12-2-93

MRID No. 415651-38

DATA EVALUATION RECORD

1. **CHEMICAL:** Acetochlor.
Shaughnessey No. 121601.

2. **TEST MATERIAL:** Acetochlor (ICIA5676); 2-chloro-N-ethoxymethyl-6'-ethylacet-o-toluidide; Reference No. 11758-43; 89.4% active ingredient w/w.

3. **STUDY TYPE:** *Daphnia magna* Life-Cycle (21-Day Renewal) Chronic Toxicity Test. Species Tested: *Daphnia magna*.

4. **CITATION:** Rapley, J.H. and M.J. Hamer. 1990. Acetochlor: *Daphnia magna* Life-Cycle Study. Report No. RJ0785B. Prepared by ICI Agrochemicals, Jealott's Hill Research Station, Bracknell, Berkshire, UK. Submitted by ICI Americas, Inc., Wilmington, DE. EPA MRID No. 415651-38.

5. **REVIEWED BY:**

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Signature: Louis M Rifici
Date: 10/4/91

6. **APPROVED BY:**

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Supervisor, EEB/EFED
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Date: H. T. Craven 12/2/93

7. **CONCLUSIONS:** This test is scientifically sound and meets the guideline requirements for a chronic toxicity test using the freshwater invertebrate, *Daphnia magna*. Based on mean measured concentrations, the 21-day LC_{50} value was 2.2 mg/l. Based on the most sensitive biological parameters, daphnid survival and reproduction, the MATC was >1.24 and <2.45 mg/l mean measured concentrations.

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

9. **BACKGROUND:**

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

11. MATERIALS AND METHODS:

A. Test Animals: The *Daphnia magna* (<24 hours old) used were taken from individual cultures maintained in reconstituted water at 20°C on a 16-hour daylight photoperiod. The cultures were fed a diet of yeast and *Chlorella vulgaris*.

B. Test System: Vessels used in the test were 250-ml glass beakers containing 200 ml of dilution water (control) or test solution. The vessels were arranged in a randomized block design in a temperature-controlled water bath (20 ±1°C). A 16-hour daylight photoperiod was provided by fluorescent tubes with a light intensity of approximately 800 lux at the surface of the test solutions.

The test dilution water was hard reconstituted water prepared by dissolving $NaHCO_3$ (192 mg/l), $CaSO_4.2H_2O$ (120 mg/l), $MgSO_4.7H_2O$ (245 mg/l), and KCl (8 mg/l) in deionized water. The same batch of reconstituted water was used throughout the test.

C. Dosage: Twenty-one-day static-renewal test. Five nominal concentrations (0.625, 1.25, 2.5, 5, and 10 mg a.i./l) and a dilution water control were used. The highest concentration was prepared by direct addition of test material to the dilution water. The lowest four concentrations were prepared by serial dilution.

D. Design: Seven replicate beakers per concentration contained one daphnid per beaker (for observations of growth and reproduction) and three beakers per concentration contained five daphnids per beaker (for observations of survival only). Assessments of survival were made on days 3, 5, 7, 10, 12, 14, 17, 19, and 21. Every Monday, Wednesday, and Friday, the first generation daphnids were transferred to freshly-prepared test solutions. After the adult had been removed from the old test solution, the solution was poured through a fine mesh screen. The trapped young were resuspended in test solution, counted, and discarded. The number of young produced in each beaker was determined on days 7, 10, 12, 14, 17, 19, and 21. At renewal, *Chlorella vulgaris* (3.5×10^8 cells/l) and yeast (7 mg/l) were added to each test solution. The lengths of the surviving first generation daphnids were determined at the end of the test using a

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stereomicroscope equipped with a calibrated graticule lens.

Dissolved oxygen concentration and pH were measured in the freshly prepared solutions on days 0, 7, and 14, and in the old solutions on days 3, 7, 14, and 21 for all test concentrations with surviving daphnids. The temperature of the water bath was recorded continuously using a minimum/maximum thermometer. The alkalinity, total hardness, and specific conductivity of the dilution water were determined on days 0 and 19.

The concentration of the test material was measured by HPLC analysis. The freshly prepared solutions were sampled on days 0, 3, 5, 7, 10, 12, 14, 17, and 19. The old solutions were sampled on days 3, 7, 14, and 21. Due to 100% mortality at 10 mg/l, no samples were analyzed past day 3.

E. **Statistics:** The median lethal concentrations (LC_{50}) and associated 95% confidence intervals (C.I.) for several study periods were calculated using probit analysis (SAS Version 5.18). The length of the first generation daphnids and the number of young per female reproductive day were analyzed using analysis of variance.

12. **REPORTED RESULTS:** The mean measured concentrations of the newly prepared test solutions were 0.61, 1.24, 2.45, 5.63, and 10.78 mg/l (Table 1, attached). These values represent 98-113% of nominal concentrations. Measured concentrations were fairly consistent between sampling periods.

Mortality data were presented in Table 6 (attached). The 21-day LC_{50} value, based on nominal concentrations, was 2.5 mg/l. Daphnid reproduction was significantly reduced at nominal concentrations greater than 1.25 mg/l (Table 3, attached). The test material had no effect on daphnid growth at levels less than 10 mg/l nominal (Table 7, attached). No daphnids survived at 10 mg/l.

Based on the most sensitive biological parameter, daphnid reproduction, the no observed effect concentration (NOEC) was 1.25 mg/l nominal concentration. The maximum acceptable toxicant concentration (MATC) was therefore >1.25 mg/l and <2.5 mg/l nominal concentrations.

Dissolved oxygen levels were ≥7.6 mg/l (83% of saturation) when measured and the pH values ranged from 7.6-8.5. The

temperature was 19.5-20.5°C. The alkalinity, hardness, and conductivity of the dilution water were 115-120 mg/l, 170 mg/l, and 560-590 µS/cm, respectively.

13. **STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:** The authors stated no conclusions other than those previously mentioned.

Good Laboratory Practice and Quality Assurance Unit statements were included in the report indicating the study was conducted in accordance with USEPA GLP Regulations.

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

A. **Test Procedure:** The test procedures were generally in accordance with the SEP and ASTM (1988), but deviated as follows:

The conductivity, hardness, and alkalinity of the exposure solutions were not determined. The SEP states that these parameters must be determined for the control and at least one test concentration once per week.

The report did not state whether the daphnids were randomly placed in the test beakers. Test daphnids must be randomly distributed to the test vessels.

Daphnid dry weight is preferable to length as the measurement of growth. Length was used in this study.

The report did not state whether the recommended 15-30 minute transition period between light and dark was used.

Observations of the daphnid cultures such as adult mortality, stress, and the presence of ephippia were not given in the report.

B. **Statistical Analysis:** The reviewer used EPA's Toxanal program and the mean measured concentrations to calculate the 21-day LC_{50} value as 2.2 mg/l (95% C.I. = 1.7-2.8 mg/l) by the probit method (see attached printout 1). The slope of the dose-response curve was 5.2. Survival in the three replicates containing 5 daphnids each was analyzed using a non-parametric procedure (Toxstat Version 3.3). Though no significant differences between the means were determined using the

Kruskal-Wallace test (see attached printouts 2 and 3), there was no survival at the two highest concentrations and less than 50% survival at 2.45 mg/l mean measured concentration. The reviewer considers survival at the three highest levels to be significantly different from the control. Reproduction (total number of young produced per daphnid) was analyzed using one-way ANOVA and Bonferroni's t-test. Reproduction at concentrations ≥2.45 mg/l (mean measured) was significantly lower than the control (see attached printouts 4 and 5). Because only a single daphnid survived and was measured at 5.63 mg/l (mean measured), growth was analyzed using the general linear models procedure (Minitab Version 7). The F-test was not significant (P = 0.946) so no means testing was attempted (printout 6, attached).

C. **Discussion/Results:** This test is scientifically sound and meets the guideline requirements for a chronic toxicity test using the freshwater invertebrate, *Daphnia magna*. Based on mean measured concentrations, the 21-day LC_{50} value was 2.2 mg/l. Based on the most sensitive biological parameters, daphnid survival and reproduction, the MATC was >1.24 and <2.45 mg/l 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, 09-23-91.

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ACETOCHLOR

Page_____ is not included in this copy.

Pages 6 through 9 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.

Printout 1

NOTE: THERE WAS CONTROL MORTALITY, BUT AT LEAST ONE
OF THE LOWER CONCENTRATIONS HAD ZERO MORTALITY.
THEREFORE, ABBOTT'S CORRECTION IS NOT APPLICABLE.

RIFICI ACETOCHLOR DAPHNIA MAGNA 9-23-91

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CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB.(PERCENT)
10.78	15	15	100	3.051758E-03
5.63	15	15	100	3.051758E-03
2.45	15	8	53.33334	50
1.24	15	2	13.33333	.3692627
.61	15	0	0	3.051758E-03

THE BINOMIAL TEST SHOWS THAT 1.24 AND 5.63 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 2.326388

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS	
4	7.132525E-02	2.174096	1.642144 — 2.804762	

2.804762

LR
9/23/91

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
6	.2023112	1	.8626531

SLOPE = 5.184553
95 PERCENT CONFIDENCE LIMITS = 2.852592 AND 7.516514

LC50 = 2.194889
95 PERCENT CONFIDENCE LIMITS = 1.74214 AND 2.827148

LC10 = 1.248689
95 PERCENT CONFIDENCE LIMITS = .748624 AND 1.599549

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Printout 2

TITLE: 415651-38, ACETOCHLOR, DAPHNID SURVIVAL, 21 DAYS
FILE: A:41565138.DAM
TRANSFORM: ARC SINE(SQUARE ROOT(Y)) NUMBER OF GROUPS: 6

GRP	IDENTIFICATION	REP	VALUE	TRANS VALUE
1	CONTROL	1	0.8000	1.1071
1	CONTROL	2	0.8000	1.1071
1	CONTROL	3	1.0000	1.3453
2	0.61	1	1.0000	1.3453
2	0.61	2	1.0000	1.3453
2	0.61	3	1.0000	1.3453
3	1.24	1	0.6000	0.8861
3	1.24	2	1.0000	1.3453
3	1.24	3	1.0000	1.3453
4	2.45	1	0.2000	0.4636
4	2.45	2	0.8000	1.1071
4	2.45	3	0.4000	0.6847
5	5.63	1	0.0000	0.2255
5	5.63	2	0.0000	0.2255
5	5.63	3	0.0000	0.2255
6	10.78	1	0.0000	0.2255
6	10.78	2	0.0000	0.2255
6	10.78	3	0.0000	0.2255

Shapiro Wilks test for normality

D = 0.392
W = 0.875
Critical W (P = 0.05) (n = 18) = 0.897
Critical W (P = 0.01) (n = 18) = 0.858

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

Hartley test for homogeneity of variance
Bartletts 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.

Printout 3

415651-38, ACETOCHLOR, DAPHNID SURVIVAL, 21 DAYS
File: A:41565138.DAM Transform: ARC SINE(SQUARE ROOT(Y))

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	1.187	0.867	37.500
2	0.61	1.345	1.000	46.500
3	1.24	1.192	0.867	40.000
4	2.45	0.752	0.467	26.000
5	5.63	0.226	0.000	10.500
6	10.78	0.226	0.000	10.500

Calculated H Value = 15.088 Critical H Value Table = 11.070
Since Calc H > Crit H REJECT Ho:All groups are equal.

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 5 6 4 1 3 2
5	5.63	0.226	0.000	\
6	10.78	0.226	0.000	. \
4	2.45	0.752	0.467	. . \
1	CONTROL	1.187	0.867	. . . \
3	1.24	1.192	0.867	. . . . \
2	0.61	1.345	1.000	. . . . . \

* = significant difference (p=0.05) . = no significant difference
Table q value (0.05,6) = 2.936 SE = 4.189

Printout 4

TITLE: 415651-38, ACETOCHLOR, DAPHNID REPRODUCTION, 21 DAYS
FILE: A:41565138.DM3
TRANSFORM: NO TRANSFORM NUMBER OF GROUPS: 5

GRP	IDENTIFICATION	REP	VALUE
1	CONTROL	1	132.0000
1	CONTROL	2	139.0000
1	CONTROL	3	125.0000
1	CONTROL	4	109.0000
1	CONTROL	5	71.0000
1	CONTROL	6	135.0000
1	CONTROL	7	28.0000
2	0.61	1	123.0000
2	0.61	2	139.0000
2	0.61	3	143.0000
2	0.61	4	109.0000
2	0.61	5	108.0000
2	0.61	6	152.0000
2	0.61	7	138.0000
3	1.24	1	124.0000
3	1.24	2	106.0000
3	1.24	3	84.0000
3	1.24	4	13.0000
3	1.24	5	72.0000
3	1.24	6	114.0000
3	1.24	7	103.0000
4	2.45	1	0.0000
4	2.45	2	23.0000
4	2.45	3	94.0000
4	2.45	4	61.0000
4	2.45	5	9.0000
4	2.45	6	118.0000
4	2.45	7	81.0000
5	5.63	1	23.0000
5	5.63	2	34.0000
5	5.63	3	56.0000
5	5.63	4	0.0000
5	5.63	5	1.0000
5	5.63	6	0.0000

Shapiro Wilks test for normality

D = 35496.000
W = 0.954
Critical W (P = 0.05) (n = 34) = 0.933
Critical W (P = 0.01) (n = 34) = 0.908

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

Printout 5

415651-38, ACETOCHLOR, DAPHNID REPRODUCTION, 21 DAYS
File: A:41565138.DM3 Transform: NO TRANSFORMATION

Bartletts test for homogeneity of variance

Calculated B statistic = 6.33
Table Chi-square value = 13.28 (alpha = 0.01)
Table Chi-square value = 9.49 (alpha = 0.05)
Average df used in calculation ==> df (avg n - 1) = 5.80
Used for Chi-square table value ==> df (#groups-1) = 4

Data PASS homogeneity test at 0.01 level. Continue analysis.

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	4	49312.029	12328.007	10.072
Within (Error)	29	35496.000	1224.000	
Total	33	84808.029		

Critical F value = 2.70 (0.05,4,29)
Since F > Critical F REJECT Ho:All groups equal

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

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	CONTROL	105.571	105.571		
2	0.61	130.286	130.286	-1.322	
3	1.24	88.000	88.000	0.940	
4	2.45	55.143	55.143	2.697	*
5	5.63	19.000	19.000	4.448	*

Bonferroni T table value = 2.36 (1 Tailed Value, P=0.05, df=29,4)

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	CONTROL	7			
2	0.61	7	44.208	41.9	-24.714
3	1.24	7	44.208	41.9	17.571
4	2.45	7	44.208	41.9	50.429
5	5.63	6	46.013	43.6	86.571

Printout 6

415651-38 RAW DATA

ROW	CONC	LENGTH
1	1	4.25
2	1	4.54
3	1	4.61
4	1	4.25
5	1	4.68
6	2	4.54
7	2	4.25
8	2	4.32
9	2	4.46
10	2	4.25
11	2	4.54
12	2	4.46
13	3	4.46
14	3	4.32
15	3	4.61
16	3	4.46
17	3	4.25
18	4	4.61
19	4	4.46
20	4	4.32
21	5	4.39

GENERAL LINEAR MODELS PROCEDURE

Factor	Levels	Values
CONC	5	1 2 3 4 5

Analysis of Variance for LENGTH

Source	DF	Seq SS	Adj SS	Adj MS	F	P
CONC	4	0.01707	0.01707	0.00427	0.18	0.946
Error	16	0.38333	0.38333	0.02396		
Total	20	0.40040				

Unusual Observations for C2

Obs.	C2	Fit	Stdev.Fit	Residual	St.Resid
21	4.39000	4.39000	0.15478	0.00000	* X

X denotes an obs. whose X value gives it large influence.