

US EPA ARCHIVE DOCUMENT

7-9-96



UNITED STATES ENVIRONMENTAL PROTECTION AGENCY  
WASHINGTON, D.C. 20460

**MEMORANDUM**

OFFICE OF  
PREVENTION, PESTICIDES AND  
TOXIC SUBSTANCES

**SUBJECT:** Isoxaflutole data review (D216391, Chemical #123000,  
Case 286745)

**FROM:** Renée Costello, Biologist *Renée Costello* 7/9/96  
Environmental Risk Characterization Branch  
Environmental Fate and Effects Division (7507C)

**THRU:** Elizabeth Leovey, Chief *Elizabeth Leovey* 7/9/96  
Environmental Risk Characterization Branch  
Environmental Fate and Effects Division

**TO:** Joanne Miller, PM 23  
Registration Division (7505C)

The Environmental Risk Characterization Branch (ERCB) has completed the review of the data submitted in support of registration of Isoxaflutole, chemical number 123000. The following is a brief summary of the data reviewed:

**Citation:** RPA 201772 Technical - Acute Toxicity to Eastern Oyster (*Crassostrea virginica*) Under Flow-Through Conditions  
**EPA MRID No.:** 435732-39

**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 3.3 ppm ai mean measured concentration, which classifies RPA 201772 as moderately toxic to the eastern oyster. The NOEC was 0.98 ppm ai.

If there are any questions regarding this data review contact Renée Costello at 305-5294.

1/7

**Results Synopsis**

EC<sub>50</sub>: 3.3 ppm ai  
 NOEC: 0.98 ppm ai

95% C.I.: 2.1-5.1 ppm ai  
 Probit Slope: 1.8  
 (regression slope)

**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	39 ±4 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	10 days

Guideline Criteria	Reported Information
<b>pH</b>	7.4-7.9
<b>Dissolved Oxygen</b> ≥ 60% throughout	Range: 61-103%
<b>Total Organic Carbon</b>	1.3 mg/L
<b>Test Aquaria</b> Should be constructed of glass or stainless steel.	Glass aquaria (49.5 X 25.5 X 29 cm) containing 18 liters of test solution.
<b>Type of Dilution System</b> Must provide reproducible supply of toxicant	Constant-flow serial diluter.
<b>Flow rate</b> Consistent flow rate	6 volume replacements/24 hours
<b>Was the loading of organisms such that each individual sits on the bottom with water flowing freely around it?</b>	Yes.
<b>Photoperiod</b> 16 hours light, 8 hours dark	16 hours light, 8 hours dark
<b>Solvents</b> Not to exceed 0.5 mL/L	Solvent: Acetone Maximum conc.: 0.5 mL/L

#### D. Test Design

Guideline Criteria	Reported Information
<b>Range Finding Test</b> If $EC_{50} > 100$ mg/L with 30 fish, then no definitive test is required.	A preliminary test showed 6 to 61% reduction in shell growth at concentration range of 0.31-5.0 mg ai/L.
<b>Nominal Concentrations of Definitive Test</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 0.65, 1.1, 1.8, 3.0, and 5.0 mg ai/L.

Guideline Criteria	Reported Information
Signs of toxicity (if any) were described?	Yes.

Shell Growth

Concentration (ppm ai)		Number Per Level	Number Dead	Mean Shell Deposition (mm)	Mean Percent Reduction
Nominal	Mean Measured				
Control	<0.056	40	0	2.2	-
Solvent Control	<0.056	40	0	1.9	-
0.65	0.58	40	0	1.6	21
1.1	0.98	40	0	1.9	7.8
1.8	1.6	40	0	1.3	37
3.0	2.6	40	1	1.3	38
5.0	4.4	40	0	0.8	62

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

Method: Linear Regression (Probit analysis)

96-hr EC<sub>50</sub>: 3.4 ppm ai  
Probit Slope: 1.52

95% C.I.: 0.43-11 ppm ai  
NOEC: 0.98 ppm ai

**14. VERIFICATION OF STATISTICAL RESULTS**

Parameter	Result
Statistical Method for EC <sub>50</sub>	Non-linear Regression
EC <sub>50</sub> (95% C.I.)	3.3 (2.1-5.1) ppm ai
Probit Slope	1.8 (Regression slope)
Statistical Method for NOEC	ANOVA with Dunnett's Test
NOEC	0.98 ppm ai

OBS	CONC	LOG_CONC	REP	Y
1	0	.	1	0
2	0	.	1	2
3	0	.	1	3
4	0	.	1	2
5	0	.	1	2
6	0	.	1	2
7	0	.	1	4
8	0	.	1	5
9	0	.	1	1
10	0	.	1	5
11	0	.	1	0
12	0	.	1	8
13	0	.	1	1
14	0	.	1	7
15	0	.	1	1
16	0	.	1	4
17	0	.	1	2
18	0	.	1	2
19	0	.	1	2
20	0	.	1	2
21	0	.	2	4
22	0	.	2	2
23	0	.	2	2
24	0	.	2	6
25	0	.	2	7
26	0	.	2	9
27	0	.	2	0
28	0	.	2	1
29	0	.	2	3
30	0	.	2	4
31	0	.	2	4
32	0	.	2	0
33	0	.	2	5
34	0	.	2	0
35	0	.	2	7
36	0	.	2	0
37	0	.	2	1
38	0	.	2	8
39	0	.	2	9
40	0	.	2	4
41	0	.	2	2
42	0	.	2	6
43	0	.	2	2
44	0	.	2	5
45	0	.	2	1
46	0	.	2	6
47	0	.	2	9
48	0	.	2	0
49	0	.	2	1
50	0	.	2	6
51	0	.	2	5
52	0	.	2	9
53	0	.	2	4
54	0	.	2	5
55	0	.	2	1
56	0	.	2	8
57	0	.	2	7
58	0	.	2	8
59	0	.	2	8
60	0	.	2	4
61	0	.	2	7
62	0	.	2	3
63	0	.	2	3
64	0	.	2	3

OBS	CONC	LOG_CONC	REP	Y
65	0	.	2	3
66	0	.	2	2
67	0	.	2	3
68	0.00	.	2	1.4
69	0.00	.	2	1.3
70	0.00	.	2	1.3
71	0.00	.	2	0.7
72	0.00	.	2	3.9
73	0.00	.	2	1.2
74	0.00	.	2	1.0
75	0.00	.	2	1.8
76	0.00	.	2	0.7
77	0.00	.	2	0.9
78	0.00	.	2	1.1
79	0.00	.	2	2.5
80	0.00	.	2	2.5
81	0.58	-0.23657	1	3.4
82	0.58	-0.23657	1	2.0
83	0.58	-0.23657	1	3.3
84	0.58	-0.23657	1	2.8
85	0.58	-0.23657	1	1.0
86	0.58	-0.23657	1	1.7
87	0.58	-0.23657	1	1.2
88	0.58	-0.23657	1	0.0
89	0.58	-0.23657	1	2.3
90	0.58	-0.23657	1	1.5
91	0.58	-0.23657	1	3.0
92	0.58	-0.23657	1	0.3
93	0.58	-0.23657	1	1.8
94	0.58	-0.23657	1	4.0
95	0.58	-0.23657	1	0.8
96	0.58	-0.23657	1	2.2
97	0.58	-0.23657	1	0.4
98	0.58	-0.23657	1	0.6
99	0.58	-0.23657	1	1.4
100	0.58	-0.23657	1	2.1
101	0.58	-0.23657	1	1.4
102	0.58	-0.23657	2	4.1
103	0.58	-0.23657	2	0.4
104	0.58	-0.23657	2	0.3
105	0.58	-0.23657	2	0.0
106	0.58	-0.23657	2	0.0
107	0.58	-0.23657	2	2.7
108	0.58	-0.23657	2	0.0
109	0.58	-0.23657	2	0.0
110	0.58	-0.23657	2	0.6
111	0.58	-0.23657	2	2.1
112	0.58	-0.23657	2	2.1
113	0.58	-0.23657	2	0.9
114	0.58	-0.23657	2	5.4
115	0.58	-0.23657	2	2.5
116	0.58	-0.23657	2	1.3
117	0.58	-0.23657	2	3.7
118	0.58	-0.23657	2	0.6
119	0.58	-0.23657	2	0.0
120	0.58	-0.23657	2	0.0
121	0.98	-0.00877	1	2.2
122	0.98	-0.00877	1	1.8
123	0.98	-0.00877	1	2.7
124	0.98	-0.00877	1	0.4
125	0.98	-0.00877	1	4.3
126	0.98	-0.00877	1	0.7
127	0.98	-0.00877	1	3.3
128	0.98	-0.00877	1	0.3

OBS	CONC	LOG_EC50	REP	Y
257	4.4	0.64345	1	0.0
258	4.4	0.64345	1	0.0
259	4.4	0.64345	1	0.0
260	4.4	0.64345	1	0.7
261	4.4	0.64345	2	0.5
262	4.4	0.64345	2	0.7
263	4.4	0.64345	2	0.7
264	4.4	0.64345	2	1.0
265	4.4	0.64345	2	1.0
266	4.4	0.64345	2	0.9
267	4.4	0.64345	2	0.0
268	4.4	0.64345	2	0.0

RPA 201772 Technical: Exposure to Eastern Oysters  
02:31 Monday, March 4, 1996

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

NOTE: Convergence criterion met.

Iter	LOG_EC50	SIGMA	Method	Weighted SS
0	0.530000	0.658000	Gauss-Newton	298.675225
1	0.512977	0.563040		303.857491
2	0.516060	0.555109		303.922551
3	0.516221	0.553292		303.946885
4	0.516264	0.552908		303.951051
5	0.516273	0.552826		303.951916
6	0.516275	0.552808		303.952101
7	0.516275	0.552805		303.952140
8	0.516275	0.552804		303.952149
9	0.516275	0.552804		303.952151

Non-Linear Least Squares Iterative Phase  
Dependent Variable COUNT

Source	DF	Weighted SS	Weighted MS
Regression	3	438.80000000	146.26666667
Residual	276	303.95215071	1.10127591
Uncorrected Total	279	742.75215071	
(Corrected Total)	278	343.70353314	

Parameter Estimate Asymptotic Std. Error Lower Confidence Interval Upper Asymptotic 95 %

Parameter	Estimate	Asymptotic Std. Error	Lower Confidence Interval	Upper Asymptotic 95 %
LOG_EC50	0.516275	0.09411106691	0.3310062880	0.7015446345
SIGMA	0.552805361	0.18051819634	0.1974313602	0.9081757622
CO	2.022667578	0.16169994721	1.7043414071	2.3409937480

Asymptotic Correlation Matrix

Corr	LOG_EC50	SIGMA	CO
LOG_EC50	1		
SIGMA	-0.044433737	1	
CO	-0.619091064	0.5003571872	1

RPA 201772 Technical: Exposure to Eastern Oysters  
02:31 Monday, March 4, 1996

MODEL: YOUNG = CO \* PROBNORM ((LOG\_EC25 - LOG\_CONC) / SIGMA)  
WEIGHTED REGRESSION

NOTE: Convergence criterion met.

Iter	LOG_EC25	SIGMA	Method	Weighted SS
0	0.230000	0.658000	Gauss-Newton	279.854274
1	0.117361	0.555254		308.811895
2	0.141346	0.555500		303.963837
3	0.142968	0.553373		303.945946
4	0.143319	0.552926		303.950865
5	0.143394	0.552830		303.951877
6	0.143411	0.552809		303.952092
7	0.143414	0.552805		303.952139
8	0.143415	0.552804		303.952148
9	0.143415	0.552804		303.952151

Non-Linear Least Squares Iterative Phase  
Dependent Variable COUNT

Source	DF	Weighted SS	Weighted MS
Regression	3	438.80000000	146.26666667
Residual	276	303.95215063	1.10127591
Uncorrected Total	279	742.75215063	
(Corrected Total)	278	343.70353296	

Parameter Estimate Asymptotic Std. Error Lower Confidence Interval Upper Asymptotic 95 %

Parameter	Estimate	Asymptotic Std. Error	Lower Confidence Interval	Upper Asymptotic 95 %
LOG_EC25	0.143414981	0.15716279913	-0.1659792572	0.4528092192
SIGMA	0.552805369	0.18051819868	0.1974313636	0.9081757748
CO	2.022667582	0.16169994770	1.7043414104	2.3409937533

Asymptotic Correlation Matrix

Corr	LOG_EC25	SIGMA	CO
LOG_EC25	1		
SIGMA	-0.801331018	1	
CO	-0.758358032	0.5003571836	1

MODEL: YOUNG = CO \* PROBNORM ((LOG\_EC25 - LOG\_CONC) / SIGMA - 0.67449)  
SUMMARY OF NONLINEAR REGRESSION  
02:31 Monday, March 4, 1996

OBS	CONC	LOG_EC25	SIGMA	CO	RESID_SS	EC25
1	0	0.14341	0.55280	2.02267	303.952	1.39128

RPA 201772 Technical: Exposure to Eastern Oysters

Dunnnett'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= 273 MSE= 1.680431  
 Critical Value of Dunnnett's T= 2.290

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

DOSE Comparison	Simultaneous Confidence Limit		Difference Between Means	Simultaneous Upper Confidence Limit		
	Lower Limit	Upper Limit		Lower Limit	Upper Limit	
0.98 - 0	-0.7336	0.4161	-0.1588	0.1461	***	
0.58 - 0	-1.0036	0.1461	-0.4288	0.1789	***	
1.6 - 0	-1.3286	-0.1944	-0.7538	-0.1944	***	
2.6 - 0	-1.3540	-0.7742	-0.7742	-0.7742	***	
4.4 - 0	-1.8511	-1.2765	-1.2765	-1.2765	***	

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