(4)

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

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

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

3. **CITATION**

Author: Machado, M.W.
Title: Metolachlor technical - acute toxicity to mysid shrimp (*Mysidopsis bahia*) under flow-through conditions.
Date: 1994
Lab. Report #: 94-7-5402
Laboratory: Springborn Laboratories, Inc., Wareham, MA
Sponsor: Ciba Crop Protection, Greensboro, NC
MRID No.: 434871-03

4. **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

6. **CONCLUSIONS:** The 96-h LC_{50} of 4.9 mg ai/l classifies technical metolachlor as moderately toxic to mysid shrimp. The NOEC is 2.3 mg ai/l.

7. **ADEQUACY OF THE STUDY:** Core.

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

10. **MATERIALS AND METHODS:**

A. Test Organisms

Guideline Criteria	Reported Information
Species Preferred species are *Mysidopsis bahia*, *Penaeus setiferus*, *P. duorarun*, *P. aztecus* and *Palaemonetes sp.*	*Mysidopsis bahia*
Age Juvenile, mysids should be ≤ 24 hours old	≤24 h old
Supplier	Springborn Laboratories culture facility
All shrimp are from same source?	Yes
All shrimp are from the same year class?	Yes

B. Source/Acclimation

Guideline Criteria	Reported Information
Acclimation Period minimum 10 days	14 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
Feeding No feeding during the study and no feeding for 24 hour before the beginning of the test if organisms are over 0.5 g each.	Time of last feeding was not reported
Pretest Mortality <3% mortality 48 hours prior to testing	% mortality prior to testing was not reported

C. **Test System**

Guideline Criteria	Reported Information
Source of dilution water Soft reconstituted water or water from a natural source, **not** dechlorinated tap water	Filtered seawater from Cape Cod Canal, Bourne, MA
Does water support test animals without observable signs of stress?	Yes
Salinity 30-34 ‰ for marine (stenohaline) shrimp and 10-17 ‰ for estuarine (euryhaline) shrimp, weekly range < 6 ‰	31-32‰
Water Temperature 22 ± 1 °C	25 ± 1 °C
Ph 8.0-8.3 for marine (stenohaline) shrimp, 7.7-8.0 for estuarine (euryhaline) shrimp, monthly range < 0.8	7.9
Dissolved Oxygen Static: ≥ 60% during 1st 48 hrs and ≥ 40% during 2nd 48 hrs, Flow-through: ≥ 60%	80-81% at 72 h at highest dosage
Total Organic Carbon	1.4 mg/L
Test Aquaria 1. Material: Glass or stainless steel 2. Size: 19.6 L is acceptable for organisms ≥ 0.5 g (e.g. pink shrimp, white shrimp, and brown shrimp), 3.9 L is acceptable for smaller organisms (e.g. mysids and grass shrimp). 3. Fill volume: 15 L is acceptable for organisms ≥ 0.5 g, 2-3 L is acceptable for smaller organisms.	 Glass 39 x 20 x 25 cm 7.0-11 l

Guideline Criteria	Reported Information
Type of Dilution System Must provide reproducible supply of toxicant	Continuous-flow serial diluter calibrated to deliver 50 ml/min of exposure solution to each replicate aquarium
Flow Rate Consistent flow rate of 5-10 vol/24 hours, meter systems calibrated before study and checked twice daily during test period	6.5 vol/24 hours
Biomass Loading Rate Static: ≤ 0.8 g/L at ≤ 17°C, ≤ 0.5 g/L at > 17°C; flow-through: ≤ 1 g/L/day	0.00014 g/L
Photoperiod 16 hours light, 8 hours dark	16 h light, 8 h dark.
Solvents Not to exceed 0.5 ml/L for static tests or 0.1 ml/L for flow-through tests	Solvent: acetone Maximum conc.: 0.1 ml/L.

D. Test Design

Guideline Criteria	Reported Information
Range Finding Test If LC_{50} >100 mg/L with 30 shrimp, then no definitive test is required.	3 preliminary tests - 100% mortality at 8.0 mg ai/l
Nominal Concentrations of Definitive Test Control & 5 treatment levels; a geometric series in which each concentration is at least 60% of the next higher one.	0.50, 1.0, 2.0, 4.0, and 8.0 mg ai/l
Number of Test Organisms Minimum 20/level, may be divided among containers	20
Test organisms randomly or impartially assigned to test vessels?	Yes
Biological observations made every 24 hours?	Yes

Guideline Criteria	Reported Information
Water Parameter Measurements 1. Temperature Measured constantly or, if water baths are used, every 6 hrs, may not vary > 1°C 2. DO and pH Measured at beginning of test and ever 48 h in the high, medium, and low doses and in the control	Temp. continuously monitored in one rep. of control and once daily in all others DO, pH, and salinity measured once daily in both reps of each treatment and control
Chemical Analysis needed if solutions were aerated, if chemical was volatile, insoluble, or known to absorb, if precipitate formed, if containers were not steel or glass, or if flow-through system was used	Samples from each replicate analyzed for metolachlor at 0 h and 96 h (except for 2.0 mg ai/l dosage, which was sampled at 24 h and 96 h due to suspected sampling error at 0 h)

11. REPORTED RESULTS

A. General Results

Guideline Criteria	Reported Information
Quality assurance and GLP compliance statements were included in the report?	Yes
Recovery of Chemical	89-120% of nominal
Control Mortality Not more than 10% of control organisms may die or show abnormal behavior.	None
Raw data included?	Yes
Signs of toxicity (if any) were described?	Yes

Mortality:

Concentration (ppm)		Number of Shrimp	Cumulative Number Dead			
			Hour of Study			
Nominal	Mean Measured		24	48	72	96
Control		20	0	0	0	0
Solvent Control		20	0	0	0	0
0.5	0.61	20	0	0	0	0
1.0	1.0	20	0	0	0	0
2.0	2.3	20	0	0	0	0
4.0	4.0	20	0	1	4	7
8.0	7.1	20	0	1	13	16

Other Findings: At the two highest dosages, surviving mysids exhibited dark pigmentation and were lethargic by the end of the study.

B. Statistical Results:

Method: **Probit Analysis** 96-h LC_{50}: **4.9 mg ai/l**

95% C.I.: **4.2-5.9 mg ai/l** NOEC: **2.3 MG AI/l**

Probit Slope: (not reported)

12. **VERIFICATION OF STATISTICAL RESULTS:**

Method: **Probit Analysis** 96-h LC_{50}: **4.9 mg ai/l**

95% CL: **4.2-5.9 mg ai/l** NOEC: **2.3 mg ai/l**

13. **REVIEWER'S COMMENTS:** The study is scientifically sound and fulfills the guideline requirement for an acute toxicity test with mysid shrimp. Technical metolachlor is classified as moderately toxic to marine/estuarine mysid shrimp.

W. ERICKSON METOLACHLOR MYSID SHRIMP ACUTE TEST

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CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
7.1	20	16	80	.5908966
4	20	7	35	13.1588
2.3	20	0	0	9.536742E-05
1	20	0	0	9.536742E-05
.61	20	0	0	9.536742E-05

THE BINOMIAL TEST SHOWS THAT 2.3 AND 7.1 CAN BE
USED AS STATISTICALLY SOUND CONSERVATIVE 95 PERCENT
CONFIDENCE LIMITS, BECAUSE THE ACTUAL CONFIDENCE LEVEL
ASSOCIATED WITH THESE LIMITS IS GREATER THAN 95 PERCENT.

AN APPROXIMATE LC50 FOR THIS SET OF DATA IS 4.813171

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS	
2	9.753802E-02	4.861711	4.172503	5.902251

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
7	.1756815	1	.8140603

SLOPE = 6.06238
95 PERCENT CONFIDENCE LIMITS = 3.521371 AND 8.603389

LC50 = 4.951274
95 PERCENT CONFIDENCE LIMITS = 4.20845 AND 5.944308

LC10 = 3.056527
95 PERCENT CONFIDENCE LIMITS = 2.106609 AND 3.684414

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