

**DATA EVALUATION RECORD**  
**72-3(B) - ACUTE EC<sub>50</sub> TEST WITH AN ESTUARINE/MARINE MOLLUSK**  
**SHELL DEPOSITION STUDY**

1. **CHEMICAL:** Orthosulfamuron

PC Code No.: 108209

2. **TEST MATERIAL:** IR5878

Purity: 98.56%

3. **CITATION**

Palmer, S., Kendall, T., and Krueger, H. 2004. IR5878: A 96-Hour Shell Deposition Test with the Eastern Oyster (*Crassostrea virginica*). Unpublished study performed by Wildlife International, Ltd, Easton, Maryland. Laboratory Report ID: 544A-106. Study submitted by ISAGRO S.p.A., Milano, Italy. Study initiated May 23, 2002 and completed January 7, 2004.

MRID No.:465789-57

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

Signature: *Rebecca L. Bryan*

Date: 2/13/06

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

Signature: *Teri S. Myers*

Date: 3/17/06

5. **APPROVED BY:** Kristina Garber, Biologist, EPA/OPP/EFED/ERBIV

Signature: *Kristina Garber*

Date: 7/27/06

6. **STUDY PARAMETERS**

**Scientific Name of Test Organism:** *Crassostrea virginica*  
**Age or Size of Test Organism:** 33.3-43.5 mm (Valve height)  
**Definitive Test Duration:** 96 hours  
**Study Method:** Flow-through  
**Type of Concentrations:** Mean measured

7. **CONCLUSIONS:**

In this 96-hour, flow-through acute EC<sub>50</sub> test with an estuarine/marine mollusk, the Eastern oyster (*C. virginica*) was exposed to Orthosulfamuron (IR5878 Technical) at nominal concentrations of 0 (negative control), 6.3, 13, 25, 50, and 100 mg/L. Mean-measured concentrations were <3.00 (<LOQ, control), 6.2, 13, 24, 49, and 97 mg a.i./L. Mean-measured concentrations were 96-100% of nominal values.

No mortality was observed in any group during the 96-hour exposure period. The shell growth was reduced 2.1 and 1.0% in the 49 and 97 mg a.i./L treatment groups, respectively, compared to the control. Based on shell deposition, the NOAEC and LOAEC are 97 and >97 mg a.i./L, respectively. The EC<sub>50</sub> was estimated as >97 mg a.i./L, which categorizes Orthosulfamuron (IR5878 Technical) as practically nontoxic to the Eastern oyster an acute toxicity basis.

This study is scientifically sound and fulfills the requirements of an acute toxicity test with an estuarine/marine mollusk. This study is classified as **ACCEPTABLE**.



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**Results Synopsis**

96-hr EC<sub>50</sub>: >97 mg a.i./L

NOAEC: 97 mg a.i./L (based on shell deposition)

**8. BACKGROUND:**

**9. GUIDELINE DEVIATIONS**

1. The amount of peripheral shell growth removed prior to testing was not specified.
2. The pretest mortality of the oysters was not reported.

**10. MATERIALS AND METHODS**

The test substance was IR 5878. It was described as a white powder with 98.56% purity. The CAS number was 213464-77-8. Test organism descriptions are located in table 1. Descriptions of test system characteristics, such as water characteristics and testing vessels are located in table 2. Test design details, including number of organisms, test substance concentrations and observations made during the test are described in table 3.

Table 1. Descriptions of test organisms.

Parameter	Reported Information
Mean valve height 25 - 50 mm along the long axis	38.5 ± 2.4 mm
Supplier	Taylor Shellfish Farms, Shelton, Washington
Are all oysters from same source?	Yes
Are all oysters from the same year class?	Not reported
Acclimation Period	11 days
Were there signs of disease or stress during the acclimation period?	No
Treated for disease?	N/A
Amount of peripheral shell growth removed prior to testing	Recently deposited shell (amount not specified).
Feeding during the acclimation	Marine microalgae suspension rate of $5.8 \times 10^9$ cells/oyster/day
Pretest Mortality	Not reported

Table 2. Descriptions of water characteristics and testing conditions.

Parameter	Reported Information
Source of dilution water Natural unfiltered seawater from an uncontaminated source.	Natural seawater collected from the Indian River Inlet, Delaware. Results from periodic analysis of the saltwater for pesticides, organics, and metals (7/31/02) indicated that levels were below concern (<50 ppb) for pesticides and organics. Arsenic was present at 0.042 ppm.
Does water support test animals without observable signs of stress?	Yes

Salinity	20 ppt (20-20 ppt; range over 4-week period preceding the test)
Water Temperature	20 + 2°C (range of 19.5-20.5°C)
pH	8.2 - 8.3
Dissolved Oxygen	Range: 6.1-7.5 mg/L (≥76% saturation)
Total Organic Carbon	Not reported
Test Aquaria	54 L stainless steel aquaria 13 L fill volume
Type of Dilution System	Continuous-flow diluter
Flow rate	39 volume additions/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
Solvents	N/A

Table 3. Descriptions of test design.

Parameter	
Range Finding Test	A range-finding study was conducted, but the results were not reported.
Nominal Concentrations	6.3, 13, 25, 50, and 100 mg/L
Mean measured Concentrations	6.2, 13, 24, 49, and 97 mg a.i./L (90.9 - 103% of nominal)
Number of Test Organisms	20 oysters/level
Number of Replications/level	1
Test organisms randomly or impartially assigned to test vessels?	Yes
Biological observations made every 24 hours?	Yes
Water Parameter Measurements Temperature	Measured in each aquarium at the beginning and end of the test and continuously in one test vessel (negative control).  DO was measured in each test chamber daily.  The pH was measured at test initiation, at 48 hours, and test termination.
DO	
pH	
Was chemical analysis performed to determine the concentration of the test material at the beginning and end of the test?	Yes

**11. REPORTED RESULTS**

**A. General Results:**

By 96 hours, no mortalities were observed in the controls or treatment groups. The shell growth was reduced 2.1 and 1.0% in the 49 and 97 mg a.i./L treatment groups, respectively, compared to the control (Table 4). No effects were observed for shell deposition when comparing results of controls and treatment groups. The resulting NOAEC was 97 mg a.i./L, the highest concentration tested. Raw data were included in the report for measured concentrations, water characteristics (temperature, dissolved oxygen, pH, salinity), mortality, clinical observations and shell deposition measures. Data were also reported for organics and metals measured in previously sampled water.

Table 4. Observations of mortality and shell deposition observed during shell deposition study of Eastern oyster.

Concentration		Mortality (%)	Mortality	Mean Shell Growth (mm)	Mean Percent Reduction <sup>a</sup>
Nominal (mg/L)	Mean Measured (mg a.i./L)				
Control	Non Detect	20	0	4.20	-
6.3	6.2	20	0	4.44	-5.7
13	13	20	0	4.57	-8.8
25	24	20	0	4.31	-2.6
50	49	20	0	4.11	2.1
120	97	20	0	4.16	1.0

<sup>a</sup> Negative percent inhibition indicates promoted growth.

**B. Statistical Results**

Method: Mean shell growth was calculated using "TOXSTAT Release 3.5" computer software. The data was evaluated for normality (Chi-square test) and homogeneity of variance (Bartlett's test). Significant differences in shell growth compared to the control were determined using ANOVA and Dunnett's test. Since no effects were observed for mortality or shell deposition in all test concentrations, the NOAEC was the highest concentration tested, and the EC<sub>50</sub> was assumed to be greater than that value.

96-hr EC<sub>50</sub>: >97 mg a.i./L  
 NOAEC: 97 mg a.i./L

**12. VERIFICATION OF STATISTICAL RESULTS**

Since no effects were observed during this test, the EC<sub>50</sub> was determined to be greater than the highest concentration tested, 97 mg a.i./L. Dunnett's Test was utilized to confirm the NOAEC.

**13. REVIEWER'S COMMENTS:**

The reviewer's conclusions were consistent with those of the study author.

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It was not clear if the concentrations were corrected for purity, but the reviewer assumed that they were.

The toxicity category, "practically non-toxic" is defined by an  $EC_{50} > 100$  mg/L. Since the  $EC_{50}$  for this test was defined as  $> 97$  mg a.i./L, there is some uncertainty regarding the suitability of this classification. However, since no sublethal effects were observed at 97 mg a.i./L, it seems reasonable that an increase in the concentration of the test substance by 3% is unlikely to result in effects resulting in mortality of 50% of tested organisms.

OECD recommends water solubility, stability in water and light, pKa, Pow, and vapor pressure of test compound. These data were not provided in the report.

#### 14. REFERENCES:

- U.S. Environmental Protection Agency. 1996. Series 850 - Ecological Effects Test Guidelines (draft), OPPTS Number 850.1025: *Oyster Acute Toxicity Test (Shell Deposition)*.
- U.S. Environmental Protection Agency. 1985. Standard Evaluation Procedure, *Acute Toxicity Test for Estuarine and Marine Organisms (Mollusc 96-Hour Flow-Through Shell Deposition Study)*. EPA-540/9-85-011.
- ASTM Standard E 729-88a. 1994. *Standard Guide for Conducting Acute Toxicity Tests with Fishes, Macroinvertebrates, and Amphibians*. American Society for Testing and Materials.
- West, Inc. and D.D. Gulley. 1996. TOXSTAT® Version 3.5. Western Ecosystems Technology, Inc. Cheyenne, Wyoming.