

US EPA ARCHIVE DOCUMENT

12-2-93

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MRID No. 427131-02

**DATA EVALUATION RECORD**

- 1. **CHEMICAL:** Acetochlor.  
Shaughnessey No. 121601.
- 2. **TEST MATERIAL:** Acetochlor; Batch/Lot/NBR No. QUE-9001-1482-T; 92.07% active ingredient; a brown liquid.
- 3. **STUDY TYPE:** 72-3. Estuarine Fish Flow-Through Acute Toxicity Test. Species Tested: Sheepshead Minnow (*Cyprinodon variegatus*).
- 4. **CITATION:** Swigert, J.P. 1992. Acetochlor: A 96-Hour Flow-Through Acute Toxicity Test with the Sheepshead Minnow (*Cyprinodon variegatus*). Project No. 139A-134. Prepared by Wildlife International Ltd., Easton, MD. Submitted by Acetochlor Registration Partnership. EPA MRID No. 427131-02.

5. **REVIEWED BY:**

Louis M. Rifici, M.S.  
Associate Scientist  
KBN Engineering and  
Applied Sciences, Inc.

Signature: *Louis M Rifici*  
Date: *5/21/93*

6. **APPROVED BY:**

Rosemary Graham Mora, M.S.  
Associate Scientist  
KBN Engineering and  
Applied Sciences, Inc.

Signature: *Rosemary Graham Mora*  
Date: *5/21/93*

Henry T. Craven, M.S.  
Supervisor, EEB/EFED  
USEPA

Signature: *William S. Robert 10/19/93*  
Date: *H.T. Craven 12/2/93*

7. **CONCLUSIONS:** This study is scientifically sound and meets the guideline requirements for an estuarine fish acute toxicity test using sheepshead minnows. The 96-hour LC<sub>50</sub> was 2.10 mg a.i./l mean measured concentration which classifies acetochlor as moderately toxic to sheepshead minnows. The NOEC was 0.93 mg a.i./l.

8. **RECOMMENDATIONS:** N/A.

9. **BACKGROUND:**

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10. DISCUSSION OF INDIVIDUAL TESTS: N/A.

11. MATERIALS AND METHODS:

- A. Test Animals: Juvenile sheepshead minnows (*Cyprinodon variegatus*) were obtained from in-house cultures. The fish were fed a commercially-available flaked food except for the final 48 hours before the test. During the 14-day period prior to test initiation, the temperature and salinity in the holding unit were 19.4-23.0°C and 23-25 parts per thousand (ppt), respectively. The fish were acclimated to the test conditions for approximately 48 hours before the test. During holding and acclimation, the fish showed no signs of disease or stress.

All fish used in the test were from the same year class. The average length of 10 control fish at the end of the test was 24 mm (22-27 mm) with an average weight of 0.49 g (0.34-0.71 g).

- B. Test System: A continuous-flow diluter system was used to prepare and deliver the test solutions. The test chambers were Teflon®-lined, 25-l polyethylene aquaria filled with 15 l of test solution. The solution depth was approximately 17 cm. Approximately 6 volume additions were delivered to the chambers every 24 hours. The diluter was preconditioned with the test material for approximately 66 hours prior to testing.

The aquaria were indiscriminately positioned in a temperature-controlled water bath (22 ±1°C) under a 16-hour light photoperiod with 30-minute dawn and dusk simulations. Light intensity at the test solution surface was approximately 431 lux.

Natural seawater, collected at Indian River Inlet, DE, was diluted with well water, aerated, and filtered before use as test dilution water. The salinity of the dilution water was 24-25 ppt and the pH was 7.9-8.4 during the 4-week period immediately preceding the test.

One stock solution was prepared for each of the five concentrations. The primary stock (80.0 mg/ml) was prepared by dissolving the test material in dimethylformamide (DMF). Aliquots of this stock were diluted with DMF to prepare four additional stocks. The stocks were injected into the diluter mixing chambers.

C. **Dosage:** Ninety-six-hour, flow-through test. Based on preliminary testing, five nominal concentrations (1.04, 1.73, 2.88, 4.8, and 8.0 mg/l), a solvent control, and a dilution water control were tested. The nominal test concentrations were mg/l of whole material (i.e., not adjusted for the percentage active ingredient). The concentration of solvent in the solvent control and treatments was 0.1 ml/l.

D. **Design:** Sheepshead minnows were impartially removed from holding tanks in groups of two and distributed to the test chambers until each contained 10 fish. Two replicates were used for the control and exposure levels. Loading during the test was 0.05 g/l/day or 0.33 g/l at any given time.

Observations of mortality and treatment-related effects were made at 3, 24, 48, 72, and 96 hours. The dissolved oxygen concentration (DO) and pH were measured in alternating replicates of each test level at the beginning of the test and at each 24-hour observation. The temperature of one of the control chambers was monitored continuously and measured in each replicate vessel at the beginning and end of the test. The salinity of the dilution water control was measured at test initiation.

Test solution samples were collected from each test chamber at 0, 48, and 96 hours. The samples were analyzed for acetochlor using gas chromatography.

E. **Statistics:** The median lethal concentration ( $LC_{50}$ ) values were calculated, if necessary, using a computer program developed by C.E. Stephan.

12. **REPORTED RESULTS:** The mean measured concentrations were 1.1, 2.0, 3.3, 5.5, and 9.3 mg/l (Table 1, attached). Measured concentrations of active ingredient were also provided.

No treatment-related mortality or sublethal effects were noted in the dilution water control, solvent control, and 1.1 mg/l test level during the study. One incidental mortality occurred in the solvent control on day 2. Mortalities and sublethal effects observed in the remaining levels were presented in Table 3 (attached).

During the test, the DO ranged from 5.3 to 6.9 mg/l (>60% of saturation). The pH values ranged from 8.1 to 8.3 and the

temperature was 21.2-22.5°C. The salinity of the dilution water control at test initiation was 25 ppt.

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

The 96-hour LC<sub>50</sub> value for sheepshead minnows was 2.4 mg/l with a 95% confidence interval of 2.0-3.3 mg/l. The no mortality concentration and no-observed-effect concentration (NOEC) were 1.1 mg/l.

Quality Assurance and Good Laboratory Practice Statements were included in the report, indicating that the study was conducted in accordance with U.S. EPA Good Laboratory Practice Standards set forth in 40 CFR Part 160. The dates and types of quality assurance audits were reported. Characterization of the test material was the responsibility of the sponsor.

**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 salinity of the dilution water in the study was 25 ppt with a pH of 8.1-8.3. The recommended salinity and pH for sheepshead minnows are 10-17 ppt and 7.7-8.0.

Acclimation of the sheepshead minnows to the test conditions (48 hours) was much shorter than recommended (2 weeks).

**B. Statistical Analysis:** The reviewer used mean measured concentrations of active ingredient (Table 1, attached) and EPA's Toxanal computer program to determine the 96-hour LC<sub>50</sub> as 2.10 mg a.i./l with a 95% C.I. of 1.71-2.9 mg a.i./l (see attached printout). The NOEC was 0.93 mg a.i./l

**C. Discussion/Results:** This study is scientifically sound and meets the guideline requirements for an estuarine fish acute toxicity test using sheepshead minnows. The 96-hour LC<sub>50</sub> was 2.10 mg a.i./l mean measured concentration which classifies acetochlor as moderately toxic to sheepshead minnows. The NOEC was 0.93 mg a.i./l.

**D. Adequacy of the Study:**

(1) **Classification:** Core.

(2) **Rationale:** N/A.

(3) Repairability: N/A.

15. COMPLETION OF ONE-LINER FOR STUDY: Yes, 05-14-93.

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ACETOCHLOR

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Pages 6 through 7 are not included.

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The material not included contains the following type of information:

- 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.
  - A draft product label.
  - The product confidential statement of formula.
  - Information about a pending registration action.
  - FIFRA registration data.
  - The document is a duplicate of page(s)         .
  - The document is not responsive to the request.
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The information not included is generally considered confidential by product registrants. If you have any questions, please contact the individual who prepared the response to your request.

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RIFICI ACETOCHLOR SHEEPSHEAD MINNOW 05-14-93

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CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
8.11	20	20	100	9.536742E-05
4.77	20	20	100	9.536742E-05
2.9	20	20	100	9.536742E-05
1.71	20	2	10	2.012253E-02
.93	20	0	0	9.536742E-05

THE BINOMIAL TEST SHOWS THAT 1.71 AND 2.9 CAN BE USED AS STATISTICALLY SOUND CONSERVATIVE 95 PERCENT CONFIDENCE LIMITS, BECAUSE THE ACTUAL CONFIDENCE LEVEL ASSOCIATED WITH THESE LIMITS IS GREATER THAN 95 PERCENT.

AN APPROXIMATE LC50 FOR THIS SET OF DATA IS 2.10296

WHEN THERE ARE LESS THAN TWO CONCENTRATIONS AT WHICH THE PERCENT DEAD IS BETWEEN 0 AND 100, NEITHER THE MOVING AVERAGE NOR THE PROBIT METHOD CAN GIVE ANY STATISTICALLY SOUND RESULTS.

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