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

1/9/04

1. **CHEMICAL:** Mesosulfuron-methyl — PC Code No.: 122009

2. **TEST MATERIAL:** AE F130060 Technical — Purity: 95.6%

3. **CITATION:**

Author: Dionne, E.

Title: AE F130060 00 1C96 004 - Acute Toxicity to Eastern Oysters (*Crassostrea virginica*) Under Flow-Through Conditions.

Study Completion Date: December 7, 2000

Laboratory: Springborn Laboratories, Inc.
790 Main Street
Wareham, MA 02571-1075

Sponsor: Aventis CropScience
2 T.W. Alexander Drive
Research Triangle Park, NC 27709

Laboratory Report ID: 13726.6125

MRID No.: 45386302

DP Barcode: D284719

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

Signature: Rebecca Bryan **Date:** 8/22/03

APPROVED BY: Christie E. Padova, B.S., Staff Scientist, Dynamac Corporation

Signature: C. E. Padova **Date:** 8/22/03

5. **APPROVED BY:** ~~Tim Bergar~~ Leo LaSota, Biologist, OPP/EFED/ERB - III

Signature: L LaS **Date:** 01/09/04

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6. STUDY PARAMETERS:

Scientific Name of Test Organism: *Crassostrea virginica*

Age or Size of Test Organism: Valve height: 37 ± 4.0 mm

Definitive Test Duration: 96 hours

Study Method: Flow-through

Type of Concentrations: Mean-measured

7. CONCLUSIONS:

In this 96-hour, flow-through acute EC_{50} test with an estuarine/marine mollusk, the Eastern oyster (*Crassostrea virginica*) was exposed to AE F130060 Technical (Mesosulfuron-methyl) at mean-measured concentrations of <2.3-2.6 (negative control), 13, 22, 36, 60, and 100 ppm a.i. Nominal concentrations were 0 (control),13, 20, 37, 57, and 100 ppm.

No mortalities or sublethal effects were observed during the test. The mean percent reductions of shell growth compared to the control were 0, 3, 10, 12, and <1% in the 13, 20, 37, 57, and 100 ppm a.i. treatment groups, respectively. The **NOEC is 100 ppm a.i.**, the highest test concentration. The **EC_{50} is >100 ppm a.i.**, which categorizes AE F130060 Technical (Mesosulfuron-methyl) as **practically non-toxic** to the Eastern oyster (*Crassostrea virginica*) on an acute toxicity basis.

This study is scientifically valid and fulfills the requirements of an acute toxicity test with an estuarine/marine mollusk [§72-3(b)]. This study is classified as **CORE**.

Results Synopsis

EC_{50}: >100 ppm a.i.
NOEC: 100 ppm a.i.
LOEC: >100 ppm a.i.

8. ADEQUACY OF THE STUDY:

A. Classification: Core

B. Rationale: The guideline deviation was considered to be minor and did not impact the

acceptability or validity of the study. Missing information should be provided to U.S. EPA.

C. Repairability: N/A

9. BACKGROUND:

10. GUIDELINE DEVIATIONS:

1. The total organic carbon measurement was not reported.

11. SUBMISSION PURPOSE: This study was submitted to provide data on the toxicity of AE F130060 Technical (Mesosulfuron-methyl) to an estuarine/marine mollusk for the purpose of chemical registration.

12. 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	37 ± 4.0 mm
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	13 days
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
Amount of peripheral shell growth removed prior to testing	3-5 mm
Feeding during the acclimation Must be fed to avoid stress.	Supplementary algal diets of *Tetraselmis* and *Isochrysis*.
Pretest Mortality <3% mortality 48 hours prior to testing	Mortality was <1% during 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 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) salinity, weekly range: <6 ‰	32-34‰
Water Temperature 15-30°C, consistent in all test vessels	20-22°C

Guideline Criteria	Reported Information
pH	7.9-8.0
Dissolved Oxygen ≥60% throughout	95-107% saturation
Total Organic Carbon	Not reported
Test Aquaria Should be constructed of glass or stainless steel.	Glass, 37 L (49.5 x 25.5 x 29 cm) 18-L fill volume
Type of Dilution System Must provide reproducible supply of toxicant	Constant-flow proportional diluter
Flow rate Consistent flow rate	6.0 turnovers/aquarium/day, or 5 L/oyster/hr.
Was the loading of organism such that each individual sits on the bottom with water flowing freely around it?	Not reported; study authors reported that oysters were spaced equidistant from one another with valve inflow openings facing toward the flow of water.
Photoperiod 16 hours light, 8 hours dark	16 hours light, 8 hours dark with a transition period
Solvents Not to exceed 0.5 mL/L	N/A

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.	A static (re-circulated) 96-hour range-finding study was performed at 0 (negative control) and 100 ppm a.i. By 96 hours, the reduction in shell growth was 26% at the 100 mg a.i./L treatment group, compared to the control.

Guideline Criteria	Reported Information
Nominal Concentrations of Definitive Test Control & 5 treatment levels; each conc. should be 60% of the next highest conc.; conc. should be in a geometric series	0 (negative control), 13, 22, 36, 60, and 100 ppm
Number of Test Organisms Minimum 20 individual per test level and in each control	40 oysters/level, divided into two replicates of 20 oysters each
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. Measured daily in each aquarium and continuously in one control vessel. 2. Measured daily in each aquarium.
Was chemical analysis performed to determine the concentration of the test material at the beginning and end of the test? (Optional)	Yes

13. 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.	No mortality occurred.
Control Shell Deposition Must be at least 2 mm.	3.4 mm
Recovery of Chemical	Based on QC samples prepared at each sampling interval at fortification levels of 10, 30, and 100 ppm and analyzed concurrently with the test samples, recoveries ranged from 95.1 to 98.7% of nominal (Table 2, p. 21).
Raw data included?	Yes
Signs of toxicity (if any) were described?	None observed.

Shell Growth

Concentration (ppm)		Number Per Level	Number Dead	Mean Shell Deposition (mm)	Mean Percent Reduction
Nominal	Mean Measured				
Negative Control	---	40	0	3.4 ± 1.0	---
13	13	40	0	3.5 ± 1.2	0 (+ 6)
22	20	40	0	3.3 ± 1.1	3
36	37	40	0	3.0 ± 0.9	10
60	57	40	0	2.9 ± 1.2	12
100	100	40	0	3.3 ± 1.2	<1

Limit of quantitation = 2.3-2.6 mg a.i./L

No mortalities or sublethal effects were observed during the test. The mean percent reductions of shell growth compared to the control were 0, 3, 10, 12, and <1% in the 13, 20, 37, 57, and 100 ppm a.i. treatment groups, respectively.

B. Statistical Results

The EC_{50} was estimated based on a visual inspection of the data. The NOEC was determined using the Williams' Test.

EC_{50}: >100 ppm a.i.
NOEC: 100 ppm a.i.

14. VERIFICATION OF STATISTICAL RESULTS:.

Shell deposition data satisfied the assumptions of ANOVA (i.e., normality and homogeneity of variances). The ANOVA revealed no significant differences, so multiple comparison tests were not necessary to determine the NOEC. Reductions in shell deposition did not exceed 50%, so the EC_{50} was visually determined to be greater than the highest concentration.

EC_{50}: >100 ppm a.i.
NOEC: 100 ppm a.i.
LOEC: >100 ppm a.i.

15. REVIEWER'S COMMENTS:

The reviewer's conclusions were identical to the study authors. The EC_{50} was >100 ppm a.i., which categorizes AE F130060 Technical (Mesosulfuron-methyl) as practically non-toxic to the Eastern oyster [72-3(b)] on an acute toxicity basis.

The oysters in each test aquarium were fed supplemental feedings of algae (*Isochrysis galbana*) at a rate of 10^7 cells/mL three times daily (p. 12).

This study was conducted in accordance with USEPA Good Laboratory Practice Regulations with the following exceptions: routine dilution water contaminant screening analyses were not collected in accordance with GLP procedures, and an in-life inspection was not conducted for this study (p. 3). A Quality Assurance Statement was included.

16. REFERENCES:

ASTM. 1998. Standard practice for conducting acute toxicity tests with fishes, microinvertebrates, and amphibians. Standard E-729-96. American Society for Testing and Materials, 100 Barr Harbor Drive, West Conshoken, PA 19428.

Benoit, D.A., *et al.* 1982. A continuous flow mini-diluter system for toxicity testing. *Water Research.* 16:457-464.

Sokal, R.R., and F.J. Rohlf. 1981. *Biometry.* 2nd Edition. W.H. Freeman and Company, New York. 859 pp.

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. Standard evaluation procedures for acute toxicity test for estuarine and marine organisms (Mollusc 96-hour flow-through shell deposition study). EPA-540/9-85-011. June 1985. Emended August 1990.

U.S. EPA. 1985. Office of Pesticide Programs. Pesticide Assessment Procedure for Acute Toxicity Test for Estuarine and Marine Organisms (Mollusc 96-hour flow-through shell deposition study). EPA-540/9-85-011. June 1985. U.S. Environmental Protection Agency, Washington, D.C.

U.S. EPA. 1989. Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA); Good Laboratory Practice Standards; Final Rule (40 CFR, Part 160); FR: 8/17/89; pp. 34052. U.S. Environmental Protection Agency, Washington, D.C.

U.S. EPA. 1996. Office of Prevention, Pesticides and Toxic Substances. Ecological Effects Test Guideline, OPPTS 850.1025. Oyster Acute Toxicity Test (Shell deposition). "Public Draft". EPA 712-C-96-115. April 1996. U.S. Environmental Protection Agency, Washington, D.C.

Williams, D.A. 1971. A test for differences between treatment means when several dose levels are compared to a zero dose control. *Biometrics* 27:103-117.

Williams, D.A. 1972. A comparison of several dose levels with a zero control. *Biometrics* 28:519-531.

17. OUTPUT OF REVIEWER'S STATISTICAL VERIFICATION:

shell deposition
File: 6302sd Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	0.480	0.096	2.526
Within (Error)	6	0.230	0.038	
Total	11	0.710		

Critical F value = 4.39 (0.05,5,6)
Since F < Critical F **FAIL TO REJECT Ho:All groups equal**

shell deposition
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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	control	3.350	3.350		
2	13	3.550	3.550	-1.026	
3	20	3.250	3.250	0.513	
4	37	3.050	3.050	1.539	
5	57	2.950	2.950	2.052	
6	100	3.350	3.350	-0.000	

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

shell deposition
File: 6302sd Transform: NO TRANSFORMATION

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	control	2			
2	13	2	0.552	16.5	-0.200

3	20	2	0.552	16.5	0.100
4	37	2	0.552	16.5	0.300
5	57	2	0.552	16.5	0.400
6	100	2	0.552	16.5	-0.000

shell deposition
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WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	control	2	3.350	3.350	3.230
2	13	2	3.550	3.550	3.230
3	20	2	3.250	3.250	3.230
4	37	2	3.050	3.050	3.230
5	57	2	2.950	2.950	3.230
6	100	2	3.350	3.350	3.350

shell deposition
File: 6302sd 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
control	3.230				
13	3.230	0.613		1.94	k= 1, v= 6
20	3.230	0.613		2.06	k= 2, v= 6
37	3.230	0.613		2.10	k= 3, v= 6
57	3.230	0.613		2.12	k= 4, v= 6
100	3.350	0.000		2.13	k= 5, v= 6

s = 0.196
Note: df used for table values are approximate when v > 20.

APPENDIX III - EXCERPTED RAW DATA

Page ___ is not included in this copy.

Pages 14 through 20 are not included in this copy.

The material not included contains the following type of information:

_____ Identity of product inert ingredients.

_____ Identity of product impurities.

_____ Description of the product manufacturing process.

_____ Description of quality control procedures.

_____ Identity of the source of product ingredients.

_____ Sales or other commercial/financial information.

_____ A draft product label.

_____ The product confidential statement of formula.

_____ Information about a pending registration action.

✓ FIFRA registration data.

_____ The document is a duplicate of page(s) _____.

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The information not included is generally considered confidential by product registrants. If you have any questions, please contact the individual who prepared the response to your request.