1-22-92

MRID No. 415651-41

DATA EVALUATION RECORD

1. **CHEMICAL:** Acetochlor.
Shaughnessey No. 121601.

2. **TEST MATERIAL:** Acetochlor technical; 2-chloro-N-ethoxymethyl-6'-ethylacet-o-toluidide; Test substance No. R1072; 89.7% w/w active ingredient; a brown liquid.

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

4. **CITATION:** Smyth, D.V., J.F. Tapp, S.A. Sankey and R.D. Stanley. 1989. Acetochlor: Determination of Toxicity to the Green Alga Selenastrum capricornutum. Laboratory ID No. R1072/I. Conducted by Imperial Chemical Industries PLC, Brixham, Devon, UK. Submitted by ICI Americas, Inc. EPA MRID No. 415651-41.

5. **REVIEWED BY:**

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7. **CONCLUSIONS:** This study is scientifically sound and meets the guideline requirements for a Tier 2 non-target aquatic plant study. The NOEC and EC_{50} of acetochlor for Selenastrum capricornutum were 1.0 and 1.43 µg/l, respectively.

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

9. **BACKGROUND:**

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7. **CONCLUSIONS:** This study is scientifically sound and meets the guideline requirements for a Tier 2 non-target aquatic plant study. The NOEC, EC_{25}, and EC_{50} of acetochlor for *Selenastrum capricornutum* were 1.0, 0.95, and 1.43 µg/l, respectively.

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

9. **BACKGROUND:**

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

11. **MATERIALS AND METHODS:**

A. **Test Species:** The alga used in the test, Selenastrum capricornutum Printz, came from laboratory stock cultures kept under axenic conditions. Stock cultures were maintained in synthetic nutrient medium (Miller et al., 1978) at a temperature of 24 ±1°C, with orbital shaking at 100 rpm. Light was supplied continuously at an intensity of 3960 lux. Cultures that were growing logarithmically were used as inoculum for the test.

B. **Test System:** Test vessels used were 250-ml conical glass flasks fitted with foam stoppers. The test medium was the same as that used for culturing, with a Ph of 7.1 ±0.1.

The test vessels were kept in an incubator with environmental conditions like those employed in culturing.

C. **Dosage:** Nominal concentrations of 0.32, 0.56, 1.0, 1.8, 3.2, 5.6, 10 µg/l, a blank set of treatments and control (no algal inoculum), and a medium control were used for the definitive test.

D. **Test Design:** A stock solution of 400 µg/l was prepared by direct addition of the test material to sterile distilled water. Aliquots of the stock were added to sterile culture medium to obtain the nominal concentrations. All solutions were clear and colorless. One-hundred milliliters of the test solution were placed in each of three replicate 250-ml flasks (3 per treatment level). The control flasks were replicated six times.

An inoculum volume of 0.155 ml was used to provide 3000 cells/ml per flask. Cell counts were performed every 24 hours for 5 days using an electronic particle counter. The flasks were randomized by rows within the incubator.

At the start of the test, samples were taken of each test solution, using the excess remaining after filling the test vessels, and were analyzed for the concentration of the test substance by gas chromatography (GC). At the end of the test, each blank solution was sampled and analyzed in the same manner.

The Ph values of the test solutions were measured at test initiation and termination. Light intensity was measured once during the experiment and temperature was monitored daily and continuously in the incubator.

E. **Statistics:** For each nominal concentration, the mean of the measured concentration on day 0 and day 5 was calculated. The mean measured concentrations were then used as the basis for the data analysis. The area under the growth curve and growth rate were examined as a function of time. Probit and Dunnett's analysis (p= 0.05) were conducted on both of these parameters at day 5.

12. **REPORTED RESULTS:** Algal cell densities for the control and the exposure concentrations throughout the test are given in Table 1 (attached).

Measured concentrations on day 0 were 100% to 107% of nominal while day 5 measured concentrations were between 88% and 104%. The means of the measured concentrations were 0.33, 0.57, 1.0, 1.7, 3.1, 5.9, and 10 µg/l.

Increasing concentrations of acetochlor had increasingly inhibitory effects upon the growth and reproduction of Selenastrum capricornutum.

By day 5, the effect of the test material on the area under the growth curve, relative to the control, ranged between 5% and 100% inhibition (Table 2, attached). The EC_{50} was 1.3 µg/l with 95% confidence limits of 0.74 and 2.30 µg/l.

By day 5, the effect of the test material on the growth rate, relative to the control, ranged between 1% and 96% inhibition (Table 3, attached). The EC_{50} was 3.1 µg/l with 95% confidence limits of 2.1 and 4.7 µg/l.

Results from Dunnett's analysis indicated that the areas under the growth curve on day 5 at the four highest concentrations were significantly less than the control. The NOEC was determined to be 1.0 µg/l. The results from the growth rate data were similar to area under the growth curve results. The NOEC was again reported as 1.0 µg/l.

The pH readings in the control and the exposure concentrations were 9.54 to 9.60 and 7.44 to 10.04, respectively, by test termination. The hourly temperatures ranged from 23.9 to 24.2°C. Light intensity is 3960 Lux.

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

A Good laboratory practice statement was included in the report indicating that this study satisfied the requirements of 40 CFR Part 160.

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 initial pH of the test solutions (7.1) was lower than recommended (7.5).

The type of lighting was not specified.

B. **Statistical Analysis:** Probit and Dunnett's tests were used to determine the EC and NOEC values, respectively. The reviewer used a computer program to perform statistical analysis (attached) of the 5 day cell density data to determine the NOEC. Area under the growth curve data were used for the determination of EC values. This parameter was more conservative than growth rate. The results from Dunnett's analysis were in agreement with the authors'. The reviewer obtained EC values that were similar to the authors'.

C. **Discussion/Results:** This study is scientifically sound and meets the guideline requirements for a Tier 2 non-target aquatic plant study. The NOEC and EC_{50} of acetochlor for Selenastrum capricornutum were 1.0 and 1.43 μg/l, respectively. Growth of S. capricornutum was increasingly inhibited by increasing amounts of acetochlor.

D. **Adequacy of the Study:**

(1) Classification: Core.

(2) Rationale: N/A.

(3) Repairability: N/A.

15. **COMPLETION OF ONE-LINER:** Yes, 9/19/91.

S

ACETOCHLOR

Page_____ is not included in this copy.

Pages 6 through 8 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.

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____ Information about a pending registration action.

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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.

selenastrum cell density

Summary Statistics and ANOVA

Transformation = None

Group (rate (mg/l))	n	Mean	s.d.	cv%
1 = control	6	4841666.6667	215630.8574	4.5
2 0.33	3	4496666.6667	41633.3200	.9
3 0.57	3	4463333.3333	325166.6240	7.3
4 1.0	3	4500000.0000	265141.4717	5.9
5* 1.7	3	2410000.0000	688258.6723	28.6
6* 3.1	3	100666.6667	18036.9990	17.9
7* 5.9	3	7333.3333	1625.8331	22.2
8* 10	3	4300.0000	1873.4994	43.6

*) the mean for this group is significantly less than
the control mean at alpha = 0.05 (1-sided) by a t - test
with Bonferroni adjustment of alpha level

NOEC = 1.0 mg/l

Raw data from Table 1 (attached)

Minumum detectable difference for
t-tests with Bonferroni adjustment = -442808.149461
This difference corresponds to -9.15 percent of control

```
************************************************
*                                              *
* Note - the above value for the minimum       *
* detectable difference is approximate as      *
* the sample sizes are not the same for all of *
* the groups.                                  *
*                                              *
************************************************
```

Between groups sum of squares =********** with 7 degrees of freedom.

Error mean square = ********** with 19 degrees of freedom.

Bartlett's test p-value for equality of variances = .001

```
************************************************
*                                              *
* Warning - the test for equality of variances *
* is significant (p less than 0.01). The       *
* results of this analysis should be inter-    *
* preted with caution.                         *
*                                              *
************************************************
```

EPA PROBIT ANALYSIS PROGRAM
USED FOR CALCULATING EC VALUES
Version 1.4

selenastrum growth rate

Conc.	Number Exposed	Number Resp.	Observed Proportion Responding	Adjusted Proportion Responding	Predicted Proportion Responding
0.3300	100	5	0.0500	0.0500	0.0083
0.5700	100	14	0.1400	0.1400	0.0665
1.0000	100	3	0.0300	0.0300	0.2805
1.7000	100	59	0.5900	0.5900	0.6133
3.1000	100	98	0.9800	0.9800	0.8983
5.9000	100	100	1.0000	1.0000	0.9900
10.0000	100	100	1.0000	1.0000	0.9993

Chi - Square Heterogeneity = 69.652

****
* WARNING *
*
* Significant heterogeneity exists. The results reported *
* for this data set may not be valid. The results should *
* be interpreted with appropriate caution. *
****

Mu = 0.154108
Sigma = 0.265113

Parameter	Estimate	Std. Err.	95% Confidence Limits
Intercept	4.418708	0.300891	(3.645116, 5.192299)
Slope	3.771977	0.941679	(1.350921, 6.193033)

Theoretical Spontaneous Response Rate = 0.0000

selenastrum growth rate

Estimated EC Values and Confidence Limits

Point	Conc.	Lower 95% Confidence Limits	Upper 95% Confidence Limits
EC 1.00	0.3446	0.0255	0.6552
EC 5.00	0.5224	0.0787	0.8756
EC10.00	0.6521	0.1414	1.0356
EC15.00	0.7574	0.2080	1.1711
EC50.00	1.4260	0.8356	2.5076
EC85.00	2.6845	1.7249	10.4478
EC90.00	3.1180	1.9452	15.4138
EC95.00	3.8922	2.2949	27.7785
EC99.00	5.8998	3.0599	85.7411

$y = 4.42 + 3.77(x)$

$EC_{25} =$ ~~0.946 mg/ml~~

0.95 µg/l