4-20-93

FINAL

DATA EVALUATION REPORT

Kathon 886 MW Biocide

Study Type: Mutagenicity: In Vivo Cytogenetic Assay with Rats

Prepared for:

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Environmental Protection Agency
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Work Assignment Number: 2-47
Clement Number: 135
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GUIDELINE SERIES 84: MUTAGENICITY
IN VIVO MAMMALIAN CYTOGENETICS

MUTAGENICITY STUDIES

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DATA EVALUATION REPORT

STUDY TYPE: Mutagenicity: In vivo chromosome aberration in mouse bone marrow cells.

EPA IDENTIFICATION Numbers:

Tox Chem. Number: 107102

MRID Number: 425380-01

TEST MATERIAL: Kathon 886 MW Biocide

SYNONYM: RH-886; chloro-2-methyl-3(2H)-isothiazolone; methyl-3(2H)-isothiazolone

SPONSOR: Rohm and Haas Company, Spring House, PA.

STUDY NUMBERS: SITEK Study No. 0202-1541; Rohm and Haas Report No. 92RC-0054.

TESTING FACILITY: SITEK Research Laboratories, Rockville, MD.

TITLE OF REPORT: Acute Test for Chemical Induction of Chromosome Aberration in Mouse Bone Marrow Cells In Vivo

AUTHOR: R. Gudi

REPORT ISSUED: October 16, 1992

CONCLUSIONS--EXECUTIVE SUMMARY: The single oral gavage administration of 3, 15 or 30 mg ai/kg Kathon 886 MW Biocide to male or female mice did not cause a significant increase in the frequency of structural chromosome aberrations in bone marrow cells harvested 6, 24, or 48 hours posttreatment. Deaths occurred in animals of both sexes administered $\geq$ 30 mg/kg. Based on these findings, we conclude that Kathon 886 MW Biocide was adequately tested over an appropriate range of doses and found to be nonclastogenic in this in vivo cytogenetic assay.

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

A. MATERIALS:

1. Test Material: Kathon 886 MW Biocide

Description: Amber-gold liquid
Identification numbers: Batch No. J70089; Sample No. TD 92-051
Purity: 14.1% active ingredient (sponsor analysis); the components in the remaining 85.9% of the test material were not specified; the analytical report accompanying the study indicated that RH-886 is composed of RH-573 and RH-651 in the approximate ratio of 25:75, respectively (see Appendix A).
Receipt date: May 1, 1992
Stability: Not reported
Contaminants: None listed
Solvent used: Deionized water
Other provided information: The test material was stored at room temperature. Test solutions were corrected for purity and prepared before use. Analytic determinations were performed to verify actual concentrations of the dosing solutions.

2. Control Materials:

Negative/route of administration: None

Vehicle/final concentration/route of administration: Sterile deionized distilled water (DH_2O) was administered once by oral gavage at a dosing volume of 10 mL/kg.

Positive/final dose(s)/route of administration: Triethylenemelamine (TEM) was administered once by intraperitoneal (i.p.) injection at a dose of 1.0 mg/kg. The preparation of TEM was not described.

3. Test Compound:

Route of administration: Oral gavage

Volume of test substance administered: 10 mL/kg; the report did not indicate if dosing volume was based on individual body weights.

Dose levels used:

•Range-finding study: 10, 20, 30, 40 and 50 mg ai/kg

•Chromosome assay 1: 3.5, 17.5 and 35 mg ai/kg

•Chromosome assay 2: 3.0, 15, 25, 30 and 35 mg ai/kg

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4. Test Animals:

a. Species: Mouse Strain: CD-1 Age: 7-9 weeks (at dosing)
Weight: 21-34 g (males); 17-30 g (females)
Sex: Male and Female Source: Charles River Laboratories, Raleigh, NC for range-finding study and chromosome aberration Assay 1; Charles River Laboratories, Portage, MI for chromosome assay 2.

b. Number animals used per dose:

- Treatment groups: 5-7 males 5-7 females per dose per sacrifice time

Note: For the chromosomal assays, 7 males and 7 females were assigned to the highest dose groups (35 mg ai/kg in assay 1; 25, 30, and 35 mg ai/kg in assay 2). Five males and five females were assigned to the other (lower) dose groups. Five animals/sex/dose/group were used in the Range Finding Study.

- Positive control: 5 males 5 females per dose at the 24-hour sacrifice only

- Vehicle control: 7 males 7 females per dose per sacrifice time

Note: Slides were scored from 5 animals/sex/treatment group/sacrifice time

c. Properly maintained? Yes.

B. TEST PERFORMANCE:

1. Treatment and Sampling Times:

Test compound
Dosing: x once _____ twice (24 hours apart)
_____ other (describe):

Sampling (after last dose): x 6 hours _____ 12 hours
x 24 hours x 48 hours _____ 72 (mark all that are appropriate)
_____ other (describe):

Negative and/or vehicle control
Dosing: x once _____ twice (24 hours apart)
_____ other (describe):

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Sampling (after last dose): __x__ 6 hours _____ 12 hours
__x__ 24 hours __x__ 48 hours _____ 72 (mark all that are appropriate)
_____ other (describe):

Positive control

Dosing: __x__ once _____ twice (24 hours apart)
_____ other (describe):

Sampling (after last dose): _____ 6 hours _____ 12 hours
__x__ 24 hours _____ 48 hours _____ 72 (mark all that are appropriate)
_____ other (describe):

2. Administration of spindle inhibitor

Inhibitor used/dose: Colchicine/1 mg/kg

Administration time: 1.5-2.5 hours prior to sacrifice

Route of administration __x__ i.p. _____ other (describe)

3. Tissues and Cells Examined:

__x__ bone marrow _____ other (list):

Number of cells per animal per treatment group examined: 50.

Number of cells per animal per control group examined: 50.

The mitotic index (MI) was determined for each animal by counting the number of mitotic cells in a sample of 100 cells.

4. Details of Cell Harvest and Slide Preparation: Animals in the treatment and vehicle control groups were sacrificed by CO_2 asphyxiation at 6, 24, and 48 hours postexposure to the appropriate dose of the test material or vehicle. Animals in the positive control group were sacrificed 24 hours posttreatment. Bone marrow was flushed from both femurs into a tube containing a small volume of Hank's balanced salt solution. Collected marrow cells were centrifuged, resuspended in 0.06 M KCl, incubated at 37°C and recentrifuged. The supernatants were discarded. Cell pellets were fixed twice in methanol:acetic acid (3:1), held overnight at 4°C, dropped onto slides, air dried, stained with Giemsa, mounted and coded.

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5. Evaluation Criteria:

   (a) Assay validity: The assay was considered valid if (1) the percentage of cells with aberrations in the vehicle control animals was $\leq$ 4%, (2) the percentage of cells with aberrations in the positive control animals was $\geq$20% and (3) at least 50% of the animals/sex/treatment group were alive at the sacrifice.

   (b) Positive response: The test material was considered positive if a dose-related increase in the percentage of cells with aberrations was observed with the increase being statistically significant ($p\leq 0.05$) for at least one dose. In the absence of a dose-response, a significant increase occurring at two or more dose levels was also considered a positive response.

6. Statistical Methods: The percentage of cells with structural aberrations was evaluated using a Chi-square test. Significance was denoted at $p<0.05$. Data for males and females were analyzed separately.

7. Protocol: See Appendix B.

C. REPORTED RESULTS:

1. Analytical Determination: The concentrations of the test material in three aqueous dosing solutions (target concentrations: low-dose: 300 ppm; mid-dose: 1500 ppm; high-dose: 3000 ppm) were analyzed by reverse phase high performance liquid chromatography. The vehicle was also analyzed. Since the test material consisted of RH-573 and RH-651 in the approximate ratio of 25/75 RH-573/RH-651 (see Appendix A), these 2 components were analyzed separately. The concentration of the test material in the low-, mid- and high-dose solutions were 116.8%, 107.4%, and 104.1% of the target concentrations, respectively.

2. Range-finding Study: Sluggishness was noted in all animals receiving 40 or 50 mg/kg approximately 2 hours after treatment. In addition, inactivity and piloerection were seen (on days 1 and 2) in animals administered 50 mg/kg. Piloerection was also observed in 4/5 males and 3/5 females administered 30 mg/kg. The incidence of death in males treated with 40 or 50 mg/kg was 2/5 and 2/5, respectively. In females, receiving the same dose levels, the incidence of death was 1/5 and 2/5, respectively. Deaths occurred between 4 hours after treatment to day 10 postdosing. Necropsy findings included bloated stomachs and/or reddish lungs. Mean body weights were increased in all dose groups over the 14-day observation period. However, overall mean body weight gains were lower in males administered 40 and 50 mg/kg (30 and 60% lower, respectively) and in females administered 50 mg/kg (64% lower) when compared to controls. Based on these data

doses selected for the first cytogenetic assay were 3.5, 17.5, and 35 mg/kg of the test material.

3. Cytogenetic Assay: Mortality occurred in animals treated with 35 mg/kg in chromosome assay 1. At the 6-hour sacrifice, 3/7 females died. At the 24-hour sacrifice, 4/7 males and 1/7 females died, while at the 48-hour sacrifice, 5/7 males and 1/7 females died. Consequently, assay 1 was aborted because of the high incidence of deaths at 35 mg/kg. Doses evaluated in the second cytogenetic assay were 3, 15, 25, 30, and 35 mg/kg. At the 6-hour sacrifice, 1/7 males administered 35 mg/kg died 5 hours after treatment. At the 24-hour sacrifice, 2/7 and 1/7 females died following treatment with 25 or 35 mg/kg, respectively, while 1/7 and 2/7 males were found dead after treatment with 30 or 35 mg/kg, respectively. At the 48-hour sacrifice, 1/7 females and 1/7 males died at 30 mg/kg, and 2/7 males died at 35 mg/kg. Based on the mortality data, slides were prepared and scored from animals treated with 3, 15 or 30 mg/kg.

   Representative results from the bone marrow cytogenetic phase of the assay are presented in Table 1. With the exception of a slight decrease in the MI of high-dose females at the 6-hour sacrifice and high-dose males 24 hours posttreatment; the data showed no evidence that Kathon 886 MW Biocide had a cytotoxic effect on the target organ. There were also no increases in the number of cells with chromosome aberrations or in the number of aberrations per cell in bone marrow cells evaluated in male and female mice treated with 3, 15 or 30 mg/kg test material. In contrast, the positive control (1.0 mg/kg TEM) induced significant ($p \leq 0.05$) clastogenic effects in both males and females.

D. REVIEWERS' DISCUSSION/CONCLUSIONS: We assess that the study was properly conducted and the study author's interpretation of the data was correct. The test material was assayed at dose levels that caused mortality in treated animals, but failed to induce a cytotoxic or clastogenic response in mouse bone marrow cells. The sensitivity of the test system to detect a positive response in male and female mouse bone marrow cells was shown by significant ($p \leq 0.05$) results obtained with the positive control (1.0 mg/kg TEM). We conclude, therefore, that Kathon 886 MW Biocide was not genotoxic in this in vivo mouse micronucleus assay.

E. QUALITY ASSURANCE MEASURES: Was the test performed under GLPs? Yes. (A quality assurance statement was signed and dated 10/16/92).

F. CBI APPENDICES: Appendix A, Results of the Analytical Determination, CBI p. 64; Appendix B, Protocol, CBI pp. 44-58; Appendix C, Materials and Methods, CBI pp. 9-16.

TABLE 1. Representative Results of the Mouse Bone Marrow Cytogenetics Assay with Kathon 886 MW Biocide

Treatment/Dose	Dose	Exposure Time[a]	No. of Animals Analyzed	No. of Metaphases Analyzed	Relative Mitotic Index	Total Number of Aberrations	Number of Aberrations/ Cell	Number of Cells with Aberrations[b]	Biologically Significant Aberrations Total No./Type
Vehicle Control									
Deionized water	10 mL/kg	6 h	5 M	250	100	2.0	0.008	2.0	2TB
		6 h	5 F	250	100	0.0	0.000	0.0	---
		24 h	5 M	250	100	1.0	0.004	2.0	1TB
		24 h	5 F	250	100	0.0	0.000	0.0	---
		48 h	5 M	250	100	1.0	0.004	1.0	1TB
		48 h	5 F	250	100	1.0	0.004	1.0	1TB
Positive Control									
Triethylenemelamine	1.0 mg/kg	24 h	5 M	250	48	338	1.35	63*	52TB; 16ISB; 48TF; 1ISF; 11TR; 21SD
		24 h	5 F	250	20	219	2.41	122*	116TB; 12ISB; 42TF; 4ISF; 1R; 3TR; 11CR; 3PU
Test Material									
Kathon 886 MW Biocide	30 mg/kg[c]	6 h	5 M	250	141	1.0	0.004	1.0	1TB
		6 h	5 F	250	67	0.0	0.000	0.0	---
		24 h	5 M	250	57	2.0	0.008	2.0	1TB; 1TE
		24 h	5 F	250	94	0.0	0.000	0.0	---
		48 h	5 M	250	218	0.0	0.000	0.0	---
		48 h	5 F	250	164	0.0	0.000	0.0	---

[a]Time after test material or vehicle exposure by oral gavage or triethylenemelamine exposure by intraperitoneal injection.
[b]Excluding gaps.
[c]Results for the low-(3 mg/kg) and mid-(15 mg/kg) treatment groups at all sacrifice times did not suggest a clastogenic effect.
Abbreviations used:

TB = Chromatid break
ISB = Isochromatid break
TF = Chromatid fragment
ISF = Isochromatid fragment
R = Ring chromosome
QR = Quadriradial
TR = Triradial
CR = Complex interchange
SD = Cells with ≥10 aberrations; counted as 10 aberrations
PU = Pulverization; counted as 10 aberrations

Note: The study authors considered "PU" and "SD" to be equal to 10 aberrations in the calculations.
*Significantly higher than the vehicle control group ($p<0.05$) by Chi-square.

Note: The data were extracted from the study; see CBI pp. 24-39.

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

APPENDIX A

RESULTS OF THE ANALYTICAL DETERMINATIONS
CBI pp. 60-65

KATHON 88L (PC # 107102)

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____ A draft product label.

____ The product confidential statement of formula.

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APPENDIX B

PROTOCOL
CBI pp. 43-59

KATHON 886 (PC # 107102)

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APPENDIX C

MATERIALS AND METHODS
CBI pp. 9-16

KATHON 886 (PC# 107102)

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