Attachment G

Guideline Series 84: MUTAGENICITY

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008478

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 Acetochlor 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 μg/ml in both the presence and absence of rat S9 mix activation and at 50 μg/ml without metabolic activation.

Dose levels tested: 10, 50, 100 μg/ml

Classification: Acceptable

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

A. MATERIALS  Acetochlor Technical

1. Test Material: Name:
   Description (e.g. technical, nature, color, stability):
   a brown liquid
   Batch #: A10169  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 µg/ml/physiological saline (0.85%)
     Activation (concentrations, solvent):
     Cyclophosphamide/100 µg/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): Na2HPO4 75 mM; KCl 25 mM;
   Glucose-6-phosphate 4 mM; NADP 3 mM; MgCl2 6 mM

4. Test compound concentrations used:
   Non-activated conditions: 10, 50, & 100 µg/ml
   Activated conditions: 10, 50, & 100 µg/ml
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? ☑ / N (circle one)

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

Cell line or strain periodically checked for karyotype
stability? ☑ / 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 on guidelines for mutagenicity testing, United
Kingdom Environmental Mutagen Society (Page 19-22).
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 µg/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 µg/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 µg/ml) was selected for the cytogenetic test with 100 µg/ml as the highest concentration.
IN VITRO MAMMALIAN CYTOGENETICS

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 µg/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 µg/ml. The positive control compounds (0.5 µg/ml Mitomycin C and 100 µg/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 clastogenic to human lymphocytes in vitro."
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):

- 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.

- 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.

- The test compound, acetochlor, was tested at cytotoxicity level (100 ug/ml).

- 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.

- 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 mutagenicity study (chromosomal aberrations).

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

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

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

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

\* 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

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

** 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.