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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

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JUL 12 1990

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: EPA ID No.: 3125-341. Evaluation of a Mutagenicity Study (In Vitro Chromosomal Aberration Assay) with SRA 5172 (Methamidophos Technical; Monitor)

Record No.: 263491
HED Project No.: 0-1165
Tox. Chem. No.: 378A

FROM: Krystyna K. Locke, Toxicologist
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THRU: Roger Gardner, Acting Section Head
Section I, Toxicology Branch I (IRS)
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Roger Gardner 7-2-90

Toxicology Branch/HED has completed an evaluation of the following study:

"Mutagenicity Test on SRA 5172 In An In Vitro Cytogenetic Assay Measuring Chromosomal Aberration Frequencies In Chinese Hamster Ovary (CHO) Cells"; Hazleton Laboratories America, Inc.; Report No. 100024; January 19, 1990.
MRID No.: 414614-01

Methamidophos (SRA 5172) was negative for inducing chromosomal aberrations in the presence of the metabolic activation system (S9), but was weakly positive under the nonactivated conditions.

Replicate cultures of CHO cells were tested with methamidophos concentrations (µg/ml of assay medium) as follows: 1) Assays involving 10-hour harvest time: 499-4990 µg/ml with S9 and 624-2500 µg/ml without S9; and 2) Assays involving 20-hour harvest time: 2570-5140 µg/ml and 2100-5250 µg/ml (repeated assay), each without S9. Relative to the negative control

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values, small (8.5 or 6.0%) but statistically significant ($p < 0.01$) increases in the percentage of cells with chromosomal aberrations were observed only at methamidophos concentrations of 5140 µg/ml (first 20-hour assay) and 4200 µg/ml (repeated 20-hour assay).

The concentrations of methamidophos tested and the harvest times used in this study were based on the results of the preliminary cytotoxicity study. Positive controls used in the chromosomal aberration study were Mitomycin C (0.04-1.0 µg/ml), without S9 and cyclophosphamide (25 and 50 µg/ml), with S9.

Because this study provided the first positive mutagenic response for methamidophos, it was submitted to EPA under Section 6(a)(2) of FIFRA.

Classification of study: Acceptable

Guideline Series 84: MUTAGENICITY

Reviewed by: Krystyna K. Locke, Toxicologist Krystyna K. Locke 6/28/90
Section I, Tox. Branch I/IRS (H7509C)
Secondary reviewer: Roger Gardner, Acting Section Head
Section I, Tox. Branch I/IRS (H7509C)
Secondary reviewer: Irving Mauer, Geneticist
Tox. Branch I/IRS (H7509C) 6/26/90

DATA EVALUATION REPORT

CHEMICAL: SRA 1572 (Methamidophos) Tox. Chem. No.: 378A

STUDY TYPE: Mammalian cells in culture cytogenetics assay in Chinese hamster ovary (CHO) cells

ACCESSION or MRID NUMBER: 414614-01

SYNONYMS/CAS No.: Monitor/10265-92-6

SPONSOR: Bayer AG/Mobay Corporation

TESTING FACILITY: Hazleton Laboratories America, Inc.

TITLE OF REPORT: Mutagenicity Test on SRA 5172 In An *In Vitro* Cytogenetic Assay Measuring Chromosomal Aberration Frequencies In Chinese Hamster Ovary (CHO) Cells

AUTHOR(S): Hemalatha Murli

STUDY NUMBER(S): 100024/HLA 10972-0-437

REPORT ISSUED: January 19, 1990

CONCLUSION(S) - Executive Summary:

SRA 5172 (Methamidophos) did not induce chromosomal aberrations in Chinese hamster ovary (CHO) cells, in the presence of metabolic activation, but the results were weakly positive under nonactivated conditions. The concentrations of SRA 5172 tested without metabolic activation were 624-2500 µg/ml of assay medium, with cells harvested 10 hours after treatment (10-hour assay) and 2570-5140 µg/ml, in a 20-hour assay. Because positive results were observed at 5140 µg/ml, this assay was repeated at SRA 5172 concentrations of 2100-5250 µg/ml. In the second 20-hour assay without metabolic activation, positive results were obtained at

4200 µg/ml but not at 5250 µg/ml of SRA 5172. No significant increase in the percentage of cells with chromosomal aberrations was observed in the presence of the metabolic activation system. In these assays, the concentrations of SRA 5172 tested ranged from 499 µg/ml to 4990 µg/ml and only 10-hour harvest time was used. The concentrations of SRA 5172 and the harvest times used in this chromosome aberration assay were based on the results of a preliminary cytotoxicity assay. Mitomycin C and cyclophosphamide were used as positive controls without and with activation, respectively. Replicate cultures were used for each concentration of SRA 5172 tested and single cultures for the negative (solvent) and positive controls.

Classification: Acceptable

A. MATERIALS

1. Test Material: Name: O,S-Dimethyl phosphoramidothioate (SRA 5172; Methamidophos; Monitor)

Description: Clear oily liquid; yellow crystals under refrigeration; readily soluble in water and McCoy's 5a culture medium; stable at room temperature in McCoy's 5a culture medium, in concentrations ranging from 1.0 mg/ml to 600 mg/ml, for at least 24 hours.

Batch No.: 233990379 Purity: 74.5%

Solvent used: McCoy's 5a culture medium, for both SRA 5172 and positive controls.

2. Control Materials:

Negative: McCoy's 5a culture medium.
Solvent/final concentration: 10µl/ml

Positive: Non-activation: Mitomycin C (MMC); 0.5 and 1.0 µg/ml of assay medium, for cells harvested 10 hours after treatment; 0.04 and 0.08 µg/ml, for cells harvested 20 hours after treatment.

Activation: Cyclophosphamide (CP); 25 and 50 µg/ml of assay medium, for cells harvested 10 hours after treatment (only time interval used with activation).

3. Activation: The S9 fraction was derived from the liver of male Sprague-Dawley rats which had been previously treated with Aroclor 1254 (dose not specified). The metabolic activation system consisted of S9 fraction (15µg/ml), NADP (1.5 µg/ml) and isocitric acid (2.7 µg/ml).

4. Test compound concentrations used:

Non-activated conditions: 624, 1250, 1870 and 2500 µg/ml, for cells harvested 10 hours after treatment (10-hour assay); 2570, 3850 and 5140 µg/ml, in 20-hour assay; and 2100, 3150, 4200 and 5250µg/ml in repeated 20-hour assay.

Activated conditions: 499, 1250, 2500, 3750 and 4990 µg/ml, in 10-hour assay (only harvest time used).

5. Test Cells: Mammalian cells in culture (Chinese hamster ovary cells; CHO-WBL). These cells were from a permanent cell line and were originally obtained from the laboratory of Dr. S. Wolff, University of California, San Francisco. The cells have since been recloned to maintain karyotypic stability. This cell line has an average cycle time of 12 to 14 hours with a modal chromosome number of 21.

The CHO cells were grown in McCoy's 5a culture medium which was supplemented with 10% fetal calf serum (FCS). 1% L-glutamine, and 1% penicillin and streptomycin (complete McCoy's culture medium), at about 37° C, in an atmosphere containing about 5% CO_2. The cells were periodically checked for Mycoplasma contamination and karyotype stability.

B. TEST PERFORMANCE

1. Cell treatment:

a. Cells exposed to test compound for: 7.25 or 17.5 hours (non-activated); 2 hours (activated).

b. Cells exposed to positive controls for: 7.25 or 17.25 hours (non-activated); 2 hours (activated).

c. Cells exposed to negative and/or solvent controls for: 7.25 or 17.25 hours (non-activated); 2 hours (activated).

2. Protocol:

This assay was designed to examine cells in the first mitosis after exposure to the test materials, without and with metabolic activation. Replicate cultures were used for each concentration of SRA 5172 tested and single cultures for the negative (solvent) and positive controls. (Details of the protocol as reported in the submission, including statistics used, are in Attachment I.)

3. Preliminary cytotoxicity (range-finding) assay:

This assay was conducted to determine not only the concentration of SRA 5172 but also the optimal harvest times for the chromosomal aberration assay. Cultures were initiated by seeding 0.3 x 10^6 cells per 25 cm^2 flask in 5 ml of complete McCoy's 5a medium. The concentrations of SRA 5172 tested, in a half-log series, ranged from 0.168 µg/ml to 5030 µg/ml, with and without metabolic activation. The range-finding assays were conducted in the same way as the aberration assays, except for the following:

Nonactivated Assay

a. The cultures were incubated with the test material in the presence of 5-bromo-2'-deoxyuridine (BrdUrd), the thymidine analog. BrdUrd was added at a final concentration of 10 µM approximately 2 hours after the initial exposure of the cells to the test material and the incubation was continued for an additional 23-24 hours.

b. Following washing of the cells with buffered saline to remove the test material, fresh complete McCoy's 5a medium containing both Colcemid (0.1 µg/ml) and BrdUrd (10 µM) was added approximately 2.75 hours before the cells were harvested.

c. The concentrations of MMC (positive control) used were 0.25 and 0.50 µg/ml.

Assay with Metabolic Activation

a. Following washing of the cells with buffered saline to remove the test material, complete McCoy's 5a medium containing BrdUrd (final concentration 10 µM) was added to the cultures which were then incubated for approximately 23 hours. Colcemid (final concentration 0.1 µg/ml) was then added for 2.5 hours to collect metaphase cells.

b. Concentrations of CP (positive control) used were 12.5 and 20 µg/ml.

The cultures treated with three highest concentrations of SRA 5172 (503, 1680 and 5030 µg/ml), MMC (0.25 µg/ml), CP (12.5 µg/ml) and negative (solvent) controls were evaluated for cytotoxicity (visual observation of monolayer confluence) and cell cycle kinetics. One hundred consecutive metaphase cells, if available, were assessed for the number of cell cycles through which the cells had progressed while in the presence of BrdUrd.

The following results were observed without metabolic activation when treated cultures were compared with negative (solvent) controls: 1) reduction in the cell monolayer confluence and in visible mitotic cells at SRA 5172 concentration of 5030 µg/ml; and 2) increases in percentage of polyploid cells and cell cycle delay at SRA 5172 concentrations of 1680 and 5030 µg/ml. (See Attachment II, TABLE 1, for details.)

The following results were observed with metabolic activation when treated cultures were compared with negative (solvent) controls: 1) reductions in the cell monolayer confluence at the highest concentrations of SRA 5172 evaluated; and 2) increases in the percentage of polyploid cells at SRA concentrations of 1680 and 5030 µg/ml. (See Attachment II, TABLE 1, for details.)

4. Cytogenetics assay:

In all instances, 200 cells per treatment were scored for cultures exposed to SRA 5172 and for negative (solvent) controls, and 25 cells for positive controls. Although 3-5 concentrations of SRA 5172 and 2 concentrations of positive controls were assayed with 10-hour or 20-hour harvest times, only the following highest concentrations were analyzed for chromosomal aberrations:

IN VITRO MAMMALIAN CYTOGENETICS

Without Activation; 10-Hour Assay:

Concentrations analyzed: 1870 and 2500 µg/ml of SRA 5172 and 0.5 µg/ml of MMC. Relative to the negative control (solvent) cultures, no significant increases in the percentage of cells with aberrations were observed in cultures treated with SRA 5172. As expected, significant ($p < 0.01$) increases in chromosomal aberrations were noted in cultures treated with MMC. Cytotoxicity was not observed in any of the test cultures. (For details, see Attachment III, TABLE 2.)

Without Activation; 20-Hour Assay:

Concentrations analyzed: 2570, 3850 and 5140 µg/ml of SRA 5172 and 0.08 µg/ml of MMC. Findings observed included 1) cytotoxicity (slight reduction in visible mitotic cells) at 5140 µg/ml; 2) large increase in the percentage of polyploid cells at 3850 µg/ml but not at 5140 µg/ml; and 3) small but statistically significant ($p < 0.01$) increase in the percentage of cells with aberrations at 5140 µg/ml. The aberrations observed were mostly complex chromatid rearrangements. (For details, see Attachment III, TABLE 4.) This assay was repeated to verify the positive response seen at 5140 µg/ml.

Without Activation; Repeated 20-Hour Assay:

Concentrations analyzed: 3150, 4200 and 5250 µg/ml of SRA 5172 and 0.08 µg/ml of MMC. Findings observed included 1) cytotoxicity (slight reduction in visible mitotic cells and in cell monolayer confluence) at 4200 and 5250 µg/ml; 2) large increase in the percentage of polyploid cells at 3150 µg/ml, but not at other concentrations; and 3) small but statistically significant ($p < 0.01$) increase in the percentage of cells with aberrations at 4200 µg/ml, but not at 5250 µg/ml. Again, the aberrations observed were mostly complex chromatid rearrangements. (For details, see Attachment III, TABLE 6.)

With Activation; 10-Hour Assay:

Concentrations analyzed: 1250, 2500, 3750 and 4990 µg/ml of SRA 5172 and 25 µg/ml of CP. The only finding noted was cytotoxicity (unhealthy cell monolayer, floating cellular debris and reduction in the cell monolayer confluence) at 3750 and 4990 µg/ml. No significant increases in the percentage of cells with aberrations were observed at the concentrations of SRA 5172 analyzed. Statistically

significant ($p < 0.01$) increases in the percentage of cells with aberrations were observed in cultures exposed to PC. (For details, see Attachment III, TABLE 8.)

5. Reviewer's discussion/conclusions:

This study 1) meets the November 7, 1989 acceptance criteria; 2) is well planned, conducted and reported; and 3) contains only one typographical error* but no obvious deficiencies. This study is, therefore, acceptable.

*The following is stated on page 17 of the submitted report:

"In the second 20-hour assay----. Results were analyzed at 3150, 4200, and 5250 µg/ml----. A weakly significant increase in cells with chromosomal aberrations was observed at 4200 µg/ml but not at 5140 µg/ml." The value of 5140 µg/ml should have been 5250 µg/ml.

6. Was test performed under GLPs (is a quality assurance statement present)? Yes

7. CBI appendix attached? No
According to the registrant (Mobay Corporation), no claim of confidentiality is made on information contained in the submitted report, although each page of the report is stamped PROPERTY OF MOBAY CORPORATION. The tables included in the review of this study (Attachments II and III), as well as parts of the MATERIALS AND METHODS section (Attachment I), are, therefore, not regarded as CBI data.

Attachment I

Methamidophos toxicology review

Page _____ is not included in this copy.

Pages 11 through 20 are not included in this copy.

The material not included contains the following type of information:

___ Identity of product inert ingredients

___ Identity of product impurities

___ Description of the product manufacturing process

___ Description of product quality control procedures

___ Identity of the source of product ingredients

___ Sales or other commercial/financial information

___ A draft product label

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