1-15-92

MRID No. 415651-36

DATA EVALUATION RECORD

1. **CHEMICAL:** Acetochlor.
Shaughnessey Number: 121601.

2. **TEST MATERIAL:** Acetochlor technical; 2-chloro-N-(ethoxymethyl)-N-(2-ethyl-6-methylphenylacetamide); 89.6% w/w active ingredient; a brown liquid.

3. **STUDY TYPE:** Mollusc 48-hour Embryo-Larval Study. Species Tested: Pacific Oyster (*Crassostrea gigas*).

4. **CITATION:** Thompson, R.S. and J.F. Tapp. 1989. Acetochlor Determination of Acute Toxicity to Larvae of the Pacific Oyster (*Crassostrea gigas*). Brixham Study No. R1072/H. Study performed by Imperial Chemical Industries PLC, Brixham Laboratory, Freshwater Quarry, Brixham, Devon, U.K. Submitted by ICI Americas, Inc. EPA MRID No. 415651-36.

5. **REVIEWED BY:**

Rosemary Graham Mora, M.S.
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Signature: Rosemary G. Mora
Date: 10/3/91

6. **APPROVED BY:**

Pim Kosalwat, Ph.D.
Senior Scientist
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Signature: P. Kosalwat
Date: 10/3/91

Henry T. Craven, M.S.
Supervisor, EEB/EFED
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Signature: Michael Craven 1-15-92
Date: for Daniel Rieder

7. **CONCLUSIONS:** This study is scientifically sound and meets the guideline requirements for an acute toxicity study using oyster larvae. The 48-hour EC_{50} values based on percentage of normal larvae and survival for *Crassostrea gigas* were 8.0 and 15.5 mg/l mean measured concentrations as test material, respectively. Based on the most sensitive parameter measured (development of larvae), acetochlor technical is classified as moderately toxic to *Crassostrea gigas*. The NOEC was 6.2 mg/l mean measured concentrations as whole test material.

8. **RECOMMENDATIONS:** N/A

9. **BACKGROUND:**

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

11. **MATERIALS AND METHODS:**

A. **Test Animals:** Embryos of the Pacific oyster (*Crassostrea gigas*) were obtained by inducing female oysters to spawn. Adult oysters, with a shell length of 88-108 mm, were obtained approximately 35 days prior to test initiation from Surfside Oysters, Paignton, Devon. The oysters were held in flowing seawater (>20 l/day/oyster) at 8 ±2°C. The temperature was increased (<2°C/day) to 18.6°C over the final 7 days prior to testing.

Individual oysters were cleaned of debris and fouling organisms and transferred to beakers containing filtered (1 µm) seawater. Females were induced to spawn by briefly raising the water temperature to 25-28°C, followed by the addition of heat-deactivated sperm suspension to the water. The egg suspension from a single female was fertilized with sperm from excised gonad of one male to provide the embryo suspension. The suspension was maintained at 20 ±1°C with gentle agitation by an orbital shaker, until used to inoculate the test solutions. Embryos (3.4 hours post-fertilization) were used to initiate the test.

B. **Test System:** The study was conducted in 250-ml glass beakers with loose fitting lids. Duplicates of each control and test substance concentration were prepared, each containing 200 ml of test solution. The test temperature was maintained at 20 ±1°C by controlling the room temperature.

The dilution water was seawater collected from Tor Bay, Devon. The dilution water was natural seawater which was adjusted to a salinity of 32 ±2 parts per thousand (ppt) with distilled water, and filtered (0.2 µm) before use.

C. **Dosage:** Mollusc 48-hour embryo-larval static test. Eight nominal concentrations were used in this study (1.0, 1.8, 3.2, 5.6, 10.0, 18.0, 32.0, and 56 mg/l). In addition, a dilution water control was also included.

The solution for the highest concentration (56 mg/l) was prepared by dissolving 0.056 g of test substance in

1 l of distilled water. The solution for the 32 mg/l concentration was prepared by dissolving 0.048 g of test substance in 1.5 l of dilution water. Appropriate aliquots of the this test solution (32 mg/l) were added to dilution water to prepare the remaining test concentrations.

D. **Design:** At the start of the test, a volume of embryo suspension (2 ml) was randomly added to each vessel. Three additional vessels containing control water were inoculated and sampled immediately for definitive determination of the inoculum density which was 19.7 embryos/ml.

After 48 hours, each vessel was mixed with a perforated plunger, and 20 ml of solution removed and fixed with 1 ml of buffered formalin. Once the larvae had settled, the sample volume was reduced (larval density increased) by a factor of 2. Subsequently, the number of normal and abnormal larvae were counted in replicate 1 ml subsamples from each sample, in ring cells mounted on Sedgewick-Rafter grid slides, under a microscope. D-veliger larvae were defined as normal if the bivalve (Prodissoconch I) shell was fully formed. All larvae observed, other than empty shells, were recorded. For the inoculum samples, all dividing embryos were counted.

The temperature was measured and recorded daily in one replicate of each treatment, and hourly in one dilution water vessel. The salinity of the dilution water and the pH of each test solution were measured at the start of the test, using the excess remaining after filling the test vessels. The dissolved oxygen concentration of the control, and the low, medium, and high test solutions was also determined at test initiation. At test termination, the pH and dissolved oxygen concentration in one replicate of each solution were measured.

Each test solution was sampled for determination of the test substance concentrations at the start and finish of the test using gas chromatography. Samples were taken from the excess solutions at the start of the test, and from one replicate of each solution at the end of the test.

E. **Statistics:** Results of the toxicity test were used to calculate the percentage reduction of normal oyster larvae from each test concentration when compared to

the control. The percentage reduction of normal 48-hour embryos was determined as follows:

$$\% \text{ Reduction} = \frac{\% \text{ normal in control minus } \% \text{ normal in treatment}}{\% \text{ normal in control}} \times 100$$

These treatment percentage reductions were used to calculate the median effective concentration (EC50) and its 95% confidence limits, defined as the concentration resulting in a 50% reduction in normal development of the larvae. This was calculated by moving average angle analysis using a computer program.

12. **REPORTED RESULTS:** The mean measured concentrations of acetochlor technical were 1.1, 2.0, 3.1, 6.2, 10.4, 18.5, 32.5, and 55.7 mg/l (Table 1, attached). These values ranged from 97% to 111% of the nominal concentrations.

The data showing the counts of normal, abnormal and total larvae in subsamples of the test solutions, and of embryos in the inoculum samples, are presented in Table 2 (attached). It should be noted that these values (and subsequent derived values) are not corrected for dilution by fixative (x 1.05) and concentration (x 2) by sedimentation of the samples.

Table 3 (attached) shows the number of normally developed larvae and the percentage of embryos developing normally. Mean normal development in the control was 92%. Table 3 also shows the mean percentage normality for each treatment and the percentage reduction in normal development compared with the control.

The 48-hour EC50 was 8.3 mg/l with a 95% confidence interval of 7.5-9.1 mg/l. The no-observed effect level (NOEL) was 6.2 mg/l.

During the test, the dissolved oxygen concentration ranged from 6.3 to 7.4 mg/l and the pH ranged from 7.84 to 8.12. The daily and hourly temperature ranged from 19.5 to 20.7°C. The salinity of the dilution water was 33.6 ppt.

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, the project manager, and a representative of the sponsor company, 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). This statement also indicates that the study satisfies the requirements of 40 CFR 160.

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

A. Test Procedure: The test procedures were generally in accordance with protocols recommended by the guidelines, but deviated from the SEP as follows:

The authors did not report percentage of mortality or the EC_{50} of acetochlor technical for the survival of oyster embryos and larvae.

This study employed only two control replicates for the length of the study; the SEP recommends 4 control replicates be used or 10% of the total number of test replicates, which ever is greater.

The test vessels used in this were 250-ml glass beakers; the SEP recommends 1-l test vessels.

The SEP states that embryos should be tested within one hour of spawning and after fertilization. This test used embryos 3.4 hours after fertilization.

The photoperiod employed during the test was not reported. The SEP recommends a 16-hour light and an 8-hour dark photoperiod with a 15-to 30-minute transition period between light and dark.

B. Statistical Analysis: The reviewer used EPA's Toxanal computer program to calculate the 48-hour EC50 of acetochlor technical to the normal development of oyster larvae (printout, attached). The moving average method provided a 48-hour EC50 value of 8.0 mg/l and a 95 percent confidence interval of 7.2-8.8 mg/l which are similar to those reported by the authors.

In order to determine the EC_{50} of acetochlor technical for the survival of oyster larvae, the reviewer calculated the percentage mortality based on the initial and final number of embryos at each treatment level.

The reviewer used EPA's Toxanal computer program to determine the EC_{50} value (95% confidence interval) which was calculated to be 15.5 mg/l (14.1-17.1 mg/l)

based on mean measured concentrations (printout, attached).

C. **Discussion/Results:** Upon checking the stock solution preparation and measured concentrations in Table 1, it appears that the measured concentrations were reported as whole test material (converted from percentage a.i.). Therefore, all results reported here are measured concentrations as whole test material.

The deviations listed above probably did not affect the results of this test. This study is scientifically sound and meets the guideline requirements for an acute toxicity study, using oyster larvae. The 48-hour EC_{50} values of acetochlor technical based on percentage of normal larvae and survival of *Crassostrea gigas* were 8.0 and 15.5 mg/l mean measured concentrations, respectively. Based on the most sensitive parameter measured (development of larvae), acetochlor technical is classified as moderately toxic to *Crassostrea gigas*. The NOEC was 6.2 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, September 19, 1991.

ACETOCHLOR

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Rosemary Graham Mora Acetochlor Crassostrea gigas 9-17-91 % NORMAL

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CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB.(PERCENT)
55.7	92	92	100	9.765625E-02
32.5	92	92	100	9.765625E-02
18.5	92	92	100	37.69531
10.4	92	86	93.47826	9.765625E-02
6.2	92	0	0	9.765625E-02
3.1	92	1	1.086957	9.765625E-02
2	92	2	2.173913	1.074219
1.1	92	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 8.395502

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS	
7	9.650081E-03	7.967435	7.21162	8.804425

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
12	67.26943	1081.372	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 = 7.26219
95 PERCENT CONFIDENCE LIMITS =-52.3008 AND 66.82518

LC50 = 7.94954
95 PERCENT CONFIDENCE LIMITS = 0 AND +INFINITY

LC10 = 5.314514
95 PERCENT CONFIDENCE LIMITS = 0 AND +INFINITY

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Rosemary Graham Mora Acetochlor (% survival) Crassostrea gigas 9-19-91

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CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB.(PERCENT)
55.7	100	99	99	0
32.5	100	83	83	0
18.5	100	64	64	0
10.4	100	45	45	0
6.2	100	0	0	0
3.1	100	4	4	0
2	100	5	5	0
1.1	100	3	3	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 12.08649

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS	
5	.0124701	15.50908	14.07835	17.13413

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
5	.2487692	11.5494	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 = 2.596315
95 PERCENT CONFIDENCE LIMITS = 1.301357 AND 3.891274

LC50 = 13.46363
95 PERCENT CONFIDENCE LIMITS = 8.211079 AND 23.10205

LC10 = 4.365234
95 PERCENT CONFIDENCE LIMITS = 1.287126 AND 7.347739

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