

DATA EVALUATION RECORD

- 1. **CHEMICAL:** Acetochlor.  
Shaughnessey Number: 121601.
- 2. **TEST MATERIAL:** 1) Technical acetochlor (ICIA5676); 2-chloro-N-(ethoxymethyl)-N-(2-ethyl-6-methyl-phenylacetamide); Batch P2; 89.4% active ingredient w/w. 2) Formulation WF2061; prepared from Technical Batch P2; 68.8% active ingredient w/w.
- 3. **STUDY TYPE:** Freshwater Invertebrate Static Toxicity Tests.  
Species Tested: Daphnids (*Daphnia magna*).
- 4. **CITATION:** Farrelly, E. and M.J. Hamer. 1989. Acetochlor: An Investigation of the Toxicity of the Technical Material and Formulation WF2061 to First Instar *Daphnia magna*. Laboratory Report No. RJ 0744B. Study performed by ICI Agrochemicals, Jealott's Hill Research Station, Bracknell, Berkshire, U.K. Submitted by ICI Americas, Inc. EPA MRID No. 415651-34.

5. **REVIEWED BY:**

Rosemary Graham Mora, M.S.  
Associate Scientist  
KBN Engineering and  
Applied Sciences, Inc.

Signature: *Rosemary Graham Mora*  
Date: *10/3/91*

6. **APPROVED BY:**

Pim Kosalwat, Ph.D.  
Senior Scientist  
KBN Engineering and  
Applied Sciences, Inc.

Signature: *P. Kosalwat*  
Date: *10/3/91*  
*Michael Davy*

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

Signature: *Henry T. Craven*  
Date: *1-15-92*  
*2-4-92*

- 7. **CONCLUSIONS:** These studies are scientifically sound and meet the guideline requirements for an acute toxicity study using freshwater invertebrates.

The 48-hour EC<sub>50</sub> of technical acetochlor for *Daphnia magna* was 8.2 mg a.i./l mean measured concentration, which classifies technical acetochlor as moderately toxic to *Daphnia magna*. The NOEC for technical acetochlor was 6.4 mg a.i./l mean measured concentration.

*10-11-91*  
*11/1/91*  
*4 tests*  
*1*

The 48-hour EC<sub>50</sub> of acetochlor formulation WF2061 for *Daphnia magna* was 7.2 mg a.i./l mean measured concentration, which classifies acetochlor formulation WF2061 as moderately toxic to *Daphnia magna*. The NOEC for acetochlor formulation WF2061 was 5.5 mg a.i./l mean measured concentration.

8. **RECOMMENDATIONS:** N/A

9. **BACKGROUND:**

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

11. **MATERIALS AND METHODS:**

A. **Test Animals:** The test organisms (*Daphnia magna*, <24 hours old) were obtained from cultures at Jealott's Hill Research Station. The daphnids were maintained in reconstituted water at 20°C on 16 hours of light per day, and fed a diet of yeast and *Chlorella vulgaris*.

B. **Test System:** The test vessels were covered 250-ml glass beakers containing 200 ml of test solution. The test vessels were held in a water bath at 20°C under fluorescent lighting (700 lux) for 16 hours per day.

The dilution water was hard reconstituted water prepared by dissolving the given reagents in deionized water.

C. **Dosage:** Forty-eight-hour static acute test. Two sets of tests (Test I and Test II) were performed using the test materials technical acetochlor and acetochlor formulation WF2061 with the following nominal concentrations: (technical acetochlor only, 0.56 and 0.93), 1.6, 2.6, 4.3, 7.2, 12.0, and 20.0 mg a.i./l for Test I; and 1.6, 2.6, 4.3, 7.2, 12.0, 20.0, and 33.0 mg a.i./l for Test II. In addition, a dilution water control was included in each set of tests.

The highest concentrations were prepared by adding appropriate amounts of test substance to reconstituted water and then serially diluted to prepare lower test concentrations.

D. **Design:** Two sets of tests were performed using both the technical and formulated test materials.

Ten daphnids were added to each triplicate vessel of each test concentration and control. The daphnids were not fed during the test.

The effect (immobilization) of the test material to the daphnids was assessed at 3, 9, 24, and 48 hours during the study.

Dissolved oxygen concentration and pH were measured at 0 and 48 hours. The temperature of the water bath was measured at each assessment time using a min/max thermometer.

Chemical analysis of each concentration was determined using high pressure liquid chromatography on samples collected at test initiation and at 48 hours.

- E. **Statistics:** The  $EC_{50}$  values were calculated using "the technique of iteratively reweighted least squares of probit response on  $\log_{10}$  (concentration). A combined  $EC_{50}$  from both tests was calculated by taking a weighted average of the individual  $\log EC_{50}$  with weight given by the inverse of the estimated variance of the  $\log EC_{50}$ ."

The no observed effects level (NOEL) for each test was calculated "by contrasting the effect at each dose with the effect in the control group using a pooled estimate of error variance from a one-way analysis of variance."

12. **REPORTED RESULTS:** Measured concentrations are given in Table 1 (attached). These mean measurements represent 79-93% of nominal concentrations of technical acetochlor, and 110-132% of nominal concentrations of acetochlor formulation WF2061. All values reported in this section are based on mean measured concentrations.

In Test I, 73-100% immobility was observed in the two highest test concentrations (10.3 and 16.6 mg a.i./l) of technical acetochlor; 0-3% immobility was observed in the remaining test concentrations (Table 4, attached). In Test II, 97-100% immobility was observed in the three highest test concentrations (10.9-29.1 mg a.i./l) of technical acetochlor; 0-7% immobility was observed in the remaining test concentrations (1.42-6.42 mg a.i./l) (Table 5, attached).

The 48-hour  $EC_{50}$  values (95% confidence interval) for Daphnia magna exposed to technical acetochlor in Tests I and

II were 9.0 (8.2-9.9) mg a.i./l and 8.1 (7.5-9.0) mg a.i./l, respectively (Table 2, attached). The NOELs for Tests I and II were 6.1 and 6.4 mg a.i./L, respectively.

In Test I, 73-100% immobility was observed in the three highest test concentrations (8.63-22.1 mg a.i./l) of acetochlor formulation WF2061; 0-7% immobility was observed in the remaining test concentrations (Table 6, attached). In Test II, 100% immobility was observed in the three highest test concentrations (15-39.4 mg a.i./l) of acetochlor formulation WF2061; 0-23% immobility was observed in the remaining test concentrations (1.92-8.66 mg a.i./l) (Table 7, attached).

The 48-hour EC<sub>50</sub> values (95% confidence interval) for Daphnia magna exposed to acetochlor formulation WF2061 in Tests I and II were 7.4 (6.7-8.1) mg a.i./l and 9.6 (9.0-13.0) mg a.i./l, respectively (Table 2, attached). The NOELs for Tests I and II were 3.1 and 5.5 mg a.i./L, respectively.

During these tests, the pH ranged from 8.1 to 8.3, the temperature ranged from 20-21°C, and the dissolved oxygen concentration ranged from 8.1 to 8.8 mg/l.

**13. STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:**  
The authors made no conclusions in the report.

A GLP compliance statement, signed by the study director and head of department, was included in the report indicating that the study conducted in accordance with the principles of Good Laboratory Practice of the United Kingdom Department of Health Compliance programme (1989). A Quality Assurance Statement was also included in the report and signed by a quality assurance officer.

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

**A. Test Procedure:** The test procedures were generally in accordance with the guidelines and the protocols recommended by the guidelines, except for the following deviations:

The report did not indicate that the reconstituted water was aged one or two weeks prior to test initiation, and intensely aerated prior to use as recommended. However, this is acceptable because the report showed dissolved oxygen concentration to be  $\geq 8.1$  mg/l.

The hardness, alkalinity, and conductivity of the dilution water was not reported, as recommended by ASTM (1980).

The test temperature was monitored at each observation period with a min/max thermometer, not every six hours as recommended for a system controlled by a water bath.

The length of time between solution preparation and test initiation was not reported.

No observations of pretest mortality or health of the source culture(s) were given in the report.

First instar test organisms should be from the fourth or later broods of a given parent. The author did not indicate which brood was the source of the test animals.

The recommended photoperiod for a freshwater invertebrates acute toxicity study is 16-hour light/8-hour dark with 15- to 30-minute transitions. Transition periods were not used in the study.

The report did not indicate whether the daphnids were randomly assigned to the test chambers as required by the SEP.

- B. Statistical Analysis:** For acetochlor formulation WF2061 Tests I and II, and for technical acetochlor Test I, EPA's Toxanal computer program was used to verify the EC<sub>50</sub> values and 95% confidence intervals presented by the authors. The EMSL computer program for probit analysis was used to determine the EC<sub>50</sub> value for technical acetochlor Test II. The data from this test (Test II of technical acetochlor) could not be analyzed using Toxanal (program aborted). The reviewer's EC<sub>50</sub> values and 95% confidence intervals are similar to those of the authors (printouts, attached). However, the reviewer does not accept the weighted average EC<sub>50</sub> presented by the authors. The lowest EC<sub>50</sub> value of the two tests performed for each test substance is accepted as the EC<sub>50</sub> value for that test material.

- C. **Discussion/Results:** The deviations listed above probably did not affect the results of these tests. These studies are scientifically sound and meet the guideline requirements for an acute static toxicity study using freshwater invertebrates.

The 48-hour EC<sub>50</sub> of technical acetochlor for *Daphnia magna* was 8.2 mg a.i./l mean measured concentrations, which classifies technical acetochlor as moderately toxic to *Daphnia magna*. The NOEC for technical acetochlor was 6.4 mg a.i./l based on mean measured concentrations.

The 48-hour EC<sub>50</sub> of acetochlor formulation WF2061 for *Daphnia magna* was 7.2 mg a.i./l mean measured concentrations, which classifies acetochlor formulation WF2061 as moderately toxic to *Daphnia magna*. The NOEC for acetochlor formulation WF2061 was 5.5 mg a.i./l based on mean measured concentrations.

- D. **Adequacy of the Study:**

- (1) **Classification:** Core.
- (2) **Rationale:** N/A.
- (3) **Repairability:** N/A.

15. **COMPLETION OF ONE-LINER FOR STUDY:** Yes, September 24, 1991.

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ACETOCHLOR

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Pages   7   through  12  are not included.

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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.
  - FIFRA registration data.
  - The document is a duplicate of page(s)           .
  - The document is not responsive to the request.
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Rosemary Graham Mora Acetochlor Daphnia magna 9-19-91

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CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB.(PERCENT)
20	30	30	100	9.313227E-08
10.3	30	22	73.33334	.8062402
6.09	30	1	3.333334	2.8871E-06
3.85	30	0	0	9.313227E-08
2.24	30	0	0	9.313227E-08
1.32	30	0	0	9.313227E-08
.77	30	0	0	9.313227E-08
.44	30	0	0	9.313227E-08

TEST I

THE BINOMIAL TEST SHOWS THAT 6.09 AND 10.3 CAN BE USED AS STATISTICALLY SOUND CONSERVATIVE 95 PERCENT CONFIDENCE LIMITS, BECAUSE THE ACTUAL CONFIDENCE LEVEL ASSOCIATED WITH THESE LIMITS IS GREATER THAN 95 PERCENT.

AN APPROXIMATE LC50 FOR THIS SET OF DATA IS 8.831817

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS	
3	.0325853	9.344828	8.363845	10.5336

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT	PROBABILITY
8	.1565914	1	1	

SLOPE = 10.79161  
 95 PERCENT CONFIDENCE LIMITS = 6.521192 AND 15.06202

LC50 = 9.014728  
 95 PERCENT CONFIDENCE LIMITS = 8.158513 AND 9.922194

LC10 = 6.874935  
 95 PERCENT CONFIDENCE LIMITS = 5.536432 AND 7.68991

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EPA PROBIT ANALYSIS PROGRAM  
 USED FOR CALCULATING EC VALUES  
 Version 1.4

ACETOCHLOR/DAPHNIA MAGNA

TEST II

Conc.	Number Exposed	Number Resp.	Observed Proportion Responding	Adjusted Proportion Responding	Predicted Proportion Responding
1.4200	30	0	0.0000	0.0000	0.0000
2.4300	30	0	0.0000	0.0000	0.0000
3.8700	30	0	0.0000	0.0000	0.0000
6.4200	30	2	0.0667	0.0667	0.0667
10.9000	30	29	0.9667	0.9667	0.9667
17.9000	30	30	1.0000	1.0000	1.0000
29.1000	30	30	1.0000	1.0000	1.0000

Chi - Square Heterogeneity = -0.000

Mu = 0.911010  
 Sigma = 0.068930

Parameter	Estimate	Std. Err.	95% Confidence Limits	
Intercept	-8.216419	2.219743	( -12.567116,	-3.865723)
Slope	14.507443	2.455727	( 9.694217,	19.320667)

Theoretical Spontaneous Response Rate = 0.0000

ACETOCHLOR/DAPHNIA MAGNA

Estimated EC Values and Confidence Limits

Point	Conc.	Lower Upper 95% Confidence Limits	
EC 1.00	5.6319	4.6267	6.3075
EC 5.00	6.2752	5.3859	6.9092
EC10.00	6.6477	5.8251	7.2721
EC15.00	6.9115	6.1323	7.5393
EC50.00	8.1472	7.4641	8.9646
EC85.00	9.6039	8.7563	11.0595
EC90.00	9.9851	9.0601	11.6656
EC95.00	10.5777	9.5150	12.6447
EC99.00	11.7859	10.3965	14.7567

Rosemary Graham Mora Acetochlor formulation WF2061 Daphnia magna 9-23-91

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CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB.(PERCENT)
22.1	30	30	100	9.313227E-08
14.2	30	30	100	9.313227E-08
8.63	30	22	73.33334	.8062402
5.11	30	2	6.666667	4.339964E-05
3.08	30	0	0	9.313227E-08
1.83	30	0	0	9.313227E-08

TEST I

THE BINOMIAL TEST SHOWS THAT 5.11 AND 8.63 CAN BE USED AS STATISTICALLY SOUND CONSERVATIVE 95 PERCENT CONFIDENCE LIMITS, BECAUSE THE ACTUAL CONFIDENCE LEVEL ASSOCIATED WITH THESE LIMITS IS GREATER THAN 95 PERCENT.

AN APPROXIMATE LC50 FOR THIS SET OF DATA IS 7.298658

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS	
5	3.258529E-02	7.225838	6.312644	8.324101

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
6	.1184818	1	.9980894

SLOPE = 9.645099  
95 PERCENT CONFIDENCE LIMITS = 6.325141 AND 12.96506

LC50 = 7.377471  
95 PERCENT CONFIDENCE LIMITS = 6.667547 AND 8.142395

LC10 = 5.447967  
95 PERCENT CONFIDENCE LIMITS = 4.461721 AND 6.116093

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Rosemary Graham Mora Acetochlor formulation WF2061 Daphnia magna 9-23-91

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CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB.(PERCENT)
39.4	30	30	100	9.313227E-08
23.4	30	30	100	9.313227E-08
15	30	30	100	9.313227E-08
8.66	30	7	23.33334	.261144
5.46	30	0	0	9.313227E-08
3.44	30	0	0	9.313227E-08
1.92	30	0	0	9.313227E-08

TEST II

THE BINOMIAL TEST SHOWS THAT 8.66 AND 15 CAN BE USED AS STATISTICALLY SOUND CONSERVATIVE 95 PERCENT CONFIDENCE LIMITS, BECAUSE THE ACTUAL CONFIDENCE LEVEL ASSOCIATED WITH THESE LIMITS IS GREATER THAN 95 PERCENT.

AN APPROXIMATE LC50 FOR THIS SET OF DATA IS 10.1043

WHEN THERE ARE LESS THAN TWO CONCENTRATIONS AT WHICH THE PERCENT DEAD IS BETWEEN 0 AND 100, NEITHER THE MOVING AVERAGE NOR THE PROBIT METHOD CAN GIVE ANY STATISTICALLY SOUND RESULTS.

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Technical Acetochlor

Study/Species/Lab/ MRID # Chemical % a.i. Results Reviewer/ Date Validation Status

48-Hour EC<sub>50</sub> 89.4% EC<sub>50</sub> - 8.2 <sup>at</sup> ppm (7.5 - 8.9) 95% C.L. Probit Control Mortality (%) - 0  
 Solvent Control Mortality (%) - NA  
 Species: Daphnia Magna Slope - 14.5 # Animals/Level - 30 Temperature - 20-21°C  
 Lab: ICI Agrochemicals 48-Hour Dose Level ppM\* / (% Effect) 9/24/91 Core  
Jealott's Hill Research 10M 1.42 (0), 2.43 (0), 3.87 (0), 6.42 (7), 10.9 (97),  
17.9 (100), 29.1 (100)  
 Comments: Based on mean measured concentrations of active ingredients

96-Hour LC<sub>50</sub> \_\_\_\_\_ LC<sub>50</sub> - pp ( \_\_\_\_\_ ) 95% C.L. Control Mortality (%) - \_\_\_\_\_  
 Solvent Control Mortality (%) - \_\_\_\_\_  
 Species: \_\_\_\_\_ Slope - \_\_\_\_\_ # Animals/Level - \_\_\_\_\_ Temperature - \_\_\_\_\_  
 Lab: \_\_\_\_\_  
 MRID # \_\_\_\_\_ 96-Hour Dose Level pp / (% Mortality) \_\_\_\_\_  
 ( \_\_\_\_\_ ), ( \_\_\_\_\_ ), ( \_\_\_\_\_ ), ( \_\_\_\_\_ )

Comments: