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

007060

MAR - 7 1989

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Kathon - Submission of Four Mutagenicity Studies in Compliance with the Data Call-In Notice for Octhilinone (Kathon 893T, EPA Registration No. 707-143)

TOX Chem No.: 613C
Project No.: 8-0908

FROM: William B. Greear, M.P.H. William B. Greear 3/6/89
Review Section II
Toxicology Branch I - Insecticide, Rodenticide Support
Health Effects Division (H7509C)

TO: Lois A. Rossi/Herman Toma, PM Team 21
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THRU: Marion P. Copley, D.V.M., Section Head M. Copley 3/6/89
Review Section II
Toxicology Branch I - Insecticide, Rodenticide Support
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and

Judith W. Hauswirth, Ph.D., Chief Judith W. Hauswirth 3/6/89
Toxicology Branch I - Insecticide, Rodenticide Support
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~~Four~~ THREE of the five mutagenicity studies are acceptable; the CHO in vitro cytogenetics study (#86RC-0048) is inconclusive. The requirement for mutagenicity testing has been fulfilled.

Background

Under a cover letter dated May 31, 1988, G.R. Ackerman of the Rohm & Haas Company has submitted four mutagenicity studies for evaluation in compliance with the Data Call-In

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Notice for octhilinone (Kathon 893T, EPA Registration No. 707-143). The results of the studies are summarized below.

| Study | Results | Classification |
|---|---|---|
| 1. Gene Mutation | | |
| a. Bacteria, Reverse Mutation | | |
| - Salmonella typhimurium Ames Strains (84R-0144) | Negative | UnAcceptable |
| b. Mammalian Cell Culture | | |
| - CHO/HGPRT Forward Mutation Assay (86A-055) | Negative | Acceptable |
| 2. Structural Chromosomal Aberration | | |
| a. Mammalian Cells in Culture | | |
| - In Vitro Cytogenetics CHO (86RC-0048) | Presumptively Positive | Inconclusive |
| b. Mammals In Vivo | | |
| - Cytogenetic Analysis (86R-218) | Negative | Acceptable |
| 3. Other Genotoxic Effects | | |
| a. Unscheduled DNA Synthesis | | |
| - Rat Hepatocyte (86A-0018) | Negative | Acceptable |

Three of the five studies listed above are acceptable. The three studies collectively fulfill the requirements for mutagenicity testing.

EPA: 68D80056
DYNAMAC No. 115-A
December 29, 1988

# DATA EVALUATION RECORD

## KATHON

## Mutagenicity--*Salmonella typhimurium* Mammalian Microsome Mutagenicity Assay

APPROVED BY:

Robert J. Weir, Ph.D.
Acting Department Manager
Dynamac Corporation

Signature: William L. McLellan (for)

Date: 12-29-88

007060

EPA: 68D80056
DYNAMAC No. 115-A
December 29, 1988

DATA EVALUATION RECORD

KATHON

Mutagenicity--*Salmonella typhimurium* Mammalian Microsome Mutagenicity Assay

REVIEWED BY:

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Principal Reviewer
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Date: 12-29-88

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Date: 1/3/89

Marion Copley, D.V.M.,
D.A.B.T., EPA Acting Section
Head, Section II
Toxicology Branch I (TS-769C)

Signature: Marion Copley
Date: 2/9/89

DATA EVALUATION RECORD

CHEMICAL: TD No. 84-122 (RH-893 process variation).

STUDY TYPE: Salmonella/mammalian microsome activation mutagenicity assay.

MRID NUMBER: 406475-01.

SYNONYMS/CAS no.: Kathon; Octhilione; Skane M-8; 2-octyl-3(2H)-isothiazolone.

SPONSOR: Rohm and Haas Company, Philadelphia, PA.

TESTING FACILITY: Rohm and Haas Company, Toxicology Department, Spring House, PA.

AUTHOR(S): Chism, E. M.

STUDY NUMBER(S): 84R-0144.

REPORT ISSUED: June 28, 1984.

CONCLUSION(S)-Executive Summary: Concentrations of TD No. 84-122 (RH-893 process variation) ranging from 0.5 to 7500 µg/mL with or without S9 activation were evaluated in the Salmonella typhimurium microsome mutagenicity assay. Results indicated that dose levels >75 µg/plate/+ or -S9 were cytotoxic to the four tester strains. At noncytotoxic doses, no appreciable increase in revertant colonies of any strain were seen. The study, however, does not provide acceptable evidence of a negative response for the following reasons:

1. The solvent control reverse-mutation frequencies for strain TA100 were too low.[1]

2. Individual plate counts were not provided and average values without the standard deviations were reported.

3. The reported results for solvent control mutant colony counts were based on the means and standard deviations from 39 replicate plates. We assume, therefore, that these results represent the historical data for the 13 most recent assays.

4. The concentration of 2-anthramine (2-AA) used to demonstrate strain sensitivity under S9-activated conditions was excessive (10 µg/plate) compared to usually accepted levels. Since direct-acting positive controls were not used, the ability of the test system, particularly with strain TA1535, TA1537, and TA100, to detect weak mutagenic action was not adequately shown.

5. No direct-acting positive controls were used.

The study is not acceptable. The study should be repeated using methods that conform with established procedures for the Salmonella/mammalian microsome assay.[2]

A. MATERIALS

1. Test Material: Name: TD No. 84-122 (RH-893 process variation)
Description: Amber liquid.
Lot No.: 7KDR42-1; purity: 76.0%.
Contaminants: See Appendix A, Analysis of Test Substance, CBI p. 4.
Solvent used: Not specified, but presumed to be dimethylsulfoxide (DMSO).

2. Control Materials:
Negative: DMSO.
Solvent/final concentration: 100 µL/plate.
Positive: With and without activation:
2-Aminoanthracene (2-anthramine) 10 µg/plate TA100, TA1535, TA1537.
2-Acetamidofluorene 50 µg/plate TA98.

[1] de Serres, F. J. and Shelby, M. D. Recommendations on data production and analysis using the Salmonella microsomal mutagenicity assay. Mutat. Res. 64 (1979): 159-165.

3. Activation: S9-derived from
__x__ Aroclor 1254 __x__ induced __x__ rat __x__ liver
____ phenobarbital _____ noninduced _____ mouse ____lung
____ none _____ other
____ other.

4. Test Organism Used: S. typhimurium strains
____ TA97 __x__ TA98 __x__ TA100 _____ TA102 ____ TA104
__x__ TA1535 __x__ TA1537 _____ TA1538; list any others.
Test organisms were properly maintained. Yes.
Checked for appropriate genetic markers (rfa mutation, R factor): Yes.

5. Test compound concentrations used: Based on the reported results S. typhimurium TA1535 and TA1537 were exposed to five doses (2.5, 7.5, 25, 75, and 250 µg/plate/+ or -S9). Strain TA98 was initially exposed to the following nonactivated and S9-activated doses: 7.5, 25, 75, 250, and 750 µg/plate and subsequently dosed with 0.5, 5.0, 7.5, 15, and 25 µg/plate/+ or -S9.

Strain TA100 was initially exposed to 75, 250, 750, 2500, and 7500 µg/plate + or -S9 and subsequently dosed with 2.5, 7.5, 25, 75, and 250 µg/plate/+ or -S9.

B. TEST PERFORMANCE:

1. Type of Salmonella Assay: __x__ Standard plate test (Incubation time: 72 hours)
_____ Pre-incubation (_) minutes
_____ "Prival" modification
_____ Spot test
_____ Other (describe).

2. Preliminary Cytotoxicity Assay: The report made no reference to the performance of a preliminary cytotoxicity assay; however, the high doses initially assayed with strain TA100 suggest that this may have served as a range-finding test.

3. Mutagenicity Assay (reported results):

a. Initial Test: As shown in Table 1, nonactivated doses ≥ 75 µg/plate were cytotoxic for all strains. Reduced revertant colonies were also seen for TA1535 and TA98 at 25 µg/plate; this level was not cytotoxic in strain TA1537, and TA100 was not assayed against doses <75.0 µg/plate/+ or -S9. S9-activated 75 µg/plate was only cytotoxic in strain TA98. At noncytotoxic doses, no

TABLE 1. Representative Results of the Initial Salmonella typhimurium Mutagenicity Assay with ID No. 84-122

| Substance | S9 Activation | Dose (µg/plate) | Revertants per Plate of Bacterial Tester Strain[a] TA1535 | TA1537 | TA98 | TA100 |
|---|---|---|---|---|---|---|
| Solvent Control | | | | | | |
| Dimethyl-sulfoxide | - | -- | 26.7 ± 6.8 | 8.9 ± 2.8 | 25.6 ± 5.5 | 68.8 ± 9.6 |
| | + | -- | 26.6 ± 4.8 | 16.9 ± 4.6 | 52.3 ± 7.4 | 62.2 ± 11.5 |
| Positive Control | | | | | | |
| 2-Anthramine | + | 10.0 | 210.8 | 134.8 | -- | 535.2 |
| 2-Acetamidofluorene | + | 50.0 | -- | -- | 937.5 | -- |
| Test Material | | | | | | |
| ID No. 84-122 | - | 7.5[b] | 17.0 | 7.7 | 28.7 | ND |
| | - | 25.0 | 13.7 | 9.3 | 7.7 | ND |
| | - | 75.0 | 6.0 | 0.0 | 0.0 | 0.0 |
| | + | 7.5 | 32.7 | 14.3 | 52.3 | ND |
| | + | 25.0 | 21.0 | 17.0 | 44.0 | ND |
| | + | 75.0 | 19.0 | 15.3 | 11.0 | 52.0 |

[a]Means and standard deviations of counts from 39 plates for the solvent control; average of triplicate plates for the positive controls and test material doses.

[b]Lowest dose with TA98; results for 2.5 µg/plate/ + or - S9 with TA1535 and TA1537 were comparable to the solvent control values.

increases in reversion to histidine prototrophy were induced in any strain either in the presence or absence of S9 activation.

b. Repeat Test: The repeat nonactivated and S9-activated assays used the following test material doses with strain TA98 or TA100:

S. typhimurium TA98: 0.5, 5, 7.5, 15, and 25 µg/plate/+ or -S9.

S. typhimurium TA100: 2.5, 7.5, 25, 75, and 250 µg/plate/+ or -S9.

In strain TA98, the five selected nonactivated and S9-activated doses were neither cytotoxic nor mutagenic (Table 2). The lack of cytotoxicity conflicts with the earlier findings showing that nonactivated 25 µg/plate caused an ≈70% reduction in revertant colonies of TA98. Results for strain TA100 compared favorably with the earlier results, which indicated that 75 µg/plate was completely cytotoxic in the absence of S9 but not cytotoxic with S9 activation. No appreciable increases in mutant colony counts were seen at the noncytotoxic levels. The author concluded that TD No. 84-122 was not mutagenic in this test system.

4. Reviewers' Discussion/Conclusions:

We assess that the following deficiencies preclude acceptance of this study as valid evidence of a nonmutagenic response for TD No. 84-122:

a. As shown in Table 2, solvent control mutation frequencies for TA100 were low following a 72-hour incubation. The normal range for a 48-hour incubation is 60-220 revertant colonies.[3] The protocol stated that genetic markers, such as ampicillin resistance, were determined "at time of transfer." The decrease in the reversion frequency of TA100, however, suggested that the R-factor was partially lost; thus, the strain would revert at a lower frequency.

b. The presentation of average revertant colony counts without some indication of variability (e.g., standard deviations) is not an acceptable practice.

[3]Ibid.

TABLE 2. Representative Results of the Second Salmonella typhimurim Mutagenicity Assay with TD No. 84-122

| Substance | S9 activation | Dose (µg/plate) | Revertants per Plate of Bacterial Tester Strain[a] TA98 | TA100 |
|---|---|---|---|---|
| Solvent Control | | | | |
| Dimethyl-sulfoxide | - | -- | 24.2 ± 4.5 | 66.8 ± 8.1 |
| | + | -- | 47.9 ± 9.1 | 55.4 ± 10.3 |
| Positive Control | | | | |
| 2-Anthramine | + | 10.0 | -- | 416.7 |
| 2-Acetamido-fluorene | + | 50.0 | 776.7 | -- |
| Test Material | | | | |
| TD No. 84-122 | - | 25[bc] | 24.3 | 59.3 |
| | - | 75[d] | ND | 0.0 |
| | + | 25 | 44.7 | 62.3 |
| | + | 75 | ND | 53.7 |

[a]Means and standard deviations of counts from 39 plates for the solvent control; average of triplicate plates for the positive controls and test material doses.
[b]Highest dose tested with TA98; results for lower levels (15, 7.5, 5, and 0.5 µg/plate/+ or -S9) were comparable to the solvent control value.
[c]Results for lower levels assayed with TA100 (2.5 and 7.5 µg/plate) were comparable to the solvent control results.
[d]Higher doses with TA100 were 100% cytotoxic.

c. Means and standard deviations presented for the solvent control cultures were based on the counts from 39 replicate plates. Since the protocol indicated that three replicates per treatment group would be used, the results for solvent control cultures probably represent the historical data for 13 experiments. We can only assume that the actual colony counts for this assay were included in the 39 replicates since solvent control counts of strain TA98 and TA100 were different in the initial and repeat assays. However, the inclusion of historical background data in lieu of concurrent negative control results is not an acceptable practice.

d. Although strains TA1535, TA1537, and TA100 responded to 2-anthramine (2AA), the concentration selected to demonstrate S9-induced mutagenicity was excessive (10 µg/plate). Generally, peak mutagenic activity for this compound occurs over a dose range of 2-5 µg/plate. Since the investigator elected not to include direct-acting positive controls in the assays, greater emphasis must be placed on strain performance with the S9-activated controls. Based on this consideration, we conclude that the use of a high concentration of 2-AA provides no assurance that the strains could respond to low level mutagenic action.

e. No direct-acting positive controls were used.

5. The test was perfcrmed under good laboratory practices (a quality assurance statement was present).

6. The CBI Appendix: Appendix A, Analysis of Test Substance, CBI p. 4, and Appendix B, Protocol, CBI pp. 11-15.

Page _____ is not included in this copy.

Pages 12 through 19 are not included in this copy.

The material not included contains the following type of information:

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_____ 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.

✓ FIFRA registration data.

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

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EPA: 68D80056
DYNAMAC No. 115-B
December 13, 1988

DATA EVALUATION RECORD

KATHON

Mutagenicity--Chinese Hamster Ovary Cell/HGPRT Forward Mutation Assay

STUDY IDENTIFICATION: Foxall, S. Skane M-8 HQ microbiocide CHO/HGPRT gene mutation assay. Guideline reference 84-2. (Unpublished study No. 86R-055 prepared by Rohm and Haas Co., Toxicology Department, Spring House, PA, for Rohm and Haas Co., Philadelphia, PA; dated August 11, 1986.) ~~Accession~~ MRID No. 406475-02.

APPROVED BY:

Robert J. Weir, Ph.D.
Acting Department Manager
Dynamac Corporation

Signature: William L. McFall (for)

Date: Dec 14, 1988



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1. CHEMICAL: Kathon; Skane M-8; 2-octyl-3(2H)-isothiazolone.

2. TEST MATERIAL: Skane M-8 from lot No. SW85-0311 was described as a yellow liquid that contained 46.7% active ingredient in 53.3% propylene glycol.

3. STUDY/ACTION TYPE: Mutagenicity--Chinese hamster ovary cell/HGPRT forward mutation assay.

4. STUDY IDENTIFICATION: Foxall, S. Skane M-8 HQ microbiocide CHO/HGPRT gene mutation assay. Guideline reference 84-2. (Unpublished study No. 86R-055 prepared by Rohm and Haas Co., Toxicology Department, Spring House, PA, for Rohm and Haas Co., Philadelphia, PA; dated August 11, 1986.) Accession No. 406475-02.

5. REVIEWED BY:

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Date:

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Acting EPA Section Head,
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(TS-769C)
Signature: Marion Copley
Date: 2/19/89



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7. CONCLUSIONS:

A. Exposure of Chinese hamster ovary (CHO) cells to five nonactivated (0.5 to 4 µg/mL) and six S9-activated (0.5 to 8 µg/mL) doses of Skane M-8 did not increase the frequency of mutation at the HGPRT locus. Severe cytotoxicity was achieved at nonactivated levels ≥ 4 µg/mL and at S9-activated levels > 5 µg/mL. It was, therefore, concluded that the test material was assayed up to a cytotoxic dose with no evidence of a mutagenic effect.

B. The study is acceptable.

Items 8 through 10--see footnote 1.

11. MATERIALS AND METHODS (PROTOCOLS):

A. Materials and Methods: (See Appendix A for details.)

1. Test Material: Skane M-8 from lot No. SW85-0311 was described as a yellow liquid that contained 46.7% active ingredient (a.i.) in 53.3% propylene glycol. Storage conditions, stability, and other characteristics that define the test material were not reported. The test material was dissolved in dimethyl-sulfoxide (DMSO); no corrections were made for the a.i. concentration. Solutions used in the assays were freshly prepared for each experiment.

2. Mammalian Cells: CHO cells, subclone CHO-K1-$BH_4$, were obtained from Dr. A. Hsie, Oak Ridge National Laboratory, Oak Ridge, TN. Stock cultures were held in liquid nitrogen. Working cultures, maintained in Ham's F-12 medium supplemented with 5 or 10% fetal calf serum, L-glutamine, and antibiotics, were grown for ≈24 hours prior to use.

[1]Only items appropriate to this DER have been included.



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3. S9 Activation: The S9 fraction used for metabolic activation was derived from the livers of male Sprague-Dawley rats induced with Aroclor 1254. The S9 cofactor mix contained either 0.3, 1.0, or 2.0 mg protein/mL of culture medium.

4. Preliminary Cytotoxicity Assay: Prepared cultures, seeded at $\approx 5 \times 10^5$ cells/plate, were treated with test material doses that spanned at least a 4-log concentration range, the solvent control (DMSO), and the positive controls [ethylmethanesulfonate (EMS), 100 nL/mL/-S9; 7,12-dimethylbenzanthracene (DMBA), 7 µg/mL/+S9]. Exposure without S9 activation lasted 18 to 20 hours; with S9 activation, however, cells were exposed for 5 hours. Following treatment, cells were washed, incubated for 2 days, replated at a density of 200 cells/dish, and reincubated for 7 days. Colonies were fixed, stained with Giemsa, and counted; survival in treatment groups relative to growth in the control was determined. Based on these results, at least four doses that spanned a cytotoxicity range of 10 to 90% survival were selected for the mutagenicity assay.

5. Mutagenicity Assay: The initial steps in the mutation assay were identical to those performed in the cytotoxicity assay. Following exposure of duplicate cultures to the selected test material doses, solvent (DMSO), and positive controls (100 nL/mL EMS/-S9 or 7 µg/mL DMBA/+S9), cells were washed, refed fresh medium, and incubated for 2 days. Cells were counted and replated at two cell densities. Four replicate plates containing 200 cells were incubated for 7 days, stained, and counted to determine cytotoxicity. Cells were also seeded at a density of $\approx 1 \times 10^6$ cells to allow expression of mutations. During the 8-day mutation expression period, cells were subcultured every 2 days and reseeded at $5 \times 10^5$ cells. Mutant selection was accomplished by plating $2 \times 10^5$ cells/dish (five dishes) in selection medium containing 10 µM 6-thioguanine. Viability at the time of selection was determined by seeding 200 cells/dish (four dishes/treatment) in complete medium. Selection and viability cultures were incubated for 7 days, fixed, stained with Giemsa, and counted. Plating efficiencies (PEs) and mutation frequencies (MFs) were calculated.

For compounds that were negative in the mutation assay, the S9-activated phase of testing was repeated with varying concentrations of the S9 liver homogenate (0.3 and 2.0 mg S9 protein/mL of the S9 cofactor mix.)



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6. Evaluation Criteria:

a. Assay Validity: For the assay to be considered valid, the following criteria must be satisfied: 1) the PE for the negative control must be ≥60%; 2) the background MF must be within the 95th percentile of historical solvent control values; 3) the MF for the positive controls must be > 3x the 95th percentile of historical solvent control values; 4) a minimum of four selection plates must be available for both control and treatment groups; and 5) a minimum of two test doses must be available for analysis.

b. Positive Response: The test material was considered positive if a significant (p value not specified) and dose-related increase in the MF with an accompanying increase in mutant colonies over the control was observed.

7. Statistical Analysis: The statistical methods used to evaluate the data, if any, were not reported. However, a reference for statistical procedures was cited.

B. Protocol: See Appendix B.

12. REPORTED RESULTS:

A. Preliminary Cytotoxicity Assay: Fourteen doses ranging from 0.2 to 1000 µg/mL were assayed in the preliminary nonactivated cytotoxicity test. Extreme cytotoxicity was induced at doses ≥ 5 µg/mL. For the remaining doses, survival increased as test material concentrations decreased. Percent survival ranged from 32% at 3 µg/mL to 105% at the lowest assayed dose (0.2 µg/mL). Based on these results, five levels were selected for the nonactivated test (0.5, 1, 2, 3, and 4 µg/mL).

Results of the preliminary nonactivated assay governed dose selection for the S9-activated cytotoxicity assessment. Accordingly, nine doses (0.01 to 10 µg/mL) were assayed. No cells survived exposure to the S9-activated test material at 10 µg/mL; at 5 µg/mL, 4% of the cells survived. For the remaining levels, survival was dose related and ranged from 31% survival at 3 µg/mL to >100% survival at ≥0.25 µg/mL. Doses selected for the S9-activated mutation assay were 0.5, 1, 2, 3, 5, and 8 µg/mL.



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B. Mutation Assay: At the end of treatment, the highest nonactivated dose (4.0 µg/mL) was not plated due to extreme cytotoxicity. As shown in Table 1, the highest S9-activated dose (8.0 µg/mL) was also severely cytotoxic. PEs at selection for all nonactivated and S9-activated levels carried through the assay were ≈70% or higher. No appreciable increases in mutant colonies or MFs were observed at any treatment level. By contrast, the positive controls (EMS at 100 nL/mL/-S9 and DMBA at 7 µg/mL/+S9) induced marked increases in mutant colonies and the corresponding MFs.

The S9-activated assay was repeated as specified by the protocol. Results presented in the report indicated that 2 µg/mL of the test material in the presence of 0.3 or 2.0 mg S9 protein/mL was not mutagenic.

13. STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:

A. The study author concluded that Skane M-8 was not mutagenic in the CHO/HGPRT gene mutation assay.

B. A quality assurance statement was signed and dated August 5, 1986.

14. REVIEWERS' DISCUSSION AND INTERPRETATION OF STUDY RESULTS:

We assess that the study was properly conducted and that the author interpreted the data correctly. The test material was assayed up to an acceptable level of cytotoxicity both in the presence and absence of S9 activation with no indication of a mutagenic response. It was noted, however, that compound information furnished in the report on the unscheduled DNA synthesis assay listed the pH of Skane M-8 at 2.4 (study No. 86R-0018, CBI p. 27); however, neither study provided information indicating that the pH of the treatment media was measured. We can only speculate that the steep toxicity-dose response curve seen between 3 and 4 µg/mL/-S9 and 5 and 8 µg/mL/+S9 could have resulted from changes in the pH of the treatment medium. Nevertheless, Skane M-8, both with and without S9 activation, was adequately tested and not found to be mutagenic.

The sensitivity of the assay to detect a mutagenic response was demonstrated by the marked increase in the MF of CHO cells exposed to direct or metabolically activated mutagens. It is, therefore, concluded that Skane M-8 was adequately evaluated and found to be nonmutagenic in a well-controlled study.

TABLE 1. Representative Results of the CHO Forward Gene Mutation Assay with Skane M-8

| | Dose/ mL | S9 Activation | Percent Survival at the End of Treatment | Average[a] Mutant Colonies/ 10 Dishes | Percent[a] Plating Efficiency at Selection | Average[a] Mutation Frequency[b] x10$^{-5}$ |
|---|---|---|---|---|---|---|
| Solvent Control | | | | | | |
| Dimethylsulfoxide | -- | - | 100 | 0.1 | 87.9 | 0.6 |
| | -- | + | 100 | 1.4 | 81.8 | 8.8 |
| Positive Control | | | | | | |
| Ethylmethane-sulfonate | 100 nL | - | 49 | 61.6 | 79.7 | 386.6* |
| 7,12-Dimethyl-benzanthracene | 7 µg | + | 98 | 25.4 | 79.1 | 161.2* |
| Test Material | | | | | | |
| Skane M-8 | 3 µg[c] | - | 48 | 0.1 | 90.4 | 0.5 |
| | 3 µg[d] | + | 55 | 0.6 | 72.7 | 4.0 |
| | 5 µg | + | 17 | 0.1 | 76.2 | 0.7 |
| | 8 µg | + | 2 | 0 | 77.9 | 0 |

[a]Combined results from duplicate cultures as averaged by our reviewers.

[b]Mutation Frequency (MF) = $\frac{\text{Average Mutant Colonies/Plate}}{\% \text{ Plating Efficiency} \times 2 \times 10^5}$.

[c]Highest nonactivated dose (4 µg/mL) was not plated due to extreme cytotoxicity. MFs for lower levels (0.5, 1, and 2 µg/mL) were comparable to the solvent control values.

[d]MFs for lower S9-activated doses (0.5, 1, and 2 µg/mL) were comparable to the solvent control value.

*Fulfills the reporting laboratory criterion for an acceptable positive control response.



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16. CBI APPENDIX: Appendix A, Materials and Methods, CBI pp. 15-21, and Appendix B, Protocol, CBI pp. 33-46.

Page _____ is not included in this copy.

Pages 38 through 50 are not included in this copy.

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007060

EPA: 68D80056
DYNAMAC No. 115-C
December 13, 1988

DATA EVALUATION RECORD

KATHON

Mutagenicity--In Vitro Cytogenetic Study
with Chinese Hamster Ovary Cells

STUDY IDENTIFICATION: Ivett, J. L., and Murli, H. Clastogenic evaluation of Skane M-8 microbiocide in an in vitro cytogenetic assay measuring chromosomal aberration frequencies in Chinese hamster ovary (CHO) cells. (Unpublished study No. 86RC-0048 prepared by Hazleton Biotechnologies Co., Kensington, MD, for Rohm and Haas Co., Spring House, PA; dated March 27, 1987.) Accession No. 406475-03. MRID

APPROVED BY:

Robert J. Weir, Ph.D.
Acting Department Manager
Dynamac Corporation

Signature: William L. McLellan Jr.

Date: Dec 13, 1988



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1. CHEMICAL: Kathon; Skane M-8; 2-octyl-3(2H)-isothiazolone.

2. TEST MATERIAL: Skane M-8 from lot No. SW 85-0311 was described as a clear liquid that contained 46.7% active ingredient in 53.3% propylene glycol.

3. STUDY/ACTION TYPE: Mutagenicity--In vitro cytogenetic study with Chinese hamster ovary cells.

4. STUDY IDENTIFICATION: Ivett, J. L., and Murli, H. Clastogenic evaluation of Skane M-8 microbiocide in an in vitro cytogenetic assay measuring chromosomal aberration frequencies in Chinese hamster ovary (CHO) cells. (Unpublished study No. 86RC-0048 prepared by Hazleton Biotechnologies Co., Kensington, MD, for Rohm and Haas Co., Spring House, PA; dated March 27, 1987.) Accession No. 406475-03.

5. REVIEWED BY:

Nancy E. McCarroll, B.S.
Principal Reviewer
Dynamac Corporation

Signature: Nancy E. McCarroll
Date: 12-13-88

I. Cecil Felkner, Ph.D.
Independent Reviewer
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6. APPROVED BY:

I. Cecil Felkner, Ph.D.
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Date:

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Signature: Marion Copley 3/6/89.
Date:



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7. CONCLUSIONS:

A. Skane M-8 was evaluated for the potential to induce chromosome aberrations in Chinese hamster ovary (CHO) cells both under nonactivated and S9-activated assays that employed 10- and 20-hour cell harvests. Under nonactivated conditions (20-hour cell harvest), the combined results from two trials showed that 4, 6, and 8 µg/mL of the test material induced significant ($p < 0.01$) increases in the percent cells with aberrations. The effect was dose related and the test material induced a wide variety of simple and complex aberrations. Similar results were achieved for cells harvested at 10 and 20 hours under S9-activated conditions. Results from the 20-hour harvest showed significant ($p < 0.01$) effects at 15, 17.5, and 20.0 µg/mL; the response was dose related. Although the findings indicated a clastogenic response, it was noted that compound information furnished with the unscheduled DNA repair assay report listed the pH of Skane M-8 at 2.4 (study No. 86R-0018, CBI p. 27). Neither study, however, provided information indicating that the pH of the treatment media was measured. Without these data, we are unable to determine if the clastogenic response is valid or resulted from a treatment-condition (i.e., low pH); see Reviewers' Discussion and Interpretation of Study Results, Section 14. We conclude, therefore, that Skane M-8 is presumptively clastogenic in this test system.

B. The study is inconclusive; however, Skane M-8 is classified as presumptively clastogenic because it is not possible to determine whether the test material, the experimental conditions, or a combination of both caused the response.

8. RECOMMENDATIONS:

The study can be upgraded provided that the study authors can present data showing that the test material did not shift the pH of the treatment medium outside the range that can support normal mammalian cell growth (pH 6.8-7.5). Otherwise, the assay should be repeated with appropriate pH measurements and adjustments, if necessary, to maintain pH levels around neutral during treatment.

Items 9 and 10--see footnote 1.

11. MATERIALS AND METHODS (PROTOCOLS):

A. Materials and Methods: (See Appendix A for details.)

1. Test Material: Skane M-8 from lot No. SW 85-0311 was described as a clear liquid that contained 46.7% active ingredient in 53.3% propylene glycol. Storage conditions, stability, and other characteristics that define the test material were not reported. The test material was soluble in dimethylsulfoxide (DMSO) at a concentration of 500 mg/mL; however, upon addition to tissue culture medium (McCoy's 5a), 5 mg/mL of the test material (final concentration) formed fine oily globules. At a final concentration of 2 mg/mL, a fine precipitate was initially formed in tissue culture medium, but the precipitate rapidly went into solution. DMSO was, therefore, selected as the solvent of choice to prepare the stock concentration of 200 mg/mL.

2. Cell Line: The Chinese hamster ovary cells (CHO-WBL) used in this assay were originally obtained from Dr. Sheldon Wolff, University of California, San Francisco. The CHO cells were grown in McCoy's 5a, supplemented with 10% fetal calf serum (FCS), L-glutamine, and antibiotics, for 24 hours prior to use.

3. S9 Activation: The S9 fraction used for metabolic activation was derived from the livers of Sprague-Dawley rats treated with Aroclor 1254. The S9 mix contained 15 µL rat liver S9/mL.

4. Preliminary Cytotoxicity Assay: Prepared cell cultures, seeded at $0.3 \times 10^6$ cells/flask, were exposed with or without S9 activation to half-log dilutions ranging from 0.067 to 2000 µg/mL of the test material, the solvent (DMSO), and the positive controls [mitomycin C (MMC) at 0.25 µg/mL/-S9 and cyclophosphamide (CP) at 20 µg/mL/+S9.]

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[1]Only items appropriate to this DER have been included.



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In the nonactivated system, cells were exposed for 2 hours to the test material; BrdUrd (10 μM) was added to the cultures, and incubation was continued for 23 hours. Cell monolayers were washed, refed with fresh complete medium containing BrdUrd, and reincubated in the presence of 0.1 μg/mL colcemid for 2.5 hours. In the presence of S9 activation, cultures were exposed for 2 hours in a culture medium without FCS. After exposure, cells were washed twice, refed with complete medium containing BrdUrd (10 μM), and reincubated for 23 hours. Colcemid (0.1 μg/mL) was added, and the cultures were incubated for an additional 2.5 hours. After incubation, monolayers were visually evaluated for confluency and dead cells. Surviving cells from four to six of the highest doses were harvested. Metaphase cells were collected by mitotic shake off, swollen in a hypotonic 0.075 M solution of KCl, and washed in a fixative (methanol:acetic acid, 3:1). Estimation of cell-cycle delay was accomplished by staining the cells with the modified fluorescent-plus Giemsa techniques of Perry and Wolff[2] and Goto et al.[3] One hundred metaphase cells per culture were examined for the percentage of first ($M_1$), between first and second ($M_1$+), and second or higher ($\geq M_2$) division metaphases. Based on the findings, doses and harvest times were selected for the cytogenetic assay.

5. Cytogenetics Assay:

   a. Treatment: Duplicate cultures were seeded at 1.2 to 1.5 x $10^5$ cells and exposed to the selected test material doses, the solvent (DMSO), or the positive controls, MMC (0.04 and 0.08 μg/mL) without S9 activation or CP (25 and 50 μg/mL or 12.5 and 17.5 μg/mL) with S9 activation. In the nonactivated system, cells were dosed for 17.5 hours (Trial 1) or 17.2 hours (Trial 2).

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[2]Perry, P., and Wolff, S. New Giemsa method for the differential staining of sister chromatids. Nature (1974) 251: 156-158.

[3]Goto, K., Maeda, S., Kano, Y., and Sugiyama, T. Factors involved in differential Giemsa-staining of sister chromatids. Chromosoma (1978) 66: 351-359.

Cultures were washed, refed medium containing colcemid, and reincubated for approximately 2.5 hours. Under S9-activated conditions, cells were exposed for 2 hours, washed, refed culture medium, and incubated an additional 7.9 hours (10-hour harvest) or 18.3 hours (20-hour harvest). Colcemid was added 2.5 hours before the cultures were harvested. Metaphase cells were collected and fixed. Slides were stained with 5% Giemsa and coded.

b. Additional Cytotoxicity Assessment: Duplicate cultures, seeded at 0.4 to 0.5 x $10^6$ cells, were exposed to similar test material concentrations and the solvent (DMSO) and assessed for cytotoxicity as described above in Section 5a. Cells were trypsinized, counted, and measured for cell viability by trypan blue exclusion, and the mitotic index was determined.

c. Metaphase Analysis: One hundred cells per culture in the negative, solvent, and treatment groups were scored for chromosome aberrations. Twenty-five cells were scored from one of each positive control culture.

6. Statistical Methods: The data were evaluated for statistical significance at $p < 0.05$ by Fisher's exact test. The negative (culture medium) and solvent controls were pooled if no statistical differences were calculated in the Fisher's exact test.

7. Evaluation Criteria: The biological significance of the results was evaluated relative to the overall chromosome aberration frequencies, percentage of cells with aberrations, percentage of cells with > 1 aberration, dose response, and the types of aberrations observed.

B. Protocol: See Appendix B.



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12. REPORTED RESULTS:

A. Preliminary Cytotoxicity Assay: The cytotoxicity assay was conducted with doses of the test material ranging from 0.067 to 2000 µg/mL, separated by half-log dilutions, in the presence or absence of S9 activation.

1. Without S9 Activation: No cells survived treatment with doses ≥ 6.67 µg/mL. At 0.667 and 2.0 µg/mL, mitotic cells were reduced; 2 µg/mL also induced cytotoxic effects on the monolayer. As shown in Table 1, mitotic suppression was apparent at doses ≥0.2 µg/mL. Based on these findings, the nonactivated cytogenetic assay used a 20-hour harvest time to evaluate 0.4 to 8 µg/mL of the test material.

2. With S9 Activation: Complete cell death was reported for S9-activated doses ≥20 µg/mL. Cell-cycle delay and reduced monolayer confluency occurred following exposure to 6.67 µg/mL (Table 1); the lower doses were not cytotoxic. For the S9-activated cytogenetic assay, the investigators selected a 10-hour harvest to assess test material doses ranging from 0.75 to 10 µg/mL and a 20-hour harvest to evaluate 10, 15, and 20 µg/mL.

B. Cytogenetic Assay

1. Nonactivated Assay: Two independent nonactivated cytogenetic assays were performed; the results were as follows:

a. Initial Assay: Reduced monolayer confluency, cell death, and an observable reduction in mitotic cells were reported for cultures exposed to the highest dose of the test material (8 µg/mL). Metaphases were scored from the 2-, 4-, 6-, and 8-µg/mL doses groups. As shown in Table 2, significant ($p < 0.01$) and dose-related increases in the percent of cells with aberrations were scored for the two highest treatment groups. At these doses, the percent cells with >1 aberrations were also increased. A wide variety of simple (chromatid and chromosome breaks) and complex aberrations were induced at both levels that gave positive



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TABLE 1. Results form the Preliminary Test for Delay of Cell-Cycle Progression with Skane M-8

| Substance | Dose/mL | S9 Activation | % Cells[a] $M_1$ | % Cells[a] $M_{1+}$ | % Cells[a] $\geq M_2$ | Monolayer Confluency (%) |
|---|---|---|---|---|---|---|
| Negative | | | | | | |
| Culture medium | -- | - | 0 | 0 | 100 | 100 |
| | -- | + | 2 | 2 | 96 | 100 |
| Solvent Control | | | | | | |
| Dimethylsulfoxide | 10 µL | - | 1 | 10 | 89 | 100 |
| | | + | 0 | 3 | 97 | 100 |
| Positive Control[3] | | | | | | |
| Mitomycin C | 0.25 µg | - | 100 | 0 | 0 | 100 |
| Cyclophosphamide | 20.0 µg | + | 8 | 57 | 35 | 100 |
| Test Material | | | | | | |
| Skane M-8 | 0.200 µg | - | 0 | 37 | 63 | 100 |
| | 0.667 µg | - | 2 | 30 | 68 | 100 |
| | 2.000 µg[b] | - | 12 | 61 | 27 | 86 |
| | 0.200 µg | + | 0 | 7 | 93 | 100 |
| | 0.667 µg | + | 0 | 6 | 94 | 100 |
| | 2.000 µg | + | 1 | 7 | 92 | 100 |
| | 6.670 µg[b] | + | 13 | 24 | 63 | 86 |

[a]Percent cells in first ($M_1$), between first and second ($M_{1+}$), and second or higher ($\geq M_2$) division.

[b]No survival was observed at doses $\geq$ 6.67 µg/mL/-S9 and $\geq$20.0 µg/mL/+S9.

Note: Lowest dose (0.067 µg/mL/+/-S9) was not cytotoxic and did not interfere with cell-cycle progression.

TABLE 2. Representative Results of the Nonactivated CHO Cell Cytogenetic Assays with Skane M-8, Harvest Time was 20.3 Hours

| Substance | Dose (µg/mL) | Cytotoxicity Assessment: % Relative Survival | Cytotoxicity Assessment: Mitotic Index | Cytogenetic Evaluation: No. of Cells Scored | Cytogenetic Evaluation: % Aberrations per Cell | Cytogenetic Evaluation: % Cells with Aberrations | Cytogenetic Evaluation: % Cells with >1 Aberrations | Cytogenetic Evaluation: Biologically Significant Aberrations[a] No./Type |
|---|---|---|---|---|---|---|---|---|
| **Pooled Negative Control** | | | | | | | | |
| Medium plus dimethylsulfoxide | --[b] | ND | ND | 200 | 0.02 | 1.5 | 0.0 | 1SB; 1TR; 1D |
| | --[c] | 100.0 | 9.0 | 200 | 0.01 | 1.0 | 0.0 | 2D |
| **Positive Control** | | | | | | | | |
| Mitomycin C | 0.08[b] | ND | ND | 25 | 1.16 | 64.0* | 28.0* | 13TB; 7SB; 5TR; 3DM; 1CI |
| | 0.08[c] | ND | ND | 25 | 0.92 | 52.0* | 20.0* | 10TB; 4SB; 3ID; 3TR; 1DR; 2CI |
| **Test Material** | | | | | | | | |
| Skane M-8 | 6.0[b,d] | ND | ND | 200 | >0.18 | 13.0* | 4.5 | 6TR; 5SR; 4ID; 6TR; 3DR; 1CR; 1D; 1CI; 1DF; 1RF; 2PU |
| | 8.0[b] | ND | ND | 200 | 0.52 | 29.5* | 15.0 | 28TR; 11SR; 9ID; 15TR; 2SDR; 3CR; 1D; 11CI. |
| | 2.0[c] | 78.2 | 6.7 | 200 | >0.03 | 3.0 | 0.5 | 1TR; 3D; 1R; 1PU |
| | 4.0[c] | 59.7 | 11.5 | 200 | >0.10 | 7.0* | 2.0 | 3TR; 1SB; 2ID; 3TR; 6DR; 2D; 1CI; 2PU |
| | 6.0[c] | 51.8 | 4.9 | 200 | 0.06 | 4.5 | 1.0 | 3TB; 1TR; 4DR; 2D; 1R |
| | 8.0[c] | 42.3 | 1.3 | 200 | >0.34 | 15.5* | 9.5* | 23TB; 13SB; 1DM; 9ID; 5TR; 8DR; 3CR; 4D; 1PU |

[a]Abbreviations Used:
TB - Chromatid break; SB - Chromosome break; D - Dicentric; CR - Complex rearrangement; QR - Quadriradial; SD - Interstitial deletion; CI - Chromosome interchange; PU - Pulverized cell; RF - Ring with acentric fragment; DM - "Double minute" fragment; TR - Triradial; DF - Dicentric with fragment

[b]Results from the first nonactivated trial.
[c]Results from the second nonactivated trial.
[d]Lower doses in the first assay (2.0 and 4.0 µg/mL) were not significantly different from the pooled negative control values.
*Significantly higher ($p < 0.01$) than the pooled negative control values as determined by Fisher's exact test.
ND = Not Done.

clastogenic effects. The majority of scored complex aberrations were triradials, quadriradials, complex rearrangements, and chromosome intrachanges. The remaining doses (2 and 4 µg/mL) were negative. It was noted, however, that one triradial was scored in the pooled negative control cultures. Based on these findings, a repeat assay was performed.

b. Repeat Assay: The repeat cytogenetic assay included a parallel cytotoxicity test to evaluate the effects of seven test material doses (0.4 to 8 µg/mL) on cell survival, viability, and the mitotic index. Cell viability for both treatment and control groups were comparable (> 90%). As shown in Table 2, the percent relative growth ranged from 42.3% at 8.0 µg/mL to 78.2% at 2.0 µg/mL. Although mitotic suppression, as indicated by lower than control mitotic indices, was seen at three test concentrations, only the high dose induced a severe effect. Metaphase analysis for the treatment groups indicated that the percent cells with aberrations and the percent cells with >1 aberrations were elevated at all dose levels. The increases were significant ($p < 0.01$) at 4 and 8 µg/mL but not at 6 µg/mL. Although a clear dose response was not uncovered in the repeat assay, the frequency and distribution of aberrations were comparable to that seen in the initial test. Based on the reproducibility of the response, the authors concluded that nonactivated Skane M-8 was clastogenic in CHO cells.

2. S9-Activated Assay: Four S9-activated trials were performed. The findings are discussed below:

a. Trial 1 (20-hour cell harvest): The three doses selected for the first 20-hour cell harvest were 10, 15 and 20 µg/mL. Severe cytotoxicity was reported for the highest dose; at 15 µg/mL, the report indicated that monolayers were unhealthy and dead cells were seen. Metaphase analysis of the 10- and 15-µg/mL dosed cultures revealed significant ($p < 0.01$) increases in the percent aberrant cells and the percent of cells with >1 aberration for the 15-µg/mL test group (Table 3). It was noted that the increases were higher than those scored for the positive control culture. In agreement with the nonactivated findings, both simple and complex aberrations were markedly increased. Although the effect at 10 µg/mL was not significant, all measured parameters were increased

TABLE 3. Representative Results of the S9-Activated CHO Cell Cytogenetic Assays with Skane M-8

| Substance | Dose (μg/mL) | Cytotoxicity Assessment: Harvest Time (hours) | Cytotoxicity Assessment: % Relative Survival | Cytogenetic Evaluation: Mitotic Index | No. of Cells Scored | % Aberrations per Cell | % Cells with Aberrations | % Cells with >1 Aberrations | Biologically Significant Aberrations[a] No./Type |
|---|---|---|---|---|---|---|---|---|---|
| Pooled Negative Control | | | | | | | | | |
| Medium plus dimethyl sulfoxide | -- | 20.3[b] | ND | ND | 200 | 0.05 | 4.5 | 0.0 | 1TB; 2SB; 1TR; 4D; 1R |
| | -- | 20.0[c] | 100 | 11.5 | 200 | 0.01 | 1.0 | 0.0 | 2D |
| | -- | 10.2[b] | ND | ND | 200 | 0.05 | 3.0 | 1.0 | 3TB; 1SB; 2DM; 11D; 2D |
| | -- | 10.0[c] | 100 | 8.6 | ND | -- | -- | -- | --- |
| Positive Control | | | | | | | | | |
| Cyclophosphamide | 12.5 | 20.3[b] | ND | ND | 25 | 0.72 | 36.0* | 16.0* | 4TB; 6SB; 5TR; 7QR; 1CI |
| | 17.5 | 20.0[c] | ND | ND | 25 | 1.12 | 52.0* | 32.0* | 4TB; 4SB; 14TR; 3QR; 1CR; 2CI |
| | 50.0 | 10.2[b] | ND | ND | 25 | 0.24 | 20.0* | 4.0 | 2SB; 1TR; 2QR; 1? |
| Test Material | | | | | | | | | |
| Skane M-8 | 10.0 | 20.3[b] | ND | ND | 200 | 0.11 | 7.5 | 2.5 | 2TB; 6SB; 2DM; 11D; 5TR; 3QR; 1CR; 2D |
| | 15.0 | 20.3 | ND | ND | 200 | 1.86 | 69.5* | 51.5* | 86TB; 84SB; 41TD; 58TR; 57QR; 20CR; 4D; 3R; 18CI |
| | 12.5 | 20.0[c] | 56.8 | 14.2 | 200 | 0.03 | 2.5 | 0.0 | 1TR; 2D; 1R; 1RF |
| | 15.0 | 20.0 | 42.4 | 7.0 | 200 | 0.11 | 5.5* | 2.5 | 1TB; 3SB; 1TD; 1DTR; 3QR; 4CI |
| | 17.5 | 20.0 | 26.5 | 2.4 | 200 | 0.23 | 13.0* | 4.5 | 1STB; 2SB; 1STR; 9QR; 1CR; 2D; 2CI |
| | 20.0 | 20.0 | 27.5 | 1.2 | 200 | 1.88 | 65.0* | 51.0* | 57TB; 26SB; 21D; 46TR; 44QR; 6CR; 2D; 2R; 3CI |
| | 7.5 | 10.2[bd] | ND | ND | 200 | 0.02 | 1.5 | 0.0 | 1SB; 1TR; 1D |
| | 10.0 | 10.2 | ND | ND | 200 | 0.07 | 7.0* | 0.0 | 3TR; 5SR; 1DM; 1TD; 1TR; 1QR; 1D; 1CI |
| | 7.5 | 10.0[ce] | 68.1 | 12.1 | ND | -- | -- | -- | --- |
| | 10.0 | 10.0[f] | 62.2 | 14.1 | ND | -- | -- | -- | --- |

[a]Abbreviations Used:

TB - Chromatid break
SB - Chromosome break
D - Dicentric
CR - Complex rearrangement
QR - Quadriradial
TD - Interstitial deletion
CI - Chromosome interchange
PU - Pulverized cell
RF - Ring with acentric fragment
DM - "Double minute" fragment
TR - Triradial
DF - Dicentric with fragment

[b]Results from the first S9-activated trials.

[c]Results from the repeat S9-activated trials.

[d]Cytogenetic evaluation of lower doses (2.5 and 5.0 μg/mL) showed no significant difference from the pooled negative control.

[e]Percent relative survival for lower doses (2.5 and 5.0 μg/mL) were ≥69%.

*Significantly higher ($p < 0.01$) than the pooled negative control values as determined by Fisher's exact test.

compared to the pooled negative control values and rare complex aberrations were similar to those scored for the 15-µg/mL dose group.

b. Trial 2 (10-hour cell harvest): Following the 10-hour cell harvest, significant ($p < 0.01$) effects were seen at 10 µg/mL; the remaining doses (2.5, 5.0, and 7.5 µg/mL) were not clastogenic.

c. Trial 3 (20-hour cell harvest): Cytotoxicity was assessed as described for the repeat nonactivated assay with a dose range of 10 to 20 µg/mL. As shown in Table 3, the two highest doses adversely affected the mitotic index; relative survival was dose related and ranged from 27.5% at 200 µg/mL to 56.8% at 12.5 µg/mL. Findings from metaphase analysis confirmed the earlier results and indicated that Skane M-8 induced a powerful clastogenic effect. The response was both significant ($p < 0.01$) and dose related.

d. Trial 4 (10-hour harvest): The cytotoxicity assessment with four test doses (2.5, 5.0, 7.5, and 100.0 µg/mL) indicated no severe cytotoxicity relative to survival, viability, or the mitotic index. Metaphases were not scored from the repeat 10-hour harvest.

Based on the combined results of the four experiments using S9 activation, the authors concluded that Skane M-8 was clastogenic.

13. STUDY AUTHORS' CONCLUSIONS/QUALITY ASSURANCE MEASURES:

A. The study authors stated, "The test article, Skane M-8 microbicide, is considered positive for inducing chromosomal aberrations in Chinese hamster ovary cells under both the metabolic activation and nonactivation conditions of this assay."

B. A quality assurance statement was signed and dated March 27, 1987.

14. REVIEWERS' DISCUSSION AND INTERPRETATION OF STUDY RESULTS:

We assess that the in vitro cytogenetic studies conducted with Skane M-8 tend to support the study authors' conclusions that the test material, both with and without S9 activation, is clastogenic. However, it was noted during the review of an unscheduled DNA synthesis assay with Skane M-8 from the identical lot number that the pH of the test material was listed as 2.4 (see study No. 86R-0018, CBI p. 27). Neither study provided information indicating

that the pH of the treatment media was measured or adjusted to maintain a neutral pH. We are, therefore, unable to assess the impact, if any, of the low pH of the test material on the outcome of the study. It was noteworthy, however, that peak clastogenic activity occurred at doses that severely reduced the mitotic index (8 µg/mL/-S9 and 20 µg/mL/+S9). Brusick* demonstrated that, during treatment, reduced pH conditions can induce cytotoxic effects and marked increases in chromosome aberrations of CHO cells. However, since the response, particularly under S9 activation, was dose related and high frequencies of rare complex aberrations were present, the results tend to suggest a clastogenic response. It is possible, therefore, that the pH change affected the ionic species and perhaps compound uptake.

We conclude that although Skane M-8 was positive under the conditions of these assays, the possibility of a "treatment-condition effect" should not be overlooked. If the study authors can establish by supporting data that the test material did not reduce the pH of the treatment medium below the normal range required to support mammalian cell growth (pH 6.8-7.5), the assay can be accepted as valid evidence of a positive clastogenic response. In addition, an explanation of how the compound pH might affect uptake should be presented since this would be relevant to our assessment. Otherwise, the assay should be repeated and steps taken, if necessary, to minimize possible pH effects on treatment conditions.

Item 15--see footnote 1.

16. CBI APPENDIX: Appendix A, Materials and Methods, CBI pp. 12-16, and Appendix B, Protocol, CBI pp. 41-57.

*Brusick, D. Genotoxic effects in cultured mammalian cells produced by low pH treatment conditions and increased ion concentrations. Environ. Mutagenesis (1986)8 (6):879-886.

Page _____ is not included in this copy.

Pages 64 through 88 are not included in this copy.

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.

✓ FIFRA registration data.

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EPA: 68D80056
DYNAMAC No. 115-D
December 13, 1988

DATA EVALUATION RECORD

KATHON

Mutagenicity--*In Vivo* Cytogenetic Study in Rats

STUDY IDENTIFICATION: Sames, J. L., and Frank, J. P. Skane M-8 HQ *in vivo* cytogenetic study in rats. (Unpublished study No. 86R-218 prepared and submitted by Rohm and Haas, Spring House, PA; dated March 30, 1987.) ~~Accession~~ MRID No. 406475-04.

APPROVED BY:

Robert J. Weir, Ph.D.
Acting Department Manager
Dynamac Corporation

Signature: William L. McLellan for)

Date: 12/13/88



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1. CHEMICAL: Kathon; Skane M-8; 2-octyl-3(2H)-isothiazolone.

2. TEST MATERIAL: Skane M-8 from lot No. SW85-0311 was described as an amber liquid that contained 42.9% active ingredient in 57.1% propylene glycol.

3. STUDY/ACTION TYPE: Mutagenicity--In vivo cytogenetic study in rats.

4. STUDY IDENTIFICATION: Sames, J. L., and Frank, J. P. Skane M-8 HQ in vivo cytogenetic study in rats. (Unpublished study No. 86R-218 prepared and submitted by Rohm and Haas, Spring House, PA; dated March 30, 1987.) Accession No. 406475-04.

5. REVIEWED BY:

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Date: 2/1/89



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7. CONCLUSIONS:

A. Under the conditions of this assay, the single oral exposure of male and female rats to the approximate maximum tolerated dose of Skane M-8 [215 mg/kg active ingredient (a.i.)] did not cause a significant increase in chromosomal aberrations of bone marrow cells sampled over the entire mitotic cycle. It was, therefore, concluded that Skane M-8 was assayed to an appropriate toxic dose with no evidence of clastogenicity.

B. The study is acceptable.

Items 8 through 10--see footnote 1.

11. MATERIALS AND METHODS (PROTOCOLS):

A. Materials and Methods: (See Appendix A for details.)

1. Test Material: Skane M-8 from lot No. SW85-0311 was described as an amber liquid that contained 42.9% a.i. in 57.1% propylene glycol. Information on physical and chemical properties, as well as safe, handling procedures were detailed in an appendix to the final report. The test material was diluted with propylene glycol (PG); prepared solutions were based on the a.i. content of the test material and were analyzed for Skane M-8 concentration.

2. Test Animal: Two hundred and six CRL:CD BR VAF/Plus rats (104 males and 102 females) weighing 100 to 125 g were obtained from Charles River Breeding Laboratories, Canada.

3. Animal Maintenance: Prior to initiation of the study, the animals were acclimated to laboratory conditions for 13 days, which included a 7-day quarantine period. Eighty-one males and 81 females were randomly selected and distributed among stainless steel cages in groups of two or three. Throughout the course of the study, animals were housed in an environment controlled for temperature (73 to 75°F), relative humidity (43 to 55%), and light (12 hours). With the exception of dosing intervals, animals were permitted Purina Rodent Lab Chow Checkers ad libitum; water was available ad libitum at all times.

[1]Only items appropriate to this DER have been included.



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4. Assignment to Groups: Animals were weighed, randomly assigned to treatment groups, ear tagged, and identified with a unique number. At initiation of dosing, animals weighed 133 to 232 g.

5. Dose Selection Rationale/Dosing Procedures:

   a. Dose Selection: The doses of the test material used in this assay (21.5, 107.5, and 215.0 mg/kg a.i.) were selected to approximate the acute oral $LD_{10}$ in rats and 0.5 and 0.1 of the $LD_{10}$. Toxicity data to support the $LD_{10}$ value were furnished by the sponsor.

   b. Dosing Procedures: Animals were fasted 18 hours prior to dosing on day 1 and were gavaged with the appropriate dosing solution or the vehicle control (PG) at a constant volume of 10 mL/kg. All dosing solutions were prepared fresh on the day of use.

6. Cytogenetic Assay:

   a. Compound Administration: Twenty-three male and 23 female rats received single oral administrations of the highest test material dose or vehicle control (PG). Fifteen animals/sex/group were administered the mid- and low-dosing solutions. The additional 16 rats in the high-dose and solvent control groups were weighed and observed for toxic effects daily for 4 days posttreatment; these animals were not used in the cytogenetic evaluation.

   Animals used for chromosome analysis were weighed prior to dosing or colchicine injection; toxic signs were monitored daily. Ten representative members of each group were sacrificed at 6, 27, and 51 hours after compound administration.

   The positive control, triethylenemelamine (TEM), was administered as a single dose (0.5 mg/kg, i.p.) to five males and five females. Animals in this group were sacrificed 21 hours postexposure.

   b. Animal Sacrifice/Bone Marrow Harvest: Colchicine (1 mg/kg, i.p.) was injected 3 hours prior to the scheduled sacrifice; animals were sacrificed by $CO_2$ asphyxiation. Bone marrow cells were collected from both femurs by aspiration into 0.65% KCl. Aspirates were incubated for 20 to 30 minutes at

37°C and centrifuged; the supernatants were discarded. The pellets were fixed three times in methanol:acetic acid (3:1), dropped onto slides, dried, stained with Giemsa, mounted, and coded. Four slides were prepared per animal.

c. Slide Analysis: A maximum of 50 well-defined metaphases per animal were scored for the presence of cytogenetic abnormalities. Chromosome aberrations were characterized as breaks, gaps, fragments, pulverized cells, translocations, or rearrangements. Gaps were not included in the final analyses.

7. Statistical Analysis: The data were evaluated for statistical significance ($p < 0.05$) by the beta binomial model[2,3] and Fisher exact test.

8. Evaluation Criteria: No criteria to evaluate assay validity, a positive response, or the biological significance of the findings were presented.

B. Protocol: See Appendix B.

12. REPORTED RESULTS:

A. Animal Observations: Four deaths occurred in the 215-mg/kg dose group (three females on day 1 and one male on day 2); animals that died before the scheduled bone marrow cell harvest were replaced. Other frequently observed toxic signs for the high-dose animals included passiveness, yellow/brown stained anogenital areas, respiratory noise, and scant/no feces. With the exception of scant/no feces (seen on day 2), these signs were reported on day 1,

[2]Williams, D. A. The analysis of binary responses from toxicological experiments involving reproduction and teratogenicity. Biometrics 31 (1975): 949-954.

[3]Stiratelli, R. G., McCarthy, K. L., and Scribner, H. E. Parametric approach to the analysis of in vivo cytogenetics studies. Environ. Mutagenesis 7, Suppl. 4 (1985): 43-54.

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persisted through day 2, but subsided by day 3. In general, animals in the mid-dose group (107.5 mg/kg) exhibited similar toxic signs on day 1; however, these signs were noted in fewer animals. Passiveness was reported for one low-dose male on day 1. High-dose animals used for body weight determinations had an ≈65 to 90% reduction in body weight gain as compared to the vehicle control group.

B. Chromosome Analysis: The percent aberrant cells in the vehicle control group at the 6- and 27-hour harvest intervals were slightly elevated but not sufficiently increased to compromise the study. Chromosome aberrations for high-dose animals, whether grouped according to sex or combined for both sexes, were either lower than or comparable to the corresponding control group at all cell harvests. Similarly, no unusual types of chromosome aberrations were scored in the treated groups. By contrast, the positive control, TEM at 0.5 mg/kg, induced a significant ($p < 0.05$) increase in the percent aberrant cells. Slides were not scored for the lower test groups. Representative results combined for both sexes are presented in Table 1.

13. STUDY AUTHORS' CONCLUSIONS/QUALITY ASSURANCE MEASURES:

A. The authors concluded, "Under these test conditions, Skane M-8 HQ did not induce chromosomal aberrations in bone marrow cells of male or female rats."

B. A quality assurance statement was signed and dated January 29, 1987.

14. REVIEWERS' DISCUSSION AND INTERPRETATION OF STUDY RESULTS:

We assess that the assay was properly conducted and that the study authors interpreted the data correctly. Compound-related deaths, adverse effects on body weight gain, and other signs of toxicity were seen in animals of both sexes exposed to the high dose (215 mg/kg). The data showed no evidence of target cell cytotoxicity; however, it was concluded that Skane M-8 was assayed to a level that approximated the maximum tolerated dose with no indication of clastogenic activity. The requirement to analyze metaphases from the lower dose groups is, therefore, waived. The statistically significant increases in chromosome aberrations in male and female rats treated with the positive control (TEM, 0.5 mg/kg, i.p.) adequately demonstrated the sensitivity of the test system to detect clastogenic agents.



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TABLE 1. Representative Results of the *in vivo* Cytogenetic Study in Rats with Skane M-8

| Substance | Dose | Exposure Time[a] (hours) | No. of Animals Scored[b] | No. of Metaphases Examined | No. of Cells with Aberrations[c] | Percent Aberrant Cells[d] |
|---|---|---|---|---|---|---|
| Vehicle Control | | | | | | |
| Propylene glycol | 10 mL/kg | 6 | 10 | 500 | 17 | 3.4 |
| | | 27 | 10 | 500 | 15 | 3.0 |
| | | 51 | 10 | 500 | 9 | 1.8 |
| Positive Control | | | | | | |
| Triethylene-melamine | 0.5 mg/kg | 21 | 10 | 483 | 211 | 43.7* |
| Test Material | | | | | | |
| Skane M-8 | 215.0 mg/kg[e] | 6 | 10 | 500 | 5 | 1.0 |
| | | 27 | 10 | 500 | 9 | 1.8 |
| | | 51 | 10 | 500 | 12 | 2.4 |

[a]Time after compound administration.

[b]Combined for both sexes (five males and five females/group).

[c]Gaps not included.

[d]Percent aberrant cells = $\frac{\text{No. of cells with aberrations}}{\text{No. of metaphases examined}} \times 100$.

[e]Highest assayed dose; four deaths occurred in this group.

*Significantly different from 27-hour control value (at $p < 0.05$) using the beta binomial model.

Note: Chromosome preparations for the 21.5- and 107.5-mg/kg dosing groups were not scored.



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Item 15--see footnote 1.

37°C and centrifuged; the supernatants were discarded. The pellets were fixed three times in methanol:acetic acid (3:1), dropped onto slides, dried, stained with Giemsa, mounted, and coded. Four slides were prepared per animal.

c. Slide Analysis: A maximum of 50 well-defined metaphases per animal were scored for the presence of cytogenetic abnormalities. Chromosome aberrations were characterized as breaks, gaps, fragments, pulverized cells, translocations, or rearrangements. Gaps were not included in the final analyses.

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B. Chromosome Analysis: The percent aberrant cells in the vehicle control group at the 6- and 27-hour harvest intervals were slightly elevated but not sufficiently increased to compromise the study. Chromosome aberrations for high-dose animals, whether grouped according to sex or combined for both sexes, were either lower than or comparable to the corresponding control group at all cell harvests. Similarly, no unusual types of chromosome aberrations were scored in the treated groups. By contrast, the positive control, TEM at 0.5 mg/kg, induced a significant ($p < 0.05$) increase in the percent aberrant cells. Slides were not scored for the lower test groups. Representative results combined for both sexes are presented in Table 1.

13. STUDY AUTHORS' CONCLUSIONS/QUALITY ASSURANCE MEASURES:

A. The authors concluded, "Under these test conditions, Skane M-8 HQ did not induce chromosomal aberrations in bone marrow cells of male or female rats."

B. A quality assurance statement was signed and dated January 29, 1987.

14. REVIEWERS' DISCUSSION AND INTERPRETATION OF STUDY RESULTS:

We assess that the assay was properly conducted and that the study authors interpreted the data correctly. Compound-related deaths, adverse effects on body weight gain, and other signs of toxicity were seen in animals of both sexes exposed to the high dose (215 mg/kg). The data showed no evidence of target cell cytotoxicity; however, it was concluded that Skane M-8 was assayed to a level that approximated the maximum tolerated dose with no indication of clastogenic activity. The requirement to analyze metaphases from the lower dose groups is, therefore, waived. The statistically significant increases in chromosome aberrations in male and female rats treated with the positive control (TEM, 0.5 mg/kg, i.p.) adequately demonstrated the sensitivity of the test system to detect clastogenic agents.



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TABLE 1. Representative Results of the *in vivo* Cytogenetic Study in Rats with Skane M-8

| Substance | Dose | Exposure Time[a] (hours) | No. of Animals Scored[b] | No. of Metaphases Examined | No. of Cells with Aberrations[c] | Percent Aberrant Cells[d] |
|---|---|---|---|---|---|---|
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| | | 27 | 10 | 500 | 15 | 3.0 |
| | | 51 | 10 | 500 | 9 | 1.8 |
| Positive Control | | | | | | |
| Triethylene-melamine | 0.5 mg/kg | 21 | 10 | 483 | 211 | 43.7* |
| Test Material | | | | | | |
| Skane M-8 | 215.0 mg/kg[e] | 6 | 10 | 500 | 5 | 1.0 |
| | | 27 | 10 | 500 | 9 | 1.8 |
| | | 51 | 10 | 500 | 12 | 2.4 |

[a]Time after compound administration.

[b]Combined for both sexes (five males and five females/group).

[c]Gaps not included.

[d]Percent aberrant cells = $\frac{\text{No. of cells with aberrations}}{\text{No. of metaphases examined}} \times 100$.

[e]Highest assayed dose; four deaths occurred in this group.

*Significantly different from 27-hour control value (at $p < 0.05$) using the beta binomial model.

Note: Chromosome preparations for the 21.5- and 107.5-mg/kg dosing groups were not scored.



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Item 15--see footnote 1.

16. CBI APPENDIX: Appendix A, Materials and Methods, CBI pp. 9-16, and Appendix B, Protocol, CBI pp. 25-35.



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Page _____ is not included in this copy.

Pages 97 through 117 are not included in this copy.

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EPA: 68D80056
DYNAMAC No. 115-E
December 13, 1988

DATA EVALUATION RECORD

VATHON

Mutagenicity--Unscheduled DNA Synthesis
in Primary Rat Hepatocytes

STUDY IDENTIFICATION: Muller, G. Skane M-8 HQ microbiocide in vitro unscheduled DNA synthesis assay. (Unpublished study No. 86R-0018 prepared by Rohm and Haas Co., Toxicology Department, Spring House, PA; submitted by Rohm and Haas Co., Philadelphia, PA; dated August 12, 1986.) Accession No. 406475-05.

APPROVED BY:

Robert J. Weir, Ph.D.
Acting Department Manager
Dynamac Corporation

Signature: Robert J. Weir for J. McLellan

Date: 12/13/88



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1. CHEMICAL: Kathon; Skane M-8; 2-octyl-3(2H)-isothiazolone.

2. TEST MATERIAL: Skane M-8 from lot No. SW85-0311 was described as an amber liquid that contained 46.7% active ingredient in 53.3% propylene glycol.

3. STUDY/ACTION TYPE: Mutagenicity.

4. STUDY IDENTIFICATION: Muller, G. Skane M-8 HQ microbiocide in vitro unscheduled DNA synthesis assay. (Unpublished study No. 86R-0018 prepared by Rohm and Haas Co., Toxicology Department, Spring House, PA; submitted by Rohm and Haas Co., Philadelphia, PA; dated August 12, 1986.) Accession No. 406475-05.

5. REVIEWED BY:

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Principal Reviewer
Dynamac Corporation
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Date: 12-13-88

I. Cecil Felkner, Ph.D.
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Dynamac Corporation
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Date: 12-13-88

6. APPROVED BY:

I. Cecil Felkner, Ph.D.
Genetic Toxicology Studies
Technical Quality Control
Dynamac Corporation
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Date: 12-13-88

William Greear, M.P.H.
EPA Reviewer, Section II
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(TS-769C)
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Date: 12/14/88

Marion Copley, D.V.M.,
D.A.B.T.
EPA Acting Section Head,
Section II
Toxicology Branch I (TS-769C)
Signature: Marion Copley
Date: 2/9/89

7. CONCLUSIONS:

A. Skane M-8 was investigated in two independent unscheduled DNA synthesis (UDS) assays in primary rat hepatocytes. Combined results from both assays indicated that doses ≥ 9 µg/mL caused a marked reduction in cell survival. Net nuclear grain counts for hepatocytes treated with 10 test material concentrations (0.05 to 8 µg/mL) showed no increase in UDS. It was concluded, therefore, that the test material was assayed to an appropriate cytotoxic level with no evidence of a genotoxic effect.

B. The study is acceptable.

Items 8-10--see footnote 1.

11. MATERIALS AND METHODS (PROTOCOLS):

A. Materials and Methods: (See Appendix A for details.)

1. Test Material: Skane M-8 from lot No. SW85-0311 was described as an amber liquid that contained 46.7% active ingredient (a.i.) in 53.3% propylene glycol. Information on physical and chemical properties, as well as safe handling procedures were detailed in an appendix to the final report. The test material was dissolved in dimethylsulfoxide (DMSO); prepared solutions were based on the a.i. content of the test material.

2. Indicator Cells: Primary rat hepatocytes were obtained by the *in situ* perfusion of the liver of an adult male Sprague-Dawley rat purchased from Charles River Laboratories, Inc., Lakeview, NJ.

3. Cell Preparation:

a. Hepatocyte Isolation: The liver was perfused with Hanks' buffered salt solution and Williams' Medium E (WME) containing 100 units/mL collagenase. The liver was excised, cleaned of extraneous tissue, placed in the collagenase perfusion solution, and combed to release the hepatocytes.

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[1] Only items appropriate to this DER have been included.



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b. Hepatocyte Harvest/Primary Culture Preparation: Recovered cells were filtered through gauze, centrifuged, stained with trypan blue, and counted. Cells were seeded at a density of 4 x $10^5$ cells into six replicate wells of Linbro dishes containing plastic coverslips. Cultures were placed in a humidified, 5% $CO_2$ incubator for a 1.5- to 2-hour attachment period.

5. UDS Assay:

a. Treatment: Prepared hepatocyte cultures were seeded onto coverslips in six wells per plate and exposed for 18.5 to 19.5 hours to doses ranging from 0.05 to 100 µg/mL of the test material, the negative control (WME), the solvent control (DMSO), or five concentrations of the positive control (0.05 to 5.0 µg/mL 2-acetylaminofluorene, 2AAF). Treatment medium contained 10 µCi/mL [$^3$H]thymidine. Dosed monolayers were washed twice with serum free WME.

b. Cytotoxicity Evaluation: One of the replicates was refed, reincubated overnight, stained with trypan blue, and fixed with 10% formalin. Cytotoxicity for dosed and control cultures was assessed by counting 1000 cells per coverslip and determining the number of viable and nonviable cells.

c. UDS Slide Preparation: Four of the remaining cultures, attached to coverslips, were swollen with 1% sodium citrate, fixed in glacial acetic acid-ethanol (1:3), dried, and mounted.

d. Preparation of Autoradiographs/Grain Development: Slides were dipped into Kodak NTB-2 emulsion, dried, and stored at -20°C for 7 days. Slides were developed in Kodak D-19, fixed, stained with hematoxylin-eosin, and coded.

e. Grain Counting: Based on the cytotoxicity results, at least five test doses were scored for UDS. One level of the positive control and the negative and solvent control groups were scored.



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The nuclear grains of 150 morphologically normal cells (50/slide) from the selected test doses, negative, solvent, and selected positive control groups were scored for incorporation of [$^3$H] thymidine into DNA. Net nuclear grain counts were determined by subtracting the nuclear grain count of each cell from the mean cytoplasmic grain count of three nuclear-sized areas adjacent to each nucleus.

5. Evaluation Criteria:

   a. Assay Validity: The assay was considered valid if: a) values for the negative or solvent control were within the normal range of historical data, b) the ability of the test system to detect UDS was demonstrated by the positive control group, and c) at least two treatment levels showed >50% survival and were available for slide analysis.

   b. Positive Response: The assay was considered positive if the test material induced reproducible "significant" increases in net nuclear grains compared to the concurrent negative and solvent controls.

6. Statistical Methods: The data were not analyzed statistically.

B. Protocol: See Appendix B.

12. REPORTED RESULTS:

Two UDS assays were performed; cytotoxicity was assessed in parallel with both assays. The results were as follows:

a. Trial 1: Eight test concentrations (0.05 to 100 µg/mL) were evaluated. Survival ranged from 97% at 1 µg/mL to 1% at 100 µg/mL. The three highest doses reduced the cell population by ≥ 50% and were, therefore, not scored for UDS activity. The slide analysis of scored doses (0.05, 0.1, 0.5, 1.0, and 5.0 µg/mL) was negative.

b. Trial 2: The author stated that the assay was repeated because of the "extremely steep toxicity dose-response curve." Accordingly, the author selected a narrower range of nine test material doses (1, 3, 4, 5, 6, 7, 8, 9, and 10 µg/mL) for the reevaluation of Skane M-8. Thirty-three percent of the hepatocytes survived treatment with 9 µg/mL of the test material; this dose was not analyzed for UDS. Below this level, ≥ 63% of the cells survived and survival increased as the dose decreased. Based on these results, all groups except the 4-µg/mL dose group were scored for nuclear grains. In agreement with the findings from trial 1, no increases in net nuclear grains accompanied exposure to the six scored treatment groups.

By contrast, marked increases in mean net nuclear grains, percent cells with >6 grains, and percent cells with >20 grains were scored for the cultures treated with varying concentrations of the positive control (0.2, 1.0, and 2.0 µg/mL 2AAF).

Representative results from both assays are presented in Table 1.

13. STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:

A. The author stated, "Under the conditions of this study, treatment of CRCD rat primary hepatocytes with Skane M-8 HQ microbicide did not result in the induction of unscheduled DNA synthesis."

B. A quality assurance statement was signed and dated August 11, 1986.

14. REVIEWERS' DISCUSSION AND INTERPRETATION OF STUDY RESULTS:

We assess that the study was well conducted and that the author correctly interpreted the data. None of the doses induced an increase in UDS. The cytotoxic effects seen at doses ≥ 8 µg/mL indicated that Skane M-8 HQ was assayed to an appropriate level with no indication of a genotoxic effect.

It was noted, however, that compound information attached to the protocol (see CBI p. 27) listed the pH of the test material as 2.4. Since the pH of the treatment medium was not determined, we can only speculate that the "steep toxicity-dose-response curve" could have resulted from changes in the pH of the treatment medium. The abrupt decrease in percent survival between 8 and 9 µg/mL tend to suggest a rapid shift in pH.

TABLE 1. Representative Results of the Unscheduled DNA Synthesis Rat Hepatocyte Assays with Skane M-8 HQ

| Treatment | Dose (μg/mL) | % Survival After Treatment | No. Cells Scored | Mean Net Nuclear Grain Counts ± Standard Deviations | % Cells with >6 Net Nuclear Grains | % Cells with > 20 Net Nuclear Grains |
|---|---|---|---|---|---|---|
| Negative Control | | | | | | |
| Culture medium | -- | 100[a] | 150 | -4.5 ± 0.10 | 0 | 0 |
| | -- | 104[b] | 150 | -4.1 ± 0.40 | 0 | 0 |
| Solvent Control | | | | | | |
| Dimethylsulfoxide | -- | 100[a] | 150 | -8.1 ± 1.77 | 1.3 | 0 |
| | -- | 100[b] | 100[b] | -5.4 ± 2.10 | 0 | 0 |
| Positive Control | | | | | | |
| 2-Acetylamino-fluorene | 0.2 | 96[a] | 150 | +37.1 ± 10.3 | 99 | 83 |
| | 2.0 | 87[b] | 150 | +20.4 ± 6.48 | 95 | 47 |
| Test Material | | | | | | |
| Skane M-8 HQ | 5.0 | 88[b c] | 150 | -10.9 ± 0.55 | 0 | 0 |
| | 8.0 | 63[b d] | 150 | - 4.2 ± 0.53 | 0 | 0 |

[a]Initial assay results. Two levels of the positive control were scored; the lowest dose was selected as representative.

[b]Repeat assay.

[c]Highest dose scored for UDS; higher levels were cytotoxic (<50% cell survival). Lower doses (1.0, 0.5, 0.1, and 0.05 μg/mL) showed no increase in UDS activity.

[d]Highest dose scored for UDS; highest assayed dose (9 μg/mL) was cytotoxic (<50% cell survival). Lower doses (7, 6, 5, 3, and 1 μg/mL) were negative for UDS activity.

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The study adequately demonstrated the sensitivity of the test system to detect UDS as shown by the findings with varying concentrations of the positive control (0.2, 1, and 2 µg/mL 2AAF).

Item 15--see footnote 1.

16. CBI APPENDIX: Appendix A, Materials and Methods, CBI pp. 11-18, and Appendix B, Protocol, CBI pp. 27-35.

Page _____ is not included in this copy.

Pages 126 through 144 are not included in this copy.

The material not included contains the following type of information:

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_____ Identity of product impurities.

_____ Description of the product manufacturing process.

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