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Attachment G

Guideline Series 84: MUTAGENICITY

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IA EVALUATION REPORT

CHEMICAL: Acetochlor **Tox. Chem. No.:** 003B

EPA File Symbol:

STUDY TYPE: **Mammalian cells in culture cytogenetics assay** in human lymphocytes

ACCESSION NUMBER:
MRID No.: 415651-22
SYNONYMS/CAS No.:

SPONSOR: ICI Americas Inc., Wilmington, Delaware 19897

TESTING FACILITY: ICI Central Toxicology Laboratory, Cheshire, UK

TITLE OF REPORT: An evaluation in the in-vitro cytogenetic assay with Acetochlo in human lymphocytes

AUTHOR(S): C.A. Howard

STUDY NUMBER(S): SV0336

REPORT ISSUED: July 20, 1989

CONCLUSION(S).- Executive Summary:

Technical acetochlor was clastogenic in cultured human lymphocytes at 100 ug/ml in both the presence and absence of rat S9 mix activation and at 50 ug/ml without metabolic activation .

Dose levels tested: 10, 50, 100 ug/ml

Classification: Acceptable

This study satisfies the Guideline Requirements, 84-3, for a mutagenicity study (chromosomal aberrations)

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IN VITRO MAMMALIAN CYTOGENETICS

A. MATERIALS Acetochlor Technical

1. Test Material: Name:
Description (e.g. technical, nature, color, stability):
a brown liquid
Batch #: A1016/9 Purity: 89.4%
Contaminants: if reported, list in CBI appendix
Solvent used: DMSO
Other comments:

2. Control Materials:
Negative: DMSO
Solvent/final concentration:
Positive: Non-activation (concentrations, solvent):
Mitomycin C/0.5 ug/ml/physiological saline (0.85%)

Activation (concentrations, solvent):
Cyclophosphamide/100 ug/ml /physiological saline (0.85%)

3. Activation: S9 derived from Alpk:APFSD albino rat

X	Aroclor 1254	___	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):
Final concentration in S9-mix (mM): Na_2HPO_4 75 mM; KCl 25 mM; Glucose-6-phosphate 4 mM; NADP 3 mM; $MgCl_2$ 6 mM

4. Test compound concentrations used:
Non-activated conditions: 10, 50, & 100 ug/ml

Activated conditions: 10, 50, & 100 ug/ml

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5. Test Cells: mammalian cells in culture
Describe cell line, cell strain or primary cell culture (if human lymphocytes, describe conditions of subjects) used:

Human blood was drawn aseptically from two healthy donors, donor 1 who is male and donor 2 who is female, both donors having a previously established low incidence of chromosomal damage. Cultures were initiated with phytohemagglutinin (0.1 mg/ml) and maintained in supplemented RPMI 1640 tissue culture medium at 37° C.

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

Cell line or strain periodically checked for Mycoplasma contamination? Y / N (circle one) Not applicable

Cell line or strain periodically checked for karyotype stability? Y / N (circle one) Not applicable

B. TEST PERFORMANCE

1. Cell treatment:
a. Cells exposed to test compound for:
2.5-~~3.5~~ hours (non-activated) 2.5-~~3.5~~ hours (activated)

b. Cells exposed to positive controls for:
2.5-~~3.5~~ hours (non-activated) 2.5-~~3.5~~ hours (activated)

c. Cells exposed to negative and/or solvent controls for:
2.5-~~3.5~~ hours (non-activated) 2.5-~~3.5~~ hours (activated)

2. Protocol (brief description, or attach copy to appendix, if appropriate; include e.g. number of cell cultures; medium; incubation times; if lymphocytes, nature of mitogen and when added; cell density during treatment; harvest times; spindle inhibitor and when used; chromosome preparation and analysis; number of cells/culture analyzed; statistics used):

The test protocol used was based on the criteria established by Scott et al. (In-vitro chromosome aberration assays: In: Brian J. Dean (Ed) Report of UKEMS Sub-Committee onguidelines for mutagenicity testing, United Kingdom Enviromental Mutagen Society (Page 19-22).

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3. Preliminary cytotoxicity assay (include concentration ranges, activation and nonactivation; reported results, e.g. cytotoxicity and solubility; rationale for determining harvest times (e.g. alterations in cell cycle) and concentration levels, if reported):

At 44 hours after culture initiation, the test sample of acetochlor was administered to duplicate cultures from donors 1 and 2 at concentrations ranging from 3-900 ug/ml growth media, from which an appropriate dose range range was selected for the main study. The top dose was determined by the toxicity of this solution to reduce the mitotic index. In the absence of metabolic activation, the mitotic index in cultures (donors 1 & 2) treated with 100 ug/ml of acetochlor was reduced to 35.1-40.8% of the concurrent control values (See results given in Table 1). Therefore, a range of dose levels (100, 50, & 10 ug/ml) was selected for the cytogenetic test with 100 ug/ml as the highest concentration.

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4. **Cytogenetics assay (reported results, e.g. induction of aberration frequency; types of aberrations, e.g. whether gaps are included in analysis or not, chromatid vs. chromosomal events, complex aberrations; positive and background aberration frequencies; number of cultures per concentration; levels of cytotoxicity obtained, e.g. effect on mitotic index or cell survival, if examined; include representative table, if appropriate):**

Technical acetochlor was found to induce significant increases($P<0.05$) in the incidences of chromosomal damage at dose level of 100 ug/ml in both the presence or absence of metabolic activation (See results provided in Tables 1 & 2). In the absence of metabolic activation, acetochlor also demonstrated significant increases in the incidences of chromosomal damage at 50 ug/ml. The positive control compounds (0.5 ug/ml Mitomycin C and 100 ug/ml cyclophosphamide) induced significant positive responses ($P<0.01$) in both the presence and absence of metabolic activation as expected (See also results given in Tables 1 & 2).

The study author concluded that"under the conditions of this assay acetochlor is clastogenetic to human lymphocytes in vitro."

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5. **Reviewer's discussion/conclusions (include e.g. rationale for acceptability or not; necessity for repeat, if appropriate; address any discrepancies with author conclusions; remember, do not include gaps in final aberration frequency analysis):**

- o The positive control compounds (Mitomycin C & Cyclophosphamide) adequately demonstrated the sensitivity of the cultured human lymphocytes with or without metabolic activation to detect a clastogenic agent.
- o The number of cells with chromosomal aberrations in the negative (solvent) control group (less than 0.5% metaphases observed) was found within the acceptable range established by the testing laboratory.
- o The test compound, acetochlor, was tested at cytotoxicity level (100 ug/ml)
- o Although the preliminary assessment of cell cycle delay was not conducted in this study, the single harvest time (22.5 hrs posttreatment) for cells exposed to acetochlor in the presence or absence of metabolic activation appeared adequate for the detection of chromosomal aberrations in the cultured human lymphocytes.
- o This study was conducted in a manner to generate valid results. We agree with the study Author's conclusion that acetochlor is clastogenic to human lymphocytes in-vitro at 100 ug/ml in both the presence or absence of metabolic activation and at 50 ug/ml without metabolic activation. This study satisfies the guideline requirements, 84-3, for a mutagencity study (chromsomal aberrations).

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

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

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ACETOCHLOR: AN EVALUATION IN THE IN VITRO CYTOGENETIC ASSAY IN HUMAN LYMPHOCYTES

TABLE 1

CHROMOSOMAL ABNORMALITIES, AND MITOTIC INDEX SHOWN AS A MEAN PERCENTAGE OF THE TOTAL NUMBER OF CELLS ANALYSED PER DOSE LEVEL WITHOUT AUXILIARY METABOLIC ACTIVATION

Treatment Atmosphere Concentration	Mean % Abnormal Cells Excluding Gaps	No. of Aberrations per Cell Excluding Gaps	Mean Mitotic Index (%)
Donor 1			
Dimethylsulphoxide 1µl/ml	0.00	0.000	14.40
Mitomycin C - 0.5µg/ml	24.00**	0.240	9.20Δ
Acetochlor - 100µg/ml	41.33**	1.000	5.05
- 50µg/ml	3.00*	0.030	14.05
- 10µg/ml	1.00	0.010	12.35
Donor 2			
Dimethylsulphoxide 1µl/ml	0.00	0.000	7.10
Mitomycin C - 0.5µg/ml	16.00**	0.160	0.60Δ
Acetochlor - 100µg/ml	9.50**	0.150	2.90
- 50µg/ml	2.50*	0.040	3.25
- 10µg/ml	1.00	0.010	12.00

** Statistically significant increase in chromosomal damage at $p<0.01$ using Fisher's Exact Test (one-sided).

Δ Positive control mitotic index is determined from a single culture.

ACETOCHLOR: AN EVALUATION IN THE IN VITRO CYTOGENETIC ASSAY IN HUMAN LYMPHOCYTES

TABLE 2

CHROMOSOMAL ABNORMALITIES, AND MITOTIC INDEX SHOWN AS A MEAN PERCENTAGE OF THE TOTAL NUMBER OF CELLS ANALYSED PER DOSE LEVEL WITH AUXILIARY METABOLIC ACTIVATION

Treatment Atmosphere Concentration	Mean % Abnormal Cells Excluding Gaps	No. of Aberrations per Cell Excluding Gaps	Mean Mitotic Index (%)
Donor 1			
Dimethylsulphoxide 1µl/ml	1.00	0.010	16.15
Cyclophosphamide - 100µg/ml	44.00**	0.720	5.20Δ
Acetochlor - 100µg/ml	12.67**	0.400	5.10
- 50µg/ml	1.00*	0.010	11.10
- 10µg/ml	0.00	0.000	12.00
Donor 2			
Dimethylsulphoxide 1µl/ml	0.00	0.000	8.15
Cyclophosphamide - 100µg/ml	32.00**	0.320	1.30Δ
Acetochlor - 100µg/ml	16.67**	0.493	5.20
- 50µg/ml	2.00	0.020	9.60
- 10µg/ml	0.00	0.000	9.45

** Statistically significant increase in chromosomal damage at $p<0.01$ using Fisher's Exact Test (one-sided).

Δ Positive control mitotic index is determined from a single culture.

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