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14 day

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SHAUGHNESSY NO.

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

DATA ACCESSION NO(S). 419635-03

PRODUCT MANAGER (NO.) Christine Rice

PRODUCT NAME(S) Kathon 886 Biocide, Methylisothiazolinone

COMPANY NAME Rohm and Haas Company

SUBMISSION PURPOSE Review data for acute flow through with rainbow trout -- 14 day prolonged test.

SHAUGHNESSY NO.

CHEMICAL & FORMULATION(S)

% A.I.

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

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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:** 96-hour Acute Flow Through Test with Rainbow Trout -- 14 Day Prolonged Test.
4. **Citation:** Ward, T.J. and R.L. Boeri. 1990. Acute Flow Through Toxicity of Kathon 886 Biocide to the Rainbow Trout, Oncorhynchus mykiss -- 14 Day Prolonged Test. Study performed by: EnviroSystems Division Resource Analysts, Inc., P.O. Box 778 One Lafayette Road, Hampton New Hampshire 03842. EnviroSystems study number: 9006-RH. Rohm and Haas Report Number: 89RC-0348. Accession number: 419635-03.
5. **REVIEWED BY:**
Regina M. Hirsch, Biologist
Ecological Effects Branch
Environmental Fate and Effects Division (H7507 C)
Regina Hirsch 12/5/91
6. **APPROVED BY:**
Les Touart, Section Head
Ecological Effects Branch
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L. T. 12/5/91
7. **CONCLUSIONS:** This study does not fulfill test requirements for an acute flow through -- 14 day prolonged test with rainbow trout because adequate sample of fish (controls and test groups) size were not provided. Test concentrations were not measured every 24-hours and temperature was higher than 12°C. Using the given data the LC₅₀ is 0.08 mg a.i./L of Kathon 886 Biocide, therefore it is considered very highly toxic to freshwater fish.

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8. MATERIALS AND METHODS:

A. Test Organisms:

Species -- rainbow trout (Oncorhynchus mykiss).

Supplier -- commercial supplier (Aquatic Research Organisms Division of Resource Analysts, Inc.).

Mean Weight -- 0.73 g (N=20) (measured from just control fish at the end of the study).

Mean Length -- 40.2 mm (N=20) (measured from just control fish at the end of the study).

Acclimation Period -- fish were maintained in 100% dilution water under flow-through conditions for 28 days. Temperature: 14.0 to 15.8^oC; Dissolved oxygen: above 8.8 mg/L and mortality equalled 0%.

B. Test System:

Fish -- 120 rainbow trout were used in this study.

Test vessels -- 17 L 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 of 50 to 250 mg/L as CaCO₃ and stored in 500 gallon polyethylene tanks, where it was aerated.

Hardness -- CaCO₃ = 160-168 mg/L

Alkalinity -- N/A

Conductivity -- N/A

Temperature -- 15 ± 2^oC

pH -- 6.0-8.5

Loading -- less than 1.0 g/L

Dissolved oxygen -- 60% saturation

Feeding -- Fish were fed a dry commercial food throughout acclimation and test periods, except for the 48 hours before the test.

C. Definitive Test:

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

Number of test organisms -- 20 (5 per replicate) rainbow trout 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 4,445 times (5.3 media exchanges per 24 hours in each test vessel).

Test concentration -- stock solutions were prepared by combining 21.2 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, seventh day and termination of the study. Nominal concentrations of test substance were 0.00 (control), 0.03, 0.05, 0.10, 0.17, and 0.30 mg a.i./L Kathon 886 Biocide.

Study duration -- 14 days of exposure to Kathon 886 Biocide.

Organism observations -- all aquaria were examined initially and at 24 hour intervals throughout the study: number of survivors; sublethal effects (loss of equilibrium, erratic behavior, loss of reflex, excitability, discoloration, or change in behavior); and dead organisms removed. After the 14 day test period control fish were weighed and measured.

Physical observations -- dissolved oxygen, pH, conductivity, and temperature were measured and recorded daily in each test chamber that contained live fish.

9. REPORTED RESULTS:

Statistics: LC₅₀ were interpreted by standard statistical techniques. The probit or binomial method was used to calculate the 24, 48, 72, 96 hour, and 14 day median LC₅₀

(See Table 5). Fourteen day survival data was statistically analyzed to determine the NOEL and LOEL after arc sin square root transformation. Because the data was not normally distributed, according to the Shapiro-Wilk's test, the NOEL and LOEL were calculated using Steel's Many-One Rank Test. The fourteen day MATC was calculated as the geometric mean of the NOEL and LOEL. All statistics were performed using the mean measured concentrations of the active ingredient.

14-Day LC₅₀: 0.07 mg/L with confidence interval of 0.06-0.09 mg/L.

NOEL: 0.05 mg/L

MATC: 0.07 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>
0.03	0.03
0.05	0.05
0.10	0.09
0.17	0.15
0.30	0.27

Observations: Sublethal effects were observed in one fish (day 3 of exposure) at the 0.05 mg/L and 15 (day 2) and 12 (days 3-6) at the 0.09 mg/L concentration. The mean percentage of fish surviving to 14 days was 90-100% at 0.03 and 0.05 mg/L, 50% at 0.09 mg/L, and 0% at 0.15, 0.27 mg/L (See Table 3).

Loading rate: approx. 0.24 g/L during the test, at any one time. 0.04 g/L at 24 hours.

pH: 7.6-8.0

Conductivity: 1100-1200 umhos/cm

Dissolved oxygen: 8.2-10.1 mg/L

Temperature: 14.6-16.3^oC

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

A. Test was conducted in 17 liter test vessel that

contained 15 liters of media instead of 19 liter vessel with 15 liters of media.

B. Turnover rate averaged 5.3 instead of 6-10 media exchanges per day.

C. Length and wet weight of a representative sample of fish was taken at study termination instead of initiation.

D. Can not be verified from the raw data that the test vessels were cleaned and siphoned 2 times per week.

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 fish.

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) temperature for coldwater fish studies is 12⁰C this study reported temperatures of 14.6-16.3⁰C.

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

3) fish size (length and weight) must be provided with raw data included in study packet to verify homogeneity of test groups. It is inadequate to provide summary data on only control fish to fulfill this requirement.

4) test concentrations for flow-through studies should be measured at 24-hour intervals to insure consistency of concentration throughout study.

B. **Statistical Analysis:** The moving average method was used to determine the LC₅₀ Kathon 886 on rainbow trout. Using the conservative 95% confidence intervals of 0.06 and 0.10 the LC₅₀ was calculated to be 0.08. This number is comparable with the LC₅₀ determined by the study authors.

C. **Discussion/Results:** Fish size was not reported properly. Test concentrations were not measured every 24-hours.

Temperature was beyond acceptable level for coldwater species. In addition, these test results were inconsistent with results from the 96-hour acute flow through test for rainbow trout. The 24, 48, 72, and 96-hour LC₅₀ values were higher in the 96-hour toxicity test than the 14 day prolonged test. Because daily concentrations were not recorded, the effects reported are equivocal for toxicity of Kathon on freshwater fish. Discrepancies between toxic levels for the acute and 14 day prolonged rainbow trout studies reinforce the uncertainty with the data. Using the submitted data the 96-hour LC₅₀ value for Kathon 886 Biocide at 14.17% a.i. is 0.08 mg/L. Kathon 886 Biocide can be classified as very highly toxic to freshwater fish.

D. Adequacy of Test:

1. **Validation Category:** Supplemental
2. **Rationale:** Fish size was improperly recorded and concentrations were not measured every 24-hours. Temperature was higher than 12°C.
3. **Repairability:** No

12. COMPLETION OF ONE-LINER FOR TEST: No

KATHON

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Pages 8 through 9 are not included.

The material not included contains the following type of information:

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