necessary for each experiment." Analytical determinations were not conducted to determine actual concentrations.

2. Control Materials:

Negative: None

Solvent/final concentration:	DMSO/100 µL/plate--plate incorporation assay 20 µL/plate--pre-incubation assay
Positive:	
N-Methyl-N'-nitro-n-nitroso-guanidine (MNNG)	1, 2, 5 µg/plate TA1535, TA100 0.5, 1, 2 µg/plate E. coli WP2, WP2 uvrA,
ICR 191	0.5, 1, 2 µg/plate TA1537
Daunomycin (DR)	0.2, 0.5, 1 µg/plate TA98
4-Nitro-o-phenylene diamine (4NPD)	1, 2, 5 µg/plate TA1538
Activation:	
2-Aminoanthracene (2-AA)	0.5, 1, 2 µg/plate TA1535,TA1537 0.2, 0.5, 1 µg/plate TA1538,TA98, TA100 5, 10, 20 µg/plate E. coli WP2 1, 2, 5 µg/plate E. coli WP2 uvrA

3. Activation: S9 derived from Alderly Park (Alpk:APfSD) rats

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

The rat liver S9 homogenate was prepared by the performing laboratory and the S9 mix contained the following components:

Component:	Concentration
Na_2HPO_4	100mM
KCl	33mM
Glucose-6-phosphate	5mM
NADP (NA salt)	4mM
$MgCl_2$	8mM
S9 homogenate	10%

4. Test Organism Used: S. typhimurium strains

_____ TA97	x TA98	x TA100	_____ TA102
_____ TA104	x TA1535	x TA1537	x TA1538

List any others: E. coli WP2 and WP2 uvrA

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

5. Test Compound Concentrations Used:

a. Preliminary genotoxicity assay: Not performed.

b. Mutation assay: Two independent nonactivated and S9-activated mutation assays were performed with six doses (100, 200, 500, 1000, 2500 and 5000 µg/plate). Triplicate plates were prepared per dose per strain per condition. Duplicate plates were prepared for each positive control set and five replicates were prepared for each negative/solvent control set.

B. TEST PERFORMANCE:

1. Type of Salmonella Assay:

- x Standard plate test (Trials 1 and nonactivated phase of Trial 2)
- x Pre-incubation (__) minutes (S9-activated phase of Trial 2)
- ___ "Prival" modification
- ___ Spot test
- ___ Other (describe)

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2. Mutation Assays:

(a) Plate Incorporation Test: Two milliliter volumes of top agar[a] were added to bijou bottles containing 0.1 mL of an overnight broth culture of the appropriate tester strain, 0.5 mL of S9 mix buffer or 0.5 mL of S9 mix and 0.1 mL of the appropriate test material dose, solvent, or positive control.

The contents of the bottles were mixed, poured over Vogel-Bonner minimal medium E, and incubated at 37°C for 3 days. At the end of incubation, the background lawn of growth was examined and revertant colonies were counted. Means and standard deviations were determined.

(b) Pre-incubation Test: Similar procedures were used for the pre-incubation phase of testing with the following exceptions:

- Test material and positive control doses and the solvent control were added to the bijou bottles in 0.02 mL volumes, and the volume was brought to 0.1 mL with phosphate buffered saline.
- Prior to the addition of top agar, reaction mixture were pre-incubated with shaking for 60 minutes at 37°C.

3. Evaluation Criteria:

(a) Assay validity: The assay was considered valid if the following criteria were met: (1) the concurrent solvent control data were "acceptable"; (2) the positive control data showed unequivocal positive results and; (3) the lowest dose showed no evidence of cytotoxicity and at least three doses showed no significant cytotoxicity.

(b) Positive response: The test material was considered positive if: (1) there was a reproducible statistically significant dose-related increase in mean revertant colonies/plate and; (2) there was a reproducible statistically significant, ≥2-fold increase in revertant colonies for at least one dose level.

4. Statistical Methods: The data were evaluated for statistical significance at $p<0.01$ using either a one-tailed Student's t-test or Donnett's t-test.

C. REPORTED RESULTS: There was no indication in the report that the test material was insoluble at any dose. Selected data from the two trials are presented in Tables 1 and 2. In general, the two highest nonactivated

[a]Top agar was supplemented with histidine and biotin for the S. typhimurium strains or with tryptophan for the E. coli strains.

TABLE 1. Representative Results of the Initial Microbial/Mammalian Microsome Mutation Assay with PJ2 Acetochlor Metabolite--Plate Incorporation Protocol

Substance	Dose per Plate	S9 Activation	Revertants per Plate of Microbial Tester Strains[a]						
			S. typhimurium					E. coli	
			TA1535	TA1537	TA1538	TA98	TA100	WP2	WP_2 uvrA
Solvent Control									
DMSO	100 µL	-	18.8±3.8	4.6±0.9	10.4±1.1	20.0±3.2	±14.2	32.2±4.7	99.8±7.2
	100 µL	+	10.0±2.5	4.4±2.3	8.2±1.9	20.2±7.8	104.6±9.4	39.4±7.9	115.2±10.8
Positive Controls[b]									
MNNG	2 µg	-	93.5±17.7	--	--	--	631.0±15.6	2380.5±72.8	2214.5±95.5
ICR 191	1 µg	-	--	93.0±2.8	--	--	--	--	--
4NPD	2 µg	-	--	--	223.5±12.0	--	--	--	--
DR	1 µg	-	--	--	--	237.5±17.7	--	--	--
2AA	1 µg	+	249.0±17.0	41.5±2.1	1041.0±62.9	2141.5±62.9	1831.0±58.0	--	673.5±191.6
	5 µg	+	--	--	--	--	--	94.0±5.7	--
Test Material									
PJ2 Acetochlor metabolite	2500 µg[c,d]	-	15.0±1.7	1.3±0.6	6.7±1.2	14.7±5.5	27.0±7.0	26.0±6.6	82.3±2.5
	5000 µg[d]	-	12.0±1.0	0.3±0.6	5.0±1.7	11.0±1.7	39.7±3.2	14.7±3.8	61.0±8.7
	2500 µg[c]	+	10.0±4.0	3.3±0.6	9.7±4.9	19.7±1.5	119.0±5.3 (1.1)[e]	49.3±3.1 (1.3)[e]	125.7±3.8
	5000 µg	+	12.0±3.5	2.7±2.1	13.0±5.3 (1.6)[f]	22.0±7.2	103.0±24.6	57.3±4.7 (1.5)[e]	110.7±7.1

[a]Means and standard deviations of counts from five plates--solvent control, duplicate plates--positive controls, and triplicate plates--test material doses

[b]Three levels of each positive control were assayed; results for all strains were generally significant ($p<0.01$) at the majority of doses. The presented data were selected as representative.

[c]Results for lower doses (100, 200, 500, or 1000 µg/plate +/-S9) did not suggest a mutagenic effect.

[d]Reduced background lawn of growth for all strains at these doses

[e]Increases at these levels were significant ($p\leq0.05$); values in () are the fold increase.

[f]Nonsignificant but 1.6-fold increase over background

Abbreviations:

DMSO = Dimethyl sulfoxide
MNNG = N-Methyl-N'-nitro-n-nitrosoguanadine
4NPD = 4-Nitro-O-phenylenediamine
DR = Daunomycin
2AA = 2-Aminoanthracene

Note: Data were extracted from the study report, pp. 17-25

TABLE 2. Representative Results of the Repeat Microbial/Mammalian Microsome Mutation Assay with PJ2 Acetochlor Metabolite--Plate Incorporation Protocol--Nonactivated Phase--Preincubation Protocol--S9-Activated Phase

Substance	Dose per Plate	S9 Activation	Revertants per Plate of Microbial Tester Strains[a] S. typhimurium TA1535	TA1537	TA1538	TA98	TA100	E. coli WP2	E. coli WP_2 uvrA
Solvent Control									
DMSO	100 µL	-	24.6±7.3	6.2±2.9	13.3±6.7	15.4±3.9	93.0±13.2	31.2±5.3	96.6±6.7
	100 µL	+	11.2±1.9	2.6±0.9	10.6±3.2	17.0±4.3	82.6±9.2	32.0±2.4	118.2±5.6
Positive Controls[b]									
MNNG	2 µg	-	557.5±345.8	--	--	--	809.5±85.6	2756.5±217.1	2008.0±70.7
ICR 191	1 µg	-	--	82.0±1.4	--	--	--	--	--
4NPD	2 µg	-	--	--	200.5±87.0	--	--	--	--
DR	1 µg	-	--	--	--	217.0±41.0	--	--	--
2AA	1 µg	+	224.0±22.6	66.0±1.4	3732.0±315.4	3732.0±270.1	4199.5±225.1	--	657.5±2.1
	5 µg	+	--	--	--	--	--	55.0±7.1	--
Test Material									
PJ2 Acetochlor metabolite	2500 µg[c,e]	-	22.3±2.9	5.7±0.6	11.5±0.7[d]	12.0±2.0	78.7±4.9	25.3±0.6	63.0±1.7
	5000 µg[e]	-	19.0±5.6	6.7±4.0	8.3±5.5	11.0±2.0	54.7±8.1	18.0±4.6	43.5±19.1[d]
	2500 µg[c]	+	10.7±7.4	2.7±1.5	15.3±1.2 (1.4)[f]	19.0±1.7	79.0±4.4	39.0±4.6 (1.2)[f]	127.7±6.7 (1.1)[f]
	5000 µg[e]	+	14.0±7.0	2.7±2.1	19.3±2.9 (1.8)[f]	19.7±1.5	85.3±4.2	44.0±1.7 (1.4)[f]	146.0±15.6 (1.2)[f]

[a]Means and standard deviations of counts from five plates--solvent control, duplicate plates--positive controls, and triplicate plates--test material doses

[b]Three levels of each positive control were assayed; results for all strains were generally significant ($p<0.01$) at the majority of doses. The presented data were selected as representative.

[c]Results for lower doses (100, 200, 500, or 1000 µg/plate +/-S9) did not suggest a mutagenic effect.

[d]One plate was reported to be contaminated, presented data are from the counts of duplicate plates.

[e]Reduced background lawn of growth for all strains at these doses

[f]Significant ($p\leq0.05$) increases noted at these levels; values in () are the fold increase over control

Abbreviations:

DMSO = Dimethyl sulfoxide
MNNG = N-Methyl-N'-nitro-n-nitrosoguanadine
4NPD = 4-Nitro-O-phenylenediamine
DR = Daunomycin
2AA = 2-Aminoanthracene

Note: Data were extracted from the study report, pp. 19, 20, and 26-31.

doses (2500 and 5000 µg/plate) induced marginal to moderate cytotoxic effects in the majority of strains. The background lawn of growth for all strains was also reported to be sparse at these nonactivated levels. With the exception of a nonreproducible reduction in revertant colonies of strain TA1537 at 5000 µg/plate + S9 (plate incorporation assay) and a thinning of the background lawn of growth of all strains at this dose in the pre-incubation assay, cytotoxicity was not clearly shown in the S9-activated phase of testing. In the first trial, significant and dose-related increases in tryptophan revertants of E. coli WP2 were noted at 2500 and 5000 µg/plate +S9 (1.3- and 1.5-fold over control, respectively). A noticeable but not significant increase in histidine revertants of S. typhimurium TA1538 (1.6 fold) was also reported at the highest S9-activated level. Using the preincubation protocol for the S9-activated phase of the repeat trial, significant increases in revertant colonies of E. coli WP2 were again noted at 2500 and 5000 µg/plate (1.4- and 1.2-fold higher than control, respectively). The earlier findings with S. typhimurium strain TA1538 were essentially duplicated in the second assay with significantly increased numbers of revertants at 2500 and 5000 µg/plate +S9 (1.4- and 1.8-fold increases, respectively). The study author stated that the apparent statistical significance observed with strains TA1538 and WP2 (+S9) was not confirmed by a full evaluation using Dunnett's method. However, the data showing the statistical analyses, which the author stated was included in the study report, were missing. Based on the results of these statistical analyses, the study author concluded that "PJ2 acetochlor metabolite did not induce any reproducible statistically-significant increases in revertant colony numbers in any tester strain, either in the presence or absence of S9."

D. REVIEWERS' DISCUSSION/CONCLUSIONS: We assess that reproducible, significant and dose-related increases in revertant colonies of E. coli WP2 and S. typhimurium TA1538 were observed at comparable doses in the S9-activated phase of both trials. However, the increases were consistently less than 2-fold over control and occurred in strains that detect either base-pair substitution mutations (E. coli WP2) or frameshift mutations (S. typhimurium TA1538). We conclude, therefore, that the findings with these two strains are equivocal. For the remaining strains, PJ2 acetochlor metabolite was assayed over an appropriate range of concentrations (100-5000 µg/plate +/- S9) and failed to induce a mutagenic response in either the plate incorporation or pre-incubation phase of testing. There was also no clearly reproducible evidence of a cytotoxic effect. In addition, the sensitivity of the test system to detect mutagenesis was adequately demonstrated by the results obtained with the positive controls in both trials. Although we assess that the results with strains WP2 and TA1538 were equivocal, we conclude that the study is acceptable.

E. QUALITY ASSURANCE MEASURES: Was the test performed under GLP? Yes. (A Quality Assurance Statement was signed and dated June 25, 1991.)

GUIDELINE §84: MUTAGENICITY
SALMONELLA/E. coli.

CORE CLASSIFICATION: Acceptable. The study satisfies the requirements for FIFRA Test Guideline 84-2 for in vitro mutagenicity (bacterial reverse gene mutation) data.