12-2-93

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MRID No. 427131-07

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; 95.1% w/w active ingredient; a red liquid.

3. **STUDY TYPE:** 123-2. Growth and Reproduction of Aquatic Plants - Tier 2. Species Tested: *Lemna gibba*.

4. **CITATION:** Smyth, D.V., S.A. Sankey, and A.J. Penwell. 1993. Acetochlor: Toxicity to the Duckweed (*Lemna gibba*). Laboratory ID No. W556/D (FT21/92). Conducted by ZENECA Limited, Brixham, Devon, UK. Submitted by ZENECA Agrochemicals, Surrey, UK. EPA MRID No. 427131-07.

5. **REVIEWED BY:**

William S. Rabert
Biologist
Ecological Effects Branch
Environmental Fate and Effects Division (7507C)

Signature: William Rabert
Date: 10/4/93

6. **APPROVED BY:**

Dan Rieder
Section Head
Ecological Effects Branch
Environmental Fate and Effects Division (7507C)

Signature: Dan Rieder
Date: 11.9.93

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

Signature: H T. Craven
Date: 12/2/93

7. **CONCLUSIONS:** This study is scientifically sound and meets the guideline requirements for a Tier 2 non-target aquatic plant study. [Based on mean measured concentrations and reduced growth (dry weight), the 14-day NOEC, LOEC, and EC_{50} for *L. gibba* exposed to acetochlor technical were 0.12, 0.22 and 3.4 µg/l, respectively.] revised

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 plants used in the test, *Lemna gibba* G3, were obtained from in-house cultures originally obtained from the University of Waterloo, Canada. Plants were maintained in M-type Hoagland's medium under 5000 lux illumination at a temperature of 25 ±1°C. Warm-white fluorescent tubes and a continuous photoperiod were used. Plants that were growing actively were used as inoculum for the test.

B. Test System: Test vessels used were glass 400-ml cylindrical dishes with loose-fitting lids. The test medium was the same as that used for culturing, with a pH of 4.6 to 4.8.

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

C. Dosage: Fourteen-day growth and reproduction study. Nominal rates of 0.25, 0.50, 1.0, 2.0, 4.0, 8.0, 16, and 32 µg/l, and a medium control were tested.

A primary stock solution (20,000 g/l) was prepared by direct addition of the test material to sterile culture medium. The test solutions were prepared by dilution of a secondary stock (640 µg/l) or the 32 µg/l test solution in sterile culture medium.

D. Test Design: One-hundred and sixty ml of the test or control solution were placed in each of three replicate dishes per treatment level and control. Test solutions were renewed on day 7. The dishes were randomized by rows within the incubator and were re-randomized after 7 days.

Three plants with four fronds each were randomly placed in each replicate dish. Frond counts were performed on test days 2, 5, 7, 9, 12, and 14. All fronds which visibly projected beyond the edge of the parent frond were counted. Toxicity symptoms were recorded. At the end of the test (14 days), the plants from each dish were rinsed with distilled water and dried to a constant weight at 60°C.

Samples were taken from the freshly-prepared solutions on days 0 and 7, and from the old test solutions on days 7 and 14. These samples were analyzed for the test material using gas chromatography.

The pH of the freshly-prepared test solutions was measured on days 0 and 7 and the pH of two replicates of the old test solutions was measured on days 7 and 14. The temperature of the incubator was measured daily by thermometer and hourly by a data logger. The light intensity was measured once during each week of the study.

E. **Statistics:** The increase in frond number over the 14 day test period was calculated by subtracting the number of fronds inoculated on day 0 (12) from the day 14 counts. Mean increase in frond number was used to determine the percent inhibition. Percent inhibition data were analyzed using the moving average angle method to estimate the 14-day EC_{50} and its associated 95% confidence interval (C.I.). Increase in frond number was examined by one-way analysis of variance, and Dunnett's test ($p \leq 0.05$) was used to identify significant differences from the control.

Increase in dry weight was calculated by subtraction of the estimated initial weight (12 fronds = 2.0 mg dry weight) from the 14 day dry weight. The mean increase for each treatment and mean percent inhibition were calculated. These data were analyzed as previously described.

12. **REPORTED RESULTS:** Mean measured concentrations were 0.12, 0.22, 0.46, 1.1, 2.3, 4.6, 8.1, and 15 µg/l and ranged from 44 to 58% of nominal (Table 1, attached). After solution preparation, a visual assessment showed the solutions to be clear and colorless with some small particles in stirred suspension.

The number of fronds and the number of plants in each vessel at each time period are presented in Table 2 (attached). Increase in frond number and percent inhibition are listed in Table 3 (attached). Plant dry weights and percent inhibition are given in Table 4 (attached).

The reported no-observed-effect concentration (NOEC) for increase in frond number was 2.3 µg/l. The EC_{50} based on frond number was 5.3 µg/l (95% C.I.= 4.8-6.0 µg/l). The NOEC and EC_{50} based on dry weight were 0.12 µg/l and 3.4 (95% C.I.= 2.7-4.3 µg/l), respectively.

From day 5 onwards in the 4.6, 8.1, and 15 µg/l mean measured test concentrations, new frond growths were visibly smaller than normal, discolored, and had less root growth. These effects were also observed in the mean measured 2.3

µg/l test concentration vessels from day 12 onwards. There were no [visually] observed symptoms at, or below, the 1.1 µg/l mean measured concentration compared with the control. added

The pH in the freshly prepared solutions ranged between 4.6 and 4.8 and between 5.1 and 5.8 in the old test solutions. Temperature ranged between 25 and 26°C. Light intensity was 5.5 klux on both day 0 and 7 of the test.

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

[The reported no-observed-effect concentration (NOEC), lowest-observed-efect concentration (LOEC) and EC_{50} based on dry weight were 0.12 µg/l, 0.22 µg/l, and 3.4 (95% C.I.= 2.7-4.3 µg/l), respectively. The NOEC for increase in frond number was 2.3 µg/l. The EC_{50} based on frond number was 5.3 µg/l (95% C.I.= 4.8-6.0 µg/l).] ~~revised~~ Added

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 procedure and the report deviated from the SEP and Subdivision J guidelines in the following areas:

Three plants with 4 fronds each were used as inoculum rather than the recommended 5 plants with 3 fronds each.

The light intensity was 5.5 klux. The recommended intensity is 5 klux.

The pH of the culture medium (4.6-4.8) was lower than the recommended 5.0 ± 0.1.

[The initial measured test concentrations were about one-half the nominal test levels. The renewed test concentrations on Day 7 were at or close to nominal concentrations. Comparison of initial and final test concentrations indicate that the test concentrations were not stable between the 7-day renewal periods. Chemical losses ranged from 50 to 93 percent with a mean of loss of 72 percent. Chemical losses of over 50 percent are of potential concern for stability and certainty about whether the toxicity is due to the parent compound or degradation products.] added

B. **Statistical Analysis:** The reviewer used EPA's Toxanal program to determine the EC_{50} value and Dunnett's test to determine the NOEC and LOEC. Frond number and mean measured concentrations were used for the analysis. The results were similar to those of authors (see attached printouts).

C. **Discussion/Results:** This study is scientifically sound and meets the guideline requirements for a Tier 2 non-target aquatic plant study. Based on mean measured concentrations and inhibition of frond number, the 14-day NOEC, LOEC, and EC_{50} for *L. gibba* exposed to acetochlor were 2.3, 4.6, and 5.3 µg/l, respectively.

[Supplemental data on plant dry weight indicate that adverse effects on biomass occur and the NOEC, LOEC, and EC_{50} values are 0.12, 0.22, and 3.4 (2.7 - 4.3) µg/l, respectively. Dry weight measurements are a method for determining ecologically important trophic level effects on plant growth and biomass. Athough dry weight is not a OPP-specified endpoint, it is an ecologically important endpoint for biomass and should be considered in a risk assessment.] Added

D. **Adequacy of the Study:**

(1) **Classification:** Core.

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

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

15. **COMPLETION OF ONE-LINER:** Yes, [4 November 1993.] Revised

DATA EVALUATION RECORD

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5. **REVIEWED BY:**

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

Signature: Louis M Rifici
Date: 5/24/93

6. **APPROVED BY:**

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

Signature:
Date: 5/24/93

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

Signature:
Date:

7. **CONCLUSIONS:** This study is scientifically sound and meets the guideline requirements for a Tier 2 non-target aquatic plant study. Based on mean measured concentrations and inhibition of frond number, the 14-day NOEC, LOEC, and EC_{50} for *L. gibba* exposed to acetochlor technical were 2.3, 4.6, and 5.3 µg/l, respectively.

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

9. **BACKGROUND:**

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

ACETOCHLOR

Page_____ is not included in this copy.

Pages 7 through 11 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) _____.

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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.

RIFICI Acetochlor LEMNA GIBBA 05-24-93

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CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
15	100	88	88	0
8.100001	100	~~100~~ 85	85	~~85~~ 0
4.6	100	62	62	0
2.3	100	0	0	0
1.1	100	0	0	0
.46	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 4.173939

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS	
4	2.045824E-02	5.321081	4.747051	5.996272

5.996272

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
6	.506902	11.81212	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 = 3.785324
95 PERCENT CONFIDENCE LIMITS = 1.090285 AND 6.480363

LC50 = 5.032217
95 PERCENT CONFIDENCE LIMITS = 2.612765 AND 9.249286

LC10 = 2.324091
95 PERCENT CONFIDENCE LIMITS = .2767759 AND 3.804265

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427131-07, Lemna gibba, Acetochlor, number of fronds
File: a:42713107.dt1 Transform: NO TRANSFORMATION

Shapiro Wilks test for normality
Data PASS normality test at P=0.01 level. Continue analysis.

Bartletts test for homogeneity of variance
Data PASS homogeneity test at 0.01 level. Continue analysis.

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	8	244700.519	30587.565	73.554
Within (Error)	18	7485.333	415.852	
Total	26	252185.852		

Critical F value = 2.51 (0.05,8,18)
Since F > Critical F REJECT Ho:All groups equal

DUNNETTS TEST - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	control	238.000	238.000		
2	0.12	217.000	217.000	1.261	
3	0.22	219.000	219.000	1.141	
4	0.46	265.667	265.667	-1.662	
5	1.1	307.667	307.667	-4.184	
6	2.3	264.667	264.667	-1.602	
7	4.6	97.000	97.000	8.468	*
8	8.1	45.333	45.333	11.571	*
9	15	38.333	38.333	11.992	*

Dunnett table value = 2.58 (1 Tailed Value, P=0.05, df=18,8)

NOEC = 2.3
LOEC = 4.6

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	control	3			
2	0.12	3	42.958	18.0	21.000
3	0.22	3	42.958	18.0	19.000
4	0.46	3	42.958	18.0	-27.667
5	1.1	3	42.958	18.0	-69.667
6	2.3	3	42.958	18.0	-26.667
7	4.6	3	42.958	18.0	141.000
8	8.1	3	42.958	18.0	192.667
9	15	3	42.958	18.0	199.667

TITLE: 427131-07, Lemna gibba, Acetochlor, number of fronds
FILE: a:42713107.dt1
TRANSFORM: NO TRANSFORMATION NUMBER OF GROUPS: 9

GRP	IDENTIFICATION	REP	VALUE	TRANS VALUE
1	control	1	280.0000	280.0000
1	control	2	204.0000	204.0000
1	control	3	230.0000	230.0000
2	0.12	1	197.0000	197.0000
2	0.12	2	208.0000	208.0000
2	0.12	3	246.0000	246.0000
3	0.22	1	213.0000	213.0000
3	0.22	2	244.0000	244.0000
3	0.22	3	200.0000	200.0000
4	0.46	1	255.0000	255.0000
4	0.46	2	266.0000	266.0000
4	0.46	3	276.0000	276.0000
5	1.1	1	331.0000	331.0000
5	1.1	2	303.0000	303.0000
5	1.1	3	289.0000	289.0000
6	2.3	1	249.0000	249.0000
6	2.3	2	267.0000	267.0000
6	2.3	3	278.0000	278.0000
7	4.6	1	91.0000	91.0000
7	4.6	2	115.0000	115.0000
7	4.6	3	85.0000	85.0000
8	8.1	1	52.0000	52.0000
8	8.1	2	41.0000	41.0000
8	8.1	3	43.0000	43.0000
9	15	1	37.0000	37.0000
9	15	2	36.0000	36.0000
9	15	3	42.0000	42.0000