DATA EVALUATION RECORD
• 72-3(B) -- ACUTE EC_{50} TEST WITH AN ESTUARINE/MARINE MOLLUSK SHELL DEPOSITION STUDY

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 Shell Deposition Test with the Eastern Oyster (*Crassostrea virginica*)
Study Completion Date:	December 10, 2007 (amended report date)
Laboratory:	Wildlife International, Ltd., Easton, MD
Sponsor:	Arkema, Inc., Philadelphia, PA
Laboratory Report ID:	524A-118
MRID No.:	473075-03
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, Bio 09/15t

Signature: Valerie Woodard **Date:** 3/30/2010

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 shell deposition in oysters. 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: 44.7±1.54 mm (n=20)
Definitive Test Duration: 96-Hours
Study Method: Flow-Through

8. CONCLUSIONS:

There was significant mortality and reduction in shell deposition in this study. The estimated 96h LC50=29mg/L using the moving average method, which categorizes dimethyl disulfide as slightly toxic on an acute toxicity basis. There were significant differences between the negative control and 15, 30 and 60 mg/L nominal concentrations.

Results Synopsis

LC_{50}: 29mg/L	95% C.I.: 24-37 mg/L
EC_{50}:	95% C.I.:
NOAEC:	
Probit Slope:	

9. ADEQUACY OF THE STUDY

A. Classification: INVALID

B. Rationale: For mean shell deposition, there was a significant difference between the negative and solvent control groups, which according to the EPA memo titled, "Interim Policy Guidance for the Use of Dilution-Water (Negative) and Solvent Controls in Statistical Data Analysis for Guideline Aquatic Toxicology Studies", dated March 30, 2006, could result in the INVALID classification of this study.

C. Repairability: NA

10. BACKGROUND

11. GUIDELINE DEVIATIONS

The following deviations from OPPTS 850.1025 were noted:

1. Shell deposition was significantly ($p<0.05$) lower in the solvent control (22%), relative to the negative control group.
2. No pretest mortality was reported.
3. It was not reported if all oysters were from the same year class.
4. The amount of peripheral shell removed prior to testing was not reported.

5. Salinity was 20 ppt rather than the suggested 30-34 ppt.

The significant difference between the two controls for mean shell deposition affects the acceptability of this study.

12. SUBMISSION PURPOSE: This study was submitted to provide data on the acute exposure of dimethyl disulfide to the eastern oyster (*Crassostrea virginica*) for the purpose of new chemical registration.

13. MATERIALS AND METHODS

A. Test Organisms

Guideline Criteria	Reported Information
Species Preferred species are the Pacific oyster (*Crassostrea gigas*) and the Eastern oyster (*Crassostrea virginica*)	*Crassostrea virginica*
Mean valve height 25 - 50 mm along the long axis	44.7±1.54 mm (n=20)
Supplier	Circle C Oyster Ranch, Ridge, Maryland
Are all oysters from same source?	Yes
Are all oysters from the same year class?	Not reported

B. Source/Acclimation

Guideline Criteria	Reported Information
Acclimation Period Minimum 10 days	10 Days
Wild caught organisms were quarantined for 7 days?	N/A

Guideline Criteria	Reported Information
Were there signs of disease or injury?	The oysters showed no sign of disease or stress during the acclimation period.
If treated for disease, was there no sign of the disease remaining during the 48 hours prior to testing?	N/A
Amount of peripheral shell growth removed prior to testing	Recently deposited shell was removed from all oysters; the actual amount was not specified.
Feeding during the acclimation Must be fed to avoid stress.	Oysters were fed a suspension of marine microalgae (Reed Mariculture, Inc., Campbell, California) at a nominal rate of $2.9x10^9$ cells/oyster/day.
Pretest Mortality <3% mortality 48 hours prior to testing	No mortality was reported.

C. Test System

Guideline Criteria	Reported Information
Source of dilution water Natural unfiltered seawater from an uncontaminated source.	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) salinity, weekly range < 6 ★	20 ★
Water Temperature 15E-30E C, consistent in all test vessels	20.5-21.0EC
pH	8.0-8.1

Guideline Criteria	Reported Information
Dissolved Oxygen ∃ 60% throughout	≥6.1 mg/L (≥76% of saturation)
Total Organic Carbon	Not Reported
Test Aquaria Should be constructed of glass or stainless steel.	Test chambers were 54 L glass aquaria filled with approximately 27 L of test water. Test chambers were indiscriminately positioned in a temperature-controlled environmental chamber.
Type of Dilution System Must provide reproducible supply of toxicant	The test concentrations, control and solvent control were delivered to the chambers using a continuous-flow diluter. Syringe pumps delivered the test substance stock solutions and solvent to mixing chambers assigned to each treatment and the solvent control.
Flow rate Consistent flow rate	19 vol/24 hours
Was the loading of organism such that each individual sits on the bottom with water flowing freely around it?	Yes
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	Solvent: DMF Maximum conc.: 0.1 ml/L

D. Test Design

Guideline Criteria	Reported Information
Range Finding Test If EC_{50} >100 mg/L with 30 or more oysters, 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; each conc. should be 60% of the next highest conc.; concentrations should be in a geometric series	0 (negative and solvent controls), 3.8, 7.5, 15, 30 and 60 mg ai/L
Number of Test Organisms Minimum 20 individual per test level and in each control	20 per level and control
Test organisms randomly or impartially assigned to test vessels?	Yes
Biological observations made every 24 hours?	Yes
Water Parameter Measurements 1. Temperature Measured hourly in at least one chamber 2. DO and pH Measured at beginning of test and every 48 h in the high, medium, and low doses and in the control	1. Temperature was measured in each test vessel at 0 and 96 hours. Temperature was also measured continuously in the negative control chamber. 2. DO was measured in each test vessel at test initiation and every 24 hours thereafter; pH was measured in each vessel at 0, 48 and 96 hours.
Was chemical analysis performed to determine the concentration of the test material at the beginning and end of the	Yes; analytical verification was conducted at 0, 48 and 96 hours using HPLC with ultraviolet detection at 200 nm.

Guideline Criteria	Reported Information
test? (Optional)	

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 seawater for potential contaminants were performed using a certified laboratory and standard U.S. EPA analytical methods.
Control Mortality Not more than 10% of control organisms may die or show abnormal behavior.	0% in both controls
Control Shell Deposition Must be at least 2 mm.	3.2 and 2.5 mm in the negative and solvent controls, respectively.
Recovery of Chemical	78.5-82.8%, based on the reviewer-calculated time-weighted average concentrations
Raw data included?	Yes
Signs of toxicity (if any) were described?	Yes

Shell Growth

Concentration (mg ai/L)		Number Per Level	Number Dead	Mean Shell Deposition (mm)	Mean Percent Reduction[1]
Nominal	TWA				
Control	<2.00	20	0	3.2±1.21	N/A
Solvent Control	<2.00	20	0	2.5±1.18	21.9
3.8	3.01	20	0	2.9±1.19	9.4
7.5	5.93	20	0	2.2±0.79	31.3
15	11.8	20	0	1.7±0.43	46.9
30	23.7	20	8	0.2±0.39	93.8
60	49.7	20	16	0.0±0.00	100

[1] Inhibitions were calculated by the reviewer relative to the negative control only.
N/A- Not Applicable

By test termination, no sub-lethal effects were observed in the controls or in the TWA 3.01 and 5.93 mg ai/L treatment groups; 4, 9 and 4 of the surviving oysters at the TWA 11.8, 23.7 and 49.7 mg ai/L treatment levels, respectively, were observed to be slow to close their valves when gently prodded.

B. Statistical Results

Method: Statistical analyses were conducted using the TOXSTAT® computer program. Negative control and solvent control shell deposition data were compared using an appropriate t-test. There were no significant differences between the control groups (p = 0.05). Therefore, growth inhibition was evaluated on the basis of the pooled control data. The EC_{50} value was calculated using linear interpolation. The data were evaluated for normality and homogeneity of variance using the Chi-Square test and Levene's test, respectively. The data met the assumption of normality but failed that of homogeneity of variance due to the lack of growth and mortality in the 23 and 50 mg ai/L treatment groups. Data were transformed using square root and passed the assumption of both normality and homogeneity of variance. The treatment groups were compared to the pooled control data using analysis of variance (ANOVA) and the Bonferroni t-test to identify any significant differences. The NOAEC was determined from the statistical analysis of the data and an assessment of the concentration-response pattern.

96-hr EC_{50}: 14 mg ai/L 95% C.I.: 11-15 mg ai/L
NOAEC: 3.0 mg ai/L
Probit Slope: Not Reported

15. VERIFICATION OF STATISTICAL RESULTS

Parameter	Result
Statistical Method for EC_{50}	Probit Analysis via Nuthatch statistical software
EC_{50} (95% C.I.)	12 (10-14) mg ai/L
Probit Slope	4.87±0.783
Statistical Method for NOAEC	Kruskal-Wallis and Williams
NOAEC	3.01 mg ai/L

16. REVIEWER'S COMMENTS:

The reviewer's results were determined using the negative control only, while those of the study authors were determined using the pooled control. The reviewer's results are reported in the Conclusions section of this DER. Additionally, the reviewer used the moving average method to determine the 96-hour LC_{50} to be 29.4 (24.2-37.4) mg ai/L due to the poor fit for the probit method.

For mean shell deposition, there was a significant difference between the negative and solvent control groups with an inhibition of 21.9% in the solvent control relative to the negative control, which according to the EPA memo titled, "Interim Policy Guidance for the Use of Dilution-Water (Negative) and Solvent Controls in Statistical Data Analysis for Guideline Aquatic Toxicology Studies", dated March 30, 2006, could result in the INVALID classification of this study. Other factors will be evaluated in addition to the significant difference between the negative control and the solvent.

The reviewer's analyses of the shell deposition data indicated that the data did not meet the assumptions of ANOVA (normality and homogeneity of variances). The non-parametric Kruskal-Wallis test identified a NOAEC value of 5.93 mg ai/L; however, the reviewer concurs with the study authors' analysis that the % reduction at this level (31%, relative to the negative control) was biologically significant. Therefore, the reviewer reported the NOAEC value to be

3.01 mg ai/L.

The in-life portion of the definitive toxicity test was conducted from October 22 to October 26, 2007. This amended report was submitted on December 10, 2007 to correct a typographical error in the study authors' original report of the EC_{50} value.

APPENDIX I. OUTPUT OF REVIEWER'S STATISTICAL VERIFICATION:

Shell deposition (mm), 96 hours; TWA- mg ai/L
File: 7503sd Transform: NO TRANSFORM

t-test of Solvent and Blank Controls Ho:GRP1 MEAN = GRP2 MEAN

GRP1 (SOLVENT CRTL) MEAN =	3.2050	CALCULATED t VALUE =	2.0232
GRP2 (BLANK CRTL) MEAN =	2.4600	DEGREES OF FREEDOM =	38
DIFFERENCE IN MEANS =	0.7450		

TABLE t VALUE (0.05 (2),40) = 2.021** SIGNIFICANT DIFFERENCE at alpha=0.05
TABLE t VALUE (0.01 (2),40) = 2.704 NO significant difference at alpha=0.01

Shell deposition (mm), 96 hours; TWA- mg ai/L
File: 7503sd Transform: NO TRANSFORMATION

Chi-square test for normality: actual and expected frequencies

INTERVAL	<-1.5	-1.5 to <-0.5	-0.5 to 0.5	>0.5 to 1.5	>1.5
EXPECTED	6.432	23.232	36.672	23.232	6.432
OBSERVED	3	26	36	25	6

Calculated Chi-Square goodness of fit test statistic = 2.3369
Table Chi-Square value (alpha = 0.01) = 13.277

Data PASS normality test. Continue analysis.

Shell deposition (mm), 96 hours; TWA- mg ai/L
File: 7503sd Transform: NO TRANSFORMATION

Shapiro-Wilks test for normality

******** Shapiro-Wilks Test is aborted ********

This test can not be performed because total number of replicates
is greater than 50.

Total number of replicates = 96

Shell deposition (mm), 96 hours; TWA- mg ai/L
File: 7503sd Transform: NO TRANSFORMATION

Hartley test for homogeneity of variance
Bartletts test for homogeneity of variance

These two tests can not be performed because at least one group has
zero variance.

Data FAIL to meet homogeneity of variance assumption.
Additional transformations are useless.

Shell deposition (mm), 96 hours; TWA- mg ai/L
File: 7503sd Transform: NO TRANSFORMATION

KRUSKAL-WALLIS ANOVA BY RANKS - TABLE 1 OF 2

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	RANK SUM
1	neg control	3.205	3.205	1427.000
2	3.01	2.850	2.850	1275.000
3	5.93	2.160	2.160	1068.500
4	11.8	1.665	1.665	737.000
5	23.7	0.167	0.167	116.500
6	49.7	0.000	0.000	32.000

Calculated H Value = 63.738 Critical H Value Table = 11.070
Since Calc H > Crit H REJECT Ho:All groups are equal.

Shell deposition (mm), 96 hours; TWA- mg ai/L
File: 7503sd Transform: NO TRANSFORMATION

DUNNS MULTIPLE COMPARISON - KRUSKAL-WALLIS - TABLE 2 OF 2

GROUP	IDENTIFICATION	TRANSFORMED MEAN	ORIGINAL MEAN	GROUP 6	5	4	3	2	1
6	49.7	0.000	0.000	\					
5	23.7	0.167	0.167	.	\				
4	11.8	1.665	1.665	.	.	\			
3	5.93	2.160	2.160	*	*	.	\		
2	3.01	2.850	2.850	*	*	*	.	\	
1	neg control	3.205	3.205	*	*	*	.	.	\

* = significant difference (p=0.05) . = no significant difference
Table q value (0.05,6) = 2.936 Unequal reps - multiple SE values

Estimates of EC%

Parameter	Estimate	95% Bounds Lower	95% Bounds Upper	Std.Err.	Lower Bound /Estimate
EC5	5.6	3.9	8.0	0.078	0.70
EC10	6.6	4.9	9.0	0.067	0.74
EC25	8.8	7.0	11.	0.050	0.80
EC50	12.	10.	14.	0.034	0.86

Slope = 4.87 Std.Err. = 0.783

!!!Poor fit: p = 0.034 based on DF= 3.0 90.

7503SD : Shell deposition (mm), 96 hours; TWA- mg ai/L

Observed vs. Predicted Treatment Group Means

Dose	#Reps.	Obs. Mean	Pred. Mean	Obs. -Pred.	Pred. %Control	%Change
0.00	20.0	3.20	2.85	0.356	100.	0.00
3.01	20.0	2.85	2.84	0.00578	99.8	0.160
5.93	20.0	2.16	2.66	-0.503	93.5	6.51
11.8	20.0	1.66	1.49	0.176	52.3	47.7
23.7	12.0	0.167	0.222	-0.0557	7.81	92.2
49.7	4.00	0.00	0.00404	-0.00404	0.142	99.9

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	**G**	**LC50**	**95 PERCENT CONFIDENCE LIMITS**	
2	**9.753802E-02**	**29.44713**	**24.24249**	**37.40059**

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
6	.1617677	1	.6686297

SLOPE = 4.591528
95 PERCENT CONFIDENCE LIMITS = 2.744799 AND 6.438257

LC50 = 30.2527
95 PERCENT CONFIDENCE LIMITS = 24.55577 AND 38.31603

LC10 = 16.00177
95 PERCENT CONFIDENCE LIMITS = 10.11995 AND 20.3425