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

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MEMORANDUM

OFFICE OF PESTICIDES AND TOXIC SUBSTANCES

SUBJECT: Zineb Host-mediated Mutagenicity Study

TO: Ms. Joanna Dizikes
Review Manager 64

FROM: Byron T. Backus, Toxicologist
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THROUGH: Marcia van Gemert, Ph.D.
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6/24/87

Project No. 7-0697

Tox. Chem. No. 930

Action Requested:

Review of a host-mediated mutagenicity study on Zineb.

Conclusions and Recommendations:

1. The Agency can accept the reported finding that a mutagenic response occurred with S. typhimurium strain TA 1537 at 2500 mg/kg Zineb dosage. However, it is noted that this finding was somewhat equivocal (although a doubling in revertants occurred, it is not certain whether this was statistically significant, and there was no conclusive indication of a similar effect at 5000 mg/kg, despite a level of viability - or recovery - for the TA 1537 similar to that at 2500 mg/kg).

2. While no effects were observed with S. typhimurium strains TA 98 or TA 100 at dosage levels of 2500 or 5000 mg/kg, these negative findings were not confirmed in a replicate assay.

3. Overall, the study is acceptable in demonstrating mutagenic activity in S. typhimurium strain TA 1537 at 2500 mg/kg, but not acceptable in demonstrating a lack of mutagenic activity in S. typhimurium strains TA 98 and TA 100 at doses of 2500 and 5000 mg/kg.

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DATA EVALUATION REPORT I

STUDY TYPE: Mutagenicity - Host (Mouse) Mediated Assay with S. typhimurium

TOX. CHEM. NO.: 930

ACCESSION NUMBER: not given

MRID NO.: not given

TEST MATERIAL: Zineb

STUDY NUMBER(S): 179001-M-00587

SPONSOR: W. R. Landis Associates, Inc.

TESTING FACILITY: Life Science Research
Roma Toxicology Centre
Via Tito Speri 14,
Pomezia (Roma), Italy

TITLE OF REPORT: Host Mediated Assay Test Substance: Zineb

AUTHOR(S): Edwards, C. N. & Forster, R.

REPORT ISSUED: April 21, 1987

Classification: Acceptable in demonstrating mutagenic activity with S. typhimurium TA1537 at 2500 mg/kg
Not acceptable in demonstrating a lack of mutagenic activity with S. typhimurium TA98 and TA 100 at doses of 2500 and 5000 mg/kg.

CONCLUSIONS:

1. The study is acceptable in showing an increase in revertants occurred with S. typhimurium strain TA 1537 in the host-mediated assay at 2500 mg/kg. However, it is noted that this finding was somewhat equivocal (although a doubling in revertants occurred, it is not certain that this was actually statistically significant), and there was no indication of an effect at 5000 mg/kg, despite a level of viability similar to that at 2500 mg/kg.

2. While no effects were observed with S. typhimurium strains TA 98 or TA 100 at 2500 or 5000 mg/kg, these negative findings were not confirmed in a replicate assay.

A. MATERIALS:

1. Test compound: Zineb: description: a fine pale buff powder, no batch number or purity given.

2. Positive controls: Dimethylnitrosamine (DMN) at a dose of 100 mg/kg. Additionally, the TA 1537 strain was exposed to 9-Aminoacridine (9-AA) at 50 ug/plate, TA 98 was exposed to 2-Nitrofluorene at 2 ug/plate, and TA 100 to sodium azide at

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1 ug/plate in in vitro assay.

2. Organisms: Male B6C3F1/Crl BR mice, 22-29 g, age(s) not specified, received from Charles River Italia S.p.A. According to the assay procedure on p. 34 they would weigh approximately 25-30 gms and be 5-6 weeks old when used in this assay. Salmonella typhimurium tester strains TA 1537, TA 98, and TA 100, obtained from Dr. B. N. Ames, University of California.

B. STUDY DESIGN:

1. Dosage selection:

According to the report (p. 12) information in the scientific literature indicated the oral LD_{50} for Zineb in mice is 7600 mg/kg. "Accordingly dose-levels of 5000 and 2500 mg/kg were selected for this study..."

2. Preparation of cultures:

"Overnight bacterial cultures were grown in nutrient broth, centrifuged at 5000 rpm for 10 minutes and resuspended in one-fifth of the original volume of Hank's balanced salts solution."

3. Administration of test material and bacteria:

Groups of 15 male B6 C3F1/Crl BR mice were orally gavaged with 2500 or 5000 mg/kg of Zineb in 0.5% carboxymethylcellulose solution. A group of 15 mice served as negative controls, receiving vehicle only. Immediately after dosage 5 mice/dose level were intraperitoneally inoculated with 0.5 ml S. typhimurium TA 1537, TA 98 or TA 100 with the following densities:

TA 1537	1.60×10^{10} cells/ml
TA 98	1.40×10^{10} cells/ml
TA 100	1.20×10^{10} cells/ml

A positive control group of 5 mice was orally dosed with 100 mg/kg DMN and intraperitoneally inoculated with TA 100.

4. Bacterial recovery:

After three hours mice were sacrificed and 2 ml of sterile Hank's solution was introduced into the peritoneal cavity and then withdrawn. Three 0.2 ml aliquots/mouse were plated out directly to obtain numbers of revertant cells, while serial dilutions were also prepared to determine numbers of viable cells. Bacterial counts used in subsequent calculations were from plate counts obtained using 0.1 ml aliquots at a 10^{-5} dilution.

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5. Direct plating:

In addition to plates prepared from individual animals, resuspended cultures of bacteria (and dilutions thereof) were plated directly onto selective agar to determine revertant numbers, and on nutrient agar to determine the numbers of viable cells.

6. Calculations:

The mutation frequency for controls was calculated using a formula similar to the following:

$$\frac{\text{Average mutant population}}{\text{Average total population}}$$

The induced mutation frequency (I.M.F.) was calculated for treated animals by a formula which was essentially the following:

$$\frac{\text{Mutants/Treatment group - Mutants/control group}}{\text{Population of treated group}}$$

"In each case, the viable colony counts obtained from the 10^{-5} dilution were used in calculating the mutation frequency."

Results from two animals, one from the vehicle control group with TA98 and one from the 5000 mg/kg Zineb treatment group with TA100, "were excluded from group means and subsequent statistical analyses as "outlier" values."

7. Criteria for a positive response:

"The test substance is considered to have a mutagenic effect if a two-fold increase in the mutation frequency is observed at any treated test-point, compared with the relevant negative control value. The internal consistency of the findings within groups and the biological significance of any observed increases is taken into consideration in evaluating the results."

8. Quality assurance:

This is provided on p. 4 and 5 (the latter page includes the signature of the Quality Assurance Manager) of the subject document.

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C. RESULTS:

1. Mutant counts:

From tables 1, 2 and 3:

	Negative Control group	Zineb 2500 mg/kg	Zineb 5000 mg/kg	Positive control
TA 1537 Total mutants	28	64	38	not done
TA 98 Total mutants	(35)*	28	20	not done
TA 100 Total mutants	698	338	(414)†	7651

*corrected value: 28 for the 4 animals without outlying value from #14. The 35 represents 28 x 1.25.

†corrected value: 331 for the 4 animals without outlying value from #88. 414 = 331 x 1.25.

2. Group mean mutation and induced mutation frequencies:

From table 5, p. 23:

	TA 1537 Mean ± S.D.	TA 98 Mean ± S.D.	TA 100 Mean ± S.D.
	(all values x 10^{-8})		
Vehicle control Mutation frequency	5.38 ± 1.49	3.79 ± 1.01	20.31 ± 2.23
2500 mg/kg Zineb Ind. mutation frequency	7.17 ± 4.80	-1.47 ± 2.65	-15.89 ± 6.82
5000 mg/kg Zineb Ind. mutation frequency	0.79 ± 3.70	-2.35 ± 3.02	-89.11 ± 48.65
100 mg/kg DMN Ind. mutation frequency	not tested	not tested	242.32 ± 116.78

3. Direct plating:

The following mean mutation frequencies were obtained from direct plating:

From table 6, p. 24:

	TA 1537	TA 98	TA 100
	(all values x 10^{-8})		
Direct plating Mutation frequency	2.69	1.17	25.65

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4. In vitro exposure to positive controls:

From table 7:

	Treatment/plate	Revertants Mean	S.E.
TA 1537	DMSO 100 ul	22.5	4.5
"	9-Aminoacridine 50 ug	57.5	19.5
TA 98	DMSO 100 ul	31.3	4.4
"	2-Nitrofluorene 2 ug	127.3	12.3
TA 100	Distilled water	141.0	4.5
"	Sodium azide 1 ug	646.0	18.8

This demonstrates that the strains were sufficiently sensitive.

D. DISCUSSION:

While no mutagenic effects were observed with S. typhimurium strains TA 98 or TA 100 at 2500 or 5000 mg/kg, these negative findings were not confirmed in a replicate assay. This part of the study is therefore classified as unacceptable.

According to the criteria used by the laboratory mutagenic activity occurred with S. typhimurium strain TA 1537 at 2500 mg/kg, since a doubling in total number of revertants occurred with respect to the negative control (although no indication is given as to whether this doubling was statistically significant).

It is noted that there was essentially no significant difference in mean viabilities for strain TA 1537 (control: 1.84×10^7; 2500 mg/kg: 1.94×10^7; 5000 mg/kg: 2.24×10^7). Despite the same (or slightly better viability) at 5000 mg/kg Zineb than at 2500 mg/kg the number of revertants actually decreased. This fact, as well as the question as to whether a statistically significant increase in revertants occurred at 2500 mg/kg, suggests that the reported study finding of mutagenicity for TA 1537 at 2500 mg/kg Zineb is somewhat equivocal. However, unless shown otherwise, the Agency can accept this positive finding.

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