6/13/89

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DATA EVALUATION RECORD

1. Chemical: RE-45601 (Clethodim)

2. Test Material: RE-45601 Technical (83.3% purity specified in protocol).

3. Study Type: Warmwater Fish LC_{50}
   Species Tested: Bluegill Sunfish (Lepomis macrochirus)

4. Study ID: Swigert, J.P. 1986. Acute Toxicity of Chevron RE-45601 Technical to Bluegill Sunfish in a Static Test System. Prepared by Analytical Biochemistry Laboratories, Inc., Columbia, MO (ABC Laboratory Project ID #34967). Study Sponsor: Chevron Environmental Health Center, Inc., Richmond, CA. EPA Accession No. 409745-29.

5. Reviewed By: David Warburton
   Wildlife Biologist
   EEB/EFED
   Signature: David Warburton
   Date: 6/13/89

6. Approved By: Douglas J. Urban
   Supervisory Biologist
   EEB/EFED
   Signature: Douglas J Urban
   Date: 6/13/89

7. Conclusions:

The study is scientifically sound and documents a 96-hour LC_{50} of greater than 33 mg/l for bluegill sunfish exposed to RE-45601 Technical. The study fulfills the Guidelines requirement for acute toxicity testing for a warmwater freshwater fish only for an LC_{50} value of up to 33 mg/l of RE-45601.

8. Recommendations:

If regulatory criteria identify a need for a documented LC_{50} value greater than 33 mg/l in order to complete a hazard assessment, the study will need to be repeated at higher exposure levels to establish an LC_{50} value and corresponding 95% confidence intervals or to document an LC_{50} greater than 100 mg/l, as per Guidelines requirements.

9. Background:

The study was submitted to support, in part, a proposed Experimental Use Permit for Select 2EC herbicide to control annual and perennial grasses in cotton (EPA Record No. 238236).

10. Discussion of Individual Test: N/A.

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11. Materials and Methods (Excerpted in part from submission):

a. Test Animals - Bluegill used in the test were obtained from Osage Catfisheries, Osage Beach, Missouri. All test fish were held in culture tanks on a 16 hour daylight photoperiod and observed for at least 14 days prior to testing. During this period the fish received a standard commercial fish food occasionally supplemented with brine shrimp daily until 48-96 hours prior to testing at which time feeding was discontinued. The bluegill used as the controls for this experiment had a mean weight of 0.53 (±0.11) g and a mean standard length of 27 (±1.6) mm. This gave a test chamber loading biomass of 0.36 g/l for the definitive study.

b. System - The static fish bioassay was conducted in five gallon glass vessels containing 15 liters of soft reconstituted water prepared to yield a total hardness of 40-45 mg/l as $CaCO_3$, a total alkalinity of 30-35 mg/l as $CaCO_3$ and an initial pH of 7.2 to 7.6. Test vessels were kept in a water bath at 20°C (±1.0).

c. Dose - Definitive test concentrations were obtained by transferring appropriate aliquots of a working standard directly to the test chambers. All test concentrations were corrected for sample purity (83.3%). Before addition to the test chambers, 1.5 ml of dimethylformamide (DMF) was added to each sample weight to increase dispersion of the compound in the dilution water. Test solutions were stirred vigorously after compound addition. The solvent control chamber received a 1.5 ml aliquot of DMF, which was equivalent to the highest amount used in any test solution. The fish were added to the test chambers by random assignment within 30 minutes after addition of test material.

A RE-45601 primary standard of 2.06 mg/ml in acetone was prepared; subsequent dilutions were prepared in 2% acetic acid in HPLC-grade acetonitrile for chromatography standards. Analysis of water samples for RE-45601 Technical at 0-, 48-, and 96-hours of testing were accomplished according to a method provided by the study sponsor. Prior to the initiation of the definitive study, a method validation in aged well water was conducted to determine the extractability of RE-45601 from aquatic test water.

d. Design - Based on the results of preliminary testing, five duplicate concentrations of the test compound, ranging in a geometric series from 10 to 100 mg/l, with 10 fish per replicate, 20 per concentration, were selected for the definitive bioassay. Also included were duplicate dilution water controls and solvent control chambers. Randomization was achieved by sequentially adding one fish per test container until all containers had their complement of test organisms. All test organisms were observed at 3 and 6 hours and once every 24 hours thereafter for mortality and abnormal (sub-lethal) effects. Any dead individuals were removed from the test chambers after each 24-hour observation.

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e. Statistics - Concentration vs. effect data (generally mortality) was analyzed by a computerized LC_{50} program developed by Stephan et al. (1978).

12. Reported Results (Excerpted in part from submission):

Results of the 96-hour static toxicity test with bluegill sunfish exposed to RE-45601 Technical are presented in attached Table 2. The 24-, 48-, and 96-hour LC_{50} values for RE-45601 Technical (active ingredient) were all >100 mg/l. On a total product basis, this corresponds to a value of >120 mg/l for the 24-, 48-, and 96-hour LC_{50}. All results were based on the active ingredient (83.3%) nominal concentrations of 10, 18, 32, 56 and 100 mg/l. All solutions had an oily surface film at 0-hour. The 32, 56 and 100 mg/l solutions also had yellow oily drops of precipitate on the surface, which increased with increasing concentration. The 100 mg/l chambers also had yellow oily drops at the bottom of the chambers. The surface film was still present in all levels after 96 hours of testing. The 56 mg/l A chamber had a large drop of yellow precipitate on the surface. The chamber had a large drop of yellow precipitate on the surface. The 100 mg/l solutions had many dark yellow drops at the bottom of the chambers at 96-hours.

The 96-hour no-observed effect concentration was estimated to be 56 mg/l, based on the lack of mortality or observed abnormal (sub-lethal) effects. The abnormal effects of surfacing and/or gulping air were observed in the 100 mg/l B test concentration during the 96-hour exposure period.

Results of the determination of RE-45601 Technical in the static toxicity test are presented in attached Table 3. The measured concentrations yielded an average percent recovery of 88 $\pm$9.0% of nominals for the low test level, 64 $\pm$22% of nominals for the mid level, and 33 $\pm$5.1% of nominals for the high test level. The average concentration of RE-45601 Technical for the low, middle and high test levels measured at 0-, 48- and 96-hours were 8.8, 20 and 33 mg/l, respectively. Fortification samples analyzed with each sample day ranged from 81 to 100%. Recoveries for the fortification levels conducted with well water were 86 to 110% for a range of 1.0 to 100 ppm RE-45601.

Nominal test concentrations, mortality rates, and water quality data are presented in attached Table 6. The dissolved oxygen concentrations ranged from 5.5 to 9.2 mg/l during the test. These values represented 61 and 102% saturation at 21°C, respectively, and were considered adequate for testing. The pH values ranged from 6.9 to 7.6. Conductivity, hardness and alkalinity were measured at 0- and 96-hours and are also reported in Table 6.

13. Study Author's Conclusions/QA Measures:

The 96-hour static fish toxicity LC_{50} was determined to be greater than 100 mg/l on an active ingredient basis for bluegill sunfish exposed to RE-45601 Technical. Also, the results indicated a 96-hour, no-observed effect concentration could be estimated at 56 mg/l nominal concentration.

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.

14. Reviewer's Discussion and Interpretation of Study:

a. Test Procedures - The study was conducted according to Agency recommended procedures.

b. Statistical Analysis - Not required due to mortality pattern.

c. Discussion/Results - Based on the results of the measured concentrations of RE-45601 Technical in the test chambers, exposure of fish at the highest nominal test level (100 mg/l) was actually 33 mg/l, apparently due to technical substance solubility limitations. Section 72-1 (b)(3)(i) of the Pesticide Assessment Guidelines, Subdivision E - Hazard Evaluation: Wildlife and Aquatic Organisms states: "Satisfactory data must establish either: (A) A 96-hour LC_{50} value with 95 percent confidence intervals; or (B) That the 96-hour LC_{50} is greater than 100 mg/l or greater than 100,000 times the MEEC or EEC." Therefore, because results of this study indicate only that the 96-hour LC_{50} value for bluegill exposed to RE-45601 is greater than 33 mg/l, the Guidelines requirement will be considered fulfilled only for toxicity testing up to 33 mg/l exposure. Should a regulatory hazard assessment ever require an LC_{50} value greater than that documented by this study, the study will have to be repeated at higher exposure levels in order to establish an LC_{50} value and corresponding 95% confidence intervals or to document an LC_{50} greater than 100 mg/l. If that becomes necessary, EEB recommends the study be repeated using the formulated (end-use) product to increase solubility, and therefore exposure, of the test substance in water. EEB does not concur with the study author's conclusions of the LC_{50} being greater than 100 mg/l due to maximum actual (measured) exposure of 33 mg/l. This study adequately documents an LC_{50} of greater than 33 mg/l; this value may be used in hazard assessments where appropriate.

d. Adequacy of Study -

1) Classification - Core for RE-45601 technical (Clethodim) up to 33 mg/l exposure.
2) Rationale - Conducted according to Agency protocol; however, dosage levels (as measured in

testing waters) were less than those identified in the Guidelines requirements.

3) Reparability - Study should be repeated as discussed only if the documented LC_{50} value (33 mg/l) is considered inadequate for use in a regulatory hazard assessment.

CLETHODIM

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Pages 6 through 10 are not included in this copy.

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_____ Identity of product inert ingredients.

_____ Identity of product impurities.

_____ Description of the product manufacturing process.

_____ Description of quality control procedures.

_____ Identity of the source of product ingredients.

_____ Sales or other commercial/financial information.

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