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DATA EVALUATION RECORD (11)

1. Chemical: CGA-64250

2. Formulation: 90.7%, Techincal

3. Citation: Iley, E.S., Jr. (1981). Flow-through Fathead Minnow Early Life Stage Toxicity Test with CGA-64250. Environmental Research and Technology Inc., Fort Collins, Colorado. Acc. #072210.

4. Reviewed by: Carol M. Natella
Wildlife Biologist
EEB/HED

5. Date Reviewed: Feb. 13, 1984

6. Test Type: Fish early life stage - fathead minnow

7. Reported Results: MATC >95 and <184 ppb, based on egg mortality, length, and weight

8. Reviewer's Conclusions: The study is scientifically sound. The study does fulfill the requirement for a fish early life stage test.

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Materials/Methods

Test Procedures

Test animals: Fertilized fathead minnow eggs (Pimephales promelas) were obtained from Sea Plantations, Inc., in Salem, Massachusetts. At ERT's request, and Ciba-Geigy's approval, these eggs were not treated with an antifungal agent (as is normally done) prior to shipment in order to eliminate the possibility of affecting test results.

Test water quality: Domestic water was used for dilutions and controls. The Fort Collins domestic water supply (34.4 ppm hardness as $CaCO_3$) was filtered through an in-house battery of five automatic backflush dechlorinating filters.

Test system: A proportional diluter was used to deliver five test concentrations and a control. For each treatment level and the control, the test solutions were delivered to two replicate test chambers. The egg cups were pint capacity glass jars with the bottoms replaced by 600 micron mesh Nitex net. In each test chamber one egg cup was suspended from a rocker arm apparatus. Newly hatched larvae were removed from the egg cup and placed in the test chamber proper. Temperature of the test container was maintained at 25°±1°C.

Exposure: Measured test concentrations were 666, 321, 184, 95 and 48 ppb. Nanograde acetone was used to prepare the stock solution, resulting in 0.14 ml/l acetone in the highest treatment level test chambers. Sixty-five healthy, fertile eggs were impartially transferred to each egg cup. An egg cup was randomly placed in each egg test chamber. At 48 hours hatching had begun in all test chambers. Test organisms were reduced to a total of 51 healthy eggs and larvae in each chamber by removing the appropriate number of eggs. Organisms were exposed to test material for a total of 32 days.

Date of testing: 7/27/81-8/28/81.

Statistical Analysis

Chi-square analysis was used to analyze the following:

a) the number of healthy fertile eggs at 48 hours.

b) the number of eggs that produced live fry.

c) the number of eggs that produced live, normal fry.

d) the number of eggs that produced live fish at end of test.

e) the number of eggs that produced live, normal fish at end of test.

f) the number of hatched fry producing live fish at end of test.

g) the number of hatched fry producing live, normal fish at end of test.

At the end of the test all surviving fish were weighed and measured. A condition coefficient was calculated for each fish according to the following equation:

$$K = \frac{Wx105}{L^3}, \quad \text{where: } W = \text{weight (g)}, \; L = \text{length (mm)}$$

For weights, lengths, and condition coefficients, two-tailed F-tests were used to determine equality or inequality of replicates. Each treatment level was compared to the control using a one-tailed Dunnett's test. Individual fish weights, lengths, and condition coefficients were used as separate data points for analysis of variance.

Author's Discussion/Results

An MATC was not obtained in the first early life stage test that was performed because all treatment levels showed an effect. A 32-day egg-larvae LC_{50} of 0.72 ppm was obtained, however. A second test was then run at much lower levels, but was terminated on Day 11 when the control survival dropped below 80%. A third test using the same levels as the second was eventually successful.

Egg viability or hatchability and fry viability data summarized as percentages for each test chamber are presented in Table 3. At the three highest levels, high egg mortality was observed on Day 3 because of an apparent CGA-64250 stimulated fungus belonging to Class Chytridiomcetes or Oomycetes. After Day 3 mortality was very low in all of the test chambers.

For all statistical tests the level of significance was chosen to be ≤ 0.05. Since no abnormal fish were observed during the test the values for tests b and c, d, and e, f and g are identical. Following are the results of the author's Chi-square analyses (See addenda for calculations):

a) The number of healthy fertile eggs at 48 hours was significantly lower in the 0.321 ppm CGA-64250 treatment level than in the control.

b and c) The eggs from the 0.666, 0.321 and 0.184 ppm CGA-64250 treatment levels produced significantly fewer numbers of live normal fry than the control (due to heavy egg mortality on Day 3).

d and e) The eggs from 0.666, 0.321, 0.184 and 0.048 ppm CGA-64250 treatment levels produced significantly fewer live normal fish at the end of the test than the control. The slightly higher mortality in the 0.048 ppm level than that in the 0.095 ppm level may be assumed to be a random effect rather than an effect caused by CGA-64250.

f and g) Results for eggs that hatched producing live normal fish at the end of the test are the same as for factors d and e.

Using a two-tailed F-test for analysis of weight, length and condition coefficient, the author found that the replicate test chambers were not significantly different. The one-way analysis of variance indicated a significant difference between two or more of the populations and provided the mean standard error, an integral factor for the Dunnett's test. A one-tailed Dunnett's test was used to determine signficant differences between the control and each treatment level. The fish from the 666, 321, and 184 ppb levels had weights and lengths significantly (Dunnett's) lower than the control fish weights and lengths, while the fish from the 95 and 48 ppb levels were the same as the control fish. The condition coefficients of the surviving fish in all treatment levels showed no significant (Dunnett's) difference from the control fish.

Based on egg mortality, length, and weight, the MATC limits were determined to be >95 and <184 ppb, with a geometric mean 132 ppb. The author based the egg mortality evaluation on the Chi-square analysis of factors b and c.

A direct relationship was observed between the concentration of CGA-64250 and the growth of fungus in the test chambers. The author believes that this fungus was definitely related to egg mortality on Day 3 and may have contributed to fish mortality.

Reviewer's Evaluation

A. Test Procedure

The test procedure generally complies with an acceptable protocol (ASTM, Draft #5). Measurements of water quality (hardness, alkalinity, pH,

conductivity), however, were made only at the end of the test. Futhermore, these values occur only on the raw data recording sheets and are illegible.

B. Statistical Analysis

The author's Chi-square calculations were verified and have been found to support the author's conclusions. The methods used for the two-tailed F-Test, the ANOVA, and the Dunnett's test were all verified and the calculations were spot checked. The results of the analyses were found to support the author's conclusions.

C. Conclusions

1. Category: Core
2. Rationale: The test water has been shown to support the growth and reproduction of fathead minnows and is therefore of acceptable quality.
3. Repairability: N/A