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

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

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

3. **CITATION**

Authors: Dionne, E.

Title: AE 0172747- Acute Toxicity to Eastern Oysters (*Crassostrea virginica*) Under Flow-Through Conditions

Study Completion Date: July 15, 2003

Laboratory: Springborn Smithers Laboratories
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Sponsor: Bayer CropScience GmbH
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Laboratory Report ID: 13798.6101/35400

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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: 7-14-06

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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 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

Scientific Name of Test Organism:	*Crassostrea virginica*
Age or Size of Test Organism:	32 ± 4 mm
Definitive Test Duration:	96 hours
Study Method:	Flow-through
Type of Concentrations:	Mean measured and Nominal

8. CONCLUSIONS:

A significant difference was detected between the negative and solvent control groups for shell deposition.

Results Synopsis

96-hr EC_{50}: 39 mg ai/L 95% C.L.: 17-93 mg ai/L
NOAEC: 14 mg ai/L
Probit Slope: 1.35±0.623

9. ADEQUACY OF THE STUDY

A. Classification: INVALID

B. Rationale: There was a significant difference between negative and solvent controls. 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, the significant difference between the negative and solvent control groups results in the INVALID classification of this study.

11. GUIDELINE DEVIATIONS

1. The mean shell deposition in the negative control (1.6 mm) was less the recommended (2.0 mm).
2. A statistically significant difference was detected between the replicate mean shell deposition data of the negative and solvent controls.

12. SUBMISSION PURPOSE: This study was submitted to provide data on the acute toxicity of AE 0172747 to oysters for the purpose of 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*)	Eastern oyster (*Crassostrea virginica*)
Mean valve height 25 - 50 mm along the long axis	32 ± 4 mm (based on a representative sample of 30 test organisms)
Supplier	Circle C Oysters, Ridge, MD
Are all oysters from same source?	Yes
Are all oysters from the same year class?	Yes

B. Source/Acclimation

Guideline Criteria	Reported Information
Acclimation Period Minimum 10 days	15 days
Wild caught organisms were quarantined for 7 days?	N/A

Guideline Criteria	Reported Information
Were there signs of disease or injury?	No (oysters unsuitable for testing were discarded).
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	3-5 mm
Feeding during the acclimation Must be fed to avoid stress.	Supplemental algae diet of *Tetraselmus maculata* and *Isochrysis galbana* prepared in seawater from a concentrate.
Pretest Mortality <3% mortality 48 hours prior to testing	<1% mortality the 7 days prior to testing.

C. Test System

Guideline Criteria	Reported Information
Source of dilution water Natural unfiltered seawater from an uncontaminated source.	Natural seawater collected from the Cape Cod Canal, Bourne, MA. Results of routine analysis of the saltwater indicate no pesticides, PCBs, or toxic metals were detected at toxic concentrations.
Does water support test animals without observable signs of stress?	Yes
Salinity 30-34 ‰ (parts per thousand) salinity, weekly range < 6 ‰	32 ‰
Water Temperature 15°-30° C, consistent in all test vessels	21-22°C
pH	7.6 – 8.1

Guideline Criteria	Reported Information
Dissolved Oxygen ≥ 60% throughout	4.6-7.2 mg/L (>60% saturation)
Total Organic Carbon	Not reported
Test Aquaria Should be constructed of glass or stainless steel.	Glass aquaria, 49.5 x 25.5 x 29 cm (fill volume of 18 L).
Type of Dilution System Must provide reproducible supply of toxicant	Constant-flow serial diluter
Flow rate Consistent flow rate	6 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	16 hours light, 8 hours dark
Solvents Not to exceed 0.5 ml/L	Solvent: DMF Maximum conc.: 0.10 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.	Two range-finding tests were conducted. The second test had concentrations of 1.9, 3.8, 7.5, 15, and 30 mg ai/L with a dilution water control. After 96 hours, there was 8, 29, 0, 54, and 60% reductions in shell growth for the 1.9, 3.8, 7.5, 15, and 30 mg ai/L treatment groups, respectively, compared to the control.
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), 3.1, 6.3, 13, 25, and 50 mg ai/L
Number of Test Organisms Minimum 20 individual per test level and in each control	40 oysters/level (20 oysters/replicate)
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	The pH, temperature, and DO were measured daily in each replicate aquarium.
Was chemical analysis performed to determine the concentration of the test material at the beginning and end of the	Yes

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
Control Mortality Not more than 10% of control organisms may die or show abnormal behavior.	0%
Control Shell Deposition Must be at least 2 mm.	Negative Control: 1.6 mm Solvent Control: 2.5 mm
Recovery of Chemical	93 - 110 % (mean recoveries)
Raw data included?	Yes
Signs of toxicity (if any) were described?	No sublethal effects were observed.

Shell Growth

Concentration (ppm)		Number Per Level	Number Dead	Mean Shell Deposition (mm)	Mean Percent Reduction
Nominal	Mean Measured				
Control	--	40	0	1.6	--
Solvent Control	--	40	0	2.5	-56
3.1	3.0	40	0	2.1	-31
6.3	5.9	40	0	1.4	13
13	14	40	0	1.3	19
25	25	40	0	0.8	50*
50	50	40	0	0.9	44*

Percent reductions were determined by the reviewer comparing treatment data to the negative control.

* Statistically different from negative control based on Dunnett's Test ($p<0.05$)

B. Statistical Results

Method: The negative and solvent control data were compared using a t-test. A significant difference between the control groups was observed, and the solvent control was used for all comparisons. Significant differences in shell growth compared to the solvent control were determined using Williams' test. The NOAEC was based on a visual interpretation of the shell growth statistical data. The EC_{50} was calculated using linear regression and inverse prediction.

96-hr EC_{50}: 14 mg ai/L 95% C.I.: 0.62 - 380 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}	Bruce and Verteeg (1992), Probit method
EC_{50} (95% C.I.)	39 (17-93) mg ai/L
Probit Slope	1.35±0.623
Statistical Method for NOAEC	Dunnett's Test
NOAEC	14 mg ai/L

16. REVIEWER'S COMMENTS:

The reviewer compared the replicate mean shell deposition data from the negative and solvent controls using a t-test for two samples assuming equal variances, via Microsoft Excel. This analysis yielded a p value of 0.01, indicating a significant difference between the two controls. The determination of the EC_{50} and NOAEC values was done by comparing replicate treatment data to the negative control. 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, the significant difference between the negative and solvent control groups would result in the INVALID classification of this study.

The reviewer's 95% C.I. associated with the EC_{50} value was narrower than the study author's and the reviewer based all comparisons on the negative control group response; therefore, the reviewer's results are reported in the Conclusions section of this DER.

This study was conducted in accordance with USEPA (40 CFR Parts 160) with the exception that the routine dilution water screening analyses were performed at a separate analytical facility (Geolabs, Inc., Braintree, MA).

The experimental start date was September 19, 2002 and the experimental termination date was September 23, 2002.

During testing, the oysters were fed algae suspensions (*Isochrysis galbana*) at a concentrated rate of 10^7 cells/mL three times daily (average concentration of 10^5 cells/mL in the test solutions).

Solubility trials were conducted and several hours were required to solubilize AE 0172747 in seawater. The highest test concentration (nominal 50 mg ai/L) was prepared in a reservoir that was stirred for approximately 24 hours before flowing to the exposure system.

The 96-hour acute toxicity of AE 0172747 to the Eastern oyster, *Crassostrea virginica*, was studied under flow-through conditions. Oysters were exposed to the test material at nominal concentrations of 3.1, 6.3, 13, 25, and 50 mg ai/L with negative and solvent (DMF at 0.10 mL/L) controls. The mean measured concentrations were <0.28-0.32 (<LOQ; controls), 3.0, 5.9, 14, 25, and 50 mg ai/L. By 96 hours, there was -56, -31, 13, 19, 50 and 44% reductions in shell growth in the solvent control and the 3.0, 5.9, 14, 25, and 50 mg ai/L treatment groups, relative to the negative control. No mortalities or other sublethal effects occurred in the controls or treatment groups

The **96-hour EC_{50} value was 39 mg ai/L**, which categorizes AE 0172747 as **slightly toxic** to the Eastern oyster, *Crassostrea virginica*, on an acute toxicity basis. Based on shell deposition, the **NOAEC and LOAEC values were 14 and 25 mg ai/L**, respectively.

This study does not satisfy the guideline requirement for an acute toxicity test with oysters. The mean shell deposition in the negative control (1.6 mm) was less the recommended (2.0 mm). According to an EPA memo dated October 29, 1992, "Reevaluation of Previously Rejected Mollusk Shell Deposition Studies", the poor performance in the control group may mask effects on shell growth caused by the pesticide and the resulting EC_{50} may not reliably indicate toxicity; because this study was conducted after the issuance of this guidance, the study should be repeated.

APPENDIX I. OUTPUT OF REVIEWER'S STATISTICAL VERIFICATION:

t-Test: Two-Sample Assuming Equal Variances

	Negative Control	*Solvent Control*
Mean	1.55	2.55
Variance	0.005	0.045
Observations	2	2
Pooled Variance	0.025	
Hypothesized Mean Difference	0	
df	2	
t Stat	-6.32455532	
P(T<=t) one-tail	**0.012049964**	
t Critical one-tail	2.91998558	
P(T<=t) two-tail	0.024099927	
t Critical two-tail	4.30265273	

Oyster mean shell deposition (mm), mg ai/L; 96-hours
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ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	2.114	0.423	22.263
Within (Error)	6	0.115	0.019	
Total	11	2.229		

Critical F value = 4.39 (0.05,5,6)
Since F > Critical F REJECT Ho:All groups equal

Oyster mean shell deposition (mm), mg ai/L; 96-hours
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DUNNETTS TEST - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	neg control	1.550	1.550		
2	3.0	2.100	2.100	-3.990	
3	5.9	1.350	1.350	1.451	
4	14	1.300	1.300	1.814	
5	25	0.850	0.850	5.078	*
6	50	0.900	0.900	4.716	*

Dunnett table value = 2.83 (1 Tailed Value, P=0.05, df=6,5)

Oyster mean shell deposition (mm), mg ai/L; 96-hours
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DUNNETTS TEST - TABLE 2 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	neg control	2			
2	3.0	2	0.390	25.2	-0.550
3	5.9	2	0.390	25.2	0.200
4	14	2	0.390	25.2	0.250
5	25	2	0.390	25.2	0.700
6	50	2	0.390	25.2	0.650

Oyster mean shell deposition (mm), mg ai/L; 96-hours
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WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	neg control	2	1.550	1.550	1.825
2	3.0	2	2.100	2.100	1.825
3	5.9	2	1.350	1.350	1.350
4	14	2	1.300	1.300	1.300
5	25	2	0.850	0.850	0.875
6	50	2	0.900	0.900	0.875

Oyster mean shell deposition (mm), mg ai/L; 96-hours
File: 5433sd Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
neg control	1.825				
3.0	1.825	1.986	*	1.94	k= 1, v= 6
5.9	1.350	1.445		2.06	k= 2, v= 6
14	1.300	1.806		2.10	k= 3, v= 6
25	0.875	4.875	*	2.12	k= 4, v= 6
50	0.875	4.875	*	2.13	k= 5, v= 6

s = 0.138
Note: df used for table values are approximate when v > 20.

Estimates of EC%

Parameter	Estimate	95% Bounds Lower	Upper	Std.Err.	Lower Bound /Estimate
EC5	2.4	0.10	56.	0.60	0.043
EC10	4.5	0.35	56.	0.49	0.080
EC25	13.	2.6	60.	0.30	0.21
EC50	39.	17.	93.	0.17	0.42

Slope = 1.35 Std.Err. = 0.623

!!!Poor fit: p = 0.0088 based on DF= 3.0 6.0

5433SD : Oyster mean shell deposition (mm), mg ai/L; 96-hours

Observed vs. Predicted Treatment Group Means

Dose	#Reps.	Obs. Mean	Pred. Mean	Obs. -Pred.	Pred. %Control	%Change
0.00	2.00	1.55	1.76	-0.208	100.	0.00
3.00	2.00	2.10	1.64	0.456	93.5	6.50
5.90	2.00	1.35	1.53	-0.176	86.8	13.2
14.0	2.00	1.30	1.28	0.0194	72.8	27.2
25.0	2.00	0.850	1.06	-0.214	60.5	39.5
50.0	2.00	0.900	0.781	0.119	44.4	55.6

!!!Warning: EC5 not bracketed by doses evaluated.