

US EPA ARCHIVE DOCUMENT

12-2-93

4

MRID No. 427131-05

DATA EVALUATION RECORD

- 1. **CHEMICAL:** Acetochlor. Shaughnessey No. 121601.
- 2. **TEST MATERIAL:** Acetochlor technical; Lot No. QUE-9001-1482T; CAS No. 34256-82-1; 92.07% active ingredient; a dark brown liquid.
- 3. **STUDY TYPE:** 72-4. Freshwater Invertebrate Life-Cycle Flow-Through Test. Species Tested: *Daphnia magna*.
- 4. **CITATION:** Blakemore, G.C. and M. Muckerman. 1993. Chronic Toxicity of Acetochlor to *Daphnia magna* Under Flow-Through Test Conditions. ABC Report No. 40048. Study conducted by Analytical Bio-Chemistry Laboratories, Inc., Columbia, MO. Submitted by Acetochlor Registration Partnership, c/o Monsanto Agricultural Company, St. Louis, MO. EPA MRID No. 427131-05.
- 5. **REVIEWED BY:**

William S. Rabert
 Biologist
 Ecological Effects Branch
 Environmental Fate and Effects Division

Signature: *William S. Rabert*

Date: *2 November 1993*
 (7507C)

6. **APPROVED BY:**

Dan Rieder
 Section Head
 Ecological Effects Branch
 Environmental Fate and Effects Division

Signature: *Dan Rieder 11.9.93*

Date: (7507C)

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

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

Date:

7. **CONCLUSIONS:** [This study is scientifically sound and meets the guideline requirements for a daphnid life-cycle test. Raw length data should have been submitted with the report, but their availability would not alter the final MATC value. *revised* The MATC of acetochlor for *Daphnia magna* was between 22.1 and 42.7 µg/l mean measured concentrations (geometric mean = 30.7 µg/l).

8. **RECOMMENDATIONS:**

9. **BACKGROUND:**

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

11. MATERIALS AND METHODS:

- A. **Test Animals:** First instar *Daphnia magna* were obtained from an in-house culture. The culture was maintained in a temperature-controlled area at 20 \pm 2°C in hard blended water (132-150 mg/l as CaCO₃). Cultured daphnids were fed green algae (*Selenastrum capricornutum* and/or *Ankistrodesmus falcatus*) with a trout chow and yeast suspension supplement.
- B. **Test System:** The test system was conducted under flow-through conditions using a 500-ml intermittent proportional diluter with a 0.5 dilution factor. Test vessels were 1-l glass beakers each with a notched drain covered with 50-mesh stainless steel screen. The diluter delivered water to each vessel at an average rate of 3.5 ml/minute which provided 5 volume replacements daily. The diluter was allowed to equilibrate for 7 days prior to test initiation.

A photoperiod of 16 hours of light and 8 hours of darkness with 30-minute dawn and dusk transition periods were provided. Light intensity at the water surface was 54-57 footcandles. Test temperature was maintained at 20 \pm 2°C by a temperature-controlled water bath.

The dilution water, a blend of well water and reverse osmosis water, had a pH of 8.0-8.4, a conductivity of 280-300 μ mhos/cm, and a hardness and alkalinity of 132-150 and 150-166 mg/l as CaCO₃, respectively.

Diluter stock solution (3500 mg/l) was prepared by weighing 0.35 g of the test material into a 100 ml volumetric flask. The flask was brought up to volume with dimethylformimide (DMF). The stock solution was placed in a 15-ml volumetric tube for use by the diluter system while the remaining solution was stored in a refrigerator.

- C. **Dosage:** Twenty-one-day, flow-through test. Based on results of preliminary testing and two previous definitive studies, five nominal concentrations (21.0, 42.0, 87.5, 175, and 350 μ g/l) were selected for this study. A dilution water control and a solvent control were also included. The solvent control had a DMF concentration of 0.05 ml/l which was equivalent to the solvent concentration in the highest test level.

- D. **Design:** Ten first-instar daphnids (<24 hours old) were randomly assigned to each of four replicate vessels per treatment (i.e., 40 daphnids/treatment). The daphnids were fed an algal suspension (*Selenastrum capricornutum* and *Ankistrodesmus falcatus*) which provided at least 4×10^8 cells/l. A trout chow and yeast suspension was provided twice daily. The test vessels were cleaned three times per week.

Observations of survival and abnormal effects were recorded daily. Adult reproduction was determined every Monday, Wednesday, and Friday during the test period at which time the young were counted and discarded. At test termination (day 21 of exposure), body length of each surviving adult was recorded. Mean adult dry weight for each replicate was also determined.

Dissolved oxygen concentration (DO), temperature, and pH were measured on days 0, 4, 7, 14, and 21 in alternating, duplicate replicates of the high, medium and low test concentrations and the dilution water control. Temperature was monitored continuously in the water bath. Hardness, alkalinity, pH, conductivity, DO, and temperature were also measured in the dilution water daily.

On test days 0, 4, 7, 14, and 21, water samples (12.5 ml) were collected from each of the four replicate vessels of each treatment for determination of acetochlor. Replicate samples were combined for each treatment level before chemical analysis. Analysis was performed using gas-liquid chromatography.

- E. **Statistics:** Quadruplicate test chambers were grouped and assigned to a treatment concentration which provided a nested experimental design. Survival was analyzed using frequency analysis coupled with a one-tailed Fisher's exact test and the chi-square statistic. A t-test was used to determine significant differences between the dilution water control and the solvent control. When no difference was indicated, the control data were pooled for comparison to the treatments. When a significant difference was demonstrated, the treatments were compared to the solvent control. Reproduction data were analyzed using a t-test to compare experimental design and Dunnett's one-tailed multiple means comparison procedure to determine significant differences between the treatments and control(s). Length and weight data were

analyzed using a one-way analysis of variance (ANOVA) coupled with Dunnett's one-tailed multiple means comparison procedure. All conclusions were based on a 95% confidence level.

The 21-day EC_{50} was calculated using a computer program developed by Stephan et al. (1978).

12. **REPORTED RESULTS:** Mean measured concentrations were 22.1, 42.7, 81.3, 160, and 341 $\mu\text{g/l}$ which represent 91-105% of nominal concentrations (Table VII, attached).

Survival of *Daphnia magna* after a 21-day exposure to acetochlor was significantly reduced at 160 $\mu\text{g/l}$ when compared to the pooled controls (Table X, attached). "Since this level did not follow the dose response of the other levels for survival, it was considered to be an outlier due to biological variability, and thus survival of daphnids is not considered to be statistically affected at any of the test levels." The 21-day EC_{50} was calculated to be >341 $\mu\text{g/l}$ using the binomial method.

Mean length of surviving adults and mean number of young per reproductive day were significantly reduced at 81.3, 160, and 341 $\mu\text{g/l}$ when compared to the pooled controls (Tables X and XI, attached). Mean dry weight was significantly reduced at 160 and 341 $\mu\text{g/l}$ when compared to the pooled controls (Table XII, attached).

"The mean measured test concentrations of 42.7, 81.3, 160, and 341 $\mu\text{g/l}$ were flagged as being significantly affected for days to first brood when compared to the pooled controls. However, it is our opinion the 42.7 and 81.3 $\mu\text{g/l}$ mean measured test concentrations were falsely flagged as being statistically affected. These levels did not follow a true dose response and the difference in days to first brood between the pooled controls and these test concentrations (42.7 and 81.3 $\mu\text{g/l}$) was slight 1.0 and 0.5 days, respectively. Based on these two facts, the affect was not believed to be compound related, but was due to biological variability. Therefore, days to first brood will be considered to affected only at the test concentrations of 160 and 341 $\mu\text{g/l}$ (Table XI [attached])."

During the study, the test solutions had a pH of 8.2-8.4, a DO of 7.2-8.2 mg/l, and a temperature of 20°C. The temperature in the water bath was 19-21°C.

13. **STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:**
"Based on the statistical analysis of survival, adult mean

length, adult mean weight, days to first brood, and young/adult/reproduction day from this 21-day *Daphnia magna* chronic toxicity study, MATC limits were determined to be 81.3 $\mu\text{g}/\text{l}$ (LOEC) and 42.7 $\mu\text{g}/\text{l}$ (NOEC). The geometric mean of the MATC limits is 58.9 $\mu\text{g}/\text{l}$. The day 21 EC_{50} (based on mortality) was calculated to be $>341 \mu\text{g}/\text{l}$."

A GLP compliance statement was included in the report indicating that this study was conducted in accordance with FIFRA Good Laboratory Practice Standards (40 CFR 160). A Quality Assurance statement was also included.

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

- A. Test Procedure:** An SEP for *Daphnia* chronic flow-through studies is not available at this time, thus the SEP for static-renewal studies was used as a general guidance in this data validation process.

Added [Significant reductions ($P=0.05$) in adult daphnid body length were reported at measured test concentrations of 81.3 $\mu\text{g}/\text{l}$ and higher. Visual comparison of mean body length at 22.1 $\mu\text{g}/\text{l}$ (4.66 mm) with the controls (4.67 mm) suggests that it is highly unlikely that it is significantly different. Omission of the raw data for body lengths from the final study prevents independent confirmation of the level of effect. While all raw data should be submitted for independent analyses, it is apparent that this missing data will not affect the overall MATC value for this study.]

- B. Statistical Analysis:** The reviewer used the computer program Toxstat® to analyze survival and reproduction data. The homogeneity of variance and normality of all data were examined using Hartley's test and the chi-square test, respectively. Survival data (arcsine-squareroot transformed) and time to first brood data did not meet these assumptions. Survival data were analyzed using Steel's Many-One Rank test (pages 1-4 of printouts, attached). The number of young per reproductive day data met the assumptions of homogeneity of variance and normality, therefore, this parameter was analyzed using William's test (pages 5-8 of printouts, attached). The reviewer's results for survival and number of young per reproductive day were the same as those of the authors. The time to first brood data were analyzed using the Kruskal-Wallis test which demonstrated a significant difference only at the highest test level when compared to the control (pages 9-11 of printouts, attached). However, upon visual

examination of the data, there is an obvious trend of increasing days to first brood with increasing test concentrations beginning at 42.7 $\mu\text{g}/\text{l}$. Therefore, the reviewer concludes an NOEC of 22.1 $\mu\text{g}/\text{l}$.

- C. Discussion/Results: [This study is scientifically sound and meets the guideline requirements for a chronic toxicity test using the freshwater invertebrate, *Daphnia magna*. The raw data on adult length data should be presented in the report, but for this study the data will not alter the final MATC value.] The MATC of acetochlor for *Daphnia magna* was between 22.1 and 42.7 $\mu\text{g}/\text{l}$ mean measured concentrations. *revised*

D. Adequacy of the Study:

- (1) Classification: [Core] *Revised*
(2) Rationale: N/A *Revised*
(3) Repairability: N/A *Revised*

15. COMPLETION OF ONE-LINER: [Yes, 2 November 1993.] *Revised*

DATA EVALUATION RECORD

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- 2. **TEST MATERIAL:** Acetochlor technical; Lot No. QUE-9001-1482T; CAS No. 34256-82-1; 92.07% active ingredient; a dark brown liquid.
- 3. **STUDY TYPE:** 72-4. Freshwater Invertebrate Life-Cycle Flow-Through Test. Species Tested: *Daphnia magna*.
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5. **REVIEWED BY:**

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

Signature:

Rosemary Graham Mora

Date:

3 June 1993

6. **APPROVED BY:**

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

Signature:

P. Kosalwat

Date:

6/3/93

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

Signature:

Date:

- 7. **CONCLUSIONS:** This study is scientifically sound but does not meet the guideline requirements for a daphnid life-cycle test. Raw length data were not submitted with the report. The MATC of acetochlor for *Daphnia magna* was between 22.1 and 42.7 µg/l mean measured concentrations (geometric mean = 30.7 µg/l).

Revised

8. **RECOMMENDATIONS:**

*H. T. Craven
2/2/93*

9. **BACKGROUND:**

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

ACETOCHLOR

Page _____ is not included in this copy.

Pages 8 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.
 - FIFRA registration data.
 - The document is a duplicate of page(s) _____.
 - The document is not responsive to the request.
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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.

Acetochlor: Survival of Exposed D.magna
File: C:\STATS\TOXSTAT\42713105.SUR

Transform: ARC SINE(SQUARE ROOT(Y))

Chi-square test for normality: actual and expected frequencies

INTERVAL	<-1.5	-1.5 to <-0.5	-0.5 to 0.5	>0.5 to 1.5	>1.5
EXPECTED	1.876	6.776	10.696	6.776	1.876
OBSERVED	0	2	25	1	0

Calculated Chi-Square goodness of fit test statistic = 31.1710
Table Chi-Square value (alpha = 0.01) = 13.277

Data FAIL normality test. Try another transformation.

Warning - The two homogeneity tests are sensitive to non-normal data and should not be performed.

Acetochlor: Survival of Exposed D.magna
File: C:\STATS\TOXSTAT\42713105.SUR

Transform: ARC SINE(SQUARE ROOT(Y))

Hartley test for homogeneity of variance
Bartlett's test for homogeneity of variance

These two tests can not be performed because at least one group has zero variance.

Data FAIL to meet homogeneity of variance assumption.
Additional transformations are useless.

TITLE: Acetochlor: Survival of Exposed D.magna
 FILE: C:\STATS\TOXSTAT\42713105.SUR
 TRANSFORM: ARC SINE(SQUARE ROOT(Y))

NUMBER OF GROUPS: 7

GRP	IDENTIFICATION	REP	VALUE	TRANS VALUE
1	Control	1	1.0000	1.4120
1	Control	2	1.0000	1.4120
1	Control	3	1.0000	1.4120
1	Control	4	1.0000	1.4120
2	Solvent Control	1	1.0000	1.4120
2	Solvent Control	2	1.0000	1.4120
2	Solvent Control	3	1.0000	1.4120
2	Solvent Control	4	1.0000	1.4120
3	22.1	1	1.0000	1.4120
3	22.1	2	1.0000	1.4120
3	22.1	3	1.0000	1.4120
3	22.1	4	1.0000	1.4120
4	42.7	1	1.0000	1.4120
4	42.7	2	1.0000	1.4120
4	42.7	3	1.0000	1.4120
4	42.7	4	1.0000	1.4120
5	81.3	1	1.0000	1.4120
5	81.3	2	1.0000	1.4120
5	81.3	3	1.0000	1.4120
5	81.3	4	1.0000	1.4120
6	160	1	0.8900	1.2327
6	160	2	0.9000	1.2490
6	160	3	0.9000	1.2490
6	160	4	1.0000	1.4120
7	341	1	0.9000	1.2490
7	341	2	1.0000	1.4120
7	341	3	1.0000	1.4120
7	341	4	1.0000	1.4120

Acetochlor: Survival of Exposed D.magna
File: C:\STATS\TOXSTAT\42713105.SUR

Transform: ARC SINE(SQUARE ROOT(Y))

STEELS MANY-ONE RANK TEST

- Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	RANK SUM	CRIT. VALUE	df	SIG
1	Control	1.412				
2	Solvent Control	1.412	18.00	10.00	4.00	
3	22.1	1.412	18.00	10.00	4.00	
4	42.7	1.412	18.00	10.00	4.00	
5	81.3	1.412	18.00	10.00	4.00	
6	160	1.286	12.00	10.00	4.00	
7	341	1.371	16.00	10.00	4.00	

Critical values use $k = 6$, are 1 tailed, and $\alpha = 0.05$

Acetochlor: Reproduction of Exposed D.magna

File: C:\STATS\TOXSTAT\42713105.REP

Transform: NO TRANSFORMATION

Hartley test for homogeneity of variance

Calculated H statistic (max Var/min Var) = 8.87

Closest, conservative, Table H statistic = 216.0 (alpha = 0.01)

Used for Table H ==> R (# groups) = 7, df (# reps-1) = 3

Actual values ==> R (# groups) = 7, df (# avg reps-1) = 3.00

Data PASS homogeneity test. Continue analysis.

NOTE: This test requires equal replicate sizes. If they are unequal but do not differ greatly, the Hartley test may still be used as an approximate test (average df are used).

Acetochlor: Reproduction of Exposed D.magna

File: C:\STATS\TOXSTAT\42713105.REP

Transform: NO TRANSFORMATION

Chi-square test for normality: actual and expected frequencies

INTERVAL	<-1.5	-1.5 to <-0.5	-0.5 to 0.5	>0.5 to 1.5	>1.5
EXPECTED	1.876	6.776	10.696	6.776	1.876
OBSERVED	0	7	11	10	0

Calculated Chi-Square goodness of fit test statistic = 5.3020

Table Chi-Square value (alpha = 0.01) = 13.277

Data PASS normality test. Continue analysis.

TITLE: Acetochlor: Reproduction of Exposed D.magna
FILE: C:\STATS\TOXSTAT\42713105.REP
TRANSFORM: NO TRANSFORMATION

NUMBER OF GROUPS: 7

GRP	IDENTIFICATION	REP	VALUE	TRANS VALUE
1	Control	1	12.7000	12.7000
1	Control	2	11.7000	11.7000
1	Control	3	12.0000	12.0000
1	Control	4	12.2000	12.2000
2	Solvent Control	1	11.9000	11.9000
2	Solvent Control	2	12.4000	12.4000
2	Solvent Control	3	12.2000	12.2000
2	Solvent Control	4	10.5000	10.5000
3	22.1	1	13.8000	13.8000
3	22.1	2	12.0000	12.0000
3	22.1	3	12.5000	12.5000
3	22.1	4	12.7000	12.7000
4	42.7	1	12.0000	12.0000
4	42.7	2	11.5000	11.5000
4	42.7	3	9.9600	9.9600
4	42.7	4	11.7000	11.7000
5	81.3	1	5.4900	5.4900
5	81.3	2	4.1700	4.1700
5	81.3	3	6.3100	6.3100
5	81.3	4	6.1300	6.1300
6	160	1	4.2500	4.2500
6	160	2	4.2800	4.2800
6	160	3	4.6400	4.6400
6	160	4	3.5500	3.5500
7	341	1	2.4300	2.4300
7	341	2	1.9900	1.9900
7	341	3	2.6800	2.6800
7	341	4	2.6800	2.6800

Acetochlor: Reproduction of Exposed D.magna

File: C:\STATS\TOXSTAT\42713105.REP

Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	Control	4	12.150	12.150	12.217
2	Solvent Control	4	11.750	11.750	12.217
3	22.1	4	12.750	12.750	12.217
4	42.7	4	11.290	11.290	11.290
5	81.3	4	5.525	5.525	5.525
6	160	4	4.180	4.180	4.180
7	341	4	2.445	2.445	2.445

Acetochlor: Reproduction of Exposed D.magna

File: C:\STATS\TOXSTAT\42713105.REP

Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
Control	12.217				
Solvent Control	12.217	0.132		1.72	k= 1, v=21
22.1	12.217	0.132		1.80	k= 2, v=21
42.7	11.290	1.703		1.83	k= 3, v=21
81.3	5.525	13.116	*	1.84	k= 4, v=21
160	4.180	15.779	*	1.85	k= 5, v=21
341	2.445	19.213	*	1.85	k= 6, v=21

s = 0.714

Note: df used for table values are approximate when v > 20.

Acetochlor: Time to 1st Brood of Exposed D.magna
File: 42713105.brd Transform: NO TRANSFORMATION

Chi-square test for normality: actual and expected frequencies

INTERVAL	<-1.5	-1.5 to <-0.5	-0.5 to 0.5	>0.5 to 1.5	>1.5
EXPECTED	1.876	6.776	10.696	6.776	1.876
OBSERVED	0	3	23	2	0

Calculated Chi-Square goodness of fit test statistic = 23.3763
Table Chi-Square value (alpha = 0.01) = 13.277

Data FAIL normality test. Try another transformation.

Warning - The two homogeneity tests are sensitive to non-normal data and should not be performed.

Acetochlor: Time to 1st Brood of Exposed D.magna
File: 42713105.brd Transform: NO TRANSFORMATION

Hartley test for homogeneity of variance
Bartlett's test for homogeneity of variance

These two tests can not be performed because at least one group has zero variance.

Data FAIL to meet homogeneity of variance assumption.
Additional transformations are useless.

Acetochlor: Time to 1st Brood of Exposed D.magna
 File: 42713105.brd Transform: NO TRANSFORM

KRUSKAL-WALLIS ANOVA BY RANKS - TABLE 1 OF 2 (p=0.05)

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	RANK SUM
1	Control	7.000	7.000	30.000
2	Solvent Control	7.000	7.000	30.000
3	22.1	7.000	7.000	30.000
4	42.7	8.000	8.000	72.000
5	81.3	7.500	7.500	51.000
6	160	8.750	8.750	87.000
7	341	10.000	10.000	106.000

Calculated H Value = 24.774 Critical H Value Table = 12.590
 Since Calc H > Crit H REJECT Ho: All groups are equal.

Acetochlor: Time to 1st Brood of Exposed D.magna
 File: 42713105.brd Transform: NO TRANSFORM

DUNNS MULTIPLE COMPARISON - KRUSKAL-WALLIS - TABLE 2 OF 2 (p=0.05)

GROUP	IDENTIFICATION	TRANSFORMED MEAN	ORIGINAL MEAN	GROUP						
				0	0	0	0	0	0	0
2	Solvent Control	7.000	7.000	0	0	0	0	0	0	0
3	22.1	7.000	7.000
1	Control	7.000	7.000
5	81.3	7.500	7.500
4	42.7	8.000	8.000
6	160	8.750	8.750
7	341	10.000	10.000	*	*	*

* = significant difference (p=0.05) . = no significant difference
 Table q value (0.05,7) = 3.038 SE = 5.383

11. MATERIALS AND METHODS:

- A. **Test Animals:** First instar *Daphnia magna* were obtained from an in-house culture. The culture was maintained in a temperature-controlled area at 20 \pm 2°C in hard blended water (132-150 mg/l as CaCO₃). Cultured daphnids were fed green algae (*Selenastrum capricornutum* and/or *Ankistrodesmus falcatus*) with a trout chow and yeast suspension supplement.
- B. **Test System:** The test system was conducted under flow-through conditions using a 500-ml intermittent proportional diluter with a 0.5 dilution factor. Test vessels were 1-l glass beakers each with a notched drain covered with 50-mesh stainless steel screen. The diluter delivered water to each vessel at an average rate of 3.5 ml/minute which provided 5 volume replacements daily. The diluter was allowed to equilibrate for 7 days prior to test initiation.

A photoperiod of 16 hours of light and 8 hours of darkness with 30-minute dawn and dusk transition periods were provided. Light intensity at the water surface was 54-57 footcandles. Test temperature was maintained at 20 \pm 2°C by a temperature-controlled water bath.

The dilution water, a blend of well water and reverse osmosis water, had a pH of 8.0-8.4, a conductivity of 280-300 μ mhos/cm, and a hardness and alkalinity of 132-150 and 150-166 mg/l as CaCO₃, respectively.

Diluter stock solution (3500 mg/l) was prepared by weighing 0.35 g of the test material into a 100 ml volumetric flask. The flask was brought up to volume with dimethylformimide (DMF). The stock solution was placed in a 15-ml volumetric tube for use by the diluter system while the remaining solution was stored in a refrigerator.

- C. **Dosage:** Twenty-one-day, flow-through test. Based on results of preliminary testing and two previous definitive studies, five nominal concentrations (21.0, 42.0, 87.5, 175, and 350 μ g/l) were selected for this study. A dilution water control and a solvent control were also included. The solvent control had a DMF concentration of 0.05 ml/l which was equivalent to the solvent concentration in the highest test level.

- D. **Design:** Ten first-instar daphnids (<24 hours old) were randomly assigned to each of four replicate vessels per treatment (i.e., 40 daphnids/treatment). The daphnids were fed an algal suspension (*Selenastrum capricornutum* and *Ankistrodesmus falcatus*) which provided at least 4×10^8 cells/l. A trout chow and yeast suspension was provided twice daily. The test vessels were cleaned three times per week.

Observations of survival and abnormal effects were recorded daily. Adult reproduction was determined every Monday, Wednesday, and Friday during the test period at which time the young were counted and discarded. At test termination (day 21 of exposure), body length of each surviving adult was recorded. Mean adult dry weight for each replicate was also determined.

Dissolved oxygen concentration (DO), temperature, and pH were measured on days 0, 4, 7, 14, and 21 in alternating, duplicate replicates of the high, medium and low test concentrations and the dilution water control. Temperature was monitored continuously in the water bath. Hardness, alkalinity, pH, conductivity, DO, and temperature were also measured in the dilution water daily.

On test days 0, 4, 7, 14, and 21, water samples (12.5 ml) were collected from each of the four replicate vessels of each treatment for determination of acetochlor. Replicate samples were combined for each treatment level before chemical analysis. Analysis was performed using gas-liquid chromatography.

- E. **Statistics:** Quadruplicate test chambers were grouped and assigned to a treatment concentration which provided a nested experimental design. Survival was analyzed using frequency analysis coupled with a one-tailed Fisher's exact test and the chi-square statistic. A t-test was used to determine significant differences between the dilution water control and the solvent control. When no difference was indicated, the control data were pooled for comparison to the treatments. When a significant difference was demonstrated, the treatments were compared to the solvent control. Reproduction data were analyzed using a t-test to compare experimental design and Dunnett's one-tailed multiple means comparison procedure to determine significant differences between the

treatments and control(s). Length and weight data were analyzed using a one-way analysis of variance (ANOVA) coupled with Dunnett's one-tailed multiple means comparison procedure. All conclusions were based on a 95% confidence level.

The 21-day EC₅₀ was calculated using a computer program developed by Stephan et al. (1978).

12. **REPORTED RESULTS:** Mean measured concentrations were 22.1, 42.7, 81.3, 160, and 341 µg/l which represent 91-105% of nominal concentrations (Table VII, attached).

Survival of *Daphnia magna* after a 21-day exposure to acetochlor was significantly reduced at 160 µg/l when compared to the pooled controls (Table X, attached). "Since this level did not follow the dose response of the other levels for survival, it was considered to be an outlier due to biological variability, and thus survival of daphnids is not considered to be statistically affected at any of the test levels." The 21-day EC₅₀ was calculated to be >341 µg/l using the binomial method.

Mean length of surviving adults and mean number of young per reproductive day were significantly reduced at 81.3, 160, and 341 µg/l when compared to the pooled controls (Tables X and XI, attached). Mean dry weight was significantly reduced at 160 and 341 µg/l when compared to the pooled controls (Table XII, attached).

"The mean measured test concentrations of 42.7, 81.3, 160, and 341 µg/l were flagged as being significantly affected for days to first brood when compared to the pooled controls. However, it is our opinion the 42.7 and 81.3 µg/l mean measured test concentrations were falsely flagged as being statistically affected. These levels did not follow a true dose response and the difference in days to first brood between the pooled controls and these test concentrations (42.7 and 81.3 µg/l) was slight 1.0 and 0.5 days, respectively. Based on these two facts, the affect was not believed to be compound related, but was due to biological variability. Therefore, days to first brood will be considered to be affected only at the test concentrations of 160 and 341 µg/l (Table XI [attached])."

During the study, the test solutions had a pH of 8.2-8.4, a DO of 7.2-8.2 mg/l, and a temperature of 20°C. The temperature in the water bath was 19-21°C.

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

"Based on the statistical analysis of survival, adult mean length, adult mean weight, days to first brood, and young/adult/reproduction day from this 21-day *Daphnia magna* chronic toxicity study, MATC limits were determined to be 81.3 µg/l (LOEC) and 42.7 µg/l (NOEC). The geometric mean of the MATC limits is 58.9 µg/l. The day 21 EC₅₀ (based on mortality) was calculated to be >341 µg/l."

A GLP compliance statement was included in the report indicating that this study was conducted in accordance with FIFRA Good Laboratory Practice Standards (40 CFR 160). A Quality Assurance statement was also included.

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

- A. **Test Procedure:** An SEP for *Daphnia* chronic flow-through studies is not available at this time, thus the SEP for static-renewal studies was used as a general guidance in this data validation process.

Expanded

Raw length data were not presented to allow independent statistical analysis.

- B. **Statistical Analysis:** The reviewer used the computer program Toxstat® to analyze survival and reproduction data. The homogeneity of variance and normality of all data were examined using Hartley's test and the chi-square test, respectively. Survival data (arcsine-squareroot transformed) and time to first brood data did not meet these assumptions. Survival data were analyzed using Steel's Many-One Rank test (pages 1-4 of printouts, attached). The number of young per reproductive day data met the assumptions of homogeneity of variance and normality, therefore, this parameter was analyzed using William's test (pages 5-8 of printouts, attached). The reviewer's results for survival and number of young per reproductive day were the same as those of the authors. The time to first brood data were analyzed using the Kruskal-Wallis test which demonstrated a significant difference only at the highest test level when compared to the control (pages 9-11 of printouts, attached). However, upon visual examination of the data, there is an obvious trend of increasing days to first brood with increasing test concentrations beginning at 42.7 µg/l. Therefore, the reviewer concludes an NOEC of 22.1 µg/l.

Changed and added.

C. Discussion/Results: This study is scientifically sound but does ~~not~~ meet the guideline requirements. No raw length data were presented in the report. The MATC of acetochlor for *Daphnia magna* was between 22.1 and 42.7 $\mu\text{g/l}$ mean measured concentrations. The geometric mean of the MATC was 30.7 $\mu\text{g/l}$.

D. Adequacy of the Study:

- (1) Classification: Supplemental. *Core*
- (2) Rationale: Raw length data were not presented in the report. *Unnecessary, for MATC determination*
- (3) Repairability: Yes, upon satisfactory review of raw length data.

15. COMPLETION OF ONE-LINER: Yes, 13 May 1993.