DATA EVALUATION RECORD

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

2. **TEST MATERIAL:** Prometon Technical; ID No.: FL-872050, ARS-12119, Batch Code: 73152-ML-5664; white powder with a reported purity of 97.7%.

3. **STUDY TYPE:** Flow-Through Life-Cycle Toxicity Test with the Cladoceran.
Species Tested: Daphnia magna.

4. **CITATION:** Ward, Timothy, J. 1991. "Acute Flow Through Life-Cycle Toxicity Test with the Cladoceran (Daphnia magna)". Study performed by Wildlife International LTD. 305 Commerce Drive, Easton, MD. 21601. Lab Report No. 108A-114E. Submitted by Ciba-Geigy Corporation, P.O. Box 18300, Greensboro, N.C. 27419. MRID No. 418109-03.

5. **REVIEWED BY:**

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

Signature: Dana Lateulere
Date: 9/13/91

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 a Daphnia magna Life-Cycle Chronic Toxicity Test. ANOVA was used to determine the NOEC, LOEC, and MATC for each of three parameters: growth, reproduction and survival. The results are as follows:

	NOEC	LOEC	MATC
Growth	6.77	13.5	9.56
Reproduction	3.45	6.77	4.84
Survival	13.5	28.5	19.5

8. **RECOMMENDATIONS:** N/A

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

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

11. **MATERIALS AND METHODS:**

A. **Test Organisms:** Neonates were obtained from cultures maintained at Wildlife International Ltd. Easton MD. Daphnids in the cultures appeared to be in good health and showed no signs of disease or stress. Daphnids were cultured under test conditions. Individual adult daphnids were transferred to dilution water in separate holding chambers 24 hours prior to test initiation. The progeny produced by three or more adults during this 24-hour period were used as test organisms.

B. **Test System:** A continuous-flow, proportional diluter was used to provide each concentration of the test substance, a negative (well water) control, and a solvent control (triethylene glycol). Syringe pumps were used to inject the test substance and solvent 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 approximately 14 volume additions of test solution every 24 hours. The delivery of test substance to test chambers was initiated approximately 20 hours prior to the beginning of the test in order to establish equilibrium concentrations of the test substance.

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 30 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.

C.Dosage: Nominal concentrations were: 0.0 (control and solvent control), 1.9, 3.8, 7.5, 15.0, and 30.0 mg/L Prometon. Mean measured concentrations were used for all calculations: 1.85, 3.45, 6.77, 13.5, and 28.5 mg/L. (See Table 1).

D. **Design:** Daphnia magna neonates (<24 hours old) were exposed to a geometric series of five test concentrations, a negative (well water) control, and a solvent control for 21 days. To begin the test, individual neonate daphnids were randomly placed in seven replicate test chambers assigned to each treatment and control group. Those daphnids were observed for survival, reproduction, and growth. In addition, groups of five daphnids were randomly placed in each of three beakers assigned to each treatment and control group to assess survival.

Observations of survival and reproduction of first-generation daphnids were made on Monday, Wednesday, and Friday throughout the test. The criteria for death included absence of heartbeat, white opaque coloration, lack of movement of appendages, and lack of response to gentle prodding. In the seven test chambers the number of live, second-generation daphnids was counted and recorded three times per week. After each observation, live first-generation daphnids were transferred to clean test compartments and second-generation daphnids were discarded. First-generation daphnids were also observed for abnormal development and aberrant behavior, such as an inability to maintain position in the water column, uncoordinated swimming, and cessation of feeding. The lengths of the first-generation daphnids surviving at the end of the test were also measured.

The test was conducted at 20 +/- 1°C. Temperature, pH, and dissolved oxygen were measured in each treatment at test initiation and at weekly intervals thereafter. Conductivity, hardness and alkalinity were measured in the negative control at test initiation and at weekly intervals, thereafter.

E. **Statistics:** Statistical analyses were performed on data concerning survival, the number of live young produced by each individually-exposed daphnid, and the length of surviving daphnids. In each analysis, comparisons were made between each treatment group and the negative control group to determine the significance of treatment-related effects.

The survival data were evaluated using Fisher's Exact Test. The reproduction and growth data passed Chi-Square tests for normality and Bartlett's tests for homogeneity of variance. Consequently, Dunnett's test was used to evaluate treatment-related differences in reproduction and growth.

12. **REPORTED RESULTS:** The monitored environmental factors for the controls and test concentrations for the duration of the test were reported as follows: the mean conductivity was 310 umhos/cm, the mean hardness as $CaCO_3$ was 150 mg/L, the alkalinity as $CaCO_3$ was 185.5 mg/L, the mean pH was 8.1, the temperature range was 19.7 - 20.1, the dissolved oxygen content ranged from 8.0 - 8.7.

The no observed effect concentration (NOEC), the lowest observed effect concentration (LOEC), and the maximum acceptable toxicant concentration (MATC) were calculated using mortality, reproduction, and growth data. Mortality at test concentrations <13.5 mg prometon/L ranged from 0 to 32% and was not statistically different from mortality in the control. Although 32% mortality occurred in the 6.77 mg prometon/L treatment, a clear concentration-response curve was not evident, and this effect was not considered to be treatment related. Mortality at the highest concentration tested, 28.5 mg prometon/L was 45% at the end of the study.; this mortality was considered treatment related. (See Table 9).

There was no reproduction of first-generation daphnids exposed to 28.5 mg prometon/L. Each of the daphnids that survived to the end of the test at 13.5 mg prometon/L released at least 2 broods in 21 days, but the broods were small. Reproduction was also significantly reduced at 6.77 mg prometon/L. Reproduction at 1.85 mg prometon/L was not significantly different from that in the controls. (See Table 10).

Growth was estimated by measuring the length of each surviving daphnid at test termination. Length was significantly reduced at test concentrations 6.77, 13.5, and 28.5 mg prometon/L. At 3.45 mg prometon/L daphnid lengths were not significantly different from those in the controls. The lengths of daphnids exposed to 1.85 mg prometon/L were statistically different from the controls, but were not considered to be biologically significant due to the lack of a clearly defined concentration-response relationship. (See Table 11).

The highest concentration tested, 28.5 mg prometon/L, induced significant mortality and eliminated reproduction. Although survival was not adversely affected at the lower test concentrations, reproduction and growth were impaired at concentrations as low as 6.77 mg prometon/L. Consequently, the LOEC was 6.77 mg prometon/L, and the NOEC was 3.45 mg prometon/L. Based on these results, the maximum

acceptable toxicant concentration (MATC) was estimated to be >3.45 mg prometon/L and <6.77 mg prometon/L (geometric mean MATC = 4.83 mg prometon/L).

13. **STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:** The study author included a Good Laboratory Practice Statement and a Quality Assurance Statement.

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:

- It is recommended that the daphnids are weighed to determine their growth during the test; their lengths were measured which has been determined not to be an accurate means of determining growth.

- 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 mortality 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 by mean measured concentrations. (See attached).

C. **Discussion/Results:** The NOEC, LOEC and MATC for growth of daphnids exposed to prometon are 6.77, 13.5 and 9.56 mg prometon/L, respectively; reproduction results are 3.45, 6.77 and 4.84 mg prometon/L, respectively; survival results are 13.5, 28.5, and 19.5 mg prometon/L, respectively. EEB has determined that for overall effects to daphnids the following values should be used as they are the most sensitive: NOEC = 3.45, LOEC = 6.77 and the MATC = 4.84 mg prometon/L.

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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OUTPUT
Command ===>

SAS 6:48 Wednesday, August 28, 1991 5

General Linear Models Procedure

Dependent Variable: GROWTH

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	6	19.26915727	3.21152621	18.90	0.0001
Error	31	5.26636905	0.16988287		
Corrected Total	37	24.53552632			

R-Square	C.V.	Root MSE	GROWTH Mean
0.785577	11.09235	0.412169	3.71578947

ZOOM I

OUTPUT
Command ===>

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General Linear Models Procedure

Duncan Grouping	Mean	N	TRT
A	4.650	6	b
A	4.283	6	a
	4.021	7	d
C	3.760	5	c
C			
C	3.333	5	e
E	2.803	6	f
E			
E	2.333	3	g

ZOOM I

OUTPUT
Command ===>

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General Linear Models Procedure

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Comparison		Simultaneous Lower Confidence Limit	Difference Means	Simultaneous Upper Confidence Limit	
c	- a	-23.67	12.17	48.01	
b	- a	-28.34	5.83	40.01	
d	- a	-49.05	-16.12	16.81	
e	- a	-61.47	-25.63	10.21	
f	- a	-75.67	-41.50	-7.33	***
g	- a	-100.69	-58.83	-16.98	***

ZOOM I

12

OUTPUT
Command ===>

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General Linear Models Procedure

Dependent Variable: REPRO

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	6	18195.98195	3032.66366	6.46	0.0002
Error	31	14561.72857	469.73318		
Corrected Total	37	32757.71053			

R-Square	C.V.	Root MSE	REPRO Mean
0.555472	49.46465	21.67333	43.8157895

ZOOM I

OUTPUT
Command ===>

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General Linear Models Procedure

Duncan Grouping			Mean	N	TRT
	A		71.00	5	c 12.5
	A				
	A		64.67	6	b
	A				
3	A		58.83	6	a Solvent
3	A				
	A	C	42.71	7	d 3.5
		C			
		C	31.00	5	e 6.75
	D	C	17.33	6	f 13.5
	D				
	D		0.00	3	g 27.5

ZOOM I

OUTPUT
Command ===>

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General Linear Models Procedure

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Comparison	Simultaneous Lower Confidence Limit	Difference Between Means	Simultaneous Upper Confidence Limit	
b - a	-0.283	0.367	1.017	
(d) - a	-0.888	-0.262	0.364	
(c) - a	-1.175	-0.493	0.188	←
e - a	-1.635	-0.953	-0.272	***
f - a	-2.125	-1.475	-0.825	***
g - a	-2.746	-1.950	-1.154	***

b = control
c = ~~1.85~~ 1.85
d = ~~3.27~~ 3.45

ZOOM I

Mortality

345

FISHERS EXACT TEST

IDENTIFICATION	NUMBER OF ALIVE	DEAD	TOTAL ANIMALS
CONTROL	14	1	15
negcontrol	13	2	15
TOTAL	27	3	30

CRITICAL FISHERS VALUE (15,15,14) (p=0.05) IS 9. b VALUE IS 13.
Since b is greater than 9 there is no significant difference between CONTROL and TREATMENT at the 0.05 level.

FISHERS EXACT TEST

IDENTIFICATION	NUMBER OF ALIVE	DEAD	TOTAL ANIMALS
CONTROL	14	1	15
1.85	13	2	15
TOTAL	27	3	30

CRITICAL FISHERS VALUE (15,15,14) (p=0.05) IS 9. b VALUE IS 13.
Since b is greater than 9 there is no significant difference between CONTROL and TREATMENT at the 0.05 level.

FISHERS EXACT TEST

IDENTIFICATION	NUMBER OF DEAD	ALIVE	TOTAL ANIMALS
CONTROL	1	14	15
3.45	0	15	15

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TOTAL	1	29	30

CRITICAL FISHERS VALUE (15,15,1) (p=0.05) IS LESS THAN 0. b VALUE IS 0.
NO SIGNIFICANT DIFFERENCE

FISHERS EXACT TEST

IDENTIFICATION	NUMBER OF ALIVE	NUMBER OF DEAD	NUMBER OF TOTAL ANIMALS
CONTROL	14	1	15
6.77	10	5	15
TOTAL	24	6	30

CRITICAL FISHERS VALUE (15,15,14) (p=0.05) IS 9. b VALUE IS 10.
Since b is greater than 9 there is no significant difference
between CONTROL and TREATMENT at the 0.05 level.

FISHERS EXACT TEST

IDENTIFICATION	NUMBER OF ALIVE	NUMBER OF DEAD	NUMBER OF TOTAL ANIMALS
CONTROL	14	1	15
13.5	13	2	15
TOTAL	27	3	30

CRITICAL FISHERS VALUE (15,15,14) (p=0.05) IS 9. b VALUE IS 13.
Since b is greater than 9 there is no significant difference
between CONTROL and TREATMENT at the 0.05 level.

FISHERS EXACT TEST

NUMBER OF

IDENTIFICATION	ALIVE	DEAD	TOTAL ANIMALS
CONTROL	14	1	15
28.5	9	6	15
TOTAL	23	7	30

CRITICAL FISHERS VALUE (15,15,14) (p=0.05) IS 9. b VALUE IS 9.
Since b is less than or equal to 9 there is a significant difference between CONTROL and TREATMENT at the 0.05 level.

SUMMARY OF FISHERS EXACT TESTS

GROUP	IDENTIFICATION	NUMBER EXPOSED	NUMBER DEAD	SIG (P=.05)
	CONTROL	15	1	
1	negcontrol	15	2	
2	1.85	15	2	
3	3.45	15	0	
4	6.77	15	5	
5	13.5	15	2	
6	28.5	15	6	*

TITLE: Prometon Survival data 3x5
FILE: promsurv
TRANSFORM: NO TRANSFORM NUMBER OF GROUPS: 6

GRP	IDENTIFICATION	REP	VALUE	TRANS VALUE
1	0	1	1.0000	1.0000
1	0	2	2.0000	2.0000
1	0	3	3.0000	3.0000
2	1.85	1	2.0000	2.0000
2	1.85	2	3.0000	3.0000
2	1.85	3	4.0000	4.0000
3	3.45	1	3.0000	3.0000
3	3.45	2	4.0000	4.0000
3	3.45	3	5.0000	5.0000
4	6.77	1	4.0000	4.0000
4	6.77	2	5.0000	5.0000
4	6.77	3	6.0000	6.0000
5	13.5	1	5.0000	5.0000
5	13.5	2	6.0000	6.0000
5	13.5	3	67.0000	67.0000