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

005054

MEMORANDUM:

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCE

SUBJECT: Ronilan Fungicide - EPA Reg. No. 7969-53
Chemical No. 323C

TO: Henry Jacoby
Product Manager (21)
Fungicide - Herbicide Branch
Registration Division (TS-767)

FROM: Carlos A. Rodriguez *CAR 4/22/86*
Review Section VI
Toxicology Branch
Hazard Evaluation Division (TS-769)

THRU: Jane E. Harris, Ph.D. *JEH 4/23/86*
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Applicant: BASF Wyandotte Corporation *4/23/86*
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Present Action:

Review two additional mutagenicity studies for Ronilan (Vinclozolin) and made them part of the files for EPA Reg. No. 7969-53.

Recommendations:

1. Two mammalian mutagenicity studies submitted by BASF Wyandotte Corporation October 23, 1985, were reviewed and evaluated as follows:

- 1) Rat Hepatocyte DNA Repair Study, Report No. 20991 (acceptable).
- 2) U5178Y TK Forward Mutation Study, Report No. E-341 (acceptable).

2. The studies are made part of the files for EPA Reg. No. 7969-53.

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Conclusions:

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Vinclozolin showed negative mutagenic activity in the Primary Rat Hepatocyte UDS assay and weakly positive only at concentrations exceeding solubility in the test medium in the mouse lymphoma forward mutation assay.

The studies are acceptable and satisfy requirements for DNA damage/repair assay in mammalian cells, and gene mutation cells in culture.

1. Study Type: (In vitro) Rat Hepatocyte DNA Repair (HPC-UDS).

Accession No.: 255329.

Sponsor: BASF Aktiengesellschaft, West Germany.

Contracting Lab: Litton Bionetics, Inc., Kensington, MD 20895.

Report No./Date: 20991, January 1984.

Authors: Maria A. Cifone, Ph.D., Marie McKenson.

Test Material: Ronilan (Vinclozolin) 3-(3,5-dichlorophenyl)-5-ethenyl-5-methyl-2,4-oxazolidinedione...>99.5%.

Procedure:

The rat liver primary cells were cultured in William's Medium E (WME). After obtaining a viable cell count, approximately 0.5×10^6 viable cells were seeded onto 25 mm round plastic coverslips in 35 mm culture dishes containing 3 ml of WME plus dexamethasone and 5% serum per disk. The cells were allowed to attach to the coverslips during an incubation period of 1.5 to 2 hours at $37 \pm 2^\circ\text{C}$ in a humidified atmosphere containing about 5% CO_2 . Unattached cells were removed and the cultures refed with WME.

The UDS assay was initiated within 3 hours by replacing the media in the culture disks with 2.5 ml WME containing 1% fetal bovine serum, $1 \mu\text{Ci/ml}^3$ H-thymidine and the test material at the desired concentrations. Eight treatments from 1000 ug/ml to 5.0 ug/ml were selected to cover adequate concentrations range for possible UDS activity. The highest two concentrations were used to measure cytotoxicity. The solvent control (DMSO), the positive control, 2-acetyl amino fluorene (2-AAF) and cytotoxicity test were run concurrently. After 18-19 hours of treatment in a 5% CO_2 incubator at 37°C , the cells were washed with WME, swelled with 1% sodium citrate, fixed in acetic acid ethanol (1:3) dried and treated for the determination of unscheduled DNA synthesis (USD) under oil immersion microscopy.

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Results: (Table No. 1 of Report)

The test material (Vinclozolin) showed to be moderately toxic from 1000 ug/ml to 250 ug/ml (53.0% to 73.3% survival) and weakly toxic at 100 ug/ml and 50 ug/ml (89.5% survival). The test material was inactive in inducing UDS activity at all doses tested and up to cytotoxic doses (500 and 1000 ug/ml).

Conclusion:

The results showed negative mutagenic activity in the UDS assay. The results obtained in this assay is evidenced by adequate general guidelines of the UDS method described by Williams (1).

Core Classification: Acceptable.

Reference:

1. William, G.M., Detection of chemical carcinogens by unscheduled DNA synthesis in rat liver primary cell culture. Cancer Res. 37:1845-1951, 1977.

2. Study Type: Mouse Lymphoma Forward Mutation Assay.

Accession No: 255329.

Sponsor: BASF Aktiengesellschaft, West Germany.

Contracting Lab: Litton Bionetics, The Netherlands.

Report No./Date: E-431, June 1984.

Author: W.F. Witterland.

Test Material: Ronilan (Vinclozolin)3-,5-dichlorophenyl)-5-ethenyl-5-methyl-2,4-oxazolidinedione>99.5%.

Procedure:

The mouse lymphoma cells line, L5178Y TK+/-3.72 clone used in this assay was derived from the Fischer L5178Y line of Dr. Donald Clive, and are maintained in liquid nitrogen and laboratory cultures and checked periodically for the absence of mycoplasma contamination.

The test material in DMSO was tested for mutagenic activity on L5178Y(TK+/-) mouse lymphoma cells in vitro. In the presence of a metabolic activation system, the material was assayed from 10 ug/ml to 600 ug/ml (72.5 to 9.8 per cent relative growth) and in the nonactivation condition from

25 ug/ml to 1,000 ug/ml (71.7 to 17.9 per cent relative growth). Controls (DMSO) treated and untreated and positive control (EMS) ethylmethane sulfonate were run concurrently.

The mutagenicity test procedure used is based on that reported by Clive and Spector, et al. 1975. The L5178Y(TK+/-) cells are treated with the selected doses at a cell density of 3×10^5 cell/ml for 4 hours, then washed and placed in growth medium for 48 to 72 hours (expression time), and cloned for TK+/- mutants in the presence of trifluorothymidine (TFT). Cells which grow to form colonies in the presence of TFT are mutated, either spontaneously or by the action of the test substance to the TK -1-genotype. The induction of viable mutants was quantitated in duplicate sample determinations for each treatment and cultures showing >10% survival evaluation for mutagenic response.

Evaluation:

Mutation Assay:

To be acceptable, the cloning efficiency of the solvent controls should be between 60 and 130%. Values greater than 100% are possible because errors in cell counts and cell divisions in delays between counting and cloning of many cell cultures. Cloning efficiencies in the range of 50% to 70% may be conditionally acceptable.

The minimum acceptable value for the average suspension growth of the average solvent controls for two days is 8.0. Generally in order to call a substance non-mutagenic it should be tested within a dose range which induces 80% to 90% toxicity at the higher limits unless prevented by insolubility.

TK+/- Mouse Lymphoma Mutation assay: a substance is considered mutagenic if it produces a dose-dependent increase in mutation frequency over 3 doses to a level at least 2.5 times the solvent/vehicle control.

Results:

1. Toxicity was seen at doses exceeding 100 ug/ml.
2. In the direct assay, none of the treatments induced significant increases above the background frequency.

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3. In the presence of metabolic activation, there was a significant reproducible increase in mutation frequency. This result was obtained at concentrations of the test material exceeding complete solubility in the assay medium, the sponsor, therefore considers this evidence of mutagenicity activity as uncertain.

Conclusions:

The assays used to evaluate the mutagenic activity of the test compound is supportable by the procedures described by Clive (1975 and 1979).

Core Classification: Acceptable.

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