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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY  
WASHINGTON, D.C. 20460

FEB 28 1994

OFFICE OF  
PREVENTION, PESTICIDES AND  
TOXIC SUBSTANCES

MEMORANDUM

D198054

**Subject:** The Ecological Effects Branch (EEB) reconsideration of our previous evaluation of MRID No. 426849-50. The DP barcode for this action is D198054 (chemical code 129034 -- Flumioxazin herbicide).

**From:** Anthony F. Maciorowski, Chief  
Ecological Effects Branch  
Environmental Fate and Effects Division  
7507C

**To:** Joanne Miller, PM 23  
(Daniel Kenny)  
Registration Division 7505C

Please find herein attached the Ecological Effects Branch (EEB) review and reevaluation of our previous data evaluation review (DER) for the daphnid test with the following citation:

**CITATION:** Reed, D. and J.P. Swigert. 1992. A 48-Hour Flow-Through Acute Toxicity Test with the Cladoceran (*Daphnia magna*). Project No. 263A-104. Prepared by Wildlife International Ltd., Easton, MD. Submitted by Valent U.S.A. Corporation, Walnut Creek, CA. EPA MRID No. 426849-50.

The registrant, Valent U.S.A. Corporation has requested that we reconsider our previous evaluation of the above cited study. The serious difficulties encountered in testing Flumioxazin because of its low water solubility (the



compound precipitated in the tests containers) and the consequent difficulty in establishing, with acceptable degree of certainty, the EC50 of the test organism and the degree to which the observed adverse effects were due to the mechanical impact of the precipitate as opposed to the aquatic toxicity of the chemical in solution led, us then to rate the test as invalid.

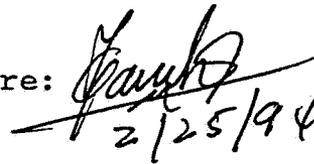
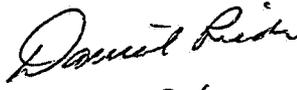
Because EEB is in the process of developing guidance for the purpose of facilitating and to some extent standardizing the handling and testing of compounds with low water solubility; because the registrant did ensure that the contract laboratory was made aware of the potential difficulties in testing this material and made available to said laboratory alternative methods/solvent systems to try to enhance the water solubility and because EPA was asked to and was involved in the problem solving process we find the registrant's request as reasonable and after reexamining the above mentioned daphnid test we have decided to upgrade it to supplemental for the following reasons:

1. because as mentioned above, the registrant did act in a responsible, reasonable and anticipatory manner in handling the unavoidable testing difficulties encountered;
2. because the testing of the material did follow scientifically acceptable techniques and the protocol deviations were either minor or necessary and did not seem to have had significant distorting effects on the results;
3. because repetition of the study would be unlikely to produce significantly better data thus lowering the level of uncertainty as far as the true toxicity to daphnid-like organisms is concerned;
4. and because the material does not appear to have toxicological characteristics that we would consider serious thus presenting probable high risk to test organisms represented by Daphnia.

EPA/OPP does however reserve the right to require additional testing in the future if warranted.

For further information on this action please contact Alvaro Yamhure of the EEB staff at (703) 305-6179.

AMENDED DATA EVALUATION RECORD

1. **CHEMICAL:** V-53482.  
Shaughnessey No. 129034.
2. **TEST MATERIAL:** S-53482; Lot No. PPG-90111-M; 94.7% active ingredient; a white to light brown crystalline solid.
3. **STUDY TYPE:** 72-2. Freshwater Invertebrate Flow-Through Acute Toxicity Test. Species Tested: *Daphnia magna*.
4. **CITATION:** Reed, D. and J.P. Swigert. 1992. A 48-Hour Flow-Through Acute Toxicity Test with the Cladoceran (*Daphnia magna*). Project No. 263A-104. Prepared by Wildlife International Ltd., Easton, MD. Submitted by Valent U.S.A. Corporation, Walnut Creek, CA. EPA MRID No. 426849-50.
5. **REVIEWED BY:**  
  
Alvaro A. Yamhure  
Aquatic Biologist, EEB/EFED  
USEPA  
  
Signature:   
Date: 2/25/94
6. **APPROVED BY:**  
  
Daniel Rieder,  
Head Section 3  
EEB/EFED  
  
Signature:   
Date: 2-25-94
7. **CONCLUSIONS:** This study is scientifically sound but does not meet the guideline requirements for a flow-through acute toxicity test using *Daphnia magna*. The presence of precipitate in the exposure solutions confounded the results of the study. Under the conditions of the test, the 48-hour EC<sub>50</sub> of 5.5 mg a.i./l mean measured concentration of supernatant classifies S-53482 as moderately toxic to *Daphnia magna*. The NOEC could not be determined due to immobility in all treatment levels. This study is rated SUPPLEMENTAL.
8. **RECOMMENDATIONS:** N/A.
9. **BACKGROUND:**
10. **DISCUSSION OF INDIVIDUAL TESTS:** N/A.
11. **MATERIALS AND METHODS:**

old

DATA EVALUATION RECORD

TGAI = 72-2(a)

1. CHEMICAL: V-53482.  
Shaughnessey No. 129034.
2. TEST MATERIAL: S-53482; Lot No. PPG-90111-M; 94.7% active ingredient; a white to light brown crystalline solid.
3. STUDY TYPE: 72-2, (a)-TGAI Freshwater Invertebrate Flow-Through Acute Toxicity Test. Species Tested: *Daphnia magna*.
4. CITATION: Reed, D. and J.P. Swigert. 1992. A 48-Hour Flow-Through Acute Toxicity Test with the Cladoceran (*Daphnia magna*). Project No. 263A-104. Prepared by Wildlife International Ltd., Easton, MD. Submitted by Valent U.S.A. Corporation, Walnut Creek, CA. EPA MRID No. 426849-50.
5. REVIEWED BY:  

Louis M. Rifici, M.S. Associate Scientist KBN Engineering and Applied Sciences, Inc.	Signature: <i>Louis M. Rifici</i> Date: 5/11/93
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6. APPROVED BY:  

Rosemary Graham Mora, M.S. Associate Scientist KBN Engineering and Applied Sciences, Inc.	Signature: <i>Rosemary Graham Mora</i> Date: 11 May 1993
Henry T. Craven, M.S. Supervisor, EEB/EFED USEPA	Signature: <i>Henry T. Craven</i> 7/26/93 Date: 7/29/93
7. CONCLUSIONS: This study is not scientifically sound and does not meet the guideline requirements for a flow-through acute toxicity test using *Daphnia magna*. The presence of precipitate in the exposure solutions confounded the results of the study. Under the conditions of the test, the 48-hour EC<sub>50</sub> of 5.5 mg a.i./l mean measured concentration of supernatant classifies S-53482 as moderately toxic to *Daphnia magna*. The NOEC could not be determined due to immobility in all treatment levels.
8. RECOMMENDATIONS: N/A.
9. BACKGROUND:

- D. **Design:** Daphnids were impartially removed in groups of two from the cultures and equally distributed to each test beaker, two beakers per concentration, for a total of 20 individuals per concentration. Observations of mortality, immobility, and signs of toxicity were made at 18, 24, and 48 hours. The daphnids were not fed during the test.

The dissolved oxygen concentration (DO) and pH were measured in alternating replicates at the beginning of the test and at each 24-hour observation. The temperature of one of the dilution water control chambers was monitored continuously and measured in each replicate vessel at the beginning and end of the test.

Test solution samples were collected from each test chamber at 0, 24, and 48 hours. The concentration of S-53482 in centrifuged and noncentrifuged samples was determined using reverse phase high performance liquid chromatography.

- E. **Statistics:** The authors used a computer program developed by Stephan to calculate the  $EC_{50}$  values (probit analysis).

12. **REPORTED RESULTS:** There were considerable differences between nominal, centrifuged-measured, and noncentrifuged-measured concentrations. "The observed differences related to the low water solubility of the compound (1.79 mg/l in distilled water), the presence of precipitated compound in the test solutions, and the rapid degradation under slightly basic conditions (half life of 21.3-25.9 minutes at pH 9)." The mean measured concentrations for centrifuged and noncentrifuged samples were presented in Table 1 (attached).

At the beginning of the test, the solutions in the mixing chambers were foamy and white with a turbidity which increased with increasing test concentration. A tan precipitate was observed in the mixing and test chambers of the three highest test concentrations. By the end of the test, precipitate was observed in the mixing cells of all treatment groups. The mixing chamber of the highest test level became clogged with precipitate two hours before the completion of the test. The solution in the chamber overflowed into the test chambers. The authors noted that the analytical results indicated no contamination had occurred as a result of the spill.

"When the combined effects of mortality and immobility are viewed with respect to the analytical data, the toxicological effects match the noncentrifuged sample measurements in a dose-response pattern (Table 4, attached)." Using the centrifuged sample analytical data, the immobility/mortality data did not follow a typical dose-response pattern. The authors suggested that the presence of precipitate in the test solutions was mechanically (a physical interaction between the precipitate and the daphnids) detrimental to the daphnids. The no-observed-effect concentration (NOEC) could not be determined due to immobility at all exposure levels.

During the test, the DO ranged from 7.8 to 8.2 mg/l (>60% of saturation). The pH values ranged from 8.1 to 8.4. The temperature was 20.1-20.5°C throughout the test.

**13. STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:**

The 48-hour EC<sub>50</sub> value was 5.9 mg a.i./l (95% C.I. = 5.4-6.5 mg a.i./l) using the centrifuged mean measured concentrations and 17 mg a.i./l (95% C.I. = 14-22 mg a.i./l) using the noncentrifuged mean measured concentrations. The soluble part of S-53482 in water did not appear to be toxic since the observed dose-response did not match the measurements of S-53482 in centrifuged water samples. Because 10% of the daphnids in the solvent control and two lowest test concentrations were affected during the test, the NOEC was the noncentrifuged concentration of 8.54 mg/l. Quality Assurance and Good Laboratory Practice statements were included in the report, indicating that the study was conducted in accordance with USEPA Good Laboratory Practice Standards set forth in 40 CFR Part 160. The dates and types of quality assurance audits performed were also presented.

**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 concentration of solvent mixture in the solvent control and exposure levels was 4 ml/l. The SEP recommends no more than 0.1 ml/l in flow through tests. Hydrogenated castor oil is not a recommended solvent but is acceptable in this study given the unusually low toxicity of the compound and that its presence in the solvent control does not suggest it to be toxic to the test organism.

It was stated in the report that alkalinity, hardness, and conductivity were measured in dilution water

control at beginning of test. The results were not provided.

The number of volume replacements was 22 per day. The SEP states that the number of volume replacements should not exceed 10 per day, but again, it is recognized that the registrant was attempting to increase the aquatic concentration of the toxicant, so in this particular case this deviation is considered to be reasonable.

First instar *Daphnia magna* used in tests should be from the fourth or later broods of a given parent. The authors did not indicate which brood was the source of the test animals.

- B. **Statistical Analysis:** The centrifuged mean measured concentration data and corresponding immobility data were ordered by increasing concentration prior to analysis. The reviewer used EPA's Toxanal computer program to determine the 48-hour EC<sub>50</sub> value 5.5 mg a.i./l (95% C.I. = 5.1-6.1 mg a.i./l; see attached printout).
- C. **Discussion/Results:** The solubility of the test material was apparently exceeded in the exposure solutions and the amount of precipitate became a factor in the behavior of the test organisms. The authors stated "The amount of precipitate sometimes made the observations of the daphnids difficult to complete." The presence of the precipitate in the test solution confounded the results of the study. Therefore, this study is not scientifically sound. The 48-hour EC<sub>50</sub> of 5.5 mg a.i./l mean measured concentration of supernatant classifies S-53482 as moderately toxic to *Daphnia magna*. The NOEC could not be determined due to immobility in all treatment levels.
- D. **Adequacy of the Study:**
- (1) **Classification:** Supplemental.
  - (2) **Rationale:** The presence of the precipitate in the test solution confounded the results of the study.
  - (3) **Repairability:** No.
15. **COMPLETION OF ONE-LINER FOR STUDY:** Yes, 04-29-93.