255987, 255988
RECORD NO.

106201
SHAUGHNESSEY NO

REVIEW NO.

EEB REVIEW

DATE: IN 12-8-89 OUT FEB 14 1990

FILE OR REG. NO. 45639-RUA

PETITION OR EXP. NO.

DATE OF SUBMISSION 10-4-89

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RD ACTION CODE/TYPE OF REVIEW 181

TYPE PRODUCT(S) insecticid/miticide

DATA ACCESSION NO(S) 412887-01, 412887-02

PRODUCT MANAGER, NO. 12

PRODUCT NAME(S) Amitraz (Ovasyn)

COMPANY NAME Nor-Am Chemical Corporation

SUBMISSION PURPOSE Review 2 Chronic Studies (72-4) in Support of Registration of Amitraz on Cotton

SHAUGHNESSEY NO.	CHEMICAL	% A.I.
106201	Amitraz	96%
106201	Amitraz	98.8%

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

February 7, 1990

MEMORANDUM

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

SUBJECT: Review of toxicity data for Amitraz (Ovasyn)

FROM: James W. Ackerman, Chief
Ecological Effects Branch
Environmental Fate and Effects Division (H-7507C)

TO: Dennis Edwards (PM 12)
Insecticide/Fungicide Branch
Registration Division (H7505C)

EEB has completed reviews of two Amitraz (Ovasyn) studies submitted by Nor-Am Chemical Corporation in response to the Amitraz Registration Standard to support the registration of Amitraz (Ovasyn) on Cotton. (copies are attached). The following is a brief summary of the reviews:

1. STUDY IDENTIFICATION: Smith, Gregory, J., 1989, (W108) Flow-Through Chronic Toxicity of Amitraz to Daphnia magna, Batelle Columbus Division Laboratory, 505 King Avenue, Columbus, Ohio 43201-2693, Submitted by Nor-Am Chemical Company, 3509 Silverside Road, P.O. Box 7495, Wilmington, Delaware 19803, MRID #412887-01

   CONCLUSIONS: This study is scientifically sound. Although, at present no SEP Guidelines exist for a 21-Day Flow-Through Daphnia magna chronic toxicity test, the test procedures were discussed with the EPA Environmental Research Laboratory, Duluth, MN, and were found to be in compliance with currently accepted methods.

   Based on the parameters of length and reproduction, the MATC, LOEC, and NOEC values of Amitraz Technical for Daphnia magna were >1.10 and <2.21, 2.21, and 1.10 µg/L respectively. Amitraz did not significantly affect survival of Daphnia magna at the concentrations used in the study. The LC_{50} of Amitraz to Daphnia magna is >8.68 µg/L (the highest level tested)

   RECOMMENDATIONS: N/A

2. STUDY IDENTIFICATION: Hill, R.W., J.E. Caunter, E. Gillings, A.M. Riddle, 1989,(W109): ^{14}C Amitraz Equivalents: Determination of Chronic Toxicity to Fathead Minnow (Pimephales promelas) Embryos and Larvae, Schering

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Agrochemical Limited, Chesterford Park Research Station, Saffron Walden, Essex CB10 1XL, Submitted by Nor-Am Chemical Company, P.O. Box 7495, Wilmington, Delaware 19803, MRID #412887-02

CONCLUSIONS: This study is scientifically sound and fulfills the SEP Guideline requirements for a Fish Early Life-Stage Test. Based on the most sensitive parameter (length) the MATC, NOEC, and LOEC values of Amitraz (98.8%) for Pimephales promelas were >1.48 and <2.71 µg/L, 1.48 µg/L, and 2.71 µg/L respectively.

RECOMMENDATIONS: N/A

DATA EVALUATION RECORD

1. **CHEMICAL:** Amitraz
Shaughnessy #106201

2. **TEST MATERIAL:** ^{14}C Amitraz, Batelle ID#EEC1656, 96% pure

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

4. **STUDY IDENTIFICATION:** Smith, Gregory, J., 1989, (W108) Flow-Through Chronic Toxicity of Amitraz to Daphnia magna, Batelle Columbus Division Laboratory, 505 King Avenue, Columbus, Ohio 43201-2693, Submitted by Nor-Am Chemical Company, 3509 Silverside Road, P.O. Box 7495, Wilmington, Delaware 19803, MRID #412887-01

5. **REVIEW BY:** Harry A. Winnik
Biologist
EFED/EEB
Signature:
Date: 2-13-90

6. **APPROVED BY:** Henry Craven
Supervisory Biologist
EFED/EEB
Signature:
Date: 2/13/90

7. **CONCLUSIONS:** This study is scientifically sound. Although, at present not SEP Guidelines exist for a 21-Day Flow-Through Daphnia magna chronic toxicity test, the test procedures were discussed with the EPA Environmental Research Laboratory, Duluth, MN, and were found to be in compliance with currently accepted methods.

Based on the parameters of length and reproduction, the MATC, LOEC, and NOEC values of Amitraz Technical for Daphnia magna were >1.10 and <2.21, 2.21, and 1.10 µg/L respectively. Amitraz did not significantly affect survival of Daphnia magna at the concentrations used in the study. The LC_{50} of Amitraz to Daphnia magna is >8.68 µg/L (the highest level tested)

8. **RECOMMENDATIONS:** N/A

9. **BACKGROUND:** The study was submitted by Nor-Am Chemical Company to support the registration of Amitraz on cotton.

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

11. **MATERIALS AND METHODS:**

A. Test Animals: (excerpted from the submission)

Daphnia magna used in testing were originally obtained from the U.S. Environmental Protection Agency (Environmental Research Laboratory, Duluth MN) and cultured in an environmental chamber under controlled conditions (temperature: 20 ± 1°C; photoperiod: 16 hours light - 8 hours dark; light intensity: 323-1076 lux).

Daphnia were cultured in 1-L glass beakers (10 Daphnia per beaker) containing 800 ml of hard reconstituted laboratory water (hardness 160-180 mg/L as $CaCO_3$, alkalinity 110-120 mg/L $CaCO_3$ and pH 7.6-8.5). Each beaker received 2.3 x 10^8 cells/liter Selenastrum capricornutum and 10 mg/L of a yeast/trout food/Cerophyl® suspension three times each week when the culture water was changed. After 28 days, the adults were discarded and new cultures started.

Twenty-four hours before the start of the test, adults were transferred to clean beakers with food to ensure that only daphnids less than 24 hours old would be used to start the test. Young daphnids (<24 hours old at test initiation) used as test animals in the chronic toxicity test were from 23-day old cultures. There was 100 percent survival of culture animals and an average of 7.7 young produced per female per reproductive day during the week before the toxicity test.

B. Test System: One liter glass Griffin beakers containing 800 ml of test solution at a depth of approximately 10 cm. were used as test vessels. An overflow covered with fine mesh Nytex® screen maintained constant volume and prevented escape of test organisms.

Deep well water, treated to remove iron and organic impurities, sequentially passed through reverse osmosis purifiers and millipore Milli-Q system to reduce ion concentrations to the µg/L range was used as the water source to prepare the test waters used. The test water was prepared by adding reagent-grade salts to a measured volume of purified water, was aerated overnight, and the hardness, alkalinity, pH and specific conductivity was measured the next day. Only those batches of test water that were within the required specifications for hardness (160-180 mg/L as $CaCO_3$), alkalinity (110-120 mg/L as $CaCO_3$) and pH (7.6-8.2)

were used. Each batch of test water was siphoned into a 20-gallon glass aquarium reservoir and was pumped to the flow-through diluter system on demand.

The test was conducted in a controlled environment room at a temperature of approximately 20° C with a photoperiod of 8-hour darkness and 16-hours light with light intensity of 792-958 lux provided by fluorescent light bulbs.

The toxicant was delivered to the test chambers using a solenoid-activated proportional diluter programmed to deliver five concentrations plus a dilution water control at a maximum rate of 100 ml per chamber per cycle. The solvent control was pumped via a peristaltic pump and gravity siphon to the test chambers.

The proportions of Amitraz and its major degradation products BTS-27271, BTS-27919, and BTS-24868 were measured by High Pressure Liquid Chromatography (HPLC) and Thin-Layer Chromatography (TLC).

C. Dosage: Stock solutions of ^{14}C-Amitraz were prepared in acetone three days prior to study initiation, in order to determine purity, and on days 7 and 14 of the study. 7.75-7.78 mg of ^{14}C-Amitraz crystals were added to 50 ml of sodium sulfate dried acetone to give a concentration of 155.00-155.6 mg/L. The stock solution was stored in a sealed glass jar containing desiccant at 4 ± 2° C in the dark until needed. A working stock solution was prepared every three to four days by transferring approximately 10 ml of the 155.2 mg/L acetone stock solution to a glass vial which was then incorporated in the test material delivery system.

Five geometrically spaced concentrations of ^{14}C-Amitraz were prepared from the working stock solution with nominal concentrations of 0.625, 1.25, 2.5, 5.0, and 10.0 µg/L. Duplicate test chambers were set up for each concentration level, dilution water control and solvent control.

D. Design: Ten immature daphnids (<24 hours old at test initiation) were placed into each test chamber (20 daphnids per concentration) using a two-step randomization procedure. After the daphnids were placed in the beakers, a fine mesh Nytex® screen was placed in each beaker so as to eliminate the occurrence of floaters. Twice daily each test chamber received an aliquot of the green alga, Selenastrum capricornutum, 1.14 x 10^8 cells/L for days 0-6 of the study and 1.44 x 10^8 cells/L for days 7-20 of the study. A yeast/trout food/Cerophyl® (YTC)suspension was also added once each day at a rate of 0.4 ml/beaker for days 0-6 of the

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study and at a rate of 0.5 ml/beaker for days 7-20 of the study.

Adult survival was noted each day. Reproduction was noted at two-to-three day intervals and at the end of the experiment. The young were counted and discarded either after they were pipetted from the test chamber or retained by a Nytex® screen. On the final day of the test, length measurements were made on all surviving adult daphnids using a binocular dissecting microscope with a calibrated ocular micrometer.

E. Statistics: (excerpted from the submission)

Two types of response data were collected during the study: quantal data (survival-live or dead) and non-quantal data (total number of young per reproducing adult female, total number of young per reproducing adult female per reproductive day, and total length). The quantal data (survival) were analyzed using Fisher's Exact Test a 2x2 contingency table). The non-quantal data (reproduction and total length) were analyzed using a one-way analysis of variance (ANOVA) followed by Bonferroni's multiple comparison test to determine which treatments were significantly different from the controls.

12. **REPORTED RESULTS:**

The hardness, alkalinity and specific conductivity of the dilution water ranged from 160 to 168 mg/L as $CaCO_3$, 112 to 116 mg/L as $CaCO_3$ and 510 µmhos/cm respectively. Dissolved oxygen and pH varied minimally among the test chambers with ranges of 6.8 to 8.5 mg/L and 7.9 to 8.2 respectively with water temperatures 20° C ± 1.2° C and mean temperatures 20.0 ± 0.55° C.

For the nominal concentrations of 0.625, 1.25, 2.5, 5.0, and 10.0 µg/L, the mean measured concentrations were 0.46, 1.10, 2.21, 4.15, and 8.68 µg Amitraz equivalents/L respectively as measured by Liquid Scintillation Counting (LSC) of the total ^{14}C activity (see attached). The overall mean recovery of the exposure solutions ranged from 93.2 to 105.6 percent. The percentage of the total radioactive material in the aqueous phase after the solvent extraction ranged from 1.7 to 5.1 percent.

The percentages and ranges of Amitraz and its degradation products as measured by HPLC and TLC were Amitraz 54.7 (40.4-68.8) and 47.3(36.4-62.2), BTS-27271 13.3(8.6-20.2) and 12.1(5.9-23.1), BTS-27919 21.0(10.7-27.0) and 14.6(9.8-20.1), and BTS-24868 2.3(0.9-3.9) and 5.1(0.0-13.5) percent respectively.

No significant reduction in adult survival ($p<0.05$) was apparent in any of the test material concentrations in comparison with the acetone/reconstituted water controls. The 21-day LC_{50} value (based on the LSC measured total ^{14}C activity due to Amitraz and its three transformation products) was greater than 8.68 µg Amitraz equivalents/L since there was 80 percent or greater survival in all test beakers.

The effect of Amitraz and its transformation products on reproduction and reproduction rate were analyzed using a one-way analysis of variance. For reproduction the overall analysis of variance results were significant ($f=147.9$; $dF=6$; $p<0.0001$), and Bonferroni's Multiple Comparison Test indicated that reproduction in the 0.46 and 1.10 µg/L test material concentrations were not significantly different from the reproduction in the controls, but reproduction in the 2.21, 4.15, and 8.68 µg/L test material concentrations were significantly reduced when compared with reproduction in the acetone/reconstituted water controls. The no-observed-effects-concentration (NOEC) and the lowest-observed-effects-concentration (LOEC) for number of young per reproducing adult female, therefore, were 1.10 and 2.21 µg/L, respectively, of test material as measured by LSC.

For reproduction rate the overall analysis of variance results were also significant ($f=>2.1$; $dF=5$; $P<0.0001$). The highest test material concentration (8.68 µg/L) was deleted from the data set to sufficiently reduce the heterogenous nature of the complete data set and satisfy the ANOVA homogeneity of variance assumption. Bonferroni's Multiple Comparison Test indicated that reproduction in the 0.46 and 1.10 µg/L test material concentrations were not significantly different from the reproduction in the acetone/reconstituted water controls, but reproduction in the 2.21, 4.15, and 8.68 µg/L test material concentrations were significantly reduced when compared with reproduction in the controls. The NOEC and LOEC for number of young per reproducing adult female per reproductive day of this smaller data set, therefore, were 1.10 and 2.21 µg/L, respectively, of test material as measured by LSC.

Length measurements on the 130 surviving adult female daphnids ranged from 3.29 to 3.99 mm. The effect of the test material concentrations on the length of the surviving adults was also analyzed by using ANOVA. As with the reproduction analysis, only the acetone/reconstituted water control data were included in the ANOVA. The overall analysis of variance was highly significant ($f=8.95$; $dF=106$; $P<0.0001$). Bonferroni's Multiple Comparison Test indicated that only the animals in the 2.21, 4.15, and 8.68 µg/L test

material concentrations were significantly smaller ($P<0.05$) than the control animals. The NOEC and LOEC for total length, therefore, were 1.10 and 2.21 µg/L, respectively, of test material as measured by LSC.

13. **STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:**

(excerpted from the submission)

Survival was not significantly reduced in any of the test material concentrations when compared to the acetone/reconstituted water controls as determined by Fisher's Exact Test.

The 21-day LC_{50} value, based on the measured total ^{14}C activity due to Amitraz and its three transformation products, was greater than 8.68 µg/L (the highest concentration tested).

The NOEC and LOEC for reproduction were 1.10 and 2.21 µg/L respectively, as determined by One-way analysis of variance.

The NOEC and LOEC for reproduction rate were 1.10 and 2.21 µg/L respectively, as determined by a one-way analysis of variance when all treatments except the highest test material concentration (8.68 µg/L) were included in the analysis.

The NOEC and LOEC for total length were 1.10 and 2.21 µg/L, respectively, as determined by a one-way analysis of variance.

The MATC was greater than 1.10 µg/L and less that 2.21 µg/L.

The HPLC and TLC results for the relative percentages of Amitraz, BTS-27271, BTS-27919, and BTS-24868 were in good agreement.

The "study was inspected by the Batelle Quality Assurance Unit and reports were submitted to management and the Study Director." "The methods described were the methods followed and the data presented accurately represent data generated during the study."

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

A. Test Procedures: This study is scientifically sound. Although, at present not SEP Guidelines exist for a 21-Day Flow-Through Daphnia magna chronic toxicity test, the test procedures were discussed with the EPA Environmental Research Laboratory, Duluth, MN, and were found to be in compliance with currently accepted methods.

B. Statistical Analysis: Mortality, reproduction, and length data were reanalyzed by the reviewer using Fisher's Exact Test, Analysis of Variance, and Bonferroni's Multiple Comparison Test (see attached printouts).

Fisher's Exact Test showed no significant difference in mortality between the dilution water control and any of the treatments or solvent control at the 0.05 level.

The results of an ANOVA and Bonferroni's Multiple Comparison Test showed that for reproduction (the total number of young per reproducing adult female) there was no significant difference between the dilution water control and the 0.46 and 1.10 µg/L treatment levels. A significant difference in reproduction was found between the dilution water control and the 2.21, 4.15, and 8.68 µg/L treatment levels. The results were the same for reproduction rate (the total number of young per reproducing adult female per reproductive day) and Length data. As such, the reviewer's results and the study author's results are in agreement.

C. Discussion of Results: The 21-day LC_{50} of technical Amitraz and its degradation products is >8.68 µg/L (the highest concentration tested).

The NOEC, LOEC, and MATC for the parameters of growth (length), and reproduction (young/adult female and young/adult female/reproduction day) are 1.10 µg/L, 2.21 µg/L, and >1.10 µg/L and <2.21 µg/L respectively.

D. Adequacy of the Study:

1. Classification: Core

2. Rationale: The study is scientifically sound and methods were found to be in compliance with currently accepted methods.

3. Repairability: N/A

15. **COMPLETION OF ONE-LINER FOR STUDY:** 01-26-1990

attachments

FISHERS EXACT TEST

IDENTIFICATION	NUMBER OF DEAD	NUMBER OF ALIVE	NUMBER OF TOTAL ANIMALS
CONTROL	2	18	20
solvent control	1	19	20
TOTAL	3	37	40

CRITICAL FISHERS VALUE (20,20,2) (p=0.05) IS LESS THAN 0. b VALUE IS 1.
NO SIGNIFICANT DIFFERENCE

IDENTIFICATION	NUMBER OF DEAD	NUMBER OF ALIVE	NUMBER OF TOTAL ANIMALS
CONTROL	2	18	20
0.46 ug/L	0	20	20
TOTAL	2	38	40

CRITICAL FISHERS VALUE (20,20,2) (p=0.05) IS LESS THAN 0. b VALUE IS 0.
NO SIGNIFICANT DIFFERENCE

IDENTIFICATION	NUMBER OF DEAD	NUMBER OF ALIVE	NUMBER OF TOTAL ANIMALS
CONTROL	2	18	20
1.10 ug/L	0	20	20
TOTAL	2	38	40

CRITICAL FISHERS VALUE (20,20,2) (p=0.05) IS LESS THAN 0. b VALUE IS 0.
NO SIGNIFICANT DIFFERENCE

IDENTIFICATION	NUMBER OF ALIVE	DEAD	TOTAL ANIMALS
CONTROL	18	2	20
2.21 ug/L	18	2	20
TOTAL	36	4	40

CRITICAL FISHERS VALUE (20,20,18) (p=0.05) IS 12. b VALUE IS 18.
Since b is greater than 12 there is no significant difference between CONTROL and TREATMENT at the 0.05 level.

IDENTIFICATION	NUMBER OF ALIVE	DEAD	TOTAL ANIMALS
CONTROL	18	2	20
4.15 ug/L	18	2	20
TOTAL	36	4	40

CRITICAL FISHERS VALUE (20,20,18) (p=0.05) IS 12. b VALUE IS 18.
Since b is greater than 12 there is no significant difference between CONTROL and TREATMENT at the 0.05 level.

IDENTIFICATION	NUMBER OF ALIVE	DEAD	TOTAL ANIMALS
CONTROL	18	2	20
8.68 ug/L	18	2	20
TOTAL	36	4	40

CRITICAL FISHERS VALUE (20,20,18) (p=0.05) IS 12. b VALUE IS 18.
Since b is greater than 12 there is no significant difference between CONTROL and TREATMENT at the 0.05 level.

GROUP	IDENTIFICATION	NUMBER EXPOSED	NUMBER DEAD	SIG (P=.05)
	CONTROL	20	2	
1	solvent control	20	1	
2	0.46 ug/L	20	0	
3	1.10 ug/L	20	0	
4	2.21 ug/L	20	2	
5	4.15 ug/L	20	2	
6	8.68 ug/L	20	2	

Class Level Information

Class	Levels	Values
TRT	7	a b c d e f g

Number of observations in data set = 14

General Linear Models Procedure

Dependent Variable: YOUNG/REPRODUCING ADULT FEMALE

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	6	1975.361886	329.226981	161.36	0.0001
Error	7	14.282000	2.040286		
Corrected Total	13	1989.643886			

R-Square	C.V.	Root MSE	YOUNG Mean
0.992822	3.166952	1.428386	45.1028571

Source	DF	Type I SS	Mean Square	F Value	Pr > F
TRT	6	1975.361886	329.226981	161.36	0.0001

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TRT	6	1975.361886	329.226981	161.36	0.0001

General Linear Models Procedure

Bonferroni (Dunn) T tests for variable: YOUNG

NOTE: This test controls the type I experimentwise error rate, but generally has a higher type II error rate than REGWQ.

Alpha= 0.05 df= 7 MSE= 2.040286
Critical Value of T= 4.64
Minimum Significant Difference= 6.6219

Means with the same letter are not significantly different.

Bon Grouping	Mean	N	TRT
A	55.350	2	b
A			
A	54.800	2	c
A			
A	54.450	2	a
A			
A	54.050	2	d
B	41.390	2	e
C	30.230	2	g
C			
C	25.450	2	f

TRT=a

N Obs	N	Minimum	Maximum	Mean	Std Dev
2	2	54.3000000	54.6000000	54.4500000	0.2121320

TRT=b

N Obs	N	Minimum	Maximum	Mean	Std Dev
2	2	54.4000000	56.3000000	55.3500000	1.3435029

TRT=c

N Obs	N	Minimum	Maximum	Mean	Std Dev
2	2	53.7000000	55.9000000	54.8000000	1.5556349

TRT=d

N Obs	N	Minimum	Maximum	Mean	Std Dev
2	2	53.9000000	54.2000000	54.0500000	0.2121320

TRT=e

N Obs	N	Minimum	Maximum	Mean	Std Dev
2	2	41.0000000	41.7800000	41.3900000	0.5515433

TRT=f

N Obs	N	Minimum	Maximum	Mean	Std Dev
2	2	23.7000000	27.2000000	25.4500000	2.4748737

TRT=g

N Obs	N	Minimum	Maximum	Mean	Std Dev
2	2	28.9000000	31.5600000	30.2300000	1.8809040

Parameter-Young/Female/Reproductive Day

General Linear Models Procedure

Class Level Information

Class	Levels	Values
TRT	7	a b c d e f g

Number of observations in data set = 14

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	6	10.49408571	1.74901429	51.57	0.0001
Error	7	0.23740000	0.03391429		
Corrected Total	13	10.73148571			

R-Square	C.V.	Root MSE	YOUNG Mean
0.977878	5.364579	0.184158	3.43285714

Source	DF	Type I SS	Mean Square	F Value	Pr > F
TRT	6	10.49408571	1.74901429	51.57	0.0001

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TRT	6	10.49408571	1.74901429	51.57	0.0001

Bonferroni (Dunn) T tests for variable: YOUNG

NOTE: This test controls the type I experimentwise error rate, but generally has a higher type II error rate than REGWQ.

Alpha= 0.05 df= 7 MSE= 0.033914
Critical Value of T= 4.64
Minimum Significant Difference= 0.8537

Means with the same letter are not significantly different.

Bon Grouping	Mean	N	TRT
A	4.340	2	a
A			
A	4.270	2	c
A			
A	4.000	2	d
A			
B A	3.885	2	b
B			
B C	3.065	2	e
C			
D C	2.545	2	g
D			
D	1.925	2	f

TRT=a

N Obs	N	Minimum	Maximum	Mean	Std Dev
2	2	4.3200000	4.3600000	4.3400000	0.0282843

TRT=b

N Obs	N	Minimum	Maximum	Mean	Std Dev
2	2	3.7500000	4.0200000	3.8850000	0.1909188

TRT=c

N Obs	N	Minimum	Maximum	Mean	Std Dev
2	2	4.1400000	4.4000000	4.2700000	0.1838478

TRT=d

N Obs	N	Minimum	Maximum	Mean	Std Dev
2	2	3.9900000	4.0100000	4.0000000	0.0141421

TRT=e

N Obs	N	Minimum	Maximum	Mean	Std Dev
2	2	3.0400000	3.0900000	3.0650000	0.0353553

TRT=f

N Obs	N	Minimum	Maximum	Mean	Std Dev
2	2	1.7600000	2.0900000	1.9250000	0.2333452

TRT=g

N Obs	N	Minimum	Maximum	Mean	Std Dev
2	2	2.3100000	2.7800000	2.5450000	0.3323402

General Linear Models Procedure
Class Level Information

Class	Levels	Values
TRT	7	a b c d e f g

Number of observations in data set = 130

General Linear Models Procedure

Dependent Variable: LENGTH

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	6	0.50139046	0.08356508	8.32	0.0001
Error	123	1.23505646	0.01004111		
Corrected Total	129	1.73644692			

R-Square	C.V.	Root MSE	LENGTH Mean
0.288745	2.810688	0.100205	3.56515385

Source	DF	Type I SS	Mean Square	F Value	Pr > F
TRT	6	0.50139046	0.08356508	8.32	0.0001

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TRT	6	0.50139046	0.08356508	8.32	0.0001

Bonferroni (Dunn) T tests for variable: LENGTH

NOTE: This test controls the type I experimentwise error rate but generally has a higher type II error rate than Tukey's for all pairwise comparisons.

Alpha= 0.05 Confidence= 0.95 df= 123 MSE= 0.010041
Critical Value of T= 3.10247

Comparisons significant at the 0.05 level are indicated by '***'.

General Linear Models Procedure

TRT Comparison		Simultaneous Lower Confidence Limit	Difference Between Means	Simultaneous Upper Confidence Limit	
b	- d	-0.0961	0.0035	0.1031	
b	- a	-0.0517	0.0506	0.1528	
b	- c	-0.0456	0.0553	0.1561	
b	- e	0.0105	0.1128	0.2150	***
b	- f	0.0455	0.1478	0.2500	***
b	- g	0.0650	0.1672	0.2695	***

d	- b	-0.1031	-0.0035	0.0961	
d	- a	-0.0539	0.0471	0.1481	
d	- c	-0.0478	0.0518	0.1514	
d	- e	0.0083	0.1093	0.2103	***
d	- f	0.0433	0.1443	0.2453	***
d	- g	0.0627	0.1637	0.2647	***
a	- b	-0.1528	-0.0506	0.0517	
a	- d	-0.1481	-0.0471	0.0539	
a	- c	-0.0975	0.0047	0.1070	
a	- e	-0.0414	0.0622	0.1659	
a	- f	-0.0064	0.0972	0.2009	
a	- g	0.0130	0.1167	0.2203	***
c	- b	-0.1561	-0.0553	0.0456	
c	- d	-0.1514	-0.0518	0.0478	
c	- a	-0.1070	-0.0047	0.0975	
c	- e	-0.0447	0.0575	0.1598	
c	- f	-0.0097	0.0925	0.1948	
c	- g	0.0097	0.1120	0.2142	***
e	- b	-0.2150	-0.1128	-0.0105	***
e	- d	-0.2103	-0.1093	-0.0083	***
e	- a	-0.1659	-0.0622	0.0414	
e	- c	-0.1598	-0.0575	0.0447	
e	- f	-0.0686	0.0350	0.1386	
e	- g	-0.0492	0.0544	0.1581	
f	- b	-0.2500	-0.1478	-0.0455	***
f	- d	-0.2453	-0.1443	-0.0433	***
f	- a	-0.2009	-0.0972	0.0064	
f	- c	-0.1948	-0.0925	0.0097	
f	- e	-0.1386	-0.0350	0.0686	
f	- g	-0.0842	0.0194	0.1231	
g	- b	-0.2695	-0.1672	-0.0650	***
g	- d	-0.2647	-0.1637	-0.0627	***
g	- a	-0.2203	-0.1167	-0.0130	***
g	- c	-0.2142	-0.1120	-0.0097	***
g	- e	-0.1581	-0.0544	0.0492	
g	- f	-0.1231	-0.0194	0.0842	

---------- TRT=a ----------

N Obs	N	Minimum	Maximum	Mean	Std Dev
18	18	3.5000000	3.7100000	3.5894444	0.0670796

---------- TRT=b ----------

N Obs	N	Minimum	Maximum	Mean	Std Dev
19	19	3.4300000	3.9900000	3.6400000	0.1419311

TRT=c

N Obs	N	Minimum	Maximum	Mean	Std Dev
19	19	3.4300000	3.7100000	3.5847368	0.0827559

TRT=d

N Obs	N	Minimum	Maximum	Mean	Std Dev
20	20	3.5000000	3.7800000	3.6365000	0.0833682

TRT=e

N Obs	N	Minimum	Maximum	Mean	Std Dev
18	18	3.3600000	3.7100000	3.5272222	0.1179634

TRT=f

N Obs	N	Minimum	Maximum	Mean	Std Dev
18	18	3.2900000	3.6400000	3.4922222	0.0986709

TRT=g

N Obs	N	Minimum	Maximum	Mean	Std Dev
18	18	3.2900000	3.6400000	3.4727778	0.0902810

DATA EVALUATION RECORD

1. **CHEMICAL:** Amitraz
   Shaughnessy #106201

2. **TEST MATERIAL:** Amitraz, 98.8%

3. **STUDY TYPE:** Fish Early Life-Cycle

4. **STUDY IDENTIFICATION:** Hill, R.W., J.E. Caunter, E. Gillings, A.M. Riddle, 1989,(W109): ^{14}C Amitraz Equivalents: Determination of Chronic Toxicity to Fathead Minnow (Pimephales promelas) Embryos and Larvae, Schering Agrochemical Limited, Chesterford Park Research Station, Saffron Walden, Essex CB10 1XL, Submitted by Nor-Am Chemical Company, P.O. Box 7495, Wilmington, Delaware 19803, MRID #412887-02

5. **REVIEW BY:** Harry A. Winnik
   Biologist
   EFED/EEB
   Signature:
   Date: 2-13-90

6. **APPROVED BY:** Henry Craven
   Supervisory Biologist
   EFED/EEB
   Signature:
   Date: 2/13/90

7. **CONCLUSIONS:** This study is scientifically sound and fulfills the SEP Guideline requirements for a Fish Early Life-Stage Test. Based on the most sensitive parameter (length) the MATC, NOEC, and LOEC values of Amitraz (98.8%) for Pimephales promelas were >1.48 and <2.71 µg/L, 1.48 µg/L, and 2.71 µg/L respectively.

8. **RECOMMENDATIONS:** N/A

9. **BACKGROUND:** The study was submitted by Nor-Am Chemical Company to support the registration of Amitraz on cotton.

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

11. **MATERIALS AND METHODS:**

A. Test Animals: (excerpted from the submission)

The fathead minnow (Pimephales promelas) embryos used in this study were obtained from brood stock held at the Brixham Laboratory. The fish were originally purchased from SP Engineering Technology, Salem, Massachusetts, USA and held in the laboratory since 4 March, 1987. The brood stock fish were fed daily on a basic diet of Promin®, a proprietary brand of tropical fish food, and brine shrimp. This was supplemented with other foods at the discretion of the operator. 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. 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: (excerpted from the submission)

A dynamic (flowthrough) test system was used for this study.

The test apparatus was constructed of glass with a minimum of other materials in contact with the test material.

The test vessels were of all glass construction, rectangular in shape with dimensions 30 x 20 x 20 cm and a capacity of 12 liters. The volume used was nominally 9.0 liters and the water depth in each tank was approximately 15 cm.

The incubation cups were made from 8 cm lengths of 5 cm o.d. glass tubing with nylon mesh 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.

C. Dosage: (excerpted from the submission)

Five nominal concentrations, 12, 6, 3, 1.5, 0.75 µg/L of ^{14}C Amitraz technical a solvent control and a dilution water control were used in the study. Replicate tanks were employed for all concentrations and the controls.

Stock solutions for each test concentration used were prepared by mixing relative weights of an inactive solution of Amitraz in basified triethylene glycol (TEG) with corresponding weights of active Amitraz in basified TEG. These solutions were then diluted with TEG to the volumes required. From the weights of the active and inactive Amitraz used, the total radioactivity and the specific activity for each stock concentrate was calculated. Stock solutions of 10,000 times the required exposure concentrations were prepared in this manner. These stock solutions were stirred continuously prior to use in the study where they were delivered at a nominal flow rate of 0.03 ml/minute to the mixing chambers. The flow rates of the stocks and of the dilution water were measured on day 0 and thereafter three times per week. The nominal dilution achieved at this stage, immediately before delivery to the exposure tanks, was 10,000 times. The stock jars were replenished at weekly intervals.

The solvent control contained 100 µL/L of triethylene glycol which was the level used in each test concentration.

The dilution water was dechlorinated mains 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 5 µm activated carbon, filtered to 1 µm to remove particulate material and preheated to 25° C in a header tank on the test rig.

D. Design: The test was begun by dosing ^{14}C Amitraz equivalents into the test system. Two incubation cups, each containing 20 eggs, were placed into each duplicate test concentration tank (giving a total of 40 eggs per duplicate and 80 eggs per test concentration), giving a nominal loading of 4.7 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 tanks divided by the number of eggs on day 0.

The "hatch day" was determined to be that day on which the greatest number of fry were released into the progeny tanks. When all eggs had hatched the number of fry was thinned to 30 per replicate by removal of any excess fry.

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. Time to swim-up started at exposure day 6, 2 days post-hatch and the majority of fish were swimming at day 4 post-hatch.

The test was undertaken at 25 $\pm$ 1°C with a photoperiod of 16 hours light alternating with 8 hours of darkness.

Dissolved oxygen, pH and temperature measurements were made in both replicate tanks at day 0 and then twice weekly throughout the study. Each weekday the general laboratory freshwater supply was monitored in terms of water hardness conductivity. Representative samples of the laboratory freshwater were analyzed for heavy metals, pesticides and other constituents.

E. Statistics: (excerpted from the submission)

The relative standard deviations (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, looking for differences at the 5% significance level.

The larval length and weight data for the solvent control and dilution water controls were tested for differences

(P=0.05) between replicates using Student's t tests. In the absence of significance 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 5% and 1% 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**: (excerpted from the submission)

The mean measured analytical levels of ^{14}C Amitraz equivalents in this study ranged from 86 to 99% of the nominal concentration.

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.

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 85 to 100% with and overall mean value of 91%.

Larval survival was not significantly affected (P=0.05) between the solvent and dilution water controls. The larval survival for all concentrations ranged from 33.3 to 100% based on the initial number of embryos exposed. The 10.63 µg/L mean measured concentration was significantly different compared to both controls.

(With respect to length) Comparison of the replicates of the dilution water control and the solvent controls showed no significant differences. Using the solvent control for further comparisons the 2.71 and 5.25 µg/L measured concentrations are significantly different from the control. There was no significant difference between the solvent control and the higher concentration tested, measured 10.63 µg/L ^{14}C Amitraz equivalents. This is thought to be due to the reduction which occurred in the fry and does not indicate that this was a safe concentration.

(With respect to weight) a significant difference (P=0.05) was seen between the solvent control and the measured 5.25 µg/L ^{14}C Amitraz equivalents. No other significant differences were seen in any other concentration compared

with the solvent and dilution water controls. Analysis of the weight data followed the same pattern as the length data in this case.

The no observed effect concentration (NOEC) of Amitraz equivalents was therefore considered to 1.48 µg/L ^{14}C Amitraz equivalents. The lowest observed effect concentration (LOEC) was considered to be 2.71 µg/L. The maximum acceptable toxicant concentration (MATC) was >1.48 <2.71 µg/L Amitraz equivalents.

13. **STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:**

Hatchability of fathead minnow embryos was not significantly affected at any concentration tested.

Survival was significantly reduced in the 10.63 µg/L mean measured ^{14}C Amitraz equivalents test concentration.

Larval growth with respect to the parameter length was significantly reduced in the 2.71 and 5.25 µg/L mean ^{14}C Amitraz equivalents test concentrations when compared to the solvent control.

Larval growth with respect to the parameter weight was significantly reduced in the 5.25 µg/L mean ^{14}C Amitraz equivalents test concentration when compared to the solvent control.

Using the mean measured concentrations and based on the above data the NOEC was determined as 1.48 µg/L Amitraz equivalents, the LOEC was 2.71 µg/L Amitraz equivalents, and the MATC was >1.48 and <2.71 µg/L Amitraz equivalents.

The 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 Procedures: This study is scientifically sound and generally meets the Guidelines for a Fish Early Life-Stage study but deviated from the SEP as follows:

The SEP states that "embryos should be 2-24 hours old at the beginning of the test." In this study the embryos were <48 hours old.

The SEP states that "fresh water parameters in a control and one concentration must be analyzed once a week. These parameters should include pH, alkalinity, hardness, and conductance." In this study the general laboratory freshwater supply was monitored in terms of water hardness

and conductivity each weekday. pH was measured twice weekly.

B. Statistical Analysis: Mortality, hatchability, length and weight data were reanalyzed by the reviewer using analysis of variance, Dunnett's test, Bonferroni's test, and Duncan's test (see attached). Due to the statistically significant difference between the dilution water control and the solvent control with respect to the growth parameters length and weight, possible indicating a solvent effect, all test concentrations were compared to the solvent control.

The results of an ANOVA, Bonferroni's and Dunnett's test showed no significant difference in hatchability between the solvent control and the different treatment concentrations.

The results of an ANOVA and Bonferroni's, Dunnett's, and Duncan's tests showed no significant difference in mortality between the solvent control and the 0.65, 1.48, 2.71, and 5.25 µg/L mean measured ^{14}C Amitraz equivalent test concentrations. There was a significant difference between the solvent control and the 10.63 µg/L concentration.

The results of an ANOVA and Bonferroni's, Dunnett's, and Duncan's tests showed no significant difference in the growth parameter, length, between the solvent control and the 0.65 and 1.48 µg/L mean measured ^{14}C Amitraz equivalent test concentrations. There was a significant difference between the solvent control and the 2.71 and 5.25 µg/L concentrations. There was no significant difference between the solvent control and the 10.63 µg/L concentration.

The results of an ANOVA and Bonferroni's, Dunnett's, and Duncan's tests showed no significant difference in the growth parameter, weight, between the solvent control and the 0.65, 1.48, and 2.71 µg/L mean measured ^{14}C Amitraz equivalent test concentrations. There was a significant difference between the solvent control and the 5.25 µg/L concentration. There was no significant difference between the solvent control and the 10.63 µg/L concentration.

Although the reviewer did not pool the controls as did the study author, the results were in good agreement and are considered acceptable.

C. Discussion of Results: Hatching was not affected by Amitraz at any concentrations tested. The test concentration of 10.63 µg/L significantly reduced larval survival when compared to the solvent control. Larval weight was significantly reduced in the 5.25 µg/L test concentration.

Larval length was significantly reduced in the 2.71 and 5.25 µg/L test concentrations.

Therefore, based on the most sensitive parameter (length), the MATC, NOEL, and LOEC values of Amitraz for the fathead minnow (Pimephales promelas) were >1.48 and <2.71 µg/L, 1.48 µg/L, and 2.71 µg/L respectively.

D. Adequacy of the Study:

(1) Classification: Core

(2) Rationale: The study was scientifically sound and fulfills the SEP Guideline requirements.

(3) Repairability: N/A

15. **COMPLETION OF ONE-LINER FOR STUDY**: 2-6-90

attachments

hatchability
File: a:\amiminha.dat Transform: NO TRANSFORM

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	145.718	29.144	1.096
Within (Error)	6	159.485	26.581	
Total	11	305.202		

Critical F value = 4.39 (0.05,5,6)
Since F < Critical F FAIL TO REJECT Ho:All groups equal

hatchability
File: a:\amiminha.dat Transform: NO TRANSFORM

DUNNETTS TEST - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	solvent control	95.250	95.250		
2	0.75	93.750	93.750	0.291	
3	1.5	97.550	97.550	-0.446	
4	3.0	95.100	95.100	0.029	
5	6.0	86.450	86.450	1.707	
6	12.0	92.450	92.450	0.543	

Dunnett table value = 2.83 (1 Tailed Value, P=0.05, df=6,5)

hatchability
File: a:\amiminha.dat Transform: NO TRANSFORM

DUNNETTS TEST - TABLE 2 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	solvent control	2			
2	0.75	2	14.591	15.3	1.500
3	1.5	2	14.591	15.3	-2.300
4	3.0	2	14.591	15.3	0.150
5	6.0	2	14.591	15.3	8.800
6	12.0	2	14.591	15.3	2.800

hatchability
File: a:\amiminha.dat Transform: NO TRANSFORM

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	145.718	29.144	1.096
Within (Error)	6	159.485	26.581	
Total	11	305.202		

Critical F value = 4.39 (0.05,5,6)
Since F < Critical F FAIL TO REJECT Ho:All groups equal

hatchability
File: a:\amiminha.dat Transform: NO TRANSFORM

BONFERRONI T-TEST - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	solvent control	95.250	95.250		
2	0.75	93.750	93.750	0.291	
3	1.5	97.550	97.550	-0.446	
4	3.0	95.100	95.100	0.029	
5	6.0	86.450	86.450	1.707	
6	12.0	92.450	92.450	0.543	

Bonferroni T table value = 3.14 (1 Tailed Value, P=0.05, df=6,5)

hatchability
File: a:\amiminha.dat Transform: NO TRANSFORM

BONFERRONI T-TEST - TABLE 2 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	solvent control	2			
2	0.75	2	16.204	17.0	1.500
3	1.5	2	16.204	17.0	-2.300
4	3.0	2	16.204	17.0	0.150
5	6.0	2	16.204	17.0	8.800
6	12.0	2	16.204	17.0	2.800

General Linear Models Procedure
Class Level Information

Class	Levels	Values
TRT	6	a b c d e f

Number of observations in data set = 12

Dependent Variable: MORTALITY

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	5	343.7500000	68.7500000	25.00	0.0006
Error	6	16.5000000	2.7500000		
Corrected Total	11	360.2500000			

R-Square	C.V.	Root MSE	MORT Mean
0.954198	24.56759	1.658312	6.75000000

Source	DF	Type I SS	Mean Square	F Value	Pr > F
TRT	5	343.7500000	68.7500000	25.00	0.0006

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TRT	5	343.7500000	68.7500000	25.00	0.0006

Bonferroni (Dunn) T tests for variable: MORT

NOTE: This test controls the type I experimentwise error rate, but generally has a higher type II error rate than REGWQ.

Alpha= 0.05 df= 6 MSE= 2.75
Critical Value of T= 4.70
Minimum Significant Difference= 7.7906

Means with the same letter are not significantly different.

Bon Grouping	Mean	N	TRT
A	17.500	2	f
B	8.000	2	e
B			
B	7.000	2	c
B			
B	4.000	2	d
B			
B	3.000	2	a
B			
B	1.000	2	b

Dunnett's T tests for variable: MORT

NOTE: This tests controls the type I experimentwise error for comparisons of all treatments against a control.

Alpha= 0.05 Confidence= 0.95 df= 6 MSE= 2.75
Critical Value of Dunnett's T= 3.389
Minimum Significant Difference= 5.6195

Comparisons significant at the 0.05 level are indicated by '***'.

TRT Comparison	Simultaneous Lower Confidence Limit	Difference Between Means	Simultaneous Upper Confidence Limit	
f - a	8.881	14.500	20.119	***
e - a	-0.619	5.000	10.619	
c - a	-1.619	4.000	9.619	
d - a	-4.619	1.000	6.619	
b - a	-7.619	-2.000	3.619	

Duncan's Multiple Range Test for variable: MORT

NOTE: This test controls the type I comparisonwise error rate, not the experimentwise error rate

Alpha= 0.05 df= 6 MSE= 2.75

Number of Means	2	3	4	5	6
Critical Range	4.058	4.206	4.274	4.309	4.325

Means with the same letter are not significantly different.

Duncan Grouping			Mean	N	TRT
	A		17.500	2	f
	B		8.000	2	e
	B				
C	B		7.000	2	c
C	B				
C	B	D	4.000	2	d
C		D			
C		D	3.000	2	a
		D			
		D	1.000	2	b

---------- TRT=a ----------

N Obs	N	Minimum	Maximum	Mean	Std Dev
2	2	3.0000000	3.0000000	3.0000000	0

TRT=b

N Obs	N	Minimum	Maximum	Mean	Std Dev
2	2	0	2.0000000	1.0000000	1.4142136

TRT=c

N Obs	N	Minimum	Maximum	Mean	Std Dev
2	2	7.0000000	7.0000000	7.0000000	0

TRT=d

N Obs	N	Minimum	Maximum	Mean	Std Dev
2	2	4.0000000	4.0000000	4.0000000	0

TRT=e

N Obs	N	Minimum	Maximum	Mean	Std Dev
2	2	7.0000000	9.0000000	8.0000000	1.4142136

TRT=f

N Obs	N	Minimum	Maximum	Mean	Std Dev
2	2	15.0000000	20.0000000	17.5000000	3.5355339

General Linear Models Procedure
Class Level Information

Class	Levels	Values
TRT	7	a b c d e f g

Number of observations in data set = 331

General Linear Models Procedure

Dependent Variable: LENGTH

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	6	78.49559419	13.08259903	2.78	0.0119
Error	324	1524.52174720	4.70531403		
Corrected Total	330	1603.01734139			

R-Square	C.V.	Root MSE	LENGTH Mean
0.048967	10.54466	2.169174	20.5712991

Source	DF	Type I SS	Mean Square	F Value	Pr > F
TRT	6	78.49559419	13.08259903	2.78	0.0119

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TRT	6	78.49559419	13.08259903	2.78	0.0119

Dunnett's T tests for variable: LENGTH

NOTE: This tests controls the type I experimentwise error for comparison of all treatments against a control.

Alpha= 0.05 Confidence= 0.95 df= 324 MSE= 4.705314
Critical Value of Dunnett's T= 2.597

Comparisons significant at the 0.05 level are indicated by '***'.

TRT Comparison		Simultaneous Lower Confidence Limit	Difference Between Means	Simultaneous Upper Confidence Limit	
g	- a	-2.119	-0.756	0.606	
c	- a	-1.918	-0.853	0.212	
d	- a	-2.190	-1.060	0.071	
e	- a	-2.272	-1.178	-0.084	***
b	- a	-2.313	-1.218	-0.124	***
f	- a	-2.789	-1.645	-0.501	***

TRT=a

N Obs	N	Minimum	Maximum	Mean	Std Dev
54	54	14.8000000	25.1000000	21.5203704	1.7127028

TRT=b

N Obs	N	Minimum	Maximum	Mean	Std Dev
52	52	11.0000000	23.8000000	20.3019231	2.1287473

TRT=c

N Obs	N	Minimum	Maximum	Mean	Std Dev
58	58	15.6000000	24.4000000	20.6672414	1.7885001

TRT=d

N Obs	N	Minimum	Maximum	Mean	Std Dev
46	46	10.5000000	25.1000000	20.4608696	2.9650916

TRT=e

N Obs	N	Minimum	Maximum	Mean	Std Dev
52	52	10.7000000	24.6000000	20.3423077	2.5060981

TRT=f

N Obs	N	Minimum	Maximum	Mean	Std Dev
44	44	14.8000000	23.4000000	19.8750000	2.0014094

TRT=g

N Obs	N	Minimum	Maximum	Mean	Std Dev
25	25	15.4000000	23.4000000	20.7640000	1.6762756

General Linear Models Procedure
Class Level Information

Class	Levels	Values
TRT	7	a b c d e f g

Number of observations in data set = 331

Dependent Variable: WEIGHT

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	6	29048.77319	4841.46220	2.86	0.0099
Error	324	547762.57593	1690.62523		
Corrected Total	330	576811.34911			

R-Square	C.V.	Root MSE	WEIGHT Mean
0.050361	29.74748	41.11721	138.220807

Source	DF	Type I SS	Mean Square	F Value	Pr > F
TRT	6	29048.77319	4841.46220	2.86	0.0099

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TRT	6	29048.77319	4841.46220	2.86	0.0099

Dunnett's T tests for variable: WEIGHT

NOTE: This tests controls the type I experimentwise error for comparisons of all treatments against a control.

Alpha= 0.05 Confidence= 0.95 df= 324 MSE= 1690.625
Critical Value of Dunnett's T= 2.597

Comparisons significant at the 0.05 level are indicated by '***'.

TRT Comparison	Simultaneous Lower Confidence Limit	Difference Between Means	Simultaneous Upper Confidence Limit	
g - a	-23.112	2.715	28.543	
d - a	-23.944	-2.522	18.900	
c - a	-29.165	-8.975	11.215	
e - a	-35.696	-14.952	5.791	
b - a	-43.139	-22.395	-1.651	***
f - a	-45.055	-23.372	-1.689	***

TRT=a

N Obs	N	Minimum	Maximum	Mean	Std Dev
54	54	46.5000000	218.8000000	148.9129630	33.6973099

TRT=b

N Obs	N	Minimum	Maximum	Mean	Std Dev
52	52	19.2000000	213.6000000	126.5180769	37.9675926

TRT=c

N Obs	N	Minimum	Maximum	Mean	Std Dev
58	58	55.7000000	225.9000000	139.9379310	35.6258571

TRT=d

N Obs	N	Minimum	Maximum	Mean	Std Dev
46	46	14.3000000	238.6000000	146.3913043	51.4562028

TRT=e

N Obs	N	Minimum	Maximum	Mean	Std Dev
52	52	14.8000000	232.1000000	133.9605192	47.2521610

TRT=f

N Obs	N	Minimum	Maximum	Mean	Std Dev
44	44	46.3000000	203.8000000	125.5409091	40.8416643

TRT=g

N Obs	N	Minimum	Maximum	Mean	Std Dev
25	25	54.3000000	223.5000000	151.6280000	39.2494429