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Attachment F

Guideline Series 84: MUTAGENICITY

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DATA EVALUATION REPORT

CHEMICAL: Acetochlor

Tox. Chem. No.: 003B

MRID No.: 415651-23

EPA File Symbol:

STUDY TYPE: In vivo micronucleus assay in mouse bone marrow

ACCESSION NUMBER:

SYNONYMS/CAS No.:

SPONSOR: ICI Americas, Inc., Agricultural Products, Wilmington, DE 19897

TESTING FACILITY: ICI Central Toxicology Laboratory
Cheshire, UK

TITLE OF REPORT: Acetochlor: An evaluation in the mouse micronucleus test

AUTHOR(S): V. Randall

STUDY NUMBER(S): SM0339

REPORT ISSUED: 7/31/89

CONCLUSION(S) - Executive Summary:

Acetochlor was not clastogenic in the mouse micronucleus test at the dose levels tested.

Dose levels tested: 898 & 1436 mg/kg (males)
1075 & 1719 mg/kg (females)

Deficiencies found: no information on stability and storage conditions of the test material; and no indication of coded slides prior to scoring.

The study may be upgraded if the above missing information can be provided.

Study: Unacceptable

This study does not satisfy the guideline requirements, 84-3, for a mutagenicity study (chromosomal aberrations)

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A. MATERIALS Acetochlor

1. Test Material: Name:
Description (e.g. technical, nature, color, stability):
Brown liquid
Batch #: SC31/88 Purity: 89.4%
Contaminants: if reported, list in CBI appendix
Solvent used: Corn oil
Other comments: CTL Reference No. Y06341/007/001

2. Control Materials:

Negative/Route of administration:

Corn oil/Intragastric route/10 ml/kg

Vehicle/Final concentration/Route of administration:

Positive/Final concentration/Route of administration:

Cyclophosphamide/ Intragastric route / 65 mg/kg (10 ml/kg)

3. Test compound:

Route of administration: Intragastric route
(single dose at volume of 10 ml/kg)

Dose levels used:

898 & 1436 mg/kg (males)
1075 & 1719 mg/kg (females)

4. Test animals:

a. Species mouse Strain C57BL/6JFCD-1/AlPK Age 9-14 weeks
Weight _____ Source: 8-11 weeks
Phase 1 & 2,

b. No. animals used per dose: 5 males 5 females respectively

c. Properly maintained? (Y) / N (circle one)

B. TEST PERFORMANCE

1. Treatment and Sampling Times:

a. Test compound

Dosing: X once _____ twice (24 hr apart)
_____ other (describe):

Sampling (after last dose): _____ 6 hr _____ 12 hr
X 24 hr X 48 hr X 72 hr (mark all
that are appropriate)
_____ other (describe):

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b. Negative and/or vehicle control

Dosing: X once _____ twice (24 hr apart)
_____ other (describe):

Sampling (after last dose): _____ 6 hr _____ 12 hr
X 24 hr X 48 hr X 72 hr (mark all
that are appropriate)
_____ other (describe):

c. Positive control

Dosing: X once _____ twice (24 hr apart)
_____ other (describe):

Sampling (after last dose): _____ 6 hr _____ 12 hr
X 24 hr _____ 48 hr _____ 72 hr (mark all
that are appropriate)
_____ other (describe):

2. Tissues and Cells Examined:

X bone marrow _____ other (list):

No. of polychromatic erythrocytes (PCE) examined per animal: 1000

No. of normochromatic erythrocytes (NCE; more mature RBCs)
examined per animal: 1000

Other (if other cell types examined, describe):

3. Details of slide preparation:

At the end of specified intervals following dosing, bone marrow smears were prepared and stained with polychrome methylene blue and eosin (details of slide preparation were not given).

4. Preliminary cytotoxicity assay (reported results, e.g. include dose range, signs of toxicity - e.g. MTD considerations, clinical signs; no. animals):

Acetochlor was initially administered as a single intragastric dose to two groups of 2 female mice at 2000 and 3000 mg/kg. Both animals survived at the 2000 mg/kg dose level whereas both died at the 3000 mg/kg level. Then, two groups of 5 male and 5 female mice were dosed at 2000 and 3000 mg/kg for the cytotoxicity study. Three males and one female died at the 2000 mg/kg level and all animals died at the 3000 mg/kg level. Again, another group of five male and 5 female mice

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were dosed at 1000 mg/kg and no deaths were observed in either sex at this dose level. Based on these results, a dose level of 1436 mg/kg or 1719 mg/kg was selected as the highest dose for males or females, respectively, in the micronucleus test.

5. Micronucleus assay (reported results, e.g. include induction of micronuclei; appropriateness of negative, solvent and positive control micronucleus frequencies; ratio of PCE/NCE; sex differences (if any); appropriateness of dose levels and route; statistical evaluation; include representative table, if appropriate):

Significant increase ($P < 0.05$) in the frequency of MPE was observed in the female mice at 1719 mg/kg dose level (24 hour sampling time) (See results given in Table 2). However, no statistically significant increases in the frequency of MPE were observed at either dose level (50% MLD: 898 mg/kg, Males; 1075 mg/kg, Females; 80% MLD: 1436 mg/kg, Males; 1719 mg/kg, Females) of acetochlor at any of the sampling times (24, 48, or 72 hours) investigated when the sexes were combined (See results given in Table 1). In order to assess the validity of this effect, a further 2000 PE were examined for females at both dose levels of acetochlor (1075 and 1719 mg/kg) and the solvent control at the 24 hour sampling time. Following the extended counts there were no statistically significant difference between the test groups and the solvent control whether the extended counts were analyzed alone (as 2000 PE) (See results given in Table 3), or combined with the original counts (as 3000 PE) (Table 4). The positive control compound (CP) induced significant increase ($P < 0.01$) in the frequency of MPE in both male and female mice as expected.

When the sexes were combined, significant reductions ($P < 0.01$) in the ratios of PCE:NCE (expressed as % PCE) were observed in the high dose group (i.e., 80% MLD) at the 24 hour sampling time and in both levels (i.e., 50% MLD & 80% MLD) of acetochlor at the 72 hour sampling time (See results given in Table 5). Significant reductions ($P < 0.01$) in the ratios of PCE:NCE (expressed as % PCE) were also observed in male mice at 1436 mg/kg dose level at the 24 hour sampling time, in female mice at 1075 mg/kg level and both sexes at the high dose level (i.e., 1436 mg/kg for males; 1719 mg/kg for females) at the 72 hour sampling time (Table 6). These results suggest that acetochlor induced a cytotoxic response in the bone marrow.

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6. 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 positive control compound, cyclophosphamide, apparently induced significant increases of the PCE with micronuclei ($P < 0.01$) in the bone marrows of both males and females, indicating the sensitivity of the assay system to detect a clastogen.

(B) The spontaneous rates of micronuclei in the PCE of the vehicle control were found from 0.26% (females) to 0.1% (males) in this study. These results are within the normal range for performing the mouse micronucleus test as recommended by Heddle et al. (Mutation Res. 123: 61-118, 1983).

(C) The test compound has been tested to cytotoxicity level (1436 mg/kg for males and 1719 mg/kg for females) as evidenced by reducing the ratios of PCE:NCE (expressed as %PCE) in the bone marrows of high dose male and female mice.

(D) Sampling times (i.e., 24, 48, & 72 hour intervals) used were adequate in this mouse micronucleus assay.

(E) However the evaluation of this study cannot be accomplished due to the following deficiencies:

- (a) Information on the stability and storage conditions of the test material were missing; and
- (b) The report did not indicate whether slides were coded prior to scoring.

Based on the above deficiencies, the study is not fully acceptable in the present form and may be upgraded on resolution of the reported deficiencies. Therefore, this study does not satisfy the guideline requirements, 84-3, for a mutagenicity study (chromosomal aberrations).

7. Was test performed under GLPs (is a quality assurance statement present)? ☒ / N (circle one)

8. CBI appendix attached ☒ / N (circle one)