

72-1(c)

96 hour

S402232
SUBMISSION #

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SHAUGHNESSY NO.

REVIEW NO.

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

DATA ACCESSION NO(S). 417188-02

PRODUCT MANAGER (NO.) Christine Rice

PRODUCT NAME(S) Kathon 886 Biocide, Methylisothiazolinone

COMPANY NAME Rohm and Haas Company

SUBMISSION PURPOSE Review data for 96-hour acute flow through test with rainbow trout.

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:** 96-hour Acute Flow Through Test with Rainbow Trout.
4. **Citation:** Ward, T.J. and R.L. Boeri. 1990. Acute Flow Through Toxicity of Kathon 886 Biocide to the Rainbow Trout, Oncorhynchus mykiss. Study performed by: EnviroSystems Division Resource Analysts, Inc., P.O. Box 778 One Lafayette Road, Hampton New Hampshire 03842. EnviroSystems study number: 9003-RH. Rohm and Haas Report Number: 89RC-0343. Accession number: 417188-02.
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
Ecological Effects Branch
Environmental Fate and Effects Division (H7507 C) *L. Touart* 12/5/91
7. **CONCLUSIONS:** This study does not fulfill the guideline requirements (72-1) for a 96-hour acute flow through test with rainbow trout. Adequate sample of fish (controls and test groups) size were not provided. Test concentrations were not measured every 24-hours. From the given data the LC₅₀ is 0.19 mg a.i./L of Kathon 886 Biocide, therefore it is considered very highly toxic to freshwater fish.

72-1 (c)

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

Mean Length -- 30.5 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 more than 14 days. Temperature: 12.1 to 13.0 °C; Dissolved oxygen: above 8.7 mg/L.

B. Test System:

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

Test vessels -- 19.6 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 and stored in 500 gallon polyethylene tanks, where it was aerated.

Hardness -- $\text{CaCO}_3 = 40-44 \text{ mg/L}$

Alkalinity -- 100-110 mg/L

Conductivity -- N/A

Temperature -- $12 \pm 1^{\circ}\text{C}$

pH -- < 0.8 pH units

Loading -- 0.5 g/L/24 hours

Dissolved oxygen -- 60-105% saturation

Feeding -- Prior to testing fish were fed a dry commercial food, once or twice per day.

C. Definitive Test:

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

Number of test organisms -- 20 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 773 times (6.4 media exchanges per 24 hours in each test vessel).

Test concentration -- A secondary stock solution was prepared by the proportional diluter which injected 0.3 ml of the initial stock solution into 3,000 ml of dilution water to yield a nominal concentration of 0.70 mg a.i./L of Kathon 886. Analytical determination of test material concentration was performed on samples from each test vessel at the initiation of the test and from one replicate of a test concentration at the conclusion of the study. Nominal concentrations of test substance were 0.00 (control), 0.10, 0.18, 0.28, 0.41, and 0.70 mg a.i./L Kathon 886 Biocide.

Study duration -- 96 hours of exposure to Kathon 886.

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.

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

9. REPORTED RESULTS:

Statistics: LC₅₀ were interpreted by standard statistical techniques. The binomial method was used to calculate the 24, 48, 72, and 96 hour median LC₅₀ (See Table 4). The slope of the dose response curve was not calculated by the probit method because mortality between 1 and 99% occurred at only 1 concentration. All statistics were performed using the

mean measured concentrations of the active ingredient.

LC₅₀: 0.19 mg/L with confidence interval of 0.13-0.31 mg/L.

NOEC: 0.13 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>
0.10	0.13
0.18	0.20
0.28	0.31
0.41	0.45
0.70	0.71

Observations: Sublethal effects were observed in two fish at the 0.20 mg/L concentration. One fish after 72-hours exhibited a loss of equilibrium and gasping at the surface. The other fish after 96-hours exhibited loss of equilibrium and erratic swimming. The mean percentage of fish surviving to 96 hours was 100% at 0.13, 45% at 0.20 mg/L, and 0% at 0.31, 0.45 and 0.71 mg/L (See Table 3).

Loading rate: approx. 0.35 g/L during the test, at any one time. 0.05 g/L at 24 hours.

pH: 7.8-8.2

Dissolved oxygen: 10.2-11.0 mg/L

Temperature: 11.5-12.8°C

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

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

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

The study author believes that the deviation was 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) 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.

2) test concentrations for flow-through studies 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 LC_{50} Kathon 886 on rainbow trout. Using the conservative 95% confidence intervals of 0.13 and 0.31 the LC_{50} was calculated to be 0.19. This number is comparable with the LC_{50} determined by the study authors.

C. Discussion/Results: Fish size was not reported properly. Test concentrations were not measured every 24-hours. In addition, these test results were inconsistent with results from the acute flow through -- 14 day prolonged test for rainbow trout. The 24, 48, 72, and 96-hour LC_{50} 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. From the given data the 96-hour LC_{50} value for Kathon 886 Biocide at 14.17% a.i. is 0.19 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.
3. **Repairability:** No

12. COMPLETION OF ONE-LINER FOR TEST: No

KATHON

Page ___ is not included in this copy.

Pages 7 through 9 are not included.

The material not included contains the following type of information:

- Identity of product inert ingredients.
 - Identity of product impurities.
 - Description of the product manufacturing process.
 - Description of quality control procedures.
 - Identity of the source of product ingredients.
 - Sales or other commercial/financial information.
 - A draft product label.
 - The product confidential statement of formula.
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NOTE: THERE WAS CONTROL MORTALITY, BUT AT LEAST ONE OF THE LOWER CONCENTRATIONS HAD ZERO MORTALITY. THEREFORE, ABBOTT'S CORRECTION IS NOT APPLICABLE.

RHIRSCH KATHON 886 BIOCIDES Acute flow through toxicity to rainbow trout

CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
.71	20	20	100	9.536742E-05
.45	20	20	100	9.536742E-05
.31	20	20	100	9.536742E-05
.2	20	11	55	41.19014
.13	20	0	0	9.536742E-05

THE BINOMIAL TEST SHOWS THAT .13 AND .31 CAN BE USED AS STATISTICALLY SOUND CONSERVATIVE 95 PERCENT CONFIDENCE LIMITS, BECAUSE THE ACTUAL CONFIDENCE LEVEL ASSOCIATED WITH THESE LIMITS IS GREATER THAN 95 PERCENT.

AN APPROXIMATE LC50 FOR THIS SET OF DATA IS .1943916

WHEN THERE ARE LESS THAN TWO CONCENTRATIONS AT WHICH THE PERCENT DEAD IS BETWEEN 0 AND 100, NEITHER THE MOVING AVERAGE NOR THE PROBIT METHOD CAN GIVE ANY STATISTICALLY SOUND RESULTS.
