(4)

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

1. **CHEMICAL:** Metolachlor
   Shaughnessey No.: 108801

2. **TEST MATERIAL:** Metolachlor technical
   Purity: 97.3%

3. **STUDY TYPE:** 72-3b - Acute toxicity study to marine/estuarine mollusk.

4. **CITATION:**

Author:	Dionne, E.
Title:	Metolachlor technical - acute toxicity to Eastern Oyster (*Crassostrea virginica*) under flow-through conditions.
Date:	1994
Lab. Report #:	94-7-5365
Laboratory:	Springborn Laboratories, Inc., Wareham, MA
Sponsor:	Ciba Crop Protection, Greensboro, NC
MRID No.:	434871-02

5. **REVIEWED BY:**

William Erickson
Biologist
EEB/EFED

Signature: W. Erickson
Date: 1/26/95

5. **APPROVED BY:**

Harry Craven
Section Head 4
EEB/EFED

Signature: Harry T. Craven
Date: 2/15/95

7. **CONCLUSIONS:** This study is scientifically sound and fulfills the guideline requirement for an acute toxicity test with a marine/estuarine mollusk. The 96-hour EC_{50} is 1.7 mg ai/l, which classifies technical metolachlor as moderately toxic to the Eastern oyster. The NOEC is 0.71 mg ai/l.

8. **CLASSIFICATION:** Core.

9. **MAJOR GUIDELINE DEVIATIONS:** None.

10. **MATERIALS AND METHODS:**

A. Test Organism: Eastern Oyster

Guideline Criteria	Reported Information
Species (Scientific Name)	*Crassostrea virginica*
Mean valve height (25 - 50 mm -- the long axis)	39 mm
Supplier	P. Cummins Oyster Co., Pasadena, MD
All oysters from same source (y/n)	Yes
All oysters from the same year class (y/n)	Yes

B. Source/Acclimation

Guideline Criteria	Reported Information
Acclimation Period (minimum 10 days)	17 days
Wild caught 7 day quarantine (y/n)	Not reported
Check for signs of disease or injury (y/n; describe)	Yes; checked for mortality and after the shell growth was grounded off, checked for stress.
If diseased it can be treated in 48-hr pretest no sign of the disease remains (Report hours prior to test in which no sign of disease or N/A)	Examined for boring sponges and/or mudworms; several opened to confirm no parasites present
Was peripheral shell growth removed prior to testing? If so how much.	Yes; 3-5 mm
Feeding during the acclimation	Fed a supplemental algal diet of *Isochrysis galbana* Parke, clone T-Iso
<3% mortality 48 hours prior to testing (% mortality, if any)	No mortality within 7 days of testing

C. **Test System:**

Guideline Criteria	Reported Information
Describe source of dilution water (natural unfiltered seawater)	Natural unfiltered seawater from Cape Cod Canal, Bourne, MA
Does water support test animals without observable signs of stress?	Yes
Salinity of Test water:	31% ppt
Water Temperature (between 15°C and 30°C -- but must be consistent)	20 ± 1°C
pH	7.9
Dissolved Oxygen (Static 1st 48 hrs 40%; 2nd 48 hrs 60%; Flow-through 60%) (% of lowest conc. & hour)	Measured daily; 62-88% saturation at 96 h in definitive test
Total Organic Carbon	1.3 mg/l
Test Aquaria 1. Material (glass or stainless steel) 2. a. Static volume (18.9 L (5 gal or 19000 cc) with 15 L solution) b. Static or flow-through volume (300x600x300 = 54000 cc.)	Glass aquaria 18 l 49.5 X 25.5 X 29 cm
Type of Dilution System (Reproducible supply of toxicant)	Harvard Apparatus peristaltic pump was calibrated to deliver 0.1875 ml/min of the 11 mg ai/ml stock solution.
Flow rate .Consistent flow rate-meter systems calibrated before study and checked 2*24 hours - 5 to 10 vol/24 hours	6 solution volume replacements per 24 hours
Biomass Loading Rate (all oysters should be able to sit on the bottom with water flowing freely around them)	Yes
Photoperiod (16 L & 8 D)	16 hours light and 8 hours dark

Solvents (Do not exceed 0.5 ml/L for flow-through)	0.5 ml/L acetone

D. Test Design:

Guideline Criteria	Reported Information
Range Finding Test (LC_{50} >100 mg/L with 30 shrimp, no definitive test required.)	Tested concentrations of 0.039 to 6.3 mg ai/l. After 96-hours, growth reduction of 0-97% was observed.
Definitive Test	
Nominal Concentrations (control+5 treatment levels; dosage should be 60% of the next highest concentration; concentrations should be geometric series)	0.71, 1.2, 2.0, 3.3, and 5.5 mg ai/l; also negative and solvent controls
Controls (Minimum control mortality; static 10%; flow-through 5%	No mortality reported
Number of Test Organisms; (Minimum 10/level can be divided among containers)	20 oysters in each test aquarium (40 per treatment level)
All organisms must be randomly assigned to test vessels (y/n)	Impartially assigned
Biological Observations (y/n)	Yes
Water Parameter Measurements 1. Temperature - record every 6 hrs;>1°C. 2. D.O. beginning,48 hrs,end for control high, medium, and low dose. 3. pH beginning,48 hrs, end for control, high, medium, and low dose.	21-22°C Yes; lowest value was 62% at 96 hours Yes; ranged was 7.5-7.9
Chemical Analysis (needed if aeration, volatile, insoluble, precipitate, not steel or glass, known to adsorb, and flow-through) (yes or no)	Yes

11. **REPORTED RESULTS:**

Guideline Criteria	Reported Information
Mean Measured Concentrations	0.71, 1.1, 1.7, 2.9, 4.5
Recovery of Chemical (% recovery)	83-100% of nominal
Mortality & Observations (Describe observations & attach mortality tables)	No mortality at any treatment level
Mean shell growth (mm) of control and treatment groups after 96 h exposure	water control 2.2 mm solvent contr 2.4 0.71 mg ai/l 2.6 1.1 1.9 1.7 1.1 2.9 0.36 4.5 0.0
Ratio of mean shell growth of treated to untreated oysters after 96 h exposure	0.71 mg ai/l +12% 1.1 -18% 1.7 -52% 2.9 -84% 4.5 -100% note: control groups pooled
Statistical Results: **EC_{50}:** **95% CL:** **NOEC:**	Regression analysis 1.8 mg ai/l 1.2-2.8 mg ai/l 0.71 mg ai/l

Other findings: Reduced feeding and reduced fecal and pseudofecal production were observed at the 24-, 48-, 72-, and 96-hour intervals at the highest treatment level (4.5 mg ai/l). These effects were not observed at the four lowest treatment levels.

12. **QUALITY ASSURANCE MEASURES:** Yes.

13. **REVIEWER'S DISCUSSION/INTERPRETATION:**

Test Procedures: Test procedures followed guideline criteria.

Statistical Analysis:

Method: **EPA's Nuthatch program** EC_{50}: **1.6 mg ai/l**

95% CL: **1.4-1.9 mg ai/l** NOEC: **0.71 mg ai/l**

Slope: **4.97**

Discussion/Results: The study is scientifically sound and satisfies the guideline requirement for an acute toxicity test with a marine/estuarine mollusk. The EC_{50} value of 1.6 mg ai/l classifies metolachlor technical as moderately toxic to the Eastern oyster.

14. **COMPLETION DATE OF ONE-LINER FOR STUDY:** 1/26/95.

OYSTER.DAT : OYSTER SHELL DEPOSITION

Williams Test

!!!Williams: not implemented for 234 degrees of freedom.

Estimates of EC%

Parameter	Estimate	95% Bounds Lower	Upper	Std.Err.	Lower Bound /Estimate
EC1	0.55	0.40	0.77	0.072	0.72
EC5	0.76	0.58	0.99	0.059	0.77
EC25	1.2	1.0	1.4	0.040	0.83
EC50	1.6	1.4	1.9	0.028	0.88

Slope = 4.97 Std.Err. = 0.522

Goodness of fit: p = 0.084 based on DF= 3.0 2.3e+02

OYSTER.DAT : OYSTER SHELL DEPOSITION

Observed vs. Predicted Treatment Group Means

Dose	#Reps.	Obs. Mean	Pred. Mean	Obs. -Pred.	Pred. %Control	%Change
0.00	40.0	2.39	2.48	-0.0871	100.	0.00
0.710	40.0	2.38	2.38	0.193	96.4	3.65
1.10	40.0	1.89	1.99	-0.0990	80.2	19.8
1.70	40.0	1.10	1.15	-0.0525	46.4	53.6
2.90	40.0	0.355	0.266	0.0889	10.7	89.3
4.50	40.0	0.00	0.0355	-0.0355	1.43	98.6

!!!Warning: EC1 not bracketed by doses evaluated.

Analysis of Weighted Variance

Source	SS	DF	MS
Residual	131.34	237.00	0.55416
Means Lack-of-Fit	3.6654	3.0000	1.2218
Within Groups	127.67	234.00	0.54560

Directly-fitted parameters and Asymptotic Covariances

Parameter		Estimate	Std.Err.	Correlations		
Control Mean	=	2.4796	0.14505	1.0000	-0.71602	0.51894
log10 EC50	=	0.21246	0.028414	-0.71602	1.0000	-0.71177
Bruce&V. SIGMA	=	0.20140	0.021162	0.51894	-0.71177	1.0000
Starting Values		2.4875	0.20020	0.23583		

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Program: NUTHATCH
Path : C:\NUTHATCH
Date : 1/22/95

Toxicity measurement for continuous endpoints, using weighted nonlinear regression, weighting proportional to predicted means.

Reference

R.D. Bruce and D.J. Versteeg. 1992. A statistical procedure for modeling continuous toxicity data. Env. Tox. and Chem. 11:1485-1494.

OYSTER.DAT : OYSTER SHELL DEPOSITION

Raw data:

OYSTER SHELL DEPOSITION
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