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Attachment E

Guideline Series 84: MUTAGENICITY

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DATA EVALUATION REPORT

CHEMICAL: Acetochlor Tox. Chem. No.: 0038

MRID No.: 415651-21 EPA File Symbol:

STUDY TYPE: **Salmonella/mammalian activation gene mutation assay**

ACCESSION NUMBER:

SYNONYMS/CAS No.:

SPONSOR: ICI Americas, Inc., Wilmington, Delaware

TESTING FACILITY: ICI Central Toxicology Laboratory, Cheshire, UK

TITLE OF REPORT: Acetochlor: An Evaluation in the Salmonella Mutation Assay

AUTHOR(S): R.D. Challander & K.P. Priestley

STUDY NUMBER(S): YV2370/VV2423

REPORT ISSUED: July 19, 1989

CONCLUSION(S) - **Executive Summary**:

Acetochlor (89.9% purity) was found to induce reproducible, positive, mutagenic response to TA1538 strains of Salmonella typhimurium with metabolic activation system at 1000 ug/plate. Although this effect was less than 2X background mutation rates in each experiment, the increases in the observed number of revertant colonies in strain TA1538 at 1000 ug/plate in the presence of metabolic activation were statistically significant ($P<0.05$). Therefore, acetochlor gave a weak positive response in this study.

Study: Acceptable

This study satisfies the guideline requirements, 84-2, for a mutagenicity study (gene mutation).

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SALMONELLA

A. **MATERIALS** Acetochlor

1. **Test Material**: Name:
Description (e.g. technical, nature, color, stability):
Brown black liquid
Batch #: B2993/15 Purity: 89.9%
Contaminants: if reported, list in CBI appendix
Solvent used: DMSO
Other comments:

2. **Control Materials**:
Negative: DMSO
Solvent/final concentration:
Positive: Non-activation:

MNNG	[redacted]	1-5	ug/plate TA100, TA1535
Dauno Rubicin	[redacted]	0.2-1	ug/plate TA98, [redacted]
ICR-191	9-Aminoacridine	0.5-2	ug/plate [redacted] TA1537
4-NPD	[redacted]	1-5	ug/plate TA1538

MNNG = N-Methyl-N'-Nitro-N-Nitrosoquanidine; 4-NPD = 4-Nitro-O-Phenylenediamine

Activation:
2-Aminoanthracene (2-anthramine) 0.2 - 1 ug/plate
usually all strains
Other (list):

3. **Activation**: S9 derived from
__X__ Aroclor 1254 __X__ induced Male__ __ rat __X__ liver
__ phenobarbital __ non-induced __ mouse __ lung
__ none __ hamster __ other
__ other __ other
If other, describe below
Describe S9 mix composition (if purchased, give details):

S9 fraction	3 ml	Co-factor solution:		
Sucrose-Tris-EDTA Buffer	7 ml	Na_2HPO_4	100 mM	$MgCl_2$ 8 r
Co-factor solution	20 ml	KCl	33 mM	
		Glucose-6-phosphate	5 mM	
		NADP	4 mM	

4. **Test organisms**: **S. typhimurium** strains
__ TA97 __X__ TA98 __X__ TA100 __ TA102 __ TA104
__X__ TA1535 __X__ TA1537 __X__ TA1538 ; list any others:
Properly maintained? (Y) / N (circle one)
Checked for appropriate genetic markers (rfa mutation, R factor)? (Y) / N (circle one)

5. **Test compound concentrations used**:
Non-activated conditions: 1.6, 8, 40, 200, 1000, & 5000 ug/plate

Activated conditions : Same as non-activated conditions

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B. TEST PERFORMANCE

1. Type of Salmonella assay:
   - x standard plate test
   - ___ pre-incubation (___ minutes)
   - ___ "Prival" modification (i.e. azo reduction method)
   - ___ spot test
   - ___ other (describe in a.)

a. Protocol (brief description, or attach copy to appendix, if appropriate; e.g. include mediums used, incubation times, assay evaluation):

The tests were carried out in accordance with the method described by Maron and Ames (the revised methods for the Salmonella mutagenicity test; Mutation Res. 113, 173-215, 1983). The experimental procedures used for this study is attached (Lab report pages 28-33).

2. Preliminary cytotoxicity assay (include concentration ranges, activation and nonactivation; strain(s) used; reported results, e.g. cytotoxicity indices (effect on background lawn; reduction in revertants) and solubility):

Although the preliminary cytotoxicity test was not performed in this study, an acceptable maximum dosage level of test material (5 mg/plate) has been used.

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3. Mutagenicity assay (reported results, e.g. induction of revertants - individual plate counts and/or summary given; appropriateness of positive and background (concurrent and/or historical) revertant levels; number of concentration levels used; number of cultures per concentration; include representative table, if appropriate):

The mutagenicity of Acetochlor technical in the Ames test was evaluated using five tester strains of S. typhimurium (TA98, TA100, TA1535, TA1537 & TA1538) at 6 concentrations (1.6 to 5000 ug/plate) either in the presence or absence of metabolic activation system from three separate experiments. Results obtained from these three experiments, the compound did not induce any significant, reproducible increases in the numbers of revertant colonies in strains TA1535, TA1537, TA98 and TA100 either in the presence or absence of metabolic activation system (S9 activation). However, significant positive responses ($P<0.05$) were repeatedly observed in strain TA1538 at 1000 ug/plate in the presence of S9 mix (See results of the 2nd and 3rd Experiments in Tables 2 and 3). Although less than 2X background mutation rates were noted in each experiment, the general effect was consistently observed between experiments. In the first experiment (See results in Table 1), the compound induced a significant increase ($P<0.01$) in the observed number of revertant colonies in strain TA1538 at 1000 ug/plate in the absence of S9 mix. This effect was not reproduced in the 2nd and 3rd experiments and was not considered to be compound-induced mutation.

The study Author concluded that "under the conditions of this assay, acetochlor gave a weak but positive, mutagenic response in the presence of auxiliary metabolising system (S9) with Salmonella typhimurium tester strain TA1538."

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4. **Reviewer's discussion/conclusions** (include e.g. rationale for acceptability or not; necessity for repeat, if appropriate; address any discrepancies with author conclusions):

(A) The spontaneous revertant colonies for each of the five tester strains of Salmonella typhimurium are found within the normal ranges of revertant colonies recommended by the Ames Salmonella mutagenicity test (Ames et al., Mutation Res. 31: 347-364, 1975).

(B) The strain specific control compounds (MNNG, ICR191, 4-Nitro--o-phnylenediamine and Dauno Rabicin) and the positive control compound (2-AA) to ensure the efficacy of the activation system have given significantly positive responses as expected. These positive control values demonstrated the sensitivity of the assay system with or without metabolic activation.

(C) Statistically significant increases ($P<0.05$; less than 2X background mutation rates) in the number of revertant colonies for the strain TA1538 were observed in the presence of metabolic activation at 1000 ug/plate in Experiments 2 and 3 (See results given in Tables 2 and 3). We agree with the study Author's conclusion that acetochlor gave a weak but positive response with strain TA1538 in the presence of S9 mix only.

(D) The study was conducted properly to generate valid reults and satisfies the guideline requirements, 84-2, for a mutagenicity study (gene mutation).

5. **Was test performed under GLPs (is a quality assurance statement present)?** (Y) / N **(circle one)**

6. **CBI appendix attached** (Y) / N **(circle one)**