12-2-93

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MRID No. 427131-09

DATA EVALUATION RECORD

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

2. **TEST MATERIAL:** Acetochlor technical; 2-chloro-N-ethoxymethyl-6'-ethylacet-*o*-toluidide; CAS No. 34256-82-1; purity of 95.1% w/w; a red liquid.

3. **STUDY TYPE:** 123-2. Growth and Reproduction of Aquatic Plants - Tier 2. Species Tested: *Anabaena flos-aquae*.

4. **CITATION:** Smyth, D.V., S.A. Sankey, and A.J. Grinell. 1992. Acetochlor: Toxicity to the Blue-Green Alga *Anabaena flos-aquae*. Laboratory ID No. W566/A (FT18/92). Conducted by Imperial Chemical Industries PLC, Devon, UK. Submitted by ICI Agrochemicals, Surrey, UK. EPA MRID No. 427131-09.

5. **REVIEWED BY:**

Mark A. Mossler, M.S.
Associate Scientist
KBN Engineering and
Applied Sciences, Inc.

Signature: [signature]
Date: 6/1/93

6. **APPROVED BY:**

Louis M. Rifici, M.S.
Associate Scientist
KBN Engineering and
Applied Sciences, Inc.

Signature: Louis M Rifici
Date: 6/1/93

Henry T. Craven, M.S.
Supervisor, EEB/EFED
USEPA

Signature: William S. Robert 10/2/93
Date: H.T. Craven 12/2/93

7. **CONCLUSIONS:** This study is scientifically sound and fulfills the guideline requirements for a Tier 2 non-target plant growth and reproduction test. Based on mean measured concentrations, the 5-day NOEC, LOEC, and EC_{50} for *A. flos-aquae* exposed to acetochlor technical were 1.9, 4.1, and 35 mg/l, respectively.

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

9. **BACKGROUND:**

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

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11. MATERIALS AND METHODS:

A. Test Species: The alga used in the test, *Anabaena flos-aquae*, came from laboratory stock cultures kept under axenic conditions. The original source was the Culture Centre of Algae and Protozoa, Ambleside, UK. Stock cultures were maintained in synthetic nutrient medium at a temperature of 24 ±1°C, with orbital shaking at 100 rpm. Cool-white illumination provided a light intensity of 3,260 lux continuously. Cultures that were in a logarithmic growth phase were used as inoculum for the test.

B. Test System: Test vessels used were 250-ml glass conical flasks fitted with foam stoppers. The test medium was the same as that used for culturing, with a pH of 7.3-7.4.

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

C. Dosage: Five-day growth and reproduction study. Nominal concentrations of 1.0, 2.0, 4.0, 8.0, 16, 32, 64, and 128 mg/l, and a medium control were selected for the definitive test.

The highest concentration solution was prepared by direct addition of the test material to sterile culture medium (1.6 l). All lower concentration treatment solutions were prepared by adding appropriate volumes of the highest concentration solution to nutrient medium.

D. Test Design: One-hundred milliliters of the test solution were placed in each of three replicate flasks per treatment level. The control flasks were replicated six times. A blank set of solutions (extra replicate of control and test solutions without added algae) was also incubated concurrently.

An inoculum volume of 0.515 ml per flask was used to provide 20,000 cells/ml. Indirect cell counts were performed every 24 hours using a spectrophotometer. The absorbances of the blank solutions were subtracted from the absorbance readings of the exposure solutions containing algae. Absorbances were then compared to a standard curve to determine the cellular density. The flasks were randomized daily by rows within the incubator.

At the start of the test, samples taken from each test solution and control were analyzed for the concentration of the test substance by gas chromatography. At the end of the test, each blank solution was sampled and analyzed in the same manner.

The pH of the test solutions were measured at test initiation and termination. Light intensity was measured once during the experiment. Temperature was monitored continuously electronically as well as manually daily.

E. **Statistics:** For each nominal concentration, the mean of the measured concentration of the day 0 and 5 samples 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. The 5-day EC_{50} and no-observed-effect concentration (NOEC) for each parameter were determined using probit analysis and Dunnett's test ($p \leq 0.05$).

12. **REPORTED RESULTS:** The exposure solutions were clear and colorless. Initial measured concentrations of the exposure solutions ranged between 89 and 100% of nominal. The measured concentrations in the blank solutions after 120 hours ranged between 91 and 103% of nominal. The mean measured concentrations were 0.90, 1.9, 4.1, 7.5, 16, 32, 59, and 130 mg/l (Table 1, attached).

Algal densities in the control and the exposure solutions throughout the test are given in Table 2 (attached).

By day 5, the effect of the test material on the area under the growth curve, relative to the control, ranged from 2% stimulation to 98% inhibition. The NOEC, lowest-observed-effect concentration (LOEC), and EC_{50} were 7.5, 16, and 32 mg/l, respectively. The 95% confidence interval for the EC_{50} was 16-86 mg/l.

By day 5, the effect of the test material on the growth rate, relative to the control, ranged from 0 to 83% inhibition. The NOEC, LOEC, and EC_{50} were 1.9, 4.1, and 110 mg/l, respectively. The 95% confidence interval for the EC_{50} was 12->130 mg/l.

The pH in the control and the exposure solutions was 7.3-7.4 at the beginning of the study and 7.4-7.8 at the conclusion. Temperature ranged from 24.0 to 24.2°C.

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

Good Laboratory Practice and Quality Assurance Unit statements were included in the report indicating compliance with EPA Good Laboratory Practice Standards as set forth in 40 CFR Part 160.

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

A. Test Procedure: The test procedures and the report were generally in accordance with SEP and Subdivision J guidelines, but deviated as follows:

The inoculum level (20,000 cells/ml) was much greater than recommended (3,000 cells/ml).

The light intensity (3.3 klux) was greater than recommended (2 klux).

The EC_{50} was computed based on growth rate and area under the growth curve, rather than cell density.

B. Statistical Analysis: Using percent inhibition based on cell density, the reviewer used EPA's Toxanal program to determine the EC_{50} value. Analysis of variance and Bonferroni's test were used to verify the NOEC and LOEC. A similar EC_{50} and a narrower confidence interval (C.I.) were calculated using the moving average angle method. The reviewer obtained the same value for the NOEC and LOEC. The 5-day EC_{50} was 35 mg/l. The 95% C.I. for the EC_{50} was 31-39 mg/l.

C. Discussion/Results: Although the light intensity was almost double the recommended intensity and the cellular inoculum was 7 times greater than recommended, the growth of the control algae proceeded in an exponential fashion. This indicated that the two exceedances might have actually offset one another (i.e., more algae resulted in less light penetration). Therefore, the reviewer does not feel that these exceedances negatively affected the outcome of the study.

This study is scientifically sound and fulfills the guideline requirements for a Tier 2 non-target plant growth and reproduction test. Based on mean measured concentrations, the 5-day NOEC, LOEC, and EC_{50} for *A.*

flos-aquae exposed to acetochlor technical were 1.9, 4.1, and 35 mg/l, respectively.

D. **Adequacy of the Study:**

(1) Classification: Core.

(2) Rationale: N/A.

(3) Repairability: N/A.

15. **COMPLETION OF ONE-LINER:** Yes, 5-26-93.

ACETOCHLOR

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Anabaena cell density
File: ana Transform: NO TRANSFORMATION

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

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	control	1.860	1.860		
2	0.9	1.879	1.879	-0.480	
3	1.9	1.837	1.837	0.598	
4	4.1	1.691	1.691	4.288	*
5	7.5	1.621	1.621	6.040	*
6	16	1.416	1.416	11.229	*
7	32	1.221	1.221	16.149	*
8	59	0.765	0.765	27.689	*
9	130	0.000	0.000	47.014	*

Bonferroni T table value = 2.73 (1 Tailed Value, P=0.05, df=21,8)

Anabaena cell density
File: ana Transform: NO TRANSFORMATION

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	control	6			
2	0.9	3	0.108	5.8	-0.019
3	1.9	3	0.108	5.8	0.024
4	4.1	3	0.108	5.8	0.170
5	7.5	3	0.108	5.8	0.239
6	16	3	0.108	5.8	0.444
7	32	3	0.108	5.8	0.639
8	59	3	0.108	5.8	1.096
9	130	3	0.108	5.8	1.860

NOEC = 1.9 mg/l

LOEC = 4.1 mg/l

MOSSLER ACETOCHLOR ANABAENA FLOS AQUAE 5-26-93

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CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
130	100	100	100	0
59	100	59	59	0
32	100	34	34	0
16	100	24	24	0
7.5	100	13	13	0
4.1	100	9	9	0
1.9	100	1	1	0
.9	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 47.41704

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS	
4	1.453399E-02	34.85237	31.10552	39.1796

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
4	.1469609	5.706253	0

A PROBABILITY OF 0 MEANS THAT IT IS LESS THAN 0.001.

SINCE THE PROBABILITY IS LESS THAN 0.05, RESULTS CALCULATED USING THE PROBIT METHOD PROBABLY SHOULD NOT BE USED.

SLOPE = 1.903888
95 PERCENT CONFIDENCE LIMITS = 1.174024 AND 2.633753

LC50 = 34.0198
95 PERCENT CONFIDENCE LIMITS = 22.21868 AND 57.26433

LC10 = 7.322778
95 PERCENT CONFIDENCE LIMITS = 2.845523 AND 12.20362

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