11/19/91

MRID No. 419848-01

DATA EVALUATION RECORD

1. **CHEMICAL:** Myclobutanil
   Shaughnessey Number: 128857.

2. **TEST MATERIAL:** RH-3866, TD# 90-127, Lot # 2-2131 93% technical grade, received from Rohm and Haas Co. on 10/10/90.

3. **STUDY TYPE:** 123-2. Non-Target Plants: Growth and Reproduction of Aquatic Plants - Tier II. Species Tested: Selenastrum capricornutum.

4. **CITATION:** Hoberg, J. R. and M. C. R. Bayne. 1991. RH-3866 Technical- Toxicity to the Freshwater Green Alga, Selenastrum capricornutum. Springborn Laboratories, Inc. Study No. 86.0990.6118.430. SLI Report # 91-2-3641. Rohm and Haas Report # 90RC-0195. Sponsor Study # 90R-195. Performed by Environmental Science Division, Springborn Laboratories, Inc., Wareham, Massachusetts. Submitted by Rohm and Haas Company, Springhouse, Pa. EPA MRID No. 419848-01.

5. **REVIEWED BY:**

   Michael W. Davy
   Agronomist
   Ecological Effects Branch
   EFED/OPP/EPA

   Signature: Michael Davy
   Date: 11-4-91

6. **APPROVED BY:**

   Daniel Rieder
   Section Head
   Ecological Effects Branch
   EFED/OPP/EPA

   Signature: Daniel Rieder
   Date: 11-19-91

7. **CONCLUSIONS:** The study is found to be scientifically sound and meets the requirements for a Tier II study of growth and reproduction of an aquatic plant, Selenastrum capricornutum. Based on the number of cells, the 120-hour EC_{50} value of RH-3866 for Selenastrum capricornutum was 0.83 mg/L (95 percent confidence level of 0.56 to 1.1). The 120-hour NOEC was 0.56 mg/L (mean measured concentration).

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

9. **BACKGROUND:** This study was resubmitted in support of reregistration and for the petition for permanent tolerances for myclobutanil (RH-3866) on pome fruit.

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

11. **MATERIALS AND METHODS:**

A. **Test Species:** Selenastrum capricornutum used in this test were originally obtained from the Carolina Biological Supply Co., Burlington, NC and maintained in stock cultures at the laboratory. The cultures were maintained under test conditions and in Marine Biological Laboratory medium (Table 1) with distilled deionized water adjusted to a pH of 7.5 after autoclaving.

B. **Test System:** The test medium is the same type as those used in culturing. The phytotoxicity test was conducted in an environmental chamber with a 250 ml flask attached to a rotary shaker adjusted to 100 rpm. Each flask contains 100 ml of test solution. The temperature was at 24-25°C with a continuous photoperiod at an intensity of 4304-5380 lux provided by cool-white and Vita-Lite fluorescent lights.

C. **Dosage:** The nominal test concentrations, based on a preliminary test, were 0.65, 1.3, 2.5, 5.0 and 10.0 mg/L. The corrected mean measured concentrations of RH-3866 were found to be 0.56, 1.1, 2.2, 5.1 and 6.6 mg A.I./L (Table 2 attached). The control contained the nutrient medium and the solvent control contained acetone and nutrient medium.

D. **Design:** Each concentration and control was replicated three times. The Selenastrum capricornutum were added to each of the vessels. These comprised of 3,000 cells/mL in each of the test vessels. Test solutions were not renewed. Observations and cell counts were recorded daily. Temperature was measured daily. The pH and concentration of test and control solutions were measured at test initiation and termination.

E. **Statistics:** A t-test was used to compare control growth with solvent growth at each 24-hour interval. Control and solvent control were statistically similar and therefore the two control were averaged as one value. The 120-hour EC_{10}, EC_{50} and EC_{90} values for Selenastrum capricornutum was "determined by linear

regression of response (percent cell density as compared with pooled control data) vs. mean measured exposure over the range of test concentrations where a clear exposure-response relationship was observed". The method of inverse prediction was then used to apply the regression equation. The NOEC was determined using one-way analysis of variance and Bonferroni's Test after the data were checked for normality using the Chi-test and for homogeneity of variance using Hartley test.

12. **REPORTED RESULTS:** Growth and reproduction data are presented in Table 3 (attached). After 120 hours of exposure, there were no intact cells present in the 6.6, 5.1 and 2.2 mg A.I./L concentrations. Few intact cells in the 1.1 mg/L concentration were observed. The 0.56 mg/L concentration appeared to be normal and similar to the control cells. The 6.6, 5.1 and 2 treatment levels were excluded from the statistical analysis due to no growth of cells. Bonferroni's test on the 1.1 and 0.56 concentration showed NOEC to be 0.56 mg/L for the 120 hour study. The 120 hour EC_{50} value was found to be 0.91 with a 95 percent confidence level of 0.55 to 1.5 mg/L.

During the test period, the pH ranged from 7.5 at initiation to 10.6 at termination. A continuous photoperiod at an intensity of 4304-5380 lux was provided by cool-white fluorescent lights. The temperature was 24 to 26°C (Table 4 attached).

13. **STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:** Authors found The EC's and 95 percent confidence limits to be as follows: EC_{10}=0.67(0.38-1.1), EC_{50}=0.91(0.55-1.5), EC_{90}=1.3(0.79-2.1) and NOEC=0.56 mg/L.

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.

B. **Statistical Analysis:** The reviewer used the EPA's Toxanal computer program to calculate the 120-hour EC_{50} values using percent inhibition of the number cells and mean measured concentrations. Percent inhibition (I) of growth compared to control was calculated for the number of cells according to the following formula:

$$\% I = \frac{C - X}{C} \times 100$$

where: C = mean cell density in the Solvent control,
X = mean cell density in test concentration.

The 120-hour EC_{50} value using cell count was 0.83 mg/L of RH-3866 with a 95 percent confidence interval of 0.56 to 1.1 mg/L (Printout 1, attached).

This EC_{50} value is different to that presented by the authors. The authors's value for EC_{50} was based on the control value used which was the pooled averages of the solvent control and control and on the concentrations of 6.6, 5.1 and 2.2 not being calculated into the statistical analysis. The reviewer used only the solvent control for the control value and included the concentrations of 6.6, 5.1 and 2.2 into the statistical analysis. Therefore, the reviewer's values should be used for the purpose of hazard assessment.

The reviewer used Toxstat Version 3.3 to determine the NOEC for this study. A square root transformation was applied to the cell density data to obtain homogeneity and normal distribution. Once the data was transformed, Bonferroni's t-test and Dunnett's Anova test was applied. This analysis indicate the NOEC for the study was 0.56 mg/L of RH-3866, based on mean measured concentrations (Printout 2, attached).

C. **Discussion/Results:** The study is found to be scientifically sound and meets the requirements for a Tier II study of growth and reproduction of an aquatic plant, Selenastrum capricornutum. Based on the number of cells, the 120-hour EC_{50} value of RH-3866 for Selenastrum capricornutum was 0.83 mg/L (95 percent confidence level of 0.56 to 1.1). The 120-hour NOEC was 0.56 mg/L (mean measured concentration).

D. **Adequacy of the Study:**

(1) **Classification:** Core

(2) **Rationale:** N/A.

(3) **Repairability:** N/A.

15. **COMPLETION OF ONE-LINER:** 10/24/91

MYCLOBUTANIL 128857

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Printout 1

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CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB.(PERCENT)
6.6	100	100	100	0
5.1	100	100	100	0
2.2	100	100	100	0
1.1	100	94	94	0
.56	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 .8293261

WHEN THERE ARE LESS THAN TWO CONCENTRATIONS AT WHICH THE PERCENT DEAD IS BETWEEN 0 AND 100, NEITHER THE MOVING AVERAGE NOR THE PROBIT METHOD CAN GIVE ANY STATISTICALLY SOUND RESULTS.

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Davy Myclobotanil SELENASTRUM

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CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB.(PERCENT)
6.6	100	100	100	0
5.1	100	100	100	0
2.2	100	100	100	0
1.1	100	94	94	0
.56	100	0	0	0

THE BINOMIAL TEST SHOWS THAT .56 AND 1.1 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 .8293261

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Davy Myclobotanil Y

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CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB.(PERCENT)
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5.1	100	100	100	0
2.2	100	100	100	0
1.1	100	94	94	0
.56	100	0	0	0

Printout 2

ELENASTRUM
ile: SELENAS.MYC Transform: NO TRANSFORM

ANOVA TABLE

OURCE	DF	SS	MS	F
etween	5	76995.892	15399.178	353.200
ithin (Error)	12	523.193	43.599	
otal	17	77519.085		

Critical F value = 3.11 (0.05,5,12)
Since F > Critical F REJECT Ho:All groups equal

ELENASTRUM
ile: SELENAS.MYC 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	SOLVENT CONTROL	140.667	140.667		
2	0.56	141.000	141.000	-0.062	
3	1.1	9.033	9.033	24.416	*
4	2.2	0.000	0.000	26.091	*
5	5.1	0.000	0.000	26.091	*
6	6.6	0.000	0.000	26.091	*

Dunnett table value = 2.50 (1 Tailed Value, P=0.05, df=12,5)

SELENASTRUM 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	SOLVENT CONTROL	3			
2	0.56	3	13.478	9.6	-0.333
3	1.1	3	13.478	9.6	131.633
4	2.2	3	13.478	9.6	140.667
5	5.1	3	13.478	9.6	140.667
6	6.6	3	13.478	9.6	140.667

SELENASTRUM
File: SELENAS.MYC Transform: NO TRANSFORM

ANOVA TABLE

SOURCE	DF	SS	MS	F

Since F > Critical F REJECT Ho:All groups equal

Printout 2

SELENASTRUM
File: SELENAS.MYC Transform: NO TRANSFORM

BONFERRONI T-TEST - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	SOLVENT CONTROL	140.667	140.667		
2	0.56	141.000	141.000	-0.062	
3	1.1	9.033	9.033	24.416	*
4	2.2	0.000	0.000	26.091	*
5	5.1	0.000	0.000	26.091	*
6	6.6	0.000	0.000	26.091	*

Bonferroni T table value = 2.68 (1 Tailed Value, P=0.05, df=12,5)

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BONFERRONI T-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	SOLVENT CONTROL	3			
2	0.56	3	14.454	10.3	-0.333
3	1.1	3	14.454	10.3	131.633
4	2.2	3	14.454	10.3	140.667
5	5.1	3	14.454	10.3	140.667
6	6.6	3	14.454	10.3	140.667

ELENASTRUM
ile: SELENAS.MYC Transform: NO TRANSFORM

ANOVA TABLE

OURCE	DF	SS	MS	F
etween	5	76995.892	15399.178	353.200
ithin (Error)	12	523.193	43.599	
otal	17	77519.085		

Critical F value = 3.11 (0.05,5,12)
Since F > Critical F REJECT Ho:All groups equal