MRID No. 421246-09

DATA EVALUATION RECORD

1. **CHEMICAL:** Amitraz. Shaughnessey Number: 106201.

2. **TEST MATERIAL:** BTS 27919; N-(2,4-dimethylphenyl) formamide; Batch No. CR 19546/1; 99.8% active ingredient; a white powder.

3. **STUDY TYPE:** Estuarine Fish Static Acute Toxicity Test. Species Tested: Sheepshead Minnow (*Cyprinodon variegatus*).

4. **CITATION:** Schupner, J.K. and B.J. Stachura. 1991. The Static Acute Toxicity of BTS 27919 Technical to the Sheepshead Minnow, *Cyprinodon variegatus*. Laboratory Project No. 508L. Study performed by NOR-AM Chemical Company, NOR-AM Research Center, Pikeville, North Carolina. Submitted by NOR-AM Chemical Company. EPA MRID No. 421246-09.

5. **REVIEWED BY:**

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Date: 1/28/92

6. **APPROVED BY:**

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7. **CONCLUSIONS:** This study is scientifically sound and meets the guideline requirements for a static acute toxicity study using estuarine fish. The 96-hour LC_{50} of BTS 27919 for *Cyprinodon variegatus* was >102 mg a.i./l mean measured concentration which classifies BTS 27919 as practically non-toxic to *Cyprinodon variegatus*. The NOEC could not be determined since sublethal effects were noted at the concentration tested.

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

9. **BACKGROUND:** Data submitted to support conditional registration on refer

6)

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

11. **MATERIALS AND METHODS:**

A. **Test Animals:** Sheepshead minnow (*Cyprinodon variegatus*) were obtained from Aquatic BioSystems, Inc., Fort Collins, Colorado. The fish were acclimated to the dilution water (17 parts per thousand [ppt]) and the test temperature (22 ±1°C) for at least 42 hours prior to test initiation. No mortality was observed during the acclimation period. The fish were not fed during the 42 hours prior to test initiation.

The fish had a mean weight of 0.343 g and a mean length of 2.2 cm. During the study, the organism loading rate was 0.34 g/l. At the time of test initiation, the fish were approximately 106 days old.

B. **Test System:** The test was conducted under static conditions in 19-l covered glass fish tanks (39.4 x 24.5 x 20.2 cm) with a solution depth of 12.6 cm.

The test chambers were randomly positioned in a water bath and exposed to a photoperiod of 16 hours of light at an intensity of 130 foot candles (approximately 1109 lux) and 8 hours darkness. Gradual transitions from light to dark and dark to light were provided. The test chambers were not aerated during the study.

The dilution water was synthetic seawater which was prepared by dissolving Instant Ocean salts in filtered well water and adjusted to 18 ppt with deionized well water. At test initiation, the dilution water had a pH of 8.2 and a dissolved oxygen (DO) concentration 8.6 mg/l.

The primary stock solution was prepared in dimethylformamide (DMF) at a concentration of 200 mg/ml. A 5.0 ml aliquot of this stock was added to 10 l of dilution water in each of the 3 test chambers and the solutions were stirred for 24 hours.

C. **Dosage:** Ninety-six-hour static acute test. The only nominal concentration of BTS 27919 chosen for this study was 100 mg/l. In addition, a dilution water control and a solvent control (0.5 ml DMF/l) were included.

D. **Design:** Ten fish were randomly distributed in groups of two to each test chamber (3 vessels/treatment and control). The fish were not fed during the test.

Observations of mortality, and abnormal behavior and appearance were noted at 24, 48, 72, and 96 hours (±1 hour).

Salinity, DO concentrations, temperature, and pH in each test chamber were measured at test initiation, at 48 hours, and at test termination (±1 hour). Temperature in the water bath was continuously monitored.

The concentration of test material in all test replicates was determined on days 0 and 4 using gas chromatography.

E. **Statistics:** No statistical analysis was necessary for this study.

12. **REPORTED RESULTS:** Mean measured concentrations of BTS 27919 in the 3 test replicates were 98, 100, 109 mg a.i./l (Table 3, attached). There was no evidence of test material residue in the solvent control or dilution water control.

No mortality was observed in the controls or the test concentration (102 mg a.i./l mean measured concentration) (Table 1, attached). Since no mortality was observed, the 96-hour LC_{50} value for sheepshead minnow exposed to BTS 27919 was >102 mg a.i./l. Sublethal effects were noted in the test concentration (102 mg a.i./l), therefore the NOEC could not be determined.

During the study, the pH was 7.7-8.3, the temperature in the test chambers was 21.0-21.9°C (21.6-23.0°C in the water bath), and the dissolved oxygen concentration was ≥5.6 mg/l. The salinity ranged from 18 to 19 ppt.

13. **STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:** "Since there was no mortality evident during the course of this study, BTS 27919 may be considered relatively non-toxic under the conditions of this test."

A GLP Compliance Statement, signed by the study director and representatives of the sponsor company, was included in the report indicating that this study was conducted in accordance with Good Laboratory Practice (40 CFR Part 160). A Quality Assurance Statement was included in the report and

was signed by an auditor/inspector of the GLP quality assurance unit of the performing laboratory.

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

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

Fish were acclimated to the dilution water and to the test temperature for a period of 42 hours; a minimum of 48 hours for acclimation to test conditions is required.

The report does not indicate whether the fish were held for at least 7 days for observation as recommended in the SEP.

The length range of the fish was not reported. The length of the longest fish should not be more than twice that of the shortest fish.

The report states that the test solutions were stirred for 24-hours prior to test initiation. The SEP recommends that the organisms be added to the test chambers within 30 minutes of the addition of the test material.

The test solution volume (10 l) was lower than recommended (15 l).

On page 5 of the report, the date of study initiation is given as 22 October 1991. On the title page, the date of test termination is given as 01 July 1991. The actual test dates are given as 3-7 December 1990. This is a discrepancy in the report.

B. **Statistical Analysis:** Since no mortality was observed, no statistical analysis was necessary.

C. **Discussion/Results:** The deviations listed above probably did not affect the results of this test. This study is scientifically sound and meets the guideline requirements for a static acute toxicity test using estuarine fish. The 96-hour LC_{50} of BTS 27919 to *Cyprinodon variegatus* was >102 mg a.i./l mean measured concentration. Based on the results of this study, BTS 27919 is practically non-toxic to *Cyprinodon variegatus*. The NOEC could not be determined since

sublethal effects were noted at the concentration tested.

D. **Adequacy of the Study:**

(1) **Classification:** Core.

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

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

15. **COMPLETION OF ONE-LINER FOR STUDY:** Yes, January 17, 1992.

TABLE 1: STUDY 508L OBSERVATION DATA

Day: 0	Date: 03 DEC 1990						Time: 1230		
Nominal Concentration (mg/l)	Control (0)			Solvent Control (0)			100		
Replicate	1	2	3	1	2	3	1	2	3
# Organisms added	10	10	10	10	10	10	10	10	10

Day: 1	Date: 04 DEC 1990						Time: 1300		
Nominal Concentration (mg/l)	Control (0)			Solvent Control (0)			100		
Replicate	1	2	3	1	2	3	1	2	3
Observation									
Mortality	0	0	0	0	0	0	0	0	0
Lethargic	0	0	0	0	0	0	10	10	10
Surfacing	0	0	0	0	0	0	3	2	9
Loss of Equilibrium	0	0	0	0	0	0	6	3	6
Normal	10	10	10	10	10	10	0	0	0

Day: 2	Date: 05 DEC 1990						Time: 1240		
Nominal Concentration (mg/l)	Control (0)			Solvent Control (0)			100		
Replicate	1	2	3	1	2	3	1	2	3
Observation									
Mortality	0	0	0	0	0	0	0	0	0
Lethargic	0	0	0	0	0	0	10	10	10
Surfacing	0	0	0	0	0	0	2	1	5
Loss of Equilibrium	0	0	0	0	0	0	1	0	1
Normal	10	10	10	10	10	10	0	0	0

TABLE 1: STUDY 508L OBSERVATION DATA

Day: 3	Date: 06 DEC 1990						Time: 1320		
Nominal Concentration (mg/l)	Control (0)			Solvent Control (0)			100		
Replicate	1	2	3	1	2	3	1	2	3
Observation									
Mortality	0	0	0	0	0	0	0	0	0
Lethargic	0	0	0	0	0	0	10	10	10
Surfacing	0	0	0	0	0	0	2	0	5
Loss of Equilibrium	0	0	0	0	0	0	3	2	3
Normal	10	10	10	10	10	10	0	0	0

Day: 4	Date: 07 DEC 1990						Time: 1232		
Nominal Concentration (mg/l)	Control (0)			Solvent Control (0)			100		
Replicate	1	2	3	1	2	3	1	2	3
Observation									
Mortality	0	0	0	0	0	0	0	0	0
Lethargic	0	0	0	0	0	0	10	10	10
Surfacing	0	0	0	0	0	0	1	0	4
Loss of Equilibrium	0	0	0	0	0	0	3	1	4
Normal	10	10	10	10	10	10	0	0	0

TABLE 3: NOMINAL AND MEASURED EXPOSURE CONCENTRATIONS

Nominal concentration (mg/l) Treatment-replicate	Measured concentrations (mg/l) 0 hour	Measured concentrations (mg/l) 96 hours	Study Mean measured concentration (mg/l)
Control - 1	ND	ND	ND
Control - 2	ND	ND	ND
Control - 3	ND	ND	ND
Solvent Control - 1	ND	ND	ND
Solvent Control - 2	ND	ND	ND
Solvent Control - 3	ND	ND	ND
100 - 1	100	96	98
100 - 2	100	100	100
100 - 3	115	103	109

ND = Not Detected