(8-19-87)

DATA EVALUATION REPORT

1. Chemical: PP321 128897 Lambda cyhalothrin

2. Test Material: PP321, 96.5% ai

3. Study/Action Type: Acute Toxicity to Larvae of Pacific Oyster - 48-Hour Study

   Species Tested: Crassostrea gigas

4. Study ID: Hill, R.W. 1985. PP321: Determination of the Acute Toxicity to Larvae of the Pacific Oyster (Crassostrea gigas), Submitted by ICI Americas, Prepared by Brixham Laboratory, Devon. Accession No. 073989.

5. Reviewed By: Candy Brassard
   Environmental Protection Specialist
   EEB/HED
   Signature: Candy Brassard
   Date: 8-17-87

6. Approved By: Douglas J. Urban
   Head, Section III
   EEB/HED
   Signature: Douglas J. Urban
   Date: 8/19/87

7. Conclusion:

Based on the submitted data it appears the LC_{50} is > 1.0 mg/L (ppm), nominal concentration, and 0.59 mg/1, mean measured concentration, for oyster embryolarvae exposed to PP321. This indicates PP321 is highly toxic to the pacific oyster. This study is classified as "~~Supplemental~~"; therefore, this study does not fulfill Guideline Reference No. 72-3.

upgraded to Core see addend

8. Recommendations:

The company should submit an acute marine or estuarine mollusc study, preferably an oyster shell deposition (96-hour acute EC_{50}) study. This type of study could be conducted under flowthrough conditions, which is more desirable with this class of chemicals.

All five treatment levels should have been evaluated for percent mortality and percent reduction per test level.

In addition, the concentrations should be measured in each replicate test vessel as the concentrations have been known to fluctuate with this class of chemical.

The number of embryos should have been 20,000 or 30,000 per liter. This study only used 10,000 embryos. See section

14. for more details.

9. Background:

This study was submitted to support the application for registration (section 3) for Karate 1EC (PP321) Insecticide.

10. Discussion of Individual Tests: N/A

11. Materials and Methods:

a. Test Animals - Test organisms were embryos of the Pacific oyster (Crassostrea gigas), 3.5 hours postfertilization at the start of the test.

Adult oysters of 94 to 118 mm shell length were obtained on August 17, 1984 from Surfside Oysters, Torquay, Devon. The original broodstock was obtained from the estuary of the River Teign, Devon. Adults were acclimated to unfiltered, 18 to 19 °C seawater, then for $\geq$ 5 days to filtered seawater with algal food addition, at 20 $\pm$ 1 °C. Females were transferred to beakers and induced to spawn at 28 to 30 °C. The egg suspension from a single female was fertilized with a sperm suspension from the excised gonad of a single male to provide the embryo suspension used for the test. The suspension was maintained at 20 $\pm$ 1 °C, with gentle agitation on an orbital shaker, until used to inoculate the test solutions.

b. Dose - The solvent control and all concentrations of the test substance contained 0.156 mL acetone/L. The control solution consisted of dilution water only. The following doses were tested:

Control, solvent control, 0.1, 0.18, 0.32, 0.56, and 1.0 mg/L.

c. Study Design - Test was conducted in 250 mL glass beakers with loose-fitting lids. Two replicates of each control and test substance concentration were prepared, each containing 200 mL of test solution maintained at 20 °C.

The embryo suspension was 50 embryos/mL. Three additional control samples indicated inoculum density was 40.2 embryos/mL.

Excerpted from submission:

"After 48 hours each vessel was mixed with a perforator plunger, and 10 ml of solution removed and fixed with 5% buffered formalin. 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 an inverted 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 defined as survivors."

The DO and salinity was measured for control, and pH of each test solution was measured at the start of the

test. At the end of the test, pH and DO of one replicate of each solution were measured.

The temperature of one replicate of each test solution was measured daily.

The concentration of the test substance was measured at the start and the finish using gas chromatography. At the start of the test, samples were taken of the excess solutions, and at the end of the test one replicate of each solution was sampled.

d. Statistical Analysis - Biological Data Analysis was evaluated as follows:

"Larval mortality

Percentage larval mortality in each control and treatment replicate was calculated as follows:

$$\text{Mortality, \%} = 100\,(1-(Nt/No))$$

where Nt = number/ml of survivors

No = number/ml introduced (inoculum)

"Nt and No were obtained by meaning the subsample counts of the test solution and inoculum samples, respectively.

"Mean mortality in the control, solvent control, and each concentration evaluated was less than 10% and therefore it was not considered necessary to correct the test substance treatment values for control mortality.

"Larval abnormality

Percentage larval abnormality was calculated for each control and treatment as follows:

Abnormality, % = 100 ($Na/\bar{N}t$)

where Na = number/ml of abnormal larvae (mean of the replicates)

$\bar{N}t$ = number/ml of survivors [mean of the replicates]

Abnormality in each control and test concentration evaluated was less than 2% and therefore it was not considered necessary to correct the test substance treatment values for control abnormality."

12. Reported Results: [excerpted from submission]

"Larval mortality and abnormality

The mean treatment mortality and abnormality results are summarised in Table 1.

"The number of larvae in the inoculum samples (number introduced), the number of surviving and abnormal larvae for each test vessel, and the calculated mortality and abnormality percentages are given in Table 2.

"The counts of normal and abnormal larvae in individual subsamples of the test solutions and counts of the inoculum samples, from which the data of Table 2 were derived, are shown in Table 3.

"It was not considered necessary to evaluate the samples from the 0.18 and 0.32 mg/l nominal concentrations since there was clearly no effect at higher concentrations. Mortality and abnormality at the two highest concentrations tested were slightly less than in the control and solvent controls. Therefore the median lethal concentration (LC50) and median effective concentration (EC50) were:

48 hour LC50 >1.0 mg/l (nominal)

48 hour EC50 >1.0 mg/l (nominal)

"The LC50 and EC50 were defined as the concentrations resulting in 50% mortality and abnormality, respectively.

"The nominal concentration of 1.0 mg/l had an initial measured concentration of 0.84 mg/l, and the mean of the initial and final measured concentrations was 0.59 mg/l.

"Dissolved oxygen, pH, temperature and salinity

Dissolved oxygen concentrations ranged from 7.00 to 7.25 mg/l and the pH ranged from 8.08 to 8.14.

"The daily temperature measurements ranged from 19.5 to 21.2 °C.

"The salinity of the dilution water was 33.6°/oo.

"Chemical analysis

The concentrations of PP321 determined in samples from the test solutions at the start and finish of the test are given in Table 4.

"The measured concentrations at the start of the test ranged from 79 to 100% of the nominal values. The measured concentrations at the end of the test ranged from 24 to 41% of the nominal values."

13. Study Author's Conclusions/QA Measures: [excerpted from submission]

"No significant effects on larval development or survival were observed up to the maximum concentration tested which was 1.0 mg/l (nominal). This was considered the maximum concentration that could be tested due to the limited solubility of the test substance.

"The 48 hour median lethal concentration (LC50), and the 48 hour median effective concentration (EC50) for abnormality were therefore:

48 hour LC50 = >1.0 mg/l

48 hour EC50 = >1.0 mg/l

"These results were based on the nominal concentration of the test substance. The nominal 1.0 mg/l concentration

had an initial measured concentration of 0.84 mg/l, and the mean of the initial and final measured concentrations was 0.59 mg/l.

"The conduct of this study has been inspected/audited in accordance with ICI's policies and procedures for Good Laboratory Practice . . . ."

14. Reviewer's Discussion and Interpretation of Study:

The following discrepancies were noted and found to detract from the study.

a. Test Procedures - The raw data were not submitted, so that the results could not be verified; specifically, percent mortality, and percent reduction.

The loading factor should have been 20 to 30 larvae/mL, instead of 50 embryos/mL, with a total of 20,000 to 30,000/L per replicate. This study only used an average of 50 embryos per mL, only a total of 10,000 embryos were used per replicate.

The chemical characteristics of the dilution water should have been indicated.

The photoperiod used should have been reported.

All five treatment levels should have been evaluated for percent mortality, percent abnormality, and subsample counts (1 mL volume).

When testing synthetic pyrethroids, the company should measure the concentrations in each replicate test vessel, as the concentration varies within each test vessel. The study author only measured one replicate for each treatment level.

The embryos used should have been $\leq$ 1 hour old; the embryos in this study were 3.5 hours old.

EEB recommends that to calculate percent mortality and percent reduction per test level, the Standard Evaluation Procedure, "Acute Toxicity Test for Estuarine and Marine Organisms (Mollusc 48-Hour Embryolarvae) Study," Daniel Rieder, June 1985, should be referenced for future studies.

b. Statistical Analysis - These data could not be validated, and the analysis could not be completed due to lack of raw data.

c. Discussion/Results - Since the study is supposed to test the effects of Karate (PP321) on an estuarine invertebrate, (oyster), it is recommended that the registrant consider testing the effects of Karate on oysters in a shell deposition study (96-hour acute). The primary concern is that this study was not conducted under flowthrough conditions. Since embryolarvae (48-hour) studies are difficult to perform under flowthrough conditions.

This study did not evaluate all five treatment levels for effects such as percent mortality and percent abnormality.

The concentrations were only measured in one replicate at each treatment level. In the future, especially since this is a synthetic pyrethroid, the concentrations should be measured within each replicate within each treatment level.

d. Adequacy of Study

1) Classification - Supplemental for 96.5% ai.

2) Rationale - The study appears to be scientifically sound, but because the study author did not report percent mortality and abnormality for all five treatment levels, it cannot be verified that indeed the 48 hr LC_{50} > 1.0 mg/L(nominal concentrations) and >0.58 mg/l for mean measured concentrations.

3) Repairability - This study cannot be repaired.

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Pages 8 through 12 are not included in this copy.

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PP321
Amendment to DER for Oyster Embryo
Larvae Acute Toxicity Study

-The raw data were submitted and reviewed.

-The chemical characteristics of the dilution water appear to be acceptable. See Table A.

-Since the recommended protocols in Subdivision E, 72-3, did not specify the photoperiod, the photoperiod used in this study, which was continuous lighting is acceptable. However, since the SEP guidelines (1985, Dan Rieder) clearly specify the photoperiod of 16 hours light, 8 hours dark, the company should be apprised that in future studies this photoperiod will be required.

-According to the SEP guidelines (1985, Dan Rieder) there should be at least five dose levels included in the experimental design. The recommended protocol in Subdivision E, 72-3, ASTM 724-80 Standard, does not specify number of dose levels but does state that there should be at least one and preferably two or more responses between 16 and 84% and one response <16% , and one greater than 84%. This study did not meet this criteria.

Subdivision E, 72-3 (b)(3) (which was published in 1982) requires that satisfactory data should establish either:

- an EC50 or LC50 with 95 percent confidence intervals; or

- that the EC50 or LC50 is greater than 100 mg/l or greater than 100,000 times the MEEC or EEC.

-Section 11.4.5 of the ASTM 724-80, 1980 Standard, which was used by the company, states the following:

"As a minimum, toxicant concentrations should be measured in one replicate of each treatment at the onset of testing. Analysis at 48 h will demonstrate whether concentrations change temporally. If so, analyses at intermediate times may be indicated if the investigator wishes to determine the rate of decline and attempt to calculate a mean value."

The reported values within the various treatment levels ranged so that the initial concentration (0 hr) was as much as 3.2 times higher than the 48 hour measured concentration within the same treatment level. Only one replicate within each treatment level was measured, and the concentrations varied considerably within the same treatment level. The concentrations should have also been measured at 24 hours as well, in order to determine a more accurate mean and the rate of decline.

The study author did not indicate which replicate test vessel was measured for residue (only the recently submitted data indicated the dilution water characteristics in the individual replicate).

The mortality varied considerably between replicates within the same treatment level. It becomes apparent that the concentrations should have been measured within each replicate due to the variability.

-Section 10.5.3 of the ASTM 724-80 Standard, which was used by the company, stated the following:

"Embryos should be tested within 1 hr of spawning and kept suspended during this period by frequent agitation ..."

Since the company could not abide by the 1985 SEP guidelines (Dan Rieder) because the study was conducted in 1984, the draft 1987 ASTM Standard could not be considered when evaluating the study. In addition, a draft ASTM Standard can not be considered acceptable in evaluating studies. However, we do use these documents to be kept apprised of the latest developments.

-The percent mortality has been reevaluated. Since the control mortality exceeded 2% (ASTM 724-80, 1980) the treatment responses were corrected for the mean level of control mortality. See Table A.

-Since the solvent control mortality was greater than the treatment levels, the LC_{50} is greater than the highest dose level.

-EEB is aware that synthetic pyrethroids as a class of chemicals are relatively water insoluble. And in addition, in this study, the results indicate that PP321 is more toxic to other aquatic organisms.

EFB recommends in future studies with compounds of this nature the study author should perhaps use higher solvent concentrations, since the level used here, 0.156 ml/l was less than 0.5 ml/l (highest acceptable dose). Also trying other solvents such as triethylene glycol or dimethyl formamide would be an alternative.

c. Discussion of Results

Since the data indicate that the LC_{50} is > 0.59 mg/l and the level of toxicity is considerably higher than the reported data for other aquatic organisms, EFB believes requiring additional data is unnecessary. A oyster shell deposition study would have been a better study to evaluate the toxicity to oysters since it can be conducted under flow-through conditions.

d. Adequacy of Study

1) Classification- Core for 96.5% ai PP321.

2) Rationale- The data indicate that the LC50 is >0.59 mg/l (measured concentrations), indicating that PP321 is highly toxic to oyster embryo larvae on an acute basis. EFB will accept these data to partially fullfill the guideline reference no. 72-3 since we believe that no additional data can be gained from another oyster embryo larvae study for use in a hazard assessment. This LC50 is considerably higher than other reported aquatic organism data (where values are as low as 6.6 ng/l).

3) Repairability - N/A.

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Table A

This table reports the percent mortality using the following method of calculations:

$$\text{larval mortality\%} = 1 - \frac{\text{no. of surviving larvae}}{\text{no. of embryos introduced (in this case 38.33 embryos/ml) before adjusted for dilution fixative}} \times 100$$

$$\text{Net treat. mort.\%} = 1 - \frac{\text{no. of surviving larvae/ml per treat. replicate}}{\text{mean no. of surviving control larvae}} \times 100$$

Net treatment mortality was also calcualted since the control mortality >2%.

Nominal Conc. mg/l		Larval Mortality%	Net Treatment Mortality%
Control	A	7.4	-----
	B	9.3	
Solvent Control	A	5.4	------
	B	11.3	
0.10	A	3.5	-5.0
	B	15.2	7.5
0.18	A	NOT EVALUATED	
	B		
0.32	A	NOT EVALUATED	
	B		
0.56	A	9.3	1.1
	B	-1.1	-10.3
1.0	A	1.5	-7.45
	B	8.7	0.37