MRID No. 416138-01

DATA EVALUATION RECORD

1. **CHEMICAL:** Benefin (or Benfluralin). Shaughnessey No. 084301.

2. **TEST MATERIAL:** Benefin (compound 054521); N-(n-butyl)-N-ethyl-2,6-dinitro-α,α,α-trifluoro-ρ-toluidine; Lot No. 231EF4; 95.88% active ingredient; a yellow-orange powder.

3. **STUDY TYPE:** 72-1(a) Freshwater Fish Static-Renewal Acute Toxicity Test. Species Tested: Bluegill Sunfish (*Lepomis macrochirus*).

4. **CITATION:** Cocke, P.J., and G.R. Koenig. 1990. The Acute Toxicity of Benefin to Bluegill (*Lepomis macrochirus*) in a Static-Renewal Test System. Study No. F00990. Performed by Lilly Research Laboratories, Greenfield, IN. Submitted by DowElanco. EPA MRID No. 416138-01.

5. **REVIEWED BY:**

ALVARO A. YAMHURE
AQUATIC BIOLOGIST, EEB/EFED
USEPA

SIGNATURE: [signature] 2/2/94
DATE:

6. **APPROVED BY:**

DANIEL RIEDER,
HEAD SECTION 3
EEB/EFED

SIGNATURE: [signature]
DATE: 2-2-94

7. **CONCLUSIONS:** This study is scientifically but does not meet the requirements for a static-renewal acute toxicity study using freshwater fish. The measured concentrations substantially decreased during each 24-hour renewal period. Given the low solubility and vapor pressure of Benefin, initial ("New") concentrations probably decreased very rapidly under test conditions so that the **mean** measured concentrations used in calculating the LC_{50} (0.013; 5.3; 6.8; 11.7; and 21.4 mg/l) were too high an estimate of the actual exposures to which the test organisms were subjected for the longest periods of time. The actual concentrations and length of time periods during which the test organisms were exposed are not well established.

The NOEC was calculated to be 0.013 mg/l mean measured concentration but again, in reality, it was probably considerably lower than this value. However, given the reasonable efforts made by the laboratory in dealing with the technical grade of a difficult-to-test compound when in

an aquous solution, the test is accepted as supplementary and the NOEC value obtained (0.013 mg/l) can be used in risk assessment. The LC50 value is too uncertain to be of use. Additional testing with the technical grade material may not render more reliable results so a new test is considered to have low value; however, the registrant is ofcourse free to develop new data. The use of typical end use product for further testing may be an acceptable alternative and may render more useful information.

8. **RECOMMENDATIONS:**

1. Establish the solubility characteristics of Benefin and its rate of loss from aquous solution prior to animal testing and on this basis determine if testing could render meaningful results.

2. If as suspected, technical grade Benefin proves to be hard to test in spite of modifications of the solvent system and/or other established and accepted measures, then use the typical end use product as tests material.

9. **BACKGROUND:**

10. **DISCUSSION OF INDIVIDUAL TESTS:** N/A.

11. **MATERIALS AND METHODS:**

A. **Test Animals:** Juvenile bluegill sunfish (*Lepomis macrochirus*) were obtained from Osage Catfisheries, Osage Beach, Missouri. The fish were maintained in a rectangular tank containing flowing conditioned well water (4.6 l/min) which had the following water quality characteristics: a total hardness of 103 mg/l as $CaCO_3$, a total alkalinity of 120 mg/l as $CaCO_3$, and a conductivity of 220 μS/cm. The mean water temperature in the stock tank during the 14-day pretest period was 21.3 (20.7-21.6°C. The fish were fed a dry commercial pelleted food daily. The photoperiod provided 16 hours of light and 8 hours of darkness.

Mean wet weight and total length of the bluegill in the solvent control were 1.1 (0.78-2.0) g and 46.3 (42-55) mm, respectively. No mortality occurred in the stock tank during the two week period prior to test initiation.

B. **Test System:** The system consisted of 56-l glass aquaria, each containing 40 l of test solution. The

photoperiod was the same as that provided in the culture area.

The dilution water was treated well water. The treatment process consisted of passing the water through birm filters (to remove iron), an electrodialysis unit (to remove approximately 50% of the minerals), and a degasser (to remove excess CO_2) in order to adjust the pH. The water was stored in underground tanks before it was pumped to the laboratory for use. The dilution water temperature was adjusted to approximately 20°C by mixing cold and tempered water. At test initiation, the dilution water had a total hardness of 103 mg/l as $CaCO_3$, an alkalinity of 140 mg/l as $CaCO_3$, and a specific conductivity of 264 µS/cm.

The test stock solution was prepared by diluting 25 g of test material with acetone to a total volume of 250 ml. Additional stocks were also prepared in acetone. The test solutions were prepared daily by mixing 20 ml of the appropriate stock with 40 l of dilution water.

C. **Dosage:** Ninety-six-hour acute static-renewal test. Five nominal concentrations (0.1, 10, 20, 30, and 50 mg/l) were tested. A dilution water control and solvent control (0.5 ml acetone/l) were also included in the test.

D. **Design:** Ten bluegill were randomly distributed to each aquarium. The biomass loading in the solvent control was 0.3 g/l. The test solutions were renewed daily. The fish were netted and transferred to aquaria containing freshly-prepared solution. Fish were not fed during the test.

Observations of mortality and sublethal effects were made every 24 hours after test initiation. Dead fish were removed from the test chambers daily. The dissolved oxygen concentration, temperature, and pH of fresh and 24-hour old test solutions were measured daily in each test chamber. Total alkalinity, total hardness, and conductivity were measured at test initiation in the dilution water control. Total ammonia was measured in the solvent control and the 10 mg/l nominal test concentration at test termination.

Water samples were collected from the freshly prepared test solutions at test initiation and on day 3. Water samples were collected from the 24-hour old test

solutions on day 1 and test termination. The samples were analyzed for benefin using gas chromatography.

E. **Statistics:** The 96-hour median lethal concentration (LC_{50}) was calculated using the Spearman-Karber method. The slope of the concentration-response curve was obtained using linear regression analysis.

12. **REPORTED RESULTS:** Mean measured concentrations were 0.013, 5.3, 6.8, 11.7, and 21.4 mg/l (Table 1, attached). The mean measured concentrations ranged from 13 to 53% of nominal concentrations. "At the end of each 24-hour renewal period, a yellow precipitate was observed on the surfaces of the test solutions."

At mean measured concentrations $\geq$6.8 mg/l, 100% mortality was observed at 24 hours (Table 7, attached). Sublethal effects (labored respiration, prostrate behavior) were observed at the 5.3 mg/l mean measured test concentration. No mortalities or abnormal effects were observed at 0.013 mg/l or in the controls. The 96-hour LC_{50} was 6.0 mg/l. The slope of the concentration-response curve was 45.87. The NOEC was 0.013 mg/l.

During the study, pH ranged from 7.6 to 8.2, the dissolved oxygen concentration ranged from 8.0 to 11.1 mg/l (88% to 122% of saturation at 20°C), and the temperature ranged from 19.9-20.5°C. Un-ionized ammonia levels were not detected (<0.003 mg/l).

13. **STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:** Other than those presented above, the authors made no conclusions.

A Good Laboratory Practice Statement was included in the report indicating that this study was conducted in accordance with U.S. EPA Good Laboratory Practice Regulations (40 CFR Part 160). In addition, several GLP quality assurance inspections were performed throughout the study and the final report was reviewed by the Quality Assurance Unit of the performing laboratory.

14. **REVIEWER'S DISCUSSION AND INTERPRETATION OF STUDY RESULTS**

A. **Test Procedure:** The test procedures were generally in accordance with the SEP except for the following:

The report did not specify the depth of test solution maintained in each test vessel or dimensions of the test aquaria.

The time between solution preparation and fish addition was not specified in this report.

Each nominal concentration was not 60% of the next highest nominal concentration.

The report did not specify whether the photoperiod contained 15- to 30-minute transition periods between light and dark.

The temperature was not monitored continuously (hourly) or every 6 hours for a system controlled by a water bath.

The fish were fed during the 48 hours immediately prior to test initiation. A fasting period just prior to test initiation is recommended.

B. **Statistical Analysis:** The reviewer used EPA's Toxanal computer program to calculate the LC_{50} value and 95% confidence interval and obtained similar results as the authors (see attached printout).

C. **Discussion/Results:** This study is scientifically sound but does not meet the requirements for a static-renewal acute toxicity study using freshwater fish. The measured concentrations substantially decreased during each 24-hour renewal period. Therefore, the actual concentrations to which the test organisms were exposed are unknown and probably considerably lower than those used for calculating toxicity parameters. A flow-through system should have been used for this study since a yellow precipitate was observed on the surfaces of the test solutions at the end of each 24-hour renewal period. The authors state that the test material had a water solubility of 0.1 mg/l and had a high affinity for the glass test vessels. Any further tests should include a conditioning period for the glassware prior to test initiation and rigorous aeration of the dilution water to avoid dissolved oxygen sags (see recommendations in section 8 of this review).

Under the conditions of the test, the 96-hour LC_{50} was 6.0 mg/l mean measured concentration, which classifies Benefin as moderately toxic to bluegill sunfish. The NOEC was 0.013 mg/l mean measured concentration.

D. **Adequacy of the Study:**

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