CASWELL FILE

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

010288

MAY 27 1993

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: **Zinc Omadine:** Review of a Mutagenicity Study.

EPA ID# 088002-001258
Case No. 815252

DP Barcode D173067, D173070, D173066
Chem. ID No. 088002

FROM: John E. Whalan, D.A.B.T., Toxicologist
Section 1, Toxicology Branch I
Health Effects Division (H7509C)

John Whalan 4-23-93

TO: Bruce Sidwell (PM Team # 53)
Special Review and Reregistration Division (H7508W)

THRU: Roger L. Gardner, Section Head
Section 1, Toxicology Branch I
Health Effects Division (H7509C)

Roger Gardner 5-19-93

KAG 5/24/93

Olin Corporation submitted the following studies for review:

Salmonella/Mammalian-Microsome Plate Incorporation Mutagenicity Assay (Ames Test) with a Confirmatory Assay; Study No. T9153.501014; October 19, 1990; MRID No. 419065-02.

CHO/HGPRT Mutation Assay with Confirmation; Study No. T9153.332001; September 6, 1990; MRID No. 419065-03.

Micronucleus Cytogenetic Assay in Mice; Study No. T9153.122; October 22, 1990; MRID No. 419065-01.

These studies were reviewed by Clement Associates and Irving Mauer and classified **Unacceptable**. Thus, they do not satisfy Guideline requirements 84-2a and 84-2b for gene mutation and structural chromosome aberrations. These studies can be upgraded if the purity of the test article is provided.

The Ames Assay showed zinc omadine was not mutagenic to *Salmonella* strains exposed up to cytotoxic doses (≥3.3 μg/plate without S9 activation, >333 μg/plate with S9 activation).

In the CHO/HGPRT Mutation Assay, there was a negative response for induction of gene mutation at the HGPRT locus of CHO cells exposed to cytotoxic doses (>1.5 μg/ml, without S9 activation; 30 μg/ml, with S9 activation).

The Micronucleus Cytogenetic Assay showed zinc omadine was not clastogenic in bone marrow cells in I.P. treated mice up to toxic doses (44 mg/kg).

FINAL

DATA EVALUATION REPORT

2-Mercaptopyridine-N-Oxide Zinc

Study Type: Mutagenicity: Salmonella typhimurium/Mammalian Microsome Mutagenicity Assay

Prepared for:

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

Prepared by:

Clement International Corporation
9300 Lee Highway
Fairfax, VA 22031-1207

Principal Reviewer	Laura Kolb, MPH	Date 4/2/93
Independent Reviewer	Nancy E. McCarroll, B.S.	Date 4/2/93
QA/QC Manager	William L. McLellan for Sharon Segal, Ph.D.	Date 4/2/93

Contract Number: 68D10075
Work Assignment Number: 2-51
Clement Number: 151
Project Officer: Caroline C. Gordon

MUTAGENICITY STUDIES

EPA Reviewer: Irving Mauer, Ph.D. Signature: 04/08/93
Immediate Office, Tox. Branch I/HED (H-7509C) Date:

EPA Section Head: Marian Copely, DVM Signature:
Review Section IV, Tox. Branch I/HED (H-7509C) Date: 4/9/93
R.Y. 5-19-93

DATA EVALUATION REPORT

STUDY TYPE: Mutagenicity: Salmonella typhimurium/mammalian microsome plate incorporation assay

EPA IDENTIFICATION Numbers:

PC Code: 088002

Tox Chem. Number: 923

MRID Number: 419065-02

TEST MATERIAL: 2-Mercaptopyridine-n-oxide zinc

SYNONYMS: Zinc Omadine

SPONSOR: The Olin Corporation, New Haven, CT

STUDY NUMBER: T9153.501014

TESTING FACILITY: Microbiological Associates, Inc., Rockville, MD

TITLE OF REPORT: Salmonella/Mammalian-Microsome Plate Incorporation Mutagenicity Assay (Ames Test) with a Confirmatory Assay

AUTHORS: R. H. C. San and J. B. Shelton .

REPORT ISSUED: October, 19, 1990

CONCLUSIONS--EXECUTIVE SUMMARY: Doses of 2-mercaptopyridine-n-oxide zinc ranging from 0.33 µg/plate without S9 activation and 10 to 333 µg/plate with S9 activation were evaluated for the potential to induce reverse gene mutations in Salmonella typhimurium. Under the conditions of two independently performed plate incorporation assays, cytotoxicity was observed at 3.3 µg/plate and above -S9, and at 333 µg/plate and above +S9. There was, however, no reproducible evidence of a mutagenic effect in S. typhimurium at any dose either with or without activation. Although we conclude that the

compound was not mutagenic in this bacterial test system, the study was incomplete because test material purity information was not provided.

STUDY CLASSIFICATION: Unacceptable. The study does not fully satisfy Guideline requirements (§84-2a) for genetic effects Category I, Gene Mutations, but can be upgraded if the missing test material information is provided.

A. MATERIALS:

1. Test Material: 2-mercaptopyridine-n-oxide zinc

Description: White milky semi-viscous liquid
Identification number: T9153-501014
Purity: Not reported
Receipt date: February 16, 1990
Stability: Not reported
Contaminants: None listed
Solvent used: Ethanol (ETOH)
Storage conditions: Room temperature
Other provided information: Dosing solutions were prepared immediately before use; analytical determinations were not conducted to determine actual concentrations used in the study. The highest dose of the test compound was tested for sterility.

2. Control Materials:

Negative: None

Solvent/final concentration: ETOH/ 50 µl/plate

Positive:

Sodium azide	1.0 µg/plate TA1535, TA100
ICR-191	2.0 µg/plate TA1537
2-Nitrofluorene	1.0 µg/plate TA1538, TA98

Activation:

2-Aminoanthracene (2-AA)	0.5 µg/plate all strains

3. Activation: S9 derived from male Sprague-Dawley rats

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

The rat liver S9 homogenate was prepared by the performing laboratory and was characterized for its ability to convert 2-AA and 7,12-dimethylbenzanthracene to mutagenic forms. The S9 mix was prepared as follows:

Component:	Amount/ml
H_2O	0.56 ml
1.0 M NaH_2PO_4/K_2HPO_4 (pH 7.4)	0.10 ml
0.05M Glucose 6-phosphate	0.10 ml
0.04M NADP	0.10 ml
0.2M $MgCl_2$/ 0.825M KCl	0.04 ml
S9	0.10 ml

4. Test Organism Used: S. typhimurium strains
_____ TA97 __x__ TA98 __x__ TA100 _____ TA102
_____ TA104 __x__ TA1535 __x__ TA1537 __x__ TA1538
list any others:

Test organisms were properly maintained? Yes.
Checked for appropriate genetic markers (rfa mutation, R factor)?
Yes, all strains were tested on the day of their use.

5. Test Compound Concentrations Used:

a. Range-finding assay: Ten nonactivated and ten S9-activated doses (10, 33, 67, 100, 333, 667, 1000, 3333, 6667, 10000 µg/plate) were assayed using tester strain TA100. Single plates were prepared per dose per condition.

b. Mutation assay:

- Initial Trial: The initial plate incorporation assay was performed with five nonactivated (0.03, 0.1, 0.3, 1.0 and 3.3 µg/plate) and five S9-activated doses (10, 33, 50, 100, and 333 µg/plate).

- Repeat Trial: Cytotoxicity was not observed for strains TA1535, TA1537 or TA98 at doses up to 3.3 µg/plate -S9; accordingly, this phase of the assay was repeated with these strains using six nonactivated doses (0.1, 0.3, 1.0, 3.3, 10, and 33 µg/plate).

- Confirmatory Assay: The confirmatory assay was performed with the following doses:

  - Nonactivated conditions: 0.1, 0.3, 1.0, and 3.3 µg/plate -- all strains; 0.03, 2.0 and 5.0 µg/plate strain TA100; and 10 µg/plate strains TA1535, TA1537, and TA98.

  - S9-activated conditions: 10, 33, 50, 100, and 333 µg/plate -- all strains.

All tester strains were evaluated in each assay and three plates were prepared per dose, per condition. Positive and solvent controls were also plated at three per dose, per strain, per condition.

B. TEST PERFORMANCE:

1. Type of Salmonella Assay:

- x Standard plate test
- ___ Pre-incubation (__) minutes
- ___ "Prival" modification
- ___ Spot test
- ___ Other (describe)

2. Preliminary Cytotoxicity/Mutation Assays: In general, similar procedures were used for the preliminary cytotoxicity and the mutation assays. To tubes containing 2.5-ml volumes of molten top agar[1], 50 µl of an overnight broth culture of the appropriate tester strain and 100 µl of the appropriate test material dose, solvent or positive controls were added. For the S9-activated test, 0.5 ml of the S9 mix was added to tubes containing 2.0 ml of top agar; tester strains and test and control solutions were added as described. The contents of the tubes were mixed, poured over Vogel-Bonner minimal medium E, and incubated at 37±2°C for ≈48 hours. At the end of incubation, plates either were immediately scored for revertant colonies or were refrigerated and subsequently counted. Means and standard deviations were determined for the mutation assay.

3. Evaluation Criteria:

a. Assay validity: The assay was considered valid if the following criteria were met: (1) the presence of the appropriate genetic markers was verified for each strain; (2) the number of spontaneous revertants of each strain fell within the reporting laboratory's acceptable range; (3) cell densities were ≥ 0.6×10^9 cells/ml; and (4) all positive controls caused at least a 3-fold increase in revertants per plate compared to the respective solvent control.

b. Positive response: The test material was considered positive if it caused a dose-related increase in the mean revertant colonies per plate in at least one strain (over at least two concentrations) and for strains TA1535, TA5137 and TA38 the increase was three times (for strains TA98 and TA100 two times) the control value at the peak of the dose response.

[1]Note: Sufficient water was added to the top agar to ensure that the amino acid supplements were equivalent with or without activation.

C. REPORTED RESULTS:

1. Preliminary Cytotoxicity Assay: Slight precipitation of the test material was observed on plates containing the three highest doses both with and without activation (3333, 6667, 10,000 µg/plate). Cytotoxicity was apparent in strain TA100 at 333 µg/plate with S9 activation and over the entire dose range (10-10,000 µg/plate) in the absence of S9 activation. Based on these findings, doses selected for the initial mutation assay ranged from 0.03 to 3.3 µg/plate -S9 and from 10 to 333 µg/plate +S9.

2. Mutation Assays:

a. Initial Assay: Representative findings from the initial assay are presented in Table 1. As shown, cytotoxicity, as indicated either by a reduction in the background lawn of growth or reduced revertant colony counts were noted only for strains TA1538 and TA100 at the highest nonactivated level (3.3 µg/plate). Although the data provided no convincing evidence of a mutagenic response in the nonactivated phase of testing, our reviewers noted that the reduction in the background lawn of growth of strain TA100 at 3.3 µg/plate was accompanied by a ~1.7-fold increase in histidine revertants (his^+). Cytotoxicity was apparent for all strains exposed to the highest S9-activated level (333 µg/plate). In agreement with the nonactivated findings, the data from noncytotoxic levels did not suggest mutagenic effects. However, our reviewers did note the slight elevations (≤1.5-fold over background) in his^+ revertants of strain TA100 at S9-activated levels ranging from 10 to 50 µg/plate.

b. Repeat Assay: Owing to the lack of cytotoxic response in strains TA1535, TA1537 and TA98 in the nonactivated phase of the initial assay, a repeat trial was conducted with these strains and six nonactivated doses (0.1, 0.3, 1.0, 3.3, 10, and 33 µg/plate). As shown in Table 2, all strains responded to the cytotoxic action of the two highest doses (10 and 33 µg/plate) and decreased his^+ colonies and/or reductions in the background lawn were seen at 3.3 µg/plate for strains TA98 and TA1537. There was, however, no appreciable increase in reversion to histidine prototrophy of any strain at the noncytotoxic concentrations of the test material.

c. Confirmatory Assay: Owing to the difference in the individual strain response to the cytotoxic effects of the nonactivated test material, strains TA1535, TA1537, and TA98 were treated with 10 µg/plate -S9 and strain TA100 was plated with 0.03, 2.0 and 5.0 µg/plate -S9. In addition, all strains were exposed to 0.1, 0.3, 1.0 and 3.3 µg/plate. In the presence of S9 activation all strains were treated with five doses ranging from 10 to 333 µg/plate.

Representative results of the confirmatory trial are presented in Table 3. As shown, cytotoxicity was achieved in all strains except strain TA100 at 3.3 µg/plate -S9; a cytotoxic response was, however, noted in S. typhimurium strain TA100 at 5 µg/plate. The highest S9-activated dose (333 µg/plate) was cytotoxic in all strains. In agreement with the earlier findings, the test material was not mutagenic in any strain at any noncytotoxic level either in the presence or absence of S9 activation. Our reviewers further noted that the marginal increases in his^+ colonies of strain TA100 that were observed in the initial trial were not reproduced in the confirmatory assay. By contrast to the negative results with the test material, the positive controls induced the expected responses in the appropriate tester strains with or without S9 activation.

The authors considered that all of their evaluation criteria for a valid study had been met with the exception of the low cell titer for the culture of strain TA1537 used in the repeat assay; however, since the other strain characteristics were deemed acceptable the data for the TA1537 was not rejected.

Based on the overall findings, the study author concluded that the test compound was not mutagenic in this bacterial test system.

D. REVIEWERS' DISCUSSION/CONCLUSIONS: We assess that the study was properly conducted and that the study authors correctly interpreted the data. 2-Mercaptopyridine-n-oxide zinc was assayed to a nonactivated level (3.3 µg/plate) that was cytotoxic to the majority of the tester strains and to an S9-activated level (333 µg/plate) that produced cytotoxicity in all strains. There was, however, no evidence of a mutagenic response under any test condition. In addition, the sensitivity of the test system to detect mutagenesis was adequately demonstrated by the results obtained with the positive controls. We conclude, therefore, that the compound was negative in this bacterial assay system. However, the study is incomplete because information on the purity of the test material was not included. The study is, therefore, considered unacceptable but can be upgraded if the missing test material is provided.

Table 1: Representative Results of the Initial Salmonella typhimurium Mutagenicity Assay with 2-Mercaptopyridine-N-Oxide Zinc

Substance	Dose/Plate	S9 Activation	Revertants per Plate of Bacterial Tester Strain[a] TA1535	TA1537	TA1538	TA98	TA100
Solvent Control							
Ethanol	50 µl	-	11 ± 5	8 ± 4	9 ± 2	24 ± 6	170 ± 26
	50 µl	+	30 ± 6	11 ± 3	18 ± 2	29 ± 1	152 ± 13
Positive Controls							
Sodium azide	1.0 µg	-	217 ± 26	--	--	--	619 ± 47
ICR-191	2.0 µg	-	--	194 ± 21	--	--	--
2-Nitrofluorene	1.0 µg	-	--	--	384 ± 30	238 ± 19	--
2-Aminoanthracene	0.5 µg	+	93 ± 13	91 ± 11	721 ± 47	580 ± 18	793 ± 42
Test Material							
	1.0 µg[b]	-	7 ± 3	10 ± 5	6 ± 4	34 ± 7	171 ± 12
	3.3 µg	-	9 ± 3	7 ± 5	4 ± 1[c]	33 ± 8	254 ± 15[c]
	50 µg[b]	+	26 ± 6	6 ± 1	9 ± 3	47 ± 6	227 ± 9
	100 µg	+	23 ± 5	5 ± 2	5 ± 3	40 ± 11	196 ± 57
	333 µg	+	11 ± 4[c]	1 ± 1[c,d]	1 ± 1[c]	14 ± 3[c]	0 ± 0[c]

[a]Means and standard deviations of counts from triplicate plates
[b]Results for lower doses (0.03, 0.1, and 0.3 µg/plate -S9 and 10 and 33 µg/plate +S9) did not suggest a mutagenic effect.
[c]Reductions in the background lawn of growth were noted at these levels.
[d]Results based on the count of two plates; due to a technical error, one of the three plates was not inoculated with the bacterial cells

Note: Data were extracted from the study report, pp. 27-36.

Table 2: Representative Results of the Repeat Assay Salmonella typhimurium Mutagenicity Assay with 2-Mercaptopyridine-N-Oxide Zinc

Substance	Dose/Plate	S9 Activation	Revertants per Plate of Bacterial Tester Strain[a] TA1535	TA1537	TA98
Solvent Control					
Ethanol	50 µl	-	8 ± 4	10 ± 1	39 ± 10
Positive Controls					
Sodium azide	1.0 µg	-	366 ± 22	--	--
ICR-191	2.0 µg	-	--	190 ± 20	--
2-Nitrofluorene	1.0 µg	-	--	--	248 ± 16
Test Material					
	3.3 µg[b]	-	7 ± 3	5 ± 1	32 ± 2[c]
	10.0 µg	-	2 ± 2[c]	0 ± 1[c]	20 ± 5[c]
	33.0 µg	-	1 ± 1[c]	0 ± 0[c]	19 ± 2[c]

[a]Means and standard deviations of counts from triplicate plates
[b]Results for lower doses (0.01, 0.3, and 1.0 µg/plate -S9) did not suggest a mutagenic effect.
[c]Reductions in the background lawn of growth were noted at these levels.

Note: Data were extracted from the study report, pp. 37-39.

Table 3: Representative Results of the Confirmatory Salmonella typhimurium Mutagenicity Assay with 2-Mercaptopyridine-N-Oxide Zinc

Substance	Dose/Plate	S9 Activation	Revertants per Plate of Bacterial Tester Strain[a] TA1535	TA1537	TA1538	TA98	TA100
Solvent Control							
Ethanol	50 µl	-	10 ± 3	7 ± 1	5 ± 5	15 ± 5	127 ± 7
	50 µl	+	14 ± 2	16 ± 2	24 ± 6	25 ± 5	157 ± 14
Positive Controls							
Sodium azide	1.0 µg	-	442 ± 13	--	--	--	464 ± 43
ICR-191	2.0 µg	-	--	74 ± 8	--	--	--
2-Nitrofluorene	1.0 µg	-	--	--	344 ± 16	237 ± 86	--
2-Aminoanthracene	0.5 µg	+	178 ± 16	282 ± 15	1619± 230	1912± 43	2210± 45
Test Material							
	1.0 µg[b]	-	6 ± 2	8 ± 2	5 ± 3	13 ± 3	141 ± 5
	3.3 µg	-	6 ± 4[c]	4 ± 4[c]	5 ± 3[c]	8 ± 2[c]	146 ± 10
	5.0 µg	-	NT	NT	NT	NT	119 ± 24[c]
	10.0 µg	-	4 ± 2[c]	3 ± 2[c]	NT	5 ± 2[c]	NT
	50 µg[d]	+	13 ± 1	13 ± 2	15 ± 1	13 ± 3	165 ± 11
	100 µg	+	9 ± 2	11 ± 3	8 ± 3[c]	11 ± 4	74 ± 41[c]
	333 µg	+	3 ± 2[c]	1 ± 2[c]	2 ± 1[c]	3 ± 1[c]	18 ± 4[c]

[a]Means and standard deviations of counts from triplicate plates
[b]Results for other doses (0.1 and 0.3 µg/plate) with all strains, 0.03 µg/plate with strains TA1538 and TA98, and 2 µg/plate with strain TA100 did not suggest a mutagenic effect.
[c]Reductions in the background lawn of growth were noted at these levels.
[d]Results for lower doses (10 and 33 µg/plate +S9) did not suggest a mutagenic effect.

Note: Data were extracted from the study report, pp. 41-50.

E. QUALITY ASSURANCE MEASURES: Was the test performed under GLP? Yes.
(A quality assurance statement was signed and dated November 19, 1990.)

CORE CLASSIFICATION: Unacceptable. The study does not fully satisfy the data Guideline requirement (§84-2a) for genetic effects Category I, Gene Mutations, but can be upgraded if the missing test material information is submitted.

FINAL

DATA EVALUATION REPORT

ZINC OMADINE

Study Type: Mutagenicity: Gene Mutation in Cultured Chinese Hamster Ovary Cells (CHO/HGPRT)

Prepared for:

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

Prepared by

Clement International Corporation
9300 Lee Highway
Fairfax, VA 22031-1207

Principal Reviewer Kristin Jacobson Date 4/5/93
Kristin Jacobson, MSPH

Independent Reviewer Nancy E. McCarroll Date 4/5/93
Nancy E. McCarroll, B.S.

QA/QC Manager Sharon Segal Date 4/6/93
Sharon Segal, Ph.D.

Contract Number: 68D10075
Work Assignment Number: 2-51
Clement Number: 152
Project Officer: Caroline Gordon

GUIDELINE § 84: MUTAGENICITY
MAMMALIAN CELLS IN CULTURE GENE MUTATION

EPA Reviewer: Irving Mauer, Ph.D. Signature: 04/09/93
Immediate Office, Toxicology Branch I Date:
Health Effects Division (H-7509C)
EPA Section Head: Marion Copley, DVM, DABT Signature:
EPA Review Section IV, Toxicology Branch I Date: 4/9/93
Health Effects Division (H-7509C)

R. Y. 5-19-93

DATA EVALUATION REPORT

STUDY TYPE: Mutagenicity: Gene mutation in cultured Chinese hamster ovary cells (CHO/HGPRT)

EPA IDENTIFICATION NUMBERS:

Caswell Number: 932

MRID Number: 419065-03

PC Code: 088002

TEST MATERIAL: 2-Mercaptopyridine-N-oxide zinc

SYNONYM: Zinc omadine

SPONSOR: The Olin Corporation, New Haven, CT

STUDY NUMBER: T9153.332001

TESTING FACILITY: Microbiological Associates, Inc., Rockville, MD

TITLE OF REPORT: CHO/HGPRT Mutation Assay with Confirmation

AUTHORS: D. Jacobson-Kram and C.I. Sigler

REPORT ISSUED: September 6, 1990

CONCLUSIONS--EXECUTIVE SUMMARY: Under the conditions of two independently conducted Chinese hamster ovary (CHO) cell HGPRT forward gene mutation assays, treatment with a suspension of 2-mercaptopyridine-N-oxide zinc, at doses of 0.25-2.0 µg/mL -S9 and 2.5-30 µg/mL +S9, failed to induce a mutagenic response. Severe cytotoxicity was noted at levels ≥1.5 µg/mL -S9 and ≥30 µg/mL +S9. Although the assay was performed in a technically acceptable manner, the study is incomplete because information on the purity of the test material was not provided.

STUDY CLASSIFICATION: Unacceptable. The study does not fully satisfy Guideline requirements (§84.2) for genetic effects, Category I, Gene Mutations, but can be upgraded if the missing test material purity information is provided.

A. MATERIALS:

1. Test Material: 2-Mercaptopyridine-N-oxide zinc

Description: Off-white aqueous dispersion (48% solids)
Identification number: Lot no. 9RC-290-109 ZP
Purity: Not reported
Receipt date: February 16, 1990
Stability: Not reported
Contaminants: None listed
Solvent used: Ethanol
Other provided information: The test material was stored at room temperature, protected from light. Dosing solutions were prepared just prior to use. Analytical determinations of achieved concentrations or test material stability were not performed.

2. Control Materials:

Negative: Culture medium (Ham's F-12 medium containing 5% dialyzed fetal bovine serum, 1% L-glutamine, and antibiotics)

Solvent: Ethanol, at a final concentration of 1%

Positive: Nonactivation (concentrations, solvent): Ethyl methanesulfonate (EMS) was prepared in dimethyl sulfoxide (DMSO) to yield a final concentration of 0.2 µL/mL.

Activation (concentrations, solvent): Benzo(a)pyrene (BaP) was prepared in DMSO to yield a final concentration of 4 µg/mL.

3. Activation: S9 derived from 200-250-g male Sprague-Dawley

__x__ Aroclor 1254	__x__ induced	__x__ rat	__x__ liver
_____ phenobarbital	_____ noninduced	_____ mouse	_____ lung
_____ none		_____ hamster	_____ other
_____ other		_____ other	

The S9 homogenate was prepared by the performing laboratory. Prior to use, the S9 fraction was characterized for its ability to metabolize 2-aminoanthracene and 7,12-dimethylbenz(a)anthracene to mutagenic forms using Salmonella typhimurium TA100. The S9 mix was prepared as follows:

Component	Final Concentration
Sodium phosphate buffer (pH 8.0)	50 mM
Glucose-6-phosphate	5 mM
NADP	4 mM
KCl	30 mM
$CaCl_2$	10 mM
$MgCl_2$	10 mM
S9 fraction	100 µL/mL

4. Test Cells: Mammalian cells in culture

_____ mouse lymphoma L5178Y cells
__x__ Chinese hamster ovary (CHO) cells
_____ V79 cells (Chinese hamster lung fibroblasts)
_____ other (list):

Properly maintained? Yes.
Periodically checked for mycoplasma contamination? Yes.
Periodically checked for karyotype stability? Not reported.
Periodically "cleansed" against high spontaneous background? Not reported.

5. Locus Examined:

_____ thymidine kinase (TK)
selection agent: _____ bromodeoxyuridine (BrdU)
(give concentration) _____ fluorodeoxyuridine (FdU)

__x__ hypoxanthine guanine phosphoribosyl transferase (HGPRT)
selection agent: _____ 8-azaguanine (8-AG)
(give concentration) __10 µM__ 6-thioguanine (6-TG)

_____ Na^+/K^+ATPase
selection agent: _____ ouabain
(give concentration)

_____ other (locus and/or selection agent; give details):

6. Test Compound Concentrations Used:

(a) Preliminary cytotoxicity assay: Nine doses (0.03, 0.06, 0.09, 0.12, 0.23, 0.45, 0.9, 1.8, and 3.6 µg/mL) were evaluated without S9 activation. The nine doses evaluated with S9 activation were 0.45, 0.9, 1.8, 2.7, 3.6, 7.2, 14.4, 28.8, and 57.5 µg/mL.

Note: Three preliminary cytotoxicity assays were conducted. Trial 1 was repeated owing to severe cytotoxicity at levels ranging from 0.24-2000 µg/mL. Trial 2 was abated because the "desired range of toxicity" was not achieved. Data presented above were from the successfully completed third trial.

(b) Mutation assay:

Initial trial:

- Nonactivated conditions: 0.25, 0.5, 0.75, 1.0, and 2.0 µg/mL
- S9-activated conditions: 5, 7.5, 10, 20, and 30 µg/mL

Repeat trial:

- Nonactivated conditions: Dose levels of 0.25, 0.5, 0.6, 0.75, 1.2, 1.5, 1.8, and 2.2 µg/mL were prepared, including duplicate flasks for the two highest dose levels; cultures exposed to ≤1.5 µg/mL and one of the 1.8-µg/mL cultures were cloned.
- S9-activated conditions: 2.5, 5, 7.5, 10, 20, and 30 µg/mL

B. TEST PERFORMANCE:

1. Cell Treatments:

   (a) Cells were exposed to the test compound, solvent or positive control for: 5 hours (nonactivated and S9-activated conditions).

   (b) After washing, cells were cultured for at least 7 days (expression period) before cell selection.

   (c) After expression, $2x10^5$ cells/dish (5 dishes/flask) were cultured for at least 7 days in selection medium to determine numbers of mutants; 100 cells/dish (3 dishes/flask) were cultured for at least 7 days in nonselection medium to determine cloning efficiency (CE).

2. Statistical Methods: The confidence interval defining a significant ($p \leq 0.05$) response was determined using Student's t-test and the performing laboratory's historical background mutant frequency (MF) ($9.8x10^{-6}$ clonable cells), following the methods of Gupta and Singh (1982)[1]. Accordingly, a significant response required an induced MF that was: (1) $>20x10^{-6}$; (2) ≥2-fold higher than the negative or solvent control; and (3) $\geq 9.8x10^{-6}$ higher than the negative or solvent control.

3. Evaluation Criteria:

   (a) Assay validity: The assay was considered valid if the following criteria were met: (1) the absolute CEs of the negative and solvent controls were >50%; (2) the spontaneous MFs in the negative and solvent controls were $\leq 20x10^{-6}$ clonable cells; and (3) the MFs of the positive controls were ≥3-fold higher than the corresponding solvent controls.

   (b) Positive response: The test material was considered positive if there was a reproducible, dose-dependent increase in the MF that included at least one dose level at which the increase was significant.

[1]Gupta, R.S. and Singh, B. (1982). Mutagenic responses of five independent genetic loci in CHO cells to a variety of mutagens: Development and characteristics of a mutagen screening system based on selection for multiple drug-resistant markers. Mutat Res 94:449-486.

C. REPORTED RESULTS

1. Test Material Solubility: The study authors indicated that the test material was insoluble in DMSO, ethanol and acetone. Ethanol was selected as the solvent because it formed the best "workable" suspension. However, the study report noted that the test material formed a film on the surface of the solvent and on the walls of the volumetric flasks; the physical behavior of the test material in the culture medium was not described.

2. Preliminary Cytotoxicity Test: In the range-finding assay, concentrations of the test material ≥1.8 µg/mL -S9 and ≥28.8 µg/mL +S9 were severely cytotoxic (Table 1). Reduced survival was noted at 0.9 µg/mL -S9 and 14.4 µg/mL +S9, with 24% and 37% relative cloning efficiencies (RCE), respectively. For the remaining nonactivated and S9-activated concentrations, RCE was ≥75%. Based on these findings, the initial mutation assay was conducted with 0.25-2.0 µg/mL -S9 and 5-30 µg/mL +S9.

3. Mutation Assays:

Nonactivated conditions: Representative results from the initial and confirmatory nonactivated mutation assays are presented in Table 2. In the initial assay, cytotoxicity (17% RCE) was evident at the highest dose (2.0 µg/mL); a slight but significant ($p \leq 0.05$) increase in the MF to 28.9×10^{-6} was also noted at this dose. Lower doses (≤1.0 µg/mL) did not result in any appreciable cytotoxicity or increases in the MF. In the confirmatory assay, additional dose levels between 1.0 and 2.0 µg/mL (i.e., 1.2, 1.5, and 1.8 µg/mL, as well as 2.2 µg/mL) were evaluated, with duplicate cultures prepared for the 1.8- and 2.2-µg/mL levels. No cells survived treatment with 2.2 µg/mL of the test material, and with the exception of a single mutant colony selection dish at 1.8 µg/mL, cytotoxicity precluded cloning this dose level for mutant selection. There were no appreciable increases in mutant colonies at any plated dose level. It was concluded that the increased MF noted at 2.0 µg/mL in the first trial was neither reproducible nor dose related, and therefore not biologically relevant. By contrast, the nonactivated positive control (0.2 µL/mL EMS) produced marked and reproducible increases in mutant colonies in the two trials.

S9-Activated conditions: Representative results from the initial and confirmatory S9-activated assays are presented in Table 3. The highest S9-activated concentration (30 µg/mL) resulted in cytotoxicity in trials 1 and 2, with 15% and 8% RCE, respectively. In the first trial, there were no appreciable increases in the MF at any dose, but in the confirmatory trial slight but significant ($p \leq 0.05$) increases in the MF were observed in both the 2.5- and 20-µg/mL cultures. There were, however, no appreciable increases in MFs at any of the intermediate doses. Based on the lack of dose dependency and reproducibility, the significant increases in MFs were not considered biologically meaningful. By contrast, the positive control compound (4 µg/mL BaP) induced marked and reproducible increases in mutant colonies.

TABLE 1. Representative Results of the Preliminary Cytotoxicity Test with 2-Mercaptopyridine-N-oxide Zinc

Substance	Dose/mL	S9 Activation	Average Number of Surviving Colonies[a]	Relative Percent Survival
Solvent Control				
Ethanol	10 µL	-	138	100 (138)[b]
	10 µL	+	121	100 (121)
Test Material				
2-Mercaptopyridine-N-oxide Zinc	0.45 µg[c]	-	103	75
	0.9 µg[d]	-	33	24
	7.2 µg[c]	+	97	80
	14.4 µg[d]	+	45	37

[a]Results from triplicate plates (100 cells/plate) in the treatment or solvent control groups

[b]Values in () are absolute percent survival for the solvent control.

[c]Relative percent survival for cultures treated with lower doses (0.03, 0.06, 0.09, 0.12, and 0.23 µg/mL -S9; 0.45, 0.9, 1.8, 2.7, and 3.6 µg/mL +S9) was ≥77%.

[d]Higher doses (1.8, 3.6 µg/mL -S9; 28.8 and 57.5 µg/mL +S9) were severely cytotoxic.

Note: Data were extracted from the study report, p. 17.

Table 2. Representative Results of the Nonactivated Chinese Hamster Ovary (CHO) Cell Forward Gene Mutation Assays with 2-Mercaptopyridine-N-oxide Zinc

Substance	Dose/mL	Relative Percent Cloning Efficiency (after treatment)[a]	Average Number of Mutant Colonies	Average Absolute Cloning Efficiency (at selection)[a]	Average Mutation Frequency x10^{-6}[b]
Negative Control					
Culture medium	--	129[c]	0	1.16	<0.9[d]
	--	108[e]	16	0.80	20.0
Solvent Control					
Ethanol	10 µL	100 (109)[c]	0	1.11	<0.9[d]
		100 (91)[e]	5	0.80	6.3
Positive Control					
Ethyl methane-sulfonate	0.2 µL	87[c]	199	1.11	179.3*
		85[e]	224	0.71	315.5*
Test Material					
2-Mercaptopyridine-N-oxide zinc	1.0 µg[f]	103[c]	9	1.18	7.6
	2.0 µg	17	24	0.83	28.9*
	0.75 µg[f]	63[e]	0	0.77	<1.3[d]
	1.2 µg	11	0	0.65	<1.5[d]
	1.5 µg[g]	5	0	0.57	<1.8[d]

[a]Based on results of triplicate plates from single flasks per treatment, solvent or positive control group. Values in () are absolute survival.

[b]Mutation Frequency (MF) = $\frac{\text{Total Mutant Colonies}}{\text{(Total Plates)[No. Cells Plated (2x10}^5\text{)](Cloning Efficiency)}}$; calculated by our reviewers

[c]Results from initial trial
[d]Calculated on the basis of <1 mutant colony
[e]Results from confirmatory trial
[f]Results for lower doses (0.25, 0.5, and 0.75 µg/mL--initial trial; 0.25, 0.5, and 0.6 µg/mL--confirmatory trial) did not suggest a mutagenic response.
[g]Higher doses (1.8 and 2.2 µg/mL in the second trial) were severely cytotoxic.

*Significantly ($p \leq 0.05$) higher than controls, by Student's t-test.
Note: Data were extracted from the study report, pp. 18, 19, 21, and 22.

Table 3. Representative Results of the S9-Activated Chinese Hamster Ovary (CHO) Cell Forward Gene Mutation Assays with 2-Mercaptopyridine-N-oxide Zinc

Substance	Dose/mL	Relative Percent Cloning Efficiency (after treatment)[a]	Average Number of Mutant Colonies	Average Absolute Cloning Efficiency (at selection)[a]	Average Mutation Frequency $x10^{-6}$[b]
Negative Control					
Culture medium	--	100[c]	11	1.22	9.0
	--	94[d]	8	0.86	9.3
Solvent Control					
Ethanol	10 µL	100 (109)[c]	1	1.35	0.7
		100 (101)[d]	10	1.04	9.6
Positive Control					
Benzo(a)pyrene	4 µg	10[c]	186	0.90	206.7*
		17[d]	145	0.71	204.2*
Test Material					
2-Mercaptopyridine-N-oxide zinc	20 µg[f]	65[c]	0	1.18	<0.8[e]
	30 µg	15	0	0.75	<1.3[e]
	2.5 µg	97[d]	27	1.09	24.8*
	5 µg	102	18	0.99	18.2
	7.5 µg	98	3	1.16	2.6
	10	98	10	0.96	10.4
	20	25	18[g]	0.84	26.8*
	30	8	0	0.63	<1.6[e]

[a]Based on results of triplicate plates from single flasks per treatment, solvent or positive control group. Values in () are absolute survival.

[b]Mutation Frequency (MF) = $\frac{\text{Total Mutant Colonies}}{\text{(Total Plates)[No. Cells Plated (2x10}^5\text{)](Cloning Efficiency)}}$; calculated by our reviewers

[c]Results from initial trial
[d]Results from confirmatory trial
[e]Calculated on the basis of <1 mutant colony
[f]Results for lower doses (5.0, 7.5, and 10 µg/mL--initial trial) did not suggest a mutagenic response.
[g]Total mutant colonies counted on four plates; the fifth plate was contaminated and, therefore, not scored.

*Significantly ($p \leq 0.05$) higher than controls, by Student's t-test.
Note: Data were extracted from the study report, pp. 18, 20, 21, and 23.

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Based on these findings, the study authors concluded that 2-mercapto-pyridine-N-oxide zinc was not mutagenic in the CHO/HGPRT forward gene mutation assay.

D. REVIEWERS' DISCUSSION AND INTERPRETATION OF RESULTS: We assess that 2-mercaptopyridine-N-oxide zinc was tested (as a suspension) over concentration ranges that included cytotoxic levels (≥1.5 µg/mL -S9 and ≥30 µg/mL +S9) but failed to induce a reproducible, dose-related mutagenic response in CHO cells. Although the poor solubility of the test material was problematical and required diligent vortexing during the preparation of dosing solutions, our reviewers noted that cytotoxicity in the assays occurred in a dose-related manner within the fairly narrow range of concentrations evaluated, and was generally consistent between the initial and confirmatory trial. Additionally, the sensitivity of the test system to detect mutagenesis was adequately demonstrated by the results obtained with the positive controls (0.2 µL/mL EMS -S9 and 4 µg/mL BaP +S9). We conclude, therefore, that 2-mercaptopyridine-N-oxide zinc was not mutagenic in this *in vitro* mammalian cell gene mutation assay. The study was incomplete, however, because information on the purity of the test material was missing.

E. QUALITY ASSURANCE MEASURES: Was the test performed under GLP? Yes. (A quality assurance statement was signed and dated September 14, 1990.)

CORE CLASSIFICATION: Unacceptable. The study does not fully satisfy Guideline requirements (§84.2) for genetic effects, Category I, Gene Mutations, but can be upgraded if the missing test material purity information is submitted.

FINAL

DATA EVALUATION REPORT

Zinc Omadine

Study Type: Mutagenicity: In Vivo Micronucleus Assay in Mice

Prepared for:

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

Prepared by:

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Independent Reviewer	Nancy E. McCarroll Nancy E. McCarroll, B.S.	Date 4/2/93
QA/QC Manager	William L McLellan for Sharon Segal, Ph.D.	Date 4/2/93

Contract Number: 68D10075
Work Assignment Number: 2-51
Clement Number: 150
Project Officer: Caroline C. Gordon

GUIDELINE SERIES 84: MUTAGENICITY
MICRONUCLEUS

MUTAGENICITY STUDIES

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Immediate Office, Tox. Branch I/
HED (H-7509C)

Signature: [signature]
Date: 04/09/93

EPA Section Head: Marion Copley, DVM, DABT
Review Section IV, Tox. Branch I/
HED (H-7509C)

Signature: [signature]
Date: 4/9/93

DATA EVALUATION REPORT

R. H. 5-19-93

STUDY TYPE: Mutagenicity: In vivo micronucleus assay in mice

EPA IDENTIFICATION Numbers:

PC Code: 088002

Tox. Chem. Number: 932

MRID Number: 419065-01

TEST MATERIAL: 2-Mercaptopyridine-n-oxide zinc

SYNONYMS: Zinc omadine

SPONSOR: The Olin Corporation, New Haven, CT

STUDY NUMBER: T9153.122

TESTING FACILITY: Microbiological Associates, Inc., Rockville, MD

TITLE OF REPORT: Micronucleus Cytogenetic Assay in Mice

AUTHORS: D. L. Putman and M. J. Morris

REPORTS ISSUED: October 22, 1990

CONCLUSIONS--EXECUTIVE SUMMARY: The single intraperitoneal (i.p.) injection of 11, 22, or 44-mg/kg of 2-mercaptopyridine-n-oxide zinc to male and female mice did not cause a significant increase in the frequency of micronucleated polychromatic erythrocytes (MPEs) in bone marrow cells harvested 24, 48, or 72 hours postexposure. In the high-dose animals, signs of toxicity (including mortality, lethargy, piloerection and diarrhea) were observed. There was, however, no evidence of a cytotoxic effect on the target organ (i.e., bone marrow cells). Based on these findings, we conclude that the compound was tested over an appropriate range of doses and found to be nongenotoxic in the mouse micronucleus assay. Although we conclude that the compound was negative in this test system, the study was incomplete because test material purity information was not provided.

GUIDELINE SERIES 84: MUTAGENICITY
MICRONUCLEUS

STUDY CLASSIFICATION: Unacceptable. The study does not fully satisfy Guideline requirements (§84-2) for genetic effects Category II, Structural Chromosome Aberrations, but can be upgraded if the missing test material information is provided.

A. MATERIALS:

1. Test Material: 2-Mercaptopyridine-n-oxide zinc

Description: Off-white, aqueous dispersion
Identification numbers: Lot Number 9RC-290-109ZP; Code number T9153
Purity: Not reported
Storage: Room temperature, light protected, protect from freezing
Receipt date: February 16, 1990
Stability: Assumed to be stable under the conditions of the assay
Contaminants: None listed
Vehicle used: Distilled water (DH_2O)
Other provided information: The frequency of the dosing solution preparation was not reported. Analytical determinations were not performed to verify test concentrations.

2. Control Materials:

Vehicle: DH_2O by intraperitoneal (i.p.) injection at a dosing volume of 10 ml/kg.

Positive: Triethylenemelamine (TEM) was prepared in DH_2O to yield a final dose of 0.25 mg/kg and was administered by i.p. injection at a dosing volume of 10 mL/kg.

3. Test Compound:

Route of administration: Single i.p. injection

Volume of test substance administered: 10 ml/kg

Dose levels used:

- Acute dose range-finding study: 59, 77, 100, 130, and 169 mg/kg
- Micronucleus assay: 11, 22, and 44 mg/kg

4. Test Animals:

(a) Species: Mouse Strain: ICR Age: 6-8 weeks (at dosing)

Weight Range:

- Acute dose range finding study (at dosing): 24-31 g (males); 18-28 g (females)

- Micronucleus assay (at dosing):
  23-38 g (males); 18-29 g (females)

Sex: Males and females

Source: Harlan Sprague-Dawley, Inc., Frederick, MD

(b) Number of animals used per dose:
Acute study: 5 (males); 5 (females) /treatment group

Micronucleus assay (dose groups/sacrifice time):

- Treatment groups: 5 males 5 females
- Positive control: 5 males 5 females
- Vehicle control: 5 males 5 females

Note 1: A secondary group of five males and five females were administered 44 mg/kg and were used to replace high-dose animals in the primary group that died prior to the scheduled sacrifice in the micronucleus assay.

Note 2: All animals were weighed immediately prior to dosing, and dose volumes were based on individual body weights.

(c) Animals were properly maintained? Yes.

B. TEST PERFORMANCE:

1. Acute Dose Range-finding Study: Animals received the selected dose of the test compound or vehicle control by single i.p. injection and were observed for clinical signs postdosing and daily for at least 7 days. Body weight data were recorded prior to compound administration and on days 1 and 3 postdosing.

2. Micronucleus Assay:

Treatment and sampling times:

(a) Test compound:
Dosing: x once _____ twice (24 hours apart)
Sampling (after last dose): _____ 6 hours _____ 12 hours
x 24 hours x 48 hours x 72 hours

(b) Vehicle control:
Dosing: x once _____ twice (24 hours apart)
Sampling (after last dose): x 24 hours x 48 hours
x 72 hours

(c) Positive control:
Dosing: __x__ once _____ twice (24 hours apart)
Sampling (after last dose): __x__ 24 hours _____ 48 hours
_____ 72 hours

3. Tissues and Cells Examined:

__x__ bone marrow _____ other (list):
Number of polychromatic erythrocytes (PCEs) examined per animal: 1000
Number of normochromatic erythrocytes (NCEs, more mature RBCs) examined per animal: Number observed while scoring 1000 polychromatic erythrocytes

4. Details of Cell Harvest and Slide Preparation: At 24, 48, and 72 hours after administration of the test material or vehicle control, the appropriate groups of animals were sacrificed by CO_2 asphyxiation. Animals in the positive control group were sacrificed 24 hours postexposure. Bone marrow cells from both femurs of each animal were aspirated into fetal bovine serum, centrifuged, resuspended and spread onto glass slides. Prepared slides were fixed in methanol, stained with May-Gruenwald-Giemsa and mounted. After coding, slides were scored for total erythrocytes, PCEs, and micronucleated PCEs (MPEs).

5. Statistical Methods: The results were evaluated for statistical significance ($p \leq 0.05$) using Kastenbaum-Bowman Tables based on the binomial distribution. Males and females were analyzed separately.

6. Evaluation Criteria:

(a) Assay validity: The assay was considered acceptable if the mean incidence of MPEs was ≤5/1000 PCEs (0.5%) in the vehicle control and the incidence of MPEs in the positive control was significantly greater than the negative control.

(b) Positive response: The test material was considered positive if a dose-dependent positive response was induced or a positive response was observed at a single dose level at adjacent sacrifice times.

C. REPORTED RESULTS:

1. Acute Dose Range-finding Study: All mice exposed to 77, 100, 130, and 169 mg/kg died within 5 days of treatment. At 59 mg/kg (lowest dose), 5/10 mice (3 males and 2 females) died within 5 days. Clinical signs (noted day 2 or thereafter) included diarrhea, crusty eyes, and piloerection at 59 mg/kg; lethargy, tremors, irregular breathing, prostration, diarrhea and piloerection at 77 mg/kg; tremors, lethargy, ataxia, crusty eyes, and diarrhea at 100 mg/kg; tremors, lethargy, diarrhea, and crusty eyes at 130 mg/kg; and tremors, lethargy, and

crusty eyes at 169 mg/kg. Based on these data, the $LD_{50/7}$ was calculated to be approximately 54 mg/kg. The high dose was, accordingly, set at 44 mg/kg (≈80% of the $LD_{50/7}$) for the micronucleus assay.

2. Micronucleus Assays: The original assay was aborted due to the poor response of the positive control (0.25 mg/kg TEM) and the results were not reported. A repeat assay was conducted and the results from the repeat assay are herein presented.

   (a) Animal observations: Animals administered the selected doses of the compound (11, 22, or 44 mg/kg) were observed for mortality and other signs of compound toxicity immediately after dosing and daily thereafter. One male in the 44 mg/kg-group died and was replaced with an animal dosed at the same level from the replacement group. Clinical signs were apparent in the 44-mg/kg dose group and included lethargy, piloerection, and diarrhea. No clinical signs were obvserved for other groups.

   (b) Micronucleus assay: Representative results from the micronucleus assay are presented in Table 1. PCE:NCE ratios for males and females of all treatment groups were generally comparable to the corresponding vehicle control group values. Although slight increases in the frequency of MPEs were scored at the 24-hour sacrifice of both sexes administered the high dose, the increases were not significant. Similarly, there was no evidence of a genotoxic response in either sex at lower dose levels. By contrast, the frequency of MPEs in male and female mice treated with the positive control (0.25 mg/kg TEM) increased significantly ($p \leq 0.05$).

Based on the overall results, the study author concluded that 2-mercapto-pyridine-n-oxide zinc was not genotoxic in this in vivo mouse micronucleus assay.

D. REVIEWERS' DISCUSSION/CONCLUSIONS: We assess that the study was properly conducted and that the study authors' interpretation of the data was correct. The test material was evaluated to a level (44 mg/kg) that caused mortality and other clinical signs of toxicity but failed to induce a genotoxic response in bone marrow cells harvested at 24, 48 or 72 hours posttreatment. In addition, the sensitivity of the test system to detect genotoxicity was demonstrated by the significant ($p \leq 0.05$) increases in MPEs in both male and female mice exposed to the positive control (0.25 mg/kg TEM).

We conclude, therefore, that the study provided acceptable evidence that 2-mercaptopyridine-n-oxide zinc was negative in this in vivo micronucleus assay. However, the study is incomplete because information on the purity of the test material was missing.

Table 1. Representative Results of the Micronucleus Assay in Mice with 2-Mercaptopyridine-N-Oxide Zinc

Substance	Dose/kg	Exposure Time[a] (hours)	Sex	Number of Animals Analyzed per Group	Number of PCEs Analyzed per Group	Number of MPEs per Group	MPEs per 1000 PCEs Mean ± S.D.	PCEs per Total Erythrocytes
Vehicle Control								
Distilled water	10 mL	24	M	5	5000	2	0.4 ± 0.55	0.46
			F	5	5000	4(6)[b]	0.8 ± 0.84(0.6)[b]	0.58
		48	M	5	5000	7	1.4 ± 0.89	0.60
			F	5	5000	4(11)	0.8 ± 0.45(1.1)	0.63
		72	M	5	5000	0	0.0 ± 0.00	0.60
			F	5	5000	1(1)	0.2 ± 0.45(0.1)	0.46
Positive Control								
Triethylene-melamine	0.25 mg	24	M	5	5000	73*	14.6 ± 4.22	0.56
			F	5	5000	91*(164)	18.2 ± 7.66(16.4)	0.56
Test Material								
2-Mercapto-pyridine-N-Oxide Zinc	44 mg[c,d]	24	M	5	5000	8	1.6 ± 0.55	0.50
			F	5	5000	6(14)	1.2 ± 0.84(1.4)	0.65
		48	M	5	5000	2	0.4 ± 0.55	0.58
			F	5	5000	3(5)	0.6 ± 0.89(0.5)	0.58
		72	M	5	5000	0	0.0 ± 0.00	0.55
			F	5	5000	1(1)	0.2 ± 0.45(0.1)	0.60

[a]Time after compound administration by intraperitoneal injection
[b]Values in ( ) are the combined results for both sexes; calculated by our reviewers.
[c]Highest assay level; no treatment-related toxic, cytotoxic, or clastogenic effects were seen in the low-(11 mg/kg) or mid-(22 mg/kg) dose groups.
[d]One male in the 44 mg/kg group was found dead on the third day and was replaced with a secondary group animal.
*Significantly higher ($p \leq 0.05$) than the corresponding vehicle control by Kastenbaum-Bowman tables

Abbreviations used: PCE = polychromatic erythocyte; MPE = micronucleated polychromatic erythrocyte; NCE = normochromatic erythrocyte

Note: Data were extracted from the study report, pp 14-17.

E. QUALITY ASSURANCE MEASURES: Was the test performed under GLPs? Yes.
(A quality assurance statement was signed and dated November 23, 1990.)

CORE CLASSIFICATION: Unacceptable. The study does not fully satisfy the Guideline requirements (§84-2) for genetic effects Category II, Structural Chromosome Aberrations but can be upgraded if the missing test material information is provided.