(10-18-85)

DATA EVALUATION RECORD

1. Chemical: Cyromazine N-cyclopropyl-1,3,5-triazine-2,4,6,-triamine

2. Test Material: Technical CGA 72662, 93.4% ai

3. Study Type: Fish Early Life Stage Toxicity Test

   Species tested: Fathead Minnow Pimephales promelas

4. Study ID: Honeycutt, R.C. (1984) Flow-through Fathead Minnow Early Life Stage Toxicity Test with CGA-72662. Performed by ERT, Inc. Ft. Collins, CO.; Submitted by Ciba-Geigy Corp. Greensboro, NC; Accession No. 073085.

5. Reviewed by: Thomas M. Armitage
   Fishery Biologist
   EEB/HED
   Signature: Thomas M. Armitage
   Date: 10-18-85

6. Approved by: Raymond W. Matheny
   Supervisory Biologist
   EEB/HED
   Signature: Raymond W. Matheny
   Date: 10-18-85

7. Conclusions:

The study is scientifically sound and indicates that the test substance can cause reductions in growth of fathead minnows with an MATC of > 14 and < 36 parts per million. The study, however, could not fulfill a guideline requirement for a fish early life stage toxicity study. This is because, on the basis of acute toxicity studies, the fathead minnow is not sensitive to cyromazine.

8. Recommendations:

Test should be repeated using a sensitive fish species (e.g., rainbow trout).

9. Background:

The study, a chronic fish early life stage study using fathead minnows with cyromazine, was submitted in support of registration.

10. Discussion of Individual Test:

N/A

11. Materials and Methods: (definitive test)

a. Test animals - (excerpted from submission)

Fathead minnows (Pimephales promelas) for the range-finding and definitive acute toxicity test were hatched on June 14 and 15, 1983 in ERT's fathead minnow culture tank. The 300-gallon culture tank, containing an undergravel biological filtration system, had been maintained at 25°C. Fertilized fathead minnow eggs (<48 hours old) for the early life stage test were taken from the culture tank on July 27, 1983. The temperature of the culture tank ranged from 23.9°-27.8°C for the period from July 20 to July 27, 1983. The eggs were examined and impartially distributed to each test egg cup. The eggs were not treated.

Test system - (excerpted from submission)

A proportional diluter (Ace Glass design modified from Mount and Brungs 1967) was used to deliver five test concentrations and a control. Stock solutions of CGA-72662 were prepared with distilled water in a 12-gallon glass carboy. The stock solution was stirred continuously and delivered to the diluter with a Cole-Parmer Masterflex Pump.

The diluter was calibrated to deliver 100%, 51%, 27%, and 6% CGA-72662 and dilution water for the controls. These percentages translated into theoretical concentrations of 290, 147, 77, 43, and 17 ppm of CGA-72662. These concentrations were based on the results of the definitive acute toxicity test. Test solution was delivered every 14.9 minutes. The range of flow varied from 230 ml per cycle in the 5-B (100%) test chamber to 200 ml per cycle in the C-A (control) test chamber. This flow provided daily volume exchanges of 6.4 to 7.2, respectively.

For each treatment level and the control, the test solutions were delivered to two replicate test chambers, each containing an egg cup. Each glass test chamber was 19.7 centimeters wide and 40.0 centimeters long with a drain hole centered 4.4 centimeters from the bottom on one end (volume = 3.5 liters). Three or four centimeters in from the drain, a screen of 400 micron mesh nitex was sealed to the glass with silicone cement. 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 driven by a 2 rpm motor having a vertical-travel distance of 2.5 cm. Without agitating the organisms too vigorously

this arrangement insured that the organisms were always submerged and that test solution regularly flowed into and out of the cup. The mesh size on the bottom of the egg cups retained the eggs but allowed some of the newly hatched larvae to escape into the test chamber, while the Nitex screen at the end of the test chamber prevented the larvae from escaping via the drain. After all eggs had hatched in a test chamber the cup was submerged and the larvae allowed to swim out. As water flowed from the diluter into the test chambers, the excess flowed through the Nitex screen and out the drain. All test chambers were exposed to a 16-hour light and 8-hour dark photoperiod.

Temperature of the test containers was regulated by a large water bath held at 25°±1°C. Dissolved oxygen concentration was maintained by compressed air via an oilless air compressor and a 6-inch airstone in each of the two 500-gallon dilution water holding tanks. No aeration was needed in the test chambers. Test chambers were labeled C-A and C-B (Controls), 1-A and 1-B (6%), 2-A and 2-B (15%), 3-A and 3-B (27%), 4-A and 4-B (51%), and 5-A and 5-B (100%).

b. Dosage Form - (excerpted from submission)

Domestic water was used for dilutions and controls in these tests. Fort Collins domestic water supply (34 ppm $CaCO_3$ hardness) was dechlorinated through an inhouse battery of five automatic backflushing charcoal and resin exchange column filters and transported to the experimental systems via polyvinlychloride and Tygon® tubing. For the early life stage test, two 500-gallon Nalgene® tanks wre used to retain the water for "aging." Water in both of these tanks was vigorously aerated.

The test chemical, CGA-72662 (a colorless powder), was 93.4% Cyromazine (N-cyclopropyl-1,3,5-triazine-2,4, 6-triamine). For all preparations, only glass distilled water was used as solvent.

c. Design - (Excerpted from submission)

2.5.2 Exposure and Observations

Fifty healthy, fertile eggs with no visible fungus were transferred to each egg cup impartially. Egg cups were placed in the test chambers randomly.

Twenty-four and 48 hours after being placed in egg cups, eggs were examined visually and all dead or fungal-infected eggs were counted and discarded. The remaining test organisms were reduced to a total of 32 healthy eggs and larvae in each test chamber by removing the appropriate number of eggs. On a daily basis dead fish were noted and removed.

From Day 3 until the end of the test, fish in each chamber were fed live, newly-hatched (<48 hours old) brine shrimp and blended (pureed) brine shrimp daily. The blended brine shrimp provided the smaller larvae with smaller sized food. Sufficient food was provided for the number and size of fish in each test chamber.

Test chambers were cleaned daily by removing debris with a large pipette. Screens and drain holes in each test chamber were kept free of debris by periodic brushing.

Organisms were exposed to test material for a total of 32 days. At the end of the test, survivors were weighed to the nearest milligram on a Mettler #51 analytical balance and measured to the nearest millimeter.

Water Quality Parameters

Test Chamber (measured CGA-72662 concentrations)	Temperature (C°)		Dissolved Oxygen (ppm)	
	Mean	Range	Mean	Range
C-A (Control)	25.1	24.3-25.7	5.8	4.4-7.6
C-B (Control)	25.2	24.4-25.8	5.5	4.3-7.0
1-A (14 ppm)	25.1	24.4-25.8	5.6	4.8-7.3
1-B (14 ppm)	25.1	24.5-25.8	5.5	4.6-6.9
2-A (36 ppm)	25.1	24.4-25.9	5.7	5.0-7.4
2-B (36 ppm)	25.2	24.5-25.9	5.6	4.8-7.0
3-A (73 ppm)	25.2	24.4-25.9	5.8	5.0-7.5
3-B (73 ppm)	25.2	24.4-26.0	5.3	4.7-6.9
4-A (127 ppm)	25.2	24.4-26.0	5.5	4.7-7.4
4-B (127 ppm)	25.2	24.2-26.0	5.5	4.9-6.9
5-A (264 ppm)	25.2	24.3-25.9	5.3	4.2-7.3
5-B (264 ppm)	25.2	24.2-25.8	5.2	4.2-6.9

d. Studies - (Excerpted from submission)

Chi-square analyses of 2 X 2 contingency tables (Zar 1974) were applied to analyze the following mortality parameters:

a. healthy, fertile eggs at 48 hours
b. eggs that produced live fry
c. eggs that produced live, normal fry
d. eggs that produced live fish at the end of the test
e. eggs that produced live, normal fish at the end of the test
f. hatched fry producing live fish at the end of the test
g. hatched fry producing live, normal fish at the end of the test.

Observed mortality and/or abnormality for combined replicates were used as data rather than percentages.

At the end of the test all surviving fish were weighed and measured. For mass and length, two-tailed F-tests were used to determine equality or inequality of replicates. Individual fish masses and length, were used as separate data points for analyses of variance. Each treatment level was then compared to the control using a two-tailed Dunnett's test.

12. Reported Results: (excerpted from submission)

Chi-square analyses of 2 X 2 contingency tables for mortality parameters show the following:

a. Healthy, fertile eggs at 48 hours
Result: no significant difference between the control and CGA-72662 treatment level

b. Eggs that produced live fry
Result: a significant difference between the control and the 73 ppm CGA-72662 treatment level

c. Eggs that produced live, normal fry
Result: a significant difference between the control and the 73 ppm CGA-72662 treatment level

d. Eggs that produced live fish at the end of the test
Result: no significant difference between the control and any CGA-72662 treatment level

e. Eggs that produced live, normal fish at the end of the test
Result: a significant difference between the control and the 127 and 264 ppm CGA-72662 treatment levels

f. Hatched fry producing live fish at the end of the test
Result: no significant difference between the control and any CGA-72662 treatment level

g. Hatched fry producing live, normal fish at the end of the test
Result: a significant difference between the control and the 264 ppm CGA-72662 treatment level

- No significant difference existed between the control and CGA-72662 treatment levels 14 and 36 ppm for any mortality parameter.

The two-tailed F-tests showed a significant difference between 3-A and 3-B (73 ppm CGA-72662) and between 4-A and 4-B (127 ppm CGA-72662).

The one-way analyses of variance provided the mean standard error, an integral factor for the Dunnett's test. Two-tailed Dunnett's tests were used to determine significant differences between the control and each treatment level for length and mass. Fish from the 36, 73, and 264 ppm CGA-72662 treatment levels had lengths significantly (Dunnett's) different that the control fish lengths, while those from the 14 and 127 ppm levels were not significantly different from the control fish lengths . For mass, the 36, 73, 127, and 264 ppm levels were significantly different than the control and the 14 ppm level was not.

13. Study Author's Conclusions/QA Measures:

Effects of CGA-72662 on egg mortality, larval and juvenile fish mortality, length, and mass were evaluated in the early-life-stage study. Based on mortality, the maximum allowable toxicant concentration (MATC) limits were > 36 and < 73 ppm CGA-72662. But length and mass proved to be more sensitive indicators with both parameters having MATC limits of > 14 and < 36 ppm CGA-72662, with a geometric mean of 22 ppm. The fish mortality MATC evaluation is based on the chi-square analysis of 2 X 2 contigency tables of factors B and C. (See 3.2.2 Statistical Analysis). The length and mass (growth) MATC evaluation is based on the Dunnett's test using individual fish as data points.

The following quality assurance measures were noted:

- Daily record keeping
- Standardized data sheets
- Fish handling procedures
- Standardized test conditions
- Replicate analysis
- Water quality monitoring

- Defined calibration
- Standard operating procedures
- Independent QA/QC checks
- Documentation

14. Reviewer's Discussion and Intepretation of Study:

a. Test Procedures: The procedures were in accordance with recommended protocol.

b. Statistical Analysis: EEB analysis confirms the reported results.

c. Discussion/Results: Based upon reductions in length and weight, the MATC of the test material to fathead minnows was > 14 < 36 ppm. This species, however, is not acceptable for use in testing this compound. This is because of the relative insensitivity of fathead minnows to the test material in acute testing.

d. Adequacy of Study:

1. Classification: Supplemental
2. Rationale: Study appears to be scientifically sound, but the species tested was not sensitive to the test material in acute toxicity studies. Therefore, the test will not fulfill a requirement for this study should one be imposed.
3. Repairability: The study cannot be repaired, it should be repeated using a sensitive test species (e.g., rainbow trout).

15. Completion of One-Liner for Study:

One-liner completed October 11, 1985.

16. CBA Appendix:

N/A