DATA EVALUATION RECORD

1. **CHEMICAL:** Amitraz.
Shaughnessey No. 106201.

2. **TEST MATERIAL:** BTS 27271-HCl (SN49844 Technical); N-(2,4-dimethylphenyl)-N'-methylmethanimidamide hydrochloride (CA); Batch No. CR19235/1; 100% active ingredient (81.65% as BTS 27271 base); a white powder.

3. **STUDY TYPE:** Estuarine Shrimp Flow-Through Acute Toxicity Test. Species Tested: Mysid Shrimp (*Mysidopsis bahia*).

4. **CITATION:** Ward, G.S. 1991. BTS 27271: Acute Toxicity to the Mysid, *Mysidopsis bahia*, Under Flow-Through Test Conditions. Laboratory Project ID. J9011002e. NOR-AM Study No. 514L. Prepared by Toxikon Environmental Sciences, Jupiter, FL. Submitted by NOR-AM Chemical Company, Pikeville, NC. EPA MRID No. 421246-12.

5. **REVIEWED BY:**

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6. **APPROVED BY:**

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Tracy R. Perry 2/18/92

7. **CONCLUSIONS:** This study is scientifically sound and meets the guideline requirements for a flow-through acute saltwater shrimp toxicity study. The 96-hour LC_{50} of 4.7 mg BTS 27271 base/l (5.81 mg/l as BTS 27271-HCl), based on mean measured concentrations, classifies BTS 27271 base as moderately toxic to mysid shrimp. The NOEC, based on the lack of mortality and sublethal effects, was 1.47 mg BTS 27271 base/l (1.80 mg/l as BTS 27271-HCl) 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:** Post-larval mysids (*Mysidopsis bahia*), ≤24 hours old, were obtained from in-house cultures. The mysids were fed live brine shrimp nauplii. No diseases were observed in the test mysids or in the adult culture population.

B. **Test System:** The test was conducted using a proportional vacuum-siphon diluter system with a dilution factor of approximately 60%. The test chambers were 24-l glass tanks (40 x 29.5 x 20 cm). The test solution depth was 13 cm and the solution volume was approximately 15 l. The mysids were held in screened retention chambers within each test chamber.

The diluter delivered 1400 ml of test solution to each vessel per cycle (3.3 cycles per hour) for a total of 7.4 volume replacements per day. The test chambers were randomly positioned in a water bath under a 16-hour light/8-hour dark photoperiod with 15-minute dawn and dusk simulations. Light intensity during the test was 333-383 lux.

Natural filtered seawater adjusted to a salinity of 20 parts per thousand (ppt) was used as test dilution water. At test initiation, the pH of the dilution water was 8.2. The water was vigorously aerated prior to use.

A stock solution (100,000 mg/l) was prepared by adding 10 g of BTS 27271-HCl to deionized water in a 0.1 l volumetric flask. The stock solution was pumped into the diluter mixing chamber providing a high nominal test concentration of 12 mg/l. The mixing chamber solution was proportionally diluted to provide the lower test concentrations.

C. **Dosage:** Ninety-six-hour flow-through test. Based on the results of preliminary tests, five nominal concentrations (1.3, 2.1, 3.5, 5.8, and 9.7 mg BTS 27271 base/l) and a dilution water control were selected.

D. **Design:** Mysids were impartially added, by twos, to 30-ml plastic cups until 20 mysids were distributed to

each. The mysids were then randomly distributed by cup to the retention chambers.

The mysids were fed live brine shrimp nauplii daily.

Observations of mortality and sublethal responses were made every 24 hours. Dead mysids were removed at each observation period. The temperature of the water bath was monitored continuously with a minimum/maximum thermometer. The salinity, dissolved oxygen, and pH of the test solutions were measured in each chamber daily. The temperature of the control chamber was measured hourly with a recording thermometer.

BTS 27271 concentrations were measured by gas chromatography from samples collected at test initiation and termination.

E. **Statistics:** The median lethal concentration (LC_{50}) and associated 95% confidence interval (C.I.) for each 24-hour interval were calculated using a computer program developed by Wheat (1989).

12. **REPORTED RESULTS:** No undissolved test material was observed in any test chamber. The mean measured concentrations were 0.7, 1.47, 2.46, 3.69, and 7.46 mg BTS 27271 base/l (Table 1, attached). These values were 54-77% of nominal concentrations.

The responses of mysid shrimp are given in Table 2 (attached). The 96-hour LC_{50} based on mean measured concentrations was 4.74 mg BTS 27271 base/l (95% C.I. = 4.05-5.74 mg BTS 27271 base/l). The slope of the concentration-response curve was 6.01. Lethal and sublethal responses were observed at concentrations above 1.47 mg/l. The no-observed-effect concentration (NOEC) was given as 1.47 mg/l.

Dissolved oxygen ranged from 7.0 to 7.6 mg/l or 92 to 100% of saturation at 22°C and 20 ppt. The pH values ranged from 8.2 to 8.3. The temperature, as recorded by a data logger, was 19.8-23.6°C. The salinity was 19-20 ppt.

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

Quality assurance and good laboratory practice statements were included in the report, indicating that the study was conducted in accordance with U.S. EPA Good Laboratory Practice Standards set forth in FIFRA 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 recommended temperature for mysid shrimp toxicity tests is 22 ±1°C. The temperature in the study was 19.8-23.6°C.

The salinity of the dilution water in the study was 20 ppt with a pH of 8.2-8.3. The recommended salinity and pH for estuarine shrimp tests are 10-17 ppt and 7.7-8.0, respectively.

The culture conditions used were not described in the report. The SEP states that the test organisms and/or parental stock must be maintained under the actual test conditions for at least 48 hours before test initiation.

On page 11 of the report, the author states that the diluter delivered "equal solvent concentrations in all test concentrations". No solvent was used in this study. This is a discrepancy in the report.

B. **Statistical Analysis:** The reviewer used EPA's Toxanal program to calculate the LC_{50} value and obtained a 96-hour LC_{50} of 4.7 mg/l with 95% confidence limits of 4.0 and 5.5 mg/l (see attached printout) using the moving average method.

C. **Discussion/Results:** The test material was the hydrochloride salt of BTS 27271. The author states that BTS 27271 is a strong base (pKa = 9.32), exists in the ionized form in solution, and that no differences in toxicity will be observed between the hydrochloride form and the basic form. The concentrations and endpoints in this test are therefore related as the basic form (mg BTS 27271/l) which constitutes 81.65% of the total test material.

The test temperature ranged from 19.8 to 23.6°C. The greatest temperature change during the test was 3.6°C on day 3. In the reviewer's opinion, the temperature changes probably did not affect the results of the test. The limit of detection for the test material was given in Table 1 as 1.0 mg/l. However, the measured concentrations for the lowest test level were reported as 0.91 and 0.82 mg/l. This discrepancy does not

appear to have had any effect on the outcome of the test.

This study is scientifically sound and meets the guideline requirements for a flow-through acute saltwater shrimp toxicity study. The 96-hour LC_{50} of 4.7 mg BTS 27271/1 (5.81 mg/l as BTS 27271-HCl), based on mean measured concentrations, classifies BTS 27271 as moderately toxic to mysid shrimp. The NOEC, based on the lack of mortality and sublethal effects, was 1.47 mg BTS 27271/1 (1.80 mg/l as BTS 27271-HCl), mean measured concentration.

D. **Adequacy of the Study:**

(1) **Classification:** Core.

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

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

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

Table 1. Measured Concentrations of BTS 27271 During a 96-Hour Exposure of Mysid, Mysidopsis bahia, Under Flow-Through Conditions

Nominal Concentration (mg/L; ppm)		***Measured BTS 27271-HCl Conc. (mg/L)				Conc. Expressed as BTS 27271	Percent of
HCL*	Base**	0 Hr	96 hr	Mean	(±SD)	Base (mg/L)	Nominal
Control	Control	ND	ND	----		-------	---
1.6	1.3	0.91	0.82	0.86	(0.06)	0.70	54
2.1	2.1	1.55	2.06	1.80	(0.36)	1.47	70
4.3	3.5	3.29	2.73	3.01	(0.40)	2.46	70
7.2	5.8	5.07	3.98	4.52	(0.77)	3.69	64
12	9.7	8.90	9.37	9.14	(0.33)	7.46	77
100,000 (stock)	81,560	120,600	120,100	120,350	(354)	98,266	120
		MATRIX SPIKE RECOVERY DATA					
MS-1		4.65	4.6	4.94	(0.52)		97
MS-2		4.80	5.70				

*BTS 27271 HCl

**BTS 27271 Base

***Results are expressed as BTS 27271-HCl. Multiply by 0.8165 to express as BTS 27271 concentration. Reported values are uncorrected for analytical recovery.

SD = Standard Deviation.

ND = Not detected; the limit of detection for the method was 1.0 mg/L.

MS = Matrix spike. Matrix spikes consisted of test substance in dilution water and were duplicated. The spike concentration was 5.10 mg/L.

Table 2. Mortality of Mysid, Mysidopsis bahia, Exposed to BTS 27271 under Flow-Through Test Conditions

Mean Measured Concentration (mg/L; ppm)		Cumulative Number Dead (Percent Mortality)							
		24 Hour		48 Hour		72 Hour		96 Hour	
HCl*	Base**								
Control		0	(0)	0	(0)	0	(0)	0	(0)
0.86	0.70	0	(0)	0	(0)	0	(0)	0	(0)
1.80	1.47	0	(0)	0	(0)	0	(0)	0	(0)
3.01	2.46	0	(0)	0	(0)[c]	0	(0)[f]	0	(0)[i]
4.52	3.69	0	(0)[a]	3	(15)[d]	6	(30)[g]	7	(35)[j]
9.14	7.46	7	(35)[b]	11	(55)[e]	11	(55)[h]	17	(85)[k]

*BTS 27271-HCl **BTS 27271 base

[a] Two mysids were lethargic.
[b] Seven mysids were lethargic.
[c] Three mysids were erratic.
[d] Seventeen mysids exhibited a partial loss of equilibrium and were gyrating.
[e] Nine mysids exhibited a partial loss of equilibriu and were gyrating.
[f] Three mysids were gyrating and one was also erratic.
[g] Six mysids were gyrating and five exhibited a partial loss of equilibrium.
[h] Six mysids were gyrating and three were also exhibiting a partial loss of equilibrium.
[i] Two mysids were gyrating.
[j] Thirteen mysids were lethargic and of that number five were gyrating.
[k] Three mysids were gyrating.

RIFICI AMITRAZ MYSIDOPSIS BAHIA 1-15-92

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CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
7.46	20	17	85	.1288414
3.69	20	7	35	13.1588
2.46	20	0	0	9.536742E-05
1.47	20	0	0	9.536742E-05
.7	20	0	0	9.536742E-05

THE BINOMIAL TEST SHOWS THAT 2.46 AND 7.46 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 4.506477

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS	
2	8.643136E-02	4.664589	4.0498	5.537598

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
6	.137588	1	.5627755

SLOPE = 6.016492

95 PERCENT CONFIDENCE LIMITS = 3.784803 AND 8.248181

LC50 = 4.743966

95 PERCENT CONFIDENCE LIMITS = 4.046919 AND 5.742267

LC10 = 2.917797

95 PERCENT CONFIDENCE LIMITS = 2.164456 AND 3.478028

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