(4)

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

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

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

3. **CITATION**

Authors: Machado, M.W.
Title: Metolachlor technical - acute toxicity to sheepshead minnow (*Cyprinodon variegatus*) under flow-through conditions
Date: 1994
Laboratory: Springborn Laboratories, Inc., Wareham, MA
Sponsor: Ciba Crop Protection, Greensboro, NC
Lab. Report No.: 94-7-5378
MRID No.: 434871-01

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 study is scientifically sound and satisfies the guideline requirement for an acute marine/estuarine fish toxicity test. The LC_{50} value of 9.8 mg ai/l classifies technical metolachlor as moderately toxic to the sheepshead minnow. The NOEC is 3.6 mg ai/l.

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

8. **MAJOR GUIDELINE DEVIATIONS:** None.

9. **MATERIALS AND METHODS:**

A. Test Organisms

Guideline Criteria	Reported Information
Species Preferred species are the sheepshead minnow (*Cyprinodon variegatus*) or the Silverside (*Menidia sp.*).	Sheepshead minnow
Mean Wet Weight 0.5 - 5 g	Mean: 0.22 g Range: 0.11-0.35 g
Mean Standard Length Longest not > 2x shortest	Mean: 23 mm Range: 15-27 mm
Supplier	Aquatic Research Organisms, Hampton, NH
All fish from same source?	Yes
All fish from the same year class?	Not reported

B. Source/Acclimation

Guideline Criteria	Reported Information
Acclimation Period minimum 14 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?	Not reported
Feeding No feeding during the study	Last fed 48 h prior to test
Pretest Mortality <3% mortality 48 hours prior to testing	No mortality within 48 h of testing

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
Does water support test animals without observable signs of stress?	Yes
Salinity 30-34 ‰ salinity, weekly range < 6 ‰	31 ‰
Water Temperature 22 ± 1 °C	21-22 °C
pH 8.0-8.3 for marine-stenohaline fishes, 7.7-8.0 for estuarine-euryhaline fishes, monthly range < 0.8	8.0
Dissolved Oxygen Static: ≥ 60% during 1st 48 hrs and ≥ 40% during 2nd 48 hrs, flow-through: ≥ 60%	Lowest % DO was 73% at 96 h
Test Aquaria 1. Material: Glass or stainless steel 2. Size: Volume of 19 L (5 gal) or 30 x 60 x 30 cm 3. Fill volume: 15-30 L of solution	**Material:** not described **Size:** 39 x 20 x 25 cm **Fill vol.:** 15 l solution
Type of Dilution System Must provide reproducible supply of toxicant	Mount and Brungs intermittent-flow proportional diluter, temp.-controlled water bath, and set of 14 aquaria
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

Guideline Criteria	Reported Information
Biomass Loading Rate Static: ≤ 0.8 g/L at ≤ 17°C, ≤ 0.5 g/L at > 17°C; flow-through: ≤ 1 g/L/day	0.023 g/L
Photoperiod 16 hours light, 8 hours dark	16 h light
Solvents Not to exceed 0.5 ml/L for static tests or 0.1 ml/L for flow-through tests	**Solvent:** acetone **Maximum conc.:** 0.092 mL/L

D. Test Design

Guideline Criteria	Reported Information
Range Finding Test If LC_{50} >100 mg/L with 30 fish, then no definitive test is required.	100% mortality at 20 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	2.6, 4.3, 7.2, 12, and 20 mg ai/L.
Number of Test Organisms Minimum 10/level, may be divided among containers	20/level 10/rep.
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 every 48 h in the high, medium, and low doses and in the control	Temp. continuously monitored in one rep; measured once daily in both reps of each level DO, pH, and salinity measured once daily in both reps of each level
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	Yes

10. **REPORTED RESULTS**

General Results:

Guideline Criteria	Reported Information
Quality assurance and GLP compliance statements were included in the report?	Yes
Recovery of Chemical % of nominal	83-110%
Control Mortality Not more than 10% of control organisms may die or show abnormal behavior.	2.5% (1 of 40)
Raw data included?	Yes
Signs of toxicity (if any) were described?	Yes

Mortality:

Concentration (ppm)		Number of Fish	Cumulative Number Dead			
			Hour of Study			
Nominal	Mean Measured		24	48	72	96
Control		20	0	1	1	1
Solvent Control		20	0	0	0	0
2.6	2.8	20	1	1	1	1
4.3	3.6	20	0	0	0	0
7.2	6.2	20	0	0	1	1
12	11	20	0	0	2	12
20	19	20	1	7	20	20

Other Findings: Sublethal effects, including lethargy and loss of equilibrium, were observed among all surviving fish exposed to the 11 mg ai/l treatment level and to one fish in the 6.2 mg ai/l treatment level.

Statistical Results

Method: **Moving Average** 96-hr LC_{50}: **9.8 mg ai/l**

95% C.I.: **8.5-11 mg ai/l** NOEC: **3.6 mg ai/l**

11. **REVIEWER'S VERIFICATION OF STATISTICAL RESULTS**: (attached)

Method: **Moving Average** 96-hr LC_{50}: **9.8 mg ai/l**

95% C.I.: **8.5-11.4 mg ai/l** NOEC: **3.6 mg ai/l**

12. **REVIEWER'S COMMENTS:** Deviations from guideline procedures include the following:

The size of the test fish (0.11-0.35 g) was smaller than recommended (0.5-5 g).

The report did not mention if any fish were diseased and if any treatment was made.

The study is scientifically sound and satisfies the guideline requirement for an acute marine/estuarine fish toxicity test. The EC_{50} value of 9.8 mg ai/l classifies technical metolachlor as moderately toxic to the sheepshead minnow.

NOTE: THERE WAS CONTROL MORTALITY, BUT AT LEAST ONE
OF THE LOWER CONCENTRATIONS HAD ZERO MORTALITY.
THEREFORE, ABBOTT'S CORRECTION IS NOT APPLICABLE.

W. ERICKSON METOLACHLOR SHEEPSHEAD MINNOW ACUTE TEST

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CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
19	20	20	100	9.536742E-05
11	20	12	60.00001	25.17223
6.2	20	1	5	2.002716E-03
3.6	20	0	0	9.536742E-05
2.8	20	1	5	2.002716E-03

THE BINOMIAL TEST SHOWS THAT 6.2 AND 19 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 10.05811

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS	
3	5.026151E-02	9.802751	8.525102	11.47825

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
5	2.539791	8.462025	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 = 5.375903
95 PERCENT CONFIDENCE LIMITS =-3.191524 AND 13.94333

LC50 = 9.560683
95 PERCENT CONFIDENCE LIMITS = 0 AND +INFINITY

LC10 = 5.549446
95 PERCENT CONFIDENCE LIMITS = 0 AND +INFINITY

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