DATA EVALUATION RECORD

1. **CHEMICAL:** Prometon  
   Shaughnessey No. 80804

2. **TEST MATERIAL:** Prometon Technical; Batch Code 73152-ML-5664; ID # FL 872050 ARS 8114; 98.5% active ingredient; a white powder.

3. **STUDY TYPE:** Freshwater Invertebrate Static Acute Toxicity Test. Species Tested: *Daphnia magna*


5. **REVIEWED BY:**  
   Louis M. Rifici, M.S.  
   Associate Scientist II  
   KBN Engineering and Applied Sciences, Inc.  
   Signature: Louis M. Rifici  
   Date: 4/16/91

6. **APPROVED BY:**  
   Pim Kosalwat, Ph.D.  
   Senior Scientist  
   KBN Engineering and Applied Sciences, Inc.  
   Signature: P. Kosalwat  
   Date: 4/17/91

   Henry T. Craven, M.S.  
   Supervisor, EEB/HED  
   USEPA  
   Signature: Henry T. Craven  
   Date: 4/23/91

7. **CONCLUSIONS:** This study is scientifically sound and meets the guideline requirements for an acute, static toxicity test for freshwater invertebrates. Based on measured concentrations, the 48-hour EC50 of Prometon for *Daphnia magna* was 25.7 mg a.i./L. Therefore, Prometon is classified as slightly toxic to *Daphnia magna*. The NOEC could not be determined from the data.

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

10. **DISCUSSION OF INDIVIDUAL TESTS:** N/A

11. **MATERIALS AND METHODS:**

   A. **Test Animals:** The daphnids (*Daphnia magna*) used in the test were obtained from in-house cultures. The adult daphnids were fed a mixture of yeast, Cerophyll®, and trout chow. Daphnids were cultured under the test conditions listed in Section 11.B. The daphnid cultures were judged as in good health and showed no signs of disease or stress. Neonates were obtained for testing by transferring individual adult daphnids to dilution water 24 hours prior to test initiation. Less than 24-hour old, first instar larvae from at least three different adults were chosen for the test.

   B. **Test System:** The test chambers were 250-mL glass beakers containing 150 mL of test solution. The test solution depth was approximately 5 cm. The test chambers were positioned in a temperature-controlled room designed to maintain 20°C ±1°C. The laboratory environment was maintained on a 16-hour daylight photoperiod with 30-minute dawn and dusk simulations. Medium-hard well water with the characteristics listed in Table 1 (attached) was aerated and filtered (0.2 μm) before use as dilution water.

   A stock solution was prepared by adding 18.2963 g of Prometon into a 100-mL volumetric flask partially filled with triethylene glycol. The mixture was stirred for 1 hour, diluted further with solvent, and stirred for 16 hours. This solution was brought to final volume and stirred an additional four hours followed by sonication for 10 minutes to obtain a clear solution. The test solutions were prepared in a graduated cylinder using appropriate amounts of the stock solution and dilution water. The final solutions were reported as clear and free of precipitates.

   C. **Dosage:** Forty-eight-hour static test. Six nominal concentrations (7.8, 13.0, 21.6, 36.0, 60.0, and 100.0 mg/L), a dilution water control and a solvent control (0.56 mL triethylene glycol/L) were used. The concentrations made were based on the percent active ingredient in the test material.
D. **Design:** Daphnids were impartially distributed to each test beaker, two beakers per concentration, for a total of 20 individuals per concentration. Observations of mortality and immobility were made every 24 hours. The daphnids were not fed during the test.

The dissolved oxygen (D.O.) and pH were measured in all concentrations and the controls at the beginning and end of the test. The temperature of the environmental chamber was monitored continuously and each replicate of the test concentrations and controls were measured 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 effective concentration (EC₅₀) 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 measured concentrations were 5.1, 11.1, 17.3, 35.2, 45.0, and 89.0 mg a.i./L. These values represent 65 to 98% of nominal concentrations (Table 1A, attached).

The responses of *Daphnia magna* are given in Table 3 (attached). Ten percent mortality occurred in the negative control in 48 hours. Mortality occurred in all exposure concentration except 17.3 mg/L. The 48-hour EC₅₀ based on measured concentrations was 25.7 mg/L (95% C.I. = 20.6–32.0 mg/L). The slope of the concentration-response curve was 3.2. The no-observed-effect concentration (NOEC) was given as 5.1 mg/L.

Dissolved oxygen ranged from 8.0 to 9.0 mg/L. The pH values ranged from 8.2 to 8.6. The temperature was 18.5°–20.2°C throughout the test.

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

The author concluded that 17.3 mg/L was the zero mortality level even though 10% mortality had occurred at 11.1 mg/L and 5% mortality had occurred at 5.1 mg/L.

Quality Assurance and Good Laboratory Practice Regulation 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 as follows:

The solvent control and one or more test solutions contained 0.56 mL/L triethylene glycol. The recommended maximum solvent concentration is 0.5 mL solvent/L of test solution. This solvent concentration did not affect the pH or D.O. of the test solutions.

The recommended temperature for daphnid tests is 20°C. The temperature during the test was as low as 18.5°C.

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 solution preparation.

B. **Statistical Analysis**: The reviewer used EPA's Toxanal program to calculate the EC₅₀ value (see attached printout). The total number of dead and immobile individuals for each concentration were summed to give the total number of affected daphnids for each test level. The effects in the exposure concentrations were compared to the solvent control.

The 48-hour EC₅₀ was 25.7 mg a.i./L (95% C.I. = 20.7-32.0) and the slope of the probit line was 3.18.

C. **Discussion/Results**: According to the SEP, no more than 10% mortality in the control of a static test is acceptable. In this study, 10% of the daphnids died in the negative control. The concentration of solvent in the control and one or more of the test concentrations exceeded the recommended solvent concentration given in the SEP. However, this does not explain the mortality in the negative control unless the two control solutions were mistaken placed in the wrong containers at test initiation. In any event, the results from the solvent control are sufficient for use as controls in this test.
The water solubility of the test material was given in the report as 750 mg/L at 20°C. Considering the lengthy procedure used to get the material into solution in triethylene glycol and the potential for complications resulting from the use of solvents in toxicity tests, the use of solvent in this test is questionable. The study report does not give any reasons why it was necessary to prepare the stock solution in triethylene glycol. Concentrated stock solutions (less than 750 mg/L) could have been made in test dilution water and thus removed solvent effects from the responses observed in the test.

This study is scientifically sound and meets the guideline requirements for a static acute freshwater invertebrate toxicity study. The 48-hour EC₅₀ of 25.7 mg a.i./L (based on measured concentrations) classifies Prometon as slightly toxic to Daphnia magna. The NOEC cannot be estimated from this data.

D. Adequacy of the Study:

(1) Classification: Core

(2) Rationale: N/A

(3) Repairability: N/A

15. COMPLETION OF ONE-LINER FOR STUDY: Yes, 04-16-91.
Page____ is not included in this copy.
Pages ____ through ____ are not included.

The material not included contains the following type of information:

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THE BINOMIAL TEST SHOWS THAT 17.3 AND 45 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 35.67405

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD
SPAN  G  LC50  95 PERCENT CONFIDENCE LIMITS
4    8.391844E-02  26.09944  20.99142  34.54114

RESULTS CALCULATED USING THE PROBIT METHOD
ITERATIONS  G  H  GOODNESS OF FIT PROBABILITY
5    9.036554E-02  1  6.192172E-02
SLOPE = 3.179865
95 PERCENT CONFIDENCE LIMITS = 2.22397 AND 4.135759

LC50 = 25.74321
95 PERCENT CONFIDENCE LIMITS = 20.66593 AND 31.99318

LC10 = 10.26301
95 PERCENT CONFIDENCE LIMITS = 6.414564 AND 13.64636

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Study/Species/Lab/Chemical Name: Prometon

Chemical Class:  

Results:

48-Hour EC$_{50}$

98.5

Species: Daphnia magna

Slope = 3.78  # Animals/Level = 20

Temperature = 20°C

Lab: Wildlife International LTD.

MRID #: 416091-09

Comments: * Measured concentrations

96-Hour LC$_{50}$

95% C.L.

LC$_{50}$ = pp M CONTROL MORTALITY (%) =

Solvent Control Mortality (%) =

Species:  

Slope =  

# Animals/Level =

Temperature =

Lab:  

96-Hour Dose Level pp M (% MORTALITY) =

MRID #:  

Comments: