

72-4 (b)

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TYPE OF PRODUCT(S) : I,D,H,F,N,R,S Microbicide

DATA ACCESSION NO(S). 419635-02

PRODUCT MANAGER (NO.) Christine Rice

PRODUCT NAME(S) Kathon 886 Biocide, Methylisothiazolinone

COMPANY NAME Rohm and Haas Company

SUBMISSION PURPOSE Review data for chronic toxicity test for
freshwater invertebrate, Daphnia magna. Daphnia life cycle.

SHAUGHNESSY NO.

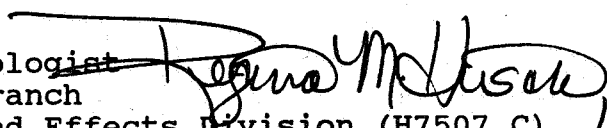
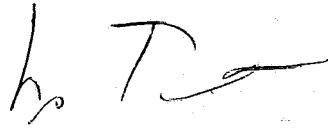
CHEMICAL & FORMULATION(S)

% A.I.

107103

5-Chloro-2-methyl-4-isothiazolin-3-one 14.17%

DATA EVALUATION RECORD

1. **CHEMICAL:** Kathon 886 Biocide (Methylisothiazolin)
2. **TEST MATERIAL:** Kathon 886 Biocide Technical (Lot No. 24088; TD No. 90-008), 14.17% active ingredient, yellow liquid.
3. **TEST TYPE:** Daphnia magna life-cycle (21-day renewal) chronic toxicity test.
4. **Citation:** Ward, T.J. and R.L. Boeri. 1991. Chronic toxicity of Kathon 886 Biocide to the Daphnid, Daphnia magna. Study performed by: EnviroSystems Division Resource Analysts, Inc., P.O. Box 778 One Lafayette Road, Hampton New Hampshire 03842. EnviroSystems study number: 9005-RH. Rohm and Haas Report Number: 89RC-0346. Accession number: 419635-02.
5. **REVIEWED BY:**

Regina M. Hirsch, Biologist
Ecological Effects Branch
Environmental Fate and Effects Division (H7507 C) 12/5/91
6. **APPROVED BY:**

Les Touart, Section Head
Ecological Effects Branch
Environmental Fate and Effects Division (H7507 C) 12/5/91
7. **CONCLUSIONS:** This study does not fulfill the guideline requirements (72-4) for an Aquatic invertebrate life cycle test with daphnia. Reproductive data for all groups were improperly reported. In addition, these test results were inconsistent with results from the acute toxicity test for daphnia. The 48-hour acute EC₅₀ value for Kathon 886 Biocide at 14.17% a.i. was 0.18 mg/L whereas, in the life cycle study daphnids were unaffected at the 0.18 mg/L level within 48 hours.

8. **MATERIALS AND METHODS:**

A. **Test Organisms:**

Species -- daphnid (Daphnia magna).

Supplier -- in-house cultures, established 15 days prior to test initiation.

Acclimation Period -- daphnids were transferred to fresh media 24 hours prior to test initiation and offspring produced were used in the study. Daphnids were not treated for any sickness, injuries, or abnormalities.

Screening Test -- was not performed. Test concentrations were based on previous testing information, Acute Daphnia magna EC₅₀; EnviroSystems Study Number 9001-RH: Rohm and Haas Report Number 89RC-0345).

B. **Test System:**

Invertebrates -- 240 daphnids, 24 hours old at test initiation, were used in this study.

Test vessels -- 270 ml glass aquaria which contained 270 ml of test solution. Test vessels were randomly arranged in a water bath during the test.

Photoperiod -- 16 hours of light and 8 hours of darkness with a 15 minute transition period.

Source of dilution water -- well water collected at EnviroSystems in Hampton, New Hampshire. Water was adjusted to hardness as CaCO₃ and stored in 500 gallon polyethylene tanks, where it was aerated.

Hardness -- CaCO₃ = 172-180 mg/L

Alkalinity -- 155-175

Conductivity -- N/A

Temperature -- 20 ± 1°C

pH -- N/A

Loading -- less than 40 animals/liter.

Dissolved oxygen -- 60 to 105% saturation (no

aeration was required throughout the study).

Feeding -- Daphnids were fed a yeast/trout chow suspension and freshwater alga, Selenastrum capricornutum at least twice per day at a rate proportional to the number of survivors in each vessel.

C. Definitive Test:

Groups -- 5 test groups and 1 control group were used in this study.

Number of test organisms -- 40 daphnids were indiscriminately and equally distributed among four replicates of each treatment concentration.

Dosage form -- test substance was supplied to the test vessels under flow through conditions by an intermittent flow proportional diluter. During the test the diluter was activated 558 times (24.6 media exchanges per 24 hours in each test vessel).

Test concentration -- Initial stock solution was prepared by the combining 14.29 g of test substance and deionized water and adjusting the final volume to 1,000 ml to result in a nominal concentration of 2,000 mg/L of the active ingredient. No solvent was used. Analytical determination of test material concentration was performed on each concentration on days 0, 7, 14, and 21. Samples were collected from replicates 1 and 3 on days 0 and 14 and from replicates 2 and 4 on days 7 and 21. Nominal concentrations of test substance were 0.00 (control), 0.03, 0.05, 0.08, 0.12, and 0.20 mg a.i./L Kathon 886 Biocide.

Study duration -- 21 days of exposure to Kathon 886 Biocide.

Organism observations -- all aquaria were examined initially and at 24 hour intervals: number of survivors; sublethal effects (loss of equilibrium, erratic behavior, loss of reflex, excitability, discoloration, or change in behavior); and dead organisms removed. The time to first brood was determined and the young produced by the adult daphnids were counted and removed at 1 to 3 day intervals.

Physical observations -- dissolved oxygen, pH, conductivity, and temperature were measured and recorded daily in each test chamber that contained live daphnids. The temperature in one test vessel was recorded continuously throughout the test (See Table

4).

9. REPORTED RESULTS:

Statistics: Only survival of first generation adult daphnids and the total number of young produced per replicate test vessel was statistically analyzed. Statistical analysis of the number of young per female, the weight of surviving daphnids, sublethal effects, and time to first brood data was not warranted. Results of the toxicity test were interpreted by standard statistical techniques. Shapiro-Wilk's test was used to determine if data were normally distributed, and Bartlett's test was used to determine if variances were homogeneous. A parametric one-way ANOVA followed by Dunnett's test was used to compare treatment to control means. Dichotomous data were transformed (arc sin square root) prior to statistical analysis. All calculations were performed using mean measured concentrations of the active ingredient.

EC₅₀: greater than 0.18 mg/L, highest concentration tested.

MATC: 0.13 mg/L.

NOEL: 0.10 mg/L

Test conditions: No insoluble material was observed in any test vessel during the test. Mean measured concentrations were 83% of the nominal concentrations (See Table A1):

<u>Nominal</u>	<u>Mean measured</u>
(mg a.i./L)	
0.03	0.02
0.05	0.04
0.08	0.06
0.12	0.10
0.20	0.18

Observations: Total number of young produced in tested concentrations was not significantly different than controls. Sublethal effects were not observed in any test groups. The percentage of surviving adults was reduced significantly in comparison to the control at 0.18 mg/L: controls had 90-100% survival; 0.18 mg/L had 50-80% survival (See Table 3 and B1). Production of first generation daphnids was observed on day 9 for all controls and concentrations tested. Control group had an average of 102 young per surviving female after 21 days of exposure (See Table 3 and B3).

Mean Weight -- 0.60 mg (N=40) (measured from just control fish at the end of the study).

Loading rate: approx. 0.024 g/L at any one time. 0.001 g/L /24 hours.

pH: 8.3 $\pm$ 0.1

Conductivity: 1100 $\pm$ 100 umhos/cm

Dissolved oxygen: 8.9-9.3 mg/L (above 75% of saturation)

Temperature: 19.6 $\pm$ 0.6°C

Protocol deviations: (taken from registrant's study document)

A. Sponsor approval of test concentrations could not be verified from raw data.

B. The fact that water flowed directly into exposure cages could not be verified from raw data.

C. Randomization of test vessels could not be verified from raw data.

D. Data regarding number of young per female was not collected during culture acclimation.

E. The 15 minute transition period included as part of the photoperiod could not be verified from the raw data.

10. **STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:**

The study author believes that the deviations were minor and did not affect the quality or integrity of the study. Quality Assurance and Good Laboratory Practice regulation Statements were included in the report.

11. **REVIEWER'S DISCUSSION AND INTERPRETATION OF THE STUDY:**

A. **Test Procedures:** Test procedures deviated from protocols recommended by the guidelines as stated in study author's "protocol deviations". In addition:

1. according to the SEP, lab reared cultures should be maintained for at least 21 days at test conditions in dilution water with renewal of culture medium at least three times per week.

2. daphnid cultures were established only 15 days

prior to test initiation and transferred to fresh media only 24 hours prior to test.

3. the alkalinity was high, 155-175, compared to recommended values of 110-120.

4. ten daphnids per concentration were in one test chamber. According to the SEP a) seven beakers at each toxicant concentration containing one daphnid each for collection of survival, growth, and reproduction data, and b) three beakers at each test concentration containing five daphnids each for collection of survival data only should be used.

5. adults weights were averaged per treatment replicate. Individual dry weight and length (preferred) data are needed to evaluate growth effects.

6. reproductive data was averaged for treatment and control groups. The average number of offspring per individual adult should be reported on a daily basis.

B. Statistical Analysis: Because of all the deficiencies in this test statistical tests were not performed. With these data a 30% difference in test group lengths would have to occur before significance could be resolved.

C. Discussion/Results: This study does not fulfill the guideline requirements (72-4) for an Aquatic invertebrate life cycle flow through test with Daphnia. Daphnid growth and reproductive data were improperly recorded. Test results were inconsistent with results from the acute toxicity test for daphnia. The 48-hour acute EC₅₀ value for Kathon 886 Biocide at 14.17% a.i. was 0.18 mg/L whereas, in the life cycle study daphnids were unaffected at the 0.18 mg/L level within 48 hours.

D. Adequacy of Test:

1. **Validation Category:** Invalid

2. **Rationale:** Inconsistencies between acute and life cycle toxicity data for Kathon 886 Biocide and because daphnid growth and reproduction data were improperly recorded.

3. **Repairability:** No

12. **COMPLETION OF ONE-LINER FOR TEST:** No

KATHON

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Pages 8 through 15 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.
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