RS-DER's
2-24-89

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RECORD NO.

106201
SHAUGHNESSEY NO.

REVIEW NO.

EEB REVIEW

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

DATA ACCESSION NO(S). 407980-01, -02

PRODUCT MANAGER NO. D. Edwards (21)

PRODUCT NAME(S) AMITRAZ

COMPANY NAME Nor-Am Chemical Company

SUBMISSION PURPOSE Submission of data in response to registration standard

SHAUGHNESSEY NO.	CHEMICAL, & FORMULATION	% A.I.

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

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Submission of Acute and Chronic Fish Data in Response to Amitraz Registration Standard

FROM: James W. Akerman, Chief H.T. Craven 2/24/89
Ecological Effects Branch
Environmenal Fate and Effects Division (H7507-C)

TO: Dennis Edwards, PM12
Insecticide-Rodenticide Branch
Registration Division (H7505-C)

The Ecological Effects Branch (EEB) has reviewed the following two fish toxicity studies on Amitraz:

1. Hill, R.W., B. J. Harland, and J.E. Caunter. 1988. W92 AMITRAZ Technical: Determination of acute toxicity to the bluegill sunfish (Lrpomis macrochirus). Study Number Q506/D. Submitted by NOR-AM Chemical Company, Wilmington, DE. Accession Number 407980-01.

2. Hill, R.W., B.J. Harland, B.G. Maddock, and A.M. Riddle. 1988. W99 Amitraz Technical: Determination of the Chronic Tozicity to Fathead Minnow (Pimephales promelas) Embryos and Larvae. Study No. ENVIR/78L. Prepared by ICI Brixham Laboratory, ICI PLC, Devon, Brixham, England. Submitted by NOR-A Chemical Company, Wilmington, DE. EPA Accession No. 407980-02.

The acute toxicity study on bluegill is scientifically sound and fulfills guideline requirements for a warmwater fish species. With a 96-hour LC_{50} of 0.34 mg/L Amitraz technical is highly toxic to freshwater fish.

The chronic toxicity study on fathead minnow is scientifically sound but does not fulfill the guideline requirements for the fish early life stage test. Survival in the solvent control was less than 70% and the range of concentrations tested did not include a no effect level (NOEC < 3.53 mg/L). The fish chronic toxicity study must therefore be repeated.

ug/l (ppb)

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DATA EVALUATION RECORD

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

2. **TEST MATERIAL:** W92 AMITRAZ Technical code BTS 27419. BX CR18645/1 Analytical Reference No. T00255. Purity 98.8 percent w/w. A white powder. N'-(2,4-dimethylphenyl)-N-[(2,4-dimethylphenyl)-imino-methyl]-N-methylmethanimidamide.

3. **STUDY TYPE:** Freshwater fish acute test.
   Species Tested: Lepomis macrochirus.

4. **CITATION:** Hill, R.W., B.J. Harland, and J.E. Caunter. 1988. W92 AMITRAZ Technical: Determination of acute toxicity to the bluegill sunfish (Lepomis macrochirus). Study Number Q506/D. Conducted by Brixham Laboratory, Brixham, Devon, England. Submitted by NOR-AM Chemical Company, Wilmington, DE. Accession Number 407980-01.

5. **REVIEWED BY:**

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

   Signature: Isabel C. Johnson
   Date: December 22, 1988

6. **APPROVED BY:**

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

   Signature: P. Kosalwat
   Date: 12/22/88

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

   Signature: Otto Gustafson
   Date: 2/23/89
   H. Craven 2/24/89

7. **CONCLUSIONS:** This study is scientifically sound and meets the Guideline requirements for a warmwater fish species. With a reported 96-hour LC_{50} of 0.34 mg/L amitraz technical (based on mean measured concentrations), is considered highly toxic to bluegill sunfish. The NOEC is less than 0.22 mg/L amitraz (active ingredient) technical.

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

9. BACKGROUND:

10. DISCUSSION OF INDIVIDUAL TESTS: N/A.

11. MATERIALS AND METHODS:

A. Test Animals: Bluegill sunfish were obtained from S.P. Engineering, Inc., Salem, Massachusetts. No sickness, injury or abnormality was observed in the fish in the two weeks prior to the test. The pretest diet was flaked aquarium 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 last medication given to the fish was a 1 ppm treatment of methylene blue two weeks prior to testing. The fish tested ranged in weight from 0.47 to 0.82 g with a mean weight of 0.63 g. The range in length was 28 to 33 mm with a mean length of 30.9 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 200 ml/minute. A 95 percent exchange of the test solutions was calculated to occur within 4.5 hours. The depth of the test solutions was 37 cm. The stock solutions were fed by a series of B Braum Perfuser VI syringe pumps and a series of peristaltic pumps was used to supply freshwater. Triethylene glycol was the solvent used. The dilution water was supplied from a 20,000 gallon reservoir and the total hardness was measured daily. Dilution water characteristics included pH range from 7.5 to 7.6, conductivity of 120 to 130 umhos/cm, hardness of 38.3 to 45.3 mg/L as $CaCO_3$, alkalinity of 24.4 to 27.7 mg/L as $CaCO_3$, and temperature of 14 °C. 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: 3.2, 1.8, 1.0, 0.56 and 0.32 mg/L amitraz technical, a freshwater control, and a solvent control. The 0.32, 0.56, 1.0, and 1.8 mg/L amitraz concentrations contained 100 uL/L trigol. The 3.2 mg/L amitraz and the solvent control contained 250 uL/L trigol. 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 and temperature. 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 technical ranged from 53 to 76 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 bluegill sunfish			
	24 Hours	48 Hours	72 Hours	96 Hours
Control	20	20	20	20
Solvent Control	20	20	20	20
0.22	20	20	20	20
0.43	20	20	8	2
0.58	20	14	9	1
0.96	15	14	14	14
1.9	5	0	0	0

The general symptoms of toxicity noted in this study were quiescence, turning dark, lying at the bottom of the tank, and loss of balance. The 96-hour LC_{50} value as amitraz technical, based on mg/L mean measured concentration was 0.45 mg/L with 95 percent confidence limits of 0.39 and 0.51 mg/L. These values were calculated using the Moving Average Method. The no-observed-effect concentration was determined to be less than 0.22 mg/L amitraz technical. Test temperature was 22 $\pm$ 1 ^{o}C. The system turnover rate calculated by the reviewer from the report data was approximately 5.3 turnovers per day.

13. STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES: The 96-hour LC_{50} value obtained in this study for amitraz technical was 0.45 mg/L (0.39 - 0.51) 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, the following 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.
- 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.
- Although the chemical test concentrations were measured daily in all test concentrations, they were not measured at test initiation.

B. Statistical Analysis: The reviewer recalculated the 96-hour LC_{50} value and obtained slightly different results of 0.34 mg/L amitraz technical (0.29 - 0.38). This difference is not significant. The reviewer's results are attached.

C. Discussion/Results: This study is scientifically sound and meets the Guideline requirements for a warmwater freshwater fish. With a 96-hour LC_{50} of 0.34 mg/L Amitraz technical is considered highly toxic to bluegill sunfish.

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

(2) Rationale: N/A.

(3) Repairability: N/A.

15. COMPLETION OF ONE-LINER: Yes, December 22, 1988.

ISABEL C. JOHNSON ANITRAZ TECHNICAL LEPOMIS MACROCHIRUS 12-22-88

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CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
1.9	20	20	100	9.536742E-05
.96	20	6	30	5.765915
.58	20	19	95	2.002716E-03
.43	20	18	90	2.012253E-02
.22	20	0	0	9.536742E-05

Mean measured concentrations

THE BINOMIAL TEST SHOWS THAT .22 AND 1.9 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 .6465292

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS	
2	6.572954E-02	.3391387	.2982791	.3778268

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
5	9.766157	19.55354	0

A PROBABILITY OF 0 MEANS THAT IT IS LESS THAN 0.001.

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

SLOPE = 2.217465
95 PERCENT CONFIDENCE LIMITS =-4.712302 AND 9.147232

LC50 = .4180972
95 PERCENT CONFIDENCE LIMITS = 0 AND +INFINITY

LC10 = .1116281
95 PERCENT CONFIDENCE LIMITS = 0 AND +INFINITY

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No. _____ Chemical Name Amitraz Tech. Chemical Class _____ Page 1 of 1

Study/Species/Lab/ Accession	Chemical % a.i.	Results	Reviewer/ Date	Validati Status
14-Day Single Dose Oral LD_{50}		LD50 = mg/kg (95% C.L.) Contr. Mort.(%)=		
Species	____	Slope= # Animals/Level= Age(Days)= Sex =	____	____
Lab		14-Day Dose Level mg/kg/(% Mortality) ( ), ( ), ( ), ( ), ( )		
Acc.		Comments:		
14-Day Single Dose Oral LD_{50}		LD50 = mg/kg. (95% C.L) Contr. Mort.(%)=		
Species	____	Slope= # Animals/Level= Age(Days)= Sex =	____	____
Lab		14-Day Dose Level mg/kg/(% Mortality) ( ), ( ), ( ), ( ), ( )		
Acc.		Comments:		
8-Day Dietary LC_{50}		LC50 = ppm (95% C.L.) Contr. Mort.(%)=		
Species		Slope= # Animals/Level= Age(Days)= Sex =	____	____
Lab	____	8-Day Dose Level ppm/(%Mortality) ( ), ( ), ( ), ( ), ( )		
Acc.		Comments:		
8-Day Dietary LC_{50}		LC50 = ppm (95% C.L) Contr. Mort.(%)=		
Species		Slope= # Animals/Level= Age(Days)= Sex =	____	____
Lab	____	8-Day Dose Level ppm/(%Mortality) ( ), ( ), ( ), ( ), ( )		
Acc.		Comments:		
48-Hour LC_{50}		LC50 = pp__ (95% C.L.) Contr. Mort.(%)= Sol. Contr. Mort.(%)=		
Species		Slope= # Animals/Level= Temperature =	____	____
Lab	____	48-Hour Dose Level pp /(%Mortality) ( ), ( ), ( ), ( ), ( )		
Acc.		Comments:		
96-Hour LC_{50}		LC50 = 0.34 ppm (95% C.L. 0.30-0.4) Con. Mor(%)= 0 Sol. Con. Mor.(%)= 0	ICJ 12/22/88	Core
Species Lepomis macrochirus 98.8		Slope= NA # Animals/Level= 20 Temp.= 22±1°C		
Lab Brixham Labs		96-Hour Dose Level pp /(%Mortality) 1.9 (100), 0.96 (30), 0.58 (95), 0.43 (90), 0.22 (0)		
Acc. 407980-01		Comments: Mean measured concentrations		
96-Hour LC_{50}		LC50 = pp__ (95% C.L) Con. Mort.(%)= Sol. Con. Mort.(%)=		
Species		Slope= # Animals/Level= Temp.=	____	____
Lab	____	96-Hour Dose Level pp /(%Mortality) ( ), ( ), ( ), ( ), ( )		
Acc.		Comments:		

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DATA EVALUATION RECORD

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

2. **TEST MATERIAL:** Q559: Amitraz technical code BTS 27 419, BX CR18645/1 analytical reference No. T00255, purity 98.8% w/w, a white powder.

3. **STUDY TYPE:** Fish Early Life-Stage Test.
Species Tested: Fathead Minnow (Pimephales promelas)

4. **CITATION:** Hill, R.W., B.J. Harland, B.G. Maddock, and A.M. Riddle. 1988. W99 Amitraz Technical: Determination of the Chronic Toxicity to Fathead Minnow (Pimephales promelas) Embryos and Larvae. Study No. ENVIR/78L. Prepared by ICI Brixham Laboratory, ICI PLC, Devon, Brixham, England. Submitted by NOR-AM Chemical Company, Wilmington, DE. EPA Accession No. 407980-02.

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

Signature: P. Kosalwat
Date: 12/22/88

6. **APPROVED BY:**

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

Signature: Isabel C. Johnson
Date: December 22, 1988

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

Signature:
Date: 2/23/89
H. Craven 2/24/89

7. **CONCLUSIONS:** This study is scientifically sound but does not fulfill the guideline requirements for fish early life-stage test. Based on the most sensitive parameter (weight), the MATC and NOEC values of amitraz technical for Pimephales promelas were determined to be less than 3.53 ug/L mean measured concentration. A more precise MATC value could not be determined due to reduction in weight observed at all test levels.

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

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9. BACKGROUND:

10. DISCUSSION OF INDIVIDUAL TESTS: N/A.

11. MATERIALS AND METHODS:

A. Test Animals: The fathead minnow (Pimephales promelas) embryos used in the test were obtained from brood stock held at the Brixham Laboratory. The fish were originally purchased from SP Engineering Technology, Salem, Massachusetts. The brood stock fish were fed daily on a basic diet of Promin, a proprietary brand of tropical fish food, and brine shrimp. No mortalities were recorded and no therapeutic treatment had been given to this batch of fish during the 14 days prior to the start of the study.

Batches of eggs from the spawnings of at least three females were pooled in a dish filled with dilution water. Each batch was less than 48 hours old, that is up to and including the tail-bud stage. Sets of five eggs were randomly selected, microscopically examined for viability and placed in incubating cups by stratified random assignment. This process was repeated until each cup contained 20 eggs.

After the embryos were distributed in the embryo cups they were treated with a 15-second exposure to malachite green at a concentration of 60 mg/L to prevent possible fungal infection. They were then rinsed with freshwater (dilution water) at 25°C.

B. Test System: The test vessels were of all glass construction, rectangular in shape with dimensions of 30 x 20 x 20 cm and a capacity of 12 liters. The volume used was nominally 9 liters and the water depth in each tank was approximately 15 cm. The incubation cups were made from 8-cm lengths of glass tubing (5-cm o.d.) with nylon mesh (0.47 mm) cemented to the bottom of each cup using silicone sealant. The cups were suspended in the test chambers and oscillated vertically over a distance of approximately 2-5 cm at a rate of 2 oscillations per minute.

The dilution water was fed from an aerated, temperature-controlled constant head tank via flow control devices to glass mixing chambers. The nominal flow rate of the dilution water to each mixing chamber was 300 ml/minute. Each chamber also received the required

amounts of test substance fed by a peristaltic pump. The mixing chambers were fitted with independent magnetic stirrers to ensure adequate mixing of the test solutions. The chambers also acted as flow-splitting devices supplying at least six tank volumes per day to each of two duplicate test vessels. The dosing system was designed so that each replicate tank received 50 ml/minute of the required test solution and a further 200 ml/minute ran to waste.

The dilution water was a dechlorinated freshwater supplied from a 100-m^3 reservoir with an average retention time of 24 hours. After dechlorination with sodium thiosulphate, the water was passed through activated carbon, filtered to 1 micrometer to remove particulate material and preheated to 25°C in a header tank on the test rig.

Alkaline triethylene glycol (TEG) was used as the solvent to prepare test solutions. The test solutions were not aerated during the study. The test was conducted at 25 ± 1°C with a photoperiod of 16 hours light alternating with 8 hours of darkness.

C. Dosage: Thirty-two-day early life stage chronic test.

D. Design: Five nominal concentrations (i.e., 6, 12, 25, 50, 100 micrograms Amitraz technical per liter), a solvent control (contained 100 uL/L of TEG) and a dilution water control were tested in the study. Replicate tanks (A and B) were employed for all concentrations and the controls.

The exposure was initiated by placing two incubation cups each containing 20 eggs into each duplicate tank (giving a total of 40 eggs per duplicate and 80 eggs per concentration). This gave a nominal loading of 4.4 eggs per liter of test solution.

The numbers of live and dead eggs were recorded daily and dead eggs and fry were discarded. Fry were released into the test chamber within 24 hours of hatching. When the hatch was complete, the number of live, deformed and dead fry in each duplicate tank was recorded. The percentage hatch was calculated as the number of live normal fry in each duplicate tank divided by the number of eggs on day 0. The "hatch day" (day 4) was determined to be that on which the greatest number of fry were released into the progeny tanks.

After releasing into the test chambers, until day 6 post-hatch, the fry were fed on un-oiled powdered Pruteen once per day. On each occasion, 0.05 ± 0.005 g of Pruteen, suspended in a small amount of water, was introduced into each tank. On days 5 and 6 post-hatch, the Pruteen feed was supplemented by one additional feed of brine shrimp (Artemia) larvae. From day 7 to day 15 post-hatch, the fry were fed solely on Artemia larvae, three times per weekday and twice per day on weekends at an estimated rate of 400 larvae per fry per feed. From day 17 post-hatch onwards, the Artemia used were approximately 72 hours old at the time of feeding and had, themselves, been fed on Pruteen. From day 25 post-hatch, one of the daily Artemia feeds was replaced with Promin. This was given at the discretion of the operator. No food was given during the last 24 hours of the test.

Daily observation of fry mortality, behavior and appearance was made and any abnormal effects recorded. The test was terminated at 32 days post-hatch and the surviving fry were counted and individually weighed and measured.

Dissolved oxygen, pH, and temperature measurements were made in both replicate tanks on day 0 and then twice weekly throughout the study. In addition, a continuous record of the temperature was kept in replicate A of the dilution water control. Water samples were taken from each A replicate on days -1, 0, 1, and 2, and from each B replicate on days -1, 0, 2, and 3 of the study. Subsequently, samples were taken at least twice weekly, alternating between each replicate, so that every tank was sampled at least once per week. The samples were analyzed for amitraz technical and its metabolites using gas-liquid chromatography.

E. Statistics: The relative standard deviation (RSD) of the weights of the larvae in both replicates of the two controls were calculated to determine the acceptability of the data according to the EPA Environmental Effects Guidelines.

The percentage hatch and survival data were analyzed by contingency table tests to compare the treatments against the controls ($p = 0.05$). The larval length and weight data for the solvent control and the dilution water controls were tested for differences ($p = 0.05$) between replicates using Student's t-tests. In the absence of significant differences, the replicates for

each treatment and the controls were pooled and a one-way analysis of variance carried out. This was followed by Dunnett's t-tests at the 95% and 99% significant levels, between each of the treatments and the controls. If significant differences were found ($p = 0.05$) between replicates, subsequent analysis was done on unpooled data.

The Maximum Acceptable Toxicant Concentration (MATC) was defined as the geometric mean between the lowest effect concentration and the highest no effect concentration.

12. REPORTED RESULTS: Water quality data during the exposure period are presented in Table 14 (attached). Dissolved oxygen levels ranged from 6.8 to 9.0 mg/L. The pH values ranged from 7.4 to 8.7, while temperature values ranged from 24.1 to 25.9°C. The mean flow rates to the individual tanks ranged from 46 to 56 ml/minute. The light intensity was 2400 Lux during the test. The analytical recoveries for the levels of amitraz in this study ranged from 53.2 to 78.1% of the nominal concentrations. The mean measured concentrations were 3.53, 7.14, 16.8, 36.6, and 57.0 ug/L.

The residual standard deviation (RSD) values of the weight of the fish which were alive at the end of the test in each control chambers were 26.5 and 40.2% for the solvent control and 29.3 and 30.0% for the dilution water control. The data were therefore considered acceptable.

There was no significant difference ($p = 0.05$) in the hatchability of the eggs or the survival of the larvae between replicates in either the dilution water control or the solvent control. All pairs of replicates were therefore pooled for subsequent analysis. No significant differences were found between weights and lengths in the replicates of the solvent control or between the replicates of the dilution water control ($p = 0.05$). The replicates were therefore pooled for subsequent analysis.

Table 4 (attached) summarizes data collected on hatchability, larval survival, and growth. The hatchability of fathead embryos was not significantly affected ($p = 0.05$) in any replicate test vessel in this study. The percentage hatchability in the individual replicates ranged from 79.5 to 97.5% with an overall mean value of 89.5%.

Larval survival was significantly affected ($p = 0.05$) at 7.14 ug/L and higher concentrations, while at 3.53 ug/L, the survival was unaffected. No fish survived in the highest test concentration (57 ug/L mean measured).

Comparison of length at each treatment level against the two controls (solvent and dilution water) showed significant differences at the 36.6- and 16.8-ug/L mean measured concentrations ($p = 0.05$ and $p = 0.01$). No significant differences were found at the 7.14-ug/L concentration, however, a significant difference was found between the 3.53 ug/L and the solvent control ($p = 0.05$).

Weights of fish in all treatments showed a significant difference ($p = 0.05$ and $p = 0.01$) compared with the solvent control. Comparison of the treatments against the dilution water control showed at $p = 0.05$, the 3.53-, 16.8-, and 36.6-ug/L treatments were significantly different. However, at $p = 0.01$, only the 16.8- and 36.6- ug/L treatments were significantly different from the dilution water control.

The no-observed-effect concentration (NOEC) of amitraz technical was therefore considered to be <3.53 ug/L. The lowest-observed-effect concentration (LOEC) was considered to be 3.53 ug/L.

13. STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES: No conclusions were made by the authors. 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, except for the following deviations:

- Time to swim-up was not reported.

- Dechlorinated water probably should not have been used as the dilution water since the analysis performed during the study showed low levels of residual chlorine in some water samples, indicating incomplete dechlorination.

- The light intensity used during the test (i.e., 2400 Lux) was much higher than the recommended intensity of 400-800 Lux.

- The range of concentrations tested did not include a no effect level. The SEP states that one concentration selected must not affect any life-stage.

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It should be noted that there is a typographical error in Table 4 (attached). The standard deviation of average weight of solvent control replicate B should have been 0.059, instead of 0.039.

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). The percent hatching and larval survival were transformed using arcsine square-root transformation before the analyses.

The results could be summarized as follows:

Mean Measured Conc. (ug/L)	% Hatching	% Larval Survival	Length (mm)	Weight (mg)
Solvent Control	93.8	66.7	20.55	0.138
Water Control	91.3	81.7	20.27	0.121
3.53	83.2	70.0	19.04	0.098[a]
7.14	89.2	56.7	20.02	0.110[a]
16.80	90.0	36.7[b]	17.15[ab]	0.076[ab]
36.60	91.4	13.4[ab]	16.69[ab]	0.073[ab]
57.00	87.7	0	-	-

[a] = Significantly different from solvent control ($p \leq 0.05$).
[b] = Significantly different from water control ($p \leq 0.05$).

C. Discussion/Results: High mortalities occurred in the solvent control (average = 33.3%). The SEP states that "a test is not acceptable if survival in any control chamber is less than 70%."

Hatching was not affected by amitraz technical at any concentrations tested. Concentration of 36.6 ug/L reduced larval survival when compared to the solvent control, and concentrations of 16.8 and 36.6 ug/L reduced the survival when compared to the water control. Amitraz technical at concentrations of 16.8 ug/L and

higher significantly reduced the length of test fish when compared to both solvent and water controls. All concentrations tested significantly reduced the weight of test fish when compared to solvent control.

Therefore, based on the most sensitive parameter (i.e., weight), the NOEC and MATC values were determined to be lower than 3.53 ug/L mean measured concentration of amitraz technical.

D. Adequacy of the Study:

(1) Classification: Supplemental.

(2) Rationale: Fish survival in both replicates of solvent control was less than 70%. Furthermore, the MATC could not be calculated due to the reduction in growth (weight) observed at all test levels.

(3) Repairability: No.

15. COMPLETION OF ONE-LINER: Yes, December 22, 1988.

TABLE 4

AMITRAZ TECHNICAL: DETERMINATION OF CHRONIC TOXICITY TO FATHEAD MINNOW EMBRYOS AND LARVAE
Hatchability and survival data

Amitraz technical nominal concn µg/l	Expo-sure tank (rep)	Mean measured concn µg/l	No of embryos at start	Number of fry hatched	Number of fry released	% hatch (indiv reps)	% hatch (pooled reps)	*No of larvae surviving (days) 11	18	25	32	Larvae % survival from hatch release	Average length mm	SD	Average weight mg	SD
100	A	53.2	40	36	30	90.0	87.7	0	0	0	0	0	-	-	-	-
	B	60.8	41	35	30	85.4	-	0	0	0	0	0	-	-	-	-
50	A	34.2	41	38	30	92.7	91.4	7	7	6	6	20.0	17.1	2.33	0.080	0.035
	B	39.1	40	36	30	90.0	-	3	3	3	2	6.7	15.4	2.62	0.054	0.038
25	A	15.4	40	36	30	90.0	90.0	11	9	9	7	23.3	17.9	1.59	0.081	0.019
	B	18.3	42	36	30	90.0	-	20	20	20	15	50.0	16.8	2.86	0.073	0.040
12	A	7.29	41	37	30	90.2	89.2	22	22	22	18	60.0	20.0	2.15	0.105	0.032
	B	6.98	42	37	30	88.1	-	21	20	20	16	53.3	20.0	2.79	0.115	0.042
6	A	3.67	38	33	30	86.8	83.2	21	21	21	21	70.0	18.7	2.66	0.093	0.033
	B	3.39	39	31	30	79.5	-	22	21	21	21	70.0	19.4	1.65	0.104	0.033
Solvent control	A	<3	40	37	30	92.5	93.8	24	24	24	20	66.7	20.3	2.21	0.131	0.035
	B	<3	40	38	30	95.0	-	26	26	26	20	66.7	20.8	3.14	0.146	0.059
Dilution water control	A	<3	40	39	30	97.5	91.3	28	28	28	25	83.3	21.1	2.23	0.132	0.039
	B	<3	40	34	30	85.0	-	27	27	27	24	80.0	19.4	2.36	0.110	0.033

*Statistical data were calculated on the hatchability and the overall survival from initial embryos

TABLE 14

AMITRAZ TECHNICAL:DETERMINATION OF CHRONIC TOXICITY TO FATHEAD MINNOW EMBRYOS AND LARVAE
Range of physical/chemical parameters determined

Date	Rep-licate	Nominal concentration (µg/l) 100			50			25			12			6			Solvent control			Dilution water control		
		O_2 mg/l	pH	Temp °C	O_2 mg/l	pH	Temp °C	O_2 mg/l	pH	Temp °C	O_2 mg/l	pH	Temp °C	O_2 mg/l	pH	Temp °C	O_2 mg/l	pH	Temp °C	O_2 mg/l	pH	Temp °C
22.10.87	A	7.6	7.4	24.6	7.8	7.6	24.7	7.6	7.5	24.6	7.4	7.4	24.9	7.2	7.5	25.2	7.4	7.4	24.6	7.4	7.5	24.9
"	B	7.8	7.4	24.4	7.4	7.5	24.7	7.6	7.4	24.7	7.4	7.3	25.0	7.6	7.4	24.9	7.4	7.5	24.3	7.4	7.4	25.0
26.10.87	A	7.8	7.7	24.8	7.8	8.0	24.9	7.6	7.9	24.6	7.6	7.8	24.6	7.6	7.8	25.1	7.8	7.8	24.4	7.8	7.9	24.7
"	B	8.0	7.8	24.4	7.6	7.8	24.8	7.8	7.9	24.5	7.8	7.8	24.7	7.6	7.9	24.6	7.8	7.8	24.2	7.6	7.9	24.5
29.10.87	A	7.8	8.0	24.3	8.0	8.0	24.5	7.8	7.9	24.6	7.6	7.8	24.8	7.8	7.7	25.5	7.8	7.8	24.7	7.6	7.9	24.8
"	B	7.6	7.4	24.8	7.6	7.8	24.5	8.0	7.9	24.6	8.0	7.8	24.8	7.8	7.9	25.4	7.4	7.5	24.7	7.8	8.0	24.8
2.11.87	A	8.0	8.2	25.4	8.0	7.8	25.4	7.6	7.7	25.6	8.0	8.0	24.8	8.0	8.1	25.8	7.6	7.8	24.6	8.2	8.0	25.3
"	B	7.0	7.6	25.2	8.2	8.3	25.5	7.8	7.9	25.4	7.8	8.1	24.8	7.6	8.1	25.7	7.2	7.8	24.7	8.2	8.1	25.7
5.11.87	A	-	-	-	8.0	7.9	25.4	7.6	7.8	25.0	7.8	7.8	25.1	8.0	8.6	24.4	7.6	7.7	24.7	7.8	7.9	25.3
"	B	-	-	-	8.2	7.8	25.1	7.8	7.7	25.0	8.0	7.8	25.4	8.2	8.5	24.3	7.4	7.6	24.7	8.0	8.0	25.2
9.11.87	A	-	-	-	8.6	8.3	25.1	8.2	8.1	24.4	8.2	8.0	25.1	8.0	8.4	25.3	8.4	7.9	24.4	8.6	8.4	25.2
"	B	-	-	-	8.8	8.5	25.2	8.0	8.0	24.5	8.4	8.3	25.1	8.2	8.2	25.5	8.4	8.0	24.5	9.0	8.6	25.3
12.11.87	A	-	-	-	7.8	8.4	25.3	7.6	8.2	24.8	7.6	8.0	24.9	7.6	8.7	25.5	7.4	8.0	25.6	8.0	8.5	25.3
"	B	-	-	-	8.0	8.5	25.5	7.8	8.0	24.9	8.0	8.2	25.2	7.8	8.2	25.6	7.8	8.0	24.6	8.0	8.6	25.2
16.11.87	A	-	-	-	7.6	7.9	25.6	8.0	7.8	25.3	8.0	7.9	25.6	8.0	8.0	25.9	8.0	7.7	25.5	8.4	8.0	25.7
"	B	-	-	-	8.4	8.4	25.8	8.0	7.8	25.3	8.6	8.1	25.4	8.0	8.1	25.8	8.0	7.9	25.3	8.4	8.5	25.2
19.11.87	A	-	-	-	8.0	8.0	25.8	7.8	7.7	25.8	7.8	8.1	25.9	7.8	7.9	26.0	7.6	7.9	25.4	7.8	8.0	25.9
"	B	-	-	-	8.8	8.7	25.8	7.2	7.7	25.7	8.4	8.3	25.8	8.0	8.2	26.0	7.6	7.8	25.1	8.2	8.6	25.7
23.11.87	A	-	-	-	7.6	8.3	25.2	7.4	7.7	24.6	7.6	7.8	24.2	7.2	8.2	25.2	6.8	7.8	24.6	7.4	7.7	24.8
"	B	-	-	-	8.2	8.6	25.5	7.2	7.6	24.6	7.8	8.1	24.6	7.8	8.1	25.0	7.2	7.8	24.6	7.4	7.8	24.8
26.11.87	A	-	-	-	7.4	8.0	25.0	7.0	8.0	24.2	7.2	7.8	24.4	7.2	7.7	24.0	6.8	7.6	24.6	6.8	7.7	24.1
"	B	-	-	-	7.6	8.3	24.9	7.2	7.9	24.2	7.2	7.8	24.6	7.4	7.9	24.2	7.0	7.9	24.4	7.2	7.8	24.4

Hatching - 1

Analysis of Variance File: amithatc Date: 12-15-1988

FILTER: None

N's, means and standard deviations based on dependent variable: HATCHING (Arcsine SQRT transformation)

* Indicates statistics are collapsed over this factor

Factors: C	Mean measured conc. (µg/L)	N	Mean	S.D.
*		14	1.2476	0.0766
1	Solvent Control	2	1.3194	0.0367
2	Water Control	2	1.2925	0.1689
3	3.53	2	1.1500	0.0694
4	7.14	2	1.2355	0.0239
5	16.80	2	1.2490	0.0000
6	36.60	2	1.2731	0.0341
7	57.00	2	1.2139	0.0497

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Fmax for testing homogeneity of between subjects variances: Not defined

DDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDD

Analysis of Variance Dependent variable: HATCHING

Source	df	SS (H)	MSS	F	P
Between Subjects	13	0.0762			
C (CONC)	6	0.0373	0.0062	1.118	0.4343
Subj w Groups	7	0.0389	0.0056		

Analysis of Variance File: amithatc Date: 12-15-1988

FILTER: None

Hatching (Arcsine SQRT transformation)

Post-hoc tests for factor C (CONC)

Level	Mean	Level	Mean
1	1.319	6	1.273
2	1.293	7	1.214
3	1.150		
4	1.236		
5	1.249		

Comparison	Scheffe'	Tukey-A*	Tukey-B*	Newman -Keuls*	Bon-ferroni	Dunnett
1 > 2						
1 > 3						
1 > 4						
1 > 5						
1 > 6						
1 > 7						
2 > 3						N.A.
2 > 4						N.A.
2 > 5						N.A.
2 > 6						N.A.
2 > 7						N.A.
3 < 4						N.A.
3 < 5						N.A.
3 < 6						N.A.
3 < 7						N.A.
4 < 5						N.A.
4 < 6						N.A.
4 > 7						N.A.
5 < 6						N.A.
5 > 7						N.A.
6 > 7						N.A.

* The only possible P-values are .01, .05 or .10 (up to 0.1000). A blank means the P-value is greater than 0.1000.

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

Analysis of Variance File: amitsurv Date: 12-15-1988

FILTER: None 32-day exposure / Larval Survival

N's, means and standard deviations based on dependent variable: SURVIVAL (Arc sine SQRT)

* Indicates statistics are collapsed over this factor

Factors: C	Mean measured Conc. (µg/L)	N	Mean	S.D.
*		12	0.8221	0.2747
1	Solvent control	2	0.9557	0.0000
2	Water Control	2	1.1284	0.0302
3	3.53	2	0.9912	0.0000
4	7.14	2	0.8500	0.0447
5	16.80	2	0.6445	0.1992
6	36.60	2	0.3627	0.1427

Fmax for testing homogeneity of between subjects variances: Not defined

Analysis of Variance Dependent variable: SURVIVAL

Source	df	SS (H)	MSS	F	P
Between Subjects	11	0.8302			
C (CONC)	5	0.7673	0.1535	14.627	0.0026
Subj w Groups	6	0.0629	0.0105		

Post-hoc tests for factor C (CONC)

Level	Mean	Level	Mean
1	0.956	6	0.363
2	1.128		
3	0.991		
4	0.850		
5	0.645		

Comparison	Scheffe'	Tukey-A*	Tukey-B*	Newman-Keuls*	Bon-ferroni	Dunnett
1 < 2						
1 < 3						
1 > 4						
1 > 5			0.1000	0.1000		
1 > 6	0.0190	0.0100	0.0100	0.0100	0.0187	0.0100
2 > 3						N.A.
2 > 4						N.A.
2 > 5	0.0478	0.0500	0.0500	0.0500	0.0506	N.A.
2 > 6	0.0053	0.0100	0.0100	0.0100	0.0051	N.A.
3 > 4						N.A.
3 > 5		0.1000	0.1000	0.1000		N.A.
3 > 6	0.0144	0.0100	0.0100	0.0100	0.0140	N.A.
4 > 5				0.1000		N.A.
4 > 6	0.0464	0.0500	0.0500	0.0100	0.0490	N.A.
5 > 6			0.1000	0.0500		N.A.

* The only possible P-values are .01, .05 or .10 (up to 0.1000). A blank means the P-value is greater than 0.1000.

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

Analysis of Variance File: amitleng Date: 12-15-1988

FILTER: None

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

* Indicates statistics are collapsed over this factor

Factors: C	Mean measured Conc. (µg/L)	N	Mean	S.D.
*		195	19.5185	2.6946
1	Solvent Control	40	20.5475	2.6900
2	Water Control	49	20.2673	2.4220
3	3.53	42	19.0381	2.2127
4	7.14	34	20.0206	2.4315
5	16.90	22	17.1500	2.5309
6	36.60	8	16.6875	2.3558

Fmax for testing homogeneity of between subjects variances: 1.48
Number of variances= 6 df per variance= 21.

Analysis of Variance Dependent variable: LENGTH

Source	df	SS (H)	MSS	F	P
Between Subjects	194	1408.5934			
C (CONC)	5	275.6277	55.1255	9.196	0.0000
Subj w Groups	189	1132.9657	5.9945		

Post-hoc tests for factor C (CONC)

Level	Mean	Level	Mean
1	20.548	6	16.688
2	20.267		
3	19.038		
4	20.021		
5	17.150		

Comparison	Scheffe'	Tukey-A*	Tukey-B*	Newman -Keuls*	Bon-ferroni	Dunnett
1 > 2						
1 > 3					0.0871	
1 > 4						
1 > 5	0.0001	0.0100	0.0100	0.0100	0.0000	0.0100
1 > 6	0.0068	0.0100	0.0100	0.0100	0.0012	0.0100
2 > 3						N.A.
2 > 4						N.A.
2 > 5	0.0003	0.0100	0.0100	0.0100	0.0000	N.A.
2 > 6	0.0139	0.0100	0.0100	0.0100	0.0027	N.A.
3 < 4						N.A.
3 > 5			0.0500	0.0500	0.0572	N.A.
3 > 6		0.0500	0.0500	0.0100		N.A.
4 > 5	0.0034	0.0100	0.0100	0.0100	0.0005	N.A.
4 > 6	0.0384	0.0100	0.0100	0.0100	0.0101	N.A.
5 > 6						N.A.

* The only possible P-values are .01, .05 or .10 (up to 0.1000). A blank means the P-value is greater than 0.1000.

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

Analysis of Variance File: amitwt Date: 12-15-1988

FILTER: None

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

* Indicates statistics are collapsed over this factor

Factors: C	Mean measured Conc. (µg/L)	N	Mean	S.D.
*		195	0.1108	0.0432
1	Solvent Control	40	0.1388	0.0481
2	Water Control	49	0.1213	0.0375
3	3.53	42	0.0983	0.0330
4	7.14	34	0.1097	0.0367
5	16.80	22	0.0755	0.0341
6	36.60	8	0.0733	0.0347

Fmax for testing homogeneity of between subjects variances: 2.12
Number of variances= 6 df per variance= 21.

Analysis of Variance Dependent variable: WEIGHT

Source	df	SS (H)	MSS	F	P
Between Subjects	194	0.3617			
C (CONC)	5	0.0821	0.0164	11.095	0.0000
Subj w Groups	189	0.2797	0.0015		

Post-hoc tests for factor C (CONC)

Level	Mean	Level	Mean
1	0.139	6	0.073
2	0.121		
3	0.098		
4	0.110		
5	0.076		

Comparison	Scheffe'	Tukey-A*	Tukey-B*	Newman -Keuls*	Bon-ferroni	Dunnett
1 > 2						
1 > 3	0.0006	0.0100	0.0100	0.0100	0.0000	0.0100
1 > 4	0.0653		0.1000	0.0500	0.0208	
1 > 5	0.0000	0.0100	0.0100	0.0100	0.0000	0.0100
1 > 6	0.0023	0.0100	0.0100	0.0100	0.0003	0.0100
2 > 3					0.0733	N.A.
2 > 4						N.A.
2 > 5	0.0010	0.0100	0.0100	0.0100	0.0000	N.A.
2 > 6	0.0608	0.0100	0.0100	0.0100	0.0188	N.A.
3 < 4						N.A.
3 > 5				0.1000		N.A.
3 > 6				0.1000		N.A.
4 > 5	0.0662	0.0500	0.0500	0.0500	0.0212	N.A.
4 > 6		0.0500	0.0500	0.0500		N.A.
5 > 6						N.A.

* The only possible P-values are .01, .05 or .10 (up to 0.1000). A blank means the P-value is greater than 0.1000.

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

Acy No. _____ Chemical Name Amitraz Chemical Class _____ Page 1 of 1

CQ 559, Amitraz technical

Study/Species/Lab/ Succession	Chemical % Active	Results				Reviewer/ Date	Validat Status
Avian Reproduction,		Group	Dose(ppm)	Effected/Parameters	Mort.(%)	%Chg Inh.	
Species:		Control					
		Treatment I					
Lab:		Treatment II					
		Treatment III					
Acc*;		Study Duration:					
		Comments:					

Field Study(Simulated/Actual)		Group	Rate(ai/a)	Treatment Interval	Total # Treatments	Mor.(%)		
Species:		Control						
		Treatment I						
Lab:		Treatment II						
Acc.*;		Treatment III						
		Crop/Site:		Study Duration:				
		Comments:						

Chronic fish,

Species Pimephales promelas 98.8

Lab: ICI Brixham Laboratory

Acc.*: 407980-02

Concentrations Tested (ppb*)= 3.53, 7.14, 16.8, 36.6, 57.0

MATC = > - < 3.53 ppb*. Effected Parameter = weight

Conc. Mort.(%)= 18.3 Sol. Conc. Mort.(%)= 33.3 PK/12-20-88 Supp

Comments: * mean measured concentration

Chronic invertebrate

Species _____

Lab _____

Acc.* _____

Concentrations Tested (pp__)= _____

MATC =>____< ____pp__. Effected Parameter(s)_____

Conc. Mort.(%)=_____ Sol. Conc. Mort.(%)=_____

Comments:

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