

DATA EVALUATION RECORD

1. **CHEMICAL:** Prometon.
Shaughnessey No. 80804.
2. **TEST MATERIAL:** Prometon Technical (2,4-bis (isopropylamino)-6-methoxy-S-triazine); Batch Code 73152-ML-5664; ID # FL-872050, ARS-8114; 98.5% active ingredient; a white powder.
3. **STUDY TYPE:** Estuarine Fish Static Acute Toxicity Test.
Species Tested: Sheepshead Minnow (Cyprinodon variegatus).
4. **CITATION:** Murphy, D. 1990. Amendment to a 96-Hour Static Acute Toxicity Test with the Sheepshead Minnow (Cyprinodon variegatus). Project No. 108A-104. Prepared by Wildlife International Ltd., Easton, MD. Submitted by Agricultural Division, Ciba-Geigy Corporation, Greensboro, NC. EPA MRID No. 417253-01.

5. **REVIEWED BY:**

Louis M. Rifici, M.S.
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Signature: *Louis M Rifici*
Date: 5/28/91

6. **APPROVED BY:**

Pim Kosalwat, Ph.D.
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Signature: *P. Kosalwat*
Date: 5/28/91

Henry T. Craven, M.S.
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Cynthia Moulton 12.3.91
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Date: 12/4/91

7. **CONCLUSIONS:** This study is scientifically sound and meets the guideline requirements for a static acute saltwater fish toxicity study. The 96-hour LC₅₀ of Prometon for sheepshead minnows was 47.3 mg/L (based on initial measured concentrations). Therefore, Prometon is classified as slightly toxic to sheepshead minnows. The NOEC was determined as 3.99 mg/L based on the lack of mortality and sublethal effects.
8. **RECOMMENDATIONS:** N/A

9. BACKGROUND:**10. DISCUSSION OF INDIVIDUAL TESTS: N/A****11. MATERIALS AND METHODS:**

A. Test Animals: Juvenile sheepshead minnows (Cyprinodon variegatus) were obtained from a commercial supplier in Fort Collins, CO, and held in the laboratory for 26 days prior to testing. Water temperature during holding ranged from 18.5° to 22.0°C and changes in water temperature did not exceed 3°C in any 72-hour period. The salinity of the holding water was 23.0-26.0 parts per thousand (ppt) and the pH ranged from 7.6 to 7.9. All fish used in the test were from the same year class and the total length of the longest fish was no more than twice that of the shortest fish. The average length of 10 control organisms was 26.5 mm (20-30 mm) with an average weight of 0.77 g (0.48-1.1 g). Frozen brine shrimp and flake food were used as food during holding. The fish were acclimated to the test conditions for approximately 50 hours before the test. Feeding was discontinued 48 hours prior to testing. No mortality occurred in the population during the 48-hour period prior to test initiation.

B. Test System: The test chambers were Teflon-lined 25-L polyethylene aquaria filled with 16 L of test solution. The test solution depth was approximately 17 cm. The test chambers were positioned in a temperature-controlled water bath set to 22°±1°C. The laboratory environment was maintained on a 16-hour daylight photoperiod with 30-minute dawn and dusk simulations. The light intensity during the test was approximately 40 ft-candles. Natural seawater, collected at Indian River Inlet, DE, was aerated and filtered (0.2 µm) before use as test dilution water. The salinity of the dilution water was 25.0 ppt and the pH was 8.2 when measured at the beginning of the test.

A stock solution was prepared by adding 18.3043 g of Prometon to 75 mL of triethylene glycol in a 100-mL volumetric flask. The mixture was stirred for 19 hours, brought to volume with solvent, stirred for an additional 2 hours and sonicated for 15 minutes. An appropriate amount of the stock solution was added to the test chambers (already containing 15 L of dilution water) and the solution volume brought to a final volume of 16 L. The resulting solutions were gently

mixed with a Teflon-coated stirring rod. No precipitates were observed in the solutions.

- C. **Dosage:** Ninety-six-hour static test. Five nominal concentrations (4.7, 9.4, 18.8, 37.5, and 75.0 mg/L), a dilution water control, and a solvent control (0.4 mL triethylene glycol/L) were used. The concentrations made were based on the percent active ingredient in the test material.
- D. **Design:** Sheepshead minnows were impartially removed in groups of two from holding tanks and distributed to the test chambers until each contained 10 fish. The instantaneous loading was 0.52 g/L.

Observations of mortality and sublethal responses (including stress and unusual behavior) were made every 24 hours. The dissolved oxygen (D.O.) and pH were measured in each replicate of all test levels 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.

Prometon concentrations were measured by gas chromatography from samples taken at test initiation.

- E. **Statistics:** The median lethal concentration (LC_{50}) and associated 95% confidence interval (C.I.) for each 24-hour interval were calculated using a computer program developed by Stephan et al. (1978).
12. **REPORTED RESULTS:** The test concentrations, measured from the initial test solutions, were 3.99, 8.12, 16.6, 37.5, and 72.6 mg/L. These values represent 85 to 100% of nominal concentrations (Table 1, attached).

The responses of sheepshead minnows are given in Table 3 (attached). The 96-hour LC_{50} , based on measured concentrations, was 47.3 mg/L (95% C.I. = 37.5-72.6 mg/L). Lethal effects were observed at 37.5 and 72.6 mg/L. The no-observed-effect concentration (NOEC), based on the lack of sublethal effects, was given as 3.99 mg/L.

Dissolved oxygen ranged from 5.4 to 7.8 mg/L. The pH values ranged from 7.6 to 8.2. The temperature, measured at test initiation and termination, was recorded as 21.4°-21.7°C, however, the results of continuous monitoring established the temperature range as 21.9° to 22.0°C.

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

The author categorized Prometon as slightly toxic to sheepshead minnows and the NOEC as 3.99 mg/L.

Quality Assurance and Good Laboratory Practice Statements were included in the report, indicating that the study was conducted in accordance with FIFRA Good 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 or Subdivision E guidelines as follows:

The test vessels were Teflon-lined polyethylene aquaria. Stainless steel or glass test vessels are recommended. In this case, the Teflon liner is probably a better substitute.

The concentration of each solution was approximately 50% of the next highest concentration. The SEP recommends that each concentration be 60% of the next highest concentration.

The salinity of the dilution water in the study was 25.0 ppt with a pH of 8.2. The recommended salinity and pH for sheepshead minnows is 10-17 ppt and 7.7-8.0 or 30-34 ppt and 8.0-8.3.

The results of preliminary studies, if any, were not given in the report.

The period between test solution preparation and the initiation of the test was not stated in the report. Tests should be initiated within 30 minutes of test solution preparation.

- B. **Statistical Analysis:** The reviewer used EPA's Toxanal program to calculate the LC₅₀ value and obtained the same results (see attached printout).
- C. **Discussion/Results:** This study is scientifically sound and meets the guideline requirements for a static acute saltwater fish toxicity study. The 96-hour LC₅₀ of 47.3 mg/L (based on initial measured concentrations) classifies Prometon as slightly toxic to sheepshead

minnows. The NOEC was determined as 3.99 mg/L based on the lack of mortality and sublethal effects.

D. Adequacy of the Study:

(1) **Classification:** Core

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

(3) **Repairability:** N/A

15. COMPLETION OF ONE-LINER FOR STUDY: Yes, 04-26-91.

RIN-0334-94 PROMETON REVIEWS (088804)

Page is not included in this copy.

Pages 6 through 8 are not included.

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.

LOUIS M. RIFICI PROMETON CYPRINODON VARIEGATUS 4-24-91

CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
72.6	20	20	100	9.536742E-05
37.5	20	3	15	.1288414
16.6	20	0	0	9.536742E-05
8.12	20	0	0	9.536742E-05
3.99	20	0	0	9.536742E-05

THE BINOMIAL TEST SHOWS THAT 37.5 AND 72.6 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 47.29605

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.

Study/Species/Lab/ MRID # _____ Chemical % a.i. _____ Results _____ Reviewer/ Date _____ Validation Status _____

48-Hour EC50 98.5 EC50 - 47.3 \times 95% C.L. Binomial Control Mortality (%) - 0
 ppm (37.5-72.6) Solvent Control Mortality (%) - 0
 Slope - N/A # Animals/Level - 20 Temperature - 22°C

Species: Cyprinodon variegatus
 Lab: Wildlife International Ltd.

LR Cre
 4/26/91

MRID # 417253-01 48-Hour Dose Level ppm / (% Effect) 3.99 (0), 8.12 (0), 16.6 (0), 31.5 (15), 72.6 (00)

Comments: * Initial measured concentration

96-Hour LC50 _____ LC50 - _____ 95% C.L. _____ Control Mortality (%) - _____
 Slope - _____ # Animals/Level - _____ Solvent Control Mortality (%) - _____
 Temperature - _____

Species: _____ 96-Hour Dose Level pp / (% Mortality) _____
 Lab: _____ (), (), (), (), ()
 MRID # _____

Comments: