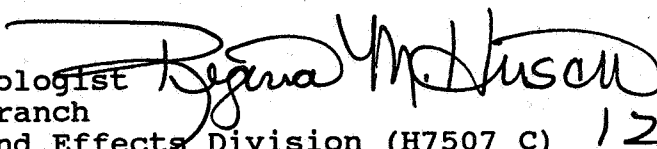
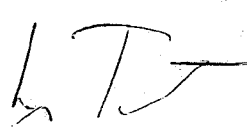


Shaughnessy # 107103

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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 Bluegill.
4. **Citation:** Ward, T.J. and R.L. Boeri. 1990. Acute Flow Through Toxicity of Kathon 886 Biocide to the Bluegill Sunfish, Lepomis macrochirus. Study performed by: EnviroSystems Division Resource Analysts, Inc., P.O. Box 778 One Lafayette Road, Hampton New Hampshire 03842. EnviroSystems study number: 9002-RH. Rohm and Haas Report Number: 89RC-0342. Accession number: 417188-01.
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 (72-1) for a 96-hour acute flow through test with bluegill sunfish. Adequate sample of fish (controls and test groups) size were not provided. In addition, test concentrations should be measured every 24-hours. From the given data the LC₅₀ is 0.30 mg a.i./L of Kathon 886 Biocide, therefore it is considered very highly toxic to freshwater fish.

72-1 (2)

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8. MATERIALS AND METHODS:

A. Test Organisms:

Species -- bluegill sunfish (Lepomis macrochirus).

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

Mean Weight -- 0.58 g (N=20) (measured from just control fish at the end of the study).

Mean Length -- 38 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 14 days. Temperature: 21.0 to 22.4 °C; Dissolved oxygen: above 8.2 mg/L.

B. Test System:

Fish -- 120 bluegill 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 -- CaCO_3 = 40-48 mg/L

Alkalinity -- 190-195 mg/L

Conductivity -- N/A

Temperature -- $22 \pm 1^{\circ}\text{C}$

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 bluegill 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 922 times (7.7 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 1.00 a.i. mg/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), 0.14, 0.22, 0.37, 0.56, and 0.93 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 moving average or binomial method was used to calculate the 24, 48, 72, and 96 hour median LC₅₀ (See Table 4). Because the probability for the 48-hour LC₅₀,

EC₅₀, or the slope response curve was less than 0.05, the probit analysis could not be used. All statistics were performed using the mean measured concentrations of the active ingredient.

LC₅₀: 0.28 mg/L with confidence interval of 0.24-0.32 mg/L.

NOEC: 0.22 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>	<u>Mean measured</u>
(mg a.i./L)	
0.14	0.14
0.24	0.22
0.40	0.37
0.61	0.56
1.00	0.93

Observations: No sublethal effects were observed in any test condition throughout the study. The mean percentage of fish surviving to 96 hours was 90-95% at 0.14 and 0.22 mg/L, 10% at 0.37 mg/L, and 0% at 0.56 and 0.93 mg/L (See Table 3).

Loading rate: approx. 0.39 g/L during the test, at any one time. 0.05 g/L at 24 hours.

pH: 7.8-8.1

Dissolved oxygen: 8.1-9.2 mg/L

Temperature: 21.8-22.9°C

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

A. Fish were not fed during the 48-hour period that immediately preceded the initiation of the test study.

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) mortality prior to study initiation should be less than 3%, study reported mortality less than 5%.

2) 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.

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

B. Statistical Analysis: Because there was a mortality in the control group and none of the lower concentrations produced zero mortalities, the data was subjected to Abbott's correction. The binomial test showed that 0.22 and 0.37 mg/L were a statistically sound conservative 95% confidence interval. Therefore, the $LC_{50} = 0.30$ mg/L using the binomial test. Using the moving average method $LC_{50} = 0.30$ mg/L with a 95% confidence interval of 0.26-0.34 mg/L.

C. Discussion/Results: Fish size was not reported properly. Test concentrations were not measured every 24-hours. 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 0.30 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 6 through 8 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.
 - ____ Information about a pending registration action.
 - X ____ FIFRA registration data.
 - ____ The document is a duplicate of page(s) _____.
 - ____ The document is not responsive to the request.
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The information not included is generally considered confidential by product registrants. If you have any questions, please contact the individual who prepared the response to your request.

NOTE: BECAUSE THERE WAS CONTROL MORTALITY, AND NONE
OF THE LOWER CONCENTRATIONS PRODUCED ZERO MORTALITY,
THE DATA HAS BEEN SUBJECTED TO ABBOTT'S CORRECTION.

rhirsch kathon 886 biocide bluegill acute toxicity LC50

CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
.93	19	19	100	1.907348E-04
.56	19	19	100	1.907348E-04
.37	19	17	89.4737	3.643036E-02
.22	19	0	0	1.907348E-04
.14	19	1	5.2632	3.814697E-03

THE BINOMIAL TEST SHOWS THAT .22 AND .37 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 .30248

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS
3	5.948356E-02	.2985601	.2623843

.3417535

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H
6	2.773711	7.069886

GOODNESS OF FIT PROBABILITY

0

A PROBABILITY OF 0 MEANS THAT IT IS LESS THAN 0.001.

SINCE THE PROBABILITY IS LESS THAN 0.05, RESULTS CALCULATED
USING THE PROBIT METHOD PROBABLY SHOULD NOT BE USED.

SLOPE = 8.886306
95 PERCENT CONFIDENCE LIMITS = -5.913357 AND 23.68597

LC50 = .2874362
95 PERCENT CONFIDENCE LIMITS = 0 AND +INFINITY

LC10 = .2068354
95 PERCENT CONFIDENCE LIMITS = 0 AND +INFINITY

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