12-2-93

MRID No. 427131-08

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: *Navicula pelliculosa*.

4. **CITATION:** Smyth, D.V., S.A. Sankey, M.M. Holland, and P.A. Johnson. 1992. Acetochlor: Toxicity to the Freshwater Diatom *Navicula pelliculosa*. Laboratory ID No. W566/C (FT20/92). Conducted by Imperial Chemical Industries PLC, Devon, UK. Submitted by ICI Agrochemicals, Surrey, UK. EPA MRID No. 427131-08.

5. **REVIEWED BY:**

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

Signature: [signature]
Date: 5/24/93

6. **APPROVED BY:**

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

Signature: Louis M Rifici
Date: 5/25/93

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

Signature: William B. Rabert 12/1/93
Date: H.T. Craven 12/2/93

7. **CONCLUSIONS:** This study is scientifically sound but does not fulfill the guideline requirements for a Tier 2 non-target plant growth and reproduction test. The test was conducted for four, rather than five days. Based on mean measured concentrations, the 4-day NOEC, LOEC, and EC_{50} for *N. pelliculosa* exposed to acetochlor technical were 0.56, 1.20, and 1.38 mg/l, respectively.

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

9. **BACKGROUND:**

1

10. DISCUSSION OF INDIVIDUAL TESTS: N/A.

11. MATERIALS AND METHODS:

A. Test Species: The diatom used in the test, *Navicula pelliculosa*, came from laboratory stock cultures kept under axenic conditions. Stock cultures were maintained in synthetic nutrient medium at a temperature of 24 ±1°C, with orbital shaking at 140 rpm. Cool-white illumination provided a light intensity of 3890 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 8.6-8.8.

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 0.125, 0.25, 0.50, 1.0, 2.0, 4.0, 8.0, and 16.0 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 (0.0259 g) 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 (3 per treatment level). The control flasks were replicated six times. A blank set of solutions (extra replicate of control and test solutions without added diatoms) was also incubated concurrently.

An inoculum volume of 0.55 ml per flask was used to provide 3,000 cells/ml. Cell counts were performed every 24 hours using an electronic particle counter. The flasks were randomized daily by rows within the incubator.

Samples of the treatment and control solutions were taken at test initiation and analyzed for the test

compound using gas chromatography. At test termination, the blank solutions were 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:** Mean measured concentrations were used as the basis for data analysis. The area under the growth curve and growth rate were examined as a function of time. Probit analysis and Dunnett's test (p= 0.05) were conducted on both of these parameters at day 4.

12. **REPORTED RESULTS:** The exposure solutions were clear and colorless. Initial measured concentrations of the exposure solutions ranged between 100 and 120% of nominal. The measured concentrations in the blank solutions after 96 hours ranged between 105 and 128% of nominal. The mean measured concentrations were 0.16, 0.27, 0.56, 1.2, 2.1, 4.6, 8.8, and 19 mg/l (Table 1, attached).

Diatom densities in the control and the exposure solutions 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 from 23% stimulation to 103% inhibition. The no-observed-effect concentration (NOEC), lowest-observed-effect concentration (LOEC), and EC_{50} were 0.56, 1.2, and 1.3 mg/l, respectively. The 95% confidence interval for the EC_{50} was 0.75-2.1 mg/l.

By day 4, the effect of the test material on the growth rate, relative to the control, ranged from 4% stimulation to 104% inhibition. The NOEC, LOEC, and EC_{50} were 2.1, 4.6, and 2.3 mg/l, respectively. The 95% confidence interval for the EC_{50} was 1.3-4.2 mg/l.

The pH in the control and the exposure solutions was 8.6-8.8 at the beginning of the study and 7.6-7.7 at the conclusion. Temperature ranged from 23.7 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 study was conducted for 4 days rather than the recommended 5 days.

The light intensity (3.9 klux) was less than recommended (4.3 klux).

The initial pH (8.6-8.8) was higher than recommended (7.5).

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

B. Statistical Analysis: 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 more conservative EC_{50} and narrower confidence interval (C.I.) were calculated using the moving average angle method. The reviewer obtained a less conservative value for the NOEC and LOEC. By combination of the results, the 4-day NOEC, LOEC, and EC_{50} were 0.56, 1.20, and 1.38 mg ai/l, respectively. The 95% C.I. for the EC_{50} was 1.23-1.55 mg/l.

C. Discussion/Results: This study is scientifically sound but does not fulfill 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 EC_{50} for *N. pelliculosa* exposed to acetochlor technical were 0.56, 1.20, and 1.38 mg/l, respectively.

D. Adequacy of the Study:

(1) Classification: Supplemental.

(2) Rationale: The test was conducted for four, rather than five days.

(3) Repairability: No.

15. COMPLETION OF ONE-LINER: Yes, 5-23-93.

ACETOCHLOR

Page_____ is not included in this copy.

Pages 5 through 7 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.

X FIFRA registration data.

____ The document is a duplicate of page(s) _____.

____ The document is not responsive to the request.

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.

Navicula cell density
File: nav Transform: NATURAL LOG(Y)

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

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	control	3.199	24.650		
2	.16	3.334	29.033	-0.481	
3	.27	3.039	20.900	0.569	
4	.56	2.910	18.433	1.030	
5	1.2	2.475	12.487	2.575	
6	2.1	2.627	14.733	2.036	
7	4.6	1.192	4.430	7.138	*
8	8.8	-1.152	0.339	15.476	*
9	19	-1.364	0.259	16.231	*

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

Navicula cell density
File: nav Transform: NATURAL LOG(Y)

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	.16	3	13.138	53.3	-4.383
3	.27	3	13.138	53.3	3.750
4	.56	3	13.138	53.3	6.217
5	1.2	3	13.138	53.3	12.163
6	2.1	3	13.138	53.3	9.917
7	4.6	3	13.138	53.3	20.220
8	8.8	3	13.138	53.3	24.311
9	19	3	13.138	53.3	24.391

NOEL = ~~2.6~~ 2.1 mg/l

LOEL = 4.6 mg/l

However - EC_{50} = 1.38, ∴

NOEL = 1.2 mg/l

LOEL = 2.1 mg/l

MOSSLER ACETOCHLOR NAVICULA PELLICULOSA 5-23-93

****

CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
8.8	100	99	99	0
4.6	100	82	82	0
2.1	100	40	40	0
1.2	100	49	49	0
.56	100	26	26	0
.27	100	15	15	0
.16	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 2.505336

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS	
6	8.651221E-03	1.381119	1.23218	1.554884

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
5	.1908323	7.273566	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 = 1.850939

95 PERCENT CONFIDENCE LIMITS = 1.042368 AND 2.65951

LC50 = 1.476478

95 PERCENT CONFIDENCE LIMITS = .8678804 AND 2.631732

LC10 = .3041506

95 PERCENT CONFIDENCE LIMITS = .0811723 AND .5667381

****