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

1. **CHEMICAL:** Ethoprop PC Code No.: 041101

2. **TEST MATERIAL:** Ethoprop technical, Batch No. 307289119, CAS Registry No. 13194-48-4, a clear liquid, Batch No. 23DEQ75.
   Purity: 98.7%

3. **CITATION**

Authors:	Emily Dionne
Title:	Ethoprop Technical - Acute Toxicity to Eastern Oyster (*Crassostrea virginica*) Under Flow-Through Conditions.
Study Completion Date:	16 May 1995
Laboratory:	Springborn Laboratories, Inc.
Sponsor:	Rhone-Poulenc Ag Company
Laboratory Report ID:	95-5-5839; SLI Study No. 10566.1294.6350.504
MRID No.:	436863-02
DP Barcode:	D218402

4. **REVIEWED BY:** Regina Hirsch, Wildlife Biologist, EEB, EFED
   Signature: [signature] **Date:** 3/18/96

5. **APPROVED BY:** Les Touart, Head of Section 1, EEB, EFED
   Signature: [signature] **Date:** 7.5.96

6. **STUDY PARAMETERS**

Scientific Name of Test Organism:	*Crassostrea virginica*
Age or Size of Test Organism:	36 ± 3 mm
Definitive Test Duration:	96-hours
Study Method:	Flow-through
Type of Concentrations:	Mean measured

7. **CONCLUSIONS:**
   Results Synopsis
   EC_{50}: 3.7 mg ai/L , 95% C.I.: 1.1 - 19 mg ai/L
   NOEL: 2.6 mg ai/L Probit Slope: 5.44

8. **ADEQUACY OF THE STUDY**

A. **Classification:** CORE

B. **Rationale:** N/A

C. **Repairability:** N/A

9. **BACKGROUND**

10. **GUIDELINE DEVIATIONS**

11. **SUBMISSION PURPOSE:** Reregistration

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	36 mm (SD 3 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	17 days
Wild caught organisms were quarantined for 7 days?	N/A
Were there signs of disease or injury?	Not reported
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.	*Isochrysis galbana* was fed to the oysters during the acclimation and testing periods.
Pretest Mortality <3% mortality 48 hours prior to testing	0% mortality prior to testing.

C. Test System

Guideline Criteria	Reported Information
Source of dilution water Natural unfiltered seawater from an uncontaminated source.	Natural unfiltered seawater, which was pumped from Cape Cod Canal, Bourne, Massachusetts
Does water support test animals without observable signs of stress?	Yes
Salinity 30-34 ‰ salinity, weekly range < 6 ‰	32 ‰

Guideline Criteria	Reported Information
Water Temperature 15°-30° C, consistent in all test vessels	20 ± 1°C
pH	7.6 - 8.0
Dissolved Oxygen ≥ 60% throughout	Range: 57-96% 57% at 72-hour
Total Organic Carbon	Not reported
Test Aquaria Should be constructed of glass or stainless steel.	49.5 x 25.5 x 29 cm glass and silicone adhesive aquaria
Type of Dilution System Must provide reproducible supply of toxicant	Continuous flow serial diluter (Benoit 1982)
Flow rate Consistent flow rate	6.0 vol/24 hours
Was the loading of organism such that each individual sits on the bottom with water flowing freely around it?	Not reported.
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.: 0.50 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.	Range finding test using 4.1, 14.0, 45.0, 150.0, and 500.0 ug ai/L. After 96-hours reduction in shell growth of 0, 0, 7.0, 2.0, and 3.0%, respectively. Additional, preliminary test showed a reduction of 33, 49, 96, 99, and 97% for oysters in 3.1, 6.3, 13.0, 25.0, and 50.0 mg ai/L, respectively.
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	1.3, 2.2, 3.6, 6.0, and 10.0 mg ai/L.
Number of Test Organisms Minimum 20 individual per test level and in each control	20 in each test aquaria, 40 per treatment level and the controls.
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	Temperature monitored continuously in one replicate (B) of the dilution water control and measured daily in each replicate exposure aquaria. measured daily in each replicate exposure aquaria.

Guideline Criteria	Reported Information
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.	0%
Control Shell Deposition Must be at least 2 mm.	control 2.7 mm (SD 1.3) solvent control 2.4 mm (SD 1.0)
Recovery of Chemical	93.7 $\pm$ 5.0 %
Raw data included?	No
Signs of toxicity (if any) were described?	Yes

Shell Growth

Concentration (ppm)		Number Per Level	Number Dead	Mean Shell Deposition (mm)	Mean Percent Reduction
Nominal	Mean Measured				
Control	--	40	0	2.7 (SD 1.3)	--
Solvent Control	--	40	0	2.4 (SD 1.0)	--
1.3	1.1	40	0	2.4 (SD 0.7)	0%
2.2	1.7	40	0	2.1 (SD 1.2)	12%
3.6	2.6	40	0	2.3 (SD 1.1)	2.9%
6.0	4.0	40	0	0.8 (SD 0.9)[a]	67%
10.0	7.8	40	0	0.1 (SD 0.3)[a]	97%

[a] Significantly different ($p \leq 0.05$) as compared to the performance of the solvent control. Reduced feeding and reduced fecal production was observed in these two concentrations.

B. Statistical Results

Method: Linear Regression

96-hr EC_{50}: 3.7 ppm ai 95% C.I.: 1.1 - 19.0 mg ai/L

Probit Slope: ___ NOEC: 2.6 mg ai/L (Using Williams' Test)

14. VERIFICATION OF STATISTICAL RESULTS

Parameter	Result
Statistical Method for EC_{50},	Moving Average
EC_{50} (95% C.I.)	3.9 mg ai/L (3.57 - 4.27) ppm ai
Probit Slope	5.4
Statistical Method for NOEC	Williams Test &, Dunnett's Test
NOEC	2.6 mg ai/L

15. REVIEWER'S COMMENTS:

Eastern Oyster Acute Test
File: c:\actions\ethoprop\dataoy Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	9.864	1.973	34.614
Within (Error)	6	0.345	0.057	
Total	11	10.209		

Critical F value = 4.39 (0.05,5,6)
Since F > Critical F REJECT Ho:All groups equal

Eastern Oyster Acute Test
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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	Contol	2.400	2.400		
2	1.1	2.400	2.400	0.000	
3	1.7	2.100	2.100	1.257	
4	2.6	2.350	2.350	0.209	
5	4.0	0.800	0.800	6.702	*
6	7.8	0.100	0.100	9.634	*

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

Eastern Oyster Acute Test
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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	Contol	2			
2	1.1	2	0.676	28.2	0.000
3	1.7	2	0.676	28.2	0.300
4	2.6	2	0.676	28.2	0.050
5	4.0	2	0.676	28.2	1.600
6	7.8	2	0.676	28.2	2.300

Eastern Oyster Acute Test
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WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	Contol	2	2.400	2.400	2.400
2	1.1	2	2.400	2.400	2.400
3	1.7	2	2.100	2.100	2.225
4	2.6	2	2.350	2.350	2.225
5	4.0	2	0.800	0.800	0.800
6	7.8	2	0.100	0.100	0.100

Eastern Oyster Acute Test
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WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
Contol	2.400				
1.1	2.400	0.000		1.94	k= 1, v= 6
1.7	2.225	0.730		2.06	k= 2, v= 6
2.6	2.225	0.730		2.10	k= 3, v= 6
4.0	0.800	6.672	*	2.12	k= 4, v= 6
7.8	0.100	9.592	*	2.13	k= 5, v= 6

s = 0.240
Note: df used for table values are approximate when v > 20.

Regina Hirsch Ethoprop Acute Toxicity to Eastern Oysters

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CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
7.8	40	39	97.5	0
4	40	27	67.5	0
2.6	40	1	2.5	0
1.7	40	5	12.5	0
1.1	40	0	0	0

BECAUSE THE NUMBER OF ORGANISMS USED WAS SO LARGE, THE 95 PERCENT CONFIDENCE INTERVALS CALCULATED FROM THE BINOMIAL PROBABILITY ARE UNRELIABLE. USE THE INTERVALS CALCULATED BY THE OTHER TESTS.

AN APPROXIMATE LC50 FOR THIS SET OF DATA IS 3.628336

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS	
2	3.341941E-02	3.916452	3.57032	4.272331

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
4	.9566912	6.145597	0

A PROBABILITY OF 0 MEANS THAT IT IS LESS THAN 0.001.

SINCE THE PROBABILITY IS LESS THAN 0.05, RESULTS CALCULATED USING THE PROBIT METHOD PROBABLY SHOULD NOT BE USED.

SLOPE = 5.441826
95 PERCENT CONFIDENCE LIMITS = .119143 AND 10.76451

LC50 = 3.605747
95 PERCENT CONFIDENCE LIMITS = 1.459519 AND 481.6214

LC10 = 2.106773
95 PERCENT CONFIDENCE LIMITS = 3.626655E-09 AND 3.236089

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