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

008498

AUG 9 1991

MEMORANDUM

OFFICE OF
PESTICIDES AND TOXIC
SUBSTANCES

SUBJECT: Kathon 886 MW Microbiocide: Submission of Two Mutagenicity Studies to Fulfill Data Call-In Requirements

TO: Valdis Goncarovs/Jim Wilson
Product Manager (31)
Registration Division (H7505C)

FROM: Linda L. Taylor, Ph.D. Linda Taylor 7/29/91
Toxicology Branch II, Section II,
Health Effects Division (H7509C)

THRU: K. Clark Swentzel K. Clark Swentzel 7/29/91
Section II Head, Toxicology Branch II
Health Effects Division (H7509C)

and

Marcia van Gemert, Ph.D. Marcia van Gemert 8/1/91
Chief, Toxicology Branch II/HFAS/HED (H7509C)

Registrant: Rohm & Haas Company
Chemical: 5-chloro-2-methyl-4-isothiazolin-3-one and 2-methyl-4-isothiazolin-3-one
Synonym: Kathon 886 MW Microbiocide
Project No.: 1-1292
Caswell No.: 195C
Record No.: Case: 022081; Submission: S396418
Identifying No.: 000707-00130
MRID No.: 418755-01 and 418755-02
Action Requested: Please review studies.

Comment: The Registrant has submitted two mutagenicity studies to fulfill the Tier I data requirements for methylisothiazolinone under the Antimicrobial Data Call-In. Both studies have been reviewed and the DER's are attached.

1. Acute Test for Chemical Induction of Chromosome Aberration in Mouse Bone Marrow Cells In Vivo. MRID # 418755-01. Under the conditions of the study, there was no indication that Kathon 886 MW Biocide produced chromosome aberrations in mouse bone marrow cells following oral administration at dose levels of 4 and 20 mg/kg, with bone marrow harvest at 6, 24, and 48 hours after dosing.

However, this assay does not meet the criteria for a valid study since less than 50% of the high-dose (40 mg/kg) females survived to study termination. Additionally, none of the high-dose animals was scored for the induction of chromosome aberration. This study is classified unacceptable, and it does not satisfy the guideline requirement [84-2(B)] for mutagenicity, Category II.

2. Test for Chemical Induction of Unscheduled DNA Synthesis in Rat Primary Hepatocyte Cultures by Autoradiography. MRID # 418755-02. Under the conditions of the study, Kathon 886 MW Biocide did not induce UDS in primary rat hepatocytes at dose levels (0.03475 to 3.475 µg a.i./mL) that were sufficiently high to adequately assess the mutagenic potential of the test material. At the highest dose levels assayed, there were sufficient levels of cytotoxicity expressed. The positive control, 2AAF, induced a significant increase in UDS, and 100% of the cells scored displayed a net nuclear grain count of 5 or more. The untreated and solvent controls were within acceptable limits. This study is classified Acceptable, and it satisfies the guideline requirements [84-2(C)] for mutagenicity, Category III.

DISCUSSION

The Registrant indicated that the test material [Kathon 886 MW] in both studies (EPA Reg. No. 707-129) is compositionally identical to the formulation grade product Kathon 886F Microbicide (EPA Reg. No. 707-130), which has been the reference registration for the Antimicrobial Data Call-In.

CONCLUSION

The UDS assay in rat primary hepatocyte cultures is acceptable, and it satisfies the guideline requirement [84-2(C)] for mutagenicity, Category III. The chromosome aberration assay in mouse bone marrow cells is unacceptable, and it does not satisfy the guideline requirement [84-2(B)] for mutagenicity, Category II.

Reviewed by: Linda L. Taylor, Ph.D. Linda Lee Taylor 7/29/91
Section II, Tox. Branch II (H7509C)
Secondary Reviewer: Byron Backus, Ph.D. Byron T. Backus 7/29/91
Section II, Tox. Branch II (H7509C)

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DATA EVALUATION REPORT

STUDY TYPE: Mutation-Chromosome Aberration TOX. CHEM. NO.: 195C

MRID NO.: 418755-01

TEST MATERIAL: Kathon 886 MW Biocide

SYNONYMS: methylisothiazolinone

STUDY NUMBER: SITEK 0159-1541

SPONSOR: Rohm and Haas Company

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: Ramadevi Gudi

REPORT ISSUED: April 29, 1991

QUALITY ASSURANCE: A quality assurance statement was provided.

CONCLUSION: Under the conditions of the study, there was no indication that Kathon 886 MW Biocide produced chromosome aberrations in mouse bone marrow cells following oral administration at dose levels of 4 and 20 mg/kg, with bone marrow harvest at 6, 24, and 48 hours after dosing. However, this assay does not meet the criteria for a valid study since less than 50% of the high-dose (40 mg/kg) females survived to study termination. Additionally, none of the high-dose animals was scored for the induction of chromosome aberration. This study is classified unacceptable, and it does not satisfy the guideline requirement [84-2](B)] for mutagenicity, Category II.

A. MATERIALS

1. Test Material: Kathon 886 MW Biocide; Description: amber-gold liquid; no data regarding stability were provided. Batch/Lot #: 53083 TD# 90-149; % Active Ingredient: 13.9%; Solvent: deionized distilled water; Lot #: 110690 & 121790.

   Comment:The Sponsor analyzed the dosing solutions, which were reported to be 97-109% of the targeted concentrations. The ratio of the two active ingredients ranged from 2.78 to 3.28.

2. Control Material: Negative: sterile, deionized, distilled water; Positive: Triethyleneamine (TEM), [1.0 mg/kg (0.1 mg/mL X 10 mL/kg)], i.p. injection, Source: Polysciences, Lot #: 84505.(Warner Graham Co., Lot #: DSP-MD-43).

3. Test Organism: (A) Species: mouse; Strain: CD-1; Age: 42 days upon receipt/7-day quarantine; Weight: males (24-36 g), females (20-28 g); Source: Charles River Laboratories, Raleigh, NC.

4. Statistical Analysis: The number of cells with aberrations for each treatment group (per sex) were analyzed using a Chi-square analysis, and significance was determined at the 95% confidence level ($p<0.05$) in comparison to the vehicle control.

B. TEST PERFORMANCE

1. Treatment and Sampling Times:

   a. Test Compound

   In the rangefinding study, the doses used were 0, 45, 50, 55, 60, 65 mg a.i./kg, which was administered by gavage once (10 mL/100 g body weight). In the chromosomal aberration assay, the doses were 0, 4, 20, and 40 mg a.i./kg. The number of animals used (randomly assigned) in the different phases of this study is shown in the following table.

Study Phase									
Dose (mg/kg)	0	4	20	40	45	50	55	60	65
Rangefinding males/females	10/10	-	-	-	10/10	10/10	5/5	5/5	5/5
Chrom. Aber. males/females									
6 hours	7/7	5/5	7/7	7/7					
24 hours	7/7	5/5	7/7	7/7					
48 hours	7/7	5/5	7/7	7/7					

The positive control at each time point (6 hour and 24/48 hour assay) consisted of 7 animals per sex. An additional 10 animals (5/sex) at the control and high-dose (40 mg/kg) levels were used for body weight determination.

The animals in the rangefinding study were observed for 14 days following exposure for clinical signs and death, and body weights were determined on days 0 and 14. All animals were necropsied and visual examinations were made of external structures, including body orifices, and organs were examined in situ.

In the chromosomal aberration assay, animals were sacrificed at 6, 24, or 48 hours after dosing.

b. Negative and Positive Controls

Dosing was by gavage for the negative control animals and i.p injection for the positive control animals. Sacrifice of the negative control animals was the same as for the treated animals, but the positive controls were sacrificed only at 24 hours post dose.

c. Administration of Spindle Inhibitor

The animals were treated with Colchicine (1 mg/kg, i.p) 1.5 to 2.5 hours prior to sacrifice.

2. Tissues and Cells Examined:

Bone marrow from the femurs of each animal was collected and placed into centrifuge tubes containing Hanks' Balanced Salt Solution (HBSS), centrifuged, and the supernatant was removed. The cells were resuspended in 0.060 M KCl, incubated at 37°C for 10 minutes, centrifuged, and the supernatant was removed. Freshly prepared chilled Carnoy's fixative [3:1 (v/v) methanol:acetic acid] was added and the cells were resuspended and maintained at 0-4°C for 30 minutes, centrifuged again, the supernatant removed and fresh fixative was added to the cells, which were stored overnight at 0-4°C. The cells were collected by centrifugation, resuspended in fresh fixative, and dropped on precleaned microslides to prepare chromosome spreads. The slides were air dried, stained in Giemsa stain, and mounted in Permount using #1 cover glasses. The slides were read "blind" and scored from 5 male and 5 female animals from each group at all harvests. Where possible, a minimum of 50 metaphases was scored from each animal (500 metaphases per dose level per harvest). Vernier readings were recorded for each aberration scored. Samples of 100 cells were scanned from each animal in order to determine the mitotic index, which was recorded on the score sheet as the % of dividing cells.

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3. Preliminary Rangefinding Assay: During the 14-day observation period, clinical signs included piloerection, weak appearance, and no movement. Deaths were as follows (see table below and graph):

Dose	# of Deaths	
(mg/kg)	Males	Females
45	5/10	5/10
50	4/10	7/10
55	0/5	5/5
60	5/5	2/5
65	4/5	3/5

Necropsy findings in those animals dying included reddish lungs and bloated stomachs. Survivors did not display any abnormalities. With the exception of one male who lost 10 g (50 mg/kg dose) and one female (60 mg/kg, no gain), all survivors gained weight during the study. Nothing else was reported for this phase, and there was no discussion of how these data were used for determining the dose levels or harvest times for use in the chromosomal aberration assay. NOTE: In the body of Table 2, which lists the results for the females, M is listed under SEX instead of F.

4. Aberration Assay: Body-weight changes in the animals fluctuated for all groups (both sexes), and there was no dose response, in general. In animals dosed for body-weight determinations only (0 and 40 mg/kg), there was no adverse effect observed on body weight with respect to survivors vs controls. It is to be noted that of the animals dying, 2 of 3 (both sexes) were animals with the lowest initial body weights.

There was 100% survival in the control, low-, and mid-dose groups (both sexes), and 71% (males)/43% (females) in the high-dose animals. Deaths were as follows:

Deaths	Males				Females			
Dose-mg/kg	0	4	20	40	0	4	20	40
n=	21	15	21	21	21	15	21	21
Harvest times (hours)								
6	0/7	0/5	0/7	0/7	0/7	0/5	0/7	0/7
24	0/7	0/5	0/7	0/7	0/7	0/5	0/7	0/7
48	0/7	0/5	0/7	2/7	0/7	0/5	0/7	4/7

Of the animals used for body-weight determinations (0 and 40 mg/kg), three per sex in the treated group died within the first two days, with a % survival at this dose of 40%.

The types of chromosome aberrations scored and the

corresponding abbreviations used in the tables of results are listed on page 13 of the study (copy appended). The number of aberrations per cell and the number of cells with one or more aberrations were calculated for each dose level. Chromatid gaps, chromosome gaps, and polyploids were scored, but they were not included in calculating the percentage of cells with aberrations and the number of aberrations per cell. Of the remaining aberrations, each aberration scored was counted as one, except pulverization (pu) and severely damaged (sd). Each of these aberrations was considered equal to 10 aberrations in calculating the number of aberrations per cell.

The results of the assay are summarized in Tables 16 and 17 of the report (copies appended). In both the test material (4 and 20 mg/kg) and negative control groups, the number of cells with aberrations ranged from 0-2 in males and 0-1 in test material (4 and 20 mg/kg) females and 0 in negative control females. The positive control showed a high, statistically significant increase ($p< 10^{-8}$) in the cells with aberrations. It was reported that, due to the high number of treatment-related deaths at the 40 mg/kg dose level, this group was not scored for the induction of chromosome aberrations.

CRITERIA FOR A VALID ASSAY

1. In the vehicle control, the number of cells with aberrations should not exceed 4%.
2. At least 20% of the cells scored in the positive control should show one or more chromosome aberrations.
3. At least 50% of the animals from each sex must be alive at the time of sacrifice for each dose level.

Positive Response: The test material is considered to have caused a positive response in this assay if: (a) the test material produces a positive dose response trend and a statistically significant increase in the number of cells with aberrations for at least one dose level; (b) in the event there is no positive dose response trend, at least two consecutive test doses show a statistically significant increase in the number of cells with aberrations at the 95% confidence level as determined by a Chi-square analysis.

Negative Response: The test material is considered to have caused a negative response if no indication of a positive dose response is observed and none of the test doses shows a significant increase in the number of aberrant cells at the 95% confidence level in a Chi-square analysis.

5. Discussion/Conclusion

Because of the number of deaths observed at the high-dose level (40 mg/kg), that dose was not scored for the induction

of chromosome aberration. However, TB II notes that in the 48-hour sacrifice group, there were 5 high-dose males that survived, which is the number of animals used for scoring at the other dose levels. In addition, all of the 40 mg a.i./kg males and females scheduled for the 6- and 24-hour sacrifices survived to these times. Further, TB II believes the three high-dose females who survived to their scheduled 48-hour sacrifices should have been scored also. It is not understood by this reviewer why the 6-hour harvest, which was performed subsequent to the 24- and 48-hour harvests, utilized a 40 mg a.i./kg dose unless the intention was to determine findings at this level, particularly since it was already known that there was approximately 50% mortality at this dose level in animals sacrificed at 48 hours.

Under the conditions of the study, there was no indication that Kathon 886 MW Biocide produced chromosome aberrations in mouse bone marrow cells at dose levels of 4 and 20 mg/kg and sacrifice times of 6, 24, and 48 hours. However, TB II is concerned that preparations from animals at the 40 mg a.i./kg dose level were not scored for chromosomal aberration, particularly as all of these animals scheduled for 6- and 24-hour sacrifices survived to these times. This study is classified unacceptable, and it does not satisfy the guideline requirement [84-2(B)] for mutagenicity, Category II.

KATHON 886 (PC 107102)

Page____ is not included in this copy.

Pages 9 through 12 are not included.

The material not included contains the following type of information:

____ Identity of product inert ingredients.

____ Identity of product impurities.

____ Description of the product manufacturing process.

____ Description of quality control procedures.

____ Identity of the source of product ingredients.

____ Sales or other commercial/financial information.

____ A draft product label.

____ The product confidential statement of formula.

____ Information about a pending registration action.

X FIFRA registration data.

____ The document is a duplicate of page(s) _____.

____ The document is not responsive to the request.

The information not included is generally considered confidential by product registrants. If you have any questions, please contact the individual who prepared the response to your request.

Reviewed by: Linda L. Taylor, Ph.D. Linda Lee Taylor 7/25/91
Section II, Tox. Branch II (H7509C)
Secondary Reviewer: Byron Backus, Ph.D. Byron T. Backus 7/25/91
Section II, Tox. Branch II (H7509C)

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DATA EVALUATION REPORT

STUDY TYPE: Mutagenicity-UDS Assay TOX. CHEM. NO.: 195C

MRID NO.: 418755-02

TEST MATERIAL: Kathon 886 MW Biocide

SYNONYMS: methylisothiazolinone

STUDY NUMBER: SITEK 0159-5100

SPONSOR: Rohm and Haas Company

TESTING FACILITY: SITEK Research Laboratories, Rockville, MD

TITLE OF REPORT: Test for Chemical Induction of Unscheduled DNA Synthesis in Rat Primary Hepatocyte Cultures by Autoradiography

AUTHOR: KJ Pant

REPORT ISSUED: January 13, 1990

QUALITY ASSURANCE: A quality assurance statement was provided.

CONCLUSIONS: Under the conditions of the study, Kathon 886 MW Biocide (13.9% a.i.) did not induce UDS in primary rat hepatocytes at dose levels (0.03475 to 3.475 µg a.i./mL) that were sufficiently high to adequately assess the mutagenic potential of the test material. At the highest dose levels assayed [3.475 µg a.i./mL (first assay); 2.224 µg a.i./mL (second assay)], there were sufficient levels of cytotoxicity (relative cell survival of 6.7 and 25.6%, respectively) expressed. The positive control, 2AAF (10 µg/mL), induced a significant increase in UDS, and 100% of the cells scored displayed a net nuclear grain count of 5 or more. The untreated and solvent controls were within acceptable limits. This study is classified Acceptable, and it satisfies the guideline requirements [84-2(C)] for mutagenicity, Category III.

A. MATERIALS

1. Test Material: Kathon 886 MW Biocide; Description: amber-gold liquid; Batch/Lot #: 53083 TD# 90-149; % Active Ingredient: 13.9%; Solvent: deionized distilled water; Lot #: 072390.

2. Control Material: Negative: untreated, ethanol, and water; Positive: 2-acetylaminofluorene (2-AAF; Sigma Chemical Co., Lot #: 58F-0313, Purity: 95-97%), dissolved in ethanol (Warner Graham Co., Lot #: DSP-MD-43).

3. Test Organism: (A) Species: male rats; Strain: Sprague-Dawley; Age: not specified; Body Weight: approximately 200-325 grams; Source: Charles River Breeding Laboratories, Raleigh, NC; quarantined at least one week after receipt; no other details provided. (B) Primary Rat Hepatocytes: obtained from the livers of the animals described above by in situ collagenase perfusion. The methods for isolation of hepatocytes are modifications of procedures of Williams (Ref. 1 & 2) and Bradlaw (Ref. 3). Separate hepatocyte preparations (one rat per preparation) were used for the Range-finding test and UDS assay. Livers were perfused with 120 mL ethylene glycol-bis-(β-aminoethyl ether)-N,N,N',N'-tetraacetic acid (EGTA) solution [0.5 mM in Ca^{++}-free and Mg^{++}-free Hanks' Balanced Salt Solution (HBSS) buffered with 0.01 M HEPES (pH 7.3)] and then with collagenase solution [100 units of collagenase type I in serum-free WME buffered with HEPES (pH 7.3), supplemented with 2 mM L-glutamine, penicillin and streptomycin] and removed from the animal. Cells were removed using a stainless steel sieve and pooled, and a viable cell count was performed using the trypan blue dye exclusion method. Only cultures having more than 80% viable cells were accepted for the assay. Cell suspensions containing 250,000 cells were seeded into pre-conditioned tissue culture plates containing culture medium (for initial seeding of hepatocytes, each culture vessel contained a total of 3 mL of medium). Cells were seeded into culture vessels without a cover glass for the cytotoxicity test and with a cover glass for the UDS assay. The cells were seeded into 6 replicate culture vessels per treatment level for the UDS assay (3 used for evaluating cytotoxicity; 3 for determining unscheduled DNA synthesis). Only 2 duplicate cultures per treatment were used in the range-finding test.

4. Range-finding Test: To determine the appropriate test material concentrations for the UDS assay, ten concentrations of the test material were evaluated (0.0139, 0.0695, 0.139, 0.695, 1.39, 6.95, 13.9, 69.5, 139, and 695 μg a.i./mL). Duplicate test cultures, seeded about 2 hours earlier, were used at each treatment level and prior to treating the cells, the cultures were washed and refed with WME buffered with HEPES (pH 7.3), supplemented with L-glutamine, penicillin and streptomycin.

Exposure was initiated by adding 20 µL of appropriate stock solution or solvent to the culture plates. After an 18-hour exposure period, the cells were washed with Ca^{++}-free and Mg^{++}-free phosphate buffered saline (PBS), dissociated with trypsin, and counted for number of viable cells using the trypan blue dye exclusion method. The average number of viable cells for the replicate cultures was determined, and the Relative Cell Survival (RCS) was calculated by comparing treated to solvent control groups. Relative Toxicity was calculated by subtracting RCS from 100%.

5. Test Material Preparation/Analysis: The test material was tested as received, and doses were expressed as active ingredient concentration of the test material (i.e., 5000 µg/ml X 13.9% a.i. = 695 µg a.i./mL). Immediately prior to use, the test material was weighed and diluted with water to the appropriate concentration (stock solution), and serial dilutions were made from this. To avoid possible problems with photoinactivation, all treatments were performed under UV-filtered lights. The stability and a.i. concentration of the test material in water was not determined at the testing facility, but was stated to have been confirmed by the Sponsor (Appendix I). NOTE: The report states that the analytical results show the samples to be within 97-129% of the expected range. It is to be noted that the Total PPM listed for R90-1835-1 should be 442.1, not 444.2.

B. TEST PERFORMANCE

Unscheduled DNA Synthesis Assays: Test cultures were prepared as described above. Three replicate cultures without cover glasses were used for the parallel cytotoxicity determination, and three replicate cultures with cover glasses were used for UDS measurements at each treatment. In the first UDS assay, the cells were treated with either 0.03475, 0.10425, 0.3475, 1.0425, or 3.475 µg a.i./mL, 10 or 2 µg/mL 2AAF (positive), 1% water or ethanol (negative), or untreated control (WME only). The treated cultures were exposed to 10 µCi/mL ^{3}H-thymidine (specific activity: 20 Ci/mM, NE Nuclear) for approximately 18 hours. Following the exposure period, the parallel cytotoxicity determination was performed as described in the range-finding test. The cultures for UDS measurement were washed 3 times in WME buffered with HEPES (pH 7.3), L-glutamine, penicillin and streptomycin, swelled in 1% sodium citrate solution, and fixed in three 15-minute changes of methanol-glacial acetic acid fixative. The cover glasses were removed, allowed to dry, and mounted cell side up on glass slides, which were dipped in NTB-2 emulsion at 43-45°C. The emulsion was allowed to drain and dry for 90 minutes at room temperature in the dark. The slides were then stored at 0-4°C in light-tight slide boxes with a desiccant for 5 days, then developed in Kodak D-19, fixed in Kodak fixer, stained in

hematoxylin stain, and mounted in Permount using #1 cover glasses. The second UDS assay was performed similarly to the first, but the dose levels were different (0.0371, 0.1112, 0.371, 1.112, and 2.224 μg a.i./mL).

The slides were scored "blind". Whenever possible, 150 randomly selected nuclei(each with three background counts) were counted per treatment. All grain counts were performed using an electronic colony counter (ARTEK 880) equipped with a microscope-mounted auxiliary television camera. Grain counts were done directly by using the "count" mode of the colony counter. Only cells that appeared normal and healthy were scored for UDS. Cells showing a severe cytotoxic effect, i.e., cells with constricted, irregularly shaped or very darkly stained nuclei, or cells having nuclei with a projected image of less than 4 mm^2 were not scored.

Incorporation of ^3H-thymidine into nuclear DNA was determined by counting the darkened grains localized over the nuclear area. The background incorporation was determined by counting at least three nucleus-size areas of cytoplasm adjacent to each nucleus. The net nuclear grain counts were determined by subtracting the average background count from the nuclear count. For each treatment, the average net nuclear grain count $\pm$ standard deviation was calculated and recorded on a summary sheet. The number of nuclei showing 5 or more net nuclear grain counts in each cover glass was also recorded. Additionally, 300 nuclei per culture were counted at random to determine the percentage of nuclei exhibiting S-Phase DNA synthesis.

C. ANALYSIS OF DATA

1. Criteria for a Valid Assay: (a) In the negative control, the average net nuclear grain count per cell should be less than 5 and the percentage of cells with net nuclear grain counts of 5 or greater should be less that 20%. (b) At least 30% of the cells scored in the positive control should show a net nuclear grain count of 5 or more and the average net nuclear grain count per cell should be 20 or more. (c) At least one of the test material concentrations scored should show more than 25% reduction in RCS. This requirement should not be applied to test materials that do not show any apparent cytotoxicity at the maximum soluble concentration or highest allowable concentration. (d) The negative control(s) should exhibit at least 0.2% nuclei in replicative DNA synthesis.

2. Evaluation of Test Results: The results were considered significant if the average net nuclear grain count for the test material was increased by at least 5 grain counts over the concurrent solvent and/or untreated controls or more than 25% of the cells scored showed a net nuclear grain count of 5

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or more. Positive Response: (a) The test material caused a dose-related response and at least one concentration exhibited a significant increase over its concurrent solvent control; (b) In the absence of a dose response, at least two successive concentrations exhibited a significant increase over the concurrent solvent control data. Marginal Positive Response: One of the test material concentrations showed a significant positive response, although there was no indication of a positive dose response observed. Negative Response:There was no indication of a positive response observed and none of the test material concentrations showed a significant positive response.

D. RESULTS

Range-finding Test: Results of the range-finding test are presented in Table 1, copy appended. Concentrations greater than or equal to 6.95 µg a.i./mL were completely toxic, as evidenced by 0% survival. The next lowest concentration, 1.39 µg ai/mL, had a relative cell survival of 72.5% and, based on these results, the concentrations chosen for the UDS assay were 0.3475-3.475 µg ai/mL.

First UDS Assay: In the parallel toxicity test, the highest concentration (3.475 µg ai/mL) showed a relative cell survival of 6.7%, with 64.4% and 80% survival being displayed by the next two lower concentrations, respectively (see Table 2, copy appended). No toxicity was displayed at the two lowest concentrations (0.10425 and 0.03475 µg ai/mL). The results of the UDS assay are summarized in Table 3, copy appended. None of the test material concentrations scored for UDS showed a significant increase in average net nuclear grain counts. The positive control induced a significant increase in UDS with an average net nuclear grain count of 48.15 at the 10 µg/mL concentration, and 100% of the cells scored displayed a net nuclear grain count of 5 or more. The untreated and solvent controls met the criteria for a negative response, and a sufficient number of nuclei were found to be in replicative DNA synthesis indicating that there was no inhibition of DNA synthesis for any of the treatments, thus fulfilling all criteria for a valid assay.

Repeat UDS Assay: Table 4 presents the results of the parallel toxicity test conducted with this assay. The relative toxicity of the test material ranged from 9.3 to 74.4%. The results of the repeat assay are shown in Table 5. There was no significant increase in the average net nuclear grain counts at any of the concentrations tested. The positive and negative controls responded as expected. NOTE: The dose range listed at the top of Table 5 (the same as that reported in the experimental procedures) is not the same as that in the table (0.0371-2.224 vs 0.035-2.09).

CONCLUSION

Kathon 886 MW was tested at 5 concentrations in two separate rat hepatocyte unscheduled DNA synthesis assays (2 different dose ranges; 0.03475-3.475 and 0.0371-2.224 µg a.i./mL). Under the conditions of the study, Kathon 886 MW Biocide did not induce DNA damage in primary rat hepatocytes at dose levels that were sufficiently high, as evidenced by levels of relative toxicity. The positive control, 2AAF at 10 µg/mL, induced a significant increase in UDS with an average net nuclear grain count of approximately 48 in both UDS assays; with 100% of the cells scored having a net nuclear grain count of 5 or more. The findings for untreated and solvent controls were within acceptable limits. There was no indication of an effect. This study is classified Acceptable, and it satisfies the guideline requirements [84-2(C)] for mutagenicity, Category III.

Comment: For the record, a new Table 5 should be submitted with the correct dose levels reflected.