

DATA EVALUATION RECORD

1. **CHEMICAL:** Amitraz.
Shaughnessey No. 106201.
2. **TEST MATERIAL:** BTS 27919; N-(2,4-dimethylphenyl) formamide;
Batch No. CR19546/1; 99.8% active ingredient; a white powder.
3. **STUDY TYPE:** Estuarine Shrimp Static Acute Toxicity Test.
Species Tested: Mysid Shrimp (*Mysidopsis bahia*).
4. **CITATION:** Schupner, J.K. and B.J. Stachura. 1991. The Static Acute Toxicity of BTS 27919 to the Mysid Shrimp, *Mysidopsis bahia*. Laboratory Project ID. 503L. Prepared and Submitted by Nor-Am Chemical Company, Pikeville, NC. EPA MRID No. 421246-13.
5. **REVIEWED BY:**

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| Louis M. Rifici, M.S. Associate Scientist KBN Engineering and Applied Sciences, Inc. | Signature: <i>Louis M. Rifici</i> Date: <i>1/28/92</i> |
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6. **APPROVED BY:**

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| Rosemary Graham Mora, M.S. Associate Scientist KBN Engineering and Applied Sciences, Inc. | Signature: <i>Rosemary Graham Mora</i> Date: <i>1/28/92</i> |
| Henry T. Craven, M.S. Supervisor, EEB/EFED USEPA | Signature: <i>Henry T. Craven</i> Date: <i>3/9/92</i> |
7. **CONCLUSIONS:** This study is scientifically sound but does not meet the guideline requirements for a static-acute saltwater shrimp toxicity study. The mysids used in the test were much older than recommended. The 96-hour LC_{50} of 25.2 mg/l (based on mean measured concentrations) classifies BTS 27919 as slightly toxic to mysid shrimp. The NOEC, based on the lack of mortality and sublethal effects, was 8.2 mg/l (mean measured concentration).
8. **RECOMMENDATIONS:** N/A.
9. **BACKGROUND:** Data submitted to support conditional registration on cotton.

10. DISCUSSION OF INDIVIDUAL TESTS: N/A.

11. MATERIALS AND METHODS:

A. Test Animals: Juvenile mysid shrimp (*Mysidopsis bahia*) were obtained from a commercial supplier in Fort Collins, CO, and were 4 days old at test initiation. They were acclimated to the test conditions (i.e., 22 \pm 1°C, salinity of 20 parts per thousand [ppt]) for at least 46 hours prior to testing. There was no mortality during acclimation. The mysids were fed live brine shrimp nauplii during acclimation and testing.

B. Test System: The test was conducted in covered, 1-l glass beakers containing 800 ml of test solution. The test solution depth was approximately 10 cm. Light was provided at an intensity of 111.5 ft-candles using cool-white fluorescent tubes on a 16-hour light/8-hour dark photoperiod. Short dawn and dusk simulation periods were used. A water bath was used to maintain temperature. No aeration was used.

Synthetic seawater, prepared using commercially-prepared sea salts and well water, was used as dilution water. The salinity was adjusted to 21 ppt with deionized water.

A stock solution (130 mg BTS 27919/ml) was prepared in dimethylformamide (DMF). An aliquot (1.0 ml) of the stock was diluted to 2 l in dilution water, giving a final concentration of 65 mg/l, and stirred for 24 hours. The resulting solution was mixed with appropriate amounts of dilution water to achieve the lower nominal test concentrations.

C. Dosage: Ninety-six-hour static test. Five nominal concentrations (8, 14, 23, 39, and 65 mg/l), a solvent control (0.5 ml DMF/l), and a dilution water control were used.

D. Design: Mysid shrimp were randomly assigned to randomly positioned test beakers. The mysids were distributed in groups of two until each beaker contained 10 mysids.

The mysids were fed live brine shrimp nauplii twice daily.

Observations of mortality and sublethal responses were made every 24 hours. The temperature of the water bath

was monitored continuously with a data acquisition system and probe. The temperature, salinity, dissolved oxygen, and pH of the test solutions were measured at 0, 48, and 96 hours.

Water samples of all treatments were taken at test initiation and termination. The concentration of BTS 27919 was determined using liquid chromatography.

E. Statistics: The median lethal concentration (LC_{50}) and associated 95% confidence interval (C.I.) were calculated using a computer program developed by Stephan (1985).

12. **REPORTED RESULTS:** The mean measured concentrations were 8.1, 14, 22, 37, and 61 mg/l (Table 3, attached). These values were 94 to 103% of nominal concentrations.

The responses of mysids are presented in Table 1 (attached). The 96-hour LC_{50} , based on mean measured concentrations, was 25 mg/l (95% C.I. = 20-33 mg/l). The slope of the concentration-response curve was 1.1. Lethal and sublethal responses were observed at concentrations above 8.2 mg/l.

Dissolved oxygen ranged from 5.1 to 7.9 mg/l or 59 to 91% of saturation. The pH values ranged from 7.7 to 8.3. The temperature, as recorded by the a data logger, was 20.8-23.0°C. The salinity was 21 ppt.

13. **STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:**
The authors presented no conclusions.

Quality Assurance and good laboratory practice statements were included in the report, indicating that the study was conducted in accordance with FIFRA Good Laboratory Practice Standards set forth in 40 CFR Part 160.

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

A. Test Procedure: The test procedures were generally in accordance with protocols recommended by the SEP, but deviated as follows:

The mysids used in this test were 4 days old at test initiation. An amendment to the SEP states that acute mysid tests must be initiated with ≤ 24 hour old mysids.

The authors did not report a no-observed-effects concentration (NOEC) for the test.

The salinity of the dilution water in the study was 21 ppt with a pH of 8.2. The recommended salinity and pH for estuarine shrimp tests are 10-17 ppt and 7.7-8.0, respectively.

- B. Statistical Analysis: The reviewer used EPA's Toxanal program to calculate the LC_{50} value and obtained the same results except for the slope of the concentration-response curve which was 4.2 (see attached printout).
- C. Discussion/Results: This study is scientifically sound but does not meet the guideline requirements for a static-acute saltwater shrimp toxicity study. The mysids used in the test were much older than recommended. The 96-hour LC_{50} of 25.2 mg/l (based on mean measured concentrations) classifies BTS 27919 as slightly toxic to mysid shrimp. The NOEC, based on the lack of mortality and sublethal effects, was 8.2 mg/l (mean measured concentration).
- D. Adequacy of the Study:
 - (1) Classification: Supplemental.
 - (2) Rationale: The mysids used in the test were much older than recommended.
 - (3) Repairability: No.

15. COMPLETION OF ONE-LINER FOR STUDY: Yes, 01-15-92.

TABLE 1: STUDY OBSERVATION DATA

| | | | | | | | |
|------------------------------|------------------------------|-----------------|-----|----|----|----|----|
| Day: 0 | Date: 29 Oct 1990 Time: 1405 | | | | | | |
| Nominal Concentration (mg/L) | Control | Solvent Control | 8.0 | 14 | 23 | 39 | 65 |
| Number of organisms | 10 | 10 | 10 | 10 | 10 | 10 | 10 |
| Day: 1 | Date: 30 Oct 1990 Time: 1430 | | | | | | |
| Nominal Concentration (mg/L) | Control | Solvent Control | 8.0 | 14 | 23 | 39 | 65 |
| Daily mortality | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Normal | 10 | 10 | 10 | 10 | 10 | 10 | 10 |
| Day: 2 | Date: 31 Oct 1990 Time: 1430 | | | | | | |
| Nominal Concentration (mg/L) | Control | Solvent Control | 8.0 | 14 | 23 | 39 | 65 |
| Daily mortality | 0 | 0 | 0 | 0 | 3 | 2 | 5 |
| Cumulative mortality | 0 | 0 | 0 | 0 | 3 | 2 | 5 |
| Lethargic | 0 | 0 | 0 | 0 | 0 | 0 | 5 |
| Normal | 10 | 10 | 10 | 10 | 7 | 8 | 0 |
| Day: 3 | Date: 01 Nov 1990 Time: 1414 | | | | | | |
| Nominal Concentration (mg/L) | Control | Solvent Control | 8.0 | 14 | 23 | 39 | 65 |
| Daily mortality | 0 | 0 | 0 | 1 | 0 | 3 | 5 |
| Cumulative mortality | 0 | 0 | 0 | 1 | 3 | 5 | 10 |
| Lethargic | 0 | 0 | 0 | 1 | 0 | 5 | 0 |
| Normal | 10 | 10 | 10 | 8 | 7 | 0 | 0 |

BLE 1: STUDY OBSERVATION DATA (continued)

| | | | | | | | |
|------------------------------|------------------------------|-----------------|-----|----|----|----|----|
| Day: 4 | Date: 02 Nov 1990 Time: 1430 | | | | | | |
| Nominal Concentration (mg/L) | Control | Solvent Control | 8.0 | 14 | 23 | 39 | 65 |
| Daily mortality | 0 | 0 | 0 | 1 | 1 | 1 | 0 |
| Cumulative mortality | 0 | 0 | 0 | 2 | 4 | 6 | 10 |
| Lethargic | 0 | 0 | 0 | 0 | 0 | 4 | 0 |
| Normal | 10 | 10 | 10 | 8 | 6 | 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 | <0.15 | <0.15 |
| Solvent Control - 1 | ND | ND | ND |
| 8.0 | 8.1 | 8.3 | 8.2 |
| 14 | 14 | 14 | 14 |
| 23 | 23 | 22 | 22 |
| 39 | 38 | 36 | 37 |
| 65 | 64 | 58 | 61 |

1 values have been adjusted for the mean recovery of 98%.
ND = Not Detected.

RIFICI AMITRAZ MYSIDOPSIS BAHIA 1-15-91

| CONC. | NUMBER EXPOSED | NUMBER DEAD | PERCENT DEAD | BINOMIAL PROB. (PERCENT) |
|-------|-------------------|----------------|-----------------|-----------------------------|
| 61 | 10 | 10 | 100 | 9.765625E-02 |
| 37 | 10 | 6 | 60.00001 | 37.69531 |
| 22 | 10 | 4 | 40 | 37.69531 |
| 14 | 10 | 2 | 20 | 5.46875 |
| 8.2 | 10 | 0 | 0 | 9.765625E-02 |

THE BINOMIAL TEST SHOWS THAT 8.2 AND 61 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 28.53069

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

| SPAN | G | LC50 | 95 PERCENT CONFIDENCE LIMITS |
|------|----------|----------|------------------------------|
| 4 | .1144045 | 25.21269 | 20.07892 32.61566 |

RESULTS CALCULATED USING THE PROBIT METHOD

| ITERATIONS | G | H | GOODNESS OF FIT PROBABILITY |
|------------|----------|---|-----------------------------|
| 5 | .2054148 | 1 | .5216005 |

SLOPE = 4.153368
95 PERCENT CONFIDENCE LIMITS = 2.270949 AND 6.035787

LC50 = 25.93941
95 PERCENT CONFIDENCE LIMITS = 19.82552 AND 34.55902

LC10 = 12.82875
95 PERCENT CONFIDENCE LIMITS = 6.733517 AND 17.29398

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