9-16-92

MRID No. 422459-02

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 Shrimp Flow-Through Toxicity Test. Species Tested: Grass Shrimp (*Palaemonetes pugio*).

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 Grass Shrimp (*Palaemonetes pugio*). Project No. 103A-102A. Prepared by Wildlife International Ltd., Easton, MD. Submitted by DowElanco, Indianapolis, IN. EPA MRID No. 422459-02.

5. **REVIEWED BY:**

Louis M. Rifici, M.S.
Associate Scientist
KBN Engineering and
Applied Sciences, Inc.

Signature: Louis M Rifici
Date: 7/9/92

Barbara T. Valente 7/13/92

6. **APPROVED BY:**

Rosemary Graham Mora, M.S.
Associate Scientist
KBN Engineering and
Applied Sciences, Inc.

Signature: Rosemary Graham Mora
Date: 7/9/92

Henry T. Craven, M.S.
Supervisor, EEB/EFED
USEPA

Signature: Henry T. Craven
Date: 9/16/92

7. **CONCLUSIONS:** This study is scientifically sound and meets the guideline requirements for an acute, flow-through, estuarine shrimp toxicity study. The 96-hour LC_{50} was 83.0 mg/l (based on mean measured concentrations) and classifies 3,5,6-Trichloro-2-pyridinol as slightly toxic to grass shrimp. The NOEC was 28.1 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 grass shrimp (*Palaemonetes pugio*) were obtained from Eastern Bay, Claiborne, MD. During the 12-day period prior to acclimation, the shrimp were fed a commercial flake food. The shrimp were acclimated to the test conditions for approximately 53 hours before the test. During acclimation, the conditions in the holding tank were a salinity of 21-25 parts per thousand (ppt), a pH of 7.5-7.7, and a temperature of 21.4°C. No mortality occurred in the population during acclimation or holding.

The average length of 10 control organisms was 26 mm (20-32 mm) and the average weight was 0.17 g (0.10-0.33 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 11 l of test solution. The solution depth was approximately 10 cm. The flow rate to each chamber provided 9.2 volume replacements per day. The test 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 370 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 25 ppt and the pH was 8.1-8.2 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 shrimp were impartially removed from a holding tank and indiscriminately distributed to the test chambers until each contained 10 shrimp. Two replicate chambers were used for a total of 20 fish per concentration. The biomass loading was 0.15 g/l or 0.02 g/l/day.

Observations of mortality and sublethal responses were made at 2, 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 probit method.

12. REPORTED RESULTS: The mean measured test concentrations were 18.6, 28.1, 48.6, 82.7, and 124 mg/l. These values represent 103-119% of nominal concentrations (Table 1, attached).

Shrimp at concentrations ≤28.1 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 83.0 mg/l (95% C.I. = 71.4-97.0 mg/l). The slope of the concentration-response curve was 6.10. The no-observed-effect concentration (NOEC) was as 28.1 mg/l.

The DO was 6.5-7.3 and remained above 60% of saturation. The pH was 7.3-8.2 and the salinity ranged from 25 to 27 ppt. The results of continuous temperature monitoring in addition to the daily individual measurements established the test temperature range as 21.7 to 22.9°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 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 25 ppt with a pH of 8.1-8.2. 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 mortality or sublethal effects were observed at 18.6 and 28.1 mg/l or in the dilution water and solvent controls and it is likely that the mortality observed at the three 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 shrimp toxicity study. The 96-hour LC_{50} was 83.0 mg/l (based on mean measured concentrations) and classifies 3,5,6-Trichloro-2-pyridinol as slightly toxic to grass shrimp. The NOEC was 28.1 mg/l mean measured concentration.

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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: Grass Shrimp, *Palaemonetes pugio*
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	16.8	19.1	19.5	18.5	18.6	119
	B	17.1	19.2	19.7	18.7		
25.9	A	28.0	28.1	28.1	28.1	28.1	108
	B	28.2	28.0	28.1	28.1		
43.2	A	48.2	48.0	48.6	48.3	48.6	112
	B	49.0	48.2	49.3	48.8		
72.0	A	77.9	94.2	81.9	84.7	82.7	115
	B	79.3	87.7	75.2	80.7		
120	A	125.6	124.3	127.0	125.6	124	103
	B	122.2	113.6	128.3	121.4		

[1] ND = Not detected at the Limit of Detection = 1.08 mg/L.

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Table 3.
Cumulative Percent Mortality and Treatment-Related Effects.*

Sponsor: The Dow Chemical Company
Test Substance: 3,5,6-Trichloro-2-pyridinol
Test Organism: Grass Shrimp, *Palaemonetes pugio*
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	0 / 20	0
	B	0 / 10	0 / 10	0 / 10	0 / 10		
Solvent Control	A	0 / 10	0 / 10	0 / 10	0 / 10	0 / 20	0
	B	0 / 10	0 / 10	0 / 10	0 / 10		
18.6	A	0 / 10	0 / 10	0 / 10	0 / 10	0 / 20	0
	B	0 / 10	0 / 10	0 / 10	0 / 10		
28.1	A	0 / 10	0 / 10	0 / 10	0 / 10	0 / 20	0
	B	0 / 10	0 / 10	0 / 10	0 / 10		
48.6	A	1 / 10	1 / 10	1 / 10	2 / 10	3 / 20	15
	B	0 / 10	0 / 10	1 / 10	1 / 10		
82.7	A	1 / 10	1 / 10	4 / 10	4 / 10	6 / 20	30
	B	0 / 10	1 / 10	1 / 10	2 / 10		
124	A	6 / 10	9 / 10	10 / 10	10 / 10	19 / 20	95
	B	5 / 10	8 / 10	9 / 10	9 / 10		

*Observed Effects: All of the surviving organisms in the control and treatment groups appeared normal throughout the test period.

The 96-hour LC50 was 83.0 mg/L with a 95% confidence interval of 71.4 to 97.0 mg/L.

RIFICI CHLORPYRIFOS DEGRADATE GRASS SHRIMP 06-30-92

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CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
124	20	19	95	2.002716E-03
82.7	20	6	30	5.765915
48.6	20	3	15	.1288414
28.1	20	0	0	9.536742E-05
18.6	20	0	0	9.536742E-05

THE BINOMIAL TEST SHOWS THAT 48.6 AND 124 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 92.40678

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS	
2	.1198138	85.35086	73.97168	100.1892

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
12	.1542862	1	.1125188

SLOPE = 6.095701
95 PERCENT CONFIDENCE LIMITS = 3.701353 AND 8.490048

LC50 = 83.03271
95 PERCENT CONFIDENCE LIMITS = 71.3553 AND 97.05662

LC10 = 51.39322
95 PERCENT CONFIDENCE LIMITS = 36.17046 AND 61.5733

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