

DATA EVALUATION RECORD

8/2/2000

ZIRAM

24

Study Type: 84-2; Unscheduled DNA Synthesis in Primary Rat Hepatocytes

Work Assignment No. 2-15C (MRID 41287801)

Prepared for

Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
1921 Jefferson Davis Highway
Arlington, VA 22202

Prepared by

Pesticides Health Effects Group
Sciences Division
Dynamac Corporation
2275 Research Boulevard
Rockville, MD 20850-3268

Primary Reviewer:
Steven Brecher, Ph.D.

Signature: _____
Date: _____

Secondary Reviewer:
William J. Spangler, Ph.D.

Signature: _____
Date: _____

Project Manager:
William J. Spangler, Ph.D.

Signature: _____
Date: _____

Quality Assurance:
Mike Norvell, Ph.D.

Signature: _____
Date: _____

Disclaimer

This Data Evaluation Record may have been altered by the Health Effects Division subsequent to signing by Dynamac Corporation personnel.

EPA Reviewer: I. Mauer, PhD, _____, Date _____
Review Section III, Toxicology Branch I (7509C)
EPA Secondary Reviewer: M. Copley, DVM, DABT _____, Date _____
Review Section IV, Toxicology Branch I (7509C)

DATA EVALUATION RECORD

STUDY TYPE: Other Genotoxicity: Unscheduled DNA Synthesis in
Primary Rat Hepatocytes

OPPTS Number: 870.5550 OPP Guideline Number: §84-2

DP BARCODE: D214220 SUBMISSION CODE: S485268
P.C. CODE: 034805 TOX. CHEM. NO.: 931

TEST MATERIAL (PURITY): Ziram (98.5% ai)

SYNONYMS: Zinc dimethyldithiocarbamate

CITATION: Proudlock, R.J. (1989). Autoradiographic Assessment of DNA Repair After In Vitro Exposure of Rat Hepatocytes to Ziram. Huntingdon Research Centre Ltd., P.O. Box 2, Huntingdon, Cambridgeshire, PE15 6ES, England. HRC Study Report No. ZIR 6/89820. September 12, 1989. MRID 41287801. Unpublished.

SPONSOR: Ziram Task Force II, Consortium No. 62416, c/o UCB Chemicals Corporation, 5365-A Robin Hood Road, Norfolk, Virginia 23513

EXECUTIVE SUMMARY:

In two trials of an unscheduled DNA synthesis assay (MRID 41287801), primary rat hepatocyte cultures were exposed to Ziram (98.5% ai) in dimethylsulfoxide (DMSO) at 12 concentrations ranging from 0.316 to 100,000 ng/mL for 19 and 20 hours. In both trials, Ziram was tested up to cytotoxic concentrations and the limit of solubility. Mutagenicity, as measured by unscheduled DNA synthesis, was determined for seven concentrations selected on the basis of cytotoxicity. In both trials, the concentrations selected were 1.0, 3.16, 10, 31.6, 100, 316, and 1,000 ng/mL. The criterion for a positive response was a substantial and reproducible dose-related and statistically significant increase in the net nuclear grain count, which was accompanied by a substantial increase in the gross nuclear grain count over concurrent solvent control values.

There was no evidence that unscheduled DNA synthesis, as determined by radioactive tracer procedures (nuclear silver grain counts) was induced. The positive control induced the appropriate response. However, the highest concentration of Ziram selected for evaluation of mutagenicity did not cause toxicity, reported

as "cell death." Since the next highest dose at 3,160 ng/mL caused cytotoxicity, the concentrations evaluated are considered adequate to conclude that Ziram is not mutagenic in this test system.

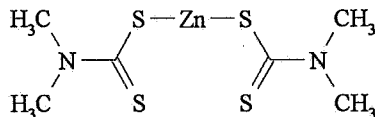
This study is classified as **acceptable** and satisfies the requirement for FIFRA Test Guideline 84-2 for other genotoxic mutagenicity data.

COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material: Ziram, technical grade
Description: Creamy white [powder] (from MRID 41287802)
Lot/Batch #: 8331 AA
Purity: 98.5% ai
Stability of compound: Stated to be "stable"
CAS #: 137-30-4 (from other sources)
Structure



Solvent used: Dimethylsulfoxide (DMSO)
Other comments: Analysis of dosing solutions not performed

2. Control Materials:
Negative: DMSO
Solvent/final concentration: DMSO (10 µL/mL culture medium)
Positive (concentrations, solvent):
2-acetylaminofluorene (AAF) dissolved in DMSO was used at final concentrations of 10, 31.6, 100, and 316 ng/mL in culture medium
3. Test compound concentrations used:

Cytotoxicity assay: 12 concentrations ranging from 0.316 to 100,000 ng/mL in half-log increments were tested in both the first and second trials, each run in conjunction with the UDS assay.

UDS assay: 7 (1, 3.16, 10., 31.6, 100, 316, and 1,000

ng/mL) of the 12 concentrations tested were evaluated in both the first and second trials.

4. Media: Williams' Medium E supplemented with glutamine and gentamicin (WEI). WEC (WEI plus 10% fetal bovine serum) was added to establish cell cultures, but was omitted during the treatment period.
5. Test Cells: Primary hepatocytes were obtained from two male Sprague-Dawley rats (one rat per trial) weighing 294 g and 238 g, supplied by Harlan Olac, Bicester.
6. Cell Preparation:
 - a. Perfusion Technique: Rat liver was perfused *in situ* for 5 minutes with Hank's balanced salt solution (Ca⁺⁺, Mg⁺⁺ free) containing EGTA (190 mg/mL in NaOH) and HEPES buffer. Perfusion continued for 10 additional minutes with WEI containing 100 units/mL of Type 1A collagenase.
 - b. Hepatocyte Harvest/Culture Preparation: Hepatocytes were obtained by mechanical dispersion of the excised liver tissue in a culture dish containing WEI and collagenase. Viable cells were counted (trypan blue exclusion), suspended in WEC at a concentration of 1×10^6 cells per mL, and plated in 2 mL aliquots onto glass coverslips in multiwell culture plates. The attachment period for cells was 1.5 hours. Unattached cells were removed and fresh WEI was added.

B. TEST PERFORMANCE

1. Cytotoxicity Assay: Each trial was run as an integral part of the UDS assay described in B.2. Signs of toxicity included cell death and sloughing of cells from the coverslip.
2. UDS Assay:
 - a. Treatment: After a 1.5 hour attachment period, the WEC was replaced with WEI containing 10 μ Ci/mL ³H-thymidine (80 Ci/mole - first trial, 84 Ci/mole - second trial). Triplicate cultures were treated with a 20 μ L aliquot of the test solution or positive control at the desired concentration. Twelve cultures were given 20 μ L aliquots of DMSO as solvent controls. After a treatment period of 20 hours - first trial or 19 hours - second trial, the cells were washed and the medium containing the test material and radioisotope was replaced

with fresh medium containing unlabelled thymidine. After a 24 hour incubation, the cell nuclei in the first trial were swollen by addition of 1% sodium citrate for 5 minutes. The cells were fixed in acetic acid:ethanol (1:3) overnight at 4 C, rinsed, and air dried. The cells were stained with orcein and the coverslips were mounted, cell side up, on glass slides. In the second trial, the cells were processed and stained in a similar manner, except that the cells were fixed in methanol, without hypotonic treatment. The slides were not stained until after autoradiography when they were stained with Mayer's Haemalum. The fixation and staining procedure used in the second assay was intended to avoid the use of acetic acid, which the laboratory found on occasion to cause deterioration of the cell cytoplasm.

- b. Preparation of Autoradiographs/Grain Development: In the dark, Kodak AR10 stripping film was applied to slides, which were then air dried overnight. The slides were stored for 13 days at 4 C in light-tight boxes containing dessicant, and developed in Kodak D-19. Following cytotoxicity determination, and prior to grain counting, slides from the 7 highest concentrations selected for evaluation were coded.
- c. Grain Counting: The net nuclear grain count was determined for at least 50 cells on each coverslip (150 cells/concentration) using an automatic counter. Where a strong positive response was obvious, only 25 cells per coverslip were counted. Net nuclear grain count was determined by counting nuclear grains and subtracting the number of grains in a nuclear-sized area adjacent to the nucleus. Nuclei in replicative DNA synthesis were excluded.
- e. Evaluation Criteria:
- Assay Validity: The criteria for a valid assay were not specified by the laboratory, except that a hepatocyte viability of at least 70% was required from each liver perfusion.

Positive Response: A positive response was indicated by a substantial and reproducible dose-related and statistically significant increase in the net nuclear grain count, which was accompanied by a substantial increase in the gross nuclear grain count over concurrent solvent control values.

- f. Statistical Analysis: Analysis of variance was used to compare the net nuclear grain counts from treated and control cultures.

II. **REPORTED RESULTS:** The viability of the hepatocytes following liver perfusion for the first and second trials was 86% and 94%, respectively. Analytical determination of dose preparations were not performed.

- A. Preliminary Cytotoxicity Assay: This assay was run as an integral part of each UDS assay. Twelve concentrations of Ziram from 0.316 to 100,000 ng/mL were tested. At 100,000 ng/mL a noticeable precipitate formed in the culture medium. At concentrations exceeding 1,000 ng/mL, toxicity precluded further analysis. Based on these results, seven concentrations (1, 3.16, 10, 31.6, 100, 316, and 1,000 ng/mL) were selected for analysis of nuclear labeling. The lowest dose level tested for cytotoxic response (0.316 ng/mL) was not selected for further analysis.
- B. UDS Assay: Two trials were performed and 7 concentrations of Ziram (1, 3.16, 10, 31.6, 100, 316, and 1000 ng/mL) were evaluated in each trial. Fifty cells per culture were analyzed and triplicate cultures per concentration were evaluated (150 cells/concentration). Twelve solvent control cultures (DMSO, 10 μ L/mL, v/v), and triplicate positive control cultures (AAF, 10, 31.6, 100, and 316 ng/mL) were evaluated. The results are shown in Appendix 1 (study report tables 1 and 2, pages 16 and 17) of this DER and are summarized as follows:

In neither trial did the test material cause a statistically significant increase in the net nuclear grain count when compared with the solvent control. In contrast, the positive control induced large, dose-related increases in the net nuclear grain count together with substantial increases in the gross nuclear grain count. Based on these results, the study author concluded that, under the conditions of the study, Ziram failed to show any evidence of DNA-damaging activity in this primary rat hepatocyte UDS assay.

III. **REVIEWER'S DISCUSSION/CONCLUSIONS:**

A. Ziram was tested to toxic concentrations and the limit of solubility, the solvent control produced the appropriate response, and the ability of the system to detect DNA damaging agents was adequately shown by the mutagenic response induced by the positive control. However, the EPA recommends that for cytotoxic chemicals, the first dose to elicit a toxic response should be the highest dose evaluated. In this study, a toxic response was indicated by cell death and occurred at 3,160 ng/mL, which was the next highest dose above 1,000 ng/mL, the highest dose evaluated for nuclear labeling. Therefore, it can be concluded that the dose levels evaluated were adequate to ascertain that Ziram is not mutagenic in this test system.

B.

STUDY DEFICIENCIES - The following deficiencies would not be expected to alter the conclusions of the study: (i) analysis of dose formulations were not performed; however, Ziram was tested to cytotoxic concentrations; and, (ii) the criteria for a valid assay were not specified, except for the viability requirement for hepatocytes following liver perfusion.

ZIRAM

Unscheduled DNA Synthesis (84-2)

SignOff Date:	8/2/00
DP Barcode:	D172447
HED DOC Number:	014277
Toxicology Branch:	RAB2