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Data Evaluation Record

Study Type: Gene mutation in CHO/HGPRT cells.

Study Identification: "CHO/HGPRT Gene Mutation Assay with MON 097."

Lab. performing study: Monsanto Environmental Health Lab.  
St. Louis, MO 63110

Sponsor: Monsanto Agricultural Products Co.  
St. Louis, MO. 63167

Study no.: ML-82-281

Project no.: EHL-830013

Accession no.: 071970

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Study director: A.P. Li, Ph.D.

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Conclusions: The submitted study demonstrated that acetochlor was weakly mutagenic in this test system, with or without metabolic activation. Although the trend for a dose-response relationship was statistically significant ( $p < 0.05$ ), the evidence for a dose-dependent effect was minimal.

Classification: Acceptable

Materials

1) Test chemicals: Acetochlor (MON 097), a "yellowish-brown liquid"; sample #T830020; 96.3% a.i.

Positive controls: Ethyl methanesulfonate (EMS)- no metabolic activation  
Benzo(a)pyrene (BP)- with metabolic activation

Metabolic activation: S-9 fraction (9,000 x g supernatant) from Arochlor 1254-induced rat livers, purchased from Litton Bionetics.

(2) Doses tested: Acetochlor- 25, 75, 100, 125, and 150 ug/ml without S-9.  
25, 50, 75, 100, and 125 ug/ml with S-9.

vehicle control- ethanol

positive control- 100 ug/ml EMS without S-9  
1 ug/ml 3-MC with S-9

(3) Test system: CHO cells, cloned K1BH4, originally obtained from Dr. A. W. Hsie of Oak Ridge National Laboratories.

Methods

A photocopy of the submitted methods is appended. The methods were reviewed, and the following point(s) were noted:

(1) The choice of ethanol as a solvent control in this assay is questionable, since incubation of cells with ethanol in the presence of S-9 fraction produced about a two-fold increase in mutation frequency in two separate experiments.

Results/Discussion

The selection of doses and concentration of S-9 fraction was based on preliminary studies which demonstrated an optimum of 10% S-9. The cytotoxicity of acetochlor increased as the concentration of S-9 increased.

Incubation of CHO cells with acetochlor in the presence or absence of S-9 caused an increase in mutation frequency when compared to untreated controls (Table 2, photocopied from submitted study report). Doses of 125 or 150 ug/ml without S-9 produced a 3.8 and 2.7x increase in mutation frequency, respectively, and were judged to be statistically significant ( $p < 0.05$ ). A substantial decrease in relative survival was noted at the highest dose of 150 ug/ml, 39% of control as compared to 71-95% of control at lower concentrations test article.

In the presence of S-9 fraction, a statistically significant increase in mutation frequency of 3x control ( $p < 0.05$ ) was observed only at the high dose of 125 ug/ml. Acetochlor was more cytotoxic in the presence of S-9, as doses of 75, 100, and 125 ug/ml produced decreases in cell survival of 34%, 30%, and 7% of control.

The investigators stated that "[t]he dose-response relationship was found to be linear ( $p < 0.05$ ) for both treatment with and without S-9."

The positive controls induced the appropriate responses, demonstrating that the test system could respond to direct and indirect mutagens.

The submitted data are considered to be evidence of a weak mutagenic potential of acetochlor, as clear evidence of mutagenicity is seen only at cytotoxic concentrations.

Classification: Acceptable