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

OCT 12 1984

MEMORANDUM

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

SUBJECT: Alachlor RPAR: Mutagenicity Studies [EPA Reg.#'s: 524-285, 524-296, 524-314, 524-316] CASWELL#11

TO: Robert Taylor, PM#25
Registration Division (TS-767C)

FROM: Irving Mauer, Geneticist
Section V, Toxicology Branch
Hazard Evaluation Division (TS-769C)

10/11/84
10/12/84

Two mutagenicity studies were evaluated:

1. Final Report - An Evaluation of the Potential of Alachlor to Induce Unscheduled DNA Synthesis in the In Vivo - In Vitro Hepatocyte DNA Repair Assay.

2. Final Report - In Vivo Bone Marrow Chromosome Study in Rats with Alachlor.

Summary and Conclusions:

Study 1: UDS/HPC - rat repair assay - ACCEPTABLE

Study 2: In vivo bone marrow chromosome aberration assay - rat - UNACCEPTABLE since evidence of systemic absorption and/or transport of effective concentration at target tissue are lacking.

See attached Toxicology Branch Data Reviews.

TOXICOLOGY BRANCH: DATA REVIEW

CHEMICAL: Alachlor

Caswell No.: 11
EPA Chem. No.: 090501

STUDY TYPE: Mutagenicity: in vivo UDS in rat HPC's

CITATION: An Evaluation of the Potential of Alachlor to Induce Unscheduled DNA Synthesis in the In Vivo - In Vitro Hepatocyte DNA Repair Assay.

ACCESSION NO./MRID NO.: 253308/NA

SPONSOR/CONTRACTING LAB.: Monsanto/SRI International
Menlo Park, CA

REPORT NO./DATE: SR-83-293/April 5, 1984

TEST MATERIAL: Technical (Lot #MDLT 1114D), purity = 95.2%.

PROCEDURES: NB: This is the newer, in vivo counterpart to the in vitro Williams UDS assay, the latter involving in vitro exposure of rat hepatocytes isolated from untreated animals. The methods for the present assay have not been standardized (see photocopy of Purpose and Methods, attached to this Review), but the end-point assayed (determination of radioactive-labelled unscheduled DNA synthesis) is the same as for the in vitro assay.

Fischer 344 male rats (presumably adults, but neither age nor body weight was stated) were given single oral doses of 0 (corn oil), 50, 100, 200 and 1,000 mg/kg in corn oil, 2 and 12 hr prior to sacrifice; the HDT is reportedly the approximate (oral ?) LD_{50} for alachlor in rats. 2-Acetylaminofluorene (2AF) served as the positive control. At sacrifice, hepatocytes were isolated, cultured with tritiated thymidine, and microscope slides prepared according to conventional radiolabelling techniques. A minimum of 50 cells per slide and 3 slides per animal per time point (3 animals per test group, and 2 per controls) were scored (i.e., a total of 450 cells/dose/time point) for net nuclear silver grain counts. A test result was considered "positive" if net counts were elevated over negative control by 5 counts or greater.

RESULTS: Compared to a significantly elevated net grain count of 18.7 ± 4.6 for 2AF-treated rat hepatocytes in situ (average % cells "in repair" = 82 ± 11), only the HDT test group which received 1000 mg/kg 12 hr prior to sacrifice showed increased repair over controls. (2.1 ± 2.4 vs - 6.0 ± 1.5, constituting 35 ± 12% of cells vs 0%).

Hence, the authors concluded that alachlor induced DNA damage in hepatocytes at the LD_{50} in this assay, i.e., was "weakly genotoxic" (positive), an assessment with which this reviewer concurs.

EVALUATION: The procedures employed were apparently adequate to generate valid (positive) results, and the study is thus ACCEPTABLE.

TOXICOLOGY BRANCH: DATA REVIEW

CHEMICAL: Alachlor

Caswell No.: 11
EPA Chem. No.: 090501

STUDY TYPE: Mutagenicity: in vivo cytogenetics in rat bone marrow (chromosome aberrations).

CITATION: In Vivo Bone Marrow Chromosome Study in Rats with Alachlor.

ACCESSION NO./MRID NO.: 253308/NA

SPONSOR/CONTRACTING LAB.: Monsanto/Hazleton Laboratories,

REPORT NO./DATE: HL-83-165/March 1, 1984

TEST MATERIAL: Technical (Lot #MDLT-08-02B), purity = 95.4%.

PROCEDURES: Alachlor was administered orally to Sprague-Dawley male and female rats at single dose levels of 0, 100, 333 and 1,000 mg/kg in corn oil, and bone marrow cells processed according to standard cytological procedures for chromosome aberration analysis (clastogenesis). (A photocopy of methods employed is attached to this review.) Cyclophosphamide (CP) served as the positive control substance.

RESULTS: In preliminary range-finding, no changes in mitotic index were apparent at dosages up to 1,300 mg/kg, but clinical effects were observed at doses of 1,000 mg/kg and above (weight loss, chromodacryorrhea, chromorhinorrhea). Compared to significant increases in both % aberrant cells and mean number of aberrations per cell for the CP-treated group, no level of alachlor induced structural or numerical chromosome aberrations when compared to corn oil controls. Hence, the authors concluded that alachlor was neither a clastogen nor an aneugen (altered chromosome modal number) at doses producing adverse clinical effects but no deaths (assuming the HDT approximates the LD_{50}). This reviewer does not concur in this evaluation, since no evidence has been presented that alachlor was absorbed from the gut, and transported to the bone marrow in effective concentrations.

DISCUSSION AND EVALUATION: This study is UNACCEPTABLE, since the following data are lacking:

Positive evidence of (1) absorption of test compound from the gastro-intestinal tract (eg, systemic effects); and/or (2) transport to target tissue (bone marrow). Toxicology Branch recommends repeating the study employing i.p. administration of test compound to assure effective concentration in bone marrow.

SUMMARY

Title: In Vivo Bone Marrow Chromosome Study in Rats with Alachlor.

Conducted by: Hazleton Laboratories, America Inc.
Vienna, VA 22180

Project No.: 241-154

Study No.: HL-83-165

Date of Report: March 1, 1984 (page correction April 16, 1984)

Date of Monsanto Staff Toxicology Review Completed: April 11, 1984

Study Director: Michael G. Farrow

The accompanying report contains the results of the referenced study with alachlor. Quality Assurance reviews were conducted by Hazleton Laboratories America Inc. A review of the data and an evaluation of the conclusions presented in this report are summarized below.

Methods

Alachlor (Lot No. MDLT-08-02B; purity 95.4%) was administered by stomach tube to three groups of 24 male and 24 female Sprague-Dawley (Crl:COBS CD®(SD)BR) rats at dosage levels of 100, 330 and 1000 mg/kg body weight. The dosage levels were determined from the results of preliminary dose range-finding studies (appendices A and B of accompanying report). A fourth group of 24 male and 24 female rats received 5 ml/kg of vehicle only (corn oil) and served as the negative control group. An additional group of six male and six female rats received 40 mg/kg cyclophosphamide and served as the positive control.

Approximately 4, 10, 22 and 46 hours after the administration of alachlor or corn oil, six males and six females of each group received a single, intraperitoneal (IP) injection of 2.0 mg/kg colchicine to inhibit mitosis and arrest the cells in metaphase. Approximately two hours after the injection of colchicine, the animals were sacrificed, the bone marrow cells were aspirated from both femurs of each animal and the cells were processed, fixed, stained and mounted on slides. The positive controls received the IP injection of colchicine at the 22 hour time point only and were sacrificed at 24 hours.

When possible, 300 cells per sex per group were analyzed at each time point for cytogenetic aberrations. Cytogenetic aberrations were classified and characterized as follows:

1) chromatid breaks - including fragments and deletions
2) chromosome breaks - including acentric fragments, deletions and minutes
3) chromatid and chromosome gaps
4) exchanges - rings, dicentrics, translocations, quadriradials and triradials
5) cells with more than 10 aberrations
6) pulverized cells.

Results

In the dosage range-finding study, dosages of alachlor up to 1300 mg/kg produced no change in the mitotic index (number of cells in metaphase/number of cells examined) but caused mild clinical signs such as depression, urine stained fur and chromodacryorrhea and chromorhinorrhea (colored discharge about the eyes and nose). Based on the clinical signs of toxicity the high dosage level was selected to be 1000 mg/kg/day. This was considered to be sufficient as it approximates the oral LD50 value for alachlor in rats.

In the definitive test, no fatalities were observed but similar clinical signs of toxicity as were seen in the range-finding study were noted. In addition, the male animals which received 1000 mg/kg alachlor exhibited statistically significant weight loss at 22 and 46 hours.

When compared with the negative control group, alachlor caused no statistically significant increases in the number of chromosome or chromatid aberrations at any dosage level at any time point. Because no mitotic delay was observed, cells from the 48 hour sacrifice period were not examined. There was no difference in the modal number (average number of chromosomes in the examined metaphases) between alachlor-treated and control animals. Cyclophosphamide, however, caused statistically significant increases in both the percent aberrant cells and the average number of aberrations per cell, thus demonstrating the validity of the assay.

SUMMARY

Title: An Evaluation of the Potential of Alachlor to Induce Unscheduled DNA Synthesis in the In Vivo-In Vitro Hepatocyte DNA Repair Assay.

Conducted by: SRI International, Menlo Park, CA 84025

Project No.: SRI LSC 83-201, 6642

Study No.: SR-83-293

Date of Report: April 5, 1984

Date Monsanto Staff Toxicology Review Completed: April 13, 1984

Study Director: J. C. Mirsalis

The accompanying report contains the results of the referenced study with alachlor. Quality assurance reviews were conducted by Stanford Research Institute (SRI) International. A review of the data and an evaluation of the conclusions presented in this report are summarized below.

Purpose

The purpose of this assay was to assess the potential of the test material to produce DNA damage in mammalian cells using an in vitro technique following in vivo administration of the test material. Hepatocyte primary cultures (HPC) were used as the cell model for the test.

Methods

Alachlor (Lot No. MDLT 1114D; purity 95.2%) was administered by stomach tube to six groups of three male Fischer-344 rats at dosage levels of 50, 200 and 1000 mg/kg body weight, at two and 12 hours prior to sacrifice. The high-dose level of 1000 mg/kg was selected because it is the approximate LD50 for alachlor in rats. Two groups of two male rats received 3 ml/kg corn oil vehicle as the negative control and 50 mg/kg 2-acetylaminofluorene as the positive control, respectively, 12 hours prior to sacrifice. At the appropriate time of sacrifice, hepatocytes were isolated from the livers.

For the DNA repair test, hepatocyte cultures were incubated with ^{3}H-thymidine for four hours, followed by incubation with unlabeled thymidine for 14 to 16 hours. After slide preparation, the slides were dipped in Kodak NTB-2 emulsion and exposed at 20°C for 12-14 days. Cells were stained with 1% methyl-green Pyronin Y.

Nuclear and cytoplasmic tritium-exposed silver grains on the radiographic films were counted using an automatic grain counter. The net nuclear grain counts were determined by subtracting the highest count of two nuclear-sized areas of the cytoplasm from the nuclear count. A minimum of 50 cells/slide, 3 slides/animal, 3 animals/dose group/time point were scored for a total of 450 cells/dose/time point. A test result was considered positive if the net grain counts and percent of cells in repair were markedly elevated above those in the vehicle control group. A cell was considered "in repair" if the net grain count was five or greater.

Results

The average net grain counts and the average number of cells in repair for each alachlor dosage level and control and for each time point are shown in the following table.

Treatment	Dose	Time (hr)	Average Net Grain Count (mean±S.E.)	Average % in Repair (mean±S.E.)
Corn Oil	3.0ml/kg	12	-6.0±1.5	0±0
2-Acetylamino fluorene	50 mg/kg	12	18.7±4.6	82±11
Alachlor	50 mg/kg	2	-6.6±0.4	1±0
		12	-6.5±1.0	3±2
Alachlor	200mg/kg	2	-2.4±4.6	19±18
		12	-5.4±0.8	5±2
Alachlor	1000mg/kg	2	-3.1±3.2	11±11
		12	2.1±2.4	35±12