

US EPA ARCHIVE DOCUMENT

of the fish but it was considered that this would not affect the validity. The fish were fed a medicated fish food for three days (0.3% tetracycline) testing. The fish were in good condition at test initiation. The mortality prior to test initiation was less than 1%.

Mean weight and length of the control fish were 0.39 g (range of 0.17-0.80 (range of 21-35 mm). Biomass loading rate in the control was 0.098 g/L.

- B. **Test System:** Vessels used in the test were 50-liter glass containers filled with water (control) or test solution. The dilution water was tap water that was activated carbon, filtered to remove particulate material and dechlorinated with sodium thiosulfate. The water had a total hardness of 62.6 mg/L as CaCO₃, a conductivity of 153 µS/cm, a chlorine level of less than 4 µg/L, and an initial pH of 7.9. The fish were kept in a temperature controlled room set to maintain 22°±1°C. The fish were exposed to the dilution water and test temperature for 39 days. The illumination was natural sunlight supplemented with artificial lights on a 16-hour light/8-hr dark cycle. Test concentrations were prepared by adding appropriate amounts of test material to the test chambers.

The bluegill sunfish were not fed during the test.

- C. **Dosage:** Ninety-six-hour static test. Seven nominal concentrations (0.10, 18.0, 32.0, 56.0, and 100 mg/L) and a dilution water control were used. Test concentrations made were based on total product.
- D. **Design:** Ten fish were randomly added to each test chamber. All chambers were observed once every 24 hours for mortality and sublethal effects. Sample solutions were taken at 0, 48, and 96 hours for chemical analysis. Dissolved oxygen (DO), pH, and temperature were recorded daily. Water hardness and conductivity were measured at the beginning of the test. Temperature was monitored continuously in the dilution water control.
- E. **Statistics:** The 96-hour median lethal concentration (LC₅₀) and associated confidence interval (C.I.) was calculated using the probit analysis.
12. **REPORTED RESULTS:** The mean measured values ranged from 100 to 115% of nominal values in the test vessels (Table 1, attached) and were fairly consistent between

The mortality responses of the bluegill sunfish are given in Table 2 (attached). The mortality, based on mean measured concentration of total product, was 24 mg/L (95% C.I. = 18-30 mg/L). The no-observed-effect concentration (NOEC), based on the lack of mortality effects (Table 3, attached), was 0.34 mg/L after 96 hours (based on mean measured concentration of total product).

At test initiation, the D.O. was 8.0 to 8.6 mg/L or 91-98% of saturation. After 24 hours, D.O. levels ranged from 70-80% of saturation. The pH values ranged from 7.6 to 7.8 and temperature was 22°±1°C throughout the test. The hardness and conductivity of water were 62.6 mg/L as CaCO₃ and 153 µS/cm, respectively.

13. **STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:**
The authors presented no conclusions.

Quality Assurance and Good Laboratory Practice Regulation Statements were included in the report, indicating that the study was conducted in accordance with

Laboratory Practice Standards set forth in 40 CFR Part 160.

14. REVIEWER'S DISCUSSION 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

Although the report stated that the temperature was monitored and the dilution water control, no raw data were submitted.

A 30-minute dawn and dusk simulation period is recommended in the transition period was not used in the study.

Each selected nominal concentration was between 55% and 57% of the highest concentration for the two sets of continuous nominal values 0.18, 0.32 mg/L and 18, 32, 56, and 100 mg/l). The SEP recommends that concentration be 60% of the next highest concentration. There is a gap of values between these two sets.

No alkalinity measurements were reported.

The report did not state the time period between test solution and pre fish addition.

- B. Statistical Analysis: The reviewer used EPA's Toxanal program to calculate the LC₅₀ value and obtained the same results (see attached printout). The reviewer noted that the authors calculated this value with a Probit analysis cannot be used when the LC value lies between two data points that are 0 and 100% mortality, respectively. Only binomial probability estimate an LC value with the upper and lower test rates as the intervals.

- C. Discussion/Results: Because all mortality occurred in the four highest concentrations, the LC value predicted is valid. However, because a large gap of concentrations exist between 0.32 and 18 mg/L, the NOEC derived herein is probably not the actual NOEC. However, since this NOEC is more conservative than that would probably be obtained if a proper concentration progression was used, it will be taken to be the correct NOEC.

This study is scientifically sound and satisfies the guideline requirements for a static acute toxicity test. The 96-hour LC₅₀ of 24 mg/L (based on measured concentration of total product) classifies Ordram 8E as slightly toxic to bluegill sunfish. The NOEC can be estimated as 0.34 mg/L (based on measured concentration of total product).

- D. Adequacy of the Study:

- (1) Classification: Core.
- (2) Rationale: N/A.
- (3) Repairability: N/A.

15. COMPLETION OF ONE-LINER FOR STUDY: Yes, 6-10-91.