DATA EVALUATION RECORD

8/2/2002

ZIRAM

23

Study Type: 84-2; *In vitro* Chromosome Aberration Assay in Chinese Hamster Ovary (CHO) Cells

Work Assignment No. 2-15D (MRID 41287802)

Prepared for

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Disclaimer

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

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Review Section III, Toxicology Branch I (7509C)
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Review Section IV, Toxicology Branch I (7509C)

DATA EVALUATION RECORD

STUDY TYPE: *In vitro* mammalian chromosome aberrations in Chinese hamster ovary (CHO) cells
OPPTS Number: 870.5375 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% active ingredient)

SYNONYMS: Zinc dimethyldithiocarbamate

CITATION: Brooker, P.C. and Akhurst, L.C. (1989). Analysis of Metaphase Chromosomes Obtained from CHO Cells Cultured In Vitro and Treated with Ziram. Huntingdon Research Centre Ltd., P.O. Box 2, Huntingdon, Cambridgeshire, PEIS 6ES, England. HRC Study Report No. ZIR 7/89675. September 5, 1989. MRID 41287802. Unpublished.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 a mammalian cell chromosome aberration assay (MRID 41287802), Chinese hamster ovary (CHO) cell cultures were exposed to Ziram (98.5% ai) in dimethylsulfoxide at concentrations ranging from 0.8 to 40,000 ng/mL without metabolic activation, and from 5 to 40,000 ng/mL with metabolic activation. The high doses used for evaluation of mutagenicity were cytotoxic concentrations from the second of two trials that provided sufficient cells for analysis, and the low and intermediate doses were the concentrations corresponding to 12.5% and 50% of the high dose. Cell cultures without metabolic activation were exposed for 21 hours prior to harvesting. Cell cultures with activation were exposed for 4 hours and harvested 17 hours following the termination of exposure.

Ziram did not produce statistically significant increases in the proportion of metaphases containing chromosomal aberrations at any dose level, with and without metabolic activation, compared with the negative controls. Positive controls induced the appropriate response.

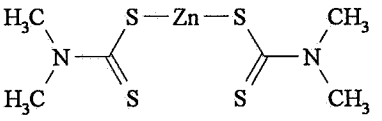
This study is classified as **acceptable** and satisfies the guideline requirement for *in vitro* cytogenetic mutagenicity studies (84-2).

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
   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: The test article was stored at room temperature in the dark and was dissolved in DMSO immediately before use. Analysis of dosing solutions was not performed.

2. Control Materials:
   Negative: DMSO
   Solvent/final concentration: DMSO (10 µL/mL in culture medium)
   Positive:
   Activation: Cyclophosphamide (20 µg/mL in distilled water)
   Nonactivation: Mitomycin C (0.2 µg/mL in distilled water)

3. Activation
   S9 was derived from Aroclor 1254 induced male Sprague-Dawley rat liver

   The S9 was prepared in advance and stored at approximately -80 C. Prior to use, the thawed S9 was tested with 7,12-dimethylbenz(a)anthracene. The S9 was

mixed with NADP (0.1M), $MgCl_2$ (0.4M), Na_2HPO_4 (0.2M), glucose-6-phosphate (1M), and distilled water, before being added to culture medium to give a final S9 concentration of 2%.

4. Test compound concentrations used

a. Cytotoxicity Assays:
(1) Nonactivated conditions: Ten dose levels of Ziram (80, 200, 300, 600, 1,300, 2,500, 5,000, 10,000, 20,000, and 40,000 ng/mL) were tested in the initial trial, and ten dose levels (0.8, 1.6, 3.1, 6.3, 12.5, 25, 50, 100, 200, and 400 ng/mL) were tested in the repeat trial.
(2) Activated conditions: Thirteen dose levels (10, 20, 40, 80, 160, 310, 630, 1,300, 2,500, 5,000, 10,000, 20,000, and 40,000 ng/mL) were tested in the initial trial, and nine dose levels (5, 50, 125, 250, 375, 500, 750, 1000, and 1500 ng/mL) were tested in the repeat trial.

For both the activated and nonactivated conditions, duplicate cultures were used for each dose level and appropriate positive control; quadruplicate cultures were used for the negative (solvent) control. Dose levels for evaluation of mutagenicity in metaphase cells were selected from the repeat trials of the cytotoxicity assays.

b. Mutagenicity Assays:
(1) Nonactivated conditions: Three dose levels (3.1, 12.5, and 25 ng/mL) were evaluated.
(2) Activated conditions: Three dose levels (125, 500, and 1,000 ng/mL) were evaluated.

5. Test cells
Chinese hamster ovary (CHO) cells, strain K_1-BH_4, were the test cells used. They were maintained in Hams F-12 medium supplemented with 5% fetal bovine serum. Cultures were incubated at 37 C in a humid atmosphere containing 5% carbon dioxide.

Properly maintained? **Yes**
Cell line or strain periodically checked for Mycoplasma contamination? **Not reported**
Cell line or strain periodically checked for karyotype stability? **Not reported**

B. TEST PERFORMANCE

1. Cytotoxicity Assay

Cells from trypsinized stock cultures were resuspended in medium to to give $8x10^4$ cells/mL. Aliquots (5 mL) were added to plastic tissue culture flasks and returned to incubation. After 24 hours, 1.25 mL of S9 mix was added to each flask from one set of cultures followed by 62.5 µL of test material in DMSO or DMSO alone. Each flask from the second set of cultures (-S9) received 50 µL of test material in DMSO or DMSO alone. Additional nonactivated cultures were treated with 50 µL of mitomycin C, and additional activated cultures were treated with 62.5 µL of cyclophosphamide. The nonactivated cultures were incubated for 21 hours. The activated cultures were incubated for 4 hours, after which the medium containing the activation mixture was removed, replaced with fresh medium, and the cultures were incubated for an additional 17 hours.

The mitotic index was determined by counting at least 2,000 cells for each dose level (4,000 cells for the negative control) and comparing the average percent of cells in metaphase with the corresponding value for the negative control.

2. Cytogenetic Assay

The dose level from the cytotoxicity assay causing a decrease in the mitotic index of 50-80% of the negative control value, or if there was no decrease, the maximum achievable concentration, was used as the highest dose level for evaluation of mutagenicity. Two dose levels, 50% and 12.5% of the highest dose level were also selected. The slides were then coded.

a. Spindle inhibition

Inhibitor used/concentration: colchicine at 0.25µg/mL
Administration time: 2 hours before cell harvest

b. Cell harvest

The experiment was terminated by detachment of the cells from the flask with 0.25% trypsin solution, resuspension in hypotonic solution (0.07 M KCL), and fixation with methanol:glacial acetic acid (3:1).

c. Details of slide preparation

Cells in fixative were dropped onto glass slides (5 slides per dose level), air dried, stained with 10% Giemsa, and after air drying, mounted in DPX.

d. Metaphase analysis

No. of cells examined per dose: 200
No. of cells examined in the solvent control: 400

No. of cells examined in the cyclophosphamide positive control: 200
No. of cells examined in the mitomycin C positive control: 200
Scored for structural aberrations: **Yes**
Scored for numerical polyploidy: **No**
Coded prior to analysis: **Yes**

e. Evaluation criteria
A positive response was claimed if a statistically significant ($p \leq 0.05$) increase was observed in the proportion of metaphase cells containing chromosome aberrations at any dose level when compared with the negative control. The test system was considered sufficiently sensitive to detect a response if both positive controls exhibited statistically significant increases in chromosomal damage.

f. Statistical analysis
Fisher's exact test was used to compare the data between the treated cultures and negative controls.

II. **REPORTED RESULTS:** Ziram was soluble in DMSO at concentrations exceeding 100 mg/mL, however, all concentrations greater than 40,000 ng/mL caused a heavy precipitate in the culture medium. Therefore, 40,000 ng/mL was considered the maximum achievable concentration in this test system.

A. Cytotoxicity assays
The initial cytotoxicity test without metabolic activation and with 10 dose levels of Ziram ranging from 80 to 40,000 ng/mL resulted in excessive toxicity at all dose levels. Very few metaphase figures were present in cultures treated with the lower dose levels and no live cells were present in cultures treated with 5000 ng/mL and above. Therefore, the cytotoxicity test was repeated with 10 dose levels ranging from 0.8 to 400 ng/mL. A dose-dependent decrease in mitotic index was seen at the five higher dose levels with the fifth dose level, 25 ng/mL, giving a mitotic index that was 22.4% of the negative control value. Cultures treated with this dose level together with two lower dose levels, 12.5 and 3.1 ng/mL, were selected for subsequent metaphase analysis. The results of the repeat cytotoxicity test are shown in Appendix 1 (study report Table 1, page 18).

The initial cytotoxicity test with metabolic activation and with 13 dose levels of Ziram ranging from 10 to 40,000 ng/mL resulted in excessive toxicity at the six higher dose levels. The mitotic index at the seventh dose level, 630 ng/mL, was

65.7% of the negative control value, and the mitotic indices of all lower dose levels were similar to this. However, a high frequency of chromosomal damage in the negative controls was observed, so the test was repeated. The repeat cytotoxicity test had nine dose levels ranging from 5 to 1,500 ng/mL. The highest dose level was excessively toxic, but the second dose level, 1,000 ng/mL produced a mitotic index that was 52.9% of the negative control value. Cultures treated with this dose level together with two lower dose levels, 500 and 125 ng/mL, were selected for subsequent metaphase analysis. The results of the repeat cytotoxicity test are shown in Appendix 1 (study report Table 1, page 20).

B. Cytogenetic assay

Cell cultures treated with Ziram at 3.1, 12.5, and 25 ng/mL, without metabolic activation, and at 125, 500, and 1,000 ng/mL with metabolic activation, were evaluated for chromosomal aberrations. There were no statistically significant increases in the proportion of metaphases containing chromosomal aberrations at any dose level, with and without metabolic activation, compared with the negative controls. The positive controls induced statistically significant increases in the proportion of aberrant metaphases. The results are shown in Appendix 2 (study report Table 2, pages 21 and 22).

III. DISCUSSION/CONCLUSIONS

A. Investigator's Conclusions

The study authors concluded that Ziram showed no evidence of clastogenic activity in this in vitro cytogenetic test system.

B. Reviewer's Discussion

Ziram was tested to toxic concentrations and the limit of solubility. The reviewer agrees with the authors'conclusion that there was no increased clastogenic response above negative control values over dose ranges that included cytotoxic levels (25 ng/mL, -S9 and 1,000 ng/mL, +S9). The sensitivity of the assay system to detect damage to chromosomes was adequately demonstrated by the results obtained with the positive controls (mitomycin C, -S9 and cyclophosphamide, +S9). The reviewer concludes, therefore, that the results of this study provide sufficient evidence to consider Ziram negative in this *in vitro* test system.

IV. STUDY DEFICIENCIES

No deficiencies that would be expected to alter the conclusions of the study were identified. Minor deficiencies that were identified included the following: (i) analysis of the dosing solutions was not performed, however Ziram was tested to cytotoxic concentrations; (ii) there was no indication that the cell line was periodically checked for mycoplasma contamination and karyotype stability; and, (iii) the cells were not scored for numerical aberrations.

APPENDIX 1

APPENDIX 2

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