RS-1DF1

232581
RECORD NUMBER

106201
PESTICIDE CHEMICAL CODE

REVIEW NUMBER

ECOLOGICAL EFFECTS BRANCH REVIEW

DATE: IN 10-03-88 OUT 12/22/88

FILE OR REG. NO. 45639-EUP-27

PETITION OR EXP NO.

DATE OF SUBMISSION 8-28-88

DATE RECEIVED BY HED 9-30-88

RD REQUESTED COMPLETION DATE 12-19-88

EEB ESTIMATED COMPLETION DATE 12-19-88

RD ACTION CODE/TYPE OF REVIEW 757

TYPE PRODUCT(S): I, D, H, F, N, R, S Miticide

DATA ACCESSION NO(S). 407805-01 thru -11

PRODUCT MANAGER NO. Lois Rossi (21)

PRODUCT NAME(S) Amitraz

COMPANY NAME Nor-Am Chemical Company

SUBMISSION PURPOSE Submission of toxicity data in response to Registration Standard

| PESTICIDE CHEMICAL CODE | CHEMICAL AND FORMULATION | % A.I. |
|---|---|---|
| | | |
| | | |

1

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: 45639-EUP-27; Amitraz Registration Standard Data Submissions; EPA Acc. Nos. 407805-01 thru -11.

TO: Lois Rossi, PM 21
Fungicide-Herbicide Branch
Registration Division (TS-767C)

FROM: Jim Ackerman, Chief
Ecological Effects Branch
Environmental Fate and Effects Division (TS-769C) — 12/22/88

Nor-Am Chemical has submitted the above referenced data in response to the Amitraz Registration Standard. EEB has reviewed the toxicity studies and the review results are indicated below.

| Guide. Ref.No. | Test Species | % AI | Test Type | Test Results | Toxicity Category | Study Status |
|---|---|---|---|---|---|---|
| 71-2 | Bobwhite Quail | 98.2 | Dietary LC50 | 3081 ppm | Slightly Toxic | Core |
| 72-1 | Rainbow Trout | 20 | 96-hour LC50 | 2.2 mg/l | Moderately Toxic | Suppl. |
| 72-2 | Daphnia magna | 20 | 48-hour EC50 | 3.38mg/l | Moderately Toxic | Core |
| 72-3 | Sheepshead minnow | 98.5 | 96-hour LC50 | none | undetermined | Suppl. |
| 72-3 | Sheepshead minnow | 20 | 96-hour LC50 | 7.9mg/l | Moderately Toxic | Core |
| 72-3 | Mysid | 20 | 96-hour LC50 | 0.48mg/l | Highly Toxic | Core (40780510) |

2

| | | | | | | |
|---|---|---|---|---|---|---|
| 72-3 | Oyster | 20.0 | 96-hour shell deposit. | 85ug/l | Very Highly Toxic | Suppl. |
| 72-4 | Daphnia magna | 96.3 | Life Cycle | MATC= <0.2mg/l 0.02mg/l | ------ | Suppl. |

The attached Data Evaluation Records will provide the necessary information concerning the repairability of each study found to be presently insufficient for Guideline fulfillment purposes. The PM is advised to forward completed review results of environmental fate studies to EEB so that determinations can be made for those ecological studies held in reserved status under the Amitraz Registration Standard.

EEB notes that the formulation testing was conducted with W95 AMITRAZ 2EC. EEB does not have any information in its files to determine if this formulation is registered. If the PM determines that the formulation is not registered, the registrant must provide an explanation for the use of this particular formulation and also provide a confidental statement of formula to demostrate that the formulation is comparable to similiar registered products. If it is determined that the formulation is not comparable, the studies may have to be repeated using registered formulations that are routinely used.

John Noles

John Noles, Biologist
Ecological Effects Branch

12/21/88

Accession No. 407805-01

# DATA EVALUATION RECORD

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

2. **TEST MATERIAL:** W81 Technical Amitraz, 98.2% (analyzed) active ingredient, Batch No. CR 20575/3, a lumpy white powder.

3. **STUDY TYPE:** Avian Dietary LC50 Test.
Species Tested: Colinus virginianus.

4. **CITATION:** Roberts, N.L. and B. Hakin. 1988. W81 Technical Amitraz: Subacute Dietary Toxicity (LC50) to the Bobwhite Quail. Prepared by Huntingdon Research Centre Ltd., Cambridgeshire, England. Study No. TOX 87261. Submitted by NOR-AM Chemical Company, Wilmington, DE. EPA Accession No. 407805-01.

5. **REVIEWED BY:**

Prapimpan Kosalwat, Ph.D.
Staff Toxicologist
KBN Engineering and
Applied Sciences, Inc.

**Signature:** P. Kosalwat
**Date:** 12/1/88

6. **APPROVED BY:**

James R. Newman, Ph.D.
Project Manager/
Principal Scientist
KBN Engineering and
Applied Sciences, Inc.

**Signature:** James R Newman
**Date:** 12/1/88

for Henry T. Craven, M.S.
Supervisor, EEB/HED
USEPA

Henry T. Craven 12/29/88

**Signature:** John Noles
**Date:** 12/20/88

7. **CONCLUSIONS:** This study is scientifically sound and meets the guideline requirements for an avian dietary LC50 test. With an LC50 value of 3081 ppm, W81 Technical Amitraz is considered slightly toxic to Bobwhite quail (Colinus virginianus), when administered through diet for five days. The NOEL was determined to be less than 250 ppm.

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



4

9. BACKGROUND:

10. DISCUSSION OF INDIVIDUAL TESTS: N/A.

11. MATERIALS AND METHODS:

A. Test Animals: Eighty, one-day old, unsexed Bobwhite quail (Colinus virginianus) from D.R. and R.E. Wise, Monkfield, Bourn, Cambridgeshire, were used. At 10 days old, the birds were allocated to treatment groups on the basis of body weight so that all groups had a similar initial mean body weight. They were acclimated for a further 3 days prior to the start of treatment.

The birds were offered ad libitum standard HRC chick diet in meal form. The diet was known to contain no added antibiotic or other growth promoter. Domestic quality drinking water was also available ad libitum.

B. Test System: The test system consisted of wooden boxes fitted with wire mesh lids, measuring 80 x 50 x 60 cm. Each box was equipped with a food and drinking trough. Ventilation fans were adjusted as necessary and continuous artificial lighting was adopted. Additional heat at bird level was provided using a 300-watt infra-red lamp suspended over each pen. The average minimum and maximum room temperatures recorded once daily were 25 (s.d. = 3°C) and 27°C (s.d. = 2°C), respectively. The relative humidity mean was 70% (s.d. = 6%).

C. Dosage: Acute dietary LC50 test. Based on a range-finding test, five nominal dietary concentrations (250, 500, 1000, 2000, and 4000 ppm W81 Technical Amitraz) were selected for the definitive study. A pre-mix was prepared by mixing test material with chick diet to give a nominal concentration of 10,000 ppm. Aliquots of this pre-mix were used to prepare 1 kg quantities of the test diets. Food in the birds' feed hoppers was replaced with newly prepared diets daily during the treatment period.

D. Design: The test consisted of five treatment and three control groups, with ten birds per group. The test was initiated when the birds were 13 days old. Test material was incorporated in the chick diet without a vehicle during the 5-day treatment period and standard HRC chick diet (control diet) was offered for the 4-day post-treatment observation period.

A sample of each treated diet and untreated (control) diet were analyzed for active ingredient concentration. Mortalities and clinical signs of toxicity were observed daily. Group mean body weights were determined on Days -3, 0, 5, 8, and 9. Group mean food consumptions were estimated on Days -3 to -1, 1 to 5 (daily), 6 to 8, and 8 to 9. Birds which died during the study were examined post-mortem. At test termination, three birds from the 4000-ppm group as well as seven birds from the 2000-ppm group were examined for gross macroscopic changes.

E. Statistics: The dietary LC50 value was determined using a method of probit line analysis with a maximum likelihood program.

12. REPORTED RESULTS: Results of the analysis of dietary samples for W81 Technical Amitraz obtained from a separate report (EPA Accession No. 407805-02) showed mean concentrations ranged from 82.3 to 93.0% of the nominal concentrations. The test for homogeneity of mixing between top, middle and bottom portions of the 250, 1000 and 4000 ppm diet mixes was reported as being satisfactory.

The distribution of mortalities during the study period and LC50 value were presented in Table 1 (attached). Marked clinical signs of toxicity, including subdued behavior, ruffled feathers, "dropped" wings and unsteadiness, were seen in all birds in the 2000- and 4000-ppm groups from the end of Day 1 up to Day 5. By Day 6, these clinical signs had moderated, the birds appearing subdued and slightly unsteady. All birds had completely recovered by the end of Day 9. The only other treatment-related clinical signs were seen on Days 4 to 6 in the 500-ppm group, where up to two birds were unsteady and either had ruffled feathers or held their head down. They had completely recovered by Day 7.

During the treatment period (Days 0 to 5), there was a dose-related reduction in bodyweight weight gain in all treatment groups, when compared to the control groups (see the attached Table 2). Mean body weight in the 4000-ppm group decreased during this period. All groups showed a similar mean body weight increase during the post-treatment observation period (Days 5 to 9). The authors stated that there was clear evidence of a dose-related reduction in the mean food consumption in all treatment groups during the treatment period (Table 3, attached). During the post-treatment observation period, food consumption in all treatment groups was comparable with the controls. No abnormalities were detected in any of the birds examined.

13. STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES: The subacute dietary LC50 value of Technical Amitraz to the Bobwhite quail was found to be 3081 ppm (95% confidence limits = 2268-4978 ppm).

A statement was included indicating that "the study was conducted in compliance with the following Good Laboratory Practice Standards: U.S. EPA (Federal Register, 29 November 1983); Organization for Economic Co-operation and Development (ISBN 92-64-12367-9, Paris 1982); Japan Ministry of Agriculture, Forestry and Fisheries (59 NogSan, Notification No. 3850); The United Kingdom Compliance Programme (Dept. of Health & Social Security 1986)." Inspections were made by the Quality Assurance Unit of Huntingdon Research Centre at various phases of the study.

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

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

o Test birds were allocated to treatment groups on the basis of body weight, rather than randomly.

o The test was conducted at the temperature range of 25-27$^{o}$C, which was lower than the recommended temperature of 35$^{o}$C.

o The range of dietary concentrations tested did not include a no-observed-effect level (NOEL).

B. Statistical Analysis: The LC50 value was recalculated using EEB's Toxanal computer program (see attach printout). The recalculated LC50 value was identical to the authors' (i.e., 3081 ppm nominal concentration). However, the 95% Confidence limits (2272-4958 ppm) were slightly different from the authors'.

C. Discussion/Results: An LC50 value of 3081 ppm classifies W81 Technical Amitraz as slightly toxic to Bobwhite quail. Due to reduction in body weight gain and food consumption in all treatment groups during the treatment period, the NOEL was determined to be less than 250 ppm.



7

D. Adequacy of the Study:

(1) Classification: Core.

(2) Rationale: Although the test procedures deviated from the SEP, they probably did not significantly affect the toxicity results of the test.

(3) Repairability: N/A.

15. COMPLETION OF ONE-LINER: Yes, December 1, 1988.

TABLE 1

Distribution of mortalities

| Group | Treatment (ppm) | No. of birds | Days of study | | | | | | | | | Total |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | -3 to -1 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | |
| 1 | Control 0 | 10 | | | | | | | | | | | 0 |
| 2 | Control 0 | 10 | | | | | | | | | | | 0 |
| 3 | Control 0 | 10 | | | | | | | | | | | 0 |
| 4 | Technical amitraz 250 | 10 | | | | | | | | | | | 0 |
| 5 | Technical amitraz 500 | 10 | | | | | | | | | | | 0 |
| 6 | Technical amitraz 1000 | 10 | | | | | | | | | | | 0 |
| 7 | Technical amitraz 2000 | 10 | | | | 1 | | 1 | | | | | 2 |
| 8 | Technical amitraz 4000 | 10 | | 1 | | 1 | 1 | 1 | 3 | | | | 7 |

| | |
|---|---|
| $LC_{50}$ value | 3081 ppm |
| 95% confidence limits | 2268 - 4978 ppm |
| Slope of the line* | 4.961 |
| Standard error of the slope* | 1.702 |

TABLE 2

Group mean bodyweights and bodyweight changes (g)

| Group | Treatment (ppm) | Days of study | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | -3 | 0 | 5 | 8 | 9 | -3 to 0 | 0 to 5 | 5 to 9 |
| 1 | Control 0 | 15.0 | 20.9 | 28.4 | 32.7 | 34.0 | +5.9 | +7.5 | +5.6 |
| 2 | Control 0 | 15.6 | 20.6 | 28.7 | 33.7 | 35.3 | +5.0 | +8.1 | +6.6 |
| 3 | Control 0 | 15.1 | 20.9 | 29.3 | 35.2 | 37.0 | +5.8 | +8.4 | +7.7 |
| 4 | Technical amitraz 250 | 15.7 | 21.3 | 25.9 | 32.5 | 33.9 | +5.6 | +4.6 | +8.0 |
| 5 | Technical amitraz 500 | 15.1 | 21.3 | 24.1 | 28.9 | 30.7 | +6.2 | +2.8 | +6.6 |
| 6 | Technical amitraz 1000 | 15.6 | 20.2 | 22.2 | 27.1 | 29.7 | +4.6 | +2.0 | +7.5 |
| 7 | Technical amitraz 2000 | 15.1 | 21.1 | 22.3[8] | 28.8[4] | 30.6[5] | +6.0 | +1.2 | +8.3 |
| 8 | Technical amitraz 4000 | 15.7 | 21.5 | 18.3[6] | 24.7[3] | 26.7[3] | +5.8 | -3.2 | +8.4 |

(where less than 10 birds were weighed, the number weighed is shown as a superscript)

TABLE 3

Group mean food consumption (g/bird/day)

| Group | Treatment (ppm) | Days of study | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | -3 to -1 | 1 | 2 | 3 | 4 | 5 | 1 to 5 | 6 to 9 |
| 1 | Control 0 | 5.3 | 5.0 | 5.5 | 6.0 | 5.5 | 4.0 | 5.2 | 5.5 |
| 2 | Control 0 | 4.9 | 5.0 | 5.1 | 5.9 | 5.2 | 5.3 | 5.3 | 5.2 |
| 3 | Control 0 | 5.3 | 5.9 | 6.6 | 5.1 | 8.0 | 4.9 | 6.1 | 5.5 |
| 4 | Technical amitraz 250 | 5.3 | 3.8 | 4.0 | 4.7 | 5.0 | 4.4 | 4.4 | 5.3 |
| 5 | Technical amitraz 500 | 5.0 | 3.6 | 4.3 | 4.3 | 4.6 | 3.7 | 4.1 | 5.0 |
| 6 | Technical amitraz 1000 | 4.9 | 3.1 | 3.9 | 4.4 | 4.6 | 3.6 | 3.9 | 5.5 |
| 7 | Technical amitraz 2000 | 5.0 | 2.6 | 2.9 | 3.2 | 4.6 | 3.3 | 3.3 | 5.1 |
| 8 | Technical amitraz 4000 | 4.9 | 2.0 | 2.4 | 1.9 | 3.9 | 4.1 | 2.8 | 6.2 |

KOSALWAT WB1 TECHNICAL AMITRAZ COLINUS VIRGINIANUS 12-01-88

**********

| CONC. | NUMBER EXPOSED | NUMBER DEAD | PERCENT DEAD | BINOMIAL PROB. (PERCENT) |
|---|---|---|---|---|
| 4000 | 10 | 7 | 70 | 17.1875 |
| 2000 | 10 | 2 | 20 | 5.46875 |
| 1000 | 10 | 0 | 0 | 9.765625E-02 |
| 500 | 10 | 0 | 0 | 9.765625E-02 |
| 250 | 10 | 0 | 0 | 9.765625E-02 |

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 3048.867

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

| SPAN | G | LC50 | 95 PERCENT CONFIDENCE LIMITS | |
|---|---|---|---|---|
| 1 | .8025485 | 3048.867 | 1901.308 | 8998.724 |

RESULTS CALCULATED USING THE PROBIT METHOD

| ITERATIONS | G | H | GOODNESS OF FIT PROBABILITY |
|---|---|---|---|
| 13 | .4389479 | 1 | .9885257 |

SLOPE = 4.961058
95 PERCENT CONFIDENCE LIMITS = 1.674201 AND 8.247915

LC50 = 3081.391
95 PERCENT CONFIDENCE LIMITS = 2271.935 AND 4957.862

LC10 = 1709.043
95 PERCENT CONFIDENCE LIMITS = 596.8986 AND 2308.024

**********

Accession Number 407805-05

DATA EVALUATION RECORD

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

2. **TEST MATERIAL:** W94 AMITRAZ 20 EC Formulation code BX CR20855/3. Amitraz content 179.5 g/L. The formulation was a straw colored clear solution. N'-(2,4-dimethylphenyl)-N-[(2,4-dimethylphenyl)-imino-methyl]-N-methylmethanimidamide.

3. **STUDY TYPE:** Freshwater fish acute test.
Species Tested: Salmo gairdneri.

4. **CITATION:** Caunter, J.E. 1988. W94 AMITRAZ 20 EC Formulation: Determination of acute toxicity to rainbow trout (Salmo gairdneri). Study Number Q711/I. Conducted by Brixham Laboratory, Brixham, Devon, England. Submitted by NOR-AM Chemical Company, Wilmington, DE. Accession Number 407805-05.

5. **REVIEWED BY:**

Isabel C. Johnson, M.S.
Principal Scientist
KBN Engineering and
Applied Sciences, Inc.

**Signature:**
**Date:**

6. **APPROVED BY:**

Prampimpan Kosalwat, Ph.D.
Staff Scientist
KBN Engineering and
Applied Sciences, Inc.

**Signature:**
**Date:**

Henry T. Craven, M.S.
Supervisor, EEB/HED
USEPA

**Signature:** John Noles
**Date:** 12/21/88

* 7. **CONCLUSIONS:** This study is scientifically sound, but does not meet the Guideline requirements for a cold water fish species due to the study deviations described herein. With a reported 96-hour $LC_{50}$ of 2.2 mg/L amitraz 20 EC formulation (based on mean measured concentrations), this formulation is considered moderately toxic to rainbow trout. The NOEC is 0.34 mg/L of this formulation.

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

* Revised to Core 8/25/89 JN

9. **BACKGROUND:** N/A.

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

11. **MATERIALS AND METHODS:**

**A. Test Animals:** Rainbow trout were obtained from Upwey Trout Farm, Upwey, Weymouth, Dorset. No sickness, injury or abnormality was observed in the fish in the two weeks prior to the test. The pretest diet was BP Mainstream Size 02. The batch of fish used for this study was held for 6 days at 12 ± 1 °C before the start of the test. The fish were held under daylight and artificial lighting. Malachite green was given to the fish three weeks prior to testing. The fish tested ranged in weight from 0.4 to 2.97 g with a mean weight of 1.16 g. The range in length was 32 to 56 mm with a mean length of 43 mm.

**B. Test System:** The apparatus used in this study was a continuous flow-through system. The test vessels, dosing lines, mixing chambers and stock vessels were all constructed of glass. Twenty liter spherical glass vessels 37 cm diameter, fitted with Quickfit glass lids and outlet lines, were used to hold the test fish. The test solutions were renewed at a rate of 125 ml/minute. A 95 percent exchange of the test solutions was calculated to occur within 9 hours. The depth of the test solutions was 37 cm. The stock solutions were fed by a series of Watson-Marlow peristaltic pumps and a further series of these peristaltic pumps was used to supply freshwater. Dilution water characteristics included pH range from 7.7 to 7.8, conductivity of 120 to 130 umhos/cm, hardness of 50 to 53 mg/L as $CaCO_3$, alkalinity of 28.5 mg/L as $CaCO_3$, temperature of 8.1 to 8.4 °C, and free chlorine of <4 ug/L. The source of the dilution water was not reported.

**C. Dosage:** Ninety-six-hour flow-through acute test.

**D. Design:** The following nominal single test exposure concentrations were used in this study: 10, 5.6, 3.2, 1.8, 1.0 and 0.56 mg/L amitraz 20 EC formulation and a freshwater control. Daily pH, dissolved oxygen (DO), and temperature readings were conducted in all test chambers in which surviving fish were found. Daily dilution water quality measurements were taken for pH, conductivity, hardness, alkalinity, temperature and free residual chlorine. Chemical concentrations were measured at the 24-, 48-, 72-, and 96-

hour exposure period, in the controls and all treatments. The photoperiod in this study was 16 hours light and 8 hours darkness.

E. **Statistics:** All $LC_{50}$ values were calculated using Stephan's computerized method. A Phillips plotter was used to draw the dose response curve.

12. **REPORTED RESULTS:** The mean measured values of amitraz 20 EC formulation ranged from 47 to 95 percent of nominal values. The levels of the two metabolites of amitraz were below the determination levels used in the study. The losses of amitraz in this study are thought to be due to adsorption, non-homogeneity in solution and precipitation. Survival is summarized below:

| Mean Measured Concentration (mg/L) | Surviving rainbow trout | | | |
|---|---|---|---|---|
| | 24 Hours | 48 Hours | 72 Hours | 96 Hours |
| Control | 20 | 20 | 20 | 20 |
| 0.339 | 20 | 20 | 20 | 20 |
| 0.669 | 20 | 20 | 20 | 20 |
| 1.48 | 20 | 20 | 19 | 18 |
| 1.93 | 19 | 13 | 7 | 6 |
| 2.69 | 20 | 20 | 12 | 10 |
| 9.47 | 0 | 0 | 0 | 0 |

The general symptoms of toxicity noted in this study were quiescence, turning dark, cessation of swimming, and loss of balance. The 96-hour $LC_{50}$ value as amitraz 20 EC formulation, based on mg/L mean measured concentration was 2.2 mg/L with 95 percent confidence limits of 1.9 and 2.5 mg/L. These values were calculated using the Moving Average Method. The no-observed-effect concentration was determined to be 0.34 mg/L amitraz 20 EC formulation. Test temperature was 12 $\pm$ 1 °C. The test compound was observed to precipitate from solution, and this was one of the reasons indicated for the difference between nominal and measured concentrations. The system turnover rate calculated by the reviewer from the report data was approximately 2.6 turnovers per day.

13. **STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:** The 96-hour $LC_{50}$ value obtained in this study for amitraz 20 EC formulation was 2.2 mg/L based on mean measured concentrations. The compound would be classified as moderately toxic according to the relevant standard evaluation procedure. "This report has been audited in accordance with ICI's policies and procedures for Good Laboratory Practice."

14. **REVIEWER'S DISCUSSION AND INTERPRETATION OF STUDY RESULTS:**
**A. Test Procedure:** Overall, the test procedures appear to be scientifically sound, but several deviations from the Guidelines were noted and are discussed below:

- The dilution water source was not described fully, other than it was supplied from a 20,000 gallon reservoir. It was not stated whether its source was ground water, surface water, or tap water. Free chlorine measurements were taken, possibly indicating that tap water may have been used. If so, method of dechlorination should have been stated. Furthermore, the Guidelines recommend that dechlorinated water should not be used for testing.

- Due to the lack of solubility of the technical grade amitraz, and the apparent solubility of the formulation, an additional inerts control should have been included.

- The percent active of the formulation was not clearly reported. However, the submitted oyster shell deposition study conducted by another laboratory indicated that the same sample lot number (code BX CR20855/3) consisted of 20% a.i..

- Temperature control method for testing was not reported, and temperature was measured daily. More frequent recording is required for both water-bath and environmental air temperature control.

- Based on the information provided in the report, the reviewer calculated that approximately 2.3 test solution turnovers per day were achieved by the flow-through system. The protocols recommend that five to ten volume additions per 24 hours be maintained.

- Although the chemical test concentrations were measured daily in all test concentrations, they were not measured at test initiation. Furthermore, the data appear to show that the test chemical was found in the control at 0.1 mg/L amitraz EC at 24 hours, and was also detected at 48



14

and 72 hours. The Amitraz metabolites were also found in the 24-hour control sample.

B. **Statistical Analysis:** The reviewer recalculated the 96-hour $LC_{50}$ value and obtained slightly different results of 2.1 mg/L amitraz 20 EC formulation (1.9 - 2.4). This difference is not significant. The reviewer's results are attached.

C. **Discussion/Results:** The study appears to be scientifically sound but due to several deviations this study does not meet the Guideline requirements. The test compound was found in the control, and the test concentrations were not measured at test initiation. With a 96-hour $LC_{50}$ of 2.2 mg/L Amitraz EC, this compound is considered moderately toxic to rainbow trout.

D. **Adequacy of the Study:**

**(1) Classification:** Supplemental.

**(2) Rationale:** The test compound was found in the control, and test concentrations were not measured at test initiation.

**(3) Repairability:** Yes, if scientifically acceptable explanations are provided for the presence of the test compound in the control are presented. Other information should include the source of the dilution water and method of temperature control during testing.

15. **COMPLETION OF ONE-LINER:** Yes, December 5, 1988.

20EC Formulation

ISABEL C. JOHNSON AMITRAZ SALMO GAIRDNERI 12-05-88

**********

| CONC. | NUMBER EXPOSED | NUMBER DEAD | PERCENT DEAD | BINOMIAL PROB. (PERCENT) |
|---|---|---|---|---|
| 9.47 | 20 | 20 | 100 | 9.536742E-05 |
| 2.69 | 20 | 10 | 50 | 58.80985 |
| 1.93 | 20 | 14 | 70 | 5.765915 |
| 1.48 | 20 | 2 | 10 | 2.012253E-02 |
| .669 | 20 | 0 | 0 | 9.536742E-05 |
| .339 | 20 | 0 | 0 | 9.536742E-05 |

THE BINOMIAL TEST SHOWS THAT 1.48 AND 9.47 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 1.777333

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

| SPAN | G | LC50 | 95 PERCENT CONFIDENCE LIMITS | |
|---|---|---|---|---|
| 3 | .2477543 | 2.104881 | 1.898792 | 2.434703 |

RESULTS CALCULATED USING THE PROBIT METHOD

| ITERATIONS | G | H | GOODNESS OF FIT PROBABILITY |
|---|---|---|---|
| 6 | 1.25096 | 2.896205 | 2.072156E-02 |

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

SLOPE = 4.62714
95 PERCENT CONFIDENCE LIMITS =-.5481453 AND 9.802424

LC50 = 2.188156
95 PERCENT CONFIDENCE LIMITS = 0 AND +INFINITY

LC10 = 1.163083
95 PERCENT CONFIDENCE LIMITS = 0 AND 1.750789

**********

Accession Number 407805-06

DATA EVALUATION RECORD

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

2. **TEST MATERIAL:** W95 AMITRAZ 20 EC Formulation code BX CR20855/3. Amitraz content 179.5 g/L. The formulation was a straw colored clear solution. N'-(2,4-dimethylphenyl)-N-[(2,4-dimethylphenyl)-imino-methyl]-N-methylmethanimidamide.

3. **STUDY TYPE:** Freshwater invertebrate acute test.
Species Tested: Daphnia magna.

4. **CITATION:** Hill, R. W., T.D. Williams, and B.J. Harland. 1988. W965 AMITRAZ 20 EC Formulation: Determination of acute toxicity to Daphnia magna. Study Number Q711/C. Conducted by Brixham Laboratory, Brixham, Devon, England. Submitted by NOR-AM Chemical Company, Wilmington, DE. Accession Number 407805-06.

5. **REVIEWED BY:**

Isabel C. Johnson, M.S.
Principal Scientist
KBN Engineering and
Applied Sciences, Inc.

**Signature:**
**Date:**

6. **APPROVED BY:**

Prampimpan Kosalwat, Ph.D.
Staff Scientist
KBN Engineering and
Applied Sciences, Inc.

**Signature:**
**Date:**

for Henry T. Craven, M.S.
Supervisor, EEB/HED
USEPA

Henry T. Craven 12/21/88

**Signature:** John Noles
**Date:** 12/21/88

7. **CONCLUSIONS:** This study is scientifically sound and fulfills the Guideline requirements for an invertebrate acute test. With a reported 96-hour $EC_{50}$ of 3.38 mg/L amitraz 20 EC formulation (based on measured concentrations), this formulation is considered moderately toxic to Daphnia magna. The NOEC was calculated to be 0.95 mg/L formulation.

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

9. **BACKGROUND:** N/A.

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

11. **MATERIALS AND METHODS:**
**A. Test Animals:** The test organism was the freshwater crustacean, Daphnia magna, obtained from continuous laboratory cultures. The stock cultures of Daphnia were maintained in a reconstituted water medium, identical to the test dilution water, at a temperature of 20 ± 2°C before the start of the test. The photoperiod was 16 hours light and 8 hours of darkness. The cultures were fed a diet of algae (Chlorella vulgaris) and yeast. Daphnia less than 24 hours old were used for testing. The broodstock was 17 days old. No symptoms of disease were observed in this culture.

12. Test System: Borosilicate glass beakers (250 ml) were used as test vessels, with four replicates per exposure concentration, each containing 200 ml of test solution. The test solutions were not aerated during testing. Dilution water was reconstituted freshwater with the following characteristics: pH 8.5, conductivity of 536 uS/cm, hardness of 166 mg/L as $CaCO_3$, alkalinity of 111 mg/L as $CaCO_3$, and total carbon of 0.7 mg/L. The water was aerated for more than 2 hours before use. The pH of the solution was 8.25 ± 0.25.

C. Dosage: Forty-eight-hour static acute test.

D. Design: The following nominal test exposure concentrations were used in this study: 32, 18, 10, 5.6, 3.2, and 1.8 mg/L amitraz 20 EC formulation and a freshwater control. When the test solutions were at test temperature, five Daphnia were randomly added to each test vessel, giving a total of 20 Daphnia per concentration. The temperature was maintained at 20 ± 1 °C and a photoperiod of 16 hours light:8 hours dark was provided. The dissolved oxygen concentration of the control was measured prior to the start of the test. The initial pH of each test solution was determined using the excess remaining after filling the test vessels. At the end of the test the pH and dissolved oxygen concentrations of two replicates of each control and treatment were measured. The temperature of water was measured at 0, 24, and 48 hours, and at hourly intervals using an automatic recording system. Chemical concentrations were measured at test initiation and termination in all test concentrations and controls.

E. Statistics: $EC_{50}$ values were calculated using a computerized probit analysis method.

12. **REPORTED RESULTS:** It was noted that the stock concentrate of the amitraz EC20 formulation was milky white in color and that some slight precipitation of the material was noted in the test vessels. The mean measured values of amitraz 20 EC formulation ranged from 77.8 to 129 percent of the nominal values; the measured concentrations at the end of the test ranged from 27.8 to 57.8 of the nominal values. The numbers of Daphnia immobilized at each concentration, after 24 and 48 hours are presented in Table 1 (attached). Where precipitation of the test material occurred in the higher concentrations, this caused impedance of Daphnia mobility. The 48-hour $EC5_0$ calculated was 3.38 mg/L amitraz EC formulation with 95-percent confidence limits of 2.52 and 4.41 mg/L. The slope of probit was 2.6. These values were calculated on the basis of the mean measured concentrations of amitraz EC 20 formulation. The formulation concentrations in the exposure samples were determined by measurement of the amitraz concentration using Q506 standards. The formulation concentrations were then calculated by multiplication using a factor of 5.504, based on the purity of the amitraz standard and the nominal amitraz content of the formulation.

    Dissolved oxygen levels ranged from 9.0 to 9.6 mg/L and the pH values ranged from 8.08 and 8.5. The range of temperatures recorded automatically at hourly intervals was 19.4 to 20.0 °C.

13. **STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:** The 48-hour $EC_{50}$ value obtained in this study for amitraz 20 EC formulation was 3.38 mg/L based on mean measured concentrations. The compound would be classified as moderately toxic according to the relevant standard evaluation procedure. "This report has been audited in accordance with ICI's policies and procedures for Good Laboratory Practice."

14. **REVIEWER'S DISCUSSION AND INTERPRETATION OF STUDY RESULTS:**
    **A. Test Procedure:** Overall the test procedures appear to be scientifically sound, but several deviations from the Guidelines were noted and are discussed below:

    - The percent active of the formulation was not clearly reported. However, it was noted elsewhere in the studies' submissions that sample batch code BX CR20855/3 consisted of 20% a.i.

- The dilution water hardness used was 166 mg/L as $CaCO_3$, which is significantly higher than that recommended by the SEP (40 to 48 mg/L as $CaCO_3$).

- The report states that a precipitate was noted in the higher test concentrations. Furthermore, the measured concentrations indicate that only 27.8 to 57.8 percent of the test chemical remained in solution after 48 hours. A flow-through test using a solvent may have been more appropriate for this compound.

- A transition period from light to dark was not reported and is recommended.

- Due to the lack of solubility of the technical grade amitraz, and the apparent solubility of the formulation, an additional inerts control should have been included.

B. **Statistical Analysis:** The reviewer recalculated the 48-hour $EC_{50}$ value and obtained similar results (attached).

C. **Discussion/Results:** The study appears to be scientifically sound albeit the deviations noted in the study. With a 96-hour $LC_{50}$ of 3.38 mg/L Amitraz EC, this compound is considered moderately toxic to Daphnia magna.

D. **Adequacy of the Study:**
   (1) **Classification:** Core

   (2) **Rationale:** Guideline fulfillment.

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

15. **COMPLETION OF ONE-LINER:** Yes, December 5, 1988.

ISABEL C. JOHNSON AMITRAZ DAPHNIA MAGNA 12-05-88

**********

| CONC. | NUMBER EXPOSED | NUMBER DEAD | PERCENT DEAD | BINOMIAL PROB. (PERCENT) |
|---|---|---|---|---|
| 27.2 | 20 | 20 | 100 | 9.536742E-05 |
| 15.6 | 20 | 20 | 100 | 9.536742E-05 |
| 8.649999 | | 20 | 14 | 70 |
| 5.765915 | | | | |
| 4.2 | 20 | 13 | 65 | 13.1588 |
| 1.9 | 20 | 8 | 40 | 25.17223 |
| .95 | 20 | 0 | 0 | 9.536742E-05 |

THE BINOMIAL TEST SHOWS THAT .95 AND 15.6 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 2.605507

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

| SPAN | G | LC50 | 95 PERCENT CONFIDENCE LIMITS | |
|---|---|---|---|---|
| 4 | 5.076996E-02 | 3.599964 | 2.841776 | 4.482166 |

RESULTS CALCULATED USING THE PROBIT METHOD

| ITERATIONS | G | H | GOODNESS OF FIT PROBABILITY |
|---|---|---|---|
| 5 | 8.510456E-02 | 1 | 6.085688E-02 |

SLOPE = 2.603688
95 PERCENT CONFIDENCE LIMITS = 1.844122 AND 3.363254

LC50 = 3.375811
95 PERCENT CONFIDENCE LIMITS = 2.518684 AND 4.411698

LC10 = 1.098015
95 PERCENT CONFIDENCE LIMITS = .5997251 AND 1.590531

**********

Accession Number 407805-07

DATA EVALUATION RECORD

1. CHEMICAL: AMITRAZ
Shaughnessey Number 106201

2. TEST MATERIAL: W96 AMITRAZ Technical code BTS 27419. N'-(2,4-dimethylphenyl)-N-[(2,4-dimethylphenyl)-imino-methyl]-N-methylmethanimidamide. Purity 98.8 percent.

3. STUDY TYPE: Saltwater fish acute test.
Species Tested: Cyprinodon variegatus.

4. CITATION: Hill, R.W., M.H.I. Comber and J.E. Caunter. 1988. W96 AMITRAZ technical: Determination of acute toxicity to sheepshead minnow (Cyprinodon variegatus). Study Number Q506/F. Conducted by Brixham Laboratory, Brixham, Devon, England. Submitted by NOR-AM Chemical Company, Wilmington, DE. Accession Number 407805-07.

5. REVIEWED BY:

Isabel C. Johnson, M.S.
Principal Scientist
KBN Engineering and
Applied Sciences, Inc.

Signature: Isabel C. Johnson
Date: December 5, 1988

6. APPROVED BY:

Prampimpan Kosalwat, Ph.D.
Staff Scientist
KBN Engineering and
Applied Sciences, Inc.

Signature: P. Kosalwat
Date: 12-5-88

for Henry T. Craven, M.S.
Supervisor, EEB/HED
USEPA

Signature: H. T. Craven 12/20/88
Date: John Noles 12/20/88

7. CONCLUSIONS: This study is scientifically sound, but does not meet the Guideline requirements for an estuarine fish species due to the study deviations described herein. A 96-hour $LC_{50}$ could not be calculated from the amitraz technical concentrations selected. The NOEC was determined to be 0.09 mg/L Amitraz Technical (based on measured concentrations).

8. RECOMMENDATIONS: N/A.

Supplemental Non-Registrable 8/25/89.

9. BACKGROUND: N/A.

10. DISCUSSION OF INDIVIDUAL TESTS: N/A.

11. MATERIALS AND METHODS:

A. Test Animals: The fish were obtained from SP Engineering, Inc., Salem, Massachusetts, USA. No sickness, injury or abnormality was observed in the fish in the seven-day acclimation period. The pretest diet was BP Mainstream Size 02 and Promin Coarse fish food. The batch of fish used for this study was held for 7 days at 22 ± 1 °C before the start of the test. The fish were held under daylight and artificial lighting. The medication given to the fish was a 2 ppm treatment of methylene blue two weeks prior to testing and Elbazin bath for 24 hours 10 days prior to testing. The fish tested ranged in weight from 0.36 to 1.87 g with a mean weight of 0.87 g. The range in length was 25 to 36 mm with a mean length of 30.4 mm.

B. Test System: The apparatus used in this study was a continuous flow-through system. The test vessels, dosing lines, mixing chambers and stock vessels were all constructed of glass. Twenty-liter spherical glass vessels 37 cm diameter, fitted with Quickfit glass lids and outlet lines, were used to hold the test fish. The test solutions were renewed at a rate of 125 ml/minute. A 95 percent exchange of the test solutions was calculated to occur within 7.5 hours. The depth of the test solutions was 37 cm. The stock solutions were fed by a series of peristaltic pumps and a further series of these peristaltic pumps was used to supply the seawater. The seawater was supplied from Tor Bay, Devon. The seawater was filtered (50 u) before use. Dilution water characteristics included pH range from 8.09 to 8.16, temperature of 8.1 to 12.1 °C, and salinity of 35 ppt.

C. Dosage: Ninety-six-hour flow-through acute test.

D. Design: Twenty sheepshead minnows were used in each test concentration and in the solvent and seawater controls. The level of the solvent (triethylene glycol) in this study in the final exposure concentrations and in the solvent control was 400 uL/L. The following nominal single test exposure concentrations were used in this study: 3.2, 1.8, 1.0, 0.56, 0.32, and 0.1 mg/L amitraz and a seawater and solvent controls. Daily pH, dissolved oxygen (DO), and temperature readings were conducted in all test chambers in which surviving fish were found. Daily dilution water quality

measurements were taken for pH, temperature and salinity. Chemical concentrations were measured at the 24-, 48-, 72-, and 96-hour exposure period, in the controls and all treatments. The photoperiod in this study was 16 hours light and 8 hours darkness.

E. Statistics: $LC_{50}$ values could not be calculated due to insufficient mortalities in all concentrations tested.

12. REPORTED RESULTS: The mean measured values of amitraz ranged from 47 to 90 percent of nominal values. The losses of amitraz in this study are thought to be due to adsorption, non-homogeneity in solution and precipitation. Survival is summarized below:

| Mean Measured Concentration (mg/L) | Surviving sheepshead minnows | | | |
|---|---|---|---|---|
| | 24 Hours | 48 Hours | 72 Hours | 96 Hours |
| Control | 20 | 20 | 20 | 20 |
| Solvent Control | 20 | 20 | 20 | 20 |
| 2.4 | 20 | 20 | 20 | 16 |
| 1.6 | 20 | 19 | 17 | 16 |
| 0.68 | 20 | 19 | 18 | 16 |
| 0.28 | 20 | 20 | 19 | 18 |
| 0.15 | 20 | 20 | 20 | 20 |
| 0.09 | 20 | 20 | 20 | 20 |

The general symptoms of toxicity noted in this study were quiescence, turning dark, cessation of swimming, and loss of balance. The 96-hour $LC_{50}$ value as amitraz technical, based on mg/L mean measured concentration is greater than 2.4 mg/L, the highest concentration tested. The no observed effect concentration was determined to be 0.09 mg/L amitraz. Test temperature was 22 $\pm$ 1 $^{o}$C. The test compound was observed to precipitate from solution, and this was one of the reasons indicated for the difference between nominal and measured concentrations. The system turnover rate calculated by the reviewer from the report data was approximately 3.2 turnovers per day.



24

13. STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES: The 96-hour $LC_{50}$ value obtained in this study for amitraz technical was determined to be greater than 2.4 mg/L based on mean measured concentrations. "This report has been audited in accordance with ICI's policies and procedures for Good Laboratory Practice."

14. REVIEWER'S DISCUSSION AND INTERPRETATION OF STUDY RESULTS:
A. Test Procedure: Overall the test procedures appear to be scientifically sound, but several deviations from the Guidelines were noted and are discussed below:

- The test conducted failed to calculate an $LC_{50}$ value due to insufficient mortalities in the highest concentration tested (2.4 mg/L amitraz technical).
- Temperature control method for testing was not reported, and temperature was measured daily. More frequent recording is required for both water-bath and environmental air temperature control.
- Based on the information provided in the report, the reviewer calculated that approximately 3.2 test solution turnovers per day were achieved by the flow-through system, although on page 14 of the report it is stated that "nine volume changes per day were used in this study." The reviewer calculated 3.2 turnovers per day based on the following statement made on the same page of the report "a 95 percent exchange of the test solution was calculated to occur within (approximately) 7.5 hours." The protocols recommend a minimum of 5 to 10 volume turnovers per day.
- Although the chemical test concentrations were measured daily in all treatment, they were not measured at test initiation.
- The percent mortalities of fish prior to testing (48 hours) was not reported.
- The test salinity was 35 ppt, the guidelines recommend 10-17 ppt for estuarine fish.

B. Statistical Analysis: Due to lack of sufficient mortalities, an $LC_{50}$ could not be calculated.

C. Discussion/Results: The study appears to be scientifically sound but due to several deviations found, this study does not meet the Guideline requirements. The major deviations include the fact that a 96-hour $LC_{50}$ could not be calculated from the test concentrations selected, and the test concentrations were not measured at test initiation. The toxicity of amitraz technical can not be categorized based on the data submitted. The NOEC was 0.09 mg/L amitraz technical.

D. Adequacy of the Study:
(1) Classification: Supplemental.

(2) Rationale: The 96-hour $LC_50$ could not be calculated from the test concentrations selected.

(3) Repairability: No.

15. COMPLETION OF ONE-LINER: Yes, December 5, 1988.

Accession Number 407805-08

# DATA EVALUATION RECORD

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

2. **TEST MATERIAL:** W98 AMITRAZ 20 EC Formulation code BX CR20855/3. Amitraz content 179.5 g/L. The formulation was a straw colored clear solution. N'-(2,4-dimethylphenyl)-N-[(2,4-dimethylphenyl)-imino-methyl]-N-methylmethanimidamide.

3. **STUDY TYPE:** Saltwater fish acute test.
Species Tested: Cyprinodon variegatus.

4. **CITATION:** Hill, R.W. and J.E. Caunter. 1988. W98 AMITRAZ 20 EC Formulation: Determination of acute toxicity to sheepshead minnow (Cyprinodon variegatus). Study Number Q711/F. Conducted by Brixham Laboratory, Brixham, Devon, England. Submitted by NOR-AM Chemical Company, Wilmington, DE. Accession Number 407805-08.

5. **REVIEWED BY:**

Isabel C. Johnson, M.S.
Principal Scientist
KBN Engineering and
Applied Sciences, Inc.

**Signature:**
**Date:**

6. **APPROVED BY:**

Prampimpan Kosalwat, Ph.D.
Staff Scientist
KBN Engineering and
Applied Sciences, Inc.

**Signature:**
**Date:**

for Henry T. Craven, M.S.
Supervisor, EEB/HED
USEPA

Henry T Craven 12/21/88

**Signature:** John Noles
**Date:** 12/21/88

7. **CONCLUSIONS:** This study is scientifically sound and meets the Guideline requirements for an estuarine fish study. With a reported 96-hour $LC_{50}$ of 7.9 mg/L 20 EC formulation (based on measured concentrations) this formulation is considered moderately toxic to sheepshead minnows. The NOEC is estimated to be 1.44 mg/L of the formulation.

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

9. **BACKGROUND:** N/A.

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

11. **MATERIALS AND METHODS:**

**A. Test Animals:** The fish were obtained from SP Engineering, Inc., Salem, Massachusetts, USA. No sickness, injury or abnormality was observed in the fish in the seven-day acclimation period. The pretest diet was aquarium flaked food. The batch of fish used for this study was held for 7 days at 22 ± 1 °C before the start of the test. The fish were held under daylight and artificial lighting. The fish tested ranged in weight from 0.49 to 1.3 g with a mean weight of 0.8 g. The range in length was 25 to 35 mm with a mean length of 28.9 mm.

**B. Test System:** The apparatus used in this study was a continuous flow-through system. The test vessels, dosing lines, mixing chambers and stock vessels were all constructed of glass. Twenty liter spherical glass vessels 37 cm diameter, fitted with Quickfit glass lids and outlet lines, were used to hold the test fish. The test solutions were renewed at a rate of 125 ml/minute. A 95 percent exchange of the test solutions was calculated to occur within 8 hours. The depth of the test solutions was 37 cm. The stock solutions were fed by a series of peristaltic pumps and a further series of these peristaltic pumps was used to supply the seawater. The seawater was supplied from Tor Bay, Devon. The flow rate was 125 ml/minute. Dilution water characteristics included pH range from 8.06 to 8.1, temperature of 12.3 to 13.8 °C, and salinity of 34.98 to 35.06 ppt.

**C. Dosage:** Ninety-six-hour flow-through acute test.

**D. Design:** The following nominal single test exposure concentrations were used in this study: 18, 10, 5.6, 3.2 and 1.8 mg/L amitraz 20 EC formulation and a seawater control. Daily pH, dissolved oxygen (DO), and temperature readings were conducted in all test chambers in which surviving fish were found. Daily dilution water quality measurements were taken for pH, temperature and salinity. Chemical concentrations were measured at the 24-, 48-, 72-, and 96-hour exposure period, in the controls and all treatments. The photoperiod in this study was 16 hours light and 8 hours darkness.

E. **Statistics**: All $LC_{50}$ values were calculated using Stephan's computerized method. A Phillips plotter was used to draw the dose response curve.

12. **REPORTED RESULTS**: The mean measured values of amitraz 20 EC formulation ranged from 72 to 136 percent of nominal values. The losses of amitraz in this study are thought to be due to adsorption, non-homogeneity in solution and precipitation. Survival is summarized below:

| Mean Measured Concentration (mg/L) | Surviving sheepshead minnows | | | |
|---|---|---|---|---|
| | 24 Hours | 48 Hours | 72 Hours | 96 Hours |
| Control | 20 | 20 | 20 | 20 |
| 1.44 | 20 | 20 | 20 | 20 |
| 2.26 | 20 | 20 | 20 | 20 |
| 7.63 | 20 | 20 | 15 | 12 |
| 10.5 | 20 | 5 | 1 | 0 |
| 14.27 | 7 | 0 | 0 | 0 |

The general symptoms of toxicity noted in this study were quiescence, turning dark, cessation of swimming, and loss of balance. The 96-hour $LC_{50}$ value as amitraz 20 EC formulation, based on mg/L mean measured concentration was 5.6 mg/L with 95 percent confidence limits of 4.7 and 6.9 mg/L. These values were calculated using the Moving Average Method. The no observed effect concentration was determined to be less than 1.44 mg/L amitraz 20 EC formulation. Test temperature was 22 $\pm$ 1 °C. The test compound was observed to precipitate from solution, and this was one of the reasons indicated for the difference between nominal and measured concentrations. The system turnover rate calculated by the reviewer from the report data was approximately 3.0 turnovers per day.

13. **STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES**: The 96-hour $LC_{50}$ value obtained in this study for amitraz 20 EC formulation was 5.6 mg/L based on mean measured concentrations. The compound would be classified as moderately toxic according to the relevant standard

evaluation procedure. "This report has been audited in accordance with ICI's policies and procedures for Good Laboratory Practice."

14. **REVIEWER'S DISCUSSION AND INTERPRETATION OF STUDY RESULTS:**
**A. Test Procedure:** Overall the test procedures appear to be scientifically sound, but several deviations from the Guidelines were noted and are discussed below:

- The percent active of the formulation was not reported. However, it was noted that elsewhere in the studies' submission that sample batch code BX CR20855/3 consisted of 20% a.i..
- Temperature control method for testing was not reported, and temperature was measured daily. More frequent recording is required for both water-bath and environmental air temperature control.
- Based on the information provided in the report, the reviewer calculated that approximately 3.0 test solution turnovers per day were achieved by the flow-through system. The Guidelines recommend a minimum of 5 to 10 daily turnovers.
- Although the chemical test concentrations were measured daily in all treatment, they were not measured at test initiation.
- The test salinity was 35 ppt, the Guidelines specify that a salinity range of 10 to 17 ppt be used for estuarine species.
- Due to the lack of solubility of the technical grade amitraz, and the apparent solubility of the formulation, an additional inerts control should have been included.

**B. Statistical Analysis:** The reviewer recalculated the 96-hour $LC_{50}$ value to be 7.94 mg/L amitraz 20 EC formulation. This value was obtained using the binomial test. The author reported a 96-hour $LC_{50}$ of 5.6 mg/L, calculated using the moving averages method. When there are less than 2 concentrations with partial mortalities, neither the moving averages method nor the probit method can give statistically sound results.

C. **Discussion/Results:** The study appears to be scientifically sound albeit the deviations found in this study. With a 96-hour $LC_{50}$ of 7.94 mg/L Amitraz EC, this formulation is considered moderately toxic to sheepshead minnows.

D. **Adequacy of the Study:**

(1) **Classification:** Core

(2) **Rationale:** Guideline fulfillment.

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

15. **COMPLETION OF ONE-LINER:** Yes, December 5, 1988.

ISABEL C. JOHNSON AMITRAZ CYPRINODON VARIEGATUS 12-05-88 Foemulation

**********

| CONC. | NUMBER EXPOSED | NUMBER DEAD | PERCENT DEAD | BINOMIAL PROB. (PERCENT) |
|---|---|---|---|---|
| 14.27 | 20 | 20 | 100 | 9.536742E-05 |
| 10.5 | 20 | 20 | 100 | 9.536742E-05 |
| 7.63 | 20 | 8 | 40 | 25.17223 |
| 2.26 | 20 | 0 | 0 | 9.536742E-05 |
| 1.44 | 20 | 0 | 0 | 9.536742E-05 |

THE BINOMIAL TEST SHOWS THAT 2.26 AND 10.5 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 7.939101

WHEN THERE ARE LESS THAN TWO CONCENTRATIONS AT WHICH THE
PERCENT DEAD IS BETWEEN 0 AND 100, NEITHER THE MOVING AVERAGE
NOR THE PROBIT METHOD CAN GIVE ANY STATISTICALLY SOUND RESULTS.

**********

Accession Number 407805-09

DATA EVALUATION RECORD

1. CHEMICAL: Amitraz
Shaughnessey Number 106201

2. TEST MATERIAL: Amitraz 20 EC, Lot #CR20855/3, a yellow liquid. The sample purity was 20 percent active ingredient. N'-(2,4-dimethyl phenyl) -N-[(2,4-dimethyl phenyl)-imino methyl]-N-methylmethanimidamide.

3. STUDY TYPE: Mollusc 96-Hour Flow-Through Shell Deposition
Eastern oyster: Crassostrea virginica

4. CITATION: Surprenant, D.C. 1988. W100 AMITRAZ: Acute toxicity of amitraz 20 EC to eastern oysters (Crassostrea virginica) under flow-through conditions. Laboratory Project ID ENVIR/88/15: 88-1-2621. Prepared by Springborn Life Sciences, Inc., Wareham, MA 02571. Submitted by NOR-AM Chemical Company, Wilmington, DE 19803. Accession Number 407805-09.

5. REVIEWED BY:

Isabel C. Johnson, M.S.
Principal Scientist
KBN Engineering and
Applied Sciences, Inc.

Signature: Isabel C. Johnson
Date: December 16, 1988

6. APPROVED BY:

Prampimpan Kosalwat, Ph.D.
Staff Scientist
KBN Engineering and
Applied Sciences, Inc.

Signature: P. Kosalwat
Date: 12/16/88

Henry T. Craven, M.S.
Supervisor, EEB/HED
USEPA

Signature: John Noles
Date: 12/20/88

* 7. CONCLUSIONS: This study is scientifically sound and but does not meet the Guideline requirements for a mollusc shell-deposition test. With a 96-hour $EC_{50}$ of 85 ug/L (a.i.), amitraz is considered very highly toxic to oysters.

8. RECOMMENDATIONS: N/A.

9. BACKGROUND: N/A.

* Revised & Core 8/25/89 JN



33

10. DISCUSSION OF INDIVIDUAL TESTS: N/A.

11. MATERIALS AND METHODS:

A. Test Animals: Eastern oysters (Crassostrea virginica) were obtained from Aquacultural Research Corporation, Dennis, Massachusetts where they were reared in natural flowing seawater from approximately the same source (Massachusetts Bay) as that used as dilution water during the toxicity test. During the 10 days prior to testing, the temperature range was 17.0 to 20.5 °C, the pH range was 7.6 to 8.0, the dissolved oxygen concentration was 87 to 95 percent saturation, and the salinity range was 28 to 32 ppt. The mortality that occurred in the test population during the period was 0.05 percent. The oysters were of similar age and had a mean valve height of 41 (± 5) mm (standard deviation). During the last 48 hours of acclimation and during testing, oysters were fed a supplementary diet of Isochrysis galbana, clone T-ISO, and Testraselmis maculata, such that the algal density was $10^5$ cells/ml in the holding tray.

B. Test System: A continuous flow serial diluter was used. Each glass aquarium measured 60 X 30 X 30 cm and was equipped with a 10-cm high drain standpipe which maintained a test solution volume of approximately 18 liters. The flow of test solution to each aquarium was 75 ml/minute, which provided approximately six volume replacements every 24 hours. In addition, the contents of each aquarium were continuously recirculated. The test solution was pumped from one end of the aquarium and returned through the other end of the aquarium and returned through the other end using a Nylon impeller pump. Return water flowed through a perforated teflon tube, situated along the entire length of the aquarium. The flow rate of the recirculating test solution was 1.75 liters per minute or about 5 liters per oyster per hour. This recirculation system aided in evenly distributing the algae fed to the oysters and in mixing the flow of fresh test solution throughout each aquarium. The test rested in a temperature controlled water bath. The water in the bath was heated to maintain a test solution temperature of 20 ± 2 °C. Illumination of the test area was 16 hours per day.

C. Dosage: Ninety-six-hour acute oyster shell deposition study.

D. Design: A diluter system with a dilution factor of 0.60 was used to deliver five test concentrations, and a seawater

control to duplicate test aquaria. Test aquaria were randomly assigned to test concentrations and controls. The nominal test concentration range was 52, 86, 140, 240, and 400 ug/L (a.i.). Natural unfiltered seawater was used as dilution and control water. Seawater was pumped from the Cape Code Canal, Bourne, Massachusetts about 4 meters offshore at a depth of approximately 0.5 meters. The seawater used during this study had a salinity of 31 to 32 ppt and a pH of 7.8 to 7.9. In conformance with EPA-GLP, routine analyses were conducted on representative samples of the seawater for the presence of pesticides and PCB's. None of these compounds have been detected in any of the water samples analyzed.

Twenty-four hours prior to testing, 2 to 5 mm of the new peripheral shell growth of each oyster was removed by grinding the shell to a blunt edge using a fine-grit grinding wheel. They were then held overnight, and carefully examined for any signs of stress which might have been caused by the removal of shell. Immediately prior to the test initiation, the outer shell edge was buffed with an emery board to remove any new shell deposition. The exposure of oysters was initiated by impartially selecting and placing 20 oysters in each test aquarium (40 per treatment). Oysters were spaced equidistantly from one another with their valve openings facing toward the flow from the teflon circulator tube. During the exposure the oysters were fed 180 ml of a concentrated algal suspension of $10^7$ cells/ml of Isochrysis galbana and or Tetraselmis maculata per test aquarium three times daily. This feeding regime resulted in an algal density of approximately $10^5$ cells/ml in each aquarium

Biological observations were made daily during the exposure in order to detect any mortality of oysters and to record any visible abnormality such as excessive mucus production or a failure to siphon and feed, as evidenced by a lack of feces and pseudofeces production. After 96 hours of exposure, the oysters were removed and the new shell growth measured microscopically to 0.1 mm using a calibrated micrometer reticle.

Prior to initiating the exposure and after the dilution system had functioned properly for 24 hours, water samples were removed from the high, middle and low test concentrations to determine that a reasonable dose gradient was established. During the definitive test, water samples

were removed at 0, 24, 48, 72, and 96 hours of exposure from each replicate including the controls.

The pH, temperature, salinity and dissolved oxygen concentration were measured daily in each aquarium. Total suspended solids were measured in a sample taken from a control aquarium after 24 hours of exposure.

E. Statistics: The biological results derived from the 96-hour test were used to statistically estimate a median effect concentration ($EC_{50}$) and the 95 percent confidence limits. The $EC_{50}$ is the estimated concentration of test material in seawater which reduced shell deposition (growth) of exposed oysters by 50 percent of the growth measured in control oysters. Thus, the individual shell growth measurements of 40 oysters for each of the five exposure concentrations were expressed as percentages of the control oyster growth.

$EC_{50}$ values and 95 percent confidence limits were determined by fitting the untransformed and transformed (i.e., growth data as percent reduction transformed to probit, concentrations transformed to logs concentration) data to a best fit linear regression curve based on least squares. Thus, a total of four linear regression curves were computed. The regression line which provided the best fit of the untransformed or transformed data was selected based on the highest associated coefficient of determination, i.e., $r^2$. This regression equation was then applied to calculate the $EC_{50}$ and its 95 percent confidence limits, using the method of inverse prediction. Results reported are based on the mean measured concentrations of the test material.

The no-observed-effect concentration (NOEC) was determined by subjecting the biological response (shell growth) data to analysis of variance and Williams' Test. The highest test concentration causing no significant reduction of shell growth was identified as the NOEC.

12. REPORTED RESULTS: Water quality was unaffected by test concentrations of amitraz 20 EC and was satisfactory for the survival and growth of the test organisms. Mean measured concentrations were: <10, 26, 41, 86, and 140 ug/L, a.i., amitraz EC, which represented a range of 29 to 36 percent of nominal concentrations. The lowest nominal concentration of 52 ug/L was below detectable levels after the 0 hour sampling. No precipitate of test material was observed

during the test in the diluter cells or the exposure aquaria. Substantial degradation of amitraz 20 EC (a.i.) was indicated over time and in the presence of the biological activity in the exposure aquaria.

During the test period, oysters exposed to mean measured concentration $\geq$ 86 ug/L exhibited reduced feeding and fecal production. Only one mortality occurred in the test population during the 96-hour exposure. The growth response data for all mean measured concentrations $\geq$ 86 ug/L (reviewer believes this to be a typographical error and that it should read $\geq$ 26) and control were subjected to analysis of variance and Williams' test. Biological data for the lowest treatment level were not included in the analysis of variance, or in the $EC_{50}$ calculation, as no mean measured concentration could be established. The mean shell growth of oysters exposed to a mean measured concentration of 41 ug/L (a.i) amitraz EC was not significantly different ($p \leq 0.05$) than the control oyster growth, which established 41 ug/L as the NOEC. The shell growth of oysters exposed to amitraz 20 EC concentration $\geq$ 86 ug/L (a.i.) was significantly reduced when compared to control growth [Table 2 (reviewer believes this to be a typographical error and that it should read Table 3) and Figure 2, attached]. The 96-hour $EC_{50}$ calculated by linear regression, was 85 ug/L (a.i.). No 95 percent confidence limit could be calculated due to the shallow nature of the dose response curve. Based on EPA (1985) criteria, the test material would be classified as very highly toxic to oysters.

13. STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES: No conclusions were presented by the author. Statements were included in the report regarding compliance with Good Laboratory Practices and data audits.

14. REVIEWER'S DISCUSSION AND INTERPRETATION OF STUDY RESULTS:
A. Test Procedure: In general, test procedures described by the author follow acceptable Guidelines with the following deviations:

- Test temperature was measured daily; Guidelines require that test temperature be measured hourly.

- The protocols referenced in the Guidelines require that a flow-through system provide 5 L of test solution per oyster per hour. This is typically understood to mean under once-through "flow-through" conditions. The author achieved the flow per oyster per hour by



37

recirculating the contents of each aquarium (under lower flow-through conditions) and by supplementing the nutrient content of natural seawater.

- Raw biological data was not provided, and it is required by the guidelines.

- Due to the apparent solubility in water of the formulated product tested, and the low solubility of amitraz technical, an inerts solvent control should have been included as part of the study design.

B. Statistical Analysis: The reviewer could not conduct statistical analysis in order to validate the results submitted by the author, because the raw biological data were not provided.

C. Discussion/Results: This study is scientifically sound. The Guideline deviations noted are not believed to have affected the test results, as the high water flow per oyster is needed to ensure appropriate nutrition and growth; both of which were achieved by this test design. The lack of biological raw data prevented the reviewer from completing this report review.

D. Adequacy of the Study:
(1) Classification: Supplemental.

(2) Rational: Raw biological data is required in order to validate test results.

(3) Repairability: Yes. Raw biological data should be submitted to allow reviewer to conduct complete statistical analyses.

15. COMPLETION OF ONE-LINER: Yes, December 15, 1988.



38

Figure 2.

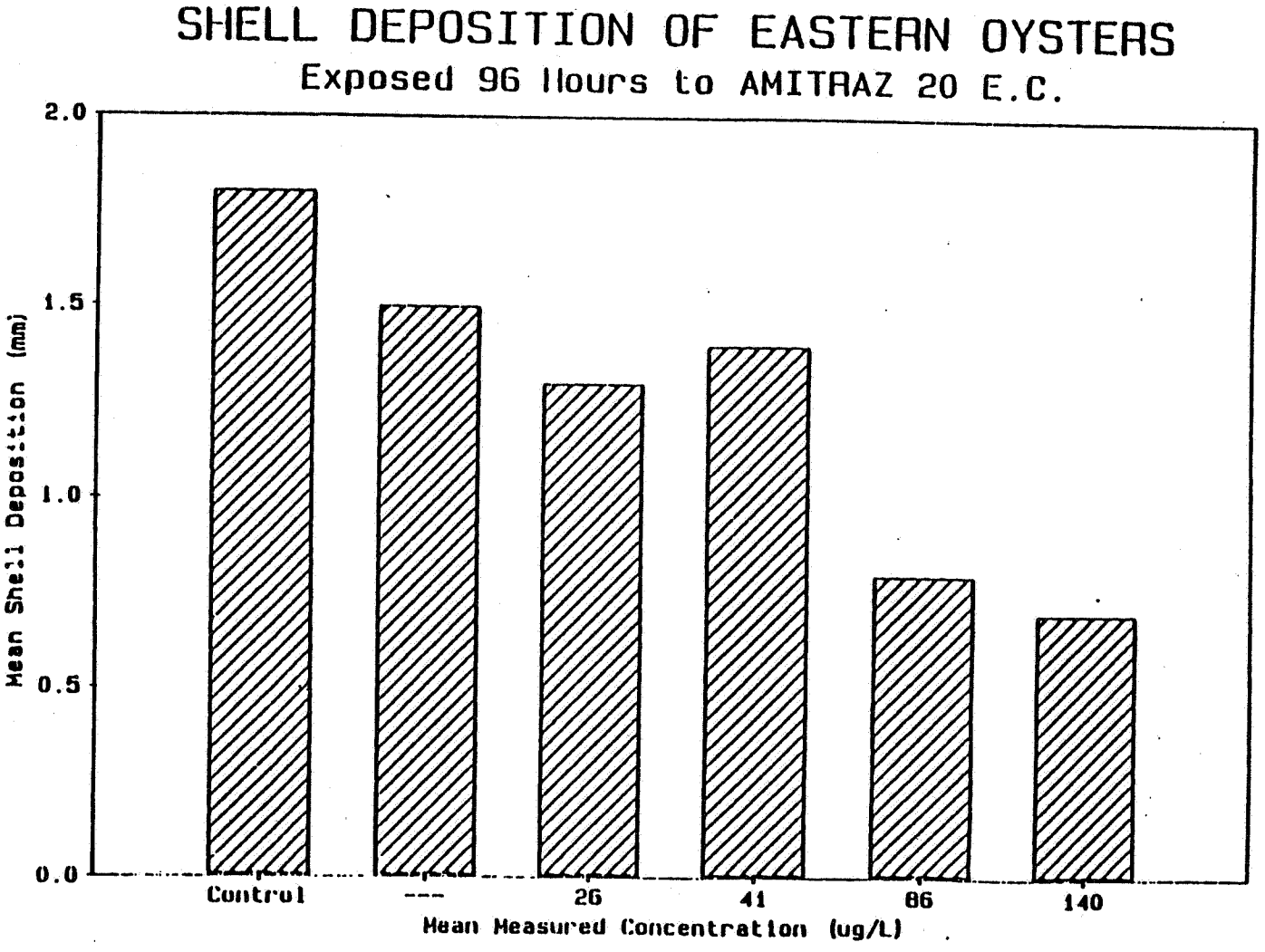
SHELL DEPOSITION OF EASTERN OYSTERS
Exposed 96 Hours to AMITRAZ 20 E.C.
Mean Shell Deposition (mm)
2.0
1.5
1.0
0.5
0.0
Control
---
26
41
86
140
Mean Measured Concentration (ug/L)

Table 3. Effects of Amitraz 20 E.C. (A.I.) on shell deposition of Eastern oysters (Crassostrea virginica) after 96 hours of exposure.

| Mean Measured Concentration (µg/L) | Mean (Standard Deviation) Shell Deposition[a] (mm) | Percentage reduction |
|---|---|---|
| 140 | 0.7 (0.1) | 61 |
| 86 | 0.8 (0.1) | 56 |
| 41 | 1.4 (0.1) | 22 |
| 26 | 1.3 (0.2) | 33 |
| <10[c] | 1.6 (0.4) | 17[d] |
| Control | 1.8 (0.4) | NA[e] |

[a] The mean represents the measurements of 40 oysters per treatment level.

[b] Percentage reduction of shell growth compared to control response.

[c] Nondetectable level.

[d] Biological data not included in the EC50 calculation or analysis of variance.

[e] Not applicable.

Accession Number 407805-10

# DATA EVALUATION RECORD

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

2. **TEST MATERIAL:** W93 AMITRAZ 20 EC Formulation code BX CR20855/3. Amitraz content 179.5 g/L. The formulation was a straw colored clear solution. N'-(2,4-dimethylphenyl)-N-[(2,4-dimethylphenyl)-imino-methyl]-N-methylmethanimidamide.

3. **STUDY TYPE:** Saltwater invertebrate acute test.
Species Tested: Mysidopsis bahia.

4. CITATION: Smyth, D.V., M.H.I. Comber, and R.W. Hill. 1988. W93 AMITRAZ 20 EC Formulation: Determination of acute toxicity to mysid shrimp (Mysidopsis bahia). Study Number Q711/G. Conducted by Brixham Laboratory, Brixham, Devon, England. Submitted by NOR-AM Chemical Company, Wilmington, DE. Accession Number 407805-10.

5. **REVIEWED BY:**

Isabel C. Johnson, M.S.
Principal Scientist
KBN Engineering and
Applied Sciences, Inc.

**Signature:**
**Date:**

6. **APPROVED BY:**

Prampimpan Kosalwat, Ph.D.
Staff Scientist
KBN Engineering and
Applied Sciences, Inc.

**Signature:**
**Date:**

for Henry T. Craven, M.S.
Supervisor, EEB/HED
USEPA

Henry T. Craven 12/21/88

**Signature:** John Noles
**Date:** 12/21/88

7. **CONCLUSIONS:** This study is scientifically sound. It fulfills the Guideline requirements for an estuarine organism (shrimp) data requirement. With a reported 96-hour $LC_{50}$ of 0.48 mg/L, amitraz 20 EC formulation is considered highly toxic to Mysidopsis bahia.

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

41

9. **BACKGROUND:** N/A.

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

11. **MATERIALS AND METHODS:**

**A. Test Animals:** The test animals , 0 to 48 hours old at the start of the test, were derived from continuous cultures at Brixham Laboratory. These cultures were established from organisms supplied by SP Engineering, Inc., Salem, Massachusetts, USA, who stated the original source to be the US EPA Laboratory, Narragansett, Rhode Island. The Brixham Laboratory cultures were maintained under the same conditions of temperature, salinity, photoperiod, and diet as described for the test. The culture system was similar to that described by Reitsema and Neff (1980). No disease treatments were employed on the test organisms or the cultures from which they were obtained, and no disease symptoms were observed.

**B. Test System:** The apparatus used in this study was a continuous flow-through system. The test vessels were rectangular tanks, of 14 liters working volume, constructed of glass and silicone rubber sealant. Each vessel contained 4 retention chambers, constructed from glass beakers of 100 mm diameter and 500 ml working volume, with a window cut in the side which was covered by nylon mesh. Each chamber had a loose-fitting glass lid.

The test vessels drained automatically to approximately one-third of working volume every 100 minutes, to ensure exchange of test solution between vessel and retention chambers. No aeration was used in the exposure vessels. Dilution water flows of 500 ml/min to each vessel were obtained by use of a fixed aperture outlets from a constant head. The test substance stock solutions were pumped peristaltically from their glass vessels, via vinyl tubing, to mix with the dilution water in glass chambers of approximately 0.4 liter working volume, and through glass lines to the test vessel. The control vessel received dilution water only. Natural seawater, obtained by continuous pumping from Tor Bay, Devon, was used as dilution water after addition of dechlorinated mains supply freshwater to adjust the salinity to 20 $\pm$ 2 ppt. Prior to mixing, both water supplies were filtered to a nominal 1 um level, including passage through an activated carbon element. Seawater, prior to mixing with freshwater, characteristics included pH range from 8.1 to 8.2, and salinity of 35 ppt.

C. **Dosage:** Ninety-six-hour flow-through acute test.

D. **Design:** Twenty mysids were randomly allocated to each concentration, distributed to give 5 mysids in each of the 4 retention chambers within each vessel. The position of the control and treatment vessels were randomly allocated with the test system. During the exposure the mysids were fed Artemia nauplii, hatched from commercially available dried cysts (San Francisco Brand, Inc.). Each chamber was fed daily with 2 ml from a 1 liter suspension of Artemia, derived from approximately 8 g of dried cysts. The following nominal single test exposure concentrations were used in this study: 3.2, 1.8, 1.0, 0.56, 0.32, 0.18 and 0.10 mg/L amitraz 20 EC formulation and a seawater control. The temperature of the test solutions was maintained at 25 $\pm$ 1 °C by control of the dilution water temperature. The photoperiod was controlled to provide 14 hours light and 10 hours darkness, with gradual transition periods of approximately 15 minutes. Daily pH, dissolved oxygen (DO), and temperature readings were conducted in all test chambers in which surviving mysids were found. Daily dilution water quality measurements were taken for pH, temperature and salinity. In addition, the temperature of the control test solution was measured at hourly intervals using an automatic recording system. Chemical concentrations were measured at the 24-, 48-, 72-, and 96-hour exposure period, in the control and all treatments.

E. **Statistics:** All $LC_{50}$ values were calculated using Stephan's computerized method. A phillips plotter was used to draw the dose response curve.

12. **REPORTED RESULTS:** The mean measured values of amitraz 20 EC formulation ranged from 21 to 54 percent of nominal values. The losses of amitraz in this study are thought to be due to adsorption, non-homogeneity in solution, precipitation, and to settlement of a proportion of the formulated product. Survival is summarized in the next table:

| Mean Measured Concentration (mg/L) | Surviving mysids 24 Hours | 48 Hours | 72 Hours | 96 Hours |
|---|---|---|---|---|
| Control | 20 | 20 | 20 | 20 |
| <0.05 | 20 | 20 | 20 | 20 |
| <0.06 | 20 | 20 | 20 | 20 |
| 0.07 | 20 | 19 | 19 | 19 |
| 0.12 | 19 | 19 | 19 | 19 |
| 0.39 | 19 | 19 | 18 | 16 |
| 0.74 | 20 | 12 | 10 | 6 |
| 1.72 | 7 | 0 | 0 | 0 |

The computation of the $LC_{50}$ values were undertaken on the five highest concentrations tested as the levels of amitraz in the two lowest concentrations were found to be near the limit of analytical detection in the study. On three occasions during the study, blockages occurred in the dosing system (1.8 mg/L and 1.0 mg/L). These were caused by precipitation of the test substance. However, the analytical measurements determined indicated that these blockages did not constitute a major problem in this study. The 96-hour $LC_{50}$ value as amitraz 20 EC formulation, based on mg/L mean measured concentration was 0.48 mg/L with 95 percent confidence limits of 0.37 and 0.65 mg/L. These values were calculated using the Moving Average Method. The no observed effect concentration was determined to be 0.12 mg/L amitraz 20 EC formulation. Test temperature was 25 ± 1 °C.

13. **STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:** The 96-hour $LC_{50}$ value obtained in this study for amitraz 20 EC formulation was 0.48 mg/L based on mean measured concentrations. The compound would be classified as highly toxic according to the relevant standard evaluation procedure. "This report has been audited in accordance with ICI's policies and procedures for Good Laboratory Practice."

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

A. **Test Procedure:** Overall the test procedures appear to be scientifically sound, but several deviations from the Guidelines were noted and are discussed below:

- The percent active ingredient of the formulation was not reported. However, it was noted elsewhere among other submitted studies that sample batch, code BX CR20855/3, consisted of 20% a.i.

- Although the chemical test concentrations were measured daily in all treatment, they were not measured at test initiation.

- Vinyl tubing was used in the diluter system. This material may absorb the test chemical, thus it is not recommended.

- The test was conducted at 20 ppt, it is recommended that when testing euryhaline species that the salinity be between 10 and 17 ppt.

- The test was conducted at 25 $\pm$ 1 °C, it is recommended that the test be conducted at 22 $\pm$ 1 °C.

- Dechlorinated freshwater was used to dilute the natural seawater; the use of dechlorinated water is discouraged by the Guidelines.

- The photoperiod used was 14 hours light : 10 hours darkness; the Guidelines recommend that a 16 : 8 photoperiod be used.

- Blockages of the diluter system were reported (three times); the report should have specified when each blockage occurred and for how long. This information would allow the reviewer to determine the effect, if any, of this anomaly.

- A typographical error was found in the Conclusions section of the report; the 96-hour $LC_{50}$ is reported as 0.48 ug/L instead of mg/L.

- Due to the lack of solubility of the technical grade amitraz, and the apparent solubility of the formulation, an additional inerts control should have been included.

B. **Statistical Analysis:** The reviewer recalculated the 96-hour $LC_{50}$ value and obtained similar results (attached).

C. **Discussion/Results:** The study appears to be scientifically sound albeit the deviations found in this study. With a 96-hour $LC_{50}$ of 0.48 mg/L Amitraz 20 EC formualtion, this formulation is considered highly toxic to mysids.

D. **Adequacy of the Study:**
(1) **Classification:** Core.

(2) **Rationale:** Guideline fulfillment.

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

15. **COMPLETION OF ONE-LINER:** Yes, December 5, 1988.

Accession No. 407805-11

**DATA EVALUATION RECORD**

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

2. **TEST MATERIAL:** A combination of the following substances:
1) Sample Q559: Amitraz technical code BTS 27 419, BX CR18645/1 analytical reference No. T00255, purity 98.8% w/w, a white powder.
2) Sample Q1048: Amitraz [ring-U-$^{14}$C], Batch CFQ 4458//RP1, specific activity 142uCi/mg (5.25 MBq/mg), radiochemical purity 96.3%.

3. **STUDY TYPE:** Daphnia magna Life-Cycle (21-Day Renewal) Chronic Toxicity Test.

4. **CITATION:** Thompson, R.S. et al. 1988. W97 Amitraz Technical: Determination of Chronic Toxicity to Daphnia magna. Study No. ENVIR/79L. Prepared by ICI Brixham Laboratory, ICI PLC, Devon, England. Submitted by NOR-AM Chemical Company, Wilmington, DE. EPA Accession No. 407805-11.

5. **REVIEWED BY:**
Prapimpan Kosalwat, Ph.D.
Staff Toxicologist
KBN Engineering and
Applied Sciences, Inc.

**Signature:** P. Kosalwat
**Date:** Dec. 5, 1988

6. **APPROVED BY:**
Isabel C. Johnson, M.S.
Principal Scientist
KBN Engineering and
Applied Sciences, Inc.

**Signature:** Isabel C. Johnson
**Date:** Dec. 5, 1988

for Henry T. Craven, M.S. Henry T. Craven 12/20/88
Supervisor, EEB/HED
USEPA

**Signature:** John Noles
**Date:** 12/20/88

7. **CONCLUSIONS:** This study is scientifically sound but does not fulfill the guideline requirements for Daphnia magna life cycle test. Based on the most sensitive parameter (length), the MATC and NOEC values of Amitraz Technical for Daphnia magna were determined to be lower than 0.02 mg/L nominal concentration. A more precise MATC value could not be determined due to reduction in growth observed at all test levels.

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



47

9. BACKGROUND:

10. DISCUSSION OF INDIVIDUAL TESTS: N/A.

11. MATERIALS AND METHODS:

A. Test Animals: Daphnia magna used in the test were obtained from continuous cultures at the Brixham Laboratory. The stock cultures were maintained in a reconstituted water medium, identical to the test dilution water, at a temperature of 20 ± 2°C, and a photoperiod of 16 hours light: 8 hours dark. The cultures were fed a defined diet of algae (Chlorella vulgaris) and yeast.

Less than 24-hour-old Daphnia, obtained from a single culture vessel, were used for testing. The parent animals were 25 ± 1 days old and had been maintained with renewal of the culture medium three times per week since birth. No disease treatments were employed on the test organisms or the culture from which they were obtained, and no disease symptoms had been observed.

B. Test System: Borosilicate glass beakers of 250 ml nominal capacity, containing 200 ml of test solution, were used as test vessels, with loose fitting rigid plastic lids. Temperature was maintained at 20 ± 1°C by control of the room temperature. A photoperiod of 16 hours light: 8 hours dark was provided at an average intensity of 412 Lux. The test solutions were not aerated.

Samples of test substances Q559 and Q1048 were dissolved in hexane. The hexane was then evaporated off and the residue redissolved in triethylene glycol to provide a nominal concentration of 3200 mg Amitraz/L. Sodium hydroxide was added to the stock solution to maintain the stability of Amitraz. Reconstituted water was used as the dilution water for testing.

C. Dosage: 21-day renewal chronic test.

D. Design: Five nominal test concentrations (i.e., 0.02, 0.04, 0.08, 0.16, 0.32 mg Amitraz/L) with 10 vessels per treatment were employed in the test. Also included in the study were 10 vessels of the control (containing only dilution water) and 10 vessels of the solvent control (containing dilution water and 0.1 mL

triethylene glycol/L). For each concentration and control, seven vessels contained one daphnid each (for survival, growth and reproduction measurements), and the remaining 3 vessels contained 5 daphnids each (for survival study only). The positions of the test treatments were randomly allocated within the test area.

The test was initiated when <24 hour old daphnids were randomly assigned to each vessel. Mortalities of the $P_o$ generation (i.e., first generation) was recorded daily. Mortality was defined as absence of any movement by the organism, when examined by eye, for a period of 15 seconds. Other symptoms of toxicity observed were also recorded. Observations were made daily from day 6 for the presence of offspring (termed the $F_1$ generation) in each vessel.

The test solutions were prepared on the day of use and were renewed every 2 days. Samples of each old and new solution were analyzed by radiochemistry for Amitraz on each renewal occasion. On four occasion, a sample of each new solution were analyzed by liquid chromatography for Amitraz and the metabolites BTS 27 919 (Q315). In addition, samples from one replicate of the corresponding old solutions (2 days later) were also analyzed.

Live and dead $F_1$ generation in each of the 7 individual animal vessels on each renewal day were recorded and removed from the vessels. At the end of the test, the length (apex of helmet to base of spine) of each surviving $P_0$ (individual) Daphnia was measured. During the test, Daphnia were fed with cultured algae (Chlorella vulgaris) and yeast at the rate of $1.2 \times 10^8$ algal cells and 1.0 mg dried yeast per vessel every 2 days (i.e., on renewal days).

The temperature and dissolved oxygen concentration (d.o.) of the dilution water were measured for each set of test solutions prepared. The pH of each newly prepared test solution was measured using the excess remaining after filling the test vessels. The pH, temperature, and d.o. of one replicate of the old test solutions were measured after transfer of the $P_o$ generation. Temperature was measured at hourly intervals in an additional replicate vessel (without Daphnia). The hardness, alkalinity, and conductivity of the highest remaining test substance concentration were measured once per week.

E. Statistics: A contingency table (exact test) procedure was used to analyze mortality data. The mean lengths as well as reproduction (number of offsprings produced) of the control and solvent control were compared using t-test analysis. The test substance treatments (for mean lengths and reproduction) were compared (one-sided) with the control and the solvent control using analysis of variance with Dunnett's procedure.

12. REPORTED RESULTS: The ranges pH, d.o., and temperature (thermometer) during the 21-day test period were 7.74-8.30, 7.3-9.4 mg/L, and 19.5-20.7°C, respectively. The overall range of the temperatures recorded automatically at hourly intervals was 19.9-20.8°C. Other water quality parameters were presented in Table 4 (attached).

The means of the new solutions measured by radiochemistry ranged from 94 to 110% of the nominal values. Excluding the highest nominal concentrations tested (0.32 mg/L), the old solutions ranged from 93 to 100% of nominal, indicating that Amitraz or its metabolites remained in solution over the 2-day period between solution renewals. At 0.32 mg/L, the radiochemical concentration had declined to 73% of nominal. By HPLC analysis, the mean Amitraz concentrations of the new solutions ranged from 69 to 90% of the nominals. The old solutions had declined to 31 to 45% of the nominals. A large proportion of the decline in the Amitraz concentration was attributed to the increase in the metabolite BTS 27 919; with the exception of the highest nominal concentration, the mean measured concentration of BTS 27 919 in the old solutions was equal to or greater than the mean measured Amitraz concentration.

Mortality after 21 days was zero in the controls and 4.5% (1 dead of 22 daphnids tested) in the solvent control. Mortalities exceeded 40% after 21 days at nominal concentrations $\geq$ 0.04 mg/L, and were significantly ($p = 0.05$) greater than that of the solvent control (Table 1, attached). There was no effect on survival at the lowest nominal concentration tested, 0.02 mg/L.

The mean lengths of the control and the solvent control were not significantly different. The mean lengths of the Daphnia at all concentrations tested were significantly ($p = 0.05$) less than that of either control (Table 2, attached). Therefore, it was not considered necessary to pool the control and solvent control for further comparison. It was concluded that the no-observed-effect concentration (NOEC) for length was <0.02 mg/L nominal.

No offsprings ($F_1$) were produced at the highest concentration tested (0.32 mg/L nominal) in which all $P_o$ were dead by day 8. All other treatments produced $F_1$. The first release of $F_1$ was observed on day 7, except at a nominal concentration of 0.16 mg/L in which the first release was on day 8. The numbers of $F_1$ produced by the control and solvent control daphnids were not significantly different ($p = 0.05$). Only reproduction (number of $F_1$ produced) of $P_o$ Daphnia surviving to the end of the test were compared to those of the control and solvent control. $F_1$ per $P_o$ was significantly reduced at all test concentrations compared with both the control and solvent control (Table 3, attached). It was concluded that the NOEC for Daphnia reproduction was <0.02 mg/L nominal.

13. STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES: Reproduction and length of Daphnia magna was affected at the lowest concentration tested. Therefore, the NOEC and the maximum acceptable toxicant concentration (MATC) for Amitraz Technical were <0.02 mg/L nominal concentration or <0.014 mg/L mean measured concentration.

The study was reported as being conducted in accordance with Good Laboratory Practice Standards as detailed in U.S. EPA, Title 40 Code of Federal Regulations Part 160, Federal Register, 29 November 1983, and Organization for Economic Co-operation and Development ISBN 92-64-12367-9, Paris 1982.

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

A. Test Procedure: The test procedures generally follow the SEP guidelines. However, the range of concentrations tested did not include a no-observed-effect concentration (NOEC).

B. Statistical Analysis: Mortality, length, and reproductive data of the first generation of daphnids were reanalyzed by the reviewer using analysis of variance with six tests (see attached printouts). Since there was only one replicate set of test animals per concentration and control, for mortality data analysis, each test animal was considered a "unit" and was assigned numbers "1" for survival and "0" for death in the analysis of variance.

The authors analyzed the reproductive data using total number of offspring (young) each daphnid produced from day 8 to day 21 (test termination). However, several daphnids died before the end of the test. Therefore, comparing total number of young/adult between treatment and control may not be appropriate. A more accurate way

would be to compare the reproductive rates (i.e., number of young/adult/reproduction day) of daphnids at each treatment level and the controls. Among the six tests chosen, Bon-ferroni's was probably the best test for length and reproductive data due to unequal numbers of observations (some first generation daphnids died before test termination or before reaching reproductive age). The results obtained were similar to those performed by the authors' and could be summarized as follows:

| Nominal Concentration (mg/L) | Mortality (%) | Length (mm) | # Young/adult/rep.day |
|---|---|---|---|
| Water Control | 0 | 4.85 | 17.6 |
| Solvent Control | 4.5 | 4.86 | 18.3 |
| 0.02 | 4.5 | $4.46^{ab}$ | 14.4 |
| 0.04 | $45.5^{ab}$ | $4.45^{ab}$ | $12.5^{ab}$ |
| 0.08 | $40.9^{ab}$ | $4.47^{ab}$ | $9.4^{ab}$ |
| 0.16 | $72.7^{ab}$ | $4.09^{ab}$ | $5.2^{ab}$ |
| 0.32 | $100.0^{ab}$ | - | - |

[a] = significantly different from water control ($p \leq 0.05$).
[b] = significantly different from solvent control ($p \leq 0.05$).
- = all test daphnids died before test termination and before reaching reproductive age.

C. Discussion/Results: Amitraz Technical concentrations of $\geq$ 0.04 mg/L significantly ($p \leq 0.05$) affected mortality of the first generation of daphnids, while all concentrations tested significantly reduced the growth (length) of test daphnids when compared to both dilution water control and solvent control. When the reproductive rates were compared, only Newman-Keuls' and T-test's showed that daphnids in the dilution water control produced more young than those in test concentration 0.02 mg/L. All tests showed that test concentrations $\geq$ 0.04 mg/L significantly reduced the reproductive rates of Daphnia magna.

Therefore, based on the most sensitive parameter (i.e., length), the MATC and NOEC values were lower than 0.02 mg/L nominal concentration of Amitraz Technical.

D. Adequacy of the Study:

(1) Classification: Supplemental.

(2) Rationale: The MATC could not be calculated due to the reduction in growth observed at all test levels.

(3) Repairability: No.

15. COMPLETION OF ONE-LINER: Yes, December 5, 1988.

Mortality 1 = Survival
0 = death.

Analysis of Variance File: AMITMORT Date: 02-01-1988

FILTER: None

N's, means and standard deviations based on dependent variable: ~~SURV~~ MORT

* Indicates statistics are collapsed over this factor

| Factors: C | N | Mean | S.D. |
|---|---|---|---|
| * | 154 | 0.6169 | 0.4877 |
| 1 | 22 | 1.0000 | 0.0000 |
| 2 | 22 | 0.9545 | 0.2132 |
| 3 | 22 | 0.9545 | 0.2132 |
| 4 | 22 | 0.5455 | 0.5096 |
| 5 | 22 | 0.5909 | 0.5032 |
| 6 | 22 | 0.2727 | 0.4558 |
| 7 | 22 | 0.0000 | 0.0000 |

---

Fmax for testing homogeneity of between subjects variances: Not defined

---

Analysis of Variance Dependent variable: ~~SURV~~ MORT

| Source | df | SS (H) | MSS | F | P |
|---|---|---|---|---|---|
| Between Subjects | 153 | 36.3961 | | | |
| C (CONC) | 6 | 19.3507 | 3.2251 | 27.813 | 0.0000 |
| Subj w Groups | 147 | 17.0455 | 0.1160 | | |

Analysis of Variance File: AMITMORT Date: 02-01-1988

FILTER: None

Post-hoc tests for factor C (CONC)

| Level | Mean | Level | Mean |
|---|---|---|---|
| 1 | 1.000 | 6 | 0.273 |
| 2 | 0.955 | 7 | 0.000 |
| 3 | 0.955 | | |
| 4 | 0.545 | | |
| 5 | 0.591 | | |

| Comparison | Scheffe' | Tukey-A* | Tukey-B* | Newman-Keuls* | Bon-ferroni | T-test | Dunnett |
|---|---|---|---|---|---|---|---|
| 1 > 2 | | | | | | | |
| 1 > 3 | | | | | | | |
| 1 > 4 | 0.0048 | 0.0100 | 0.0100 | 0.0100 | 0.0005 | 0.0000 | 0.0100 |
| 1 > 5 | 0.0181 | 0.0100 | 0.0100 | 0.0100 | 0.0024 | 0.0001 | 0.0100 |
| 1 > 6 | 0.0000 | 0.0100 | 0.0100 | 0.0100 | 0.0000 | 0.0000 | 0.0100 |
| 1 > 7 | 0.0000 | 0.0100 | 0.0100 | 0.0100 | 0.0000 | 0.0000 | 0.0100 |
| 2 = 3 | | | | | | | N.A. |
| 2 > 4 | 0.0181 | 0.0100 | 0.0100 | 0.0100 | 0.0024 | 0.0001 | N.A. |
| 2 > 5 | | 0.0500 | 0.0100 | 0.0100 | 0.0115 | 0.0005 | N.A. |
| 2 > 6 | 0.0000 | 0.0100 | 0.0100 | 0.0100 | 0.0000 | 0.0000 | N.A. |
| 2 > 7 | 0.0000 | 0.0100 | 0.0100 | 0.0100 | 0.0000 | 0.0000 | N.A. |
| 3 > 4 | 0.0181 | 0.0100 | 0.0100 | 0.0100 | 0.0024 | 0.0001 | N.A. |
| 3 > 5 | | 0.0500 | 0.0100 | 0.0100 | 0.0115 | 0.0005 | N.A. |
| 3 > 6 | 0.0000 | 0.0100 | 0.0100 | 0.0100 | 0.0000 | 0.0000 | N.A. |
| 3 > 7 | 0.0000 | 0.0100 | 0.0100 | 0.0100 | 0.0000 | 0.0000 | N.A. |
| 4 < 5 | | | | | | | N.A. |
| 4 > 6 | | | 0.0500 | 0.0100 | | 0.0088 | N.A. |
| 4 > 7 | 0.0002 | 0.0100 | 0.0100 | 0.0100 | 0.0000 | 0.0000 | N.A. |
| 5 > 6 | | 0.0500 | 0.0500 | 0.0100 | 0.0491 | 0.0023 | N.A. |
| 5 > 7 | 0.0000 | 0.0100 | 0.0100 | 0.0100 | 0.0000 | 0.0000 | N.A. |
| 6 > 7 | | | 0.0500 | 0.0100 | | 0.0088 | N.A. |

* The only possible P-values are .01, .05 or .10 (up to 0.0500).
A blank means the P-value is greater than 0.0500.

For Dunnett's test only the P-values .05 and .01 are possible
and only for comparisons with the control mean (level 1).

Growth (Length) in mm.

Analysis of Variance File: amitleng Date: 02-01-1988

FILTER: None

N's, means and standard deviations based on dependent variable: LENGTH

* Indicates statistics are collapsed over this factor

| Factors: C | N | Mean | S.D. |
|---|---|---|---|
| * | 30 | 4.6047 | 0.2752 |
| 1 | 7 | 4.8500 | 0.0885 |
| 2 | 7 | 4.8586 | 0.0840 |
| 3 | 7 | 4.4586 | 0.0934 |
| 4 | 4 | 4.4450 | 0.1542 |
| 5 | 2 | 4.4650 | 0.1202 |
| 6 | 3 | 4.0867 | 0.0115 |

Fmax for testing homogeneity of between subjects variances: 178.26
Number of variances= 6 df per variance= 3.

Analysis of Variance Dependent variable: LENGTH

| Source | df | SS (H) | MSS | F | P |
|---|---|---|---|---|---|
| Between Subjects | 29 | 2.1955 | | | |
| C (CONC) | 5 | 1.9680 | 0.3936 | 41.507 | 0.0000 |
| Subj w Groups | 24 | 0.2276 | 0.0095 | | |

Post-hoc tests for factor C (CONC)

| Level | Mean | Level | Mean |
|---|---|---|---|
| 1 | 4.850 | 6 | 4.087 |
| 2 | 4.859 | | |
| 3 | 4.459 | | |
| 4 | 4.445 | | |
| 5 | 4.465 | | |

| Comparison | Scheffe' | Tukey-A* | Tukey-B* | Newman -Keuls* | Bon-ferroni | T-test | Dunnett |
|---|---|---|---|---|---|---|---|
| 1 < 2 | | | | | | | |
| 1 > 3 | 0.0000 | 0.0100 | 0.0100 | 0.0100 | 0.0000 | 0.0000 | 0.0100 |
| 1 > 4 | 0.0001 | 0.0100 | 0.0100 | 0.0100 | 0.0000 | 0.0000 | 0.0100 |
| 1 > 5 | 0.0032 | 0.0100 | 0.0100 | 0.0100 | 0.0009 | 0.0001 | 0.0100 |
| 1 > 6 | 0.0000 | 0.0100 | 0.0100 | 0.0100 | 0.0000 | 0.0000 | 0.0100 |
| 2 > 3 | 0.0000 | 0.0100 | 0.0100 | 0.0100 | 0.0000 | 0.0000 | N.A. |
| 2 > 4 | 0.0001 | 0.0100 | 0.0100 | 0.0100 | 0.0000 | 0.0000 | N.A. |
| 2 > 5 | 0.0025 | 0.0100 | 0.0100 | 0.0100 | 0.0007 | 0.0000 | N.A. |
| 2 > 6 | 0.0000 | 0.0100 | 0.0100 | 0.0100 | 0.0000 | 0.0000 | N.A. |
| 3 > 4 | | | | | | | N.A. |
| 3 < 5 | | | | | | | N.A. |
| 3 > 6 | 0.0008 | 0.0100 | 0.0100 | 0.0100 | 0.0000 | 0.0000 | N.A. |
| 4 < 5 | | | | | | | N.A. |
| 4 > 6 | 0.0041 | 0.0100 | 0.0100 | 0.0100 | 0.0011 | 0.0001 | N.A. |
| 5 > 6 | 0.0138 | 0.0100 | 0.0100 | 0.0100 | 0.0044 | 0.0003 | N.A. |

* The only possible P-values are .01, .05 or .10 (up to 0.0500).
A blank means the P-value is greater than 0.0500.

For Dunnett's test only the P-values .05 and .01 are possible and only for comparisons with the control mean (level 1).

Reproduction
# youngs/adult/reprod. day

Analysis of Variance File: amityad Date: 02-04-1988

FILTER: None

N's, means and standard deviations based on dependent variable: YAD

* Indicates statistics are collapsed over this factor

| Factors: C | N | Mean | S.D. |
|---|---|---|---|
| * | 41 | 13.0927 | 5.0463 |
| 1 | 7 | 17.6286 | 1.2244 |
| 2 | 7 | 18.3000 | 1.2369 |
| 3 | 7 | 14.3857 | 1.0074 |
| 4 | 7 | 12.4571 | 3.3004 |
| 5 | 7 | 9.4429 | 4.1956 |
| 6 | 6 | 5.2167 | 1.3819 |

---

Fmax for testing homogeneity of between subjects variances: 17.35
Number of variances= 6 df per variance= 6.

---

Analysis of Variance Dependent variable: YAD

| Source | df | SS (H) | MSS | F | P |
|---|---|---|---|---|---|
| Between Subjects | 40 | 1018.5876 | | | |
| C (CONC) | 5 | 813.8025 | 162.7605 | 27.818 | 0.0000 |
| Subj w Groups | 35 | 204.7851 | 5.8510 | | |

---

Post-hoc tests for factor C (CONC)

| Level | Mean | Level | Mean |
|---|---|---|---|
| 1 | 17.629 | 6 | 5.217 |
| 2 | 18.300 | | |
| 3 | 14.386 | | |
| 4 | 12.457 | | |
| 5 | 9.443 | | |

| Comparison | Scheffe' | Tukey-A* | Tukey-B* | Newman -Keuls* | Bon-ferroni | T-test | Dunnett |
|---|---|---|---|---|---|---|---|
| 1 < 2 | | | | | | | |
| 1 > 3 | | | | 0.0500 | | 0.0169 | |
| 1 > 4 | 0.0174 | 0.0100 | 0.0100 | 0.0100 | 0.0049 | 0.0003 | 0.0100 |
| 1 > 5 | 0.0000 | 0.0100 | 0.0100 | 0.0100 | 0.0000 | 0.0000 | 0.0100 |
| 1 > 6 | 0.0000 | 0.0100 | 0.0100 | 0.0100 | 0.0000 | 0.0000 | 0.0100 |
| 2 > 3 | | | 0.0500 | 0.0500 | | 0.0046 | N.A. |
| 2 > 4 | 0.0050 | 0.0100 | 0.0100 | 0.0100 | 0.0011 | 0.0001 | N.A. |
| 2 > 5 | 0.0000 | 0.0100 | 0.0100 | 0.0100 | 0.0000 | 0.0000 | N.A. |
| 2 > 6 | 0.0000 | 0.0100 | 0.0100 | 0.0100 | 0.0000 | 0.0000 | N.A. |
| 3 > 4 | | | | | | | N.A. |
| 3 > 5 | 0.0260 | 0.0100 | 0.0100 | 0.0100 | 0.0080 | 0.0005 | N.A. |
| 3 > 6 | 0.0000 | 0.0100 | 0.0100 | 0.0100 | 0.0000 | 0.0000 | N.A. |
| 4 > 5 | | | | 0.0500 | | 0.0256 | N.A. |
| 4 > 6 | 0.0005 | 0.0100 | 0.0100 | 0.0100 | 0.0000 | 0.0000 | N.A. |
| 5 > 6 | | 0.0500 | 0.0500 | 0.0100 | | 0.0034 | N.A. |

* The only possible P-values are .01, .05 or .10 (up to 0.0500).
A blank means the P-value is greater than 0.0500.

For Dunnett's test only the P-values .05 and .01 are possible
and only for comparisons with the control mean (level 1).