07/17/2006

DP Barcode: D325337 MRID No.: 466954-34

DATA EVALUATION RECORD
ACUTE LC_{50} TEST WITH AN ESTUARINE/MARINE SHRIMP
• 72-3(C)

1. **CHEMICAL**: Bayer AE0172747 PC Code No.: 012801

2. **TEST MATERIAL**: Bayer AE 0172747 Purity: 94.0%.

3. **CITATION**

Authors: Lima, W.

Title: AE 0172747- Acute Toxicity to Mysids (*Americamysis bahia*) Under Flow-Through Conditions

Study Completion Date: January 22, 2003

Laboratory: Springborn Smithers Laboratories
790 Main Street
Wareham, Massachusetts 02571-1075

Sponsor: Bayer CropScience GmbH
Frankfurt, Germany

Laboratory Report ID: 13798.6102/35401

MRID No.: 466954-34

DP Barcode: D325337

4. **REVIEWED BY**: Rebecca Bryan, Staff Scientist, Dynamac Corporation

Signature: Rebecca L. Bryan **Date:** 4/12/06

APPROVED BY: John Marton, Staff Scientist, Cambridge Environmental Inc.

Signature: **Date:** 4/24/06

5. **APPROVED BY**: Jeannette Martinez

Signature: **Date:** 7/17/06

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6. DISCLAIMER: This document provides guidance for EPA and PMRA reviewers on how to complete a data evaluation record after reviewing a scientific study concerning the acute toxicity of a pesticide to shrimp. It is not intended to prescribe conditions to any external party for conducting this study nor to establish absolute criteria regarding the assessment of whether the study is scientifically sound and whether the study satisfies any applicable data requirements. Reviewers are expected to review and to determine for each study, on a case-by-case basis, whether it is scientifically sound and provides sufficient information to satisfy applicable data requirements. Studies that fail to meet any of the conditions may be accepted, if appropriate; similarly, studies that meet all of the conditions may be rejected, if appropriate. In sum, the reviewer is to take into account the totality of factors related to the test methodology and results in determining the acceptability of the study.

7. STUDY PARAMETERS

Age or Size of Test Organism:	≤24 hours old
Definitive Test Duration:	96 hours
Study Method:	Flow-through
Type of Concentrations:	Mean measured

8. CONCLUSIONS:

Results Synopsis
96-hr LC_{50}: 100 µg a.i./L 95% C.I.: 79-130 µg a.i./L
NOAEC: 46 µg a.i./L
Probit Slope: N/A

9. ADEQUACY OF THE STUDY

A. Classification: This study is scientifically sound and does satisfy the guideline requirement for an acute toxicity test with mysid shrimp. Classification is ACCEPTABLE.

B. Rationale: N/A

C. Repairability: N/A

10. BACKGROUND

11. GUIDELINE DEVIATIONS

1. The salinity of 20-22‰ was greater than recommended for an estuarine mysid (10-17‰).
2. The reported pH of the dilution water (7.6-8.0) ranged lower than recommended (7.7-8.0).
3. The reported temperature (24-25°C) was higher than recommended (22±1°C).
4. The size and fill volume of the test vessels (1.6 and 1.4 L, respectively) were smaller than recommended (3.9 and 2-3 L, respectively).

12. SUBMISSION PURPOSE: This study was submitted to provide data on the acute toxicity of AE 0172747 to mysid shrimp for the purpose of chemical registration.

13. MATERIALS AND METHODS

A. Test Organisms

Guideline Criteria	Reported Information
Species Preferred species are *Mysidopsis bahia*, *Penaeus setiferus*, *P. duorarun*, *P. aztecus* and *Palaemonetes sp.*	*Americamysis bahia*
Age Juvenile, mysids should be ≤ 24 hours old	≤24 hours old
Supplier	In-house laboratory culture
All shrimp are from same source?	Yes
All shrimp are from the same year class?	Yes

B. Source/Acclimation

Guideline Criteria	Reported Information
Acclimation Period minimum 10 days	Continuous culture (originally from Aquatic BioSystems, Inc., Ft. Collins, Colorado).
Wild caught organisms were quarantined for 7 days?	N/A
Were there signs of disease or injury?	No
If treated for disease, was there no sign of the disease remaining during the 48 hours prior to testing?	N/A
Feeding No feeding during the study and no feeding for 24 hour before the beginning of the test if organisms are over 0.5 g each. Mysids should be fed throughout the study.	Brine shrimp (*Artemia salina*) nauplii was provided at least twice daily, *ad libitum*.
Pretest Mortality <3% mortality 48 hours prior to testing	No mortality was reported prior to testing.

C. Test System

Guideline Criteria	Reported Information
Source of dilution water Soft reconstituted water or water from a natural source, **not** dechlorinated tap water	Natural unfiltered seawater collected directly from the Cape Cod Canal, Bourne, Massachusetts.
Does water support test animals without observable signs of stress?	Yes
Salinity 30-34 ‰ (parts per thousand) for marine (stenohaline) shrimp and 10-17 ‰ for estuarine (euryhaline) shrimp, weekly range < 6 ‰	20-22‰
Water Temperature Approx. 22 ± 1 °C	24-25°C

Guideline Criteria	Reported Information
pH 8.0-8.3 for marine (stenohaline) shrimp, 7.7-8.0 for estuarine (euryhaline) shrimp, monthly range < 0.8	7.6-8.0
Dissolved Oxygen Static: ≥ 60% during 1st 48 hrs and ≥ 40% during 2nd 48 hrs, Flow-through: ≥ 60%	4.6-7.9 mg/L (>60% saturation)
Total Organic Carbon Should be <5 mg/L in reconstituted seawater	Not reported
Test Aquaria 1. Material: Glass or stainless steel 2. Size: 19.6 L is acceptable for organisms ≥ 0.5 g (e.g. pink shrimp, white shrimp, and brown shrimp), 3.9 L is acceptable for smaller organisms (e.g. mysids and grass shrimp). 3. Fill volume: 15 L is acceptable for organisms ≥ 0.5 g, 2-3 L is acceptable for smaller organisms.	Glass battery jars, equipped with two drain holes covered with 40 mesh Nitex® screen. 1.6 L 1.4 L
Type of Dilution System Must provide reproducible supply of toxicant	Intermittent-flow proportional diluter
Flow Rate Consistent flow rate of 5-10 vol/24 hours, meter systems calibrated before study and checked twice daily during test period	9 volume additions/24 hours Diluter system function was monitored for normal operation twice daily (visual check).
Biomass Loading Rate Static: ≤ 0.8 g/L at ≤ 17°C, ≤ 0.5 g/L at > 17°C; flow-through: ≤ 1 g/L/day (N/A for mysids)	N/A for mysids
Photoperiod 16 hours light, 8 hours dark	16 h light, 8 h dark.

Guideline Criteria	Reported Information
Solvents Not to exceed 0.5 ml/L for static tests or 0.1 ml/L for flow-through tests	DMF at 0.096 mL/L

D. Test Design

Guideline Criteria	Reported Information
Range Finding Test If LC_{50} >100 mg/L with 30 shrimp, then no definitive test is required.	Two static and four flow-through range-finding studies were conducted. The final flow-through test was conducted at 17, 28, 47, 78, and 130 µg a.i./L with a negative control. By 96 hours, there was 40% mortality in both the 78 and 130 µg a.i./L treatment groups. No other mortalities were observed in the control or treatment groups.
Nominal Concentrations of Definitive Test Control & 5 treatment levels; a geometric series in which each concentration is at least 60% of the next higher one.	17, 28, 47, 78, and 130 µg a.i./L (mean measured concentrations were 14, 28, 46, 79, and 130 µg a.i./L)
Number of Test Organisms Minimum 20/level, may be divided among containers	20/level (10/replicate with 2 replicates)
Test organisms randomly or impartially assigned to test vessels?	Yes
Biological observations made every 24 hours?	Yes
Water Parameter Measurements 1. Temperature Measured constantly or, if water baths are used, every 6 hrs, may not vary > 1 °C 2. DO and pH Measured at beginning of test and ever	Every 24 hours and continuously in one control replicate. Every 24 hours.

Guideline Criteria	Reported Information
48 h in the high, medium, and low doses and in the control	
Chemical Analysis needed if solutions were aerated, if chemical was volatile, insoluble, or known to absorb, if precipitate formed, if containers were not steel or glass, or if flow-through system was used	Analytical determinations were performed on samples collected from replicate A at the beginning (0-hour) and from replicate B at end of the test (96-hours).

14. REPORTED RESULTS

A. General Results

Guideline Criteria	Reported Information
Quality assurance and GLP compliance statements were included in the report?	Yes
Recovery of Chemical	83-100 % (mean of 0 and 96 hour recoveries)
Control Mortality Not more than 10% of control organisms may die or show abnormal behavior.	0%
Raw data included?	Yes, replicate data were provided.
Signs of toxicity (if any) were described?	Signs of toxicity included lethargy, partial loss of equilibrium, and at the surface of the test solution.

Mortality

Concentration (µg a.i./L)		Number of Shrimp	Cumulative Percent Mortality			
			Hour of Study			
Nominal	Mean Measured		24	48	72	96
Control	<LOQ (<2.3-2.8 µg a.i./L)	20	0	0	0	0
Solvent Control	<LOQ (<2.3-2.8 µg a.i./L))	20	0	0	0	0
17	14	20	0	0	0	0
28	28	20	0	0	0	0
47	46	20	0	0	0	0
78	79	20	0	0	0	0
130	130	20	0	0	40	95

Observations: By 96 hours, there was 95% mortality in the 130 µg a.i./L treatment group. No mortalities occurred in the controls or the 14, 28, 46, and 79 µg a.i./L treatment groups. At test termination, the signs of toxicity included lethargy and/or partial loss of equilibrium 79 and 130 µg a.i./L treatment groups.

B. Statistical Results

Method: Using a computer program (Stephan, 1982), the 96-hour LC_{50} value (with 95% C.I.) was calculated using nonlinear interpolation (and binomial probability). The 96-hour NOAEC was estimated by visual interpretation of the mortality and clinical observation data. The mean-measured concentrations were used in all estimations.

96-hr LC_{50}: 100 µg a.i./L 95% C.I.: 79-130 µg a.i./L
NOAEC: 46 µg a.i./L
Probit Slope: Not reported

15. VERIFICATION OF STATISTICAL RESULTS

Parameter	Result
Binomial Test LC_{50} (C.I.)	105 (79-130) µg ai/L
Moving Average Angle LC_{50} (95% C.I.)	Not determined
Probit LC_{50} (95% C.I.)	Not determined
Probit Slope	N/A
NOAEC	46 µg ai/L

16. REVIEWER'S COMMENTS:

The reviewer determined the 96-hour LC_{50} value using the binomial test via Toxanal statistical software. Mean-measured concentrations were used to determine the 96-hour LC_{50} (and 95% C.I.). The reviewer determined the NOAEC visually.

The reviewer's 96-hour LC_{50} was less conservative than the study author's, while the 95% C.I. was identical; therefore, the study author's LC_{50} value is reported in the Conclusions section of this DER. The reviewer's NOAEC value was identical to the study author's.

Mortality was defined as a complete lack of movement after gentle prodding with a glass pipette. Sub-lethal effects were determined by a comparison of the performance and appearance of the exposed mysids to that of control mysids.

Analytical verification of unfiltered seawater fortified with the test material yielded percent recoveries of 85.6-111% of nominal, with a mean of 102%±9.41.

The size and fill volume of the test vessels (1.6 and 1.4 L, respectively) were smaller than recommended (3.9 and 2-3 L, respectively). However, since there was no mortality or sub-lethal effects observed in the control, the reviewer feels that crowding was not an issue and this deviation did not deleteriously affect the study.

Signed and dated GLP, Quality Assurance and No Data Confidentiality statements were provided. This study was conducted in accordance with U.S. EPA (40 CFR Part 160)

Good Laboratory Practices with the exception of the routine water contaminant screening analyses.

The experimental start date was September 9, 2002 and the experimental termination date was September 13, 2002.

The 96-hour acute toxicity of AE 0172747 to the saltwater mysid, *Americamysis bahia*, was studied under flow-through conditions. Mysids were exposed to the test material at nominal concentrations of 0 (negative and solvent controls), 17, 28, 47, 78, and 130 µg a.i./L with negative and solvent (DMF at 0.096 mL/L) controls. The mean measured concentrations were <2.3-2.8 (<LOQ; negative and solvent controls), 14, 28, 46, 79, and 130 µg a.i./L. By 96 hours, there was 95% mortality in the 130 µg a.i./L treatment group. No mortalities occurred in the controls or the 14, 28, 46, and 79 µg a.i./L treatment groups. At test termination, the signs of toxicity included lethargic and/or partial loss of equilibrium 79 and 130 µg a.i./L treatment groups. The **96-hour LC_{50} value was 100 µg a.i./L**, which categorizes AE 0172747 as **highly toxic** to the saltwater mysid, *Americamysis bahia*, on an acute toxicity basis. Based on mortality and behavioral observations, the **NOAEC and LOAEC values were 46 and 79 µg a.i./L**, respectively.

This study is scientifically sound and does satisfy the guideline requirement for an acute toxicity test with mysid shrimp. This study is ACCEPTABLE.

17. REFERENCES:

APHA, AWWA, WPCF. 1992. Standard Methods for the Examination of Water and Wastewater. 18th Edition, Washington, DC.

ASTM. 2000. Standard practice for conducting acute toxicity tests with fishes, microinvertebrates, and amphibians. Standard E729-96. American Society for Testing and Substances, 100 Barr Harbor Drive, West Conshohocken, PA 19428.

Mount. D.I. and W.A. Brungs. 1967. A simplified dosing apparatus for fish toxicological studies. *Water Research.* 1:21-29.

Reitsema, L.A. and J.M. Neff. 1980. A recirculating artificial seawater system for the laboratory culture of *Mysidopsis bahia* (Crustacea; Pericaridae). *Estuaries* 3: 321-323.

Stephan, C.E. 1982. U.S. EPA, Environmental Research Laboratory, Duluth, Minnesota. Personal Communication to Dr. Lowell Bahner, Chairman ASTM Task Group (E-47) on Calculating LC50's.

U.S. EPA. 40 CFR, Part 160. Federal Insecticide, Fungicide, and Rodenticide Act. Good Laboratory Practices Standards; Final Rule. Office of the Federal Register, National Archives and Records Administration. U.S. Government Printing Office, Washington, D.C.

U.S. EPA. 1982. Office of Pesticide Programs. Pesticide Assessment Guidelines. Subdivision E, Hazard Evaluation: Wildlife and Aquatic Organisms. EPA-540/9-85-024. October 1982. U.S. Environmental Protection Agency, Washington, D.C.

U.S. EPA. 1985. Office of Pesticide Programs. Standard Evaluation Procedure for Acute Toxicity Test for Estuarine and Marine Organisms. EPA-540/9-85-010. June 1985. U.S. Environmental Protection Agency, Washington, D.C.

U.S. EPA. 1996. Office of Prevention, Pesticides and Toxic Substances. Ecological Effects Test Guideline, OPPTS 850.1035. Mysid Acute Toxicity Test. "Public Draft". EPA 712-C-96-136. April 1996. U.S. Environmental Protection Agency. Washington, D.C.

APPENDIX I. OUTPUT OF REVIEWER'S STATISTICAL VERIFICATION:

CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
130	20	19	95	2.002716E-03
79	20	0	0	9.536742E-05
46	20	0	0	9.536742E-05
28	20	0	0	9.536742E-05
14	20	0	0	9.536742E-05

THE BINOMIAL TEST SHOWS THAT 79 AND 130 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 104.7117

WHEN THERE ARE LESS THAN TWO CONCENTRATIONS AT WHICH THE
PERCENT DEAD IS BETWEEN 0 AND 100, NEITHER THE MOVING AVERAGE NOR THE
PROBIT METHOD CAN GIVE ANY STATISTICALLY SOUND RESULTS.