DATA EVALUATION RECORD

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

2. **TEST MATERIAL:** Prometon Technical; ID No.: FL-872050, ARS-9776, Batch Code: 73152-ML-5664; white powder with a reported purity of 98.5%.

3. **STUDY TYPE:** Early Life-Stage Toxicity Test, 72-4. Species Tested: Pimephales promelas, Fathead minnow.

4. **CITATION:** Graves, W. and G. Peters. 1991. "An Early Life-Stage Toxicity Test with the Fathead Minnow (Pimephales promelas)". Study performed by Wildlife International LTD. 305 Commerce Drive, Easton, MD. 21601. Lab Report No. 108A-115A. Submitted by Ciba-Geigy Corporation, P.O. Box 18200, Greensboro, N.C. 27419. MRID No. 418109-02.

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:** The study is sound but does not satisfy the guidelines of the Fish Early Life Stage Toxicity Study because all raw data was not submitted. When raw data for length and weight is submitted statistical analysis may be performed and then the study may be upgraded to Core. The values determined with the information given thus far are as follows:

	NOEC	LOEC	MATC
Survival	9.49 mg	19.7 mg	13.8 mg
Hatching Success	9.49 mg	19.7 mg	13.8 mg

8. **RECOMMENDATIONS:** The raw data for length and weight need to be submitted in order for this study to fulfill the guideline requirements.

9. **BACKGROUND:** This study has been submitted as part of reregestration requirements.

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

11. **MATERIALS AND METHODS:**

A. **Test Organisms:** Fathead minnow (Pimephales promelas) embryos used to start the test were approximately 2 to 24 hours old and were obtained from cultures maintained in the laboratory at Wildlife International Ltd. Embryos were removed from spawning substrates using a gently rolling motion with the index finger or with a clean razor blade. Embryos were examined under the microscope to select healthy specimens in approximately the same stage of development. Embryos from a minimum of three spawns were combined and used to initiate testing. If more than 50% of the eggs from a given substrate were fungused or were not fertilized, all embryos from that substrate were discarded. Newly hatched larvae were fed live brine shrimp nauplii 3 times per day during the first 7 days post-hatch. On Days 8 through 26 post-hatch, all fish were fed live brine shrimp nauplii 3 times daily on weekdays and 2 times daily on weekends.

B. **Test System:** A proportional diluter was used to provide each concentration of the test substance and the negative (well water) control. Syringe pumps were used to inject the test substance and solvent (triethylene glycol) into mixing chambers where they were mixed with dilution water. The flow of dilution water into the mixing chambers was controlled using rotameters. The diluter was adjusted so that each test chamber received 6 volume additions of test solution every 24 hours. The delivery of test substance to test chambers was initiated approximately 24 hours prior to the beginning of the test in order to establish equilibrium concentrations of the test substance.

Embryo incubation cups were constructed from glass cylinders approximately 50 mm in diameter with 425 um nylon or Teflon screen attached to the bottom using silicone sealant. Test chambers wee Teflon-lined, 25-L polyethylene aquaria filled with 15 L of test solution. The depth of the test solution in each test chamber was

approximately 18 cm. The test chambers were randomly positioned in a temperature-controlled water bath designed to maintained a temperature of 25+/-1°C. The water bath was enclosed in a plexiglass ventilation hood in order to minimize potential cross-contamination between test systems.

Fluorescent tubes that emit wavelengths similar to natural sunlight were controlled by an automatic timer to provide a photoperiod of 16 hours of light and 8 hours of darkness. The light intensity was approximately 45 footcandles at the surface of the water. A 30 minute transition period of low light intensity was provided at dawn and dusk to avoid sudden changes in light intensity.

The hardness, alkalinity, and conductivity of the negative control water were measured at the beginning and end of the test and at weekly intervals during the test. The pH of one replicate of each treatment and control group was measured at the beginning and end of the test and at weekly intervals during the test. The dissolved oxygen content of the water in alternate replicates of each control and treatment group were measured daily during the first 7 days of the test and weekly thereafter.

C. **Dosage:** Nominal concentrations were: 0.0 (control and solvent control), 6.25, 12.5, 25, 50, and 100 mg a.i./L. Mean measured concentrations were used for all calculations: 4.85, 9.49, 19.7, 37.0, and 82.5 mg prometon/L.

D. **Design:** Fathead minnow embryos were exposed to a series of five test concentrations, a negative (well water) control, and a triethylene glycol solvent control for a period of 33 days. The test was begun when groups of newly-fertilized embryos wee placed in incubation cups and exposed to the test water. Two incubation cups, each containing 20 embryos, were placed in each of two replicate test chambers per treatment. After hatching, larvae were released from the incubation cups into larger test chambers, where the exposure continued and growth and mortality were observed over a 28-day period.

Observations of the effects of the test substance on hatching success, growth, and survival were used to determine the no observed effect concentration (NOEC) and the lowest observed effect concentration (LOEC). The NOEC and LOEC were used to define the minimum acceptable toxicant concentration (MATC).

E. **Statistics:** Statistical analyses were performed on data representing hatching success, survival of juvenile fish, and length and weight of surviving fish. All data were subjected to chi-square tests of normality and Bartlett's tests for homogeneity of variance. Dunnett's test was used to evaluate differences between the treatment and negative control groups.

12. **REPORTED RESULTS:** The monitored environmental factors for controls and test concentrations for the duration of the test were as follows: the mean conductivity was 355 umhos/cm, the mean hardness as $CaCO_3$ was 142 mg/L, the alkalinity as $CaCO_3$ was 198.3 mg/L; the pH range was 8.1 - 8.3, the dissolved oxygen content was 7.4 - 8.4, the temperature range was 24.5 - 25.0 ^{o}C.

Hatching success ranged from 72.6 to 68.6% in the negative and solvent control groups, respectively, while hatching success in the treatment groups ranged from 1.25 to 83.6%. Only one of the eggs exposed to 82.5 mg prometon/L concentration survived for more than 24 hours. In the 37.0 mg prometon/L treatment hatching success was significantly reduced ranging from 2.5 to 20%. Hatching success in all treatment groups <19.7 mg prometon/L was not statistically different from that observed in the negative control exposure. (See Table 9).

None of the fish in the 37.0 or 82.5 mg prometon/L treatment survived more than 4 days post-hatch, while survival in the negative and solvent control replicates from hatching until the end of the test ranged from 86.0 to 86.2%, respectively. Mean survival in the 19.7 mg prometon/L treatment was slightly reduced, but the results were not statistically significant and may not have been treatment-related. (See Table 10).

The lengths of the 45 negative control fish alive at the end of the test ranged from 16 to 27 mm. The solvent control fish wee slightly shorter, ranging from 15 to 26 mm. The mean length of fish exposed to test concentrations of 4.85,

9.49, and 19.7 mg prometon/L were 21, 21, and 20 mm, respectively. Although the fish exposed at these test concentrations were statistically shorter than those in the negative controls, they were not significantly shorter than the fish in the solvent controls. Therefore, the results were not considered to be a reflection of a biologically significant, treatment-related effect. The lengths of fish exposed in the 37.0 and 82.5 mg prometon/L treatment could not be evaluated, since none of these fish survived to the end of the test. (See Table 11).

In the negative and solvent control groups, the wet weight of fish surviving at the end of the test ranged from 12 to 167 ug. In the two lowest test concentrations, mean weights were 89 and 93 ug, respectively. There were no significant differences between the wet weights of fish in these treatment groups and the negative control group. The weights of fish in the 19.7 mg prometon/L treatment were slightly lower than those in the controls. Although the results were not statistically significant, the 19.7 mg prometon/L treatment may have elicited a slight reduction in growth.

Similarly, dry weights did not appear to be adversely affected at the two lowest test concentrations; the mean dry weights were slightly lower than the controls but were not statistically significant. The dry weights of fish exposed to the 19.7 mg prometon/L treatment were also reduced, but were not statistically different from those of the controls.

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

The hatching success and survival of fathead minnows exposed to prometon concentration $\geq$37.0 mg prometon/L were clearly reduced. The hatching success, survival, length, wet weight and dry weight of fish exposed to the 19.7 mg prometon/L treatment were also reduced; but, not statistically significant. However, the concentration-response relationship and the consistent effects upon hatching success, survival, and growth at 19.7 mg prometon/L suggest that a significant biological effect may have occurred at that concentration.

Based on these results, the NOEC for the experiment was 9.49 mg prometon/L, the LOEC was 19.7 mg prometon/L, the MATC was >9.49 and <19.7 mg prometon/L (geometric mean MATC = 13.7 mg prometon/L).

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:

- A LOEC, NOEC and MATC should have been reported for each of the parameters tested: mortality, reproduction, and growth; one value was reported as a combined LOEC, NOEC and MATC.

- Raw length and weight data was not given.

- Statistical analysis was carried out comparing the negative control with the treatment groups, the solvent control should have been used for statistical comparison.

B. **Statistical Analysis:** The reviewer used ANOVA and Dunnett's tests to determine the LOEC and NOEC; geometric mean was used to determine the MATC. All values were determined using mean measured concentrations. (See attached).

C. **Discussion/Results:** The LOEC, NOEC and MATC were determined for hatching success and survival data; no length or weight raw data was given, therefore, no statistical analysis could be carried out on these parameters.

The LOEC for survival was statistically determined to be 37.0 mg prometon/L; the NOEC was determined to be 19.7 mg prometon/L. The study author states that although the influence of the test substance was not statistically significant, the concentration-response relationship and the consistent effects upon hatching success, survival, and growth at 19.7 mg prometon/L suggest that a significant biological effect may have occurred at that concentration, EEB agrees with this observation and conclusion; therefore, the NOEC is 9.49 mg prometon/L and the LOEC is 19.7 mg prometon/L.

Hatching success data gave the same statistical results: NOEC = 19.7 mg/L and LOEC = 37.0 mg/L. However, as with the survival data and the reasons noted above, the decided usable values are: NOEC = 9.49 mg/L and LOEC = 19.7 mg/L.

The MATC for both parameters was determined geometrically to be 13.8 mg prometon/L.

D. **Adequacy of the Study:**

(1) **Classification:** Supplemental.

(2) **Rationale:** Length and weight raw growth data was not submitted.

(3) **Repairability:** If raw data is submitted the study may be upgraded.

RIN-0334-94 PROMETON REVIEWS (080804)

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PROMETON FATHEAD HASTCHING SUCCESS
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ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	1736.417	347.283	25.884
Within (Error)	6	80.500	13.417	
Total	11	1816.917		

Critical F value = 4.39 (0.05,5,6)
Since F > Critical F REJECT Ho:All groups equal

PROMETON FATHEAD HASTCHING SUCCESS
File: HATCHPRO Transform: NO TRANSFORM

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

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	0	25.500	25.500		
2	4.85	30.000	30.000	-1.229	
3	9.49	30.500	30.500	-1.365	
4	19.7	23.500	23.500	0.546	
5	37.0	4.500	4.500	5.733	*
6	82.5	0.500	0.500	6.825	*

Dunnett table value = 2.83 (1 Tailed Value, P=0.05, df=6,5)

PROMETON FATHEAD HASTCHING SUCCESS
File: HATCHPRO Transform: NO TRANSFORM

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	2			
2	4.85	2	10.366	40.7	-4.500
3	9.49	2	10.366	40.7	-5.000
4	19.7	2	10.366	40.7	2.000
5	37.0	2	10.366	40.7	21.000
6	82.5	2	10.366	40.7	25.000

PROMETON SURVIVAL FATHEAD MINNOW
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ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	18804.037	3760.807	108.088
Within (Error)	6	208.765	34.794	
Total	11	19012.802		

Critical F value = 4.39 (0.05,5,6)
Since F > Critical F REJECT Ho:All groups equal

PROMETON SURVIVAL FATHEAD MINNOW
File: PROSURV Transform: NO TRANSFORM

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

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	0	86.200	86.200		
2	4.85	93.550	93.550	-1.246	
3	9.49	78.700	78.700	1.271	
4	19.7	73.200	73.200	2.204	
5	37.0	0.000	0.000	14.614	*
6	82.5	0.000	0.000	14.614	*

Dunnett table value = 2.83 (1 Tailed Value, P=0.05, df=6,5)

PROMETON SURVIVAL FATHEAD MINNOW
File: PROSURV Transform: NO TRANSFORM

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	2			
2	4.85	2	16.693	19.4	-7.350
3	9.49	2	16.693	19.4	7.500
4	19.7	2	16.693	19.4	13.000
5	37.0	2	16.693	19.4	86.200
6	82.5	2	16.693	19.4	86.200