

US EPA ARCHIVE DOCUMENT

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: *Skeletonema costatum*.
- 4. **CITATION:** Smyth, D.V., N.C.D. Craig, S.A. Sankey, and A.J. Penwell. 1992. Acetochlor: Toxicity to the Marine Alga *Skeletonema costatum*. Laboratory ID No. W566/B (FT19/92). Conducted by Imperial Chemical Industries PLC, Devon, UK. Submitted by ICI Agrochemicals, Surrey, UK. EPA MRID No. 427131-10.

5. **REVIEWED BY:**

 Mark A. Mossler, M.S.
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Signature: 
 Date: 6/1/93

6. **APPROVED BY:**

 Louis M. Rifici, M.S.
 Associate Scientist
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 Date:  12/2/93

7. **CONCLUSIONS:** This study is scientifically sound but does not meet the guideline requirements for a Tier 2 non-target plant growth and reproduction test. The test was conducted for 4 days rather than the recommended 5 days. Based on mean measured concentrations, the 4-day NOEC, LOEC, and EC₅₀ for *S. costatum* exposed to acetochlor technical were 1.6, 3.3, and 3.4 µg/l, respectively.

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

9. **BACKGROUND:**

10. DISCUSSION OF INDIVIDUAL TESTS: N/A.

11. MATERIALS AND METHODS:

A. Test Species: The diatom used in the test, *Skeletonema costatum*, originally came from the Culture Centre of Algae and Protozoa, Ambleside, Cumbria, UK. The culture had been kept under axenic conditions since April, 1986. Stock cultures were maintained in synthetic nutrient medium at a temperature of $20 \pm 1^\circ\text{C}$ with orbital shaking at 100 rpm. Cool-white lighting at an intensity of 3.9 klux provided a 16-hour photoperiod. Cultures that were growing logarithmically were used as inoculum for the test.

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

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

C. Dosage: Four-day growth and reproduction study. Nominal rates of 0.75, 1.5, 3, 6, 12, 24, 48, and 96 $\mu\text{g/l}$, and a medium control were selected for the definitive test.

A stock solution (20 mg/l) was prepared by direct addition of the test material to sterile culture medium. The highest concentration treatment solution (96 $\mu\text{g/l}$) was prepared by addition of the stock solution to sterile culture medium. Aliquots of this solution were added to sterile culture medium to obtain the lower concentration treatment solutions.

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 set of control and test solutions without added diatoms) was also incubated concurrently.

An inoculum volume of 0.865 ml per flask was used to provide 10,000 cells/ml. Cell counts were performed every 24 hours using an electronic particle counter. The flasks were randomized daily by rows within the incubator.

At the start of the test, samples taken from each treatment and control solution were analyzed for the concentration of the test substance using 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 was measured at test initiation and termination. Light intensity was measured once during the experiment. Temperature was monitored continuously electronically as well as manually daily. Salinity was measured at test initiation.

- E. **Statistics:** For each nominal concentration, the mean of the measured concentration of the day 0 and 4 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 4-day EC_{50} and no-observed-effect concentration (NOEC) were determined for both of these parameters using probit analysis and Dunnett's test ($p \leq 0.05$).

12. **REPORTED RESULTS:** Measured concentrations on day 0 were from 96 to 113% of nominal while day 4 measured concentrations were from 100 to 108% of nominal (Table 1, attached). The means of the measured concentrations were 0.75, 1.6, 3.3, 6.6, 12, 24, 50, and 95 $\mu\text{g}/\text{l}$. The control and exposure solutions were clear and colorless.

Diatom cell densities for the control and the exposure concentrations throughout the test are given in Table 2 (attached).

By day 4, the effect of the test material on the area under the growth curve, relative to the control, ranged between 2% stimulation and 98% inhibition. The NOEC, lowest-observed-effect concentration (LOEC), and EC_{50} were 1.6, 3.3, and 4.3 $\mu\text{g}/\text{l}$, respectively. The 95% confidence interval for the EC_{50} was 0.79-8.8 $\mu\text{g}/\text{l}$.

By day 4, the effect of the test material on the growth rate, relative to the control, ranged between 1% stimulation and 90% inhibition. The NOEC, LOEC, and EC_{50} were 1.6, 3.3, and 10 $\mu\text{g}/\text{l}$, respectively. The 95% confidence interval for the EC_{50} was 3.27-29 $\mu\text{g}/\text{l}$.

The pH in the control and the exposure concentrations was 8.1-8.2 at the beginning of the study and 8.3-9.2 at the

conclusion. Temperature ranged from 20.0 to 20.6°C. The salinity was 31 parts per thousand.

13. STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:

No conclusions were made by the authors.

Good Laboratory Practice and Quality Assurance 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 study was conducted for 4 days rather than the recommended 5 days.

The EC₅₀ was computed based on growth rate and area under the growth curve, rather than cell density.

- B. Statistical Analysis:** Using cell density data, the reviewer used EPA's Toxanal program to determine the EC₅₀ value. Analysis of variance and Bonferroni's test were used to determine LOEC and NOEC values. The same NOEC and LOEC values were determined using cell density as were determined using area under the growth curve and growth rate. A similar EC₅₀ was calculated, but the reviewer obtained a narrower confidence interval (C.I.) using the moving average angle method. The 4-day EC₅₀ was determined to be 3.4 µg/l (95% C.I. = 3.1-3.8 µg/l).

- C. Discussion/Results:** This study is scientifically sound but does not meet the guideline requirements for a Tier 2 non-target plant growth and reproduction test. Based on mean measured concentrations, the 4-day NOEC, LOEC, and EC₅₀ for *S. costatum* exposed to acetochlor technical were 1.6, 3.3, and 3.4 µg/l, respectively.

D. Adequacy of the Study:

- (1) **Classification:** Supplemental.
- (2) **Rationale:** The test was conducted for 4 days rather than the recommended 5 days.

(3) Repairability: No.

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

ACETOCHLOR

Page _____ is not included in this copy.

Pages 6 through 8 are not included.

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Skeletonema costatum

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ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	8	53572.028	6696.503	218.317
Within (Error)	21	644.139	30.673	
Total	29	54216.167		

Critical F value = 2.42 (0.05,8,21)
 Since F > Critical F REJECT Ho:All groups equal

Skeletonema costatum

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

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	control	92.667	92.667		
2	0.75	96.167	96.167	-0.894	
3	1.6	89.267	89.267	0.868	
4	3.3	42.800	42.800	12.733	*
5	6.6	10.140	10.140	21.073	*
6	12	2.383	2.383	23.054	*
7	24	1.687	1.687	23.232	*
8	50	1.027	1.027	23.400	*
9	95	1.067	1.067	23.390	*

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

NEC = 1.6 µg/l

LOEC = 33 µg/l

Skeletonema costatum

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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	control	6			
2	0.75	3	10.699	11.5	-3.500
3	1.6	3	10.699	11.5	3.400
4	3.3	3	10.699	11.5	49.867
5	6.6	3	10.699	11.5	82.527
6	12	3	10.699	11.5	90.283
7	24	3	10.699	11.5	90.980
8	50	3	10.699	11.5	91.640
9	95	3	10.699	11.5	91.600

MOSSLER ACETOCHLOR SKELETONEMA COSTATUM 5-26-93

CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
12	100	97	97	0
6.6	100	89	89	0
3.3	100	54	54	0
1.6	100	4	4	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 3.148975

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS	
3	1.392242E-02	3.443418	3.117502	3.77313

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
4	.4628465	3.791985	.022551

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

SLOPE = 4.234551
95 PERCENT CONFIDENCE LIMITS = 1.353666 AND 7.115435

LC50 = 3.451613
95 PERCENT CONFIDENCE LIMITS = 1.924699 AND 5.598674

LC10 = 1.730248
95 PERCENT CONFIDENCE LIMITS = .3064096 AND 2.688236
