



UNITED STATES ENVIRONMENTAL PROTECTION AGENCY  
WASHINGTON, D.C. 20460

3/15/96

**MEMORANDUM**

**SUBJECT:** Reregistration, Data Evaluation of three Acute Estuarine Studies, Ethoprop (041101), D218402, REREG Case #0106, ID#041101, Sponsor: Rhone-Poulenc.

**TO:** Larry Schnaubelt, PM 72  
Special Review and Reregistration Division (7508W)

**FROM:** Anthony F. Maciorowski, Chief  
Ecological Effects Branch  
Environmental Fate and Effects Division (7507C)

GUIDELINE	MRID	TOXICITY	ACCEPTABILITY
72-3(A)	436863-01	0.958 ppm	Core
72-3(B)	436863-02	3.7 mg ai/L	Core
72-3(C)	436863-03	18.78 ppb	Core

These studies indicate that Ethoprop is very highly toxic to Estuarine/Marine fish and shrimp and moderately toxic to Estuarine/Marine mollusks. The results from this study triggers an additional data requirements, Estuarine/Marine chronic studies for fish and aquatic invertebrates.

If you have any questions concerning this review please, contact Regina Hirsch (414-695-9796) or Les Touart (305-6134).

**DATA EVALUATION RECORD**  
**ACUTE LC<sub>50</sub> TEST WITH AN ESTUARINE/MARINE FISH**  
**§ 72-3(A)**

1. **CHEMICAL:** Ethoprop PC Code No.: 041101
2. **TEST MATERIAL:** Ethoprop Technical, Batch No. 307289119, CAS Registry No. 13194-48-4, a clear liquid. Purity: 96.8%

3. **CITATION**

Authors: Mark W. Machado  
Title: Ethoprop Technical -- Acute Toxicity to Sheepshead Minnow (*Cyprinodon variegatus*) Under Flow-Through Conditions.

Study Completion Date: 16 May 1995

Laboratory: Springborn Laboratories, Inc.

Sponsor: Rhone-Poulenc Ag Company

Laboratory Report ID: 95-4-5811; 10566.1294.6348.505

MRID No.: 436863-01

DP Barcode: D218402

4. **REVIEWED BY:** Regina Hirsch, Wildlife Biologist, EEB, EFED

Signature: 

Date: 3/15/96

5. **APPROVED BY:** Les Touart, Head of Section 1, EEB, EFED

Signature: 

Date: 3.5.96

6. **STUDY PARAMETERS**

Scientific Name of Test Organism:	<i>Cyprinodon variegatus</i>
Age or Size of Test Organism:	5-9 mm; and 0.01-0.06 g, N=30
Definitive Test Duration:	96-hours
Study Method:	Flow-through
Type of Concentrations:	Mean measured

7. **CONCLUSIONS:**

**Results Synopsis**

LC<sub>50</sub>: 0.958 ppm ai

NOEL: 0.340 ppm ai

✓ 95% C.I.: 0.761 - 1.45 ppm ai

Probit Slope: 0.340

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**8. ADEQUACY OF THE STUDY**

A. Classification: Core.

B. Rationale: N/A

C. Repairability: N/A

**9. Guideline Deviations**

1. Weight of fish was below what is recommended for this type of study.
2. Test aquaria were smaller (11 L volume) than what is recommended (19 L volume).

**10. SUBMISSION PURPOSE: Reregistration**

**11. MATERIALS AND METHODS**

**A. Test Organisms**

Guideline Criteria	Reported Information
<b><u>Species</u></b> Preferred species are the sheepshead minnow ( <i>Cyprinodon variegatus</i> ) or the Silverside ( <i>Menidia sp.</i> ).	<i>Cyprinodon variegatus</i>
<b><u>Mean Weight</u></b> 0.5 - 5 g	0.024 g (0.01-0.06)
<b><u>Mean Standard Length</u></b> Longest not > 2x shortest	Mean: 6.1 mm Range: 5.0-9.0 mm
<b><u>Supplier</u></b>	Springborn Laboratories brood stock (SLI Lot #94A44)
All fish from same source?	Yes
All fish from the same year class?	Yes

**B. Source/Acclimation**

Guideline Criteria	Reported Information
<b><u>Acclimation Period</u></b> minimum 14 days	minimum of 14 days
<b>Wild caught organisms were quarantined for 7 days?</b>	N/A
<b>Were there signs of disease or injury?</b>	Not reported
<b>If treated for disease, was there no sign of the disease remaining during the 48 hours prior to testing?</b>	N/A
<b><u>Feeding</u></b> No feeding during the study	48 hours prior to testing
<b><u>Pretest Mortality</u></b> <3% mortality 48 hours prior to testing	0% mortality prior to testing.

**C. Test System**

Guideline Criteria	Reported Information
<b><u>Source of dilution water</u></b> Soft reconstituted water or water from a natural source, not dechlorinated tap water	Cape Cod Canal, Bourne, Massachusetts.
<b>Does water support test animals without observable signs of stress?</b>	Yes
<b><u>Salinity</u></b> 30-34 ‰ salinity, weekly range <6 ‰	31 to 32 ‰
<b><u>Water Temperature</u></b> 22 ± 1 °C	23 °C

Guideline Criteria	Reported Information
<b>pH</b> 8.0-8.3 for marine-stenohaline fishes, 7.7-8.0 for estuarine-euryhaline fishes, monthly range < 0.8	7.8-8.0
<b>Dissolved Oxygen</b> Static: $\geq 60\%$ during 1 <sup>st</sup> 48 hrs and $\geq 40\%$ during 2 <sup>nd</sup> 48 hrs, flow-through: $\geq 60\%$	80% at 48 hours
<b>Test Aquaria</b> 1. <b>Material:</b> Glass or stainless steel 2. <b>Size:</b> Volume of 19 L (5 gal) or 30 x 60 x 30 cm 3. <b>Fill volume:</b> 15-30 L of solution	Glass  39 x 20 x 25 cm  volume of 11 L
<b>Type of Dilution System</b> Must provide reproducible supply of toxicant	A continuous-flow serial dilute with a dilution factor of 60%, a modification of the diluter system described by Benoit et al (1982).
<b>Flow Rate</b> Consistent flow rate of 5-10 vol/24 hours, meter systems calibrated before study and checked twice daily during test period	6.5 vol replacements/24 hours
<b>Biomass Loading Rate</b> Static: $\leq 0.8$ g/L at $\leq 17^\circ\text{C}$ , $\leq 0.5$ g/L at $> 17^\circ\text{C}$ ; flow-through: $\leq 1$ g/L/day	0.0033 g/L/day
<b>Photoperiod</b> 16 hours light, 8 hours dark	16 h light, 8 h dark.
<b>Solvents</b> 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
<b><u>Range Finding Test</u></b> If $LC_{50} > 100$ mg/L with 30 fish, then no definitive test is required.	Preliminary testing was the basis for definitive test concentrations.
<b><u>Nominal Concentrations of Definitive Test</u></b> Control & 5 treatment levels; each conc. should be 60% of the next highest conc.; concentrations should be in a geometric series	210, 350, 580, 960, and 1600 ug ai/L.
<b><u>Number of Test Organisms</u></b> Minimum 10/level, may be divided among containers	20/treatment level and controls
<b>Test organisms randomly or impartially assigned to test vessels?</b>	Yes
<b>Biological observations made every 24 hours?</b>	Yes
<b><u>Water Parameter Measurements</u></b> 1. <b><u>Temperature</u></b> Measured constantly or, if water baths are used, every 6 hrs, may not vary $> 1^{\circ}C$ 2. <b><u>DO and pH</u></b> Measured at beginning of test and ever 48 h in the high, medium, and low doses and in the control	Test solution temperature was monitored in one replicate (B) of the control solution throughout the study.  DO, pH, and salinity and temperature were measured once daily in both replicates of each treatment level and controls.
<b><u>Chemical Analysis</u></b> 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	Sample were taken from each replicate test solution of each treatment level and controls at 0 and 96-hours of exposure for analysis of Ethoprop concentration.

**12. REPORTED RESULTS****A. General Results**

Guideline Criteria	Reported Information
Quality assurance and GLP compliance statements were included in the report?	Yes
<u>Recovery of Chemical</u>	87.2 to 103%
<u>Control Mortality</u> Not more than 10% of control organisms may die or show abnormal behavior.	0%
Raw data included?	No
Signs of toxicity (if any) were described?	Yes

**Mortality**

Concentration (ppb)		Number of Fish	Cumulative Number Dead			
Nominal	Mean Measured		Hour of Study			
			24	48	72	96
Control	0	20	0	0	0	0
Solvent Control	0	20	0	0	0	0
210	340	20	0	0	0	1
350	430	20	0	0 <sup>g</sup>	1 <sup>bd</sup>	4 <sup>e</sup>
580	620	20	1 <sup>be</sup>	2 <sup>ad</sup>	5 <sup>d</sup>	5 <sup>abcd</sup>
960	620	20	0 <sup>bcd</sup>	0 <sup>abcdf</sup>	7 <sup>cd</sup>	7 <sup>cd</sup>
1600	1500	20	2 <sup>abcd</sup>	6 <sup>acdf</sup>	13 <sup>cdh</sup>	14 <sup>h</sup>

<sup>a</sup> 1-2 surviving fish exhibited some mortality

<sup>a</sup> 1-2 surviving fish exhibited complete loss of equilibrium.<sup>b</sup> 1-2 surviving fish exhibited partial loss of equilibrium.

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- <sup>c</sup> 1 surviving fish exhibited partial loss of equilibrium and was observed at the surface of the test solution.
- <sup>d</sup> Several to all surviving fish were lethargic.
- <sup>e</sup> 1-2 surviving fish were lethargic.
- <sup>f</sup> 1-2 surviving fish exhibited complete loss of equilibrium and was at the surface of the test solution.
- <sup>g</sup> One Surviving fish was at the surface of the test solution.
- <sup>h</sup> Several to all surviving fish exhibited partial loss of equilibrium.

**Other Significant Results:**

**B. Statistical Results**

Method: Probit Analysis

96-hr LC<sub>50</sub>: 0.95 ppm ai

95% C.I.: 0.76 -1.40 ppm ai

Probit Slope: \_\_\_\_

NOEC: 0.34 ppm ai

**13. VERIFICATION OF STATISTICAL RESULTS**

Parameter	Result
Binomial Test LC <sub>50</sub> (C.I.)	0.904 (0.620 - infinity) ppm ai
Moving Average Angle LC <sub>50</sub> (95% C.I.)	0.918 (0.698 - 1.642) ppm ai
Probit LC <sub>50</sub> (95% C.I.)	0.958 (0.761 - 1.447) ppm ai
Probit Slope	2.921
NOEC	0.340 ppm ai

**14. REVIEWER'S COMMENTS:**



RHirsch Ethoprop Acute Sheepshead Minnow

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CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
1500	20	14	70	5.765915
621	20	7	35	13.1588
620	20	5	25	2.069473
430	20	4	20	.5908966
340	20	1	5	2.002716E-03

THE BINOMIAL TEST SHOWS THAT 620 AND +INFINITY 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 903.9385

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS
2	.4747728	917.7565	698.5878 1641.074

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H
3	.1954565	1

GOODNESS OF FIT PROBABILITY  
.7311044

SLOPE = 2.921231  
95 PERCENT CONFIDENCE LIMITS = 1.629741 AND 4.212721

LC50 = 957.9741  
95 PERCENT CONFIDENCE LIMITS = 761.63 AND 1447.263

LC10 = 352.051  
95 PERCENT CONFIDENCE LIMITS = 198.4942 AND 461.1175

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RHirsch Ethoprop Acute Flow-through with Sheepshead Minnow

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CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
1.5	20	14	70	5.765915
.621	20	7	35	13.1588
.619	20	5	25	2.069473
.43	20	4	20	.5908966
.34	20	1	5	2.002716E-03

THE BINOMIAL TEST SHOWS THAT .619 AND +INFINITY 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 .903939

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS	
2	.4747728	.9176729	.6981755	1.642667

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H
3	.1953965	1

GOODNESS OF FIT PROBABILITY  
.7326001

SLOPE = 2.921422  
95 PERCENT CONFIDENCE LIMITS = 1.630046 AND 4.212798

LC50 = .9576008  
95 PERCENT CONFIDENCE LIMITS = .7613357 AND 1.446652

LC10 = .3519369  
95 PERCENT CONFIDENCE LIMITS = .1984764 AND .4609458

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