

US EPA ARCHIVE DOCUMENT

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

1. **CHEMICAL:** Oryzalin PC Code No.: 104201
2. **TEST MATERIAL:** Oryzalin Purity: 96.9%
3. **CITATION:**
- Author: R.L. Boeri, P.L. Kowalski, and T.J. Ward  
Title: Oryzalin: Acute Flow-Through Mollusc Shell Deposition Test
- Study Completion Date: December 15, 1995  
Laboratory: T.R. Wilbury Laboratories, Inc.; Marblehead, MA  
Sponsor: DowElanco, Midland, MI  
Laboratory Report ID: 653-DO  
MRID No.: 438877-02  
DP Barcode: D223419

4. **REVIEWED BY:** Rosemary Graham Mora, M.S., Aquatic Toxicologist, KBN Engineering and Applied Sciences, Inc.

**Signature:** *[Handwritten Signature]*

**Date:** 5/22/96

**APPROVED BY:** Pim Kosalwat, Ph.D., Senior Scientist, KBN Engineering and Applied Sciences, Inc.

**Signature:** P. Kosalwat

**Date:** 5/22/96

5. **APPROVED BY:**

**Signature:** *[Handwritten Signature]*

**Date:** May 22 1997

6. **STUDY PARAMETERS:**

Age or Size of Test Organism:	27-50 mm
Definitive Test Duration:	96 hours
Study Method:	Flow-Through
Type of Concentrations:	Mean Measured

7. **CONCLUSIONS:** This study is scientifically sound and meets the guideline requirements for a 96-hour oyster shell deposition study. The 96-hour EC<sub>50</sub> value was 0.285 ppm mean measured concentration, which classifies Oryzalin as highly toxic to the eastern oyster. The NOEC was 0.0994 ppm.

**Results Synopsis**

EC<sub>50</sub>: 0.285 ppm  
 NOEC: 0.0994 ppm

95% C.I.: 0.171-0.477 ppm  
 Probit Slope: N/A

**8. ADEQUACY OF THE STUDY:**

- A. Classification: Core.
- B. Rationale: Fulfills requirement.
- C. Repairability: N/A.

**9. BACKGROUND:**

**10. GUIDELINE DEVIATIONS: None.**

**11. SUBMISSION PURPOSE:**

**12. MATERIALS AND METHODS:**

**A. Test Organisms**

Guideline Criteria	Reported Information
<b><u>Species</u></b> Preferred species are the Pacific oyster ( <i>Crassostrea gigas</i> ) and the Eastern oyster ( <i>Crassostrea virginica</i> )	<i>Crassostrea virginica</i>
<b><u>Mean valve height</u></b> 25 - 50 mm along the long axis	27-50 mm
<b><u>Supplier</u></b>	P. Cummins Oyster Co., Pasadena, MD
<b>Are all oysters from same source?</b>	Yes.
<b>Are all oysters from the same year class?</b>	Yes.

**B. Source/Acclimation**

Guideline Criteria	Reported Information
<b><u>Acclimation Period</u></b> Minimum 10 days	13 days

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Guideline Criteria	Reported Information
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.
<u>Amount of peripheral shell growth removed prior to testing</u>	3-5 mm.
<u>Feeding during the acclimation</u> Must be fed to avoid stress.	100% unfiltered seawater
<u>Pretest Mortality</u> <3% mortality 48 hours prior to testing	0%

### C. Test System

Guideline Criteria	Reported Information
<u>Source of dilution water</u> Natural unfiltered seawater from an uncontaminated source.	Unfiltered, natural seawater collected from T.R. Wilbury, Marblehead, MA. The water was analyzed for pesticides, PCBs, and toxic metals.
Does water support test animals without observable signs of stress?	Yes.
<u>Salinity</u> 30-34 % salinity, weekly range < 6 %	33%
<u>Water Temperature</u> 15°-30° C, consistent in all test vessels	18.1-18.8°C
<u>pH</u>	7.9-8.2
<u>Dissolved Oxygen</u> ≥ 60% throughout	Range: 77-99% of saturation
<u>Total Organic Carbon</u>	Not reported.

Guideline Criteria	Reported Information
<b><u>Test Aquaria</u></b> Should be constructed of glass or stainless steel.	20-L glass aquaria containing 15 L of test solution at a depth of 18 cm.
<b><u>Type of Dilution System</u></b> Must provide reproducible supply of toxicant	Intermittent-flow proportional diluter.
<b><u>Flow rate</u></b> Consistent flow rate	10.8 volume additions/24 hours
Was the loading of organisms such that each individual sits on the bottom with water flowing freely around it?	Not reported.
<b><u>Photoperiod</u></b> 16 hours light, 8 hours dark	16 hours light, 8 hours dark
<b><u>Solvents</u></b> Not to exceed 0.5 mL/L	Solvent: DMF Maximum conc.: 0.1 mL/L

## D. Test Design

Guideline Criteria	Reported Information
<b><u>Range Finding Test</u></b> If EC <sub>50</sub> >100 mg/L with 30 fish, then no definitive test is required.	A preliminary test showed 8% to 100% reduction in shell growth compared to the solvent control at concentrations ranging from 0.010-10 mg/L.
<b><u>Nominal Concentrations of Definitive Test</u></b> Control & 5 treatment levels; each conc. should be 60% of the next highest conc.; concentrations should be in a geometric series	Dilution water control, solvent control, and five nominal concentrations (0.12, 0.20, 0.32, 0.48, 0.80 mg/L).
<b><u>Number of Test Organisms</u></b> Minimum 20 individual per test level and in each control	10 oysters per test aquarium; two aquaria per treatment or control.
Test organisms randomly or impartially assigned to test vessels?	Yes.

Guideline Criteria	Reported Information
Biological observations made every 24 hours?	Yes.
<u>Water Parameter Measurements</u> 1. <u>Temperature</u> Measured hourly in at least one chamber 2. <u>DO and pH</u> Measured at beginning of test and every 48 h in the high, medium, and low doses and in the control	1. Temperature was measured daily in each aquarium and continuously in one test replicate.  2. DO and pH were 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.
<u>Control Mortality</u> Not more than 10% of control organisms may die or show abnormal behavior.	0%
<u>Control Shell Deposition</u> Must be at least 2 mm.	2.5 mm in the control and 2.4 mm in the solvent control.
<u>Recovery of Chemical</u>	83-88%
Raw data included?	Yes.
Signs of toxicity (if any) were described?	None.

Shell Growth

Concentration (ppm ai)		Number Per Level	Number Dead	Mean Shell Deposition (mm)	Mean Percent Reduction*
Nominal	Mean Measured				
Control	<0.0526	20	0	2.5	-
Solvent Control	<0.0526	20	0	2.4	-
0.12	0.0994	20	0	2.3	6
0.20	0.172	20	0	2.0	18
0.32	0.267	20	0	1.4	43
0.48	0.424	20	0	0.9	63
0.80	0.706	20	0	0.5	80

\*Compared to the pooled control.

- B. **Statistical Results** Percent shell-growth reduction was calculated based on the pooled control growth.

Method: Weighted Least Squares Non-Linear Regression for EC<sub>50</sub> and Bonferroni's t-test for NOEC.

96-hr EC<sub>50</sub>: 0.285 ppm      95% C.I.: 0.171-0.477 ppm  
 Probit Slope: Not reported.      NOEC: 0.0994 ppm

**14. VERIFICATION OF STATISTICAL RESULTS:**

Parameter	Result
Statistical Method for EC <sub>50</sub>	Non-Linear Regression
EC <sub>50</sub> (95% C.I.)	0.327 (0.270-0.397) ppm
Probit Slope	N/A
Statistical Method for NOEC	ANOVA with Dunnett's Test
NOEC	0.0994 ppm

15. **REVIEWER'S COMMENTS:** This study is scientifically sound, meets the guideline requirements, and is classified as Core. The 96-hour  $EC_{50}$  for the Eastern oyster exposed to Oryzalin is 0.285 ppm, which classifies the test material as highly toxic to Eastern oysters. The NOEC was 0.0994 ppm based on the lack of a significant reduction in new shell growth at this test concentration.



OBS	CONC	LOG_CONC	REP	Y
1	0.0000	.	1	2.7
2	0.0000	.	2	1.7
3	0.0000	.	3	2.6
4	0.0000	.	4	3.3
5	0.0000	.	5	2.6
6	0.0000	.	6	1.3
7	0.0000	.	7	4.3
8	0.0000	.	8	3.8
9	0.0000	.	9	2.0
10	0.0000	.	10	1.6
11	0.0000	.	11	2.7
12	0.0000	.	12	2.5
13	0.0000	.	13	2.5
14	0.0000	.	14	2.4
15	0.0000	.	15	2.4
16	0.0000	.	16	1.7
17	0.0000	.	17	2.3
18	0.0000	.	18	2.4
19	0.0000	.	19	2.7
20	0.0000	.	20	2.9
21	0.0000	.	1	2.6
22	0.0000	.	2	1.4
23	0.0000	.	3	3.2
24	0.0000	.	4	1.6
25	0.0000	.	5	2.8
26	0.0000	.	6	1.0
27	0.0000	.	7	2.8
28	0.0000	.	8	3.1
29	0.0000	.	9	1.4
30	0.0000	.	10	2.7
31	0.0000	.	11	2.8
32	0.0000	.	12	5.2
33	0.0000	.	13	3.6
34	0.0000	.	14	1.0
35	0.0000	.	15	2.8
36	0.0000	.	16	5.2
37	0.0000	.	17	1.5
38	0.0000	.	18	2.7
39	0.0000	.	19	1.0
40	0.0000	.	20	3.5
41	0.0994	-1.00261	1	3.5
42	0.0994	-1.00261	2	2.5
43	0.0994	-1.00261	3	1.8
44	0.0994	-1.00261	4	2.1
45	0.0994	-1.00261	5	1.6
46	0.0994	-1.00261	6	2.5
47	0.0994	-1.00261	7	2.5
48	0.0994	-1.00261	8	3.1
49	0.0994	-1.00261	9	5.1
50	0.0994	-1.00261	10	1.7
51	0.0994	-1.00261	11	2.3
52	0.0994	-1.00261	12	2.2
53	0.0994	-1.00261	13	2.7
54	0.0994	-1.00261	14	2.8
55	0.0994	-1.00261	15	2.9
56	0.0994	-1.00261	16	1.8
57	0.0994	-1.00261	17	2.5
58	0.0994	-1.00261	18	2.5
59	0.0994	-1.00261	19	2.3
60	0.0994	-1.00261	20	1.3
61	0.1720	-0.76447	1	2.5
62	0.1720	-0.76447	2	0.9
63	0.1720	-0.76447	3	1.4
64	0.1720	-0.76447	4	2.1
65	0.1720	-0.76447	5	2.1

OBS	CONC	LOG_CONC	REP	Y
66	0.1720	-0.76447	9	1.9
67	0.1720	-0.76447	10	3.2
68	0.1720	-0.76447	11	1.6
69	0.172	-0.76447	12	2.1
70	0.172	-0.76447	13	2.5
71	0.172	-0.76447	14	2.6
72	0.172	-0.76447	15	2.9
73	0.172	-0.76447	16	2.8
74	0.172	-0.76447	17	2.0
75	0.172	-0.76447	18	1.1
76	0.172	-0.76447	19	1.0
77	0.172	-0.76447	20	1.2
78	0.172	-0.76447	1	1.2
79	0.172	-0.76447	2	1.2
80	0.172	-0.76447	3	1.5
81	0.267	-0.57349	4	1.0
82	0.267	-0.57349	5	0.0
83	0.267	-0.57349	6	2.5
84	0.267	-0.57349	7	1.4
85	0.267	-0.57349	8	1.4
86	0.267	-0.57349	9	0.9
87	0.267	-0.57349	10	1.4
88	0.267	-0.57349	11	1.4
89	0.267	-0.57349	12	1.4
90	0.267	-0.57349	13	2.6
91	0.267	-0.57349	14	2.6
92	0.267	-0.57349	15	1.7
93	0.267	-0.57349	16	1.3
94	0.267	-0.57349	17	2.2
95	0.267	-0.57349	18	1.4
96	0.267	-0.57349	19	0.9
97	0.267	-0.57349	20	0.0
98	0.267	-0.57349	1	0.9
99	0.267	-0.57349	2	1.3
100	0.267	-0.57349	3	0.8
101	0.424	-0.37263	4	1.2
102	0.424	-0.37263	5	1.9
103	0.424	-0.37263	6	0.0
104	0.424	-0.37263	7	0.0
105	0.424	-0.37263	8	0.0
106	0.424	-0.37263	9	0.0
107	0.424	-0.37263	10	2.0
108	0.424	-0.37263	11	1.5
109	0.424	-0.37263	12	0.9
110	0.424	-0.37263	13	1.8
111	0.424	-0.37263	14	1.3
112	0.424	-0.37263	15	1.1
113	0.424	-0.37263	16	2.0
114	0.424	-0.37263	17	0.9
115	0.424	-0.37263	18	0.0
116	0.424	-0.37263	19	0.0
117	0.424	-0.37263	20	1.3
118	0.424	-0.37263	1	0.8
119	0.424	-0.37263	2	0.0
120	0.424	-0.37263	3	0.0
121	0.706	-0.15120	4	0.5
122	0.706	-0.15120	5	0.9
123	0.706	-0.15120	6	1.1
124	0.706	-0.15120	7	0.0
125	0.706	-0.15120	8	0.0
126	0.706	-0.15120	9	0.6
127	0.706	-0.15120	10	0.0
128	0.706	-0.15120	1	0.0
129	0.706	-0.15120	2	0.0
130	0.706	-0.15120	3	0.0

Iter	LOG_EC25	SIGMA	Non-Linear Least Squares Iterative Phase	Dependent Variable COUNT	Method: Gauss-Newton	Weighted SS
0	-0.845000	0.670000	2.500000	CO	42.890209	
1	-0.652846	0.171001	2.474255	CO	329.165888	
2	-0.871932	0.465909	2.659525	CO	43.349632	
3	-0.709907	0.352925	2.474475	CO	42.161742	
4	-0.733427	0.367687	2.492977	CO	42.427294	
5	-0.731955	0.366356	2.491224	CO	42.454809	
6	-0.732100	0.366469	2.491395	CO	42.453815	
7	-0.732088	0.366459	2.491380	CO	42.453905	
8	-0.732089	0.366460	2.491381	CO	42.453897	
9	-0.732089	0.366460	2.491381	CO	42.453898	
10	-0.732089	0.366460	2.491381	CO	42.453898	

NOTE: Convergence criterion met.

Source	DF	Weighted SS	Dependent Variable COUNT
Regression	3	241.90000000	80.63333333
Residual	137	42.45389799	0.30988247
Uncorrected Total	140	284.35389799	
(Corrected Total)	139	111.88767488	

Parameter	Estimate	Asymptotic Std. Error	Asymptotic 95% Confidence Interval Lower	Upper
LOG_EC25	-0.732088620	0.06859183526	-0.8677254609	-0.5964517782
SIGMA	0.366459716	0.05150836881	0.2646045548	0.4683148775
CO	2.491380729	0.13046998770	2.2333830100	2.7493784475

Asymptotic Correlation Matrix

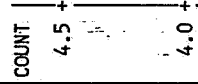
Corr	LOG_EC25	SIGMA	CO
LOG_EC25	1		
SIGMA	-0.865097292	1	
CO	-0.693317685	0.4851846148	1

MODEL: YOUNG = CO \* PROB NORM ((LOG\_EC25 - LOG\_CONC) / SIGMA - 0.67449)  
SUMMARY OF NONLINEAR REGRESSION

OBS	CONC	LOG_EC25	SIGMA	CO	RESID_SS	EC25
1	0	-0.73209	0.36646	2.49138	42.4539	0.18532

MODEL: YOUNG = CO \* PROB NORM ((LOG\_EC25 - LOG\_CONC) / SIGMA - 0.67449)

Plot of YOUNG\*LOG\_CONC. Symbol used is 'O'.  
Plot of PRED\*LOG\_CONC. Symbol used is 'I'.



131	0.706	-0.15120	11	0.0
132	0.706	-0.15120	12	0.0
133	0.706	-0.15120	13	0.7
134	0.706	-0.15120	14	1.3
135	0.706	-0.15120	15	0.0
136	0.706	-0.15120	16	0.0

OBS	CONC	LOG_CONC	REP	Y
137	0.706	-0.15120	17	1.1
138	0.706	-0.15120	18	0.8
139	0.706	-0.15120	19	0.9
140	0.706	-0.15120	20	0.6

MODEL: COUNT = CO \* PROB NORM ((LOG\_EC50 - LOG\_CONC) / SIGMA)  
WEIGHTED REGRESSION

Iter	LOG_EC50	SIGMA	Non-Linear Least Squares Iterative Phase	Dependent Variable COUNT	Method: Gauss-Newton	Weighted SS
0	-0.545000	0.670000	2.500000	CO	47.423068	
1	-0.442154	0.180460	2.467644	CO	84.045402	
2	-0.449036	0.330093	2.442644	CO	43.508631	
3	-0.485770	0.367836	2.494041	CO	42.432587	
4	-0.484839	0.366337	2.491193	CO	42.449956	
5	-0.484922	0.366471	2.491397	CO	42.453800	
6	-0.484915	0.366459	2.491379	CO	42.453907	
7	-0.484915	0.366460	2.491381	CO	42.453897	
8	-0.484915	0.366460	2.491381	CO	42.453898	
9	-0.484915	0.366460	2.491381	CO	42.453898	

NOTE: Convergence criterion met.

Non-Linear Least Squares Summary Statistics

Source	DF	Weighted SS	Dependent Variable COUNT
Regression	3	241.90000000	80.63333333
Residual	137	42.45389799	0.30988247
Uncorrected Total	140	284.35389799	
(Corrected Total)	139	111.88767487	

Parameter	Estimate	Asymptotic Std. Error	Asymptotic 95% Confidence Interval Lower	Upper
LOG_EC50	-0.484915206	0.04229383295	-0.5685490943	-0.4012813171
SIGMA	0.366459716	0.05150836882	0.2646045549	0.4683148776
CO	2.491380729	0.13046998771	2.2333830101	2.7493784476

Asymptotic Correlation Matrix

Corr	LOG_EC50	SIGMA	CO
LOG_EC50	1		
SIGMA	-0.581567797	1	
CO	-0.72586722	0.4851846147	1

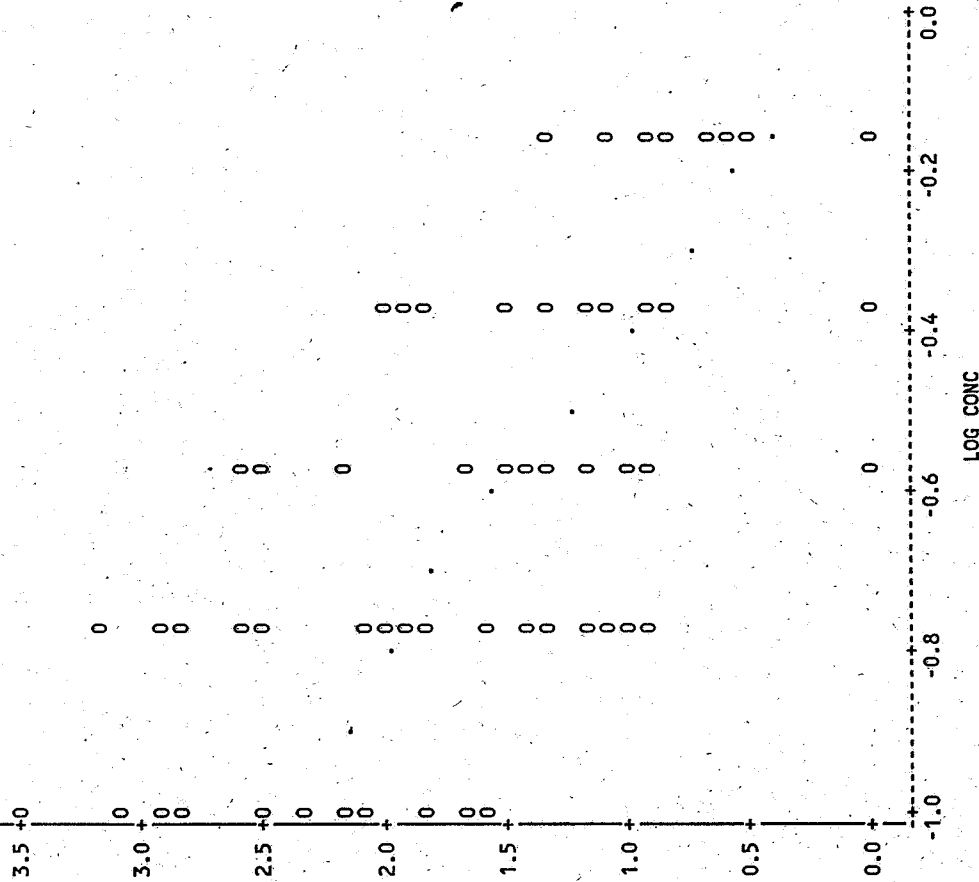
MODEL: COUNT = CO \* PROB NORM ((LOG\_EC50 - LOG\_CONC) / SIGMA)  
SUMMARY OF NONLINEAR REGRESSION

OBS	CONC	LOG_EC50	SIGMA	CO	RESID_SS	EC50
1	0	-0.48492	0.36646	2.49138	42.4539	0.32740

MODEL: YOUNG = CO \* PROB NORM ((LOG\_EC25 - LOG\_CONC) / SIGMA - 0.67449)

*Handwritten notes:*  
C.I.  
0.397  
0.270

*Handwritten notes:*  
slope  
2.7



NOTE: 89 obs had missing values. 144 obs hidden.

COMPARISON OF MEANS FOR NOEL DETERMINATION  
 TEST IF TREATMENT IS LESS THAN CONTROL

General Linear Models Procedure  
 Class Level Information

Class	Levels	Values
DOSE	6	0 0.172 0.267 0.424 0.706 0.0994

Number of observations in data set = 140

General Linear Models Procedure

Dependent Variable: RESPONSE

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	5	77.21960714	15.44392143	34.03	0.0001
Error	134	60.82175000	0.45389366		
Corrected Total	139	138.04135714			

R-Square	C.V.	Root MSE	RESPONSE Mean
0.559395	38.99144	0.673716	1.727857

Source	DF	Type I SS	Mean Square	F Value	Pr > F
DOSE	5	77.21960714	15.44392143	34.03	0.0001

COMPARISON OF MEANS FOR NOEL DETERMINATION  
 TEST IF TREATMENT IS LESS THAN CONTROL

General Linear Models Procedure

Level of DOSE	N	Mean	SD
0	40	2.46750000	0.77140814
0.172	20	2.00000000	0.69206023
0.267	20	1.40500000	0.70373066
0.424	20	0.94500000	0.72654554
0.706	20	0.46500000	0.46710193
0.0994	20	2.34500000	0.50935976

COMPARISON OF MEANS FOR NOEL DETERMINATION  
 TEST IF TREATMENT IS LESS THAN CONTROL

General Linear Models Procedure

Dunnett's One-tailed T tests for variable: RESPONSE

NOTE: This tests controls the type I experimentwise error for comparisons of all treatments against a control.

Alpha= 0.05 Confidence= 0.95 df= 134 MSE= 0.453894  
 Critical Value of Dunnett's T= 2.302

Comparisons significant at the 0.05 level are indicated by \*\*\*\*.

DOSE Comparison	Simultaneous Lower Confidence Limit	Difference Between Means	Simultaneous Upper Confidence Limit
0.0994 - 0	-0.5472	-0.1225	0.3022
0.172 - 0	-0.8922	-0.4675	-0.0428
0.267 - 0	-1.4872	-1.0625	-0.6378

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0.424 - 0 -1.9472 -1.5225 -1.0978 \*\*\*  
0.706 - 0 -2.4272 -2.0025 -1.5778 \*\*\*