DP Barcode: D348310 MRID No.: 473075-05

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

1. **CHEMICAL:** Dimethyl Disulfide PC Code No.: 029088

2. **TEST MATERIAL:** Dimethyl Disulfide Purity: 99.6%

3. **CITATION**

Authors:	Minderhout, Tui, T.Z. Kendall and H.O. Krueger
Title:	Dimethyl Disulfide: A 96-Hour Static-Renewal Acute Toxicity Test with the Saltwater Mysid (*Americamysis bahia*)
Study Completion Date:	October 18, 2007
Laboratory:	Wildlife International, Ltd., Easton, MD
Sponsor:	Arkema, Inc., Philadelphia, PA
Laboratory Report ID:	524A-116A
MRID No.:	473075-05
DP Barcode:	D348310

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

Signature: John Marton **Date:** 04/23/08

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

Signature: Teri S Myers **Date:** 04/24/08

5. **APPROVED BY:** ~~Allen Vaughan, {Specialty},~~ OPP/EFED/ERB-{Section}
Valerie Woodard, Biologist
Signature: Valerie Woodard **Date:** 03/30/2010

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:	Juveniles (<24 Hours)
Definitive Test Duration:	96-Hours
Study Method:	Static-Renewal
Type of Concentrations:	Mean-Measured

8. CONCLUSIONS:

Based on the moving average LC_{50} in this study (4.9 mg ai/L), dimethyl disulfide would be categorized as moderately toxic to mysid shrimp on an acute toxicity basis.

Results Synopsis

LC_{50}: 4.9 mg ai/L 95% C.I.: 3.9-6.1 mg ai/L
NOAEC: 2.5 mg ai/L
Probit Slope: N/A

9. ADEQUACY OF THE STUDY

A. Classification: Acceptable

B. Rationale:

C. Repairability: NA

10. BACKGROUND

11. GUIDELINE DEVIATIONS

The following deviation from OPPTS 850.1035 was noted:

The TOC of the dilution water was not reported.

This deviation does impact the acceptability of the study.

12. SUBMISSION PURPOSE: This study was submitted to provide data on the acute exposure of dimethyl disulfide to the saltwater mysid (*Americamysis bahia*) for the purpose of new 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	Juveniles (<24 Hours)
Supplier	In-house 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	Juveniles used in the test were obtained from adults cultured under test conditions for approximately 2 weeks.
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

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 in the culture were fed live brine shrimp (*Artemia sp.*) nauplii (Brine Shrimp Direct, Ogden, Utah) daily, occasionally enriched with ALGAMAC-2000 (Aquafauna, Hawthorn, California) to prevent cannibalism.
Pretest Mortality <3% mortality 48 hours prior to testing	None 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	Natural seawater collected at Indian River Inlet, Delaware. The water was filtered and diluted to a salinity of approximately 20‰ with well water.
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 ★	19-20★
Water Temperature Approx. 22 ± 1 EC	24.0-25.2EC
pH 8.0-8.3 for marine (stenohaline) shrimp, 7.7-8.0 for estuarine (euryhaline) shrimp, monthly range < 0.8	7.9-8.3
Dissolved Oxygen Static: ∃ 60% during 1st 48 hrs and ∃ 40% during 2nd 48 hrs, Flow-through: ∃ 60%	≥4.5 mg/L (≥61% of saturation)

Guideline Criteria	Reported Information
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.	1. Glass 2. 500 mL 3. 500 mL Test chambers were placed into an environmental chamber.
Type of Dilution System Must provide reproducible supply of toxicant	N/A
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
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)	N/A
Photoperiod 16 hours light, 8 hours dark	16L:8D; a 30-minute period of low-light intensity was provided to avoid sudden changes in lighting
Solvents Not to exceed 0.5 ml/L for static tests or 0.1 ml/L for flow-through tests	N/A; a solvent was not used

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.	The nominal concentrations for use in the definitive test were selected in consultation with the Sponsor, and were based on results of exploratory range finding toxicity data. However, no details pertaining to the range finding test were provided.
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), 1.1, 2.3, 4.5, 9.0 and 18 mg ai/L.
Number of Test Organisms Minimum 20/level, may be divided among containers	20 per control and treatment level, equally divided among two 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 > 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	1. Temperature was measured in each test vessel at test initiation, before and after the renewal at 48 hours, and at test termination in all test vessels. Temperature was also measured continuously in a container of water placed adjacent to the test chambers in the environmental chamber. 2. DO and pH were measured in each test vessel at test initiation, before and after the renewal at 48 hours, and at test termination in all test vessels.

Guideline Criteria	Reported Information
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	Samples were collected from each test vessel containing new solutions at 0 and 48 hours, and from aged solutions at 48 and 96 hours. Samples were analyzed using HPLC with ultraviolet detection.

14. REPORTED RESULTS

A. General Results

Guideline Criteria	Reported Information
Quality assurance and GLP compliance statements were included in the report?	Yes. This study was conducted in compliance with Good Laboratory Practice Standards as published by the U.S. Environmental Protection Agency (40 CFR Parts 160 and 792, 17 August 1989); OECD Principles of Good Laboratory Practice (ENV/MC/CHEM (89) 17); and Japan MAFF (11 NohSan, Notification No. 6283, Agricultural Production Bureau, 1 October 1999), with the following exceptions: periodic screening analyses of saltwater for potential contaminants were performed using a certified laboratory and standard U.S. EPA analytical methods.
Recovery of Chemical	Recoveries of the individual samples ranged from 74.4-115% of nominal, with the exception of the new 2.3 mg ai/L solution at test initiation, which yielded a recovery of 166% of nominal. Mean-measured concentrations yielded recoveries of 96-111% of nominal.
Control Mortality	0%

Guideline Criteria	Reported Information
Not more than 10% of control organisms may die or show abnormal behavior.	
Raw data included?	Yes
Signs of toxicity (if any) were described?	Yes

Mortality

Concentration (mg ai/L)		Number of Mysids	Cumulative Number Dead			
Nominal	Mean Measured		Hour of Study			
			24	48	72	96
Negative Control	<0.500	20	0	0	0	0
1.1	1.1	20	0	0	0	1
2.3	2.5	20	0	0	0	0
4.5	4.3	20	0	6	6	7
9.0	10	20	1	20	20	20
18	19	20	20	20	20	20

Other Significant Results:

One mysid in the negative control and two mysids in the mean-measured 4.3 mg ai/L were observed to be swimming erratically at test termination. The one mortality at the mean-measured 1.1 mg ai/L treatment level was actually a missing mysid, which was assumed to be dead.

B. Statistical Results

Method: The mortality data were analyzed using the computer program of C.E. Stephan. The program was designed to calculate the LC_{50} value and the 95% confidence interval by probit analysis, the moving average method, and binomial probability with nonlinear interpolation. The 96-hour LC_{50} value was determined using the binomial probability method. The NOAEC value was determined by visual interpretation of the mortality and observation data. All toxicity values were based on the mean-measured concentrations.

96-hr LC_{50}: 5.0 mg ai/L 95% C.I.: 2.5-10 mg ai/L
NOAEC: 2.5 mg ai/L
Probit Slope: N/A

15. VERIFICATION OF STATISTICAL RESULTS

Parameter	Result
Binomial Test LC_{50} (C.I.)	5.0 (2.5-10) mg ai/L
Moving Average Angle LC_{50} (95% C.I.)	4.9 (3.9-6.1) mg ai/L
Probit LC_{50} (95% C.I.)	4.7 (0-∞) mg ai/L
Probit Slope	4.8 (-5.5-15.0)
NOAEC	2.5 mg ai/L

16. REVIEWER'S COMMENTS:

The moving average method was used due to the poor fit for the probit method (<0.001). The reviewer's LC_{50} value and 95% confidence interval obtained using the moving average method were slightly lower and narrower, respectively, than those estimates obtained using the binomial method (the same method used by the study authors). Therefore, the reviewer's results based on the moving average method are reported in the Conclusions sections of this DER.

The reviewer used the mean-measured concentrations for the statistical analysis of the mortality data instead of the time-weighted average concentrations. Test solutions were only analytically sampled and verified at 0 and 48 hours for the nominal 9.0 mg ai/L treatment level and at 0 and 24 hours for the nominal 18 mg ai/L treatment level. Because only one renewal period was analyzed at these levels, the reviewer was unable to determine the time-weighted averages.

Therefore, to remain consistent, the reviewer used mean-measured concentrations for all levels. With the exception of the new solution at the nominal 2.3 mg ai/L level at 0 hours which yielded a recovery of 166% of nominal, test solutions remained stable during both renewal periods with recoveries ranging from 74.4 to 115% of nominal, so the mean-measured concentrations were indicative of actual exposure concentrations.

An initial trial was conducted from September 4 to 6, 2007 but was terminated due to unacceptable control mortality. The in-life portion of the final definitive toxicity test was conducted from September 12 to 16, 2007.

17. REFERENCES:

U.S. Environmental Protection Agency. 1996. Series 850- Ecological Effects Test Guidelines (*draft*), OPPTS Number 850.1035: *Mysid Acute Toxicity Test.*

U.S. Environmental Protection Agency. 1985. Standard Evaluation Procedure: *Acute Toxicity Test for Estuarine and Marine Organisms (Shrimp 96-Hour Acute Toxicity Test*). Hazard Evaluation Division. Office of Pesticide Programs. EPA-540/9-85-010. Washington, DC.

ASTM Standard E729-96. 1996. *Standard Guide for Conducting Acute Toxicity Tests on Test Materials with Fishes, Macroinvertebrates and Amphibians.* American Society for Testing and Materials.

Stephan, C.E. 1978. U.S. EPA Environmental Research Laboratory, Duluth, Minnesota. Personal Communication.

Finney, D.J. 1971. *Statistical Methods in Biological Assay.* Second Edition. Griffin Press, London.

Thompson, W.R. 1947. *Bacteriological Reviews.* Vol. II. No. 2. Pp. 115-145.

Stephan, C.E. 1977. "Methods for Calculating an LC50", *Aquatic Toxicology and Hazard Evaluations.* American Society for Testing and Materials. Publication Number STP 634, pp. 65-84.

APPENDIX I. OUTPUT OF REVIEWER'S STATISTICAL VERIFICATION:

CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
19	20	20	100	9.536742E-05
10	20	20	100	9.536742E-05
4.3	20	7	35	13.1588
2.5	20	0	0	9.536742E-05
1.1	20	1	5	2.002716E-03

THE BINOMIAL TEST SHOWS THAT 2.5 AND 10 CAN BE USED AS STATISTICALLY SOUND CONSERVATIVE 95 PERCENT CONFIDENCE LIMITS, BECAUSE THE ACUTAL CONFIDENCE LEVEL ASSOCIATED WITH THESE LIMITS IS GREATER THAN 95 PERCENT.

AN APPROXIMATE LC50 FOR THIS SET OF DATA IS 4.991972

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS
4	4.546307E-03	4.867368	3.927636-6.064012

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
6	4.627617	13.56421	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 = 4.751509
95 PERCENT CONFIDENCE LIMITS =-5.469888 AND 14.97291

LC50 = 4.739612
95 PERCENT CONFIDENCE LIMITS = 0 AND +INFINITY

LC10 = 2.561293
95 PERCENT CONFIDENCE LIMITS = 0 AND +INFINITY

SUMMARY OF FISHERS EXACT TESTS

GROUP	IDENTIFICATION	NUMBER EXPOSED	NUMBER DEAD	SIG (P=.05)
	CONTROL	20	0	
1	1.1	20	1	
2	2.5	20	0	
3	4.3	20	7	*
4	10	20	20	*
5	19	20	20	*