**DATA EVALUATION RECORD**

1. **CHEMICAL:** Prometon Technical; Shaughnessey # 080804

2. **TEST MATERIAL:** Prometon Technical; ID No.: FL-872050, ARS-12654, Batch Code: 73152-ML-5664; white powder with a reported purity of 97.7%.

3. **STUDY TYPE:** 96-Hour Acute Flow Through Mollusc Shell Deposition.
Species Tested: Eastern Oyster (Crassostrea virginica).

4. **CITATION:** Ward, Timothy, J. 1991. "Acute Flow Through Mollusc Shell Deposition Test with Prometon". Study performed by Envirosystems Division Resource Analysts, Incorporated. One Lafayette Road, Hampton, New Hampshire 03842. Submitted by Ciba-Geigy Corporation, P.O. Box 18300, Greensboro, N.C. 27419. MRID No. 418109-01.

5. **REVIEWED BY:**

Dana Lateulere, Biologist
Ecological Effects Branch
Environmental Fate
and Effects Division

**Signature:** Dana Lateulere
**Date:** 9/10/91

6. **APPROVED BY:**

Ann Stavola, Section Head, 5
Ecological Effects Branch
Environmental Fate and
Effects Division

**Signature:** Ann Stavola
**Date:** 9/13/91

7. **CONCLUSIONS:** This study fulfills the requirements for an Acute Toxicity Test for Estuarine and Marine Organisms (Mollusc 96-Hour Flow-Through Shell Deposition Study). The 96 hour $EC_{50}$ is 27.50 mg a.i./L as determined using the Probit method with mean measured concentrations. The NOEC was determined using ANOVA and Dunnett's test to be 8.61 mg a.i./L. Prometon is categorized as slightly toxic to Eastern Oysters (S.E.P.; EPA-540/9-85011).

8. **RECOMMENDATIONS:** N/A

9. **BACKGROUND:**

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

11. **MATERIALS AND METHODS:**

A. **Test Organisms:** Juvenile oysters from Aquatic Research Organisms division of Resource Analyst Inc, Hampton, VA were used as test organisms. The test oysters were acclimated for more than 10 days to test conditions. During acclimation oysters were not treated for disease and they were free of apparent sickness, injury, and abnormality at the beginning of the test. Oysters were supplied with live marine phytoplankton to supplement the available food in the unfiltered seawater that was used as dilution water and for acclimation. Oysters were 25 to 50 mm in height (measured along the long axis). At test initiation each oyster was ground to remove approximately 3 to 5 mm of shell and to form a smooth edge.

B. **Test System:** Twenty oysters were randomly and equally distributed to a single replicate of each treatment. The test was performed in 20 liter glass aquaria that contained 15 liters of test solution. The test substance was supplied to the test vessels under flow through conditions by an intermittent flow proportional diluter. An average of 35.0 media exchanges per 24 hours in each test vessel and an average of 1.1 L per oyster per hour were delivered during the test.

A 16 hour light and 8 hour dark photoperiod was automatically maintained with cool-white fluorescent lights. A 15 minute transition period was provided between dark and light. Aeration was not required to maintain dissolved oxygen concentrations above acceptable levels.

C. **Dosage:** Nominal concentrations were: 0.0 (control and solvent control), 7.0, 12.0, 25.0, 50.0, and 100.0 mg/l Prometon. Mean measured concentrations were used for all calculations: 3.58, 8.61, 22.9, 49.4, and 100 mg/L.

D. **Design:** A screening test was not conducted, historic data was used to choose the range of concentrations for the definitive test. The test was conducted at a target temperature of 15 - 30 $^{o}$C with five concentrations of test substance, a solvent control (dimethylformamide), and a dilution water control (natural seawater).

The number of surviving organisms and the occurrence of sublethal effects were determined visually and recorded initially and after 24, 48, 72 and 96 hours. At the end of the study oysters were removed from test vessels and the longest finger of new growth was measured to the nearest 0.1 mm with a Manostat caliper. Dissolved oxygen, pH, salinity and temperature were measured and recorded daily in each test chamber that contained live animals. The temperature in one test vessel was recorded continuously during the test.

E. **Statistics:** Results of the toxicity test were interpreted by standard statistical techniques. Mean measured concentrations of test substance and the percent of control shell deposition were used by the author to calculate the $EC_{50}$ value using the moving average method. The shell growth at the highest tested concentration of Prometon was considered to be 0% of the control growth for the purposes of the $EC_{50}$ calculation. No sublethal effects were noted during the 96 hour study.

12. **REPORTED RESULTS:** The monitored environmental factors for the controls and test concentrations for the duration of the test were reported as follows: the salinity was 26 ppt, the pH ranged from 7.4 to 7.9, the temperature ranged from 19 to 21°C, the dissolved oxygen content ranged from 7.4 to 7.8 except on day 2 where all were 7.3 mg/L.

Survival equal to or greater than 95% occurred at all tested concentrations of Prometon and no sublethal effects were noted during the 96 hour study. Exposure of oysters caused a reduction in shell deposition resulting in a 96 hour $EC_{50}$ of 24.7 mg/L Prometon with a 95% confidence interval of 22.3 to 27.3 mg/L. Exposure of oysters caused a reduction in shell deposition to below 50% of control deposition at two of the five tested concentrations. Figure 1 (attached) shows the dose response curve of shell deposition versus measured concentrations of Prometon over the 96 hour study. Table 2 (attached) shows the mean measured concentrations and the percent of nominal for each test concentration. Table 3 (attached) lists the shell growth data for the twenty oysters of each test concentration.

13. **STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:** The 96-hour $EC_{50}$ was found to be 24.7 mg/L with a confidence interval of 22.3 to 27.3 mg/L. The study director and author has included a Good Laboratory Practice Statement of adherence to defined laboratory practices in 40 CFR part 160, Federal Register publication 8/17/89. A Quality Assurance Statement was included stating adherence to EnviroSystems Protocol and Standard Operating Procedures Manual. All data was checked for accuracy and verified by Quality Assurance Auditors.

14. **REVIEWER'S DISCUSSION AND INTERPRETATION OF STUDY RESULTS:**

A. **Test Procedure:** The test procedures were in accordance with Subdivision E, ASTM, and SEP guidelines except for the following deviations:

- Guidelines state that a NOEC is to be reported; none was reported, nor was an explanation as to why.

- Guidelines state that the chemical characteristics of the dilution/sea-water be listed; they were not reported.

B. **Statistical Analysis:** The reviewer used the Probit Method to determine the $EC_{50}$ value. ANOVA and Dunnett's tests were used to determine the NOEC. Both values were determined by mean measured concentrations.

C. **Discussion/Results:** The 96 hour $EC_{50}$ was determined to be 27.50 mg Prometon/L. The NOEC was determined to be 8.61 mg Prometon/L. Prometon is classified as slightly toxic to Eastern Oysters.

D. **Adequacy of the Study:**

(1) **Classification:** Core.

(2) **Rationale:** n/a.

(3) **Repairability:** n/a.

RIN-0334-94 PROMETON REVIEWS (080804)

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| CONC. | NUMBER EXPOSED | NUMBER DEAD | PERCENT DEAD | BINOMIAL PROB. (PERCENT) |
|---|---|---|---|---|
| 100 | 100 | 95 | 95 | 0 |
| 49.4 | 100 | 72 | 72 | 0 |
| 22.9 | 100 | 43 | 43 | 0 |
| 8.609999 | | 100 | 10 | 10 |
| 0 | | | | |
| 3.58 | 100 | 0 | 0 | 0 |

BECAUSE THE NUMBER OF ORGANISMS USED WAS SO LARGE, THE 95 PERCENT CONFIDENCE INTERVALS CALCULATED FROM THE BINOMIAL PROBABILITY ARE UNRELIABLE. USE THE INTERVALS CALCULATED BY THE OTHER TESTS.

AN APPROXIMATE LC50 FOR THIS SET OF DATA IS 27.45091

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

| SPAN | G | LC50 | 95 PERCENT CONFIDENCE LIMITS | |
|---|---|---|---|---|
| 4 | 1.219001E-02 | 26.6929 | 23.72758 | 30.12744 |

RESULTS CALCULATED USING THE PROBIT METHOD

| ITERATIONS | G | H | GOODNESS OF FIT PROBABILITY |
|---|---|---|---|
| 3 | 2.103085E-02 | 1 | .5281301 |

SLOPE = 2.733072
95 PERCENT CONFIDENCE LIMITS = 2.336722 AND 3.129422

LC50 = 27.49138
95 PERCENT CONFIDENCE LIMITS = 24.24679 AND 31.13122

LC10 = 9.430136
95 PERCENT CONFIDENCE LIMITS = 7.501529 AND 11.30781

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LATEULERE PROMETON ACUTE SHELL DEPOSITION

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| CONC. | NUMBER EXPOSED | NUMBER DEAD | PERCENT DEAD | BINOMIAL PROB. (PERCENT) |
|---|---|---|---|---|
| 100 | 100 | 95 | 95 | 0 |
| 49.4 | 100 | 72 | 72 | 0 |
| 22.9 | 100 | 43 | 43 | 0 |
| 8.609999 | | 100 | 10 | 10 |
| 0 | | | | |
| 3.58 | 100 | 0 | 0 | 0 |

THE BINOMIAL TEST SHOWS THAT 22.9 AND 49.4 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 27.45091

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

30.12744

RESULTS CALCULATED USING THE PROBIT METHOD

| ITERATIONS | G | H | GOODNESS OF FIT PROBABILITY |
|---|---|---|---|
| 3 | 2.103085E-02 | 1 | .5281301 |

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LC50 = 27.49138

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LC10 = 9.430136

95 PERCENT CONFIDENCE LIMITS = 7.501529 AND 11.30781

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| | | |
|---|---|---|
| Total | 119 | 78.659 |

Critical F value = 2.37 (0.05,5,60)
Since F > Critical F REJECT Ho:All groups equal

PROMETON MOLLUSC 96HOUR ACUTE
File: PROMETONOYSTER Transform: NO TRANSFORMATION

DUNNETTS TEST - TABLE 1 OF 2 Ho:Control<Treatment

| GROUP | IDENTIFICATION | TRANSFORMED MEAN | MEAN CALCULATED IN ORIGINAL UNITS | T STAT | SIG |
|---|---|---|---|---|---|
| 1 | 0 | 2.050 | 2.050 | | |
| 2 | 3.58 | 2.060 | 2.060 | -0.158 | |
| 3 | 8.61 | 1.910 | 1.910 | 2.214 | |
| 4 | 22.9 | 1.215 | 1.215 | 13.203 | * |
| 5 | 49.4 | 0.570 | 0.570 | 23.401 | * |
| 6 | 100 | 0.015 | 0.015 | 32.176 | * |

Dunnett table value = 2.28 (1 Tailed Value, P=0.05, df=60,5)

PROMETON MOLLUSC 96HOUR ACUTE
File: PROMETONOYSTER Transform: NO TRANSFORMATION

DUNNETTS TEST - TABLE 2 OF 2 Ho:Control<Treatment

| GROUP | IDENTIFICATION | NUM OF REPS | Minimum Sig Diff (IN ORIG. UNITS) | % of CONTROL | DIFFERENCE FROM CONTROL |
|---|---|---|---|---|---|
| 1 | 0 | 20 | | | |
| 2 | 3.58 | 20 | 0.144 | 7.0 | -0.010 |
| 3 | 8.61 | 20 | 0.144 | 7.0 | 0.140 |
| 4 | 22.9 | 20 | 0.144 | 7.0 | 0.835 |
| 5 | 49.4 | 20 | 0.144 | 7.0 | 1.480 |
| 6 | 100 | 20 | 0.144 | 7.0 | 2.035 |