7-9-96

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
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_{50} 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.

Results Synopsis

EC_{50}: 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
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	39 ±4 mm
Supplier	P. Cummins Oyster Co., Pasadena, 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	10 days

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Guideline Criteria	Reported Information
pH	7.4-7.9
Dissolved Oxygen ≥ 60% throughout	Range: 61-103%
Total Organic Carbon	1.3 mg/L
Test Aquaria Should be constructed of glass or stainless steel.	Glass aquaria (49.5 X 25.5 X 29 cm) containing 18 liters of test solution.
Type of Dilution System Must provide reproducible supply of toxicant	Constant-flow serial diluter.
Flow rate Consistent flow rate	6 volume replacements/24 hours
Was the loading of organisms 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: Acetone Maximum conc.: 0.5 mL/L

D. Test Design

Guideline Criteria	Reported Information
Range Finding Test 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.
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	Dilution water control, solvent control; and 0.65, 1.1, 1.8, 3.0, and 5.0 mg ai/L.

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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_{50}: 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_{50}	Non-linear Regression
EC_{50}(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

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OBS	CONC	LOG_CONC	REP	Y
1	0	.	1	1.9
2	0	.	1	2.3
3	0	.	1	2.3
4	0	.	1	2.2
5	0	.	1	5.1
6	0	.	1	1.2
7	0	.	1	4.5
8	0	.	1	1.4
9	0	.	1	5.1
10	0	.	1	0.5
11	0	.	1	3.8
12	0	.	1	0.0
13	0	.	1	3.1
14	0	.	1	2.7
15	0	.	1	2.1
16	0	.	1	0.4
17	0	.	1	5.2
18	0	.	1	1.2
19	0	.	1	3.9
20	0	.	1	2.2
21	0	.	2	4.2
22	0	.	2	0.6
23	0	.	2	1.5
24	0	.	2	6.7
25	0	.	2	0.6
26	0	.	2	0.9
27	0	.	2	0.5
28	0	.	2	1.7
29	0	.	2	1.1
30	0	.	2	1.3
31	0	.	2	2.4
32	0	.	2	0.4
33	0	.	2	1.0
34	0	.	2	0.5
35	0	.	2	2.0
36	0	.	2	1.7
37	0	.	2	1.0
38	0	.	2	1.4
39	0	.	2	1.8
40	0	.	2	4.9
41	0	.	1	2.4
42	0	.	1	2.6
43	0	.	1	3.2
44	0	.	1	1.5
45	0	.	1	1.1
46	0	.	1	1.6
47	0	.	1	0.9
48	0	.	1	0.0
49	0	.	1	4.1
50	0	.	1	0.6
51	0	.	1	2.5
52	0	.	1	1.9
53	0	.	1	0.4
54	0	.	1	1.5
55	0	.	1	2.7
56	0	.	1	3.8
57	0	.	1	5.1
58	0	.	1	3.3
59	0	.	1	3.4
60	0	.	1	1.3
61	0	.	2	0.0
62	0	.	2	1.7
63	0	.	2	1.3
64	0	.	2	2.3

65	0	.	2	2.3
66	0	.	2	2.2
67	0	.	2	0.3

RPA 201772 Technical: Exposure to Eastern Oysters
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OBS	CONC	LOG_CONC	REP	Y
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	3.1
79	0.00	.	2	2.5
80	0.00	.	2	3.4
81	0.58	-0.23657	1	2.0
82	0.58	-0.23657	1	3.3
83	0.58	-0.23657	1	2.8
84	0.58	-0.23657	1	1.0
85	0.58	-0.23657	1	3.7
86	0.58	-0.23657	1	1.2
87	0.58	-0.23657	1	0.0
88	0.58	-0.23657	1	2.3
89	0.58	-0.23657	1	1.5
90	0.58	-0.23657	1	3.0
91	0.58	-0.23657	1	0.3
92	0.58	-0.23657	1	1.5
93	0.58	-0.23657	1	0.8
94	0.58	-0.23657	1	4.0
95	0.58	-0.23657	1	2.2
96	0.58	-0.23657	1	0.4
97	0.58	-0.23657	1	0.6
98	0.58	-0.23657	1	1.4
99	0.58	-0.23657	1	2.1
100	0.58	-0.23657	1	4.1
101	0.58	-0.23657	2	0.4
102	0.58	-0.23657	2	0.3
103	0.58	-0.23657	2	0.0
104	0.58	-0.23657	2	0.0
105	0.58	-0.23657	2	2.7
106	0.58	-0.23657	2	0.0
107	0.58	-0.23657	2	3.9
108	0.58	-0.23657	2	0.6
109	0.58	-0.23657	2	0.6
110	0.58	-0.23657	2	2.1
111	0.58	-0.23657	2	0.9
112	0.58	-0.23657	2	5.4
113	0.58	-0.23657	2	2.5
114	0.58	-0.23657	2	1.3
115	0.58	-0.23657	2	0.7
116	0.58	-0.23657	2	0.6
117	0.58	-0.23657	2	0.0
118	0.58	-0.23657	2	0.0
119	0.58	-0.23657	2	2.2
120	0.58	-0.23657	2	2.7
121	0.98	-0.00877	1	2.8
122	0.98	-0.00877	1	1.8
123	0.98	-0.00877	1	0.4
124	0.98	-0.00877	1	4.3
125	0.98	-0.00877	1	0.7
126	0.98	-0.00877	1	0.3
127	0.98	-0.00877	1	0.3
128	0.98	-0.00877	1	3.4

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	0.0
263	4.4	0.64345	2	0.7
264	4.4	0.64345	2	0.7
265	4.4	0.64345	2	1.0
266	4.4	0.64345	2	0.0
267	4.4	0.64345	2	0.9
268	4.4	0.64345	2	0.0

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OBS	CONC	LOG_CONC	REP	Y
269	4.4	0.64345	2	1.5
270	4.4	0.64345	2	0.0
271	4.4	0.64345	2	0.0
272	4.4	0.64345	2	1.1
273	4.4	0.64345	2	0.0
274	4.4	0.64345	2	0.3
275	4.4	0.64345	2	0.0
276	4.4	0.64345	2	1.0
277	4.4	0.64345	2	1.0
278	4.4	0.64345	2	3.3
279	4.4	0.64345	2	0.0
280	4.4	0.64345	2	0.0

RPA 201772 Technical: Exposure to Eastern Oysters
MODEL: COUNT = C0 * PROBNORM ((LOG_EC50 - LOG_CONC) / SIGMA)
WEIGHTED REGRESSION 02:31 Monday, March 4, 1996

Non-Linear Least Squares Iterative Phase
Dependent Variable COUNT Method: Gauss-Newton

Iter	LOG_EC50	SIGMA	C0	Weighted SS
0	0.530000	0.658000	2.075000	298.675225
1	0.512977	0.563040	2.031422	303.837491
2	0.516060	0.555109	2.023886	303.922551
3	0.516221	0.553292	2.022931	303.946885
4	0.516264	0.552908	2.022724	303.951051
5	0.516273	0.552826	2.022680	303.951916
6	0.516275	0.552808	2.022670	303.952101
7	0.516275	0.552805	2.022668	303.952140
8	0.516275	0.552804	2.022668	303.952149
9	0.516275	0.552804	2.022668	303.952151

NOTE: Convergence criterion met.

Non-Linear Least Squares Summary Statistics 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	Asymptotic 95 % Confidence Interval Lower	Upper
LOG_EC50	0.516275461	0.09411106691	0.3310062880	0.7015446345
SIGMA	0.552803561	0.18051819634	0.1974313602	0.9081757622
C0	2.022667578	0.16169994721	1.7043414071	2.3409937480

Asymptotic Correlation Matrix

Corr	LOG_EC50	SIGMA	C0
LOG_EC50	1	-0.044433737	-0.619091064
SIGMA	-0.044433737	1	0.5003571872
C0	-0.619091064	0.5003571872	1

RPA 201772 Technical: Exposure to Eastern Oysters
MODEL: COUNT = C0 * PROBNORM ((LOG_EC50 - LOG_CONC) / SIGMA)
SUMMARY OF NONLINEAR REGRESSION
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OBS	CONC	LOG_EC50	SIGMA	C0	RESID_SS	EC50
1	0	0.51628	0.55280	2.02267	303.952	3.28303

RPA 201772 Technical: Exposure to Eastern Oysters
MODEL: YOUNG = C0 * PROBNORM ((LOG_EC25 - LOG_CONC) / SIGMA - 0.67449)
WEIGHTED REGRESSION 02:31 Monday, March 4, 1996

Non-Linear Least Squares Iterative Phase
Dependent Variable COUNT Method: Gauss-Newton

Iter	LOG_EC25	SIGMA	C0	Weighted SS
0	0.230000	0.658000	2.075000	279.854274
1	0.117361	0.555254	2.029046	308.811895
2	0.141346	0.555500	2.023782	303.963837
3	0.142968	0.553373	2.022976	303.945946
4	0.143319	0.552926	2.022733	303.950865
5	0.143394	0.552830	2.022682	303.951877
6	0.143411	0.552809	2.022671	303.952092
7	0.143414	0.552805	2.022668	303.952139
8	0.143415	0.552804	2.022668	303.952148
9	0.143415	0.552804	2.022668	303.952151

NOTE: Convergence criterion met.

Non-Linear Least Squares Summary Statistics 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	Asymptotic 95 % Confidence Interval Lower	Upper
LOG_EC25	0.143414981	0.15716279913	-0.1659792572	0.4528092192
SIGMA	0.552803569	0.18051819868	0.1974313636	0.9081757748
C0	2.022667582	0.16169994770	1.7043414104	2.3409937533

Asymptotic Correlation Matrix

Corr	LOG_EC25	SIGMA	C0
LOG_EC25	1	-0.801331018	-0.758358032
SIGMA	-0.801331018	1	0.5003571836
C0	-0.758358032	0.5003571836	1

RPA 201772 Technical: Exposure to Eastern Oysters
MODEL: YOUNG = C0 * PROBNORM ((LOG_EC25 - LOG_CONC) / SIGMA - 0.67449)
SUMMARY OF NONLINEAR REGRESSION
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OBS	CONC	LOG_EC25	SIGMA	C0	RESID_SS	EC25
1	0	0.14341	0.55280	2.02267	303.952	1.39128

RPA 201772 Technical: Exposure to Eastern Oysters

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

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

DOSE Comparison	Simultaneous Lower Confidence Limit	Difference Between Means	Simultaneous Upper Confidence Limit	
0.98 - 0	-0.7336	-0.1588	0.4161	
0.58 - 0	-1.0036	-0.4288	0.1461	
1.6 - 0	-1.3286	-0.7538	-0.1789	***
2.6 - 0	-1.3540	-0.7742	-0.1944	***
4.4 - 0	-1.8511	-1.2763	-0.7014	***