6/13/89

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DATA EVALUATION RECORD

1. Chemical: RE-45601 (Clethodim)

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

3. Study Type: Freshwater Invertebrate Acute Toxicity LC_{50}
   Species Tested: Daphnid (Daphnia magna).

4. Study ID: Forbis, A.D. 1986. Acute Toxicity of CHEVRON RE-45601 Technical to Daphnia magna in a Static Test System. Prepared by Analytical Biochemistry Laboratories, Inc., Columbia, MO (ABC Laboratory Project ID #34969).Study Sponsor: Chevron Environmental Health Center, Inc., Richmond, CA. EPA Accession No. 409745-30.

5. Reviewed By: David Warburton
   Wildlife Biologist
   EEB/EFED
   Signature: Dail Walton
   Date: 6/8/89

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

7. Conclusions:

The study is classified as "Invalid" due to the absence of a verifiable LC_{50} value. Also, it cannot be determined from this study what the actual maximum toxicant exposure concentration was due to test substance insolubility. The study therefore does not fulfill the Guidelines requirement for acute toxicity testing of clethodim using a freshwater invertebrate species.

8. Recommendations: The study should be repeated using the formulated end-use product (refer to Section 14 of this review for discussion).

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 (Review Record No. 238236).

10. Discussion of Individual Test: N/A.

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

a. Test Animals - Test specimens of Daphnia magna were obtained from an in-house daphnid culture maintained by ABC since 1977. The adult Daphnia were fed algae (Selenastrum capricornutum) at least every three days prior to testing and supplemented with a

suspension of fish food. First instar daphnids (less than 24 hours old) were selected for the bioassay.

b. System - The static Daphnia bioassay was conducted in 250 ml glass beakers containing 200 ml of ABC aged well water with chemical characteristics listed in attached Table 1. The beakers were kept at 20 (±1.0) °C in a temperature controlled area. The lighting was maintained at 50-70 foot-candles on a 16-hour daylight photoperiod, with 30 minute simulated dawn and dusk periods.

c. Dose - A 2-liter volume of the highest test concentration (100 mg/l) was prepared by injecting an appropriate aliquot of test material into a flask of daphnid culture water. An aliquot of 0.20 ml of acetone was also added to the solution. The test material was observed to be out of solution on the bottom of the vessel. This solution was then agitated for approximately 21 hours using a magnetic stirring apparatus. Test material was still visible on the bottom and surface areas of the test solution. The 6.0, 12, 25 and 50 mg/l nominal test concentrations were then prepared as serial dilutions of the highest test concentration. The solvent control received an aliquot (0.10 ml) of acetone equivalent to that of the highest test concentration.

A RE-45601 Technical 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 at 0- and 48-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 was conducted to determine the extractability of RE-45601 Technical from aquatic test water.

d. Design - An initial range finding experiment was conducted using 10 Daphnia each in exposure concentrations of 0.1, 1.0, and 10 mg/l. A second preliminary study with analytical testing was conducted with concentrations of 2.0, 10 and 20 mg/l tested against control and solvent control chambers. From this information, five concentrations in duplicate of the test compound with 10 randomly selected Daphnia per beaker were selected for the definitive bioassay. These nominal concentrations were a logarithmic series ranging from 6.0 to 100 mg/l (corrected for active ingredient) and included a control and solvent control. All concentrations were observed once every 24 hours for mortality and abnormal effects such as surfacing, clumping of the daphnids together and daphnids lying on the bottom of test chambers.

e. Statistics - The 24- and 48-hour LC_{50} values and corresponding 95-percent confidence limits were determined by an LC_{50} computer program developed by Stephan et al. (1978).

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

The 24- and 48-hour LC_{50} values for RE-45601 Technical (corrected for active ingredient) were both >100 mg/l. The nominal concentrations were 6.0, 12, 25, 50 and 100 mg/l based on RE-45601 active ingredient. The mean measured values of the low, middle and high concentrations were 5.4, 23 and 104 mg/l, respectively (Table 4, attached). These measured concentrations averaged 96 ±8.2% of nominal over the course of the 48-hour study. The no-effect concentration based on the lack of mortality and abnormal effects was 50 mg/l (active ingredient) after 48 hours since abnormal effects of daphnids lying on the bottom of test vessels were observed in the highest test concentration. Due to the close agreement between nominal and measured concentrations, these results accurately indicate the toxicity of RE-45601 Technical. Table 3 (attached) presents the mortality rate and water quality parameters measured during the test. Dissolved oxygen concentrations ranged between 8.0 and 8.8 mg/l. These values represented 87- and 96-percent saturation at 20°C, respectively, and were considered adequate for testing.

13. Study Author's Conclusions/QA Measures:

The 48-hour static Daphnia magna toxicity LC_{50} was determined to be >100 mg/l RE-45601 Technical on an active ingredient basis. The 48-hour no-effect level observed was determined to be 50 mg/l.

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 - Although the study, for the most part, followed recommended procedures, the test substance apparently was not completely in solution during the test. EEB questions how test material can be "visible on the bottom and surface areas of the test solution" immediately following preparation of the highest concentration level (100 mg/l), yet maintain a mean measured concentration of 104 mg/l during the 48-hour test. Such measured values would be possible only if the aliquot to be analyzed was taken during a period of agitation or mixing, when all test material was in suspension; however, this then would not represent actual levels of exposure. The registrant should provide an explanation for this apparent discrepancy. EEB also questions why acetone was used as the solvent for this test when DMF was used in the freshwater fish toxicity tests performed by the same laboratory using the same test compound.

b. Statistical Analysis - N/A.

c. Discussion/Results - Due to the reported solubility problems associated with the technical compound, EEB recommends the study be repeated using the formulated product. Also, chemical analysis of each test level solution (sampled to be representative of that to which the organisms are exposed) is recommended to verify actual exposure of the daphnids to the test substance. Given the data as reported in this study, EEB cannot concur with the study author's conclusions. Since the study has not documented 1) an LC_{50} value with 95 percent confidence limits, or 2) that the LC_{50} value is greater than 100 mg/l, Guidelines requirements for freshwater invertebrate toxicity testing have not been fulfilled.

d. Adequacy of Study -

1) Classification - Invalid for RE-45601 technical.
2) Rationale - Neither a definitive LC_{50} value nor one greater than 100 mg/l was documented as required by the Guidelines.
3) Reparability - The study should be repeated as discussed above.

CLETHODIM

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