DATA EVALUATION RECORD

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

2. **TEST MATERIAL:** Prometon (2,4-bis(isopropyl(amino)-6-methoxy-s-triazine)); FL #872050; CAS No. 1610-18-0; 97% active ingredient; a white crystalline solid.

3. **STUDY TYPE:** Growth and Reproduction of Aquatic Plants -- Tier 2. Species Tested: Selenastrum capricornutum.

4. **CITATION:** Hughes, J.S. 1990. The Toxicity of Prometon to Selenastrum capricornutum. Laboratory Project No. B267-47-1. Conducted by Malcolm Pirnie, Inc., Elmsford, NY. Submitted by CIBA-GEIGY Corporation, Greensboro, NC. EPA MRID No. 417253-05.

5. **REVIEWED BY:**

Louis M. Rifici, M.S.
Associate Scientist II
KBN Engineering and
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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.
Supervisor, EEB/HED
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Cynthia Moulton 12.3.91
Signature:
Date: Henry T. Craven, 12/4/91

7. **CONCLUSIONS:** This study is scientifically sound but does not meet the guideline requirements for a Tier 2 aquatic plant growth and reproduction test. The maximum application rate was not given in the report. The 120-hour EC_{50} of Prometon for Selenastrum capricornutum was 0.098 mg/L (mean measured concentration). The NOEC was 0.032 mg/L.

8. **RECOMMENDATIONS:** See Section 14.D.(3).

9. **BACKGROUND:**

6 hrs

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

11. **MATERIALS AND METHODS:**

A. **Test Species:** The alga used in the test, Selenastrum capricornutum, came from laboratory stock cultures originally obtained from the University of Texas Culture Collection, Austin, TX. Stock cultures were maintained in synthetic algal assay procedure nutrient medium (AAP; Miller et al., 1978) under 4306 lux illumination, and a temperature of 24°±2°C. The culture flasks were continuously shaken at 100 oscillations per minute. Transfers were made into fresh medium to maintain cultures in the logarithmic phase of growth. The culture used as inoculum had been transferred to fresh medium seven days before test initiation.

B. **Test System:** All glassware was cleaned according to EPA methods and autoclaved before use. Test vessels used were 250-mL Erlenmeyer flasks fitted with foam stoppers which permitted gas exchange. The test medium was the same as that used for culturing with the pH adjusted to 7.5 ±0.1 and filtered through a 0.22-µm membrane filter.

A 0.1 mg/mL stock solution was prepared by diluting 0.0515 g of Prometon to 500 mL with ASTM Type I water. The solution was sonicated for 45 minutes to facilitate dissolution. A secondary stock (0.001 mg/mL) was prepared by serial dilution. Appropriate volumes of primary or secondary stock were diluted in sterile medium to give the desired concentrations. A control was also prepared. Fifty mL of the appropriate test solution were placed into each of three replicate 250-mL flasks (3 per treatment level and the control).

The test vessels were kept in an incubator with environmental conditions similar to those employed in culturing. Light was provided continuously at an intensity of 4306 ±646 lux.

C. **Dosage:** Seven-day growth and reproduction test. Based on the results of a preliminary test, nine nominal concentrations of 0.0156, 0.0312, 0.0625, 0.125, 0.25, 0.5, 1.0, 2.0, 4.0 mg/L, and a control were selected for the definitive test.

D. **Test Design:** An inoculum of Selenastrum capricornutum

cells calculated to provide 0.3 x 10^4 cells/mL was aseptically introduced into each flask. The inoculum volume was 0.1616 mL per flask.

Cell counts were performed using an electronic particle counter on test days 3, 4, 5, and 7. Three counts per replicate were made on each counting day. The sample volumes ranged from 0.1 to 2.0 mL. To minimize spatial differences in the incubator, the flasks were randomly repositioned each working day.

The temperature was recorded daily. The pH of each test solution was determined at test initiation and at test termination.

The actual concentrations of Prometon present in the test solutions on days 0 and 7 were determined by gas chromatography. Analytical samples taken at termination (day 7) were filtered (0.8 μm) prior to analysis to remove algal cells.

E. **Statistics:** The percent effect on mean standing crops for each level was determined using the following equation:

$$\%E = \frac{C - T}{C} \times 100$$

where: C = mean standing crop in the control
T = mean standing crop in the treated cultures

The 7-day EC_{50} values were determined using weighted least squares nonlinear regression of the log of the test concentration versus cell counts. Standing crop data were analyzed for significant differences by analysis of variance (ANOVA) and Dunnett's test.

12. **REPORTED RESULTS:** The mean measured concentrations are given in Table 3 (attached). Measured concentrations averaged 64 to 123% of nominal and were fairly consistent between sampling days. The control solution was not measured at termination.

Mean standing crops and percent inhibition for each concentration and the control are given in Table 5 (attached). After seven days, growth inhibition ranged from 12.9 to 99.9%. The percent inhibition appeared linear and increased with increasing Prometon concentration.

The 7-day EC_{25} was 0.140 mg/L with a 95% confidence interval

of 0.074-0.267. The 7-day EC_{50} was 0.210 mg/L with a 95% confidence interval of 0.133-0.334 mg/L. Mean growth in all test cultures were significantly lower than the mean of the control cultures, therefore the NOEC is lower than the lowest concentration tested, 0.010 mg/L (mean measured).

Test solution pH ranged from 7.20 to 7.34 at initiation and 7.56 to 7.84 at termination. The pH of the control solution was not measured at termination.

13. **STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:** No conclusions were made by the author.

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 (Federal Register 54(158):8/17/89)

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

A. **Test Procedure:** The test procedure and the report were generally in accordance with the SEP and Subdivision J guidelines, except for the following deviations:

The dissolved oxygen and conductivity of the test solutions were not measured.

Light intensity during the test was 4.306 ±646 klux. The recommended light intensity is 4 klux.

The temperature in the incubator was given as 24°±2°C The report stated that the temperature was measured during the test period but did not give the results.

Cell growth measurements were not taken daily. Measurements were made on days 3, 4, 5, and 7 only.

B. **Statistical Analysis:** The reviewer used a computer program (Toxstat Version 3.0 and EPA's Toxanal) to analyze the day 5 cell density data (Table 4, attached). All but the lowest two test levels had significantly lower growth compared to the control (see attached printout 1 and 2).

Based on cell growth after 5 days, exposure to Prometon caused -0.4, 5.9, 18.2, 68.8, 91.7, 98.3, 99.5, 99.7 and 99.8% inhibition to Selenastrum capricornutum. The 5-day EC_{50}, determined using the percent inhibition

results, was 0.098 mg/L with a 95% confidence interval of 0.088-0.108 mg/L (see attached printout 3). The slope of the probit line was 3.41.

C. **Discussion/Results:** The author chose to use the standing crop results after 7-days rather than those after 5-days. The 7-day EC_{50} (prepared by the author) was 0.112 mg/L higher than the 5-day EC_{50}. This seems odd because frequently EC_{50} values decrease with time as organisms become more susceptible to toxicants with increasing lengths of exposure. Since algal cultures are actively growing and replacing dead cells, some growth recovery may have taken place. In any event, Figure 1 (attached) indicates that the effect of the Prometon exposure was apparent after 5 days and continuing the test for 2 additional days is not warranted.

This study is scientifically sound but does not meet the guideline requirements for a Tier 2 aquatic plant growth and reproduction test. The maximum application rate was not given in the report, therefore, the EC_{50} of Prometon cannot be compared to expected environmental concentrations. Under the conditions of the test, the 120-hour EC_{50} of Prometon for Selenastrum capricornutum was 0.098 mg/L (mean measured concentration). The NOEC was 0.032 mg/L.

D. **Adequacy of the Study:**

(1) **Classification:** Supplemental

(2) **Rationale:** The maximum application rate was not given in the report.

(3) **Repairability:** The registrant should submit the missing information.

15. **COMPLETION OF ONE-LINER:** Yes, 04/29/91

RIN-0334-94 PROMETON REVIEWS (08804)

Page_____ is not included in this copy.

Pages 6 through 10 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.

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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.

Printout 1

417253-05 Prometon, Selenastrum day 5 growth, Rifici
File: 41725305.sel Transform: NO TRANSFORMATION

Chi-square test for normality: actual and expected frequencies

INTERVAL	<-1.5	-1.5 to <-0.5	-0.5 to 0.5	>0.5 to 1.5	>1.5
EXPECTED	2.010	7.260	11.460	7.260	2.010
OBSERVED	0	10	10	10	0

Calculated Chi-Square goodness of fit test statistic = 6.2742
Table Chi-Square value (alpha = 0.01) = 13.277

Data PASS normality test. Continue analysis.

Hartley test for homogeneity of variance
Bartletts test for homogeneity of variance

These two tests can not be performed because at least one group has zero variance.

Data FAIL to meet homogeneity of variance assumption.
Additional transformations are useless.

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	9	75285163.366	8365018.152	754.968
Within (Error)	20	221599.333	11079.967	
Total	29	75506762.700		

Critical F value = 2.39 (0.05,9,20)
Since F > Critical F REJECT Ho:All groups equal

Printout 2

417253-05 Prometon, Selenastrum day 5 growth, Rifici
File: 41725305.sel 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	control	3620.000	3620.000		
2	0.01 mg/L meas	3633.333	3633.333	-0.155	
3	0.032	3406.667	3406.667	2.482	
4	0.0613	2960.000	2960.000	7.679	*
5	0.122	1130.000	1130.000	28.972	*
6	0.235	300.667	300.667	38.621	*
7	0.569	63.000	63.000	41.387	*
8	1.154	19.667	19.667	41.891	*
9	2.408	9.667	9.667	42.007	*
10	3.781	6.000	6.000	42.050	*

Dunnett table value = 2.60 (1 Tailed Value, P=0.05, df=20,9)

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	control	3			
2	0.01 mg/L meas	3	223.459	6.2	-13.333
3	0.032	3	223.459	6.2	213.333
4	0.0613	3	223.459	6.2	660.000
5	0.122	3	223.459	6.2	2490.000
6	0.235	3	223.459	6.2	3319.333
7	0.569	3	223.459	6.2	3557.000
8	1.154	3	223.459	6.2	3600.333
9	2.408	3	223.459	6.2	3610.333
10	3.781	3	223.459	6.2	3614.000

Printout 3

LOUIS M. RIFICI PROMETON SELENASTRUM CAPRICORNUTUM 4-13-91

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CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
3.781	100	100	100	0
2.408	100	100	100	0
1.154	100	100	100	0
.569	100	98	98	0
.235	100	92	92	0
.122	100	69	69	0
.0613	100	18	18	0
.032	100	6	6	0
.01	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 9.523843E-02

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS	
6	7.282268E-03	9.890615E-02	8.762559E-02	.1113748

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
6	2.087298E-02	1	.1996216

SLOPE = 3.411101

95 PERCENT CONFIDENCE LIMITS = 2.918282 AND 3.903919

LC50 = 9.774584E-02

95 PERCENT CONFIDENCE LIMITS = 8.835021E-02 AND .1081493

LC10 = 4.147481E-02

95 PERCENT CONFIDENCE LIMITS = 3.464423E-02 AND 4.787387E-02

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Shaughnessey # 80804 Chemical Name Prometon Chemical Class _____ Page 1 of 1

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

5-Day EC_{50}

97%

EC_{50} = 0.098 * ppm (95% C.L. Probit: 0.088 - 0.108)

Cells/ml = 0.3×10^4

Slope = 3.41

Species: Selenastrum capricornutum

Temperature = 24 ± 2°C

Lab: Malcolm Pirnie, Inc.

5-Day Dose Level pp * m/(% Effect)

0.01 (-0.4), 0.032 (5.9), ~~0.061~~ 0.0613 (18.2), 0.122 (68.8), 0.235 (91.7), 0.569 (98.3), 1.154 (99.5), 2.408 (99.7), 3.781 (99.8)

LR 4/29/91 Supplemental

MRID # 417253-05

Comments: * mean measured concentration

% effect = inhibition

5-Day EC_{50}

_____

EC_{50} = pp (95% C.L. ( ))

Cells/ml =

Slope =

Species:

Temperature =

Lab:

5-Day Dose Level pp /(% Effect)

( ), ( ), ( ), ( ), ( )

MRID #

Comments:

_____ _____
