US EPA ARCHIVE DOCUMENT

9-16-92

MRID No. 422459-01

DATA EVALUATION RECORD

1. **CHEMICAL:** Chlorpyrifos Degradate.
Shaughnessey No. 059101.

2. **TEST MATERIAL:** 3,5,6-Trichloro-2-pyridinol; CAS No. 6515-38-4; AGR 143197; 99.9% active ingredient; a white powder.

3. **STUDY TYPE:** Estuarine Fish Flow-Through Toxicity Test. Species Tested: Atlantic Silverside (*Menidia menidia*).

4. **CITATION:** Graves, W.C. and G.J. Smith. 1991. 3,5,6-Trichloro-2-Pyridinol: A 96-Hour Flow-Through Acute Toxicity Test with the Atlantic Silverside (*Menidia menidia*). Project No. 103A-101A. Prepared by Wildlife International Ltd., Easton, MD. Submitted by DowElanco, Indianapolis, IN. EPA MRID No. 422459-01.

5. **REVIEWED BY:**

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Signature: Louis M Rifici
Date: 7/8/92

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6. **APPROVED BY:**

Rosemary Graham Mora, M.S.
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Signature: Rosemary Graham Mora
Date: 7/9/92

Henry T. Craven, M.S.
Supervisor, EEB/EFED
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Date: 8/16/92

7. **CONCLUSIONS:** This study is scientifically sound and meets the guideline requirements for an acute, flow-through, estuarine fish toxicity study. The 96-hour LC_{50} was 58.4 mg/l (based on mean measured concentrations) and classifies 3,5,6-Trichloro-2-pyridinol as slightly toxic to the Atlantic silverside. The NOEC was 44.5 mg/l mean measured concentration.

8. **RECOMMENDATIONS:** N/A.

9. **BACKGROUND:**

10. **DISCUSSION OF INDIVIDUAL TESTS:** N/A.

11. **MATERIALS AND METHODS:**

A. **Test Animals:** Juvenile Atlantic silversides (*Menidia menidia*) were obtained from Oak Creek, Newcomb, MD. The fish were held in the laboratory for 14 days. They were fed a commercial flake food and brine shrimp nauplii. The fish were fasted for 48 hours and acclimated to the test conditions for approximately 54 hours before the test. No mortality occurred in the population during holding or acclimation. During acclimation, the salinity was 25-26 parts per thousand (ppt), the temperature was 21.9°C, and the pH was 7.6-7.7. The average length of 10 control organisms was 25 mm (23-27 mm) and the average weight was 0.14 g (0.12-0.18 g) when measured at the end of the test.

B. **Test System:** A continuous-flow proportional diluter was used to mix and dispense the test solutions to the test chambers. The chambers were Teflon®-lined, 25-l polyethylene aquaria filled with 15 l of test solution. The solution depth was approximately 15 cm. The flow rate to each chamber provided 6.7 volume replacements per day. Pairs of replicate chambers were randomly positioned in a temperature-controlled water bath set to 22 ±1°C. The laboratory environment was maintained on a 16-hour daylight photoperiod with 30-minute dawn and dusk simulations. The light intensity during the test was approximately 970 lux.

Natural seawater, collected at Indian River Inlet, DE, was aerated and filtered (25 and 0.2 µm) before use as test dilution water. The salinity of the dilution water was 26 ppt and the pH was 8.2-8.3 when measured at test initiation.

The test material was dissolved in methanol. The primary stock (0.100 g/ml) was diluted in methanol to prepare 4 additional stocks. The stocks were delivered to the diluter and mixed with dilution water to achieve the desired test concentrations. The resulting methanol concentrations in the treatment and solvent control groups was 1.2 ml/l.

C. **Dosage:** Ninety-six-hour flow-through test. Five nominal concentrations (15.6, 25.9, 43.2, 72.0, and 120 mg a.i./l) a methanol control (1.2 ml/l), and a dilution water control were used.

D. **Design:** The fish were impartially removed in groups of two from holding tanks and indiscriminately distributed

to the test chambers until each contained 10 fish. Two replicate chambers were used for a total of 20 fish per concentration. The biomass loading was 0.10 g/l or 0.014 g/l/day.

Observations of mortality and sublethal responses were made at 1.5, 24, 48, 72, and 96 hours. The dissolved oxygen concentration (DO), temperature, salinity, and pH were measured in each replicate daily. The temperature of a dilution water control chamber was monitored continuously.

The concentration of the test material in water samples taken at test initiation, after 48 hours, and at termination was measured using ultra-violet spectroscopy.

E. Statistics: The 96-hour LC_{50} and associated 95% confidence interval were determined using the binomial probability method.

12. REPORTED RESULTS: The mean measured test concentrations were 16.6, 26.7, 44.5, 76.7, and 120 mg/l. These values represent 100 to 106% of nominal concentrations (Table 1, attached). "Within 24 hours of test initiation, a slight precipitate was observed in the two highest test concentrations (76.7 and 120 mg/l)."

Three dilution water control fish died as a result of being trapped behind the Teflon liner. Fish at concentrations ≤44.5 mg/l appeared normal throughout the test. Mortality during the test was presented in Table 3 (attached). The 96-hour LC_{50}, based on mean measured concentrations, was 58.4 mg/l (95% C.I. = 44.5-76.7 mg/l). The no-observed-effect concentration (NOEC) was as 44.5 mg/l.

The DO was 4.7-7.2 and remained above 60% of saturation. The pH was 7.3-8.4 and the salinity ranged from 24 to 28 ppt. The results of continuous temperature monitoring in addition to the daily individual measurements established the test temperature range as 21.3 to 22.5°C.

13. STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES: The authors made no conclusions other than those mentioned above.

Quality Assurance and Good Laboratory Practice Compliance Statements were included in the report, indicating that the study was conducted in accordance with EPA Good Laboratory

Practice Standards (40 CFR Part 160). The dates and types of quality assurance audits performed were also presented.

14. **REVIEWER'S DISCUSSION AND INTERPRETATION OF STUDY RESULTS:**

A. **Test Procedure:** The test procedures were generally in accordance with the SEP except for the following:

The solvent concentration used (1.2 ml/l) was much higher than recommended (0.5 ml/l).

The acclimation period used (54 hours) was shorter than recommended (2 weeks).

The test vessels were Teflon®-lined, polyethylene aquaria. Stainless steel or glass test vessels are recommended. In this case, the Teflon® liner is probably a better substitute.

The salinity of the dilution water in the study was 26 ppt with a pH of 8.2-8.3. The recommended salinity and pH for Atlantic silverside is 10-17 ppt and 7.7-8.0 or 30-34 ppt and 8.0-8.3.

The results of preliminary studies, if any, were not given in the report.

B. **Statistical Analysis:** The reviewer used EPA's toxanal program to calculate the 96-hour LC_{50} and obtained the same results as the author (printout, attached).

C. **Discussion/Results:** The laboratory used a high solvent concentration to increase the solubility of the test material. The concentration used (1.2 ml/l) was much higher than recommended (0.5 ml/l) in the SEP, but did not appear to affect the organisms. No unexplained mortality or sublethal effects were observed at 16.6, 26.7, and 44.5 mg/l or in the dilution water and solvent controls and it is likely that the mortality observed at the two highest concentrations was treatment-related. Therefore, the results of the study are acceptable.

This study is scientifically sound and meets the guideline requirements for an acute, flow-through, estuarine fish toxicity study. The 96-hour LC_{50} was 58.4 mg/l (based on mean measured concentrations) and classifies 3,5,6-Trichloro-2-pyridinol as slightly toxic to the Atlantic silverside. The NOEC was 44.5 mg/l based on the lack of mortality and sublethal effects.

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D. **Adequacy of the Study:**

(1) **Classification:** Core.

(2) **Rationale:** N/A.

(3) **Repairability:** N/A.

15. **COMPLETION OF ONE-LINER FOR STUDY:** Yes, 06-30-92.

Table 1.
Summary of Analytical Chemistry Data

Sponsor: The Dow Chemical Company
Test Substance: 3,5,6-Trichloro-2-pyridinol
Test Organism: Atlantic Silverside, *Menidia menidia*
Dilution Water: Saltwater

Nominal Concentration (mg/L)	Replicate	Measured Concentration (mg/L)					Percent of Nominal
		0 Hours	48 Hours	96 Hours	Mean Within Replicate	Grand Mean	
Negative Control	A	ND[1]	ND	ND	ND	ND	--
	B	ND	ND	ND	ND		
Solvent Control	A	ND	ND	ND	ND	ND	--
	B	ND	ND	ND	ND		
15.6	A	17.2	15.9	16.6	16.6	16.6	106
	B	17.4	15.9	16.6	16.6		
25.9	A	26.9	26.9	26.5	26.8	26.7	103
	B	26.7	26.8	26.5	26.7		
43.2	A	45.0	43.8	44.2	44.3	44.5	103
	B	45.5	43.9	44.6	44.7		
72.0	A	81.8	80.4[2]	NA[3]	81.1	76.7	106
	B	71.9	72.9[2]	NA	72.4		
120	A	120	116[2]	NA	118	120	100
	B	122	123[2]	NA	122		

[1] ND = Not detected at the Limit of Detection = 1.08 mg/L.
[2] Analytical samples used to verify test substance concentrations in these test solutions were collected at 24 hours, the first time interval during the study that 100% mortality was observed.
[3] NA = Not applicable.

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Table 3.
Cumulative Percent Mortality and Treatment-Related Effects.[1]

Sponsor: The Dow Chemical Company
Test Substance: 3,5,6-Trichloro-2-pyridinol
Test Organism: Atlantic Silverside, *Menidia menidia*
Dilution Water: Saltwater

Mean Measured Concentration (mg/L)	Replicate	Number of Dead / Number Exposed					Total % Mortality
		24 Hours	48 Hours	72 Hours	96 Hours	Cumulative Total	
Negative Control	A	0 / 10	0 / 10	0 / 10	0 / 10	3[2] / 20	15[2]
	B	3[2] / 10	3[2] / 10	3[2] / 10	3[2] / 10		
Solvent Control	A	0 / 10	0 / 10	0 / 10	0 / 10	0 / 20	0
	B	0 / 10	0 / 10	0 / 10	0 / 10		
16.6	A	0 / 10	0 / 10	0 / 10	0 / 10	0 / 20	0
	B	0 / 10	0 / 10	0 / 10	0 / 10		
26.7	A	0 / 10	0 / 10	0 / 10	0 / 10	0 / 20	0
	B	0 / 10	0 / 10	0 / 10	0 / 10		
44.5	A	0 / 10	0 / 10	0 / 10	0 / 10	0 / 20	0
	B	0 / 10	0 / 10	0 / 10	0 / 10		
76.7	A	10[1] / 10	10 / 10	10 / 10	10 / 10	20 / 20	100
	B	10[1] / 10	10 / 10	10 / 10	10 / 10		
120	A	10 / 10	10 / 10	10 / 10	10 / 10	20 / 20	100
	B	10 / 10	10 / 10	10 / 10	10 / 10		

[1] Observed Effects: All of the organisms in the control and treatment groups at 44.5 mg/L and below appeared normal throughout the test. In the 76.7 mg/L group, 2 and 8 fish were observed moribund at 1.5 hours in the A and B replicates, respectively.

[2] Three fish died as a result of being trapped behind the Teflon® liner in the test chamber.

The 96-hour LC50 was 58.4 mg/L with a 95% confidence interval of 44.5 to 76.7 mg/L.

RIFICI CHLORPYRIFOS DEGRADATE ATLANTIC SILVERSIDES 06-30-92

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CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB.(PERCENT)
120	20	20	100	9.536742E-05
76.7	20	20	100	9.536742E-05
44.5	20	0	0	9.536742E-05
26.7	20	0	0	9.536742E-05
16.6	20	0	0	9.536742E-05

THE BINOMIAL TEST SHOWS THAT 44.5 AND 76.7 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 58.42219

WHEN THERE ARE LESS THAN TWO CONCENTRATIONS AT WHICH THE PERCENT DEAD IS BETWEEN 0 AND 100, NEITHER THE MOVING AVERAGE NOR THE PROBIT METHOD CAN GIVE ANY STATISTICALLY SOUND RESULTS.

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