DATA EVALUATION RECORD

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

2. **TEST MATERIAL:** BTS 27271-HCl (SN49844. Technical); [N-(2,4-dimethylphenyl)-N'-methylmethanimidamide hydrochloride (CA)]; Lot No. 19621/01/91303; 99.6% active ingredient; a white powder.

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

4. **CITATION:** Ward, G.S. 1991. BTS 27271: Acute Toxicity to the Sheepshead Minnow, *Cyprinodon variegatus*, Under Flow-Through Test Conditions. NOR-AM Study No. 516L. Study performed by Toxikon Environmental Sciences, Jupiter, Florida. Submitted by NOR-AM Chemical Company, Pikeville, North Carolina. EPA MRID No. 421246-08.

5. **REVIEWED BY:**

Rosemary Graham Mora, M.S.
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Date: 1/28/92

6. **APPROVED BY:**

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Date: 3/8/92

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7. **CONCLUSIONS:** This study is scientifically sound and meets the guideline requirements for a flow-through acute toxicity study using estuarine fish. The 96-hour LC_{50} of BTS 27271-HCl for *Cyprinodon variegatus* was 11.5 mg a.i./l (8.9 mg/l as BTS 27271 base) measured concentration which classifies BTS 27271-HCl as slightly toxic to *Cyprinodon variegatus*. The NOEC was 2.36 mg a.i./l (1.93 mg/l BTS 27271 base) 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:** Sheepshead minnow (*Cyprinodon variegatus*) were obtained from S P Inc., Salem, Massachusetts, and held for approximately 2 months in the laboratory in natural seawater. For 7 days prior to test initiation, the fish were maintained at a temperature of 25.8-27.6°C and a salinity of 18-19 parts per thousand (ppt). No diseases were observed during the holding period and less than 1% mortality was observed in the 48 hours prior to test initiation. The fish were not fed during the 24 hours prior to test initiation.

The fish had a mean wet weight of 0.44 g (range of 0.22-0.76 g) and a mean standard length of 2.4 cm (range of 2.0-2.7 cm). At the time of test initiation, the fish were approximately 6-months old.

B. **Test System:** The test was conducted under flow-through conditions using a proportional vacuum-system diluter. The system was calibrated to provide a selected concentration series with 60% dilutions. A solution volume of 1400 ml/cycle was delivered to each test chamber. The cycle rate of the diluter was 4.2 cycles/hour providing 9.4 volume additions/day to each chamber.

The test chambers were 24-l glass tanks (40 x 29.5 x 20 cm) designed to maintain a maximum depth of 13 cm resulting in a volume capacity of approximately 15 l.

The test chambers were randomly positioned in a water bath and exposed to a photoperiod of 16 hours of light at an intensity of 342-525 lux and 8 hours darkness. A 15-minute transition period from light to dark and dark to light was provided. The test chambers were not aerated during the study.

The dilution water was natural filtered saltwater (20 ppt) which was vigorously aerated prior to use. At test initiation, the dilution water had a pH of 8.2 and a dissolved oxygen (DO) concentration 7.0 mg/l.

"BTS 27271 is a strong base with a pKa of 9.32 (20°C). It will, therefore, exist predominately in the ionized

form in the aquatic environment. The hydrochloride salt of BTS 27271 was, therefore, used as the test material because it is more stable in the solid form and easier to solubilize than BTS 27271 free base. When added to the test water, both the hydrochloride salt and the free base will protonate to yield exactly the same species in solution. No difference, therefore, in toxicity will be observed." On a molecular weight basis, BTS 27271 comprises 81.65% of the BTS 27271-HCl molecule.

The primary stock solution (100,000 mg a.i./l) was prepared by combining 10 g of BTS 27271-HCl with deionized water to a total volume of 100 ml. This primary stock solution was diluted in the chemical mixing chamber to provide the highest test concentration. Subsequent dilutions of this concentration in the diluter system provided the four lowest nominal test concentrations.

C. **Dosage:** Ninety-six-hour flow-through acute test. The five nominal concentrations of BTS 27271-HCl chosen for this study were 2.1, 3.5, 5.8, 9.6, and 16.0 mg a.i./l (for BTS 27271 base, 1.7, 2.8, 4.7, 7.8, and 13.0 mg/l). In addition, a dilution water control was included.

D. **Design:** Twenty fish were impartially distributed in groups of two to each test concentration and control (one vessel/treatment and control). The fish were not fed during the test.

Survival was noted daily during the study and dead fish removed. Behavioral and physical abnormalities were also noted.

Dissolved oxygen concentrations and pH were measured daily in all test solutions. Temperature in the water bath was continuously monitored and the minimum and maximum measurements were recorded. Temperature in the control chamber was measured hourly throughout the test.

The concentration of test material in the stock solution and at all test levels was determined on days -1, 0, and 4 using gas chromatography.

E. **Statistics:** The LC_{50} values were estimated by a computer program (Wheat, 1989) using the logit method.

12. **REPORTED RESULTS:** Mean measured concentrations of BTS 27271-HCl were 104,599 mg a.i./l in the stock solutions and 1.24, 2.36, 4.91, 7.64, and 15.4 mg a.i./l in the test concentrations. These measured concentrations represent 104% and 59-96% of nominal concentrations, respectively. Mean measured concentrations of BTS 27271 base were 1.01, 1.93, 4.01, 6.24, and 12.6 mg/l. There was no evidence of undissolved test material in the test vessels during the study and measured concentrations were fairly consistent throughout the test period.

No mortality was observed in the control or the three lowest test concentrations (1.24-4.91 mg a.i./l mean measured concentrations of BTS 27271-HCl) (Table 2, attached). Thirty-five percent mortality was observed at 7.64 mg a.i./l mean measured concentration and 65% mortality was observed at 15.4 mg a.i./l mean measured concentration. The 96-hour LC_{50} value and 95% confidence interval for sheepshead minnow exposed to BTS 27271-HCl were 13.2 and 9.19-22.0 mg a.i./l measured concentrations, respectively, and the slope was 4.9. The NOEC was 2.36 mg a.i./l mean measured concentration based on the lack of sublethal effects at that level. The 96-hour LC_{50} value and 95% confidence interval for sheepshead minnow exposed to BTS 27271 base were 10.8 and 7.5-18.0 mg/l measured concentrations, respectively. The NOEC for BTS 27271 base was 1.93 mg/l.

During the study, the pH was 8.2, the test temperature was 21.4-23.4°C, and the dissolved oxygen concentration was $\geq$5.8 mg/l. The salinity of the dilution water was 18-20 ppt.

13. **STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:** The author made no conclusions in the report.

A Statement of GLP Compliance, 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 U.S. EPA GLP Regulations (40 CFR Part 160). A Statement of Quality Assurance was included in the report and was signed by the quality assurance manager 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 protocols recommended by the guidelines, except for the following deviations:

Fish were maintained at a salinity of 18-19 ppt and a temperature of 25.8-27.6°C for a period of 7 days; a minimum of 48 hours for acclimation to test conditions is required. Test salinity and temperature were 18-20 ppt and 21-23°C, respectively.

B. **Statistical Analysis:** EPA's Toxanal computer program was used to verify the LC_{50} value and 95% confidence interval presented by the author. The 96-hour LC_{50} for BTS 27271-HCl (11.5 mg a.i./l measured concentration) and 95% confidence interval (9.4-15.6 mg a.i./l) obtained by the probit method were slightly lower than those of the author (printout, attached). The 96-hour LC_{50} and 95% confidence interval for BTS 27271 base was 8.9 and 7.3-11.8 mg/l measured concentrations, respectively.

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 flow-through toxicity study using estuarine fish. The 96-hour LC_{50} of BTS 27271-HCl to *Cyprinodon variegatus* was 11.5 mg a.i./l (8.9 mg/l as BTS 27271 base) measured concentration. Based on the results of this study, BTS 27271-HCl is slightly toxic to *Cyprinodon variegatus*. The NOEC was 2.36 mg a.i./l (1.93 mg/l as BTS 27271 base) 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, January 16, 1992.

Table 2. Mortality of Sheepshead Minnow, Cyprinodon variegatus, Exposed to BTS 27271 under Flow-Through Test Conditions

Mean Measured Concentration (mg/L; ppm) HCl*	Base**	Cumulative Number Dead (Percent Mortality) 24 Hour		48 Hour		72 Hour		96 Hour	
Control		0	(0)	0	(0)	0	(0)	0	(0)
1.24	1.01	0	(0)	0	(0)	0	(0)	0	(0)
2.36	1.93	0	(0)	0	(0)	0	(0)	0	(0)
4.91	4.01	0	(0)	0	(0)	0	(0)	0	(0)[a]
7.64	6.24	0	(0)[e]	0	(0)[e]	0	(0)[e]	7	(35)[h]
15.4	12.6	0	(0)[b]	11	(55)[d]	12	(60)[f]	13	(65)[i]

*BTS 27271-HCl **BTS 27271 Base

[a] Ten fish exhibited a complete loss of equilibrium and were immobile; five fish were at the surface and five on the bottom.
[b] All twenty fish were on the bottom and immobile; only minor fin and opercula movements unless prodded.
[c] Nine fish were at the surface and lethargic to immobile; one fish observed on the bottom and immobile.
[d] Nine fish were immobile and five observed at the surface.
[e] Four fish exhibited a complete loss of equilibrium and six a partial loss of equibrium; ten were at the surface.
[f] Two fish were immobile at the surface and three immobile on the bottom.
[g] One fish was lethargic.
[h] All fish were lethargic; eleven were at the surface and four were immobile.
[i] Four fish were immobile at the surface.

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CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB.(PERCENT)
15.4	20	13	65	13.1588
7.64	20	7	35	13.1588
4.91	20	0	0	9.536742E-05
2.36	20	0	0	9.536742E-05
1.24	20	0	0	9.536742E-05

THE BINOMIAL TEST SHOWS THAT 0 AND +INFINITY 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 10.84693

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS	
2	.1391905	10.84693	8.985963	14.36103

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
6	.1878445	1	.340671

SLOPE = 4.237495
95 PERCENT CONFIDENCE LIMITS = 2.400921 AND 6.074069

LC50 = 11.53243
95 PERCENT CONFIDENCE LIMITS = 9.397768 AND 15.60487

LC10 = 5.783829
95 PERCENT CONFIDENCE LIMITS = 3.67829 AND 7.287685

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CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
12.6	20	13	65	13.1588
6.24	20	7	35	13.1588
4.01	20	0	0	9.536742E-05
1.93	20	0	0	9.536742E-05
1.01	20	0	0	9.536742E-05

THE BINOMIAL TEST SHOWS THAT 0 AND +INFINITY 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 8.867015

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS	
2	.1391905	8.867018	7.343543	11.7449

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
6	.187578	1	.3393577

SLOPE = 4.229282
95 PERCENT CONFIDENCE LIMITS = 2.397568 AND 6.060996

LC50 = 9.429802
95 PERCENT CONFIDENCE LIMITS = 7.681469 AND 12.76694

LC10 = 4.72297
95 PERCENT CONFIDENCE LIMITS = 3.003 AND 5.952999

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