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. CR19621/01/910303; 99.6% active ingredient; a white powder.

3. **STUDY TYPE:** Mollusc 96-Hour, Flow-Through Shell Deposition Study. Species Tested: Eastern Oyster (Crassostrea virginica Gmelin).

4. **CITATION:** K.A. Carr and G.S. Ward. 1991. BTS 27271: Acute Effect on New Shell Growth of the Eastern Oyster, Crassostrea virginica. Laboratory Project ID No. J9011002g. Performed by Toxikon Environmental Sciences, Jupiter, Florida. Submitted by NOR-AM Chemical Company, Pikeville, North Carolina. EPA MRID No. 421246-10.

5. **REVIEWED BY:**

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

Signature: Rosemary Graham Mora
Date: 1/31/92

6. **APPROVED BY:**

Isabel C. Johnson, M.S.
Principal Scientist
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Signature: Isabel C. Johnson
Date: 1/31/92

Henry T. Craven, M.S.
Supervisor, EEB/EFED
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Signature: Henry T. Craven
Date: 3/9/92

7. **CONCLUSIONS:** This study is scientifically sound and does not fulfill the guideline requirements for a 96-hour flow-through mollusc shell deposition acute toxicity test, since the number of organisms used in each treatment was not the same and the flow rate was lower than recommended. Based on the conditions of the study, the 96-hour EC_{50} value for eastern oysters was 13.1 mg a.i./l measured concentration for BTS 27271-HCl (10.7 mg a.i./l measured concentration for BTS 27271 base). Therefore, BTS 27271-HCl is classified as slightly toxic to Crassostrea virginica. The NOEC could not be determined, since new shell growth was affected at all test levels.

8. **RECOMMENDATIONS:**

9. **BACKGROUND:** Data submitted to support conditional registration action.

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

11. **MATERIALS AND METHODS:**

A. **Test Animals:** Eastern oysters (Crassostrea virginica Gmelin) were obtained from Aquacultural Research Corporation, Dennis, Massachusetts. Upon receipt at the laboratory, 2-5 mm of the shell of the oysters was removed. The oysters were maintained at 23.8-26.5°C in a holding tank of the test system for approximately 24 hours prior to test initiation. New shell growth and good physical condition were exhibited by the test oysters prior to test initiation. The oysters had a mean umbo to distal valve edge length of 23.6 ±1.6 mm.

B. **Test System:** The test was conducted in a continuous flow diluter system. For test solution preparation, stock solutions were continuously delivered to mixing chambers which were positioned below the dilution water head box. Test concentrations were mixed constantly by magnetic stirrers. This system provided a flow rate of 400 ml/minute to each chamber providing 31 volume additions every 24 hours. The glass test aquaria (49.5 X 29 X 20 cm) were each equipped with a standpipe to regulate solution depth at 13 cm at a constant volume of 19 l. The minimum flow-through volume of test solution was approximately 1.2 l/oyster/hour.

Test aquaria (one aquarium per test concentration and the control) were positioned in a water bath to stabilize water temperature. The test was conducted under fluorescent lighting (intensity of approximately 400-475 lux) on a 16-hour light and 8-hour dark photoperiod. No aeration was used.

The dilution water was natural unfiltered seawater collected from the Jupiter River, Florida. The seawater had a pH of 7.7, and a salinity of 30-31 parts per thousand (ppt).

"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.

Stock solutions were prepared by combining the appropriate amount of test material to a volume of 100 ml with deionized water.

C. **Dosage:** 96-hour flow-through acute test. Based on a preliminary test, the nominal test concentrations of BTS 27271-HCl were 2.6, 4.3, 7.2, 12.0, and 20.0 mg a.i./l. A dilution water control was also included.

D. **Design:** Just prior to test initiation, oysters demonstrating new shell growth were selected for the study and the new shell growth of each oyster was removed. Each aquarium was impartially stocked with 20 oysters, except for the control and nominal concentrations of 7.2 and 20 mg a.i./l which were loaded with 25 oysters.

The survival of oysters and the test solutions were observed daily. The new shell growth of each oyster was measured at 96 hours.

Dissolved oxygen concentration and pH were measured daily in each aquarium. Temperature was monitored hourly in the control. Salinity was measured daily in the control.

Analytical determination of BTS 27271-HCl was performed on the low, middle, and high test levels on test day -1 and on the control and all test concentrations on days 0 and 4 using gas chromatography.

E. **Statistics:** The mean shell growth measurement of individual oysters for each of the five exposure concentrations were expressed as a percentage of the control oyster growth.

EC_{50} values (with 95 percent confidence limits) were determined by a computer program (Wheat, 1989) using the following methods: moving average angle, probit, logit, and non-linear interpolation. Analysis of variance (ANOVA) and Dunnett's procedure were used to determine the NOEC.

12. **REPORTED RESULTS:** Mean measured concentrations were 2.11, 3.84, 6.95, 9.83, and 18.4 mg a.i./l BTS 27271-HCl, based on analysis of test solutions at test initiation and test termination (Table 1, attached). The mean measured concentrations averaged 81-96% of nominal concentrations.

No mortality was observed in any treatment group or the control at test termination. Following 96 hours of exposure, shell growth at treatment levels 2.11-18.4 mg a.i./l was reduced by 29, 26, 25, 37, and 61%, respectively, when compared to the control (Table 3, attached). Mean shell growth range at these levels was 0.96-1.85 mm. The control oysters deposited a mean shell length of 2.47 mm. The 96-hour EC_{50} (95% confidence interval) was 13.1 (6.95-18.4) mg a.i./l BTS 27271-HCl measured concentration. New shell growth was significantly ($p < 0.05$) reduced at all test concentrations when compared to the control (Table 2, attached). Therefore, the NOEC could not be determined. Results expressed as BTS 27271 base are: 96-hour EC_{50} of 10.7 (5.67-15.0) mg a.i./l.

During the test period, the pH was 7.5-7.9, the dissolved oxygen concentration was $\geq$4.6 mg/l, the temperature was 23-27.3°C, and the salinity was 30-31 ppt.

13. **STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:** No conclusions, other than those presented above, were made by the author.

A Compliance with Good Laboratory Practice Standards statement was included in the report, indicating that the study was conducted in accordance with the EPA Good Laboratory Practice Standards. This statement was signed by of the study director and representatives of the study sponsor.

A Quality Assurance Statement was also included and was signed by the quality assurance manager of the performing laboratory. A list of protocol deviations was included (attached).

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

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

The same number of test organisms was not used in each treatment level and the control. Twenty-five oysters

were exposed in the control and 6.95 and 18.4 mg a.i./l mean measured concentrations. Twenty oysters were exposed in the remaining concentrations. The organism loading rate was different. The SEP requires that the same number of organisms be used at each test level.

In this study, the minimum flow rate of the test solution was 1.2 l/oyster/hour and no supplementary diet was provided. According to protocols recommended by the SEP (APHA, 1981 and Anonymous, 1976) each oyster should receive a minimum of 5 l of "once-through" flow through test solution per hour.

An NOEC was not determined by this study.

The length of the test organisms (mean=23.6 mm) was smaller than recommended (25-50 mm).

The test organisms were not acclimated and observed for 10 days and were not held at test conditions for 48 hours as required by the SEP. Oysters were tested within 24 hours of receipt at the laboratory.

A transition period between light/darkness and darkness/light were not mentioned in the report. Such transition periods are recommended by the SEP.

B. **Statistical Analysis:** The reviewer used EPA's Toxanal computer program to calculate the 96-hour EC_{50} value (printout, attached). The EC_{50} was 13.6 mg a.i./l with a 95% confidence interval of 10.9-17.4 mg a.i./l measured concentrations. These results are similar to those presented by the authors.

Multiple comparisons of means with the control were determined using ANOVA with Bonferroni's test. New shell growth was affected at all test levels, therefore the NOEC could not be determined. These results were the same as those of the authors.

C. **Discussion/Results:** The study is scientifically sound and does not meet the guideline requirements for a 96-hour mollusc shell deposition acute toxicity test. The deviations from test protocol (Section 14A) may have altered the test results. The number of oysters exposed at each test level was not the same. The flow of the test solutions to the test vessels was lower than recommended and no supplemental food was provided. The test organisms were not exposed to the same or

optimum test conditions (i.e., liters of solution/oyster/hour, stocking density, food availability). An NOEC was not determined by this study.

Although new shell deposition of control oysters met the minimum requirement (2 mm) by test termination, it is difficult to determine whether new shell deposition of control and test organisms would have been the same or improved at a lower stocking density (20/treatment) and increased flow rate (5 l/oyster/hour).

The 96-hour EC_{50} value was 13.1 mg a.i./l based on mean measured concentrations of BTS 27271-HCl (10.7 mg a.i./l for BTS 27271 base). Therefore, BTS 27271-HCl is classified as slightly toxic to Crassostrea virginica. The NOEC could not be determined since new shell growth was affected at all test levels when compared to the control.

D. **Adequacy of the Study:**

(1) **Classification:** Supplemental.

(2) **Rationale:** The same number of test organisms was not used in each treatment level and the control and no supplemental food was provided. The flow rate was lower than recommended. An NOEC was not determined.

(3) **Repairability:** No.

15. **COMPLETION OF ONE-LINER:** Yes, January 31, 1992.

ROSEMARY GRAHAM MORA AMITRAZ CRASSOSTREA VIRGINICA 1-22-92

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CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
18.4	100	61	61	0
9.83	100	38	38	0
6.95	100	25	25	0
3.84	100	26	26	0
2.11	100	29	29	0

BECAUSE THE NUMBER OF ORGANISMS USED WAS SO LARGE, THE 95 PERCENT CONFIDENCE INTERVALS CALCULATED FROM THE BINOMIAL PROBABILITY ARE UNRELIABLE. USE THE INTERVALS CALCULATED BY THE OTHER TESTS.

AN APPROXIMATE LC50 FOR THIS SET OF DATA IS 13.63674

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS	
1	.3620409	13.63674	10.84977	17.4116

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
3	2.077027	4.697461	2.781689E-03

SINCE THE PROBABILITY IS LESS THAN 0.05, RESULTS CALCULATED USING THE PROBIT METHOD PROBABLY SHOULD NOT BE USED.

SLOPE = .8744988

95 PERCENT CONFIDENCE LIMITS =-.3858196 AND 2.134817

LC50 = 17.08248

95 PERCENT CONFIDENCE LIMITS = 5.797232 AND +INFINITY

LC10 = .6029626

95 PERCENT CONFIDENCE LIMITS = 0 AND 2.953827

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TITLE: AMITRAZ: GROWTH OF EXPOSED EASTERN OYSTERS
FILE: B:42124610.OYS
TRANSFORM: NO TRANSFORMATION NUMBER OF GROUPS: 6

GRP	IDENTIFICATION	REP	VALUE	TRANS VALUE
1	CONTROL	1	1.5000	1.5000
1	CONTROL	2	2.6000	2.6000
1	CONTROL	3	2.0000	2.0000
1	CONTROL	4	2.3000	2.3000
1	CONTROL	5	1.6000	1.6000
1	CONTROL	6	1.6000	1.6000
1	CONTROL	7	6.0000	6.0000
1	CONTROL	8	3.0000	3.0000
1	CONTROL	9	3.7000	3.7000
1	CONTROL	10	3.5000	3.5000
1	CONTROL	11	1.2000	1.2000
1	CONTROL	12	1.8000	1.8000
1	CONTROL	13	5.1000	5.1000
1	CONTROL	14	2.8000	2.8000
1	CONTROL	15	1.6000	1.6000
1	CONTROL	16	2.1000	2.1000
1	CONTROL	17	2.2000	2.2000
1	CONTROL	18	2.4000	2.4000
1	CONTROL	19	2.7000	2.7000
1	CONTROL	20	2.7000	2.7000
1	CONTROL	21	1.2000	1.2000
1	CONTROL	22	2.0000	2.0000
1	CONTROL	23	2.0000	2.0000
1	CONTROL	24	2.4000	2.4000
1	CONTROL	25	1.8000	1.8000
2	2.11 mg/l	1	2.1000	2.1000
2	2.11 mg/l	2	1.6000	1.6000
2	2.11 mg/l	3	2.1000	2.1000
2	2.11 mg/l	4	0.0000	0.0000
2	2.11 mg/l	5	2.5000	2.5000
2	2.11 mg/l	6	2.0000	2.0000
2	2.11 mg/l	7	1.4000	1.4000
2	2.11 mg/l	8	1.4000	1.4000
2	2.11 mg/l	9	1.4000	1.4000
2	2.11 mg/l	10	2.9000	2.9000
2	2.11 mg/l	11	1.9000	1.9000
2	2.11 mg/l	12	2.0000	2.0000
2	2.11 mg/l	13	1.4000	1.4000
2	2.11 mg/l	14	1.5000	1.5000
2	2.11 mg/l	15	2.4000	2.4000
2	2.11 mg/l	16	1.8000	1.8000
2	2.11 mg/l	17	1.8000	1.8000
2	2.11 mg/l	18	1.2000	1.2000
2	2.11 mg/l	19	2.1000	2.1000
2	2.11 mg/l	20	1.4000	1.4000
3	3.84 mg/l	1	1.2000	1.2000
3	3.84 mg/l	2	1.1000	1.1000
3	3.84 mg/l	3	2.5000	2.5000
3	3.84 mg/l	4	2.1000	2.1000
3	3.84 mg/l	5	2.6000	2.6000

3	3.84 mg/l	6	1.0000	1.0000
3	3.84 mg/l	7	2.6000	2.6000
3	3.84 mg/l	8	2.0000	2.0000
3	3.84 mg/l	9	2.5000	2.5000
3	3.84 mg/l	10	2.2000	2.2000
3	3.84 mg/l	11	1.6000	1.6000
3	3.84 mg/l	12	2.6000	2.6000
3	3.84 mg/l	13	2.3000	2.3000
3	3.84 mg/l	14	2.0000	2.0000
3	3.84 mg/l	15	1.5000	1.5000
3	3.84 mg/l	16	0.0000	0.0000
3	3.84 mg/l	17	1.8000	1.8000
3	3.84 mg/l	18	1.0000	1.0000
3	3.84 mg/l	19	1.6000	1.6000
3	3.84 mg/l	20	2.5000	2.5000
4	6.95 mg/l	1	1.0000	1.0000
4	6.95 mg/l	2	1.0000	1.0000
4	6.95 mg/l	3	1.7000	1.7000
4	6.95 mg/l	4	1.6000	1.6000
4	6.95 mg/l	5	1.4000	1.4000
4	6.95 mg/l	6	1.8000	1.8000
4	6.95 mg/l	7	2.2000	2.2000
4	6.95 mg/l	8	2.5000	2.5000
4	6.95 mg/l	9	1.6000	1.6000
4	6.95 mg/l	10	1.8000	1.8000
4	6.95 mg/l	11	2.4000	2.4000
4	6.95 mg/l	12	2.0000	2.0000
4	6.95 mg/l	13	2.8000	2.8000
4	6.95 mg/l	14	2.6000	2.6000
4	6.95 mg/l	15	2.6000	2.6000
4	6.95 mg/l	16	1.8000	1.8000
4	6.95 mg/l	17	1.8000	1.8000
4	6.95 mg/l	18	2.3000	2.3000
4	6.95 mg/l	19	2.3000	2.3000
4	6.95 mg/l	20	1.8000	1.8000
4	6.95 mg/l	21	1.5000	1.5000
4	6.95 mg/l	22	0.9000	0.9000
4	6.95 mg/l	23	1.2000	1.2000
4	6.95 mg/l	24	1.6000	1.6000
4	6.95 mg/l	25	2.0000	2.0000
5	9.83 mg/l	1	1.6000	1.6000
5	9.83 mg/l	2	2.3000	2.3000
5	9.83 mg/l	3	2.2000	2.2000
5	9.83 mg/l	4	2.3000	2.3000
5	9.83 mg/l	5	0.6000	0.6000
5	9.83 mg/l	6	1.5000	1.5000
5	9.83 mg/l	7	1.8000	1.8000
5	9.83 mg/l	8	1.5000	1.5000
5	9.83 mg/l	9	1.4000	1.4000
5	9.83 mg/l	10	2.1000	2.1000
5	9.83 mg/l	11	1.0000	1.0000
5	9.83 mg/l	12	1.7000	1.7000
5	9.83 mg/l	13	1.7000	1.7000
5	9.83 mg/l	14	1.7000	1.7000
5	9.83 mg/l	15	2.6000	2.6000
5	9.83 mg/l	16	0.0000	0.0000
5	9.83 mg/l	17	0.8000	0.8000
5	9.83 mg/l	18	1.1000	1.1000
5	9.83 mg/l	19	1.6000	1.6000
5	9.83 mg/l	20	1.4000	1.4000

6	18.4 mg/L	1	0.9000	0.9000
6	18.4 mg/L	2	0.0000	0.0000
6	18.4 mg/L	3	0.0000	0.0000
6	18.4 mg/L	4	0.0000	0.0000
6	18.4 mg/L	5	0.9000	0.9000
6	18.4 mg/L	6	1.1000	1.1000
6	18.4 mg/L	7	2.5000	2.5000
6	18.4 mg/L	8	1.0000	1.0000
6	18.4 mg/L	9	1.0000	1.0000
6	18.4 mg/L	10	0.6000	0.6000
6	18.4 mg/L	11	1.4000	1.4000
6	18.4 mg/L	12	0.4000	0.4000
6	18.4 mg/L	13	1.0000	1.0000
6	18.4 mg/L	14	0.6000	0.6000
6	18.4 mg/L	15	1.5000	1.5000
6	18.4 mg/L	16	0.5000	0.5000
6	18.4 mg/L	17	1.5000	1.5000
6	18.4 mg/L	18	1.5000	1.5000
6	18.4 mg/L	19	1.3000	1.3000
6	18.4 mg/L	20	1.8000	1.8000
6	18.4 mg/L	21	0.4000	0.4000
6	18.4 mg/L	22	1.6000	1.6000
6	18.4 mg/L	23	1.1000	1.1000
6	18.4 mg/L	24	0.8000	0.8000
6	18.4 mg/L	25	0.5000	0.5000

AMITRAZ: GROWTH OF EXPOSED EASTERN OYSTERS
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ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	29.993	5.999	11.045
Within (Error)	129	70.059	0.543	
Total	134	100.052		

Critical F value = 2.29 (0.05,5,120)
Since F > Critical F REJECT Ho:All groups equal

AMITRAZ: GROWTH OF EXPOSED EASTERN OYSTERS
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BONFERRONI T-TEST - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	CONTROL	2.472	2.472		
2	2.11 mg/l	1.745	1.745	3.288	*
3	3.84 mg/l	1.835	1.835	2.881	*
4	6.95 mg/l	1.848	1.848	2.994	*
5	9.83 mg/l	1.545	1.545	4.193	*
6	18.4 mg/L	0.956	0.956	7.273	*

Bonferroni T table value = 2.36 (1 Tailed Value, P=0.05, df=120,5)

AMITRAZ: GROWTH OF EXPOSED EASTERN OYSTERS
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BONFERRONI T-TEST - TABLE 2 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	CONTROL	25			
2	2.11 mg/l	20	0.521	21.1	0.727
3	3.84 mg/l	20	0.521	21.1	0.637
4	6.95 mg/l	25	0.492	19.9	0.624
5	9.83 mg/l	20	0.521	21.1	0.927
6	18.4 mg/L	25	0.492	19.9	1.516