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72-2(a)

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

DATA ACCESSION NO(S). 417188-03

PRODUCT MANAGER (NO.) Christine Rice

PRODUCT NAME(S) Kathon 886 Biocide, Methylisothiazolinone

COMPANY NAME Rohm and Haas Company
SUBMISSION PURPOSE Review data for acute toxicity test for
freshwater invertebrate, Daphnia magna.

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:** Acute Toxicity Test for Freshwater Invertebrates using Daphnia magna.
4. **Citation:** Ward, T.J. and R.L. Boeri. 1990. Acute Flow Through 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: 9001-RH. Rohm and Haas Report Number: 89P-345. Accession number: 417188-03.
5. **REVIEWED BY:**
Regina M. Hirsch, Biologist
Ecological Effects Branch
Environmental Fate and Effects Division (H7507 C) *Regina M. Hirsch* 12/5/91
6. **APPROVED BY:**
Les Touart, Section Head
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7. **CONCLUSIONS:** This study does not fulfill the guideline requirements (72-2) for an Acute flow through test with daphnia. Daphnid size (controls and test groups) were not provided. Test concentrations were not measured every 24-hours. In addition, these test results were inconsistent with results from the life cycle toxicity test for daphnia. The 48-hour acute LC₅₀ 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.

72-2 (a)

8. MATERIALS AND METHODS:

A. Test Organisms:

Species -- daphnid (Daphnia magna).

Supplier -- in-house cultures (originally obtained from a commercial supplier, the Aquatic Research Organisms Division of Resource Analysts, Inc.).

Mean Weight -- 0.0004 g (N=20) (measured from just control fish at the end of the study).

Acclimation Period -- daphnids were maintained in 100% dilution water under static conditions for 14 days. They were free of apparent sickness, injuries, and abnormalities. Temperature: 19.7 to 20.0°C (temperature was not recorded on the 12th day).

B. Test System:

Invertebrates -- 120 juvenile daphnids, less than 24 hours old at test initiation, were used in this study.

Test vessels -- 19.6 liter glass aquaria which contained 15 L 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.

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 = 40-48 mg/L

Alkalinity -- N/A

Conductivity -- N/A

Temperature -- 20 ± 1°C

pH -- N/A

Loading -- less than 0.5 g/L

Dissolved oxygen -- 60 to 100% saturation

Feeding -- Daphnids were fed a yeast/trout chow and

freshwater alga, Selenastrum capricornutum once per day before the test (feeding was not recorded on the 12th day prior to the test initiation).

C. **Definitive Test:**

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

Number of test organisms -- 20 (10 per replicate) daphnids per treatment group and control group were used.

Dosage form -- test substance was supplied to the test vessels under flow through conditions by an intermittent flow proportional diluter which was observed twice daily for normal operation. During the test the diluter was activated 424 times (7.1 media exchanges per 24 hours in each test vessel). Secondary stock solution was prepared by the proportional diluter which injects 0.3 ml of the initial stock solution into 3,000 ml of dilution water yield a nominal concentration of 0.30 mg/L.

Test concentration -- stock solutions were prepared by combining 21.43 g of test substance and deionized water and adjusting the mixture to 1,000 ml. No solvent was used. Analytical determination of test material concentration was performed on each concentration at initiation of the study and from one replicate of each concentration at the end of the study. Nominal concentrations of test substance were 0.00 (control), 0.04, 0.08, 0.12, 0.18, and 0.30 mg a.i./L Kathon 886 Biocide.

Study duration -- 48 hours 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. Control daphnids were weighed at the end of the study.

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.

9. REPORTED RESULTS:

Statistics: EC₅₀ were interpreted by standard statistical techniques. The probit, moving average, or binomial method was used to calculate the 24 and 48 hour median LC₅₀ and EC₅₀ (See Table 4). Because the probability was less than 0.05, the probit method could not be used to calculate the 48 hour LC₅₀, EC₅₀, or the slope of the dose response curve. All statistics were performed using the mean measured concentrations of the active ingredient.

48-hour LC₅₀: 0.18 mg/L with confidence interval of 0.12-0.30 mg/L.

EC₅₀: 0.16 mg/L with confidence interval of 0.14-0.19 mg/L

Test conditions: No insoluble material was observed in any test vessel during the test. Nominal concentrations and mean measured concentrations were comparable:

<u>Nominal</u>	(mg a.i./L)	<u>Mean measured</u>
0.04		0.05
0.08		0.08
0.12		0.12
0.18		0.18
0.30		0.30

Observations: Seven of the 20 daphnids at the 0.18 mg/L concentration were immobilized at the end of the study (after 48-hours). The mean percentage of daphnids surviving to 48 hours was 95% for controls, greater than 90% at 0.05 and 0.08 mg/L, and 0.12 mg/L, 50% at 0.18 mg/L, and 5% at 0.30 mg/L (See Table 3).

Loading rate: approx. 0.0002 g/L at any one time. 0.00004 g/L /24 hours.

pH: 8.0

Conductivity: 1300-1400 umhos/cm

Dissolved oxygen: 9.1-9.3 mg/L

Temperature: 20.2-20.9°C

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

A. The screening test was conducted prior to approval of

the test protocol.

B. Test organisms were indiscriminately added to test vessels which were arranged in random order.

C. Sponsor approval of analytical method validation can not be verified from the raw data.

D. Mortality, feeding, and water quality data were not collected on day 12 of the acclimation period.

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. Therefore, based on the results of the study Kathon 886 Biocide is considered to be highly toxic to freshwater invertebrates.

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 the protocols recommended by the guidelines as stated in the study author's "protocol deviations". In addition:

1) the test containers were rather large for a daphnid study. Test containers should be 3.9 liter with 2 to 3 liters of solution.

2) temperature should be monitored continuously throughout the test in at least one test vessel throughout the study.

3) test concentrations should be measured at 24-hour intervals to insure consistency of concentration throughout study.

B. **Statistical Analysis:** The binomial test was used to determine the LC₅₀ Kathon 886 on Daphnia magna. Using the conservative 95% confidence intervals of 0.3 and 0.12 the LC₅₀ was calculated to be 0.18. This number is comparable with the LC₅₀ determined by the study authors.

C. **Discussion/Results:** Test concentrations were not measured every 24-hours. In addition, the results from this study is inconsistent with the results from the life cycle toxicity test for daphnids. The 48-hour acute LC₅₀ 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:** Concentrations were not measured every 24-hours. Inconsistent results between acute and life cycle toxicity data for Kathon 886 Biocide.
3. **Repairability:** No

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

KATHON

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Pages 8 through 10 are not included.

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