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

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OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: **Terrazole:** Review of a Mutagenicity Study.

EPA ID# 084701
Case No. 819299

DP Barcode D163836
Chem. ID No. 084701

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

John E Whalan
4-23-93

TO: Lois Rossi (PM Team # 74)
Special Review and Reregistration Division (H7508W)

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

Roger Gardner
5-3-93

KB
5/4/93

Uniroyal Chemical Company, Inc. submitted the following study for review:

Mouse Micronucleus Assay with Terrazole Technical; Study No. 053651; October 30, 1985; MRID No. 418375-01.

The study was reviewed by Clement Associates and Irving Mauer and classified **Acceptable.** Thus, this study satisfies Guideline requirement 84-2b for structural chromosome aberrations. There was a negative response for inducing micronuclei in mice treated orally up to toxic doses (1000 mg/kg).

FINAL

DATA EVALUATION REPORT

TERRAZOLE

Study Type: Mutagenicity: Micronucleus Assay with Mice

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	Dean Walton, Ph.D.	Date 4/5/93
Independent Reviewer	Nancy E. McCarroll, B.S.	Date 4/5/93
QA/QC Manager	Sharon Segal, Ph.D.	Date 4/5/93

Contract Number: 68D10075
Work Assignment Number: 2-63
Clement Number: 63-176
Project Officer: Caroline Gordon

MUTAGENICITY STUDIES

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

DATA EVALUATION REPORT

STUDY TYPE: Mutagenicity: Acute dose range-finding study/micronucleus assay with mice

EPA IDENTIFICATION Numbers:

PC Code: 084701

Tox. Chem. Number: 428

MRID Number: 418375-01

TEST MATERIAL: Terrazole technical

SYNONYM/CAS NUMBER: 5-Ethoxy-3-trichloromethyl-1-2-4-thiadazole

SPONSOR: Uniroyal Chemical Company, Inc., Bethany, CT

STUDY NUMBERS: Laboratory Project ID # 053651

TESTING FACILITY: RCC Research & Consulting Company AG, Itingen, Switzerland

TITLE OF REPORT: Mouse Micronucleus Assay with Terrazole Technical

AUTHOR: N. Banduhn

REPORT ISSUED: October 30, 1985

CONCLUSIONS--EXECUTIVE SUMMARY: The single oral gavage administration of 1000 mg/kg terrazole technical (1000 mg/kg) did not cause a significant increase in the frequency of micronucleated polychromatic erythrocytes in bone marrow cells harvested from male and female mice 24, 48, or 72 hours posttreatment. In the range-finding study, 6/6 mice exposed to 2500 mg/kg terrazole technical died. No deaths were recorded in the two lower dose groups (700 and 1000 mg/kg). Based on these results, an LD_{50} was estimated to be 1467 mg/kg and 80% of the LD_{50} was selected as the maximum tolerated dose.

In the micronucleus assay, mice treated with terrazole technical (1000 mg/kg) showed signs of toxicity including mortality, ruffled fur, ataxia, tremor, ptoses of the eyes, and sedation. Based on these findings, we conclude that

terrazole technical was adequately tested at an appropriate dose and found to be nongenotoxic in the mouse micronucleus assay.

STUDY CLASSIFICATION: Acceptable. The study satisfies Guideline requirements (§84-2) for genetic effects Category II, Structural Chromosome Aberrations.

A. MATERIALS:

1. Test Material: Terrazole technical

Description: Liquid
Identification number: Lot number CM-8561 S045017
Purity: 95%
Receipt date: August 8, 1985
Stability: Stable indefinitely at or below 45°C
Contaminants: None listed
Vehicle used: Aqueous solution with 2% w/v carboxymethylcellulose, sodium salt (CMC).
Other provided information: The test material was stored in the dark at 4°C. Test solutions were prepared immediately prior to each dosing. The report did not indicate that dosing selections were verified for actual concentrations.

2. Control Materials:

(a) Acute dose range-finding study:

Vehicle/final concentration/route of administration: 2% CMC was administered by oral gavage at a dosing volume 20 mL/kg.

(b) Cytogenetic assay:

Vehicle/route of administration: As above for the acute dose range-finding study.

Positive/final dose/route of administration: Cyclophosphamide (50 mg/kg) was prepared in a 0.9% w/v saline solution immediately before administration and given by oral gavage.

3. Test Compound:

Route of administration: Single oral gavage

Volume of test substance administered: 20 mL/kg of body weight

Dose levels used:

(a) Acute dose range-finding study: 700, 1000, and 2500 mg/kg/day

(b) Micronucleus assay: 1000 mg/kg/day

Note: Dosing was based on individual body weight.

4. Test Animals:

(a) Species: Mouse Strain: NMRI KFM (outbred SPF) Age (at initiation): 5-7 weeks Weight Range (at study initiation): 29-36 g (males); 24-32 g (females); Sex: Males and females Source: Kleintierfarm Madoerin AG, Fuellinsdorf, Switzerland.

(b) Number of animals used per dose:
Acute study: 3 males; 3 females

Micronucleus assay : 18 males; 18 females (5 males and 5 females/group/sacrifice time.)

Note: The additional male and female in each group were used as replacement animals in the event of death prior to the scheduled sacrifice.

(c) Properly maintained? Yes. The study author stated that during the acclimation period "some male animals were of poor health and had injuries due to biting. Some animals with a high degree of injuries were replaced by animals of the reserve." Our reviewers assume, therefore, that the animals used in the study following the 7-day acclimation period were those referred to as the animals without clinical findings.

B. TEST PERFORMANCE:

1. Acute Dose Range-finding Study: Eighteen animals (3/sex/dose) were dosed with 700, 1000, or 2500 mg/kg. Animals were observed for 7 days and the estimated acute oral LD_{50} was determined.

2. Micronucleus Assay:

(a) Treatment and sampling times:

- 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

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

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

3. Tissues and Cells Examined: __x__ bone marrow ____ other (list):
   Number of polychromatic erythrocytes (PCEs) examined per animal: 1000
   Number of erythrocytes (PCE + normochromatic, NCE) examined per animal: 1000

4. Details of Cell Harvest and Slide Preparation: At 24, 48, and 72 hours posttreatment with the test material, vehicle or positive control, the appropriate groups of animals were euthanized by cervical dislocation. The femoral bone marrow of each animal was aspirated, dispersed in fetal bovine serum and centrifuged. The supernatant was discarded; cell pellets were resuspended in residual serum and spread onto slides. The slides were air-dried overnight, stained using the differential stain method of Pappenheim[1] and coded prior to analysis. Two slides from each animal were produced to ensure an artifact-free sample and the first slide, if free from defects, was scored for micronucleated PCEs (MPEs) and the ratio of PCEs to total erythrocytes (RBC; i.e., PCE + NCE) was determined.

5. Statistical Methods: The frequencies of MPEs in the treated animals were compared to those of the vehicle (negative) control animals using a regression model assuming a Poisson distribution.[2] Analysis for "estimation and testing" were performed by the maximum likelihood method. Level of significance was based on $p<0.05$.

6. Evaluation Criteria: The test material was considered negative if no statistically significant positive responses were observed compared to the negative controls.

C. REPORTED RESULTS:

1. Acute Dose Range-finding Study: Details of the acute dose ranging-finding study were not provided. Mortality after a 7-day

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[1]Queisser, W. 1978. Das Knochenmark, Georg Theime Verlag, Stuttgart, p.12

[2]Armitage, P. 1971. Statistical Methods in Medical Research. Blackwell Scientific Publications, p. 214.

observation period following the single oral gavage administration of the selected doses of the test material was as follows:

	Number Dead/Number Tested		
Dose (mg/kg)	Males	Females	Combined
700	0/3	0/3	0/6
1000	0/3	0/3	0/6
2500	3/3	3/3	6/6

The approximate LD_{50} was estimated to be 1467 mg/kg with the 95% confidence level ranging from 1079 mg/kg to 2392 mg/kg. Based on this finding, the MTD selected for the micronucleus assay was 80% of the approximated LD_{50} or 1000 mg/kg.

2. Micronucleus Assay: Two deaths were reported following administration of the test material (one male at 48 hours and a second at 56 hours). Other signs of compound toxicity in the animals treated with 1000 mg/kg included ataxia, tremor, ptoses of the eyes, ruffled fur, and sedation. No clinical findings were observed in the negative and positive control groups.

Suggestive evidence of a cytotoxic effect on the target organ was observed at 72 hours in the terrazole-treated animals as indicated by the reduced PCE:NCE ratios compared to concurrent controls. However, since the reduction only occurred at 72 hours and only one dose was assayed, the data are insufficient to conclude that terrazole technical had an adverse effect on erythropoiesis. Similarly, there was no convincing evidence that the test material induced a genotoxic effect. Although the combined incidence of MPEs was slightly increased in the treated animals at all sacrifice times, the values were either within or only slightly higher than the overall combined background range for the vehicle control groups (0.11-0.18% MPEs).

By contrast, the positive control, cyclophosphamide (50 mg/kg), produced a statistically significant ($p<0.01$) increase in MPEs in bone marrow cells harvested from males and females 24, 48, and 72 hours posttreatment.

Based on the overall results, the study author concluded that terrazole technical was not genotoxic in this in vivo mouse micronucleus assay.

TABLE 1. Representative Results of the Micronucleus Assay in Mice Treated with Terrazole Technical

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	Mean Percent MPEs ±S.D.	Mean PCE:NCE Ratio ±S.D.
Vehicle Control								
2% Carboxymethyl cellulose	10 mL[b]	24	M	5	5000	5	0.10±0.12	1.55±0.59
		24	F	5	5000	6 (11)[c]	0.12±0.08 (0.11±0.09)[b]	1.48±0.38
Positive Control								
Cyclophosphamide	50 mg[b]	24	M	5	5000	246	4.92±1.59[*]	1.07±0.33
		24	F	5	5000	165 (411)	3.30±0.73[*] (4.11±1.45)	0.88±0.24
Test Material								
Terrazole Technical	1000 mg[d]	24	M	5	5000	12	0.24±0.21	1.10±0.60
		24	F	5	5000	8 (20)	0.16±0.13 (0.20±0.17)	1.39±0.58
		48	M	5	5000	8	0.16±0.15	1.13±0.33
		48	F	5	5000	6 (14)	0.12±0.13 (0.14±0.14)	1.49±0.65
		72	M	4[e]	4000	4	0.10±0.12	0.59±0.42
		72	F	5	5000	10 (14)	0.20±0.26 (0.16±0.20)	0.74±0.33

[a]Time after compound administration by oral gavage
[b]Data were presented for animals in these groups sacrificed 48 and 72 hours postexposure; the findings for the 24-hour cell harvest were selected as representative.
[c]Values in () are the combined results for both sexes.
[d]Signs of compound toxicity including mortality were reported at this dose level.
[e]Two males, including one reserve male, died during the study.

[*]Significantly higher ($p<0.01$) than the corresponding vehicle control.

Abbreviations used:

PCE = Polychromatic erythrocytes
MPE = Micronucleated polychromatic erythrocytes
NCE = Normochromatic erythrocytes

Data extracted from Study report; p. 18-20

D. REVIEWERS' DISCUSSION/CONCLUSIONS: We assess that the study was properly conducted and that the study author's interpretation of the data was correct. The dose investigated produced clear signs of overt toxicity and suggestive evidence of target cell cytotoxicity. There was, however, no appreciable increase in the frequencies of micronuclei in the bone marrow erythrocytes of male and female mice harvested 24, 48, and 72 hours posttreatment. Furthermore, assay sensitivity to detect a genotoxic response was demonstrated by the significant ($p<0.001$) results obtained in mice exposed to the positive control (50 mg/kg cyclophosphamide) at the three postexposure periods.

We conclude, therefore, that terrazole technical was tested at the maximum tolerated dose (1000 mg/kg) and failed to induce a genotoxic response in the mouse micronucleus assay.

E. QUALITY ASSURANCE MEASURES: Was the test performed under GLPs? Yes. (A quality assurance statement was signed and dated November 26, 1985.)

CORE CLASSIFICATION: Acceptable. The study satisfies Guideline requirements (§84-2) for genetic effects Category II, Structural Chromosome Aberrations.