DATA EVALUATION RECORD

22

ZIRAM

Study Type: 84-2; Salmonella/Mammalian-Microsome Plate Incorporation Mutagenicity Assay (Ames Test)

Work Assignment No. 2-15B (MRID 41642901)

8/2/2000

Prepared for

Health Effects Division
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U.S. Environmental Protection Agency
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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 IV, Toxicology Branch I (7509C)

DATA EVALUATION RECORD

STUDY TYPE: Salmonella typhimurium/mammalian activation gene mutation assay
OPPTS Number: 870.5265 OPP Guideline Number: [§84-2]

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

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

SYNONYMS: Zinc dimethyldithiocarbamate

CITATION: Jones, E., Cook, P. G. S., Grant, R. A. and Kitchings, J. (1990) Ziram Technical: Bacterial Mutation Assay. Huntingdon Research Centre Ltd, Huntingdon, Cambridgeshire, England. Laboratory Report Number ZIR 25/891914. August 22, 1990. MRID 41642901. Unpublished

SPONSOR: Ziram Tack Force II, UCB Chemicals Corporation, Norfolk, VA

EXECUTIVE SUMMARY:

In a reverse gene mutation assay in bacteria (MRID 41642901), strains TA98, TA100, TA1535, TA1537 and TA1538 of Salmonella typhimurium were exposed to Ziram technical (98.5% ai) in dimethylsulfoxide in the presence and absence of S9 mammalian metabolic activation. The five S. typhimurium strains were evaluated with Ziram technical at concentrations of 0.5, 1.5, 5, 15, and 50 µg/plate (+/-S9) and the TA100 strain was evaluated at concentrations of 15, 25, 50, 100, and 150 µg/plate in the presence of 5%, 10%, and 20% of the S9 fraction and at 25, 50, 75, and 100 µg/plate in the presence of 20% and 30% of the S9 fraction.

Ziram technical (98.5% ai) was tested up to the limit dose of 5,000 µg/plate. Cytotoxicity was observed at a concentration > 500 µg/plate. The positive controls induced the appropriate responses in the corresponding strains.

Ziram technical was mutagenic under the conditions of this microbial gene mutation assay when tested above 50 µg/plate in the presence of S9 mix. A dose related genotoxic response in the TA100 tester strain with S9 metabolic activation was observed with 2.6, 2.2, and 1.9 fold increases in mutant colonies over background at 100, 75, and 50 µg/plate, respectively. These data

confirm previously reviewed data (DP barcode D214220) concerning this compound.

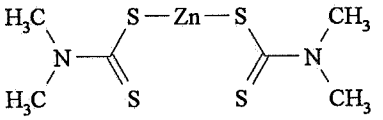
This study is classified as **acceptable**, and satisfies the requirements for FIFRA Test Guideline 84-2 for in vitro mutagenicity bacterial reverse gene mutation data.

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

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material: Ziram, technical
   Description: Creamy white powder
   Lot/Batch #: 8331 AA
   Purity: 98.5% ai
   Stability of compound: Stable for two years when stored at room temperature in the dark.
   CAS #: 137-30-4
   Structure:



   Solvent used: Dimethylsulfoxide (DMSO)

2. Control Materials:
   Negative: DMSO
   Solvent/final concentration: DMSO/100 µL per plate
   Positive: Non-activation:

Positive control	µg/plate	Strain
9-Aminoacridine	80	TA1537
N-Ethyl-N'-nitro-N-nitrosoguanidine	3	TA100
N-Ethyl-N'-nitro-N-nitrosoguanidine	5	TA1535
2-Nitrofluorene	1	TA98
2-Nitrofluorene	2	TA1538

Activation:

Positive control	µg/plate	Strain
2-Aminoanthracene	2	TA1535, 1537
2-Aminoanthracene	1	TA100
2-Aminoanthracene	0.5	TA98, 1538

3. Activation: S9 derived from

x Aroclor 1254	x induced	x rat	x liver
___ phenobarbital	___ non-induced	___ mouse	___ lung
___ none		___ hamster	___ other
___ other			___ other

The S9 mix contained:S9 fraction (5%, 10%, 20%, or 30% v/v), 8mM $MgCl_2$, 33 mM KCl, 4 mM NADP, 5 mM glucose-6-phosphate, and 100 mM Na orthophosphate buffer (pH 7.4). All cofactors were filter sterilized prior to use and 0.5 mL of the S9 mix was used per plate.

4. Test organisms: S. typhimurium strains
___ TA97 x TA98 x TA100 ___ TA102 ___ TA104
x TA1535 x TA1537 x TA1538 ; list any others:

Properly maintained? **Yes**
Checked for appropriate genetic markers (rfa mutation, R factor)? **Yes**

5. Test compound concentrations used

Preliminary cytotoxicity test: Four dose levels (5, 50, 500, and 5,000 µg/plate) were evaluated with the S. typhimurium strains TA98, TA100, TA1535, TA1537 and TA1538 with and without S9 activation (10% v/v of the S9 fraction).

Mutagenicity assay: S. typhimurium strains were evaluated with Ziram technical in three assays as follows:

Mutagenicity assay: Five dose levels (0.5, 1.5, 5, 15, and 50 µg/plate) were evaluated with the S. typhimurium strains TA98, TA100, TA1535, TA1537 and TA1538 in the presence of 10% of the S9 fraction and also without metabolic activation. This assay was performed twice on two different dates.

Repeat mutagenicity assay: One set of samples included the following: five dose levels (15, 25, 50, 100, and 150 µg/plate) that were evaluated with the S. typhimurium strain TA100 in the presence of 5%, 10%, and 20% of the S9 fraction in the S9 mix. A second set of samples, that was identical to the first set, was incubated for 60 minutes at 37 C prior to the addition of the soft agar overlay.

Second repeat mutagenicity assay: Four dose levels (25, 50, 75, and 100 µg/plate) were evaluated with the S. typhimurium strain TA100 in the presence of 20% and 30% of the S9 fraction. The pre-incubation step was omitted.

B. TEST PERFORMANCE

1. Type of Salmonella assay:
   _x_ standard plate test
   _x_ pre-incubation (60 minutes), second set (see above)
   ___ "Prival" modification *(i.e. azo-reduction method)*
   ___ spot test
   ___ other *(describe)*

2. Protocol: Test substance was diluted in DMSO to specified concentrations; dilutions of the positive control substances were not described. For the activation conditions, 0.1 mL of the appropriate tester strain culture containing approximately 2 x 10^9 cells/ml, 0.5 mL of the S9 mix, and 0.1 mL of test material solution, solvent, or positive control were mixed with 2.0 mL of melted top agar supplemented with a minimal amount of histidine. The mixture was poured over minimal agar (25 mL) per plate. For nonactivation conditions, 0.5 mL of sodium orthophosphate buffer was added. For the cytotoxicity test, single plates were prepared for each dose. For the mutagenicity assays, triplicate plates were prepared for each dose, strain, and condition and incubated for approximately 3 days at 37 C. Plates were evaluated for toxicity, total revertant colonies per plate, and mean ± standard deviations for each dose point.

3. Evaluation Criteria

(a) Assay validity: The assay was considered acceptable if the results produced a clear positive or a negative response. If a clear response was not obtained, the assay could be repeated using method modifications which may include the use of a narrower

dose range than that already tested and/or the use of different concentrations of S9 mix. If no clear positive response is observed after repeating the assay, the data may be subjected to analysis of variance followed by the Student's t test.

(b) Positive response: The test material was considered mutagenic if there was a dose-related increase in the number of revertants and the number of revertants per plate was ≥ 2x the vehicle controls in two separate experiments, with any bacterial strain, either with or without metabolic activation. The test material was considered non-mutagenic (negative response) if treatment did not produce reproducible increases of ≥1.5 the vehicle controls at any dose with any bacterial strain.

II. REPORTED RESULTS

A. Analytical determinations: No data were presented indicating the actual concentrations of the test stock solutions.

B. Preliminary cytotoxicity assay: The number of colonies/plate for the preliminary assay are presented in Attachment 1 (study report Table 1, page 20) of this DER. Four dose levels (5-5,000 µg/plate) were evaluated with the S. typhimurium strains TA98, TA100, TA1535, TA1537 and TA1538 with and without S9 activation. Single plates were used per dose and per condition. A solvent control was run. Cytotoxicity apparent as inhibition of growth was observed in the cultures treated at 500 and 5,000 µg/plate.

Based on these results, all strains of S. typhimurium were initially assayed with the test substance at 0.5-50 µg/plate (+/-S9; S9 at 10%). Additionally, the TA100 bacterial strain was assayed with the test substance at 15-150 µg/plate and at 25-100 µg/plate in the presence of 5-30% of the S9 fraction.

C. Mutagenicity assay: Summaries of the mutagenicity assay results are presented in Attachment 2 (study report Tables 2, 3, 4, 6, and 8; pages 21-25, 27-28, and 30) of this DER. In the initial assay (performed twice), the test substance ranging from 0.5-50 µg/plate (+/-S9) was evaluated in triplicate plate cultures using strains TA98, TA100, TA1535, TA1537, and TA1538. In a repeat assay, the bacterial strain TA100 was tested in the presence of 5-20% S9 fraction using 15-

150 µg/plate of the test substance; a pre-incubation procedure was also employed. A second repeat assay using the TA100 strain was run with Ziram technical at 25-100 µg/plate in the presence of 20% or 30% S9.

The positive controls induced marked increases in revertant colonies in their respective strains. There were no marked increases relative to the vehicle controls in the number of revertant colonies in any tester strain at any dose level/condition in the initial assay. When the assay was repeated, there was evidence of induced mutant colonies over background in the TA100 tester strain with Ziram technical at 15 and 50 µg/plate with metabolic activation. In the repeat assay with tester strain TA100, there were increases in mutant colonies over background following treatment with Ziram technical; the greatest increase was observed at 50 µg/plate. The differences were greater with increasing level of the S9 fraction. In the second repeat assay, a dose response relationship was observed with 2.6, 2.2, and 1.9 fold increases in mutant colonies over background at 100, 75, and 50 µg/plate, respectively, in the presence of 20% S9 fraction; similar results were obtain in the presence of the S9 fraction at 30%.

Based on these results, the study author concluded that Ziram technical was mutagenic in this microbial gene mutation assay when tested above 50 µg/plate in the presence of S9 mix.

III. REVIEWER'S DISCUSSION/CONCLUSIONS:

A. The reviewer agrees with the study author's conclusion that Ziram technical was mutagenic under the conditions of this microbial gene mutation assay and induced a genotoxic response in the TA100 tester strain with S9 metabolic activation. Ziram was assayed over an appropriate dose range as it was tested to twice the limit concentration with the S. typhimurium strains. The sensitivity of the test system to detect mutagenesis was adequately demonstrated by the response obtained with the nonactivated and S9-activated positive controls. The study is classified as acceptable.

B.
Study deficiencies - None.

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