12/6/88

Accession No. 408409-03

DATA EVALUATION RECORD

1. **CHEMICAL:** Chlorpyrifos Technical
Shaughnessey No. 059101

2. **TEST MATERIAL:** Chlorpyrifos Technical; Lot No. 389318
95.9% Active Ingredient

3. **STUDY TYPE:** Flow-through Acute Toxicity Test for Freshwater Fish. Species Tested: Salmo gairdneri

4. **CITATION:** Bowman, Jane H. (1988); Acute Flow-through Toxicity of Chlorpyrifos Technical to Rainbow Trout (Salmo gairdneri), Report No. 37188; prepared by Analytical Bio-Chemistry Laboratories, Inc. Columbia, Missouri; submitted by Makhteshim-Agan (America) Inc. New York, New York; Accession No. 408409-03.

5. **REVIEWED BY:**

Kimberly D. Rhodes
Aquatic Toxicologist
Hunter/ESE

Signature: Kimberly D. Rhodes
Date:

6. **APPROVED BY:**

Prapimpan Kosalwat, Ph.D.
Staff Toxicologist
KBN Engineering and
Applied Sciences, Inc.

Signature: P. Kosalwat
Date: 11/28/88

for Henry T. Craven, M.S.
Supervisor, EEB/HED
USEPA

Signature: John Noles
Date: 12/6/88

7. **CONCLUSIONS:** This study appears scientifically sound and fulfills the Guideline requirements for a 96-hour acute flow-through toxicity test of freshwater fish. The 96-hour LC50 based upon mean measured concentrations of Chlorpyrifos Technical to rainbow trout (Salmo gairdneri) was 0.027 mg/L, which classifies it as very highly toxic to rainbow trout. The NOEC was determined to be <0.0042 mg/L after 96 hours.

8. **RECOMMENDATIONS:** N/A

25 (20 - 32) ppb
per author.

9. BACKGROUND:

10. DISCUSSION OF INDIVIDUAL TESTS: N/A

11. MATERIALS AND METHODS:

A. Test Animals: Rainbow trout (Salmo gairdneri) were obtained from a commercial supplier in California. The fish were reared and maintained at ABC Laboratories in ABC well water and were fed newly hatched brine shrimp or a commercially available fish food daily. Seventy-two hours before the initiation of the test, rainbow trout were removed from the culture and placed in the temperature acclimation unit. During this time, the fish were held without food. The rainbow trout used as the control group during this study had a mean weight of 0.74 (±0.25) grams and a mean length of 38 (±3.9) millimeters at test termination. The biomass loading rate was 0.49 g/L. The laboratory environment was maintained on a 16-hour daylight photoperiod.

B. Test System: A proportional diluter system described by Mount and Brungs, utilizing a Hamilton Micro Lab 420 syringe dispenser, was used for the intermittent introduction of Chlorpyrifos Technical test solutions and diluent water into each test chamber. The proportional diluter system used for the project was set to provide test levels approximately 50 percent dilutions of each other. The diluter delivered one liter of test solution or control water to the test vessels at an average rate of 7.2 times per hour over the course of the study. This flow rate was sufficient to replace the 30-liter volume within the test chambers 5.8 times per day. Five concentrations of the test material with dilution water and solvent controls were tested. The test chambers were immersed in a temperature controlled water bath held at 12 ± 1°C. The lighting was maintained on a 16-hour daylight photoperiod.

Dilution water for the rainbow trout test was a blend of reverse osmosis water and ABC well water characterized as having a pH of 7.1 - 7.9, total hardness of 40 - 48 mg/L as $CaCO_3$, total alkalinity of 44 - 56 mg/L as $CaCO_3$ and specific conductance of 100 - 160 umhos/cm.

C. Dosage: 96-hour flow-through acute test.

D. Design: Two static range-finding tests were conducted with Chlorpyrifos. Based on the results of the preliminary testing, five concentrations were selected for

definitive testing. Twenty rainbow trout were tested per concentration. A control, solvent control, and nominal Chlorpyrifos Technical concentrations of 0.080, 0.040, 0.020, 0.010, and 0.0050 mg/L were tested. The nominal concentrations were not corrected for active ingredient. The concentration of acetone in the solvent control (0.1 mL of acetone in 1 liter of water) was approximately equivalent to that received by the highest test concentration. The mean measured concentrations of Chlorpyrifos Technical were 0.072, 0.037, 0.016, 0.0081 and 0.0042 mg/L as active ingredient. All concentrations were observed once every 24 hours for mortality and abnormal effects. The water quality parameters (temperature, dissolved oxygen and pH) were measured in the control, low, middle, and high test levels at 0, 48, and 96 hours of testing. Analytical samples were collected from each test level and the diluter stock at 0 and 96 hours.

E. Statistics: The concentration of toxicant lethal to 50% of the population (LC50's) and 95% confidence intervals was determined at 24-, 48-, 72-, and 96-hour exposure periods by the computer program developed by Stephan et al., 1978.

12. REPORTED RESULTS: The 24-, 48-, 72-, and 96-hour LC50 values for Chlorpyrifos were >0.072, 0.041, 0.029 and 0.025 mg/L, respectively, based upon mean measured concentrations. Slightly different values were reported in Table 4 of the author's report. The slope of the 96-hour dose-response line was 3.3 as calculated by probit computer analysis. Behavioral/sublethal effects observed in all concentrations included fish on bottom of test chamber, quiescence, loss of equilibrium, labored respiration, vertical orientation, surfacing, dark discoloration, curved spine, forward curved pectoral fins and excitability. Given these behavioral/sublethal effects in all test concentrations, a no-effect concentration of Chlorpyrifos Technical to rainbow trout was determined to be <0.0042 mg/L. Table 5 presents the cumulative mortality and behavioral observations made during the test (attached). There was no mortality in the control or solvent control during the study. The dissolved oxygen concentrations ranged between 8.5 and 9.2 mg/L (79 to 85% saturation at 12°C) during the test.

13. STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES: The 96-hour LC50 value for Chlorpyrifos Technical was calculated by the moving average method to be 0.025 mg/L with 95 percent confidence limits of 0.020 and 0.032 mg/L based upon mean measured concentrations. The NOEC (No-Observed-Effect Concentration) was <0.0042 mg/L after 96 hours.

"The study was conducted following the intent of the Good Laboratory Practice Regulations and the final report was reviewed by Analytical Bio-Chemistry Laboratories' Quality Assurance Unit." A Quality Assurance Statement was included in the report.

14. REVIEWER'S DISCU klHSSSSION AND INTERPRETATION OF STUDY RESULTS:

A. Test Procedure: The test procedures were generally in accordance with protocols recommended by the Guidelines, but deviated from the SEP as follows:

- The SEP recommends that fish be acclimated to study conditions for at least two weeks prior to testing. The rainbow trout were removed from the culture tank and placed in the temperature acclimation unit seventy-two hours before the test.

- Six-hour temperature measurements were not recorded as required by the SEP for tests conducted in a water bath.

- The SEP states that each designated treatment group should be exposed to a concentration of toxicant that is at least 60% of the next highest concentration. Each designated treatment group for the test was only 50% of the next highest concentration.

- The SEP recommends a 16-hour light/8-hour dark photoperiod with a 15- to 30-minute transition period between light and dark. The report did not state whether a 15- to 30-minute transition period between light and dark was maintained.

B. Statistical Analysis: The reviewer used the Toxanal computer program to calculate the LC50 values. These calculations are attached. The probit method provides a 96-hour LC50 value of 0.027 mg/L with a 95 percent confidence interval of 0.021 to 0.035 mg/L which is similar to that reported by the author. The slope of the toxicity curve was estimated to be 3.3.

C. Discussion/Results: The study results appear to be scientifically valid. The 96-hour LC50 value based upon mean measured concentrations was estimated to be 0.027 mg/L. Therefore, Chlorpyrifos Technical is classified as very highly toxic to rainbow trout (Salmo gairdneri).

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D. **Adequacy of the Study:**

(1) **Classification:** Core

(2) **Rationale:** N/A

(3) **Repairability:** N/A

15. **COMPLETION OF ONE-LINER FOR STUDY:** Yes, 11-17-88.

KIMBERLY RHODES CHLORPYRIFOS TECHNICAL SALMO GAIRDNERI 11-07-85

CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
.072	20	20	100	9.536743E-05
.037	20	10	50	58.80995
.019	20	4	20	5.765915
.0081	20	1	5	2.002716E-07
.0042	20	0	0	9.536743E-05

THE BINOMIAL TEST SHOWS THAT .0081 AND .072 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 .037

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	S	LC50	95 PERCENT CONFIDENCE LIMITS	
3	.572761E-02	2.466984E-02	2.109324E-02	3.254648E-02

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	S	H	GOODNESS OF FIT PROBABILITY
4	.1047093	1	.1540357

SLOPE = 3.309418
95 PERCENT CONFIDENCE LIMITS = 2.33953 AND 4.280106

LC50 = 2.690721E-02
95 PERCENT CONFIDENCE LIMITS = 2.104091E-02 AND 3.486465E-02

LC10 = 1.112027E-02
95 PERCENT CONFIDENCE LIMITS = 6.829292E-03 AND 1.492204E-02

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____ Description of quality control procedures.

____ Identity of the source of product ingredients.

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____ A draft product label.

____ The product confidential statement of formula.

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✓ FIFRA registration data.

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