DP Barcode: D328639 MRID No.: 468017-23

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

1. **CHEMICAL:** Pyrasulfotole PC Code No.: 000692

2. **TEST MATERIAL:** AE 0317309 Purity: 95.4%

3. **CITATION**

Authors:	Hoberg, James R.
Title:	AE 0317309- Acute Toxicity to Mysids (*Americamysis bahia*) Under Static Conditions
Study Completion Date:	August 5, 2004
Laboratory:	Springborn Smithers Laboratories, Wareham, MA
Sponsor:	Bayer CropScience, Stilwell, KS
Laboratory Report ID:	13798.6158
MRID No.:	468017-23
DP Barcode:	D328639

4. **REVIEWED BY:** John Marton, Staff Scientist, Cambridge Environmental Inc.

Signature: **Date:** 5/08/06

APPROVED BY: Teri S. Myers, Senior Scientist, Cambridge Environmental Inc.

Signature: **Date:** 5/21/06

5. **REVIEWED BY:** Melissa Panger, Biologist, OPP/EFED/ERB-4

Signature: **Date:** 11/29/06

REVIEWED BY: Martin LeMay, Biologist, Officer No. 1629, PMRA

Signature: **Date:** 11/02/06

REVIEWED BY: David McAdam, Australian Commonwealth Department of the Environment and Heritage (DEH).

Signature: **Date:** 11/02/06

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
Definitive Test Duration:	96-hours
Study Method:	Static
Type of Concentrations:	Mean-Measured

8. CONCLUSIONS:

Results Synopsis

LC_{50}: 1.1 mg ai/L 95% C.I.: 0.84-1.5 mg ai/L
NOAEC: 0.37 mg ai/L
Probit Slope: 2.23

9. ADEQUACY OF THE STUDY

A. Classification: Acceptable

B. Rationale: The deviations noted below did not appear to affect the results of the study.

C. Repairability: N/A

10. BACKGROUND

11. GUIDELINE DEVIATIONS

1. Pre-test health and mortality of the test organisms were not reported.
2. The salinity of the dilution water used (32-34 ppt) is higher than in the Guideline of 20 ppt.

These deviations did not appear to affect the results of the study.

12. **SUBMISSION PURPOSE:** This study was submitted for the purposes of new chemical registration for Pyrasulfotole (AE 0317309).

13. MATERIALS AND METHODS

A. Test Organisms

Guideline Criteria	Reported Information
Species Preferred species are ***Americamysis bahia*** (formerly *Mysidopsis bahia*), *Penaeus setiferus*, *P. duorarun*, *P. aztecus* and *Palaemonetes sp.*	*Americamysis bahia*
Age Juvenile, mysids should be # 24 hours old	≤24 Hours
Supplier	In-house laboratory cultures
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	14 days
Wild caught organisms were quarantined for 7 days?	N/A
Were there signs of disease or injury?	Not reported
If treated for disease, was there no sign of the disease remaining during the 48 hours prior to testing?	Not reported

Guideline Criteria	Reported Information
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.	Mysids were fed live brine shrimp (*Artemia salina*) nauplii once daily during the acclimation period and definitive test. The size of the test organisms was not provided.
Pretest Mortality <3% mortality 48 hours prior to testing	Not reported

C. Test System

Guideline Criteria	Reported Information
Source of dilution water Soft reconstituted water or water from a natural source, **not** dechlorinated tap water	Filtered natural sea water collected 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 ‰	32-34‰ The salinity of the dilution water used is higher than in the Guideline of 20%.
Water Temperature Approx. 22 ± 1 EC	24-26°C
pH 8.0-8.3 for marine (stenohaline) shrimp, 7.7-8.0 for estuarine (euryhaline) shrimp, monthly range < 0.8	7.9-8.1
Dissolved Oxygen Static: ∃ 60% during 1st 48 hrs and ∃ 40% during 2nd 48 hrs, Flow-through: ∃ 60%	5.5-7.5 mg/L (>60% throughout definitive test)

Guideline Criteria	Reported Information
Total Organic Carbon Should be <5 mg/L in reconstituted seawater	<2.0 mg/L for the month of June 2004.
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 1 L 900 mL
Type of Dilution System Must provide reproducible supply of toxicant	N/A; static conditions were used
Flow Rate Consistent flow rate of 5-10 vol/24 hours, meter systems calibrated before study and checked twice daily during test period	N/A; static conditions were used
Biomass Loading Rate Static: # 0.8 g/L at # 17EC, # 0.5 g/L at > 17EC; flow-through: # 1 g/L/day (N/A for mysids)	Not reported
Photoperiod 16 hours light, 8 hours dark	16 h light, 8 h dark; sudden transitions from light to dark were avoided
Solvents Not to exceed 0.5 ml/L for static tests or 0.1 ml/L for flow-through tests	Solvent: N/A; a solvent was not used Maximum conc.: N/A

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.	A 96-hour static range-finding test was conducted by exposing 20 mysids (2 reps of 10 mysids/rep) to nominal concentrations of 0 (negative control), 0.10, 1.0, 10.0 and 100 mg ai/L, with the exception of the 1.0 mg ai/L treatment level which only contained one replicate of 10 mysids. At test termination, mortality was 40, 95 and 100% at the 1.0, 10 and 100 mg ai/L, respectively. No mortality or sub-lethal effects were observed at the control or 0.10 mg ai/L treatment level. Based on these results and after consultation with the study sponsor, nominal concentrations of 0 (negative control), 0.10, 0.20, 0.40, 0.80, 1.6, 3.2, 6.4 and 13 mg ai/L were selected for the definitive toxicity test.
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.	0 (negative control), 0.10, 0.20, 0.40, 0.80, 1.6, 3.2, 6.4 and 13 mg ai/L
Number of Test Organisms Minimum 20/level, may be divided among containers	20 mysids/level; 10 mysids/rep, 2 reps/level
Test organisms randomly or impartially assigned to test vessels?	Yes
Biological observations made every 24 hours?	Yes

Guideline Criteria	Reported Information
Water Parameter Measurements 1. Temperature Measured constantly or, if water baths are used, every 6 hrs, may not vary > 1EC 2. DO and pH Measured at beginning of test and ever 48 h in the high, medium, and low doses and in the control	Temperature was measured daily in each test vessel and continuously in replicate B of the 0.80 mg ai/L treatment level. DO and pH were measured daily in each replicate test vessel.
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	One sample was removed and analyzed from each treatment level and the control at each sampling interval. At test initiation, samples were removed from the intermediate vessel prior to division into replicate test vessels, and at test termination samples were removed from a composite of replicates A and B.

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	92-99% of nominal
Control Mortality Not more than 10% of control organisms may die or show abnormal behavior.	0%
Raw data included?	Yes
Signs of toxicity (if any) were described?	Yes

Mortality

Concentration (mg ai/L)		Number of Shrimp	Cumulative Number Dead			
Nominal	Mean Measured		Hour of Study			
			24	48	72	96
Control	<0.0099	20	0	0	0	0
0.10	0.093	20	0	1	1	1
0.20	0.20	20	0	1	1	1
0.40	0.37	20	0	0	1	1
0.80	0.75	20	0	0	8	8
1.6	1.5	20	0	2	10	10
3.2	2.9	20	0	3	15	18
6.4	6.2	20	0	5	14	19
13	12	20	0	6	18	20

Other Significant Results:

At the 24-hour observation interval, lethargy was observed in the mean-measured 1.5, 2.9 and 6.2 mg ai/L treatment levels and erratic swimming was observed in the mean-measured 6.2 and 12 mg ai/L treatment levels. At the 48-hour observation interval, one mysid in the mean-measured 1.5 mg ai/L treatment level was lethargic and on the bottom of the test vessel, several mysids were lethargic in the mean-measured 6.2 mg ai/L treatment level and all surviving mysids in the mean-measured 12 mg ai/L treatment level were swimming erratically. At 72- and 96-hours, no sub-lethal effects were observed in the control or any of the treatment levels.

B. Statistical Results

Method: The 96-hour LC_{50} value (and 95% C.I.) was determined by comparing the cumulative mortality data of the treatment levels to the control using the probit analysis. The NOAEC value was determined by visual enumeration.

96-hr LC_{50}: 1.1 mg ai/L 95% C.I.: 0.84-1.5 mg ai/L
NOAEC: 0.37 mg ai/L
Probit Slope: Not reported

15. VERIFICATION OF STATISTICAL RESULTS

Parameter	Result
Binomial Test LC_{50} (C.I.)	1.5 (0.37-2.9) mg ai/L
Moving Average Angle LC_{50} (95% C.I.)	1.1 (0.83-1.5) mg ai/L
Probit LC_{50} (95% C.I.)	1.1 (0.84-1.5) mg ai/L
Probit Slope	2.23
NOAEC	0.37 mg ai/L

16. REVIEWERS' COMMENTS:

The reviewers' results were identical to those of the study author. The study author's results were based on the mean-measured concentrations which had been previously corrected for the purity of the test material (95.4%).

Analytical verification of the nominal concentrations yielded mean-measured concentrations of <0.0099 (<LOQ; negative control), 0.093, 0.20, 0.37, 0.75, 1.5, 2.9, 6.2 and 12 mg ai/L.

All test solutions were observed to be clear and colorless with no undissolved test material, with the exception of the nominal 13 mg ai/L exposure solution which was observed to be slightly beige in color.

The criteria used for determining death were the absence of mobility and failure to respond to gentle prodding.

The in-life portion of the 96-hour acute toxicity test was conducted between June 4 to June 8, 2004.

17. REFERENCES:

ASTM. 2002. Standard practice for conducting acute toxicity tests with fishes, macroinvertebrates and amphibians. Standard E729-96. American Society for Testing and Materials, 100 Barr Harbor Road, West Conshohocken, PA 19428.

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

Stephan, C.E. 1977. Methods for calculating an LC50. Aquatic Toxicology and Hazard Evaluation, ASTM STP 634, F.L. Mayer and J.L. Hamelink, Eds., American Society for Testing and Materials, Philadelphia, PA. pp 65-84.

U.S. EPA. Federal Insecticide, Fungicide and Rodenticide Act (FIFRA); Good Laboratory Practice Standards, Final Rule (40 CFR, Part 160). U.S. Environmental Protection Agency, Washington, D.C.

U.S. EPA. 1982. Office of Pesticide Programs. Pesticide Assessment Guidelines, Sundivision E, Hazard Evaluation: Wildlife and Aquatic Organisms. EPA-540/9-82-024. 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.

APPENDIX I. OUTPUT OF REVIEWER'S STATISTICAL VERIFICATION:

CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
12	20	20	100	9.536742E-05
6.2	20	19	95	2.002716E-03
2.9	20	18	90	2.012253E-02
1.5	20	10	50	58.80985
.75	20	8	40	25.17223
.37	20	1	5	2.002716E-03
.2	20	1	5	2.002716E-03
.093	20	1	5	2.002716E-03

THE BINOMIAL TEST SHOWS THAT .37 AND 2.9 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 1.5

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS
7	4.283236E-02	1.126335	.8313546 - 1.537068

RESULTS CALCULATED USING THE **PROBIT METHOD**

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
4	6.464742E-02	1	.2311946

SLOPE = 2.230896
95 PERCENT CONFIDENCE LIMITS = 1.663671 AND 2.79812

LC50 = 1.110686
95 PERCENT CONFIDENCE LIMITS = .835206 AND 1.480244

LC10 = .2994425
95 PERCENT CONFIDENCE LIMITS = .1739494 AND .4309787

SUMMARY OF FISHERS EXACT TESTS

GROUP	IDENTIFICATION	NUMBER EXPOSED	NUMBER DEAD	SIG (P=.05)
	CONTROL	20	0	
1	0.093	20	1	
2	0.20	20	1	
3	**0.37**	**20**	**1**	
4	0.75	20	8	*
5	1.5	20	10	*
6	2.9	20	18	*
7	6.2	20	19	*
8	12	20	20	*

FISHERS EXACT TEST

IDENTIFICATION	NUMBER OF ALIVE	DEAD	TOTAL ANIMALS
CONTROL	20	0	20
0.093	19	1	20
TOTAL	39	1	40

CRITICAL FISHERS VALUE (20,20,20) (p=0.05) IS 15. b VALUE IS 19.
Since b is greater than 15 there is no significant difference between CONTROL and TREATMENT at the 0.05 level.

FISHERS EXACT TEST

IDENTIFICATION	NUMBER OF ALIVE	DEAD	TOTAL ANIMALS
CONTROL	20	0	20
0.20	19	1	20
TOTAL	39	1	40

CRITICAL FISHERS VALUE (20,20,20) (p=0.05) IS 15. b VALUE IS 19.
Since b is greater than 15 there is no significant difference between CONTROL and TREATMENT at the 0.05 level.

FISHERS EXACT TEST

IDENTIFICATION	NUMBER OF ALIVE	DEAD	TOTAL ANIMALS
CONTROL	20	0	20

0.37	19	1	20
TOTAL	39	1	40

CRITICAL FISHERS VALUE (20,20,20) (p=0.05) IS 15. b VALUE IS 19.
Since b is greater than 15 there is no significant difference between CONTROL and TREATMENT at the 0.05 level.

FISHERS EXACT TEST

	NUMBER OF		
IDENTIFICATION	ALIVE	DEAD	TOTAL ANIMALS
CONTROL	20	0	20
0.75	12	8	20
TOTAL	32	8	40

CRITICAL FISHERS VALUE (20,20,20) (p=0.05) IS 15. b VALUE IS 12.
Since b is less than or equal to 15 there is a significant difference between CONTROL and TREATMENT at the 0.05 level.

FISHERS EXACT TEST

	NUMBER OF		
IDENTIFICATION	ALIVE	DEAD	TOTAL ANIMALS
CONTROL	20	0	20
1.5	10	10	20
TOTAL	30	10	40

CRITICAL FISHERS VALUE (20,20,20) (p=0.05) IS 15. b VALUE IS 10.
Since b is less than or equal to 15 there is a significant difference between CONTROL and TREATMENT at the 0.05 level.

FISHERS EXACT TEST

IDENTIFICATION	NUMBER OF ALIVE	DEAD	TOTAL ANIMALS
CONTROL	20	0	20
2.9	2	18	20
TOTAL	22	18	40

CRITICAL FISHERS VALUE (20,20,20) (p=0.05) IS 15. b VALUE IS 2.
Since b is less than or equal to 15 there is a significant difference between CONTROL and TREATMENT at the 0.05 level.

FISHERS EXACT TEST

IDENTIFICATION	NUMBER OF ALIVE	DEAD	TOTAL ANIMALS
CONTROL	20	0	20
6.2	1	19	20
TOTAL	21	19	40

CRITICAL FISHERS VALUE (20,20,20) (p=0.05) IS 15. b VALUE IS 1.
Since b is less than or equal to 15 there is a significant difference between CONTROL and TREATMENT at the 0.05 level.

FISHERS EXACT TEST

IDENTIFICATION	NUMBER OF ALIVE	DEAD	TOTAL ANIMALS
CONTROL	20	0	20

12	0	20	20
TOTAL	20	20	40

CRITICAL FISHERS VALUE (20,20,20) (p=0.05) IS 15. b VALUE IS 0.
Since b is less than or equal to 15 there is a significant difference between CONTROL and TREATMENT at the 0.05 level.

SUMMARY OF FISHERS EXACT TESTS

GROUP	IDENTIFICATION	NUMBER EXPOSED	NUMBER DEAD	SIG (P=.05)
	CONTROL	20	0	
1	0.093	20	1	
2	0.20	20	1	
3	0.37	20	1	
4	0.75	20	8	*
5	1.5	20	10	*
6	2.9	20	18	*
7	6.2	20	19	*
8	12	20	20	*