DATA EVALUATION RECORD

1. Chemical: PP321

2. Test Material: PP321 (^{14}C-Labeled) Purity > 97% ai

3. Study Type: Acute Toxicity for Estuarine and Marine Organisms (Shrimp 96-Hour Acute Toxicity Test) -- Flow Through

   Species Tested: Mysidopsis bahia

4. Study ID: Thompson, R.S .1985. PP321: Determination of Acute Toxicity to Mysid Shrimp (Mysidopsis bahia). Submitted by ICI Americas, Inc. Prepared by Brixham Laboratories. EPA 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:

   This study is scientifically invalid. The high percent mortality for the solvent control and the control causes serious concern. In addition, the measured concentrations fluctuated so that the actual concentration that caused mortality could not be accurately ascertained.

8. Recommendation:

   This study may be upgraded to Supplemental if the raw data are submitted and the causes of microbial growth, observations of the mysids, etc., are reported.

   upgraded to CORE SEE ADDENDUM LC50 = 49 PPT

9. Background:

   This mysid acute toxicity study is submitted prior to registration for Karate or PP321 on cotton.

10. Discussion of Individual Test: N/A

11. Materials and Methods:

a. Test Animals - Mysidopsis bahia, < 48 hours old at the start of the test, were derived from continuous laboratory cultures. The original source of these cultures was the USEPA Laboratory, Narragansett, RI, by the suppliers, Sea Plantations, Inc., Salem, MA.

The test organisms were obtained from cultures that were also under the same conditions of dilution water, temperature, and photoperiod used in the test.

The mysids were fed Artemia salina nauplii.

b. Test System - The test vessels consisted of 14 L glass rectangular tanks. Each vessel contained four retention chambers constructed from rigid plastic beakers of 90 mm diameter and 400 mL working volume, with a "window" cut in the side, covered by nylon mesh. Each chamber had a loose-fitting glass lid.

The vessels were drained every 100 minutes to 1/3 the volume to ensure exchange of test solution between vessel and retention chamber.

Flow rate of dilution water was 500 mL/min (using fixed aperture outlets from a constant head tank). Flow rate of stock solutions was 0.0108 mL/min. The solvent control received acetone in the same manner.

The temperature was 25 $\pm$ 1 °C. The photoperiod was 14 hours light:10 hours dark. The dissolved oxygen ranged from 6.6 to 7.4 mg/L, pH ranged from 8.12 to 8.22, and the salinty of control solution ranged from 18.2 to 20.9 0/00.

c. Dose - The solvent control, acetone, was used at a rate of 0.022 mL/L. The following eight levels were tested: control, solvent control, 0.0032, 0.0056, 0.010, 0.018, 0.032, and 0.056 ug/L (ppb).

d. Study Design - Twenty mysids were randomly allocated to each control and test concentration, distributed to give five mysids in each of the four retention chambers within each vessel.

Mysids were added 15 minutes after test solution was added to test vessels.

Each test solution was sampled daily for determination of the concentration of the test substance (as ^{14}C

activity) using a liquid scintillation counter. The recovery efficiencies for four spiked samples of the solvent control solution ranged from 87 to 98 percent. No correction for recovery was applied to the test data.

The concentrations ranged from 29 to 81 percent of the nominal concentrations. See Table 2. Some microbial growth was reported in the test vessels. The dosing system had problems at 92.5 hours and was corrected immediately.

12. Reported Results:

The study author reported the following median lethal concentrations (LC_{50}):

24 hours:	> 0.017 ug/L
48 hours:	0.0075 ug/L
72 hours:	0.0049 ug/L
96 hours:	0.0041 ug/L

These values were based on the mean measured concentrations of the test substance as determined by radiochemical analysis.

See Table 1 for mortality data.

13. Study Author's Conclusions/QA Measures:

The 96-hour acute toxicity LC_{50} for mysids is 0.0049 ug/L (ppb) of PP321.

"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 the Study:

Based on the following major discrepancies, the study is scientifically unsound.

A. Test Procedures

- The percent mortality of the solvent control was as high as 15 percent and percent mortality of the control was 10 percent. The SEP guidelines (Reider 1985) clearly state that the study is unacceptable if the percent mortality is > 5 percent in a flowthrough study. ASTM 1980 clearly states that if the control mortality is more than 10 percent, the study is unacceptable.

- The study author did not indicate where the samples were taken from in the test vessels. Synthetic pyrethroids have been found to vary in concentrations.

- A no-observed-effect level (NOEL) was not observed, as the lowest concentration tested reported mortality as great as 15 percent.

- For each treatment, the highest of all the measured concentrations obtained during the test divided by the lowest should be less than 1.5. The measured concentrations within each treatment level varied from 1.58 to 4.06. In addition, the measured concentrations should not be greater than 30 percent higher or lower than the nominal concentration (ASTM 1980). The measured concentrations varied from 29 to 81 percent of the nominal. Five of the six treatment levels (the five highest) ranged from 29 to 59 percent of the nominal. These levels clearly do not meet these criteria.

- The loading factor was not reported.

- Chemical characteristics of the dilution water were not reported.

- The raw data were not submitted; therefore, the mortality could not be verified.

- Observations of the organisms were not reported, i.e., when the mysids appeared dark, stressed, or diseased.

- The cause for microbial growth should be reported.

b. Statistical Analysis - The Stephans program was conducted on the submitted data. It appears that the LC_{50} is 0.0049 ug/L (0.00413 to 0.0058 ug/L). See Attachment A.

A NOEL could not be determined.

c. Discussion/Results - There were major discrepancies that caused concern for the validity of the data. These are as follows:

- The high mortality rate for the solvent control.

- The raw data were not submitted. Therefore, observations of the mysids were not reported and the mortality could not be verified.

- The microbial growth reported in the test vessels causes concern.

- The measured concentrations fluctuated, so that the actual concentration at which these test organisms were affected could not be ascertained.

d. Adequacy of Data

1) Classification - Invalid for 97% ai.

2) Rationale - Due to the concerns identified in sections 14a and 14c.

3) Repairability - The study may be upgraded to Supplemental depending on the raw data being submitted.

15. Completion of One-Liner for Study: August 12, 1987.

Page _____ is not included in this copy.

Pages 6 through 7 are not included in this copy.

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.

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The information not included is generally considered confi
by product registrants. If you have any questions, please
the individual who prepared the response to your request.

Amendment for DER
Acute Toxicity Test for the Mysid Shrimp

-Since ASTM (1980) "Standard Practice for Conducting Acute Toxicity Tests with Fishes, Macroinvertebrates, and Amphibians" requires only $\leq$ 10% control mortality, these data are within the acceptable limits.

-The study author reported that the samples were taken middepth, and central within test vessel. This appears to be adequate.

-Once again, the NOEL for this study cannot be extrapolated from another study, specifically the mysid life cycle. And again, the mysid life cycle study has not been submitted for review.

-We cannot omit data as suggested by ICI Americas, Inc. in order to accommodate variabilities in the study. The measured concentrations showed considerable variability within each treatment level, where the lowest concentration within a treatment level was as much as 4.06 less than the highest.

A concern is the limited dose-response relationship, since the mean measured concentrations are the same for the two lowest concentrations, yet the mortality differs by 15 percent.

-The study author reported the loading factor to be 0.001 g/L, which is within the recommended limits of 1 g/L.

-The chemical characteristics of the dilution water appear to be satisfactory.

-The data discrepancies with regard to percent mortality. Specifically, at test concentration (nominal) 0.018 ug/L, 16/20 (80%) mortality was reported at 48 hours and at 96 hours 14/20 (70%) was reported. This data discrepency has been corrected to read 30 % mortality at 48 hours.

-In addition, it appears from the recent submission that a number of survivors were stressed. It should be clarified how many test organisms at each treatment level every 24 hours were showing signs of stress.

-The study author reported that the microbial growth was due to the use of acetone as the solvent.

Additional Comments

-The use of plastic retention chambers within glass aquaria causes concern for absorption or leaching of test substance.

-Another concern is that the first four doses are essentially just 2 doses since the concentrations are so close (i.e. 0.0026, 0.0026 and 0.0059, .0052).

-Control and solvent control should have been measured for residue. This would be to ensure there was no contamination in the controls.

Discussion of Results

The study author responded to the concerns identified in the DER dated August 19, 1987. The data discrepancies with regard to mortality within each treatment have been clarified. The study appears to be scientifically sound. There are discrepenices such as the variability in the measured concentrations. However, they do not seem to significantly detract from the study. It is important for the study author to understand that this decision is made on a case by case basis, and the laboratory testing compounds such as these should be extremely cautious with dilution of the test material. The concerns have been basically satisfied.

Adequacy of Study

1. Classification--Core - 97% ai

2. Rationale-See discussion of results.
   In addition, EEB has received a mysid life-cycle study that indicates the mysid chronic NOEL may be as low as 0.22 ng/l .

3. Repairability--NA

Conclusion

Based on the results submitted, PP321 is very highly toxic to the mysid shrimp with an LC_{50}= 0.0049 ug/l.

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9

LC10 = 4.282446E-03
95 PERCENT CONFIDENCE LIMITS = 2.514483E-03 AND 5.875356E-03
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Attachment A

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NOTE: BECAUSE THERE WAS CONTROL MORTALITY, AND NONE OF THE LOWER CONCENTRATIONS PRODUCED ZERO MORTALITY, THE DATA HAS BEEN SUBJECTED TO ABBOTT'S CORRECTION.

Candy Brassard Karate mysidopsis bahia
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CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
.0166	17	17	100	7.629392E-04
.011	17	15	88.2353	.1174926
.0059	17	11	64.7059	16.61529
.0052	17	11	64.7059	16.61529
.0026	17	3	17.6471	.6362914
.002599		17	0	0

7.629392E-04

THE BINOMIAL TEST SHOWS THAT .0026 AND .011 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 4.225076E-03

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS	
5	.0511085	5.151448E-03	4.386161E-03	5.972415E-03

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
5	.1047971	1	.3139707

SLOPE = 4.432684
95 PERCENT CONFIDENCE LIMITS = 2.997719 AND 5.867649

LC50 = 4.91166E-03
95 PERCENT CONFIDENCE LIMITS = 4.138237E-03 AND 5.844472E-03

LC10 = 2.539338E-03
95 PERCENT CONFIDENCE LIMITS = 1.758886E-03 AND 3.149392E-03
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10