Ecological Effects Branch Review

1. Chemical: Triclopyr TEA Salts

2. Test Material: 44.7% a.i., A Brown liquid

3. Study Type: Acute Toxicity, Estuarine/Marine Organism

4. Study Identification: Ward, T.J. Boeri R.L. (1989) Acute Flow-through Toxicity of Triclopyr TEA Salt to the Tidewater Silverside, Menidia beryllina. Envirosystems Study Number #8990-D. Prepared by Envirosystems Division, Resource Analysts, Inc., Hampton, New Hampshire. Submitted by Dow Chemical, Midland, Michigan. Acc #416337-03. 116002 72-3(a)§ (d)

5. Reviewed By:

   Jeffrey Bigler
   Fishery Biologist
   Ecological Effects Branch
   Environmental Fate and Effects Division (H7507C)
   Signature: JBB
   Date: 3-6-91

   Approved By:

   Mike Rexrode
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   Ecological Effects Branch
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   Signature: Michael Rexrode
   Date: 3/14/91

6. Conclusions: This study appears to have been conducted in a scientific manner and therefore meets guideline requirements for an acute toxicity study for estuarine fish. The LC_{50} of 130 mg/l (114 - 154 mg/l) characterizes [redacted] triclopyr AS (N) practically non toxic to the Tidewater Silverside minnow.

7. Recommendations: N/A

8. Background: Submitted in support of reregistration of Triclopyr TEA Salts.

9. Discussion of Individual Results: N/A

10. Materials and Methods:

A. Test Animals: Menidia beryllina were supplied by Aquatic Research Organisms, Hampton, New Hampshire. The silversides were acclimated under test conditions for 14 days. The test fish were fed a commercial fish food daily up to 48 hours prior to the definitive test at which time the feeding was discontinued. There were no signs of sickness or injuries to the test fish during the acclimation period. The control fish had an average length of 27.0 mm (25-29 mm) and an average weight of 0.10 g (0.09-0.13 g).

B. Dose: A dilution water control and nominal test doses of 26, 42, 66, 94, and 160 mg/l.

C. Test System: The test was conducted in 19.6 liter glass aquaria (20cmx40cmx25cm) containing 15 liters of test solution with a depth of 19 cm. The test vessels were placed randomly in a water bath controlled at 22 ±1°C. The test media was delivered to each aquaria by an intermittent flow proportional diluter. Volume exchanges averaged 7.0 exchanges per day. The photoperiod was 16 hours light and 8 hours dark. Aeration was begun 48 hours after test initiation tin order to maintain adequate DO levels. The dilution water was natural seawater, aerated, and adjusted to a salinity of 11-17 ppt.

D. Design: Twenty Menidia beryllina were randomly distributed to two aquaria per treatment level for a total of 12 aquaria and 120 test organisms for the definitive test. The loading rate was 0.07 g/l/day. The photoperiod was 16 hours light and 8 hours dark. The temperature, DO, pH, and salinity were measured in the control and each vessel containing living test organisms. Chemical concentrations were measured at 0, 48, and 96 hours in all test aquaria. The water temperature during acclimation was 21.0 - 22.3 C.

E. Statistics: The mean measured concentrations and the corresponding mortality data were used to determine the LC_{50} using Stephans' computer program.

11. Reported Results: (Excerpted from study) "No insoluble material was observed in any test vessel during the test. Measured concentrations of test substance agreed well with nominal concentrations (Table 2).

Biological and water quality data generated by the acute toxicity test are presented in Table 3 and Appendix A. One hundred percent survival occurred in the control exposure. Control fish had an average wet weight of 0.10 g (range = 0.09-0.13 g) and an average total length of 27.0 mm (range = 25-29 mm) at the end of the test. Loading rate was approximately 0.07 g/l.

The dose-response curve for organisms exposed to the test substance for 96 hours is present in Figure 1. The 24, 48, 72, and 96 hour LC_{50} values for fish exposed to triclopyr TEA Salt are presented in Table 4. The 96 hour LC_{50} (95% confidence limit) is 130 (114-154) mg/l triclopyr TEA Salt. The 96 hour no observed effect level concentration is 61 mg/l."

12. Study Authors' Conclusions/QA Measures: "Exposure of Fish to the test material resulted in a 96 hour LC_{50} of 130 mg/l triclopyr TEA Salt, with a 95% confidence interval of 114-154 mg/l. The 96 hour no observed effect level is 61 mg/l."

"This study was conducted according to, and complies with, the EPA Good Laboratory Practices Regulations (40 CRF 160)."

13. Reviewers' Discussion and Interpretation of the Study:

A. Test Procedures: The test procedures were generally in accordance with accepted guidelines, but deviated from these guidelines as follows:

- The recommended pH range for this type of study is 7.7 to 8.0; the actual range in this study was 7.3 to 7.9.

- The preferred photoperiod for this type of study is 16 hours light, 8 hours dark and a 30 minute transition period; no mention of a 30 minute transition period was made during the course of this study.

B. Statistical Analysis: The Ecological Effects Branch determined the LC_{50} (95% confidence limits), based on measured concentrations, to be 130 mg/l (114 - 154 ug/l) using the Stephans' computer program. The NOEL for this study is 61 mg/l based on results of the definitive study.

C. Discussion and Results: The 96 hour LC_{50} of 130 mg/l characterizes [redacted] technical as practically non toxic estuarine fishes. triclopyr

(12)

D. Adequacy of Study:

Classification - Core.

Rational - The guideline deviations are not expected to have a significant effect on the usefulness of the study.

Repairability - N/A.

15. Completion of One-liner: Yes.

BIGLER TRICLOPYR SILVERSIDE 10-23-90

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CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB.(PERCENT)
152	20	14	70	5.765915
88	20	2	10	2.012253E-02
61	20	0	0	9.536742E-05
37	20	0	0	9.536742E-05
23	20	0	0	9.536742E-05

THE BINOMIAL TEST SHOWS THAT 0 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 128.2819

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS	
1	.235757	128.2819	112.2528	156.2781

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
8	.2000986	1	.9886774

SLOPE = 7.95167

95 PERCENT CONFIDENCE LIMITS = 4.394699 AND 11.50864

LC50 = 130.0044

95 PERCENT CONFIDENCE LIMITS = 113.6143 AND 154.0224

LC10 = 90.0001

95 PERCENT CONFIDENCE LIMITS = 66.87403 AND 104.3333

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Table B.1. Analytical data from toxicity test with triclopyr TEA salt

Nominal Concentration (mg/L)	Rep.	Measured Concentration mg/L as Acid Equivalent (mg/L as Triclopyr TEA Salt) 0 hour	48 hour	96 hour
0 (control)	1	ND (ND)	ND (ND)	ND (ND)
	2	ND (ND)	ND (ND)	ND (ND)
26	1	15 (21)	17 (24)	17 (24)
	2	14 (19)	17 (24)	17 (24)
42	1	24 (33)	28 (39)	27 (38)
	2	24 (33)	28 (39)	27 (38)
66	1	40 (56)	45 (63)	45 (63)
	2	42 (58)	45 (63)	45 (63)
94	1	59 (82)	64 (89)	64 (89)
	2	61 (85)	65 (91)	63 (88)
160	1	104 (145)	111 (155)	109 (152)
	2	107 (149)	111 (155)	111 (155)
Diluter Stock Solution	1	111 (155)	112 (156)	111 (155)

Note: 1. ND = none detected (detection limit = 5 mg/L as acid equivalent and 7 mg/L as triclopyr TEA salt)

2. The measured concentration of acid equivalent is converted to the concentration of triclopyr TEA salt by division by 71.8%

Table 3. Survival data from toxicity test with triclopyr TEA salt

Mean Measured Concentration (mg/L)	rep.	Number Alive					Number Affected				
		0hr	24hr	48hr	72hr	96hr	0hr	24hr	48hr	72hr	96hr
ND (control)	1	10	10	10	10	10	0	0	0	0	0
	2	10	10	10	10	10	0	0	0	0	0
22	1	10	10	10	10	10	0	0	0	0	0
	2	10	10	10	10	10	0	0	0	0	0
37	1	10	10	10	10	10	0	0	0	0	0
	2	10	10	10	10	10	0	0	0	0	0
61	1	10	10	10	10	10	0	0	0	0	0
	2	10	10	10	10	10	0	0	0	0	0
88	1	10	10	10	10	9	0	0	0	0	9[a]
	2	10	10	10	10	9	0	0	0	0	5[a]
152	1	10	10	8	6	3	0	5[b]	8[b]	6[b]	3[b]
	2	10	10	9	7	3	0	7[b]	9[b]	7[b]	3[b]

Note: ND = none detected (detection limit = 7 mg/L)

[a] Affected organisms exhibited lethargy
[b] Affected organisms exhibited a loss of equilibrium and lethargy

Table 2. [illegible] concentrations of test substance during toxicity [illegible] triclopyr TEA salt

Nominal Concentration (mg/L)	Measured Concentration of Triclopyr TEA Salt(mg/L)							
	0 hour		48 hour		96 hour		Mean
	rep.1	rep.2	rep.1	rep.2	rep.1	rep.2	
0 (control)	ND	ND	ND	ND	ND	ND	ND
26	21	19	24	24	24	24	23
42	33	33	39	39	38	38	37
66	56	58	63	63	63	63	61
94	82	85	89	91	89	88	88
160	145	149	155	155	152	155	152

Note: ND = none detected (detection limit = 7 mg/L)