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

006784

JUL 18 1988

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Mancozeb - Mutagenicity Study Submitted Under Accession No. 406117-01

TB Project No.: 8-0824
Caswell No.: 913A

FROM: Irving Mauer, Ph.D.
Toxicology Branch
Hazard Evaluation Division (TS-769C)

TO: Lois A. Rossi/Susan Lewis, PM Team 21
Fungicide-Herbicide Branch
Registration Division (TS-767C)

THRU: Judith W. Hauswirth, Ph.D., Head
Section VI, Toxicology Branch
Hazard Evaluation Division (TS-769C)

Registrant: Rohm & Haas
Philadelphia, PA

Request

Review and evaluate the following in vitro repair assay:

Mancozeb: In-Vitro Unscheduled DNA Synthesis Assay in Fisher 344 Rat Hepatocytes, conducted by P.J. O'Neill and J.P. Frank of Rohm and Haas' Toxicology Department, R&H Report #88R079, dated April 29, 1988.

TB Conclusion

The study is ACCEPTABLE in demonstrating that the sample of mancozeb (TD87-139) used in this assay was negative for the induction of unscheduled DNA synthesis (UDS) in primary rat hepatocytes at concentrations into the toxic range (2 to 10 ug/mL). Detailed review is attached.

1 of 6

Reviewed By: Irving Mauer, Ph.D., Geneticist
Section VI, Toxicology Branch/HED (TS-769C)
Secondary Reviewer: Judith W. Hauswirth, Ph.D. Head
Section VI, Toxicology Branch/HED (TS-769C)

DATA EVALUATION REPORT

006784

I. SUMMARY

TB Proj. No.: 8-0824
Caswell No.: 913A
MRID No.: N/A
Shaugh. No.: 014504

Study Type: Mutagenicity - DNA Repair in vitro (HPC/UDS)

Accession No.: 406117-01

Chemical: Mancozeb

CAS: 8018-01-7

Synonyms: Dithane® M-45; Manzate® 200

Sponsor: Rohm & Haas
Philadelphia, PA

Testing Facility: Rohm & Haas
Toxicology Department
Spring House, PA

Title of Report: Mancozeb: In-Vitro Unscheduled DNA Synthesis Assay in Fischer 344 Rat Hepatocytes

Authors: P.J. O'Neill and J.P. Frank

Study Number: 88R-079

Date of Issue: April 29, 1988

TB Evaluation/Conclusions:

Negative for the induction of unscheduled DNA synthesis up to toxic concentrations (2 to 10 ug/mL).

Classification (Core-Grade): ACCEPTABLE

2

INFORMATION ON PRODUCT IMPURITIES NOT INCLUDED

II. DETAILED REVIEW

A. Test Material - Mancozeb

Description: (Not given)
Batch (Lot): TD87-139, Lot 76777 [redacted]
Purity (%): 82.4%
Solvent/carrier/diluent: William's Medium E, supplemented with 200 mM glutamine, 50 ug/mL gentamicin and 10% heat-inactivated fetal bovine serum (to constitute William's Medium E Complete, WEC).

B. Test Organisms - Rodent

Species: Rat
Strain: Fischer-344
Age: Adult
Weights: Males (not given)
Females (N/A, only males used)
Source: Charles River Breeding Laboratories

C. Study Design (Protocol) - A formal protocol was not included in the Final Report, but reference was made to "Protocol Number 88P-097" (on file) for this type of study.

Quality assurance measures were included, in a signed statement by H.B. Mathason of the QA Unit, dated April 29, 1988, attesting to periodic audits throughout the course of the study, as well as adherence to GLP.

D. Procedures/Methods of Analysis - Hepatocytes were isolated from an adult Fischer-344 male by perfusing the liver with EGTA and collagenase, according to standardized (referenced) methods. The cells were placed on glass coverslips and incubated with WEC medium for 2 hours at 37 °C to allow for attachment. The WEC was replaced by medium containing 10 uCi/mL tritiated-thymidine (^{3}H-TdR, spec. act. 41 Ci/mmol), following which test substance, or positive control, or solvent control was added. All cultures were then incubated for 18 to 20 hours. The positive control article was 2-acetylaminofluorene (2-AAF), dissolved in 1 percent dimethylsulfoxide (DMSO).

Six coverslips per treatment were exposed to each of 11 concentrations of test article ranging from 0.1 to 1000 ug/mL (corrected for % of active ingredient), or to 4 concentrations of 2-AAF (0.3, 0.6, 1 and 3 ug/mL), or to solvent control (media or DMSO). After the incubation treatment period, one coverslip culture per treatment was refed with fresh (untreated) medium for 2 hours and

assessed for cytotoxicity (cell morphology). The remaining cell cultures were washed, cells swollen by a 10-minute treatment with 1 percent sodium citrate, fixed in Carnoy's Fluid (3 ETOH:1 HAc), and mounted on microscope slides (cell-side out). The slides were dipped in Kodak's NTB-2 nuclear track photographic emulsion, stored in light-tight slide boxes at freezer temperatures (-20 °C) for 7 days, following which they were developed with standard photographic chemicals, dried and stained with H+E. Unscheduled DNA synthesis (UDS) was measured by counting the developed silver grain markers of radiolabeled thymidine incorporation.

All slide preparations were coded, hence were scored "blind." Fifty cells per slide were located, scanned by an automatic counter (Biotran) adjusted to subtract the maximum number of silver grains over a cytoplasmic area the size of a nucleus (i.e., the background) from the nuclear grain count (NNG), and thus calculate the NNG for each cell. The mean NNG + SE for three slides per data point were calculated. [No other statistics were considered necessary by the authors to interpret the results of this assay.]

Cells in scheduled (replicative) DNA synthesis (evident by dense nuclear grains TNTC) were not included in UDS determinations, but were recorded for individual slides.

According to the authors, for an assay to be considered acceptable:

1. The test article must be evaluated at concentrations up to 1000 ug/mL, or the limit of solubility, or the limit of toxicity, whichever is lower.

2. At least three test concentrations must have scorable slides, defined as the presence of at least 50 hepatocytes without excessive cytotoxicity.

3. At least one concentration of the positive control must induce a mean NNG > 0, with at least 20 percent of cells exhibiting a mean NNG $\geq$ 5.

Further, unless all of the following criteria for a positive response are met, the test article would be considered negative for UDS:

1. A dose-related increase over control in mean NNG at at least three concentrations (with all values > 0), and

2. At least one concentration demonstrating a mean NNG $\geq$ 5 in at least 20 percent of cells.

006784

Results:

Hepatocytes were exposed to mancozeb at concentrations of 0.1, 0.2, 0.3, 0.5, 1, 2, 3, 5, 10, 100 and 1000 ug/mL.

Chemical analysis of four stock dose solutions of the test compound (made 2X the final concentrations, i.e., 0.2, 20, 200 and 2000 ppm) revealed concentrations within 4 percent of target (Report Appendix II). Cell viability assessment (by morphological evaluation as well as trypan blue exclusion) of fresh hepatocytes prior to treatment revealed a value of 88 percent viable.

Treatments of 100 and 1000 ug/mL mancozeb exceeded solubility limits (precipitates were evident), while lower doses (2, 3, 5 and 10 ug/mL were toxic (reduced number of viable cells and increased pyknosis).

Nine concentrations of test article ranging from 0.1 to 10 ug/mL, one concentration of 2-AAF (3 ug/mL), and respective controls were scored for UDS (3 slides per treatment). The results were recorded in summary tabulation (Report Table 1, attached) and one illustration (Figure 1), as well as by individual slide data (Report Appendix I). Compared with the marked increase in NNG/cell following treatment with the positive control, 2-AAF (mean, 25.4), none of the test doses (range, -1.3 to 1.0) induced UDS above the control value (1.2). The numbers of cells in replicative (S-phase) DNA synthesis were comparable for all treatments, ranging from 0 to 2.6% for mancozeb vs. 2.0% for media control, and 2.2% for 2-AAF vs. 1.5% for 1% DMSO.

TB Conclusion:

This study was performed with adequate procedures and controls as to generate valid results.

This sample of mancozeb TD87-139, 82.4%, did not induce UDS as evidenced by no increased net nuclear silver grain counts when tested to up to toxic concentrations (2 to 10 ug/mL).

Attachment

TABLE I (Mancozeb Summary Data)

TREATMENT	CONCENTRATION ug/ml	NNG[a]	SE[b]	% S-Phase	SE
Dithane M-45	10	-1.3	c	0.0	c
	5	-0.6	0.5	0.6	0.3
	3	-0.6	0.6	0.2	0.2
	2	-0.6	c	1.1	c
	1	0.4	0.3	1.2	0.4
	0.5	1.0	1.1	1.5	0.5
	0.3	0.1	0.3	1.5	0.3
	0.2	-1.1	0.4	2.6	0.9
	0.1	-0.5	0.7	1.4	0.3
Media Control		1.2	0.5	2.0	1.0
2AAF	3	25.4	6.7	2.2	0.4
DMSO	1%	-1.3	0.3	1.5	0.2

a NNG - Net Nuclear Grains
b SE - Standard Error
c Since there was only 1 scorable slide, there was no SE.