9-14-94

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

SEP 14 1994

MEMORANDUM

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

SUBJECT: EEB Review of TPTH Bobwhite Quail and Mallard Duck Reproduction Studies (D201655) and Review of an Acute LC_{50} for Mysid and Sheepshead Minnow, and a Shell Deposition Study with Eastern Oyster (D203510).

FROM: Anthony Maciorowski, Chief
Ecological Effects Branch
Environmental Fate and Effects Division (7507C) 9/13/94

TO: Eric Ferris, Product Manager Team Reviewer,
Team 71
Special Review and Reregistration Division (7508W)

As requested through the list A reregistration process, Atochem North America, Inc. has provided subject studies. The following are the conclusions for the avian reproduction studies from the Data Evaluation Records (DER):

BOBWHITE QUAIL MRID No. 43178501

6. CONCLUSION: This study is scientifically sound but does not fulfill the guideline requirements pertinent items need more explanation and information (see 7. Major Guideline Deviations). The no-effect-level and the lowest effect level are 3 ppm and 30 ppm, respectively. Statistically significant items are listed below under the appropriate dose level (see following Table 1).

Table 1
Tabulation of Affected Parameters by Concentration

@ 90 ppm	@ 30 ppm
1. Egg shell thickness	1. Day-14 survivors of set (%)
2. Normal eggs/hen	2. Normal hatchlings of day-18 live embryos
3. Viable embryos/hen	3. Day-14 survivors of normal hatchlings (%)
4. Live embryos/hen	4. Food consumption/pen
5. Normal hatchlings/hen	
6. Hatchling survivors/hen	

1

7. Viable embryos of set (%)	
8. Day-18 live embryos of set (%)	
9. Normal hatchlings of set (%)	
10. Day-14 survivors of set (%)	
11. Day-18 live embryos of viable embryos (%)	
12. Normal hatchlings of viable embryos (%)	
13. Normal hatchlings of day-18 live embryos	
14. Day-14 survivors of normal hatchlings (%)	
15. Female body weight/pen	
16. Food consumption/pen	

Table 3 under 12. Statistical Analysis below shows that major reproductive effects persisted throughout the 5 week recovery period. In addition, the ovary weights were statistically from the control for the 3 ppm and 90 ppm level. EEB was unable to determine if this effect was related to the TPTH exposure. Testes weight analysis showed no statistical differences.

MALLARD DUCK MRID No. 43178502

CONCLUSION: This study is scientifically sound but does not fulfill the guideline requirements pertinent items need more explanation and information. (see 7. Major Guideline Deviations). TPTH at 30 and 90 ppm cause many reproductive effects. The no-effect-level and the lowest effect level are 3 ppm and 30 ppm, respectively. Statistically significant items are listed below under the appropriate dose level. (see following table)

Table 1
Listing of Affected Parameters for 90 and 30 ppm Dose Levels

@ 90 ppm	@ 30 ppm
Eggs Laid/Pen	Live Embryos
Eggs Set/Pen	Normal Hatchlings
Viable Embryos/Pen	Hatchling Survivors
Live Embryos/Pen	14-Day-Old Survivors
Normal Hatchlings/Pen	Eggshell Thickness
Hatchlings Survivors/Pen	
Egg Shell Thickness/Pen	
14 Day Old Survivors/Pen	

Eggs Set/Eggs Laid.	
Viable Embryos/Eggs Set	
Live Embryos/Viable Embryos	
Normal Hatchlings/Eggs Laid	
Eggs Cracked/ Eggs Laid	
Normal Hatchlings/Eggs Set	
Hatchling Survivor/Eggs Set	
Male Body Weight	

Also as shown in Table 3 below several of the reproductive effects were still detectable after the 5 week recovery period. In addition, the ovary weights were not effected. On the other hand, testes weight were statistically different at the 30 ppm level but not the 90. EEB was unable to determine if this effect was due to TPTH.

Based on these results neither the bobwhite quail or mallard duck study fulfills the guideline requirements, but both may be upgraded if pertinent information is provided. The no-observable-effect-level and lowest observable effect levels were the same for both species, 3 ppm and 30 ppm, respectively.

In addition to the avian reproduction studies, three subject estuarine/marine studies were submitted by Griffin Corp (D203510 with a letter dated 4/2/94; MRID No.:43212700). The following are the conclusions to EEB's Data Evaluation Records:

Sheepshead Minnow (*Cyprinodon variegatus*) MRID No.43212702

7. CONCLUSION
This study fulfills the guideline requirements for an acute toxicity test using sheepshead minnows. Under the conditions of the test, the 96-hour LC_{50} was 25.5 (21-33) µg ai/L, which classifies TPTH as very highly toxic to sheepshead minnows.

Mysid Shrimp (*Mysidopsis bahia*) MRID No.43211703

7. CONCLUSION This study fulfills the guideline requirements. The reported moving average angle was 4.3 (3.7-5.0) µg/L which will place TPTH in the very highly toxic category (<100 µg/L).

Eastern Oyster (*Crassostrea virginica*) MRID No. 43212701

7. CONCLUSION
This test is scientifically sound but the control oysters did not grow the minimum 2 mm of shell necessary to demonstrate acceptable test conditions. Better performance of the controls would result in a more definitive EC_{50}. Based on this, the study does not fulfill the guideline requirements for an acute toxicity test using the eastern oyster. Under the conditions of the test, the 96-hour EC_{50} was 0.36 µg/L which classifies TPTH as very highly

toxic (<100 µg/L) to eastern oysters.

Of the three studies only the eastern oyster does not fulfill the guideline requirements. Because the eastern oyster shell deposition for TPTH is the most sensitive variable yet tested it is considered critical in the risk assessment. Risk assessments with a chemical toxicologically active in the parts per trillon and with low application rates inherently require more precision than less toxic and higher use rate compounds. Due to poor growth of the control shells unequivocal results were not obtained.

Therefore, in addition to the upgrade information for the avian reproduction study a shell deposition study with good shell growth in the controls is needed in order to complete a risk assessment.

In addition to the submitted studies performed by EPA's Environmental Research Laboratory in Duluth were reviewed and added to the TPTH file. The follow is the conclusion of the DER for this work:

> **CONCLUSIONS:** These studies do not fulfill the guideline requirements. This article reported the results of an acute static (single pulse) LC_{50} study for the following time periods 12, 24, 48, 72, and 96 hours, and continuous exposure survival and growth study for the following the time periods 24, 48, 72 hours. The following results were excerpted from the study:
>
> "... 96-hour LC_{50} value for continuously exposed larval fathead minnows was 7.1µg/L and the 96-hour EC_{50} value was 3.7 µg/L." See attached Table 1 for LC_{50} and EC_{50} for a range of exposure periods.
>
> "The concentrations that caused a significant reduction in survival and growth for these exposures were 13.0, 13.0 and 6.0 µg/liter, respectively. Growth, however, was a more sensitive parameter than survival during the continuous exposure studies. Survival was significantly reduced at 2.0 µg/liter, whereas, a significant reduction in growth occurred at 0.23 µg/l. The weight of the fish at this concentration was 15% lower than that for the control fish. The weight of the fish at the lowest concentration tested (0.15 µg/liter) was 9% lower than that for the control fish but this difference was not statistically significant. Approximately, a 68-h LC_{50} value would be required to cause a significant reduction in fathead minnow survival and growth within 30 days (Fig. 2)."
>
> Based on these values TPTH is very highly toxic.

Please contact Dennis McLane of EEB if any further information is needed (305-5096).

DATA EVALUATION RECORD
§ 71-4 Avian Reproduction Study
Bobwhite Quail

1. CHEMICAL: TPTH

2. TEST MATERIAL: TPTH 97.9% (Batch No. GFRAM 911K; 97.9%; CAS No. 76-87-9) was a fine, white powder with a characteristic odor.

3. CITATION:

Author:	Carol A. Pederson, Connie L. Lesar
Title:	Toxicity and Reproduction Study in Bobwhite Quail
Date:	January 24, 1994
Laboratory Report #:	BLAL No. 106-009-07
Any Other Study #:	N/A
Sponsor:	Elf Atochem North America, Inc., Philadelphia, PA
Laboratory:	Bio-life Associates, Ltd
MRID No.:	43178501

4. REVIEWED BY:

Dennis J. McLane, Wildlife Biologist
Ecological Effects Branch
Environmental Fate and Effects Division (7507 C)
9-12-94

5. APPROVED BY:

Les Touart, Section Head
Ecological Effects Branch
Environmental Fate and Effects Division (7507 C)
9-12-94

6. CONCLUSION: This study is scientifically sound but does not fulfill the guideline requirements pertinent items need more explanation and information (see 7. Major Guideline Deviations). The no-effect-level and the lowest effect level are 3 ppm and 30 ppm, respectively. Statistically significant items are listed below under the appropriate dose level (see following Table 1).

Table 1
Tabulation of Affected Parameters by Concentration

@ 90 ppm	@ 30 ppm

1. Egg shell thickness, 2. Normal eggs/hen, 3. Viable embryos/hen 4. Live embryos/hen 5. Normal hatchlings/hen 6. Hatchling survivors 7. Viable embryos of set%, 8. Day-18 live embryos of set (%), 9. Normal hatchlings of set (%), 10. Day-14 survivors of set(%), 11. Day-18 live embryos of viable embryos (%), 12. Normal hatchlings of viable embryos (%), 13. Normal hatchlings of day-18 live embryos, 14. Day-14 survivors of normal hatchlings (%), 15. Female Body weight 16. Food consumption/ pen	1. Day-14 survivors of set (%), 2. Normal hatchlings of day-18 live embryos, 3. Day-14 survivors of normal hatchlings (%), 4. Food consumption/pen

Table 3 under 12. Statistical Analysis below shows that major reproductive effects persisted throughout the 5 week recovery period. In addition, the ovary weights were statistically from the control for the 3 ppm and 90 ppm level. EEB was unable to determine if this effect was related to the exposure to TPTH. Testes weight analysis showed no statistical differences.

7. MAJOR GUIDELINE DEVIATIONS:

A. The report omitted the scientific explanation for removing the small eggs.

B. It was reported that the birds were treated with an antibiotic but the illness was not reported.

C. The dosage levels were separated by a factor of three rather than five.

D. The rational for using more than 2% total vehicle was not included. The guidelines indicate only 2% total vehicle, in this study 2% corn oil was used and 1% acetone or a total of 3%.

8. Background: Submitted as result of the Reregistration Standard.

9. MATERIALS AND METHODS:

A. Test Organisms:

Guideline Requirements	Result Results
1. Species: a. Supplier: b. Same hatch: c. Same source: d. Approaching the first breeding season: e. Indistinguishable from wild birds: f. Age: g. Pen reared:	 a. Sand Prairie Quail Farm b. Not reported c. Yes d. Yes e. Yes f. Minimum of 14 weeks approx. 15 week old g. Not reported
2. Health: a. Weight loss: b. Sickness: c. Mortality: d. History:	 a. Not reported b. Given virginiamycin by supplier c. Not reported d. Only the antibiotic reported above
3. Acclimation period:	Yes 21 days

B. Test System:	
1. Test design:	1.
a. No. of dose levels (including number of controls and vehicle controls):	a. 4
b. Factor used to separate dosage levels:	b. 10
c. No. of pens per level:	c. 16
d. Male to female ratio per pen:	d. 1 to 1
2. Pen Facilities	
a. Parents	a.
1. Temperature	1. 22°C
2. Relative humidity	2. 49%
3. Minimize cross contamination:	3. Not reported???
4. Pen materials:	4. Wire pens over galvanized dropping pans
5. Ventilation:	5. "Adequate throughout the study"
b.Chicks	b.
1. Temperature:	1. 22°C
2. Relative Humidity:	2. 49%
3. Pen materials:	3. Not reported
4. Ventilation:	4. "Adequate throughout the study"
5. Photoperiod:	5. 24-hours
3. Photoperiod:	3.
a. Parent-	a. 17-hour L - 7-hour D
b. Chick-	b. 24-hour L
4. Bodyweight	4.
a. Parent-	a. day 1 and biweekly through test week 10 but not during egg laying period
b. Chick-	b. days 1 and 14

5. Food consumption	5. Twice weekly and calculated on a weekly basis.
6. Dose preparation- a. Rational as why or how the dosage level were selected for the preliminary study. The levels for the main study were the same as the preliminary study.	a. Not reported
b. Vehicle: % of the diet	b. 2% corn oil and 1% acetone
c. Evaporative vehicle should be allowed to evaporate stored to maintain stability	c. 20 minutes of mixing after acetone was added. No additional evaporative period was mentioned before freezing.
7. Feeding and Husbandry	7.
a. appropriate diet	a. Purina Custom Game Bird Layena
b. water	b. 1-quart plastic waterers
c. test diet for at least 10 wks	c. Through week 10
d. Commercial game bird breeder ration	d. see a.
e. Store to maintain stability	e. "...kept in freezer when not being fed."
8. Egg collection, Storage, and Incubation	8.
a. collected daily	a. Yes
b. stored at 16° C and 65% relative humidity	b. Not reported
c. set at weekly intervals	c. Yes
d. candled day 0 cracks, day 11 for bobwhite, and day 14 for mallard for fertility day 18 for bobwhite and day 21 for mallards	d. Yes
e. Temp for hatching 39° and relative humidity 70%	e. Temp 37.4-37.5°C HR 70 - 73%

9. Observations a. Bobwhite chicks day 24 hatchability recorded b. On control diet for 14 days c. Mallard Ducklings day 27 hatchability d. Control diet for 14 days	9. a. Day 24 (or 25) b. Untreated diet for 14 days c. N/A d. N/A
10. Eggshell Thickness a. One day every two weeks newly laid eggs should be collected. weeks 1,3,5,7,9, b. air dry for 48 hours c. measure 3 to 4 points in 0.01 mm	10. a. Yes b. Yes c. Yes
11. Withdrawal if reduced reproduction is evident add withdrawal period not to exceed 3 wks.	11. F_0 5 week recovery period

10. REPORTED RESULTS: (see part 12. Statistical Analysis below)

11. STUDY AUTHORS'S CONCLUSIONS / QUALITY ASSURANCE MEASURES: The author signed a quality assurance statement on page 4.

"The ingestion of TPTH at the highest level, 90 ppm, by the parental generation statistically reduced and adversely affected adult female body weights, eggshell thickness, and ovary weights. The reproductive success and hatchling survivability of the 30 ppm (normal hatchling of live 18-day embryos and 14-day-old survivors) and 90 ppm (eggs laid per hen, viable embryos of eggs set, live 18-day embryos, and 14-day-old survivors of normal hatchlings) ppm test groups were also statistically reduced and adversely affected. With the exception of the viable embryos, live 18-day embryos of viable embryos and normal hatchlings of live 18-day embryos in the 90 ppm test group, all other statistically significant decreases in reproductive effects were reversible or not statistically significant during the five-week recovery period. The mid-level (30 ppm) and high level (90 ppm) were considered to be adverse effects levels. The no-observed-effect level (NOEL) was determined to be the low level (3 ppm)."

12. Statistical Analysis

The following is the reported procedure for statistical analysis: "First a Levene's test (Milliken & Johnson, Analysis of Messy Data, Vol. 1, 1984, pg 22, Lifetime Learning Publications, Belmont, Calif.) is conducted to determine whether the variances across the treatment groups are homogeneous (at the 0.01 level of significance). If there is not a significant difference in the group variances, a parametric one-way analysis of variance is conducted along with a Dunnett's test, comparing each treatment group to the control group. A one-tailed test is performed since the concern is with the detrimental (not both detrimental and beneficial) effects of the overall F test is not of interest, since the Dunnett's comparison to control (which involves the only comparisons in which there is interest) already controls for an overall controls Type I error rate of 0.05."

EEB disagrees that the overall F test is not of interest. For example, when normal hatchlings of eggs laid were analyzed using ANOVA and Bonferroni's T test (see printout File:a\tpth\bobwp.out), the ANOVA indicated Pr > F of 0.0001, however, Bonferroni's T test did not indicate which level or level were significant. The mean normal hatchlings per eggs values are as follows: Control 0.7787, 3 ppm 0.8133, 30 ppm 0.6451, and 90 ppm 0.1269. Based on this we believe statistically and biologically 90 ppm level revealed effects.

//

The following Table 2 lists the parameters which were found to be statistically significant for the test period by Bio-life Associates, Ltd:

Table 2
Biolife's Statistical Results by Concentration

Parameter	Vehicle Control	3 ppm	30 ppm	90 ppm
Eggshell Thickness	0.228	0.223	0.218	0.207*
Eggs Laid/ Hen	37	43	51	30*
Normal Eggs/Hen	36	42	50	27*
Viable Embryos of Set (%)	91	89	86	38*
Day-18 Live Embryos of Set (%)	90	89	83	26*
Normal Hatchlings of Set (%)	85	85	71	11*
Day-14 Survivors of Set (%)	79	71	54*	2*
Day-18 Live Embryos of Viable Embryos (%)	99	100	97	65*
Normal Hatchlings of Viable Embryos (%)	93	96	82	25*

Normal Hatchlings ofDay-18 Live Embryos	94	96	84*	36*
Day-14 Survivors of Day-18 of Live Embryos	94	96	84*	36*
Mean 14-Day-Old Survivors/ Hen	26	30	25	1
Ovarian Weight	8.98	6.91	8.51	6.85*
Body Weight Female @ 4 Weeks	216.7	215.3	216.6	207.0*
Body Weight Female @ 20 Weeks	263.2	255.9	261.8	242.4*

* Statistical different from controls.

In order to review the statistical results EEB used the SAS program Birdrepr.sas which is an ANOVA (reproductive parameters) and ANCOVA (body weight) with Dunnett's and Bonferroni's tests. Based on this, the following items EEB found statistically significant.

Table 3
Summary of EEB's Statistical Calculations

Parameter	Vehicle Control	3 ppm	30 ppm	90 ppm
Eggs Laid/pen	36.438	43.188	51.063*	29.562
Viable Embryos	30.812	36.625	39.938	11.063*
Live embryos	30.562	36.625	38.688	7.812*
Normal Hatchlings	28.375	35.125	32.938	3.75*
Hatchling Survivors	26.062	29.5	25.187	0.812*
Eggshell Thickness	.22775	.22353	.218	0.207*
Food Consumption	352.37	384.31	397.12*	405.25*
Male Body Weight	212-234	213.06-232.06	211.3-229.6	211-221.2*
Female Body Weight	212.9-263.2	211.5-255.9	339.3-261.8	207.9-242.4

* Statistically significant items.

Both the EEB and Biolife analysis agree the embryo and hatchling success were affected at 30 ppm and 90 ppm. It should be mentioned that the control birds did not appear to perform as well as the 3 ppm birds. Also, it was noticed that the food consumption data was a dose response relationship. As the dose concentration increased the consumption increased. On the other hand, the eggs laid parameter was in consisted eggs laid increased up to the 30 ppm level and then 90 ppm produced less than any of the other levels. Although the EEB statistically significant level for eggs laid was 30 ppm, EEB believes biologically significant level is 90 ppm. Biolife found 90 ppm level statistically significant.

In addition to these parameters the ovary and testicular weights were analyzed by Biolife. The average ovarian weights were 8.98, 6.91, 8.51, and 6.85 grams for 3, 30, and 90 ppm levels. The 90 ppm level was statistically significant but the 30 ppm level was not in their analysis. Biolife points out that if 3 low ovary weight hens in the 30 ppm level were removed and 2 in the 90 ppm level were removed the averages would be like 8.56 and 7.65 grams for the 30 ppm and 90 ppm levels, respectively. However EEB's used SAS GLM ANOVA and the probability of F was 0.0644. EEB's SAS Dunnett's one-tailed T test indicated that both the 3 and 90 ppm levels are significantly different. The lack of a dose response relationship indicates some other variable may be affecting the results.

Table 4.
Comparison of Test Period, Recovery Period, and Combination of Both

Parameter	Test Period	Recovery Period	Both
Egg Laid per Hen	90 ppm	none	none
Normal Eggs per Hen	90 ppm	none	none
Normal Eggs of Eggs Laid (%)	None	None	None
Abnormal/ Reduced of Eggs Laid (%)	None	None	None
Cracked/ Broken of Eggs Laid (%)	None	None	None
Defective of Eggs Laid (%)	None	None	None
Infertile Eggs of Set (%)	None	None	None
Viable Embryos of Set (%)	90 ppm	90 ppm	90 ppm
Embryo Mortalities @ 1 week of Set (%)	None	None	None

Embryo Mortalities @ Midterm of Set (%)	None	None	None
Day-18 Live Embryos of Set (%)	90 ppm	90 ppm	90 ppm
Embryo Mortalities @ Fullterm of Set (%)	None	None	None
Pipped Not Liberated of Set (%)	None	None	None
Normal Hatchlings of Set (%)	90 ppm	90 ppm	90 ppm
Day-14 Survivors of Set (%)	30 ppm, 90 ppm	90 ppm	30 ppm, 90 ppm
Day-18 Live Embryos of Viable Embryos (%)	90 ppm	90 ppm	90 ppm
Normal Hatchlings of Viable Embryos (%)	90 ppm	90 ppm	90 ppm
Normal Hatchlings of Day-18 Live Embryos (%)	30 ppm, 90 ppm	90 ppm	90 ppm
Day-14 Survivors of Normal Hatchlings (%)	30 ppm, 90 ppm	None	90 ppm

The above Table 4 shows reproductive effects which persisted throughout the recovery.

12. ADEQUACY OF THE STUDY:

A. Category-Supplemental

B. Rational-The following items were not addressed:
The illness the antibiotic was used to treat.
The scientific basis for removing the small eggs.

C. Reparability-The following items should be reported:
The illness the antibiotic was used to treat and number of birds infected. The scientific basis for removing the small eggs. The rational as why the dosage levels were not separated by a factor of five. The total test period food consumption per pen.

14. COMPLETION OF ONE-LINER FOR STUDY: 8/17/94

DATA EVALUATION RECORD
§ 71-4 Avian Reproduction Study
Mallard

1. CHEMICAL: TPTH

2. TEST MATERIAL: TPTH (Batch No. GFRAM 911K; 97.9%; CAS No. 76-87-9) was a fine, white powder with a characteristic odor.

3. CITATION:

Author:	Carol A. Pederson, Connie L. Lesar
Title:	Toxicity and Reproduction Study in Mallard Ducks
Date:	January 24, 1994
Laboratory Report #:	BLAL No. 106-010-08
Any Other Study #:	N/A
Sponsor:	Elf Atochem North America, Inc., Philadelphia, PA
Laboratory:	Bio-life Associates, Ltd
MRID No.:	43178502

4. REVIEWED BY:

Dennis J. McLane, Wildlife Biologist
Ecological Effects Branch
Environmental Fate and Effects Division (7507 C) 9-12-94

5. APPROVED BY:

Les Touart, Section Head
Ecological Effects Branch
Environmental Fate and Effects Division (7507 C) 9-12-94

6. CONCLUSION: This study is scientifically sound but does not fulfill the guideline requirements pertinent items need more explanation and information.(see 7. Major Guideline Deviations). TPTH at 30 and 90 ppm cause many reproductive effects. The no-effect-level and the lowest effect level are 3 ppm and 30 ppm, respectively. Statistically significant items are listed below under the appropriate dose level. (see following table)

Table 1
Listing of Affected Parameters for 90 and 30 ppm Dose Levels

@ 90 ppm	@ 30 ppm
Eggs Laid/Pen	Live Embryos
Eggs Set/Pen	Normal Hatchlings
Viable Embryos/Pen	Hatchling Survivors

Live Embryos/Pen	14-Day-Old Survivors
Normal Hatchlings/Pen	Eggshell Thickness
Hatchlings Survivors/Pen	
Egg Shell Thickness/Pen	
14 Day Old Survivors/Pen	
Eggs Set/Eggs Laid	
Viable Embryos/Eggs Set	
Live Embryos/Viable Embryos	
Normal Hatchlings/Eggs Laid	
Eggs Cracked/ Eggs Laid	
Normal Hatchlings/Eggs Set	
Hatchling Survivor/Eggs Set	
Male Body Weight	

The no-effect-level and the lowest effect level are 3 ppm and 30 ppm, respectively. Also as shown in Table 3 below several of the reproductive effects were still detectable after the 5 week recovery period. In addition, the ovary weights were not effected. On the other hand, testes weight were statistically different at the 30 ppm level but not the 90. EEB was unable to determine if this effect was due to TPTH.

7. MAJOR GUIDELINE DEVIATIONS:

A. The report omitted the scientific explanation for removing the small eggs.

B. It was reported that the birds were treated with an antibiotic but the illness was not reported.

C. The dosage levels were separated by a factor of three rather than five.

D. The rational for using more than 2% total vehicle was not included. The guidelines indicate only 2% total vehicle, in this study 2% corn oil was used and 1% acetone or a total of 3%.

E. Food consumption weight per pen (replicate) was not submitted.

8. Background: Submitted as result of the Reregistration Standard.

9. MATERIALS AND METHODS:

A. Test Organisms:

Guideline Requirements	Result Results
1. Species: a. Supplier: b. Same hatch: c. Same source: d. Approaching the first breeding season: e. Indistinguishable from wild birds: f. Age: g. Pen reared:	 a. Whistling Wings, inc. Hanover, IL 61041 b. No other source reported c. Yes d. Yes e. Yes f. Approx. 15 week old g. Not reported
2. Health: a. Weight loss: b. Sickness: c. Mortality: d. History:	 a. Not reported b. Given P.A. Bacterin at 7 and 14 days c. Not reported d. Only the treatment reported above
3. Acclimation period:	Yes 3 weeks
B. Test System: 1. Test design: a. No. of dose levels (including number of controls and vehicle controls): b. Factor used to separate dosage levels: c. No. of pens per level: d. Male to female ratio per pen:	 1. a. 4 b. 10 c. 16 d. 1 to 1

2. Pen Facilities	
a. Parents	
1. Temperature	1. 20°C
2. Relative humidity	2. 70%
3. Minimize cross contamination:	3. Not reported
4. Pen materials:	4. "61 x 121.9 x 61 cm wire pens constructed of 1" wire mesh... over concrete..."
5. Ventilation:	5. "Adequate ventilation was maintained throughout the study"
b.Chicks	
1. Temperature:	1. 36.6°C
2. Relative Humidity:	2. 37.9%
3. Pen materials:	3. Not reported
4. Ventilation:	4. Not reported
5. Photoperiod:	5. Not reported
3. Photoperiod:	
a. Parent- 1st 8 wks 7 L increased to 16-17 L; 6 footcandles or 65 lux	a. 7 hours of light until week 9 then 17-hour L - 7-hour D; @ 6 footcandles
b. Chick or Duckling- hour of light per day	b. 24-hour L
4. Bodyweight	
a. Parent- Day 1 and biweekly through test week 10 but not during egg laying period	Day 1 and biweekly through test week 10 but not during egg laying period
b. Chick- Day 1 and day 14	Days 1 and 14
5. Food consumption (Twice a week)	Twice weekly and calculated on a weekly basis.

6. Dose preparation- a. Rational as why or how the dosage level were selected for the preliminary study. The levels for the main study were the same as the preliminary study.	a. Not reported
b. Vehicle: 2% of the diet	b. 2% corn oil and 1% acetone
c. Evaporative vehicle should be allowed to evaporate stored to maintain stability	c. 20 minutes of mixing after acetone was added. No additional evaporative period was mentioned before freezing.
7. Feeding and Husbandry	
a. Appropriate diet	a. Purina Custom Game Bird Layena
b. Water	b. Automatic waterer and a small bowl
c. Test diet for at least 10 wks	c. Through week 20
d. Commercial game bird breeder ration	d. see a.
e. Store to maintain stability (prepare weekly and freeze)	e. "...prepared fresh weekly and frozen prior to feeding"
8. Egg collection, Storage, and Incubation	
a. collected daily	a. Yes
b. stored at 16° C and 65% relative humidity	b. 19°C-15°C Temp. & 60% RH
c. set at weekly intervals	c. Yes
d. candled day 0 cracks, day 11 for bobwhite, and day 14 for mallard for fertility day 18 for bobwhite and day 21 for mallards	d. Yes
e. Temp for hatching 39°C and relative humidity 70%	e. Temp 37.5-37.6°C HR 61 - 64%

9. Observations a. Bobwhite chicks hatch day (day 2) b. On control diet for 14 days c. Mallard Ducklings hatch day (day 27) d. F_1 on control diet for 14 days	 a. N/A b. N/A c. Day 27 d. Yes
10. Eggshell Thickness a. One day every two weeks newly laid eggs should be collected. weeks 1,3,5,7,9, b. Air dry for 48 hours c. Measure 3 to 4 points in 0.01 mm	 a. Yes b. Yes c. 3/egg to 0.01 mm
11. Withdrawal if reduced reproduction is evident add withdrawal period not to exceed 3 wks.	F_0 5 week recovery period

10. REPORTED RESULTS: (see part 12. Statistical Analysis below)

11. STUDY AUTHORS'S CONCLUSIONS / QUALITY ASSURANCE MEASURES: The author signed a quality assurance statement on page 4.

"TPTH was administered for 20 consecutive weeks in the diet to four groups of approximately 18-week-old mallard ducks as follows: Vehicle Control (0ppm). T-I (3 ppm), T-II (30 ppm), and T-III (90 ppm). A five-week recovery period followed the 20-week test period.

"Survival of mallard ducks at dietary levels used in this study was excellent. Clinical signs appearing to be neurological in nature were seen in two females and one male in the high level (90 ppm) group and their possible effect as a compound-related finding cannot be excluded. At the high dietary level, 90 ppm, there appeared to be initial but reversible decreases in body weight and feed consumption.

"The ingestion of TPTH at levels of 3, 30, or 90 ppm by the parental generation also appeared to adversely affect eggshell thickness in the 30 and 90 ppm test groups, as well as the reproductive success of the 30 (viable embryos of eggs set and live 21-day embryos of viable embryos) and 90 (mean eggs laid per hen, viable embryos, and normal hatchlings of live 21-day embryos of viable embryos, and normal hatchlings of live 21-day embryos) ppm test groups. With the exception of the percentages of normal hatchlings of 21-day embryos in the 90 ppm test group, all other adverse reproductive effects were readily reversible during the five-week recovery period. The mid-level (30 ppm) and high level (90 ppm) were considered to be adverse effect levels. The no-observed-effect level (NOEL) was determined to be the low level (3 ppm)."

Report provided a Quality Assurance Statement and Good Laboratory Practice Exception statement.

12. Statistical Analysis:

The following is the reported procedure for statistical analysis: "First a Levene's test (Milliken & Johnson, Analysis of Messy Data, Vol. 1, 1984, pg 22, Lifetime Learning Publications, Belmont, Calif.) is conducted to determine whether the variances across the treatment groups are homogeneous (at the 0.01 level of significance). If there is not a significant difference in the group variances, a parametric one-way analysis of variances, a parametric one-way analysis of variance is conducted along with a Dunnett's test, comparing each treatment group to the control group. A one-tailed test is performed since the concern is with the detrimental (not both detrimental and beneficial) effects of

the overall F test is not of interest, since the Dunnett's comparison to control (which involves the only comparisons in which there is interest) already controls for an overall controls Type I error rate of 0.05."

The following lists of parameters were found to be statistically significant by Bio-life Associates, Ltd:

Table 2
Results of Biolife's Statistical Analysis

Parameter	Vehicle Control	3 ppm	30 ppm	90 ppm
Mean Eggs Laid per Hen in 11 Weeks	40	43	31	11*
Eggs Cracked or Broken eggs of Eggs Laid (%)	3	6	8	24
Viable Embryos of Set (%)	92	90	71	26*
Day-21 Live Embryos of Set (%)	94	85	74	3*
Normal Hatchlings of Live 21-Day Embryos(%)	91	85	82	0*
Day-14 Survivors of Normal Hatchlings (%)	96	95	94	-
Mean 14-Day-Old Survivors/ Hen	26	26	13	0

Eggshell Thickness	0.387	0.388	0.363*	0.312*
Ovarian Weight	8.98	6.91	8.51	6.85*
Body Weight Female @ 4 Weeks	216.7	215.3	216.6	207.0*
Body Weight Female @ 20 Weeks	263.2	255.9	261.8	242.4*

* Statistical different from controls .

It does not appear that Biolife did a statistical analysis for eggs cracked/pen, eggs set per pen, viable embryos/pen, live 21-day embryos/pen, normal hatchlings/pen, and 14-day old survivors per pen. EEB has provided these in Table 3 below.

In order to review the statistical results EEB used SAS program Birdrepr.sas which an ANOVA (reproductive parameters) and ANCOVA (body weight) with Dunnett's and Bonferroni's Tests. Based on this, the following items EEB found statistically significant.

Table 3
Means for TPTH Mallard Reproduction Study Parameters

Parameter	Vehicle Control	3 ppm	30 ppm	90 ppm
Eggs Laid/Pen	39.56	43.25	31.25	11.0*
Eggs set /Pen	35.5	38.188	27.375	8.313*
Viable Embryos/ Pen	32.75	34.875	20.0*	2.063*
Live Embryos/ Pen	29.75	30.5	15.688*	0.063*
Normal Hatchlings/ Pen	27.375	27.25	12.875*	0.00*
Hatchling Survivors/ Pen	27.8	26.062	12.563*	0.00*
Eggshell Thickness	0.3782	0.3882	0.3635	0.316*
14-Day Survivor Weight	275.715	262.956	298.808*	---
Eggs Set/ Eggs Laid[1]	71.53	70.38	69.66	62.17
Live Embryos/ Viable Embryos[1]	75.34	73.43	64.85	3.63
Normal Hatchlings/ Eggs Laid[1]	56.56	52.46	38.7	0.0

Eggs Cracked/ Eggs Laid[1]	7.62	10.15	11.15	23.65
Viable Embryos/ Eggs Set[1]	73.78	74.6	60.19	28.73
Normal Hatchlings/ Eggs Set[1]	62.27	58.76	42.46	0.00
Hatchlings Survivors/ Eggs Set[1]	59.62	56.36	41.78	0.00
Male Body Weight[1]	1252.75	1298.81	1218.69	1275.19

[1] Although the ANOVA and ANCOVA show significant Dunnett's and Bonferroni's tests did not show which dosage levels were statistically significant. However, for all these variables the 90 ppm level was biologically significant.

In addition to these parameters ovary and testes weights were also analyzed. The ovary data did not show any statistical or biological significance. The testes data shows a statistical difference between the 30 ppm level and the other levels (see attached printout c:\chem\tpth\testmal.out). The variation was so great, and the 90 ppm level nearly identical to the control EEB does not believe this is related to the dosage.

Both the EEB and Biolife analysis agree that the embryo, hatchling, and 14 day old survivors were affected at 30 ppm and 90 ppm.

Table 3
Based on Biolife's Analysis Comparison of Test Period, Recovery Period, and Combination of Both

Parameter	Test Period	Recovery Period	Both
Egg Laid per Hen	90 ppm	none	90 ppm
Normal Eggs per Hen	90 ppm	none	90 ppm
Normal Eggs of Eggs Laid (%)	90 ppm	None	90 ppm
Abnormal/ Reduced of Eggs Laid (%)	None	None	None

Cracked/ Broken of Eggs Laid (%)	None	None	None
Defective of Eggs Laid (%)	None	None	None
Infertile Eggs of Set (%)	None	None	None
Viable Embryos of Set (%)	30 ppm, 90 ppm	30 ppm	30 ppm, 90 ppm
Embryo Mortalities @ 1 week of Set (%)	None	None	None
Embryo Mortalities @ Midterm of Set (%)	None	None	None
Day-21 Live Embryos of Set (%)	30 ppm, 90 ppm	30 ppm	30 ppm, 90 ppm
Embryo Mortalities @ Fullterm of Set (%)	None	None	None
Pipped Not Liberated of Set (%)	None	None	None
Normal Hatchlings of Set (%)	30 ppm, 90 ppm	30 ppm	30 ppm, 90 ppm
Day-14 Survivors of Set (%)	30 ppm, 90 ppm	30 ppm	30 ppm, 90 ppm
Day-21 Live Embryos of Viable Embryos (%)	30 ppm, 90 ppm	None	30 ppm, 90 ppm
Normal Hatchlings of Viable Embryos (%)	30 ppm, 90 ppm	None	30 ppm, 90 ppm

Normal Hatchlings of Day-21 Live Embryos (%)	90 ppm	90 ppm	90 ppm
Day-14 Survivors of Normal Hatchlings (%)	--	None	None

The above table shows reproductive effects which persisted throughout the recovery.

12. ADEQUACY OF THE STUDY:

A. Category-Supplemental

B. Rational-The following items were not addressed: the illness the antibiotic was used to treat, the scientific basis for removing the small eggs, and the total food consumption per pen.

C. Reparability-The following items should be reported: The illness the antibiotic was used to treat and number of birds infected. The scientific basis for removing the small eggs. The rational as why the dosage levels were not separated by a factor of five was not used. The total food consumption per pen.

14. COMPLETION OF ONE-LINER FOR STUDY: 8/17/94

SAS 14:30 Tuesday, August 9, 1994
1

OBS	TRT	EL	EC	ES	VE	LE	NH	HS	THICK	HATWT	SURVWT	FOOD	PREM	POSTM	PREF	POSTF
1	A	33	1	30	28	27	25	24	0.397	34.9	287.0	.	1129	1267	1069	1202
2	A	8	1	7	7	7	7	7	.	34.7	308.4	.	1232	1416	1133	1222
3	A	48	3	40	37	24	24	24	0.344	32.9	292.1	.	1152	1113	1042	1279
4	A	0	0	0	0	0	0	.	.	.	.	.	1220	1316	904	1122
5	A	62	0	59	44	43	43	43	0.410	32.3	293.9	.	1190	1181	972	1350
6	A	47	1	44	41	36	34	32	0.395	32.1	262.3	.	1075	1225	987	1139
7	A	56	0	52	50	45	31	31	0.384	31.8	270.3	.	1262	1400	954	1114
8	A	42	0	37	36	35	33	33	0.299	34.2	243.1	.	1070	1118	1066	1060
9	A	35	0	32	30	30	29	29	0.366	31.5	256.8	.	1172	1360	988	1024
10	A	53	2	47	44	41	40	39	0.383	37.5	293.7	.	1406	1513	1070	1205
11	A	52	3	45	44	44	41	36	0.373	34.4	258.6	.	1130	1164	1001	1231
12	A	0	0	0	0	0	0	0	.	.	.	.	1337	1150	860	846
13	A	70	2	63	61	48	37	32	0.376	34.8	274.7	.	1124	1201	990	1156
14	A	0	0	0	0	0	0	0	.	.	.	.	1268	1253	1077	1195
15	A	68	1	62	54	54	53	48	0.411	37.4	278.5	.	1066	1240	1080	1429
16	A	59	3	50	42	42	41	39	0.401	35.5	264.9	.	1146	1127	1100	1434
17	B	28	1	25	24	23	22	22	0.345	29.6	257.6	.	1134	1245	1014	1178
18	B	25	2	21	18	14	8	8	0.410	38.2	275.2	.	1065	994	1162	1256
19	B	63	1	55	52	50	48	48	0.353	35.8	272.1	.	953	1435	1028	1162
20	B	33	1	30	19	18	15	15	0.412	35.8	282.9	.	1430	1528	999	1103
21	B	69	1	64	63	61	54	54	0.402	34.4	278.4	.	1235	1332	877	1185
22	B	41	1	38	37	29	22	21	0.385	33.8	241.1	.	1059	1123	996	1136
23	B	59	0	55	53	48	43	42	0.379	34.4	279.6	.	1370	1583	902	1162
24	B	18	5	12	12	9	8	8	0.403	35.2	251.4	.	1036	1122	1189	1342
25	B	36	0	33	30	30	30	30	0.410	32.7	252.5	.	1255	1370	986	1120
26	B	52	4	44	43	43	43	38	0.394	32.3	270.8	.	1299	1371	1103	1274
27	B	61	2	55	53	45	37	33	0.419	35.1	255.2	.	1124	1080	960	1119
28	B	31	3	25	23	18	16	15	0.389	27.6	283.3	.	1146	1485	1025	1037
29	B	47	4	40	37	37	36	34	0.377	34.7	294.1	.	1164	1254	964	1269
30	B	46	5	37	29	2	1	1	0.359	36.0	146.0	.	1120	1316	1060	1088
31	B	37	0	35	26	22	15	10	0.413	31.1	291.2	.	1165	1184	1092	1392
32	B	46	1	42	39	39	38	38	0.362	34.1	275.9	.	1303	1359	906	1234
33	C	47	1	43	41	38	34	34	0.342	34.8	286.3	.	1099	1160	975	1250
34	C	49	1	44	27	26	23	22	0.387	33.3	304.5	.	1257	1490	1030	1250
35	C	0	0	0	0	0	0	0	.	.	.	.	1220	875	993	1046
36	C	38	1	34	31	27	25	24	0.339	34.3	311.3	.	1144	1316	1017	1276
37	C	21	2	18	16	16	13	13	0.350	33.7	312.1	.	1111	1313	879	1083
38	C	8	1	6	3	2	2	2	0.400	30.0	286.5	.	1009	1418	1111	1384
39	C	19	6	12	6	0	0	0	.	.	.	.	1150	1159	1010	1321
40	C	58	0	52	23	11	5	5	0.347	39.8	308.2	.	1218	1102	938	1197
41	C	5	1	4	3	3	3	1	.	27.0	263.0	.	1099	1156	879	968
42	C	43	1	39	18	12	9	9	0.379	35.1	285.1	.	1151	1192	1049	1136
43	C	26	3	22	18	8	6	6	0.390	33.9	302.6	.	1130	966	970	901
44	C	56	3	49	37	22	17	17	0.380	38.2	326.0	.	1193	1200	1124	1274
45	C	70	2	63	56	50	43	41	0.383	33.3	315.1	.	1070	1300	893	1301
46	C	23	1	21	13	13	12	12	0.337	33.1	286.2	.	1049	1340	1155	1132
47	C	0	0	0	0	0	0	0	.	.	.	.	1260	1156	1072	916
48	C	37	2	31	28	23	15	15	0.328	40.2	297.6	.	1155	1356	1043	1297
49	D	16	5	10	1	0	0	0	0.330	.	.	.	1005	1245	1121	1177
50	D	17	0	17	5	0	0	0	.	.	.	.	1239	1324	960	1160
51	D	0	0	0	0	0	0	0	.	.	.	.	1150	1099	1060	1040
52	D	0	0	0	0	0	0	0	.	.	.	.	1177	1230	938	937
53	D	0	0	0	0	0	0	0	.	.	.	.	1146	1225	1149	.
54	D	19	3	15	1	0	0	0	0.293	.	.	.	1236	1392	1144	1249
55	D	0	0	0	0	0	0	0	.	.	.	.	1105	1300	1016	1106
56	D	28	3	23	4	0	0	0	0.303	.	.	.	1166	1385	1010	1126
57	D	19	3	15	7	0	0	0	0.327	.	.	.	1269	1215	1090	1362
58	D	11	4	6	2	0	0	0	0.320	.	.	.	1352	1554	1149	1340

31

59	D	7	3	4	0	0	0	0	.	.	.	.	1298	1377	1090	1120
60	D	5	2	2	1	0	0	0	.	.	.	.	1179	1310	892	1203
61	D	19	7	11	4	1	0	0	0.293	.	.	.	1355	1088	963	1726
62	D	35	3	30	8	0	0	0	0.315	.	.	.	1328	1301	1150	1268
63	D	0	0	0	0	0	0	0	.	.	.	.	1080	1163	896	974
64	D	0	0	0	0	0	0	0	.	.	.	.	1120	1195	1111	1145

SAS 14:30 Tuesday, August 9, 1994
2

TRT=A

N Obs	Variable	N	Minimum	Maximum	Mean
16	EL	16	0	70.0000000	39.5625000
	EC	16	0	3.0000000	1.0625000
	ES	16	0	63.0000000	35.5000000
	VE	16	0	61.0000000	32.3750000
	LE	16	0	54.0000000	29.7500000
	NH	16	0	53.0000000	27.3750000
	HS	15	0	48.0000000	27.8000000
	THICK	12	0.2990000	0.4110000	0.3782500
	HATWT	13	31.5000000	37.5000000	34.1538462
	SURVWT	13	243.1000000	308.4000000	275.7153846
	FOOD	0	.	.	.
	PREM	16	1066.00	1406.00	1186.19
	POSTM	16	1113.00	1513.00	1252.75
	PREF	16	860.0000000	1133.00	1018.31
	POSTF	16	846.0000000	1434.00	1188.00

N Obs	Variable	Std Dev
16	EL	24.6440493
	EC	1.1814539
	ES	22.2800359
	VE	20.0295615
	LE	18.4191929
	NH	16.9228642
	HS	14.7318896
	THICK	0.0315108
	HATWT	1.9725683
	SURVWT	18.6363375
	FOOD	.
	PREM	96.7286367
	POSTM	119.0134446
	PREF	74.1904924
	POSTF	148.3603721

TRT=B

N Obs	Variable	N	Minimum	Maximum	Mean
16	EL	16	18.0000000	69.0000000	43.2500000
	EC	16	0	5.0000000	1.9375000
	ES	16	12.0000000	64.0000000	38.1875000
	VE	16	12.0000000	63.0000000	34.8750000
	LE	16	2.0000000	61.0000000	30.5000000
	NH	16	1.0000000	54.0000000	27.2500000
	HS	16	1.0000000	54.0000000	26.0625000
	THICK	16	0.3450000	0.4190000	0.3882500
	HATWT	16	27.6000000	38.2000000	33.8000000
	SURVWT	16	146.0000000	294.1000000	262.9562500
	FOOD	0	.	.	.
	PREM	16	953.0000000	1430.00	1178.63
	POSTM	16	994.00	1583.00	1298.81

PREF	16	877.0000000	1189.00	1016.44
POSTF	16	1037.00	1392.00	1191.06

N Obs	Variable	Std Dev
16	EL	14.7806179
	EC	1.7308476
	ES	14.1431668
	VE	14.8093439
	LE	16.5085836
	NH	16.0187390
	HS	15.8175799
	THICK	0.0236488
	HATWT	2.6369174
	SURVWT	34.6848280
	FOOD	.
	PREM	128.3562101
	POSTM	167.8984688
	PREF	88.9186660
	POSTF	96.4209305

SAS 14:30 Tuesday, August 9, 1994
3

TRT=C

N Obs	Variable	N	Minimum	Maximum	Mean
16	EL	16	0	70.0000000	31.2500000
	EC	16	0	6.0000000	1.5625000
	ES	16	0	63.0000000	27.3750000
	VE	16	0	56.0000000	20.0000000
	LE	16	0	50.0000000	15.6875000
	NH	16	0	43.0000000	12.9375000
	HS	16	0	41.0000000	12.5625000
	THICK	12	0.3280000	0.4000000	0.3635000
	HATWT	13	27.0000000	40.2000000	34.3615385
	SURVWT	13	263.0000000	326.0000000	298.8076923
	FOOD	0	.	.	.
	PREM	16	1009.00	1260.00	1144.69
	POSTM	16	875.0000000	1490.00	1218.69
	PREF	16	879.0000000	1155.00	1008.62
	POSTF	16	901.0000000	1384.00	1170.75

N Obs	Variable	Std Dev
16	EL	21.8220072
	EC	1.5041609
	ES	19.9194210
	VE	16.1575576
	LE	14.5863806
	NH	12.8138922
	HS	12.5058320
	THICK	0.0251523
	HATWT	3.6153696
	SURVWT	16.8597875
	FOOD	.
	PREM	72.1745281
	POSTM	159.8748339
	PREF	84.6182604
	POSTF	150.7061158

TRT=D

N Obs	Variable	N	Minimum	Maximum	Mean
16	EL	16	0	35.0000000	11.0000000
	EC	16	0	7.0000000	2.0625000
	ES	16	0	30.0000000	8.3125000
	VE	16	0	8.0000000	2.0625000
	LE	16	0	1.0000000	0.0625000
	NH	16	0	0	0
	HS	16	0	0	0
	THICK	7	0.2930000	0.3300000	0.3115714
	HATWT	0	.	.	.
	SURVWT	0	.	.	.
	FOOD	0	.	.	.
	PREM	16	1005.00	1355.00	1200.31
	POSTM	16	1088.00	1554.00	1275.19
	PREF	16	892.0000000	1150.00	1046.19
	POSTF	15	937.0000000	1726.00	1195.53

N Obs	Variable	Std Dev
16	EL	11.2427755
	EC	2.1746647
	ES	9.4496473
	VE	2.6949026
	LE	0.2500000
	NH	0
	HS	0
	THICK	0.0154041
	HATWT	.
	SURVWT	.
	FOOD	.
	PREM	102.0083126
	POSTM	119.1286245
	PREF	93.0650086
	POSTF	189.1646549

1. ANALYSIS OF EL DATA 4
****
14:30 Tuesday, August 9, 1994

General Linear Models Procedure
Class Level Information

Class	Levels	Values
TRT	4	A B C D

Number of observations in data set = 64

1. ANALYSIS OF EL DATA 5
****
14:30 Tuesday, August 9, 1994

General Linear Models Procedure

Dependent Variable: RESP

Source	DF	Sum of Squares	F Value	Pr > F
Model	3	9970.54687500	9.31	0.0001
Error	60	21425.93750000		

Corrected Total	63	31396.48437500		

R-Square	C.V.	RESP Mean
0.317569	60.44038	31.26562500

Source	DF	Type I SS	F Value	Pr > F
TRT	3	9970.54687500	9.31	0.0001

Source	DF	Type III SS	F Value	Pr > F
TRT	3	9970.54687500	9.31	0.0001

1. ANALYSIS OF EL DATA 6
****
14:30 Tuesday, August 9, 1994

General Linear Models Procedure

Dunnett's T tests for variable: RESP

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

Alpha= 0.05 Confidence= 0.95 df= 60 MSE= 357.099
Critical Value of Dunnett's T= 2.410
Minimum Significant Difference= 16.101

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

TRT Comparison	Simultaneous Lower Confidence Limit	Difference Between Means	Simultaneous Upper Confidence Limit	
B - A	-12.414	3.688	19.789	
C - A	-24.414	-8.313	7.789	
D - A	-44.664	-28.562	-12.461	***

1. ANALYSIS OF EL DATA 7
****
14:30 Tuesday, August 9, 1994

General Linear Models Procedure

Bonferroni (Dunn) T tests for variable: RESP

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= 60 MSE= 357.099
Critical Value of T= 2.73
Minimum Significant Difference= 18.23

Means with the same letter are not significantly different.

Bon Grouping	Mean	N	TRT
A	43.250	16	B
A			
A	39.563	16	A
A			

A	31.250	16	C
B	11.000	16	D

2. ANALYSIS OF EC DATA 8
****
14:30 Tuesday, August 9, 1994

General Linear Models Procedure
Class Level Information

Class	Levels	Values
TRT	4	A B C D

Number of observations in data set = 64

2. ANALYSIS OF EC DATA 9
****
14:30 Tuesday, August 9, 1994

General Linear Models Procedure

Dependent Variable: RESP

Source	DF	Sum of Squares	F Value	Pr > F
Model	3	9.68750000	1.13	0.3423
Error	60	170.75000000		
Corrected Total	63	180.43750000		

R-Square	C.V.	RESP Mean
0.053689	101.8542	1.65625000

Source	DF	Type I SS	F Value	Pr > F
TRT	3	9.68750000	1.13	0.3423

Source	DF	Type III SS	F Value	Pr > F
TRT	3	9.68750000	1.13	0.3423

2. ANALYSIS OF EC DATA 10
****
14:30 Tuesday, August 9, 1994

General Linear Models Procedure

Dunnett's T tests for variable: RESP

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

Alpha= 0.05 Confidence= 0.95 df= 60 MSE= 2.845833
Critical Value of Dunnett's T= 2.410
Minimum Significant Difference= 1.4374

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

TRT Comparison	Simultaneous Lower Confidence Limit	Difference Between Means	Simultaneous Upper Confidence Limit
D - A	-0.437	1.000	2.437
B - A	-0.562	0.875	2.312
C - A	-0.937	0.500	1.937

2. ANALYSIS OF EC DATA 11
****
14:30 Tuesday, August 9, 1994

General Linear Models Procedure

Bonferroni (Dunn) T tests for variable: RESP

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= 60 MSE= 2.845833
Critical Value of T= 2.73
Minimum Significant Difference= 1.6274

Means with the same letter are not significantly different.

Bon Grouping	Mean	N	TRT
A	2.063	16	D
A			
A	1.937	16	B
A			
A	1.562	16	C
A			
A	1.062	16	A

3. ANALYSIS OF ES DATA 12
****
14:30 Tuesday, August 9, 1994

General Linear Models Procedure
Class Level Information

Class	Levels	Values
TRT	4	A B C D

Number of observations in data set = 64

3. ANALYSIS OF ES DATA 13
****
14:30 Tuesday, August 9, 1994

General Linear Models Procedure

Dependent Variable: RESP

Source	DF	Sum of Squares	F Value	Pr > F
Model	3	8740.81250000	9.86	0.0001
Error	60	17737.62500000		
Corrected Total	63	26478.43750000		

R-Square	C.V.	RESP Mean
0.330111	62.88022	27.34375000

Source	DF	Type I SS	F Value	Pr > F
TRT	3	8740.81250000	9.86	0.0001

Source	DF	Type III SS	F Value	Pr > F
TRT	3	8740.81250000	9.86	0.0001

3. ANALYSIS OF ES DATA 14
****
14:30 Tuesday, August 9, 1994

General Linear Models Procedure

Dunnett's T tests for variable: RESP

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

Alpha= 0.05 Confidence= 0.95 df= 60 MSE= 295.6271
Critical Value of Dunnett's T= 2.410
Minimum Significant Difference= 14.65

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

TRT Comparison	Simultaneous Lower Confidence Limit	Difference Between Means	Simultaneous Upper Confidence Limit	
B - A	-11.962	2.688	17.337	
C - A	-22.775	-8.125	6.525	
D - A	-41.837	-27.187	-12.538	***

3. ANALYSIS OF ES DATA 15
****
14:30 Tuesday, August 9, 1994

General Linear Models Procedure

Bonferroni (Dunn) T tests for variable: RESP

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= 60 MSE= 295.6271
Critical Value of T= 2.73
Minimum Significant Difference= 16.587

Means with the same letter are not significantly different.

Bon Grouping	Mean	N	TRT

A	38.188	16	B
A			
A	35.500	16	A
A			
A	27.375	16	C
B	8.313	16	D

4. ANALYSIS OF VE DATA 16
14:30 Tuesday, August 9, 1994

General Linear Models Procedure
Class Level Information

Class	Levels	Values
TRT	4	A B C D

Number of observations in data set = 64

4. ANALYSIS OF VE DATA 17
14:30 Tuesday, August 9, 1994

General Linear Models Procedure

Dependent Variable: RESP

Source	DF	Sum of Squares	F Value	Pr > F
Model	3	10791.6718750	16.19	0.0001
Error	60	13332.4375000		
Corrected Total	63	24124.1093750		

R-Square	C.V.	RESP Mean
0.447340	66.76162	22.32812500

Source	DF	Type I SS	F Value	Pr > F
TRT	3	10791.6718750	16.19	0.0001

Source	DF	Type III SS	F Value	Pr > F
TRT	3	10791.6718750	16.19	0.0001

4. ANALYSIS OF VE DATA 18
14:30 Tuesday, August 9, 1994

General Linear Models Procedure

Dunnett's T tests for variable: RESP

NOTE: This tests controls the type I experimentwise error for

comparisons of all treatments against a control.

Alpha= 0.05 Confidence= 0.95 df= 60 MSE= 222.2073
Critical Value of Dunnett's T= 2.410
Minimum Significant Difference= 12.701

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

TRT Comparison	Simultaneous Lower Confidence Limit	Difference Between Means	Simultaneous Upper Confidence Limit	
B - A	-10.201	2.500	15.201	
C - A	-25.076	-12.375	0.326	
D - A	-43.014	-30.312	-17.611	***

4. ANALYSIS OF VE DATA 19
14:30 Tuesday, August 9, 1994

General Linear Models Procedure

Bonferroni (Dunn) T tests for variable: RESP

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= 60 MSE= 222.2073
Critical Value of T= 2.73
Minimum Significant Difference= 14.38

Means with the same letter are not significantly different.

Bon Grouping	Mean	N	TRT
A	34.875	16	B
A			
B A	32.375	16	A
B			
B	20.000	16	C
C	2.063	16	D

5. ANALYSIS OF LE DATA 20
14:30 Tuesday, August 9, 1994

General Linear Models Procedure
Class Level Information

Class	Levels	Values
TRT	4	A B C D

Number of observations in data set = 64

5. ANALYSIS OF LE DATA 21
14:30 Tuesday, August 9, 1994

General Linear Models Procedure

Dependent Variable: RESP

Source	DF	Sum of Squares	F Value	Pr > F
Model	3	9878.62500000	15.97	0.0001
Error	60	12369.37500000		
Corrected Total	63	22248.00000000		

R-Square	C.V.	RESP Mean
0.444023	75.56917	19.00000000

Source	DF	Type I SS	F Value	Pr > F
TRT	3	9878.62500000	15.97	0.0001

Source	DF	Type III SS	F Value	Pr > F
TRT	3	9878.62500000	15.97	0.0001

General Linear Models Procedure

Dunnett's T tests for variable: RESP

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

Alpha= 0.05 Confidence= 0.95 df= 60 MSE= 206.1563
Critical Value of Dunnett's T= 2.410
Minimum Significant Difference= 12.234

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

TRT Comparison	Simultaneous Lower Confidence Limit	Difference Between Means	Simultaneous Upper Confidence Limit	
B - A	-11.484	0.750	12.984	
C - A	-26.296	-14.063	-1.829	***
D - A	-41.921	-29.687	-17.454	***

General Linear Models Procedure

Bonferroni (Dunn) T tests for variable: RESP

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= 60 MSE= 206.1563
Critical Value of T= 2.73

Minimum Significant Difference= 13.851

Means with the same letter are not significantly different.

Bon Grouping	Mean	N	TRT
A	30.500	16	B
A			
A	29.750	16	A
B	15.688	16	C
C	0.063	16	D

General Linear Models Procedure
Class Level Information

Class	Levels	Values
TRT	4	A B C D

Number of observations in data set = 64

General Linear Models Procedure

Dependent Variable: RESP

Source	DF	Sum of Squares	F Value	Pr > F
Model	3	8290.54687500	15.63	0.0001
Error	60	10607.68750000		
Corrected Total	63	18898.23437500		

R-Square	C.V.	RESP Mean
0.438694	78.72071	16.89062500

Source	DF	Type I SS	F Value	Pr > F
TRT	3	8290.54687500	15.63	0.0001

Source	DF	Type III SS	F Value	Pr > F
TRT	3	8290.54687500	15.63	0.0001

General Linear Models Procedure

Dunnett's T tests for variable: RESP

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

Alpha= 0.05 Confidence= 0.95 df= 60 MSE= 176.7948
Critical Value of Dunnett's T= 2.410
Minimum Significant Difference= 11.329

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

TRT Comparison	Simultaneous Lower Confidence Limit	Difference Between Means	Simultaneous Upper Confidence Limit	
B - A	-11.454	-0.125	11.204	
C - A	-25.767	-14.438	-3.108	***
D - A	-38.704	-27.375	-16.046	***

General Linear Models Procedure

Bonferroni (Dunn) T tests for variable: RESP

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= 60 MSE= 176.7948
Critical Value of T= 2.73
Minimum Significant Difference= 12.827

Means with the same letter are not significantly different.

Bon Grouping	Mean	N	TRT
A	27.375	16	A
A			
A	27.250	16	B
B	12.938	16	C
C	0.000	16	D

General Linear Models Procedure
Class Level Information

Class	Levels	Values
TRT	4	A B C D

Number of observations in data set = 64

NOTE: Due to missing values, only 63 observations can be used in this analysis.

General Linear Models Procedure

Dependent Variable: RESP

Source	DF	Sum of Squares	F Value	Pr > F
Model	3	7982.15357143	17.18	0.0001
Error	59	9137.27500000		
Corrected Total	62	17119.42857143		

R-Square	C.V.	RESP Mean
0.466263	75.74998	16.42857143

Source	DF	Type I SS	F Value	Pr > F
TRT	3	7982.15357143	17.18	0.0001

Source	DF	Type III SS	F Value	Pr > F
TRT	3	7982.15357143	17.18	0.0001

General Linear Models Procedure

Dunnett's T tests for variable: RESP

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

Alpha= 0.05 Confidence= 0.95 df= 59 MSE= 154.8691
Critical Value of Dunnett's T= 2.408

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

TRT Comparison	Simultaneous Lower Confidence Limit	Difference Between Means	Simultaneous Upper Confidence Limit	
B - A	-12.506	-1.738	9.031	
C - A	-26.006	-15.238	-4.469	***
D - A	-38.568	-27.800	-17.032	***

General Linear Models Procedure

Bonferroni (Dunn) T tests for variable: RESP

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= 59 MSE= 154.8691
Critical Value of T= 2.73013

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

TRT Comparison		Simultaneous Lower Confidence Limit	Difference Between Means	Simultaneous Upper Confidence Limit	
A	- B	-10.473	1.738	13.948	
A	- C	3.027	15.238	27.448	***
A	- D	15.589	27.800	40.011	***
B	- A	-13.948	-1.738	10.473	
B	- C	1.488	13.500	25.512	***
B	- D	14.050	26.062	38.075	***
C	- A	-27.448	-15.238	-3.027	***
C	- B	-25.512	-13.500	-1.488	***
C	- D	0.550	12.563	24.575	***
D	- A	-40.011	-27.800	-15.589	***
D	- B	-38.075	-26.062	-14.050	***
D	- C	-24.575	-12.563	-0.550	***

General Linear Models Procedure
Class Level Information

Class	Levels	Values
TRT	4	A B C D

Number of observations in data set = 64

NOTE: Due to missing values, only 47 observations can be used in this analysis.

General Linear Models Procedure

Dependent Variable: RESP

Source	DF	Sum of Squares	F Value	Pr > F
Model	3	0.03035395	15.71	0.0001
Error	43	0.02769396		
Corrected Total	46	0.05804791		

R-Square	C.V.	RESP Mean
0.522912	6.897008	0.36795745

Source	DF	Type I SS	F Value	Pr > F
TRT	3	0.03035395	15.71	0.0001

Source	DF	Type III SS	F Value	Pr > F
TRT	3	0.03035395	15.71	0.0001

General Linear Models Procedure

Dunnett's T tests for variable: RESP

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

Alpha= 0.05 Confidence= 0.95 df= 43 MSE= 0.000644
Critical Value of Dunnett's T= 2.442

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

TRT Comparison		Simultaneous Lower Confidence Limit	Difference Between Means	Simultaneous Upper Confidence Limit	
B	- A	-0.01366	0.01000	0.03366	
C	- A	-0.04005	-0.01475	0.01055	
D	- A	-0.09615	-0.06668	-0.03721	***

General Linear Models Procedure

Bonferroni (Dunn) T tests for variable: RESP

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= 43 MSE= 0.000644
Critical Value of T= 2.76584

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

TRT Comparison		Simultaneous Lower Confidence Limit	Difference Between Means	Simultaneous Upper Confidence Limit	
B	- A	-0.01680	0.01000	0.03680	
B	- C	-0.00205	0.02475	0.05155	
B	- D	0.04487	0.07668	0.10849	***

A	- B	-0.03680	-0.01000	0.01680	
A	- C	-0.01391	0.01475	0.04341	
A	- D	0.03330	0.06668	0.10006	***
C	- B	-0.05155	-0.02475	0.00205	
C	- A	-0.04341	-0.01475	0.01391	
C	- D	0.01855	0.05193	0.08531	***
D	- B	-0.10849	-0.07668	-0.04487	***
D	- A	-0.10006	-0.06668	-0.03330	***
D	- C	-0.08531	-0.05193	-0.01855	***

9. ANALYSIS OF HATCHLING WEIGHT DATA 36
****
14:30 Tuesday, August 9, 1994

General Linear Models Procedure
Class Level Information

Class	Levels	Values
TRT	4	A B C D

Number of observations in data set = 64

NOTE: Due to missing values, only 42 observations can be used in this analysis.

9. ANALYSIS OF HATCHLING WEIGHT DATA 37
****
14:30 Tuesday, August 9, 1994

General Linear Models Procedure

Dependent Variable: RESP

Source	DF	Sum of Squares	F Value	Pr > F
Model	2	2.35525641	0.15	0.8619
Error	39	307.84307692		
Corrected Total	41	310.19833333		

R-Square	C.V.	RESP Mean
0.007593	8.243095	34.08333333

Source	DF	Type I SS	F Value	Pr > F
TRT	2	2.35525641	0.15	0.8619

Source	DF	Type III SS	F Value	Pr > F
TRT	2	2.35525641	0.15	0.8619

9. ANALYSIS OF HATCHLING WEIGHT DATA 38
****
14:30 Tuesday, August 9, 1994

General Linear Models Procedure

Dunnett's T tests for variable: RESP

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

Alpha= 0.05 Confidence= 0.95 df= 39 MSE= 7.893412
Critical Value of Dunnett's T= 2.292

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

TRT Comparison	Simultaneous Lower Confidence Limit	Difference Between Means	Simultaneous Upper Confidence Limit
C - A	-2.318	0.208	2.733
B - A	-2.758	-0.354	2.050

9. ANALYSIS OF HATCHLING WEIGHT DATA 39
****
14:30 Tuesday, August 9, 1994

General Linear Models Procedure

Bonferroni (Dunn) T tests for variable: RESP

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= 39 MSE= 7.893412
Critical Value of T= 2.50166

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

TRT Comparison	Simultaneous Lower Confidence Limit	Difference Between Means	Simultaneous Upper Confidence Limit
C - A	-2.549	0.208	2.964
C - B	-2.063	0.562	3.186
A - C	-2.964	-0.208	2.549
A - B	-2.271	0.354	2.978
B - C	-3.186	-0.562	2.063
B - A	-2.978	-0.354	2.271

10. ANALYSIS OF 14-DAY SURVIVOR WEIGHT DATA 40
****
14:30 Tuesday, August 9, 1994

General Linear Models Procedure
Class Level Information

Class	Levels	Values
TRT	4	A B C D

Number of observations in data set = 64

NOTE: Due to missing values, only 42 observations can be used in this analysis.

10. ANALYSIS OF 14-DAY SURVIVOR WEIGHT DATA 41
****
14:30 Tuesday, August 9, 1994

General Linear Models Procedure

Dependent Variable: RESP

Source	DF	Sum of Squares	F Value	Pr > F
Model	2	9317.36423306	7.09	0.0024
Error	39	25624.34552885		
Corrected Total	41	34941.70976190		

R-Square	C.V.	RESP Mean
0.266655	9.220311	278.00238095

Source	DF	Type I SS	F Value	Pr > F
TRT	2	9317.36423306	7.09	0.0024

Source	DF	Type III SS	F Value	Pr > F
TRT	2	9317.36423306	7.09	0.0024

10. ANALYSIS OF 14-DAY SURVIVOR WEIGHT DATA 42
****
14:30 Tuesday, August 9, 1994

General Linear Models Procedure

Dunnett's T tests for variable: RESP

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

Alpha= 0.05 Confidence= 0.95 df= 39 MSE= 657.0345
Critical Value of Dunnett's T= 2.292

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

TRT Comparison	Simultaneous Lower Confidence Limit	Difference Between Means	Simultaneous Upper Confidence Limit	
C - A	0.053	23.092	46.131	***
B - A	-34.692	-12.759	9.173	

10. ANALYSIS OF 14-DAY SURVIVOR WEIGHT DATA 43
****
14:30 Tuesday, August 9, 1994

General Linear Models Procedure

Bonferroni (Dunn) T tests for variable: RESP

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= 39 MSE= 657.0345
Critical Value of T= 2.50166

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

TRT Comparison	Simultaneous Lower Confidence Limit	Difference Between Means	Simultaneous Upper Confidence Limit	
C - A	-2.059	23.092	48.244	
C - B	11.908	35.851	59.795	***
A - C	-48.244	-23.092	2.059	
A - B	-11.184	12.759	36.703	
B - C	-59.795	-35.851	-11.908	***
B - A	-36.703	-12.759	11.184	

12. ANALYSIS OF ES/EL DATA 44
****
14:30 Tuesday, August 9, 1994

General Linear Models Procedure
Class Level Information

Class	Levels	Values
TRT	4	A B C D

Number of observations in data set = 64

NOTE: Due to missing values, only 53 observations can be used in this analysis.

12. ANALYSIS OF ES/EL DATA 45
****
14:30 Tuesday, August 9, 1994

General Linear Models Procedure

Dependent Variable: RESPONSE
Weight: WT

Source	DF	Sum of Squares	F Value	Pr > F
Model	3	12396.8255482	3.65	0.0187
Error	49	55433.5613452		
Corrected Total	52	67830.3868934		

R-Square	C.V.	RESPONSE Mean
0.182762	48.15659	69.84456233

Source	DF	Type I SS	F Value	Pr > F

Source	DF	Type III SS	F Value	Pr > F
TRT	3	12396.8255482	3.65	0.0187

Source	DF	Type III SS	F Value	Pr > F
TRT	3	12396.8255482	3.65	0.0187

12. ANALYSIS OF ES/EL DATA 46
****
14:30 Tuesday, August 9, 1994

General Linear Models Procedure

Dunnett's T tests for variable: RESPONSE

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

Alpha= 0.05 Confidence= 0.95 df= 49 MSE= 1131.297
Critical Value of Dunnett's T= 2.425

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

TRT Comparison	Simultaneous Lower Confidence Limit	Difference Between Means	Simultaneous Upper Confidence Limit
B - A	-31.600	-1.148	29.303
C - A	-33.280	-1.868	29.544
D - A	-43.670	-9.366	24.937

12. ANALYSIS OF ES/EL DATA 47
****
14:30 Tuesday, August 9, 1994

General Linear Models Procedure

Bonferroni (Dunn) T tests for variable: RESPONSE

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= 49 MSE= 1131.297
Critical Value of T= 2.74961

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

TRT Comparison	Simultaneous Lower Confidence Limit	Difference Between Means	Simultaneous Upper Confidence Limit
A - B	-33.384	1.148	35.681
A - C	-33.753	1.868	37.489
A - D	-29.534	9.366	48.266
B - A	-35.681	-1.148	33.384
B - C	-33.126	0.720	34.565
B - D	-29.063	8.218	45.498
C - A	-37.489	-1.868	33.753
C - B	-34.565	-0.720	33.126

14

C	- D	-30.794	7.498	45.789
D	- A	-48.266	-9.366	29.534
D	- B	-45.498	-8.218	29.063
D	- C	-45.789	-7.498	30.794

13 ANALYSIS OF VE/ES DATA 48
****
14:30 Tuesday, August 9, 1994

General Linear Models Procedure
Class Level Information

Class	Levels	Values
TRT	4	A B C D

Number of observations in data set = 64

NOTE: Due to missing values, only 53 observations can be used in this analysis.

13 ANALYSIS OF VE/ES DATA 49
****
14:30 Tuesday, August 9, 1994

General Linear Models Procedure

Dependent Variable: RESPONSE
Weight: WT

Source	DF	Sum of Squares	F Value	Pr > F
Model	3	277121.660350	30.02	0.0001
Error	49	150792.042189		
Corrected Total	52	427913.702539		

R-Square	C.V.	RESPONSE Mean
0.647611	82.46138	67.27296644

Source	DF	Type I SS	F Value	Pr > F
TRT	3	277121.660350	30.02	0.0001

Source	DF	Type III SS	F Value	Pr > F
TRT	3	277121.660350	30.02	0.0001

13 ANALYSIS OF VE/ES DATA 50
****
14:30 Tuesday, August 9, 1994

General Linear Models Procedure

Dunnett's T tests for variable: RESPONSE

NOTE: This tests controls the type I experimentwise error for

comparisons of all treatments against a control.

Alpha= 0.05 Confidence= 0.95 df= 49 MSE= 3077.389
Critical Value of Dunnett's T= 2.425

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

TRT Comparison		Simultaneous Lower Confidence Limit	Difference Between Means	Simultaneous Upper Confidence Limit
B	- A	-49.49	0.73	50.95
C	- A	-65.49	-13.68	38.13
D	- A	-101.72	-45.14	11.43

General Linear Models Procedure

Bonferroni (Dunn) T tests for variable: RESPONSE

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= 49 MSE= 3077.389
Critical Value of T= 2.74961

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

TRT Comparison		Simultaneous Lower Confidence Limit	Difference Between Means	Simultaneous Upper Confidence Limit
B	- A	-56.23	0.73	57.68
B	- C	-41.41	14.41	70.23
B	- D	-15.61	45.87	107.36
A	- B	-57.68	-0.73	56.23
A	- C	-45.07	13.68	72.43
A	- D	-19.01	45.14	109.30
C	- B	-70.23	-14.41	41.41
C	- A	-72.43	-13.68	45.07
C	- D	-31.69	31.46	94.62
D	- B	-107.36	-45.87	15.61
D	- A	-109.30	-45.14	19.01
D	- C	-94.62	-31.46	31.69

General Linear Models Procedure
Class Level Information

Class	Levels	Values
TRT	4	A B C D

42

Number of observations in data set = 64

NOTE: Due to missing values, only 52 observations can be used in this analysis.

General Linear Models Procedure

Dependent Variable: RESPONSE
Weight: WT

Source	DF	Sum of Squares	F Value	Pr > F
Model	3	186598.923818	9.42	0.0001
Error	48	316859.847338		
Corrected Total	51	503458.771155		

R-Square	C.V.	RESPONSE Mean
0.370634	113.8604	71.35761218

Source	DF	Type I SS	F Value	Pr > F
TRT	3	186598.923818	9.42	0.0001

Source	DF	Type III SS	F Value	Pr > F
TRT	3	186598.923818	9.42	0.0001

General Linear Models Procedure

Dunnett's T tests for variable: RESPONSE

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

Alpha= 0.05 Confidence= 0.95 df= 48 MSE= 6601.247
Critical Value of Dunnett's T= 2.429

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

TRT Comparison		Simultaneous Lower Confidence Limit	Difference Between Means	Simultaneous Upper Confidence Limit
B	- A	-77.71	-4.03	69.65
C	- A	-88.61	-12.61	63.39
D	- A	-159.39	-73.82	11.74

****

14:30 Tuesday, August 9, 1994

General Linear Models Procedure

Bonferroni (Dunn) T tests for variable: RESPONSE

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= 48 MSE= 6601.247
Critical Value of T= 2.75202

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

TRT Comparison		Simultaneous Lower Confidence Limit	Difference Between Means	Simultaneous Upper Confidence Limit
A	- B	-79.46	4.03	87.52
A	- C	-73.51	12.61	98.73
A	- D	-23.13	73.82	170.78
B	- A	-87.52	-4.03	79.46
B	- C	-73.24	8.58	90.41
B	- D	-23.37	69.80	162.96
C	- A	-98.73	-12.61	73.51
C	- B	-90.41	-8.58	73.24
C	- D	-34.32	61.21	156.74
D	- A	-170.78	-73.82	23.13
D	- B	-162.96	-69.80	23.37
D	- C	-156.74	-61.21	34.32

****

14:30 Tuesday, August 9, 1994

General Linear Models Procedure
Class Level Information

Class	Levels	Values
TRT	4	A B C D

Number of observations in data set = 64

NOTE: Due to missing values, only 43 observations can be used in this analysis.

****

14:30 Tuesday, August 9, 1994

General Linear Models Procedure

Dependent Variable: RESPONSE
Weight: WT

Source	DF	Sum of Squares	F Value	Pr > F
Model	3	22585.6948496	2.50	0.0739
Error	39	117593.2833528		
Corrected Total	42	140178.9782024		

R-Square	C.V.	RESPONSE Mean
0.161120	75.12594	73.09186698

Source	DF	Type I SS	F Value	Pr > F
TRT	3	22585.6948496	2.50	0.0739

Source	DF	Type III SS	F Value	Pr > F
TRT	3	22585.6948496	2.50	0.0739

****

14:30 Tuesday, August 9, 1994

General Linear Models Procedure

Dunnett's T tests for variable: RESPONSE

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

Alpha= 0.05 Confidence= 0.95 df= 39 MSE= 3015.212
Critical Value of Dunnett's T= 2.486

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

TRT Comparison		Simultaneous Lower Confidence Limit	Difference Between Means	Simultaneous Upper Confidence Limit
B	- A	-53.87	-2.91	48.05
C	- A	-63.74	-10.21	43.32
D	- A	-218.06	-76.43	65.21

****

14:30 Tuesday, August 9, 1994

General Linear Models Procedure

Bonferroni (Dunn) T tests for variable: RESPONSE

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= 39 MSE= 3015.212
Critical Value of T= 2.77957

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

TRT	Simultaneous Lower Confidence	Difference Between	Simultaneous Upper Confidence

Comparison		Limit	Means	Limit
A	- B	-54.08	2.91	59.90
A	- C	-49.66	10.21	70.07
A	- D	-81.96	76.43	234.82
B	- A	-59.90	-2.91	54.08
B	- C	-49.69	7.30	64.29
B	- D	-83.81	73.52	230.85
C	- A	-70.07	-10.21	49.66
C	- B	-64.29	-7.30	49.69
C	- D	-92.17	66.22	224.61
D	- A	-234.82	-76.43	81.96
D	- B	-230.85	-73.52	83.81
D	- C	-224.61	-66.22	92.17

16. ANALYSIS OF NH/EL DATA 60
****
14:30 Tuesday, August 9, 1994

General Linear Models Procedure
Class Level Information

Class	Levels	Values
TRT	4	A B C D

Number of observations in data set = 64

NOTE: Due to missing values, only 53 observations can be used in this analysis.

16. ANALYSIS OF NH/EL DATA 61
****
14:30 Tuesday, August 9, 1994

General Linear Models Procedure

Dependent Variable: RESPONSE
Weight: WT

Source	DF	Sum of Squares	F Value	Pr > F
Model	3	497556.552860	27.73	0.0001
Error	49	293052.721009		
Corrected Total	52	790609.273869		

R-Square	C.V.	RESPONSE Mean
0.629333	169.0825	45.73790367

Source	DF	Type I SS	F Value	Pr > F
TRT	3	497556.552860	27.73	0.0001

Source	DF	Type III SS	F Value	Pr > F
TRT	3	497556.552860	27.73	0.0001

16. ANALYSIS OF NH/EL DATA 62
****
14:30 Tuesday, August 9, 1994

General Linear Models Procedure

Dunnett's T tests for variable: RESPONSE

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

Alpha= 0.05 Confidence= 0.95 df= 49 MSE= 5980.668
Critical Value of Dunnett's T= 2.425

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

TRT Comparison		Simultaneous Lower Confidence Limit	Difference Between Means	Simultaneous Upper Confidence Limit
B	- A	-74.11	-4.10	65.92
C	- A	-89.96	-17.74	54.49
D	- A	-135.43	-56.56	22.31

16. ANALYSIS OF NH/EL DATA 63
****
14:30 Tuesday, August 9, 1994

General Linear Models Procedure

Bonferroni (Dunn) T tests for variable: RESPONSE

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= 49 MSE= 5980.668
Critical Value of T= 2.74961

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

TRT Comparison		Simultaneous Lower Confidence Limit	Difference Between Means	Simultaneous Upper Confidence Limit
A	- B	-75.30	4.10	83.50
A	- C	-64.16	17.74	99.64
A	- D	-32.88	56.56	146.00
B	- A	-83.50	-4.10	75.30
B	- C	-64.18	13.64	91.46
B	- D	-33.25	52.46	138.18
C	- A	-99.64	-17.74	64.16
C	- B	-91.46	-13.64	64.18
C	- D	-49.22	38.82	126.87
D	- A	-146.00	-56.56	32.88
D	- B	-138.18	-52.46	33.25
D	- C	-126.87	-38.82	49.22

17. ANALYSIS OF HS/NH DATA 64
14:30 Tuesday, August 9, 1994

General Linear Models Procedure
Class Level Information

Class	Levels	Values
TRT	4	A B C D

Number of observations in data set = 64

NOTE: Due to missing values, only 42 observations can be used in this analysis.

17. ANALYSIS OF HS/NH DATA 65
14:30 Tuesday, August 9, 1994

General Linear Models Procedure

Dependent Variable: RESPONSE
Weight: WT

Source	DF	Sum of Squares	F Value	Pr > F
Model	2	1868.86151157	0.44	0.6489
Error	39	83345.95845918		
Corrected Total	41	85214.81997075		

R-Square	C.V.	RESPONSE Mean
0.021931	56.60373	81.67043749

Source	DF	Type I SS	F Value	Pr > F
TRT	2	1868.86151157	0.44	0.6489

Source	DF	Type III SS	F Value	Pr > F
TRT	2	1868.86151157	0.44	0.6489

17. ANALYSIS OF HS/NH DATA 66
14:30 Tuesday, August 9, 1994

General Linear Models Procedure

Dunnett's T tests for variable: RESPONSE

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

Alpha= 0.05 Confidence= 0.95 df= 39 MSE= 2137.076
Critical Value of Dunnett's T= 2.292

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

TRT Comparison	Simultaneous Lower Confidence Limit	Difference Between Means	Simultaneous Upper Confidence Limit
C - A	-37.990	3.560	45.111
B - A	-37.835	1.721	41.276

17. ANALYSIS OF HS/NH DATA 67
14:30 Tuesday, August 9, 1994

General Linear Models Procedure

Bonferroni (Dunn) T tests for variable: RESPONSE

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= 39 MSE= 2137.076
Critical Value of T= 2.50166

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

TRT Comparison	Simultaneous Lower Confidence Limit	Difference Between Means	Simultaneous Upper Confidence Limit
C - B	-41.342	1.840	45.022
C - A	-41.800	3.560	48.921
B - C	-45.022	-1.840	41.342
B - A	-41.462	1.721	44.903
A - C	-48.921	-3.560	41.800
A - B	-44.903	-1.721	41.462

18. ANALYSIS OF EC/EL DATA 68
14:30 Tuesday, August 9, 1994

General Linear Models Procedure
Class Level Information

Class	Levels	Values
TRT	4	A B C D

Number of observations in data set = 64

NOTE: Due to missing values, only 53 observations can be used in this analysis.

18. ANALYSIS OF EC/EL DATA 69
14:30 Tuesday, August 9, 1994

General Linear Models Procedure

Dependent Variable: RESPONSE

Weight: WT

Source	DF	Sum of Squares	F Value	Pr > F
Model	3	35827.3838251	5.83	0.0017
Error	49	100319.1663309		
Corrected Total	52	136146.5501560		

R-Square	C.V.	RESPONSE Mean
0.263153	419.4413	10.78754872

Source	DF	Type I SS	F Value	Pr > F
TRT	3	35827.3838251	5.83	0.0017

Source	DF	Type III SS	F Value	Pr > F
TRT	3	35827.3838251	5.83	0.0017

18. ANALYSIS OF EC/EL DATA 70

14:30 Tuesday, August 9, 1994

General Linear Models Procedure

Dunnett's T tests for variable: RESPONSE

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

Alpha= 0.05 Confidence= 0.95 df= 49 MSE= 2047.33
Critical Value of Dunnett's T= 2.425

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

TRT Comparison	Simultaneous Lower Confidence Limit	Difference Between Means	Simultaneous Upper Confidence Limit
D - A	-30.11	16.03	62.18
C - A	-38.73	3.53	45.78
B - A	-38.43	2.53	43.50

18. ANALYSIS OF EC/EL DATA 71

14:30 Tuesday, August 9, 1994

General Linear Models Procedure

Bonferroni (Dunn) T tests for variable: RESPONSE

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= 49 MSE= 2047.33
Critical Value of T= 2.74961

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

TRT Comparison	Simultaneous Lower Confidence Limit	Difference Between Means	Simultaneous Upper Confidence Limit
D - C	-39.00	12.51	64.02
D - B	-36.65	13.50	63.65
D - A	-36.30	16.03	68.36
C - D	-64.02	-12.51	39.00
C - B	-44.54	0.99	46.52
C - A	-44.39	3.53	51.45
B - D	-63.65	-13.50	36.65
B - C	-46.52	-0.99	44.54
B - A	-43.92	2.53	48.99
A - D	-68.36	-16.03	36.30
A - C	-51.45	-3.53	44.39
A - B	-48.99	-2.53	43.92

19. ANALYSIS OF NH/ES DATA 72

14:30 Tuesday, August 9, 1994

General Linear Models Procedure
Class Level Information

Class	Levels	Values
TRT	4	A B C D

Number of observations in data set = 64

NOTE: Due to missing values, only 53 observations can be used in this analysis.

19. ANALYSIS OF NH/ES DATA 73

14:30 Tuesday, August 9, 1994

General Linear Models Procedure

Dependent Variable: RESPONSE
Weight: WT

Source	DF	Sum of Squares	F Value	Pr > F
Model	3	485437.073479	24.07	0.0001
Error	49	329366.419835		
Corrected Total	52	814803.493314		

R-Square	C.V.	RESPONSE Mean
0.595772	159.5340	51.39115882

Source	DF	Type I SS	F Value	Pr > F
TRT	3	485437.073479	24.07	0.0001

Source	DF	Type III SS	F Value	Pr > F
TRT	3	485437.073479	24.07	0.0001

19. ANALYSIS OF NH/ES DATA 74

14:30 Tuesday, August 9, 1994

General Linear Models Procedure

Dunnett's T tests for variable: RESPONSE

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

Alpha= 0.05 Confidence= 0.95 df= 49 MSE= 6721.764
Critical Value of Dunnett's T= 2.425

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

TRT Comparison	Simultaneous Lower Confidence Limit	Difference Between Means	Simultaneous Upper Confidence Limit
B - A	-77.73	-3.50	70.73
C - A	-96.23	-19.66	56.91
D - A	-145.88	-62.27	21.35

19. ANALYSIS OF NH/ES DATA 75

14:30 Tuesday, August 9, 1994

General Linear Models Procedure

Bonferroni (Dunn) T tests for variable: RESPONSE

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= 49 MSE= 6721.764
Critical Value of T= 2.74961

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

TRT Comparison	Simultaneous Lower Confidence Limit	Difference Between Means	Simultaneous Upper Confidence Limit
A - B	-80.67	3.50	87.68
A - C	-67.17	19.66	106.49
A - D	-32.55	62.27	157.09
B - A	-87.68	-3.50	80.67
B - C	-66.34	16.16	98.65
B - D	-32.11	58.76	149.64
C - A	-106.49	-19.66	67.17
C - B	-98.65	-16.16	66.34
C - D	-50.73	42.61	135.95
D - A	-157.09	-62.27	32.55

D	- B	-149.64	-58.76	32.11
D	- C	-135.95	-42.61	50.73

20. ANALYSIS OF HS/ES DATA 76

14:30 Tuesday, August 9, 1994

General Linear Models Procedure
Class Level Information

Class	Levels	Values
TRT	4	A B C D

Number of observations in data set = 64

NOTE: Due to missing values, only 53 observations can be used in this analysis.

20. ANALYSIS OF HS/ES DATA 77

14:30 Tuesday, August 9, 1994

General Linear Models Procedure

Dependent Variable: RESPONSE
Weight: WT

Source	DF	Sum of Squares	F Value	Pr > F
Model	3	438940.545433	22.94	0.0001
Error	49	312544.620151		
Corrected Total	52	751485.165584		

R-Square	C.V.	RESPONSE Mean
0.584097	161.3882	49.48644329

Source	DF	Type I SS	F Value	Pr > F
TRT	3	438940.545433	22.94	0.0001

Source	DF	Type III SS	F Value	Pr > F
TRT	3	438940.545433	22.94	0.0001

20. ANALYSIS OF HS/ES DATA 78

14:30 Tuesday, August 9, 1994

General Linear Models Procedure

Dunnett's T tests for variable: RESPONSE

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

Alpha= 0.05 Confidence= 0.95 df= 49 MSE= 6378.462

Critical Value of Dunnett's T= 2.425

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

TRT Comparison		Simultaneous Lower Confidence Limit	Difference Between Means	Simultaneous Upper Confidence Limit
B	- A	-75.57	-3.26	69.04
C	- A	-92.43	-17.84	56.74
D	- A	-141.08	-59.62	21.83

General Linear Models Procedure

Bonferroni (Dunn) T tests for variable: RESPONSE

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= 49 MSE= 6378.462
Critical Value of T= 2.74961

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

TRT Comparison		Simultaneous Lower Confidence Limit	Difference Between Means	Simultaneous Upper Confidence Limit
A	- B	-78.73	3.26	85.26
A	- C	-66.74	17.84	102.42
A	- D	-32.74	59.62	151.99
B	- A	-85.26	-3.26	78.73
B	- C	-65.79	14.58	94.94
B	- D	-32.16	56.36	144.88
C	- A	-102.42	-17.84	66.74
C	- B	-94.94	-14.58	65.79
C	- D	-49.14	41.78	132.70
D	- A	-151.99	-59.62	32.74
D	- B	-144.88	-56.36	32.16
D	- C	-132.70	-41.78	49.14

General Linear Models Procedure
Class Level Information

Class	Levels	Values
TRT	4	A B C D

Number of observations in data set = 64

NOTE: Due to missing values, only 53 observations can be used in this analysis.

General Linear Models Procedure

Dependent Variable: RESPONSE
Weight: WT

Source	DF	Sum of Squares	F Value	Pr > F
Model	3	535619.419479	28.20	0.0001
Error	49	310213.203691		
Corrected Total	52	845832.623170		

R-Square	C.V.	RESPONSE Mean
0.633245	139.6373	56.98107071

Source	DF	Type I SS	F Value	Pr > F
TRT	3	535619.419479	28.20	0.0001

Source	DF	Type III SS	F Value	Pr > F
TRT	3	535619.419479	28.20	0.0001

General Linear Models Procedure

Dunnett's T tests for variable: RESPONSE

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

Alpha= 0.05 Confidence= 0.95 df= 49 MSE= 6330.882
Critical Value of Dunnett's T= 2.425

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

TRT Comparison		Simultaneous Lower Confidence Limit	Difference Between Means	Simultaneous Upper Confidence Limit
B	- A	-74.42	-2.39	69.65
C	- A	-92.34	-18.03	56.28
D	- A	-147.03	-65.88	15.26

General Linear Models Procedure

Bonferroni (Dunn) T tests for variable: RESPONSE

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= 49 MSE= 6330.882
Critical Value of T= 2.74961

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

TRT Comparison		Simultaneous Lower Confidence Limit	Difference Between Means	Simultaneous Upper Confidence Limit
A	- B	-79.30	2.39	84.08
A	- C	-66.23	18.03	102.30
A	- D	-26.14	65.88	157.91
B	- A	-84.08	-2.39	79.30
B	- C	-64.42	15.65	95.71
B	- D	-24.70	63.50	151.69
C	- A	-102.30	-18.03	66.23
C	- B	-95.71	-15.65	64.42
C	- D	-42.73	47.85	138.43
D	- A	-157.91	-65.88	26.14
D	- B	-151.69	-63.50	24.70
D	- C	-138.43	-47.85	42.73

22. COVARIATE ANALYSIS OF MALE BODY WEIGHT DATA 84
14:30 Tuesday, August 9, 1994

General Linear Models Procedure
Class Level Information

Class	Levels	Values
TRT	4	A B C D

Number of observations in data set = 64

22. COVARIATE ANALYSIS OF MALE BODY WEIGHT DATA 85
14:30 Tuesday, August 9, 1994

General Linear Models Procedure

Dependent Variable: POSTM

Source	DF	Sum of Squares	F Value	Pr > F
Model	4	204049.132150	2.78	0.0349
Error	59	1083359.602225		
Corrected Total	63	1287408.734375		

R-Square	C.V.	POSTM Mean
0.158496	10.74290	1261.3593750

Source	DF	Type I SS	F Value	Pr > F
TRT	3	55823.421875	1.01	0.3933
PREM	1	148225.710275	8.07	0.0062

Source	DF	Type III SS	F Value	Pr > F
TRT	3	35616.230778	0.65	0.5882
PREM	1	148225.710275	8.07	0.0062

Parameter		Estimate	T for H0: Parameter=0	Pr > |T|	Std Error of Estimate
INTERCEPT		689.0952311 B	3.30	0.0017	209.04668775
TRT	A	-15.5405017 B	-0.32	0.7471	47.97026303
	B	34.2146390 B	0.71	0.4793	48.05356618
	C	-29.3392544 B	-0.60	0.5504	48.85324515
	D	0.0000000 B	.	.	.
PREM		0.4882831	2.84	0.0062	0.17185818

NOTE: The X'X matrix has been found to be singular and a generalized inverse was used to solve the normal equations. Estimates followed by the letter 'B' are biased, and are not unique estimators of the parameters.

22. COVARIATE ANALYSIS OF MALE BODY WEIGHT DATA 86
14:30 Tuesday, August 9, 1994

General Linear Models Procedure
Least Squares Means
Coefficients for TRT Least Square Means

TRT		A	B	C
Effect		Coefficients		
INTERCEPT		1	1	1
TRT	A	1	0	0
	B	0	1	0
	C	0	0	1
	D	0	0	0
PREM		1177.453125	1177.453125	1177.453125

TRT		D
Effect		Coefficients
INTERCEPT		1
TRT	A	0
	B	0
	C	0
	D	1
PREM		1177.453125

22. COVARIATE ANALYSIS OF MALE BODY WEIGHT DATA 87

14:30 Tuesday, August 9, 1994

General Linear Models Procedure
Least Squares Means

TRT	POSTM LSMEAN	Std Err LSMEAN	Pr > |T| H0:LSMEAN=0	LSMEAN Number
A	1248.48515	33.90988	0.0001	1
B	1298.24029	33.87724	0.0001	2
C	1234.68640	34.34145	0.0001	3
D	1264.02565	34.10367	0.0001	4

Pr > |T| H0: LSMEAN(i)=LSMEAN(j)

i/j	1	2	3	4
1	.	0.3034	0.7767	0.7471
2	0.3034	.	0.1930	0.4793
3	0.7767	0.1930	.	0.5504
4	0.7471	0.4793	0.5504	.

NOTE: To ensure overall protection level, only probabilities associated with pre-planned comparisons should be used.

22. COVARIATE ANALYSIS OF MALE BODY WEIGHT DATA 88

14:30 Tuesday, August 9, 1994

General Linear Models Procedure

Dunnett's T tests for variable: POSTM

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

Alpha= 0.05 Confidence= 0.95 df= 59 MSE= 18362.03
Critical Value of Dunnett's T= 2.411
Minimum Significant Difference= 115.51

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

TRT Comparison	Simultaneous Lower Confidence Limit	Difference Between Means	Simultaneous Upper Confidence Limit
B - A	-69.45	46.06	161.57
D - A	-93.07	22.44	137.95
C - A	-149.57	-34.06	81.45

22. COVARIATE ANALYSIS OF MALE BODY WEIGHT DATA 89

14:30 Tuesday, August 9, 1994

General Linear Models Procedure

Bonferroni (Dunn) T tests for variable: POSTM

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= 59 MSE= 18362.03
Critical Value of T= 2.73
Minimum Significant Difference= 130.8

Means with the same letter are not significantly different.

Bon Grouping	Mean	N	TRT
A	1298.81	16	B
A			
A	1275.19	16	D
A			
A	1252.75	16	A
A			
A	1218.69	16	C

23. COVARIATE ANALYSIS OF FEMALE BODY WEIGHT DATA 90

14:30 Tuesday, August 9, 1994

General Linear Models Procedure
Class Level Information

Class	Levels	Values
TRT	4	A B C D

Number of observations in data set = 64

NOTE: Due to missing values, only 63 observations can be used in this analysis.

23. COVARIATE ANALYSIS OF FEMALE BODY WEIGHT DATA 91

14:30 Tuesday, August 9, 1994

General Linear Models Procedure

Dependent Variable: POSTF

Source	DF	Sum of Squares	F Value	Pr > F
Model	4	158506.155164	1.98	0.1089
Error	58	1158317.559121		
Corrected Total	62	1316823.714286		

R-Square	C.V.	POSTF Mean
0.120370	11.91367	1186.1904762

Source	DF	Type I SS	F Value	Pr > F
TRT	3	5556.043452	0.09	0.9638
PREF	1	152950.111712	7.66	0.0076

Source	DF	Type III SS	F Value	Pr > F
TRT	3	2175.312551	0.04	0.9906
PREF	1	152950.111712	7.66	0.0076

Parameter		Estimate	T for H0: Parameter=0	Pr > |T|	Std Error of Estimate
INTERCEPT		573.6472322 B	2.52	0.0145	227.66004000
TRT	A	5.0445025 B	0.10	0.9215	50.99258038
	B	9.2289106 B	0.18	0.8571	51.03031108
	C	-6.4089725 B	-0.13	0.9009	51.22177117
	D	0.0000000 B	.	.	.
PREF		0.5983510	2.77	0.0076	0.21621256

NOTE: The X'X matrix has been found to be singular and a generalized inverse was used to solve the normal equations. Estimates followed by the letter 'B' are biased, and are not unique estimators of the parameters.

General Linear Models Procedure
Least Squares Means
Coefficients for TRT Least Square Means

TRT		A	B	C
Effect		Coefficients		
INTERCEPT		1	1	1
TRT	A	1	0	0
	B	0	1	0
	C	0	0	1
	D	0	0	0
PREF		1020.3809524	1020.3809524	1020.3809524

TRT		D
Effect		Coefficients
INTERCEPT		1
TRT	A	0
	B	0
	C	0
	D	1
PREF		1020.3809524

General Linear Models Procedure
Least Squares Means

TRT	POSTF LSMEAN	Std Err LSMEAN	Pr > |T| H0:LSMEAN=0	LSMEAN Number
A	1189.23766	35.33252	0.0001	1
B	1193.42207	35.33998	0.0001	2
C	1177.78419	35.42101	0.0001	3
D	1184.19316	36.71772	0.0001	4

Pr > |T| H0: LSMEAN(i)=LSMEAN(j)

i/j	1	2	3	4
1	.	0.9335	0.8196	0.9215
2	0.9335	.	0.7556	0.8571
3	0.8196	0.7556	.	0.9009
4	0.9215	0.8571	0.9009	.

NOTE: To ensure overall protection level, only probabilities associated with pre-planned comparisons should be used.

General Linear Models Procedure

Dunnett's T tests for variable: POSTF

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

Alpha= 0.05 Confidence= 0.95 df= 58 MSE= 19970.99
Critical Value of Dunnett's T= 2.413

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

TRT Comparison	Simultaneous Lower Confidence Limit	Difference Between Means	Simultaneous Upper Confidence Limit
D - A	-115.03	7.53	130.09
B - A	-117.50	3.06	123.63
C - A	-137.82	-17.25	103.32

General Linear Models Procedure

Bonferroni (Dunn) T tests for variable: POSTF

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= 58 MSE= 19970.99
Critical Value of T= 2.73177

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

TRT Comparison		Simultaneous Lower Confidence Limit	Difference Between Means	Simultaneous Upper Confidence Limit
D	- B	-134.27	4.47	143.22
D	- A	-131.21	7.53	146.28
D	- C	-113.96	24.78	163.53
B	- D	-143.22	-4.47	134.27
B	- A	-133.43	3.06	139.55
B	- C	-116.18	20.31	156.80
A	- D	-146.28	-7.53	131.21
A	- B	-139.55	-3.06	133.43
A	- C	-119.24	17.25	153.74
C	- D	-163.53	-24.78	113.96
C	- B	-156.80	-20.31	116.18
C	- A	-153.74	-17.25	119.24

SAS 12:22 Monday, August 15, 1994 1

OBS	TRT	RESP
1	a	25.38
2	a	43.94
3	a	4.16
4	a	16.21
5	a	1.12
6	a	2.90
7	a	12.33
8	a	31.02
9	a	29.43
10	a	13.36
11	a	14.88
12	a	18.26
13	a	43.24
14	a	7.80
15	a	55.48
16	a	26.15
17	b	41.38
18	b	23.67
19	b	18.13
20	b	1.03
21	b	29.24
22	b	6.75
23	b	20.98
24	b	27.75
25	b	6.87
26	b	23.57
27	b	34.58
28	b	29.21
29	b	25.72
30	b	32.71
31	b	29.71
32	b	37.44
33	c	7.06
34	c	17.05
35	c	0.30
36	c	8.76
37	c	31.55
38	c	9.62
39	c	29.38
40	c	1.31
41	c	0.90
42	c	3.46
43	c	0.62
44	c	23.54
45	c	27.60
46	c	13.21
47	c	3.40
48	c	1.69
49	d	33.44
50	d	32.06
51	d	10.40
52	d	26.90
53	d	1.76
54	d	22.15
55	d	22.06
56	d	39.71
57	d	0.65
58	d	37.23
59	d	5.21
60	d	30.13
61	d	27.38
62	d	22.29
63	d	14.89

64 d 21.66

SAS 12:22 Monday, August 15, 1994 2

General Linear Models Procedure
Class Level Information

Class	Levels	Values
TRT	4	a b c d

Number of observations in data set = 64

SAS 12:22 Monday, August 15, 1994 3

General Linear Models Procedure

Dependent Variable: RESP

Source	DF	Sum of Squares	F Value	Pr > F
Model	3	1614.64805469	3.28	0.0269
Error	60	9842.94074375		
Corrected Total	63	11457.58879844		

R-Square	C.V.	RESP Mean
0.140924	64.96607	19.71515625

Source	DF	Type I SS	F Value	Pr > F
TRT	3	1614.64805469	3.28	0.0269

Source	DF	Type III SS	F Value	Pr > F
TRT	3	1614.64805469	3.28	0.0269

SAS 12:22 Monday, August 15, 1994 4

General Linear Models Procedure

Duncan's Multiple Range Test for variable: RESP

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

Alpha= 0.05 df= 60 MSE= 164.049

Number of Means 2 3 4
Critical Range 9.064 9.531 9.838

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	TRT
A	24.296	16	b
A			

A	21.745	16	d
A			
A	21.604	16	a
B	11.216	16	c

SAS 12:22 Monday, August 15, 1994
5

General Linear Models Procedure

Dunnett's T tests for variable: RESP

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

Alpha= 0.05 Confidence= 0.95 df= 60 MSE= 164.049
Critical Value of Dunnett's T= 2.410
Minimum Significant Difference= 10.913

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

TRT Comparison	Simultaneous Lower Confidence Limit	Difference Between Means	Simultaneous Upper Confidence Limit
b - a	-8.221	2.692	13.606
d - a	-10.772	0.141	11.054
c - a	-21.301	-10.388	0.525

SAS 12:22 Monday, August 15, 1994
6

General Linear Models Procedure

Dunnett's One-tailed T tests for variable: RESP

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

Alpha= 0.05 Confidence= 0.95 df= 60 MSE= 164.049
Critical Value of Dunnett's T= 2.104
Minimum Significant Difference= 9.5273

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

TRT Comparison	Simultaneous Lower Confidence Limit	Difference Between Means	Simultaneous Upper Confidence Limit	
b - a	-6.835	2.692	12.220	
d - a	-9.386	0.141	9.669	
c - a	-19.915	-10.388	-0.861	***

SAS 12:22 Monday, August 15, 1994
7

General Linear Models Procedure

Dunnett's One-tailed T tests for variable: RESP

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

Alpha= 0.05 Confidence= 0.95 df= 60 MSE= 164.049
Critical Value of Dunnett's T= 2.104
Minimum Significant Difference= 9.5273

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

TRT Comparison	Simultaneous Lower Confidence Limit	Difference Between Means	Simultaneous Upper Confidence Limit
b - a	-6.835	2.692	12.220
d - a	-9.386	0.141	9.669
c - a	-19.915	-10.388	-0.861

mclard

SAS 5
8:11 Wednesday, August 10, 1994

OBS	TRT	RESP
1	a	3.17
2	a	0.73
3	a	0.77
4	a	1.20
5	a	0.71
6	a	0.42
7	a	0.88
8	a	43.46
9	a	0.78
10	a	57.50
11	a	0.86
12	a	0.53
13	a	45.95
14	a	0.99
15	a	43.88
16	a	46.22
17	b	75.56
18	b	48.09
19	b	0.51
20	b	1.43
21	b	48.54
22	b	1.05
23	b	37.47
24	b	39.73
25	b	42.65
26	b	1.92
27	b	45.47
28	b	4.18
29	b	37.86
30	b	0.60
31	b	50.13
32	b	32.66
33	c	0.93
34	c	0.85
35	c	2.38
36	c	0.75
37	c	0.35
38	c	0.93
39	c	100.51
40	c	53.58
41	c	26.76
42	c	0.60
43	c	0.51
44	c	52.32
45	c	40.63
46	c	43.44
47	c	0.62
48	c	1.11
49	d	12.81
50	d	3.28
51	d	1.62
52	d	67.38
53	d	.
54	d	59.48
55	d	3.81
56	d	0.61
57	d	1.91
58	d	1.54
59	d	2.77
60	d	4.02
61	d	305.11
62	d	6.52
63	d	5.35

55

64	d	49.90

SAS 6
8:11 Wednesday, August 10, 1994

General Linear Models Procedure
Class Level Information

Class	Levels	Values
TRT	4	a b c d

Number of observations in data set = 64

NOTE: Due to missing values, only 63 observations can be used in this analysis.

SAS 7
8:11 Wednesday, August 10, 1994

General Linear Models Procedure

Dependent Variable: RESP

Source	DF	Sum of Squares	F Value	Pr > F
Model	3	3592.09895875	0.61	0.6083
Error	59	114966.97384125		
Corrected Total	62	118559.07280000		

R-Square	C.V.	RESP Mean
0.030298	177.3281	24.89333333

Source	DF	Type I SS	F Value	Pr > F
TRT	3	3592.09895875	0.61	0.6083

Source	DF	Type III SS	F Value	Pr > F
TRT	3	3592.09895875	0.61	0.6083

SAS 8
8:11 Wednesday, August 10, 1994

General Linear Models Procedure

Duncan's Multiple Range Test for variable: RESP

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

Alpha= 0.05 df= 59 MSE= 1948.593
WARNING: Cell sizes are not equal.
Harmonic Mean of cell sizes= 15.7377

Number of Means	2	3	4
Critical Range	31.51	33.13	34.20

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	TRT
A	35.07	15	d
A			
A	29.24	16	b
A			
A	20.39	16	c
A			
A	15.50	16	a

General Linear Models Procedure

Dunnett's T tests for variable: RESP

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

Alpha= 0.05 Confidence= 0.95 df= 59 MSE= 1948.593
Critical Value of Dunnett's T= 2.412

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

TRT Comparison	Simultaneous Lower Confidence Limit	Difference Between Means	Simultaneous Upper Confidence Limit
d - a	-18.70	19.57	57.84
b - a	-23.91	13.74	51.38
c - a	-32.75	4.89	42.53

General Linear Models Procedure

Dunnett's One-tailed T tests for variable: RESP

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

Alpha= 0.05 Confidence= 0.95 df= 59 MSE= 1948.593
Critical Value of Dunnett's T= 2.106

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

TRT Comparison	Simultaneous Lower Confidence Limit	Difference Between Means	Simultaneous Upper Confidence Limit
d - a	-13.84	19.57	52.98
b - a	-19.13	13.74	46.61
c - a	-27.98	4.89	37.76

95

General Linear Models Procedure

Dunnett's One-tailed T tests for variable: RESP

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

Alpha= 0.05 Confidence= 0.95 df= 59 MSE= 1948.593
Critical Value of Dunnett's T= 2.106

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

TRT Comparison	Simultaneous Lower Confidence Limit	Difference Between Means	Simultaneous Upper Confidence Limit
d - a	-13.84	19.57	52.98
b - a	-19.13	13.74	46.61
c - a	-27.98	4.89	37.76

MRID No.: 43212702

DATA EVALUATION RECORD

1. CHEMICAL: Triphenyltin Hydroxide (TPTH)

2. TEST MATERIAL: 97.23% TGAI, white powder

3. STUDY TYPE: §72-3 Estuarine Fish 96-hour Acute Toxicity Test.

4. CITATION:

Author: Mark W. Machado
Title: Triphenyltin Hydroxide (TPTH)-Acute Toxicity to Sheephead Minnow (*Cyprinodon variegatus*) under Flow-Through Conditions
Date: March 15, 1994
Laboratory Report #: 93-10-4952
Any Other Study #: 11117.0593.6102.505
Sponsor: Griffin Corp.
Sponsor #: N/A
Laboratory: Springborn Laboratories, Inc.
MRID No.: 43212702

5. REVIEWED BY:

Dennis J. McLane, Wildlife Biologist Signature: Dennis McLane
Ecological Effects Branch
Environmental Fate and Effects Division (7507 C) Date: 9-14-94

6. APPROVED BY:

Les Touart, Chief, Section 1 Signature:
Ecological Effects Branch
Environmental Fate and Effects Division (7507C) Date: 9/15/94

7. CONCLUSION
This study fulfills the guideline requirements for an acute toxicity test using sheepshead minnows. Under the conditions of the test, the 96-hour LC_{50} was 25.5 (21-33) µg ai/L, which classifies TPTH as very highly toxic to sheepshead minnows.

8. RECOMMENDATIONS N/A

9. BACKGROUND Submitted in response to list A process.

10. MATERIALS AND METHODS

A. Test Organisms: Sheepshead minnow

Guideline Criteria	Reported Information
Species (Scientific Name)	*Cyprinodon variegatus*
Mean Weight (0.5-5 grams)	0.28 (0.20-0.34) grams
Mean Length(S.L. longest not > 2x shortest	26 (22-32) mm
Supplier	Aquatic Biosystems
All fish from same source (yes or no)	yes
All fish from the same year class (yes or no)	Not reported
Other Comments	

B. Source/Acclimation

Guideline Criteria	Reported Information
Acclimation Period (minimum 14 days)	14 days
Wild caught 7 day quarantine (yes or no)	no
Check for signs of disease or injury (yes or no, if yes describe)	no, only checked for mortality
If diseased it can be treated in 48-hr pretest no sign of the disease remains (Report hours prior to test in which no sign of disease or N/A)	no mortality in fish 48 hours prior to start of the test.
No feeding during the study (When last fed)	48 hours prior to testing.
<3% mortality 48 hours prior to testing (% mortality, if any)	0% mortality prior to testing.

C. Test System:

Guideline Criteria	Reported Information
Describe source of dilution water (prefer soft reconstituted water)	Collected from Cape Cod Canal, Bourne, MA.Seawater was then passed through a series of polypropylene core filters & then recirculated within an epoxy-lined concrete reservoir prior to use.
Does water support test animals without observable signs of stress?	yes
Salinity of water used. (reconstituted seawater of 30-34% salinity) (weekly range of salinity is less than 6%)	31-32‰
Water Temperature (22 ± 1)	22 ± 1
pH (8.0-8.3 for marine-stenohaline fish and 7.7-8.0 for estuarine-euryhaline fish species) (monthly range is less than 0.8 of a pH unit)	7.7-8.0
Dissolved Oxygen (Static 1st 48 hrs 40%; 2nd 48 hrs 60%; Flow-through 60%) (% of lowest conc. & hour)	>71% in all test and controls vessels over 96 hours of testing.
Test Aquaria 1. Material (glass or stainless steel) 2. a. Static volume (18.9 L (5 gal or 19000 cc) with 15 L solution) b. Static or flow-through volume (300x600x300 = 54000 cc.)	1. each glass test aquaria and silicone sealant a. N/A b. measured 39X20X25 cm with a 14.5 cm standpipe to maintain a volume of 11 L.
Type of Dilution System (Reproducible supply of toxicant)	flow-through, reproducible.

Flow rate 1. Consistent flow rate-meter systems calibrated before study 2. Checked 2*24 hours - 5 to 10 vol/24 hours	1. Tested and verified 6 days and 2 days prior to start of the test. 2. Monitored daily and checked twice visually each day. Flow of exposure to each test aquarium was approx. 50 ml/min, which equaled approx. 6.5 volume replacements per 24 hours per aquarium.
Biomass Loading Rate (Static no > 0.8 g/L ≤ 17°C; >17°C 0.5g/L; Flow-through 1 g/L/24	0.039 g of biomass/L of flowing test solution per day.
Photoperiod (16 L & 8 D)	16 hours light, 8 hours dark.
Solvents 1. (Do not exceed 0.5 ml/L for static tests) 2. (Do not exceed 0.1 ml/L for flow-through)	1.N/A 2. 0.092 ml/L
Other Comments	

D. Test Design:

Guideline Criteria	Reported Information
Range Finding Test (LC_{50} >100 mg/L with 30 fish, no definitive test required.)	13, 36, 100µg a.i./L 100% mortality @ 36 and 100 µg/L levels and no effects seen @ the 13 µg/L level.
Definitive Test	
Nominal Concentrations (control+5 treatment levels; dosage should be 60% of the next highest concentration; concentrations should be geometric series)	13, 22, 36, 60, 100 µg ai/L + control and solvent control. Dosage levels were 60% of the next highest level.
Controls (Minimum control mortality; static 10%; flow-through 5%	0% mortality observed in the solvent control and control.

Number of Test Organisms; (Minimum 10/level can be divided among containers)	20 fish (10 per test aquaria) per concentration and controls were used. 140 fish total.
All organisms must be randomly assigned to test vessels. (yes or no, describe if no)	impartially selected.
Biological Observations (yes or no)	yes, at test initiation and every 24 hours.
Water Parameter Measurements 1. Temperature - record every 6 hrs;>1°C. 2. D.O. beginning,48 hrs,end for control high, medium, and low dose. 3. pH beginning,48 hrs, end for control, high, medium, and low dose.	recorded continuously in one control aquaria. pH, temperature, and DO were measured in each replicate vessel once daily throughout exposure period.
Chemical Analysis (needed if aeration, volatile, insoluble, precipitate, not steel or glass, known to adsorb, and flow-through) (yes or no)	yes, sample from each replicate solution of high, medium, and low treatment levels and dilution water control were taken twice prior to definitive test. In addition, water samples were taken both replicate test solutions of each treatment level and the controls at 0-hour and 96 -hours of exposure for analysis.
Other Comments	

11. REPORTED RESULTS:

Guideline Criteria	Reported Information
Mean Measured Concentrations (report conc.)	16, 21, 33, 61, 110 µg ai/L
Recovery of Chemical (% recovery)	104% of nominal concentrations
Mortality & Observations (Describe observations & attach mortality tables)	See attached Table 3.

Author's Comments	

12. STUDY AUTHOR'S CONCLUSIONS / QUALITY ASSURANCE MEASURES:

Author provided no conclusions. Quality assurance unit statement was provided and signed by the Patricia D. Royal the Regulatory Affairs and Quality Assurance Manager. Also a good laboratory practice compliance statement was include with signature from the Study director, Sponsor study monitor, and Applicant/Submitter.

13. REVIEWER'S DISCUSSION AND INTERPRETATION

A. Test Procedure:

The following items did not meet the guideline criteria:

1. Study fish weighed less than what is generally recommended (0.12-0.56), should be between 0.5-5.0 grams.

2. Small amount of precipitate was observed in the mixing chamber of diluter system, however analyticl data indicates the test concentrations were maintained to be 81% on nominal.

B. Statistical Analysis

Guideline Criteria	Reported Information
Binomial (yes, no, or not reported)	yes, 96-hour LC_{50} = 26 (C.I. 21 - 33) µg ai/L
Moving Average Angle (yes, no, or not reported)	no
Probit (yes, no, or not reported)	no
Other Comments	The EEB Toxanal program indicates that the binomial is correct 25.55 (21-33) µg/L. (See the attached printout)

C. Discussion/Results:

This study is scientifically sound and fulfills the guideline requirements for an acute toxicity test using sheepshead

minnows. Under the conditions of the test, the 96-hour LC_{50} was 25.5 µg ai/L, which classifies TPTH as very highly toxic to sheepshead minnows.

D. Adequacy of the Study:

1. Classification: Core

2. Rational: Fulfills guideline requirements.

3. Reparability: N/A

14. COMPLETION DATE OF ONE-LINER FOR STUDY:

MRID No:43212703

DATA EVALUATION RECORD

1. CHEMICAL: Triphenyltin Hydroxide (TPTH)

2. TEST MATERIAL: 97.23 TGAI, white powder, Lot # RFRAM909K

3. STUDY TYPE: §72-3 (b) Acute Estuarine/Marine Toxicity Mollusk

4. CITATION:

Author:	Emily Dionne
Title:	Triphenyltin Hydroxide (TPTH) - Acute Toxicity to Eastern Oyster (*Crassostrea virginica*) under Flow-through Conditions.
Date:	November 4, 1994
Laboratory Report #:	93-11-5057
Any Other Study #:	11117.0593.6104.504
Sponsor:	Griffin Corp.
Sponsor #:	N/A
Laboratory:	Springborn Laboratories, Inc.
MRID No.:	43212703

5. REVIEWED BY:

Dennis J. McLane, Wildlife Biologist Signature: Dennis J McLane
Ecological Effects Branch
Environmental Fate and Effects Division (7507 C) Date: 9-12-94

6. APPROVED BY:

Les Touart, Chief, Section 1 Signature:
Ecological Effects Branch
Environmental Fate and Effects Division (7507C) Date: 9-12-94

7. CONCLUSION: This test is scientifically sound but the control oysters did not grow the minimum 2 mm of shell necessary to demonstrate acceptable test conditions. Better performance of the controls would result in a more definitive EC_{50}. Based on this, the study does not fulfill the guideline requirements for an acute toxicity test using the eastern oyster. Under the conditions of the test, the 96-hour EC_{50} was 0.36 µg/L which classifies TPTH as very highly toxic (<100 µg/L) to eastern oysters.

8. RECOMMENDATIONS Provide another study.

9. BACKGROUND Submitted in response to the list A process.

64

A. Test Organisms: Eastern Oysters

Guideline Criteria	Reported Information
Species (Scientific Name)	*Crassostrea virginica*
Mean valve height (25 - 50 mm -- the long axis)	33±4 mm
Supplier	P. Cummins Oyster Company, Pasadena, MD
All oysters from same source (yes or no)	yes
All oysters from the same year class (yes or no)	similar age
Other Comments	

B. Source/Acclimation

Guideline Criteria	Reported Information
Acclimation Period (minimum 10 days)	28 days
Wild caught 7 day quarantine (yes or no)	no
Check for signs of disease or injury (yes or no, if yes describe)	yes, checked parasites, determined to be reproductively mature, and checked for mortality
If diseased it can be treated in 48-hr pretest no sign of the disease remains (Report hours prior to test in which no sign of disease or N/A)	N/A
Was peripheral shell growth removed prior to testing? If so how much.	yes, 3-5 mm
Feeding during the acclimation	Fed a supplemental algal diet of Isochrysis galbana Parke, clone T-ISO and Tetraselmis maculata.

<3% mortality 48 hours prior to testing (% mortality, if any)	yes; <1% mortality

C. Test System:

Guideline Criteria	Reported Information
Describe source of dilution water (natural unfiltered seawater)	Natural unfiltered seawater from Cape Cod Canal, Bourne, MA
Does water support test animals without observable signs of stress?	yes
What was the salinity of the test water?	31-32‰ ppt
Water Temperature (between 15°C and 30°C -- but must be consistent)	21-22°C
pH	7.5-7.8
Dissolved Oxygen (Static 1st 48 hrs 40%; 2nd 48 hrs 60%; Flow-through 60%) (% of lowest conc. & hour)	72 hour 0.36 µg/L Replicate A D.O.dropped to 51%; Replicate B was 65%; and the 96 hour 0.36 µg/L D.O. levels were 68 and 64%, respectively. All other levels were also adequate. (see attached Table 1)
Total Organic Carbon	3.0 mg/L
Test Aquaria 1. Material (glass or stainless steel) 2. a. Static volume (18.9 L (5 gal or 19000 cc) with 15 L solution) b. Static or flow-through volume (300x600x300 = 54000 cc.)	 1. glass 2. 11 L 3. 39.5 X 20.5 X 26 cm 13.5 standpipe maintaining a volume of 11 L
Type of Dilution System (Reproducible supply of toxicant)	A constant-flow serial diluter (Benoit *et al.* 1982)

68

Flow rate Consistent flow rate-meter systems calibrated before study and checked 2*24 hours - 5 to 10 vol/24 hours	6 solution volume replacements per 24 hours
Biomass Loading Rate (all oysters should be able to sit on the bottom with water flowing freely around them)	yes
Photoperiod (16 L & 8 D)	16 hours light and 8 hours dark
Solvents (Do not exceed 0.5 ml/L for flow-through)	0.5 ml/L acetone
Other Comments	

D. Test Design:

Guideline Criteria	Reported Information
Range Finding Test (LC_{50} >100 mg/L with 30 shrimp, no definitive test required.)	tested nominal concentrations of 0.41 to 50 µg/L. After 96-hours growth reduction of 37-87% was observed in oysters in 0.13 and 1.0 µg ai/L.
Definitive Test	
Nominal Concentrations (control+5 treatment levels; dosage should be 60% of the next highest concentration; concentrations should be geometric series)	0.13, 0.22, 0.36, 0.60, 1.0 µg ai/L
Controls (Minimum control mortality; static 10%; flow-through 5%	no mortality observed
Number of Test Organisms; (Minimum 10/level can be divided among containers)	15 oysters in each test aquarium (30 per treatment level)
All organisms must be randomly assigned to test vessels. (yes or no, describe if no)	"impartially selected"

Biological Observations (yes or no)	yes, every 24 hours
Water Parameter Measurements 1. Temperature - record every 6 hrs;>1°C. 2. D.O. beginning,48 hrs,end for control high, medium, and low dose. 3. pH beginning,48 hrs, end for control, high, medium, and low dose.	21-22°C See Table 1 See Table 1
Chemical Analysis (needed if aeration, volatile, insoluble, precipitate, not steel or glass, known to adsorb, and flow-through) (yes or no)	No precipitate observed
Other Comments	

11. REPORTED RESULTS:

Guideline Criteria	Reported Information
Mean Measured Concentrations (report conc.)	0.14, 0.24, 0.30, 0.46, and 0.77 µg ai/L
Recovery of Chemical (% recovery)	0.14 - 110%, 0.24-110%, 0.30 - 83%, 0.46-76%, and 0.77-77% µg ai/L
Mortality & Observations (Describe observations & Minimum shell growth is 2 mm.)	No mortality at any treatment level; "The growth of dilution water control organisms during this study fell below this minimum:" 2 mm is the minimum and they achieve only 1.3.
Measurements of shell increments per control and test concentration.	See Table 3
Ratio of mean growth of test concentration to mean growth of controls. (provides percentage index of the response of the mollusks to toxicant)	See Table 4

EC_{50} = reduced shell deposition by 50% compared to the controls	EC_{50} = µg/L
Author's Comments	

12. STUDY AUTHOR'S CONCLUSIONS / QUALITY ASSURANCE MEASURES:

Based on the test data, the 96-hour EC_{50} was calculated by linear regression to be µg ai/L (95% CI of to µg ai/L). Based on these results and criteria established be the U.S. Environmental Protection Agency (1985), TPTH Technical would be classified as moderately toxic to *Crassostrea virginica*.

Quality assurance and good laboratory practice statements were included in the report, indicating that the study was conducted in accordance with U.S. EPA Good Laboratory Practices Regulations set forth in FIFRA 40 CFR Part 160.

13. REVIEWER'S DISCUSSION AND INTERPRETATION

A. Test Procedure:

The following items did not meet the guideline criteria:

1. The controls failed to meet the minimum required growth of 2 mm.
2. The study did not report the goodness of fit information for their statistical analysis.

B. Statistical Analysis

Guideline Criteria	Reported Information
Binomial (yes, no, or not reported)	N/A
Moving Average Angle (yes, no, or not reported)	N/A
Probit (yes, no, or not reported)	yes, 0.32 (0.11-0.92) µg a.i./L
Williams Test	no

Comments:	The EEB's probit method(EC_{50} .34 (0.27-0.44) and EC_{10} 0.062 µg/L) (toxanal) and Farrar's method (EC_{50} .025 (0.14-0.43) µg/L and EC_{10} 0.018 µg/L) did not meet the goodness of fit criteria. Therefore, the moving average angle is more appropriate. The moving average angle EC_{50} is 0.36 (0.34-.38)µg/L and. The submitted EC_{50} 0.32 (0.11-0.92) µg/L and the EC_{10} is 0.64. However the report did not provide the goodness of fit information for their analysis.

C. Discussion/Results:

The control oysters did not grow the minimum 2 mm of shell. Poor performance of the controls may have resulted in a higher EEC. Based on this, the study does not fulfills the guideline requirements for an acute toxicity test using the eastern oyster. Under the conditions of the test, the 96-hour EC_{50} was 0.36 µg/L which classifies TPTH as very highly toxic (<100 µg/L) to eastern oysters.

D. Adequacy of the Study:

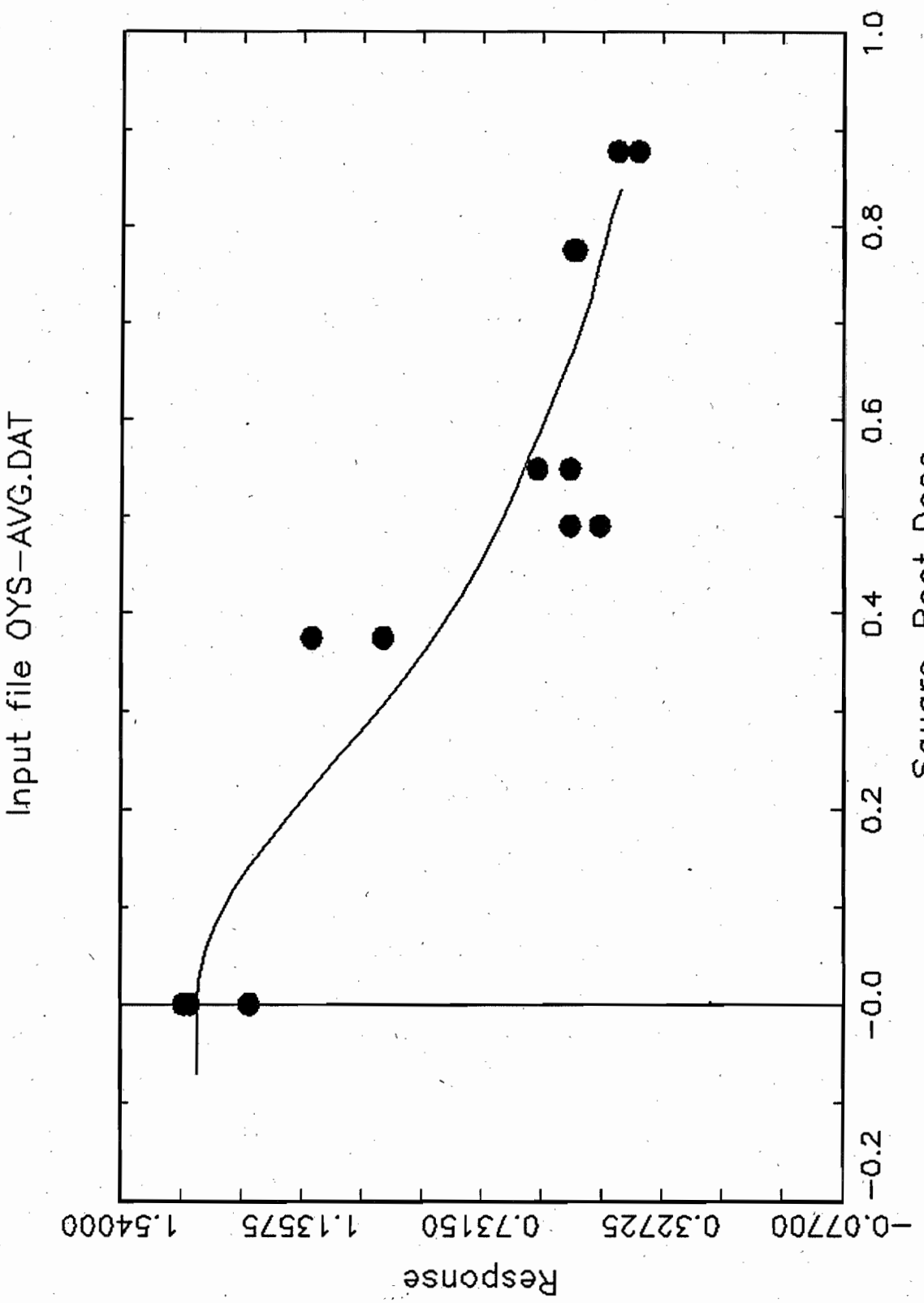
1. Classification: Supplemental

2. Rational: The control oyster failed to grow 2 mm which is considered necessary to demonstrate acceptable test conditions.

3. Reparability: No

14. COMPLETION DATE OF ONE-LINER FOR STUDY: 8/25/94

Measured Concentrations



Input file OYS-AVG.DAT

Raw data:

0	1.25333333	1.40000000	1.40000000	1.38666667
0.14	1.11333333	0.95333333	. .	
0.24	0.53333333	0.46666667	. .	
0.3	0.53333333	0.60666667	. .	
0.6	0.52000000	0.52666667	. .	
0.77	0.38000000	0.42666667	. .	

Williams Test

[One-Sided Test for Decrease]

Dose	Isotone Means	T-bar	P-value	Significance
0	1.36			
0.14	1.03	5.777	<0.005	*
0.24	0.535	14.59	<0.005	*
0.3	0.535	14.59	<0.005	*
0.6	0.523	14.8	<0.005	*
0.77	0.403	16.92	<0.005	*

"*"=Significant (p L/E 0.05) "N.S."=Not Sigificant.

Estimates of EC%

Parameter	Estimate	95% Bounds Lower	95% Bounds Upper	Std.Err.	Lower Bound /Estimate
EC10	0.018	0.0029	0.11	0.36	0.16
EC25	0.063	0.019	0.20	0.23	0.31
EC50	0.25	0.14	0.43	0.11	0.57

Slope = 1.13 Std.Err. = 0.287

Observed vs. Predicted Treatment Group Means

Dose	#Reps.	Obs. Mean	Pred. Mean	Obs. -Pred.	Pred. %Control	%Change
0.00	4.00	1.36	1.37	-0.00865	100.	0.00
0.140	2.00	1.03	0.837	0.197	61.1	38.9
0.240	2.00	0.500	0.694	-0.194	50.7	49.3
0.300	2.00	0.570	0.634	-0.0643	46.3	53.7
0.600	2.00	0.523	0.455	0.0679	33.3	66.7
0.770	2.00	0.403	0.396	0.00712	28.9	71.1

!!!Warning: EC10 not bracketed by doses evaluated.

!!!Warning: EC25 not bracketed by doses evaluated.

Analysis of Weighted Variance

Source	SS	DF	MS
Residual	0.27147	11.000	0.024679
Means Lack-of-Fit	0.23476	3.0000	0.078254
Within Groups	0.036706	8.0000	0.0045883

!!!Poor fit: p < 0.001 based on DF= 3.0000 8.0000

Directly-fitted parameters and Asymptotic Covariances

Parameter		Estimate	Std.Err.	Correlations		
Control Mean	=	1.3687	0.091837	1.0000	-0.69560	0.22465
log10 EC50	=	-0.60391	0.10990	-0.69560	1.0000	-0.56061
Bruce&V. SIGMA	=	0.88382	0.22455	0.22465	-0.56061	1.0000
Starting Values		1.3600	-0.69999	1.1235		

MCLANE TPTH SHELL DEPOSITION EC50

****

CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
.77	1000	741	74.1	0
.46	1000	617	61.7	0
.3	1000	409	40.9	0
.24	1000	375	37.5	0
.14	1000	289	28.9	0

THE BINOMIAL TEST SHOWS THAT .3 AND .46 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 .3615492

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS	
3	1.353148E-02	.3772194	.3592427	.3416722

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
2	.156681	8.045513	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 = 1.719909
95 PERCENT CONFIDENCE LIMITS = 1.039118 AND 2.4007

LC50 = .3397097
95 PERCENT CONFIDENCE LIMITS = .2688117 AND .4364069

LC10 = 6.204358E-02
95 PERCENT CONFIDENCE LIMITS = 2.021041E-02 AND .1029348

****

TITLE: TPTH shell deposition
FILE: a:\tpth\toxshell
TRANSFORM: NO TRANSFORM NUMBER OF GROUPS: 7

GRP	IDENTIFICATION	REP	VALUE	TRANS VALUE
1	control	1	1.8000	1.8000
1	control	2	0.9000	0.9000
1	control	3	1.7000	1.7000
1	control	4	1.1000	1.1000
1	control	5	1.5000	1.5000
1	control	6	0.6000	0.6000
1	control	7	0.6000	0.6000
1	control	8	2.1000	2.1000
1	control	9	1.5000	1.5000
1	control	10	1.2000	1.2000
1	control	11	0.6000	0.6000
1	control	12	0.9000	0.9000
1	control	13	2.9000	2.9000
1	control	14	0.6000	0.6000
1	control	15	0.8000	0.8000
1	control	16	1.5000	1.5000
1	control	17	3.1000	3.1000
1	control	18	1.0000	1.0000
1	control	19	1.7000	1.7000
1	control	20	1.8000	1.8000
1	control	21	1.0000	1.0000
1	control	22	1.1000	1.1000
1	control	23	2.2000	2.2000
1	control	24	0.6000	0.6000
1	control	25	0.9000	0.9000
1	control	26	1.0000	1.0000
1	control	27	1.8000	1.8000
1	control	28	0.4000	0.4000
1	control	29	0.8000	0.8000
1	control	30	2.1000	2.1000
2	S.control	1	2.7000	2.7000
2	S.control	2	1.1000	1.1000
2	S.control	3	1.3000	1.3000
2	S.control	4	0.3000	0.3000
2	S.control	5	2.0000	2.0000
2	S.control	6	0.5000	0.5000
2	S.control	7	1.9000	1.9000
2	S.control	8	0.9000	0.9000
2	S.control	9	1.4000	1.4000
2	S.control	10	0.9000	0.9000
2	S.control	11	1.1000	1.1000
2	S.control	12	0.3000	0.3000
2	S.control	13	1.5000	1.5000
2	S.control	14	3.8000	3.8000
2	S.control	15	1.3000	1.3000
2	S.control	16	0.2000	0.2000
2	S.control	17	0.9000	0.9000
2	S.control	18	1.9000	1.9000
2	S.control	19	2.1000	2.1000

2	S.control	20	1.6000	1.6000
2	S.control	21	1.6000	1.6000
2	S.control	22	0.8000	0.8000
2	S.control	23	1.5000	1.5000
2	S.control	24	1.4000	1.4000
2	S.control	25	1.8000	1.8000
2	S.control	26	0.4000	0.4000
2	S.control	27	2.0000	2.0000
2	S.control	28	0.4000	0.4000
2	S.control	29	0.3000	0.3000
2	S.control	30	3.9000	3.9000
3	.14	1	1.5000	1.5000
3	.14	2	1.1000	1.1000
3	.14	3	1.2000	1.2000
3	.14	4	1.4000	1.4000
3	.14	5	1.8000	1.8000
3	.14	6	0.6000	0.6000
3	.14	7	0.9000	0.9000
3	.14	8	1.1000	1.1000
3	.14	9	0.6000	0.6000
3	.14	10	1.3000	1.3000
3	.14	11	0.9000	0.9000
3	.14	12	1.4000	1.4000
3	.14	13	1.6000	1.6000
3	.14	14	1.0000	1.0000
3	.14	15	0.3000	0.3000
3	.14	16	1.5000	1.5000
3	.14	17	0.6000	0.6000
3	.14	18	0.8000	0.8000
3	.14	19	0.8000	0.8000
3	.14	20	0.4000	0.4000
3	.14	21	0.9000	0.9000
3	.14	22	1.6000	1.6000
3	.14	23	1.8000	1.8000
3	.14	24	1.0000	1.0000
3	.14	25	1.2000	1.2000
3	.14	26	1.0000	1.0000
3	.14	27	0.0000	0.0000
3	.14	28	0.5000	0.5000
3	.14	29	1.9000	1.9000
3	.14	30	0.3000	0.3000
4	.24	1	1.0000	1.0000
4	.24	2	1.1000	1.1000
4	.24	3	0.3000	0.3000
4	.24	4	1.2000	1.2000
4	.24	5	1.1000	1.1000
4	.24	6	2.4000	2.4000
4	.24	7	0.3000	0.3000
4	.24	8	1.6000	1.6000
4	.24	9	0.9000	0.9000
4	.24	10	0.0000	0.0000
4	.24	11	0.2000	0.2000
4	.24	12	0.0000	0.0000
4	.24	13	0.7000	0.7000
4	.24	14	0.5000	0.5000
4	.24	15	1.3000	1.3000
4	.24	16	2.0000	2.0000
4	.24	17	2.3000	2.3000
4	.24	18	0.3000	0.3000
4	.24	19	1.1000	1.1000

4	.24	20	0.6000	0.6000
4	.24	21	0.5000	0.5000
4	.24	22	0.9000	0.9000
4	.24	23	0.4000	0.4000
4	.24	24	0.6000	0.6000
4	.24	25	0.4000	0.4000
4	.24	26	0.3000	0.3000
4	.24	27	1.9000	1.9000
4	.24	28	0.4000	0.4000
4	.24	29	0.2000	0.2000
4	.24	30	1.3000	1.3000
5	.3	1	0.9000	0.9000
5	.3	2	0.0000	0.0000
5	.3	3	0.0000	0.0000
5	.3	4	0.0000	0.0000
5	.3	5	1.2000	1.2000
5	.3	6	1.2000	1.2000
5	.3	7	0.9000	0.9000
5	.3	8	0.5000	0.5000
5	.3	9	0.4000	0.4000
5	.3	10	0.0000	0.0000
5	.3	11	0.0000	0.0000
5	.3	12	0.0000	0.0000
5	.3	13	1.4000	1.4000
5	.3	14	0.8000	0.8000
5	.3	15	0.7000	0.7000
5	.3	16	1.8000	1.8000
5	.3	17	0.6000	0.6000
5	.3	18	0.7000	0.7000
5	.3	19	0.0000	0.0000
5	.3	20	0.0000	0.0000
5	.3	21	0.6000	0.6000
5	.3	22	0.0000	0.0000
5	.3	23	0.3000	0.3000
5	.3	24	1.1000	1.1000
5	.3	25	0.0000	0.0000
5	.3	26	0.6000	0.6000
5	.3	27	1.2000	1.2000
5	.3	28	0.2000	0.2000
5	.3	29	0.4000	0.4000
5	.3	30	1.6000	1.6000
6	.46	1	0.4000	0.4000
6	.46	2	0.7000	0.7000
6	.46	3	1.0000	1.0000
6	.46	4	1.1000	1.1000
6	.46	5	1.0000	1.0000
6	.46	6	0.0000	0.0000
6	.46	7	0.9000	0.9000
6	.46	8	0.0000	0.0000
6	.46	9	0.6000	0.6000
6	.46	10	0.3000	0.3000
6	.46	11	1.0000	1.0000
6	.46	12	0.0000	0.0000
6	.46	13	0.0000	0.0000
6	.46	14	0.8000	0.8000
6	.46	15	0.0000	0.0000
6	.46	16	0.4000	0.4000
6	.46	17	0.7000	0.7000
6	.46	18	1.9000	1.9000
6	.46	19	0.2000	0.2000

6	.46	20	1.1000	1.1000
6	.46	21	0.0000	0.0000
6	.46	22	0.4000	0.4000
6	.46	23	0.6000	0.6000
6	.46	24	0.0000	0.0000
6	.46	25	0.0000	0.0000
6	.46	26	0.6000	0.6000
6	.46	27	0.8000	0.8000
6	.46	28	0.0000	0.0000
6	.46	29	0.7000	0.7000
6	.46	30	0.5000	0.5000
7	.77	1	0.1000	0.1000
7	.77	2	0.0000	0.0000
7	.77	3	0.7000	0.7000
7	.77	4	0.4000	0.4000
7	.77	5	0.0000	0.0000
7	.77	6	0.0000	0.0000
7	.77	7	0.5000	0.5000
7	.77	8	0.0000	0.0000
7	.77	9	0.7000	0.7000
7	.77	10	0.0000	0.0000
7	.77	11	0.5000	0.5000
7	.77	12	1.1000	1.1000
7	.77	13	0.6000	0.6000
7	.77	14	0.2000	0.2000
7	.77	15	0.9000	0.9000
7	.77	16	0.7000	0.7000
7	.77	17	0.0000	0.0000
7	.77	18	0.7000	0.7000
7	.77	19	0.5000	0.5000
7	.77	20	1.3000	1.3000
7	.77	21	0.3000	0.3000
7	.77	22	0.8000	0.8000
7	.77	23	0.8000	0.8000
7	.77	24	0.6000	0.6000
7	.77	25	0.3000	0.3000
7	.77	26	0.0000	0.0000
7	.77	27	0.0000	0.0000
7	.77	28	0.0000	0.0000
7	.77	29	0.0000	0.0000
7	.77	30	0.4000	0.4000

TPTH shell deposition
File: a:\tpth\toxshell Transform: NO TRANSFORM

SUMMARY STATISTICS ON TRANSFORMED DATA TABLE 1 of 2

GRP	IDENTIFICATION	N	MIN	MAX	MEAN	
1	control	30	0.400	3.100	1.327	1.3
2	S.control	30	0.200	3.900	1.393	1.4
3	.14	30	0.000	1.900	1.033	1.0
4	.24	30	0.000	2.400	0.860	0.9
5	.3	30	0.000	1.800	0.570	0.6
6	.46	30	0.000	1.900	0.523	0.5
7	.77	30	0.000	1.300	0.403	0.4

TPTH shell deposition
File: a:\tpth\toxshell Transform: NO TRANSFORM

SUMMARY STATISTICS ON TRANSFORMED DATA TABLE 2 of 2

GRP	IDENTIFICATION	VARIANCE	SD	SEM
1	control	0.471	0.686	0.125
2	S.control	0.857	0.926	0.169
3	.14	0.240	0.490	0.089
4	.24	0.439	0.663	0.121
5	.3	0.295	0.543	0.099
6	.46	0.218	0.467	0.085
7	.77	0.141	0.376	0.069

TPTH shell deposition
File: a:\tpth\toxshell Transform: NO TRANSFORM

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	6	28.113	4.685	12.329
Within (Error)	203	77.182	0.380	
Total	209	105.295		

Critical F value = 2.18 (0.05,6,120)
Since F > Critical F REJECT Ho:All groups equal

TPTH shell deposition
File: a:\tpth\toxshell Transform: NO TRANSFORM

TUKEY method of multiple comparisons

GROUP	IDENTIFICATION	TRANSFORMED MEAN	ORIGINAL MEAN	GROUP 0 7	0 6	0 5	0 4	0 3	0 1	0 2
7	.77	0.403	0.403	\						
6	.46	0.523	0.523	.	\					
5	.3	0.570	0.570	.	.	\				
4	.24	0.860	0.860	.	.	.	\			
3	.14	1.033	1.033	*	*	.	.	\		
1	control	1.327	1.327	*	*	*	.	.	\	
2	S.control	1.393	1.393	*	*	*	*	.	.	\

* = significant difference (p=0.05) . = no significant difference

Tukey value (7,203) = 4.24 s = 0.380

TPTH shell deposition
File: a:\tpth\toxshell Transform: NO TRANSFORM

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	control	30	1.327	1.327	1.360
2	S.control	30	1.393	1.393	1.360
3	.14	30	1.033	1.033	1.033
4	.24	30	0.860	0.860	0.860
5	.3	30	0.570	0.570	0.570
6	.46	30	0.523	0.523	0.523
7	.77	30	0.403	0.403	0.403

TPTH shell deposition
File: a:\tpth\toxshell Transform: NO TRANSFORM

WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
control	1.360				
S.control	1.360	0.209		1.66	k= 1, v=203
.14	1.033	1.842	*	1.73	k= 2, v=203
.24	0.860	2.931	*	1.75	k= 3, v=203
.3	0.570	4.753	*	1.77	k= 4, v=203
.46	0.523	5.046	*	1.77	k= 5, v=203
.77	0.403	5.800	*	1.78	k= 6, v=203

s = 0.617
Note: df used for table values are approximate when v > 20.

MCALNE TPTH SHEEPSHEAD MINNOW 96 LC50

****

CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
110	20	20	100	9.536742E-05
61	20	20	100	9.536742E-05
33	20	20	100	9.536742E-05
21	20	1	5	2.002716E-03
16	20	0	0	9.536742E-05

THE BINOMIAL TEST SHOWS THAT 21 AND 33 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 25.55475

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.

****

DATA EVALUATION RECORD

1. **CHEMICAL:** Triphenyltin Hydroxide (TPTH)
Shaughnessey #:083601

2. **TEST MATERIAL:** Technical Purity: 96%

3. **CITATION:**

Author:	Jarvinen, A. W., Taner, D. K., Kline, E. R., Knuth, M. L.
Title:	Acute and Chronic Toxicity of Triphenyltin Hydroxide to Fathead Minnows (*Pimephales promelas*)
Date:	1987
Laboratory Report #:	7246A
Any Other Study #:	N/A
Sponsor:	USEPA
Sponsor #:	7246A
Laboratory:	Environmental Research Laboratory - Duluth
MRID No.:	43349601

4. **REVIEWED BY:**

Dennis J. McLane
Wildlife Biologist
Ecological Effects Branch (7507C)
U.S.E.P.A.

Signature: Dennis J. McLane
Date: 9-14-94

5. **APPROVED BY:**

Les W.Touart
Section 1, Chief
Ecological Effects Branch (7507C)
U.S.E.P.A.

Signature: L. Touart
Date: 9-15-94

6. **CONCLUSIONS:** These studies are scientifically sound but do not fulfill the guideline requirements. This article reported the results of an acute static (single pulse) LC_{50} study for the following time periods 12, 24, 48, 72, and 96 hours, and continuous exposure survival and growth study for the following the time periods 24, 48, 72 hours. The following results were excerpted from the study:

> "... 96-hour LC_{50} value for continuously exposed larval fathead minnows was 7.1µg/L and the 96-hour EC_{50} value was 3.7 µg/L." See attached Table 1 for LC_{50} and EC_{50} for a range of exposure periods.
>
> "The concentrations that caused a significant reduction in survival and growth for these exposures were 13.0, 13.0 and 6.0 µg/liter,

respectively. Growth, however, was a more sensitive parameter than survival during the continuous exposure studies. Survival was significantly reduced at 2.0 µg/liter, whereas, a significant reduction in growth occurred at 0.23 µg/l. The weight of the fish at this concentration was 15% lower than that for the control fish. The weight of the fish at the lowest concentration tested (0.15 µg/liter) was 9% lower than that for the control fish but this difference was not statistically significant. Approximately, a 68-h LC_{50} value would be required to cause a significant reduction in fathead minnow survival and growth within 30 days (Fig. 2)."

Based on these values TPTH is very highly toxic.

7. **ADEQUACY OF THE STUDY:**

A. Classification: Supplemental

B. Rationale: Incomplete study report.

C. Reparability: no

8. RECOMMENDATIONS

9. BACKGROUND Provided to EEB by the Environmental Research Laboratory - Duluth with a letter dated October 7, 1987. Subject: Deliverable 7246 A' - Report on toxicity-time relationships for fish exposed to pesticides.

10. MATERIALS AND METHODS

A. Test Organisms:

Guideline Criteria	Reported Information
Species (Scientific Name)	*Pimephales promelas*
Mean Weight (0.5-5 grams)	see Table 2 for flow-through tests; static test weights were not reported.
Mean Length(S.L. longest not > 2x shortest	not reported
Supplier	Environmental Research Laboratory- Duluth
All fish from same source (yes or no)	Yes
All fish from the same year class (yes or no)	<24 hours old

84

Other Comments	

B. Source/Acclimation

Guideline Criteria	Reported Information
Acclimation Period (minimum 14 days)	Not reported
Wild caught 7 day quarantine (yes or no)	no
Check for signs of disease or injury (yes or no, if yes describe)	Yes, the second 30 day study tail rot was seen in some fish. Their weights were omitted: 3 in one of the controls, 1 @ 0.23 µg/L, and 1@0.40 µg/L.
If diseased it can be treated in 48-hr pretest no sign of the disease remains (Report hours prior to test in which no sign of disease or N/A)	n/a
No feeding during the study (When last fed)	Not reported
<3% mortality 48 hours prior to testing (% mortality, if any)	not reported

C. Test System:

Guideline Criteria	Reported Information
Describe source of dilution water (prefer soft reconstituted water)	Sand filtered Lake Superior water sterilized with ultraviolet light
Does water support test animals without observable signs of stress?	Controls show no mortality in 30 day flow-through portion of the test for 24 hours, 48 hours, and 72 hours. for the 30 days control mortality was 16.5%
Was dechlorinated water used (not recommended)	No

Water Temperature (Warm water-17°C or 22°C) (Cold water-12°C)	ranged from 24.2 and 25.3°C
pH	6.8 and 8.0
Dissolved Oxygen (Static 1st 48 hrs 40%; 2nd 48 hrs 60%; Flow-through 60%) (% of lowest conc. & hour)	6.8±0.6 to 7.0±0.8 mg/liter
Total hardness (40 to 48 mg/L as $CaCO_3$ well water)	46.6 mg/L
Total Alkalinity	42.0 mg/L
Specific Conductance	not reported
Total Organic Carbon	not reported
Test Aquaria 1. Material (glass or stainless steel) 2. a. Static volume (18.9 L (5 gal or 19000 cc) with 15 L solution) b. Static or flow-through volume (300x600x300 = 54000 cc.)	1. Static test glass 2. a. 250 ml Static b. 500 ml Flow-through
Type of Dilution System (Reproducible supply of toxicant)	Mini diluter system (Benoit et al., 1982)
Flow rate Consistent flow rate-meter systems calibrated before study and checked 2*24 hours - 5 to 10 vol/24 hours	14 ml/min or 40.3 vol/24 hours
Biomáss Loading Rate (Static no > 0.8 g/L ≤ 17°C; >17°C 0.5g/L; Flow-through 1 g/L/24	Not reported
Photoperiod (16 L & 8 D)	Flow-through 16-h daylight 28 to 50 lumens at the water surface.

Solvents 1. (Do not exceed 0.5 ml/L for static tests) 2. (Do not exceed 0.1 ml/L for flow-through)	None
Other Comments	

D. Test Design:

Guideline Criteria	Reported Information
Range Finding Test (LC_{50} >100 mg/L with 30 fish, no definitive test required.)	none
Definitive Test	
Nominal Concentrations (control+5 treatment levels; dosage should be 60% of the next highest concentration; concentrations should be geometric series)	Static "duplicates of five test conc. and a control."; Flow-through (see Table 2 attached)
Controls (Minimum control mortality; static 10%; flow-through 5%	Static not reported;flow-through no control mortality for 24-h, 48-h, 72-h, but 30 day continuous exposure only 83.4 % survived however the two lowest levels 0.6 and 1.2 had 96.7 and 93.1 % survival.
Number of Test Organisms; (Minimum 10/level can be divided among containers)	Static 10; Flow-through - 15
All organisms must be randomly assigned to test vessels. (yes or no, describe if no)	Not reported
Biological Observations (yes or no)	Yes

Water Parameter Measurements 1. Temperature - record every 6 hrs;>1°C. 2. D.O. beginning,48 hrs,end for control high, medium, and low dose. 3. pH beginning,48 hrs, end for control, high, medium, and low dose.	1. Daily all tests 2. Not reported 3. Not reported
Chemical Analysis (needed if aeration, volatile, insoluble, precipitate, not steel or glass, known to adsorb, and flow-through) (yes or no)	Yes
Other Comments	

11. REPORTED RESULTS:

Guideline Criteria	Reported Information
Mean Measured Concentrations (report conc.)	yes (for flow-through see Table 2)
Recovery of Chemical (% recovery)	96.4% for static; 95.6% for flow-through tests
Mortality & Observations (Describe observations & attach mortality tables)	see tables 1 & 2
Author's Comments	see quote under conclusions

12. STUDY AUTHOR'S CONCLUSIONS / QUALITY ASSURANCE MEASURES:

Not reported

13. REVIEWER'S DISCUSSION AND INTERPRETATION

A. Test Procedure:

The following items did not meet the guideline criteria:

1. Weight of fish at start of study not reported.

2. Length of fish not reported.

88

3. Acclimation period not reported.

4. Tail rot was found in a few individuals in the second 30 day study.

5. Feeding schedule not reported.

6. Control mortality not reported, if any, for 96-hour LC_{50} and EC_{50} tests.
7. Control mortality of 16.5% in 30 day continuous exposure test.
8. Specific conductance and total organic carbon not reported.
9. Static and flow-through test aquaria to small.

10. The flow rate was higher than 10 volumes/24 hour at 40.3 vol/24 hours.
11. Loading rate not reported.

12. Concentrations not reported for the static tests.

13. Control mortality rate not reported for the static tests.
14. Not reported if fish were randomly assigned to test vessels.
15. Schedule for D.O. and pH measurements not reported.

B. Statistical Analysis

Guideline Criteria	Reported Information
Binomial (yes, no, or not reported)	No
Moving Average Angle (yes, no, or not reported)	No
Probit (yes, no, or not reported)	No
Other Comments	The 96 hour LC_{50} and EC_{50} values used Trimmed-Spearman Karber Method; 30-day studies survival data was transformed to arcsin square root %, Survival and growth data used one-way ANOVA to determine the TPTH effect. Dunnett's was used to compare treatment to control.

14. COMPLETION DATE OF ONE-LINER FOR STUDY:

90

TPTH

Page _____ is not included in this copy.

Pages 91 through 92 are not included in this copy.

The material not included contains the following type of information:

_____ Identity of product inert ingredients.

_____ Identity of product impurities.

_____ Description of the product manufacturing process.

_____ Description of quality control procedures.

_____ Identity of the source of product ingredients.

_____ Sales or other commercial/financial information.

_____ A draft product label.

_____ The product confidential statement of formula.

_____ Information about a pending registration action.

X FIFRA registration data.

_____ The document is a duplicate of page(s) _____.

_____ The document is not responsive to the request.

The information not included is generally considered confidential by product registrants. If you have any questions, please contact the individual who prepared the response to your request.

MRID No: 43212701

DATA EVALUATION RECORD

1. CHEMICAL: Triphenyltin Hydroxide (TPTH)

2. TEST MATERIAL: Triphenyltin (TPTH) Technical, Lot#RFRAM909K
Purity: 97.23%

3. STUDY TYPE: §72-3 (c) Acute toxicity test for estuarine and marine organisms: Mysid Shrimp (*Mysidopsis bahia*)

4. CITATION:

Author:	Machado, M. W.
Title:	Triphenyltin (TPTH) - Acute Toxicity to Mysid Shrimp (*Mysidopsis bahia*) under Flow-Through Conditions
Date:	March 10, 1994
Laboratory Report #:	94-1-5124
Any Other Study #:	11117.0593.6103.515
Sponsor:	Griffin Corp. Valdosta, GA
Sponsor #:	N/A
Laboratory:	Springborn Laboratories, Inc.
MRID No.:	43212701

5. REVIEWED BY:

Dennis J. McLane, Wildlife Biologist Signature: Dennis M Lane
Ecological Effects Branch
Environmental Fate and Effects Division (7507 C) Date: 8-26-94

6. APPROVED BY:

Les Touart, Chief, Section 1 Signature: L/T
Ecological Effects Branch
Environmental Fate and Effects Division (7507C) Date: 9-12-94

7. CONCLUSION This study fulfills the guideline requirements. The reported moving average angle was 4.3 (3.7-5.0) µg/L which will place TPTH in the very highly toxic category (<100 µg/L).

8. RECOMMENDATIONS N/A

9. BACKGROUND Submitted in response to the list A process.

10. MATERIALS AND METHODS

A. Test Organisms: Mysid Shrimp

93

Guideline Criteria	Reported Information
Species (Scientific Name)	*Mysidopsis bahia*
Mean Weight (> 0.5 grams)	0.001 g
Supplier	Springborn Laboratories
All shrimp from same source (yes or no)	yes
All shrimp from the same year class (yes or no)	yes ≤24 hours old
Other Comments	

B. Source/Acclimation

Guideline Criteria	Reported Information
Acclimation Period (minimum 10 days)	Cultured at this laboratory
Wild caught 7 day quarantine (yes or no)	no
Check for signs of disease or injury (yes or no, if yes describe)	Not reported
If diseased it can be treated in 48-hr pretest no sign of the disease remains (Report hours prior to test in which no sign of disease or N/A)	N/A
No feeding during the study (When last fed)	twice daily
<3% mortality 48 hours prior to testing (% mortality, if any)	Not reported

C. Test System:

Guideline Criteria	Reported Information
Describe source of dilution water	Cape Cod Canal, Bourne, Massachusetts

Does water support test animals without observable signs of stress?	Yes
What was the salinity of the water used? (30-34% ppt for marine (stenohaline) shrimp and 10-17% ppt for estuarine (euryhaline) shrimp.	32‰
Water Temperature (22°C)	25°C
pH 8.0-8.3 marine (stenohaline) shrimp 7.7-8.0 estuarine (euryhaline) shrimp	7.7-8.0
Dissolved Oxygen (Static 1st 48 hrs 40%; 2nd 48 hrs 60%; Flow-through 60%) (% of lowest conc. & hour)	90-103%
Total Organic Carbon	1.1-1.5 mg/l
Test Aquaria 1. Material (glass or stainless steel) 2. a. Static volume (18.9 L (5 gal or 19000 cc) with 15 L solution) b. Static or flow-through volume (300x600x300 = 54000 cc.)	1. glass and silicone sealant a. N/A b. 39 x 20 x 25 cm depth limited by a 14.5 cm standpipe for a volume of 11 L
Type of Dilution System (Reproducible supply of toxicant)	modified serial diluter system
Flow rate Consistent flow rate-meter systems calibrated before study and checked 2*24 hours - 5 to 10 vol/24 hours	6.5 volume replacements per aquarium every 24 hours

95

Biomass Loading Rate (Static no > 0.8 g/L ≤ 17°C; >17°C 0.5 g/L; Flow-through 1 g/L/24	0.00014 g
Photoperiod (16 L & 8 D)	16 L & 8 D
Solvents 1. (Do not exceed 0.5 ml/L for static tests) 2. (Do not exceed 0.1 ml/L for flow-through)	1. N/A 2. 0.1 ml/L
Other Comments	

D. Test Design:

Guideline Criteria	Reported Information
Range Finding Test (LC_{50} >100 mg/L with 30 shrimp, no definitive test required.)	Mortality at 11 µg/L
Definitive Test	
Nominal Concentrations (control+5 treatment levels; dosage should be 60% of the next highest concentration; concentrations should be geometric series)	control, solvent control, 1.6, 2.6, 4,3, 7.2, and 12 µg/L
Controls (Minimum control mortality; static 10%; flow-through 5%	0% mortality for both control and solvent control
Number of Test Organisms; (Minimum 20/level can be divided among containers)	20/level
All organisms must be randomly assigned to test vessels. (yes or no, describe if no)	"impartially selected and distributed"
Biological Observations (yes or no)	Yes

96

Water Parameter Measurements 1. Temperature - record every 6 hrs;>1°C. 2. D.O. beginning,48 hrs,end for control high, medium, and low dose. 3. pH beginning,48 hrs, end for control, high, medium, and low dose.	1. Continuous in replicate A range 24-25°C, others were daily and 25°C 2.daily in each aquarium 3.daily in each aquarium
Chemical Analysis (needed if aeration, volatile, insoluble, precipitate,. not steel or glass, known to adsorb, and flow-through) (yes)	yes
Other Comments	

11. REPORTED RESULTS:

Guideline Criteria	Reported Information
Mean Measured Concentrations (report conc.)	1.4, 2.2, 3.8, 6.3, 10
Recovery of Chemical (% recovery)	90, 83, 89, 88, 87
Mortality & Observations (Describe observations & attach mortality tables)	See attached Table 3 for both mortality and observations
Author's Comments	

12. STUDY AUTHOR'S CONCLUSIONS / QUALITY ASSURANCE MEASURES:
Author provided no conclusions. Quality assurance unit statement was provided and signed by the Patricia D. Royal the Regulatory Affairs and Quality Assurance Manager. Also a good laboratory practice compliance statement was include with signature from the Study director, Sponsor study monitor, and Applicant/Submitter.

13. REVIEWER'S DISCUSSION AND INTERPRETATION

A. Test Procedure:

The following items did not meet the guideline

97

criteria:

1. Rather than randomly assigned to test vessels the shrimp were "impartially selected and distributed to test vessels.

2. Disease in the shrimp was not discussed.

3. The percent mortality 48 prior to the test was not reported.

B. Statistical Analysis

Guideline Criteria	Reported Information
Binomial (yes, no, or not reported)	No
Moving Average Angle (yes, no, or not reported)	Yes, 4.3 (3.7-5.0) µg/L
Probit (yes, no, or not reported)	No
Other Comments --	EEB used the Toxanal program which gives LC_{50} values from binomial, moving average, and probit methods. They were as follows: binomial 4.7 (3.8-6.3) µg/L, moving average 4.57 (3.96-5.336) µg/L, and probit 4.74 (4.26-5.31) µg/L. (see attached printout).

C. Discussion/Results: The items under 13. REVIEWER'S DISCUSSION AND INTERPRETATION are not expected in this case to effect the results of the study.

D. Adequacy of the Study:

1. Classification: Core

2. Rational: Fulfills guideline requirements.

3. Reparability: N/A

14. COMPLETION DATE OF ONE-LINER FOR STUDY:

MCLANE TPTH MYSID 96-HOUR FLOW-THROOUGH ACUTE

****

CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
10	20	20	100	9.536742E-05
6.3	20	19	95	2.002716E-03
3.8	20	2	10	2.012253E-02
2.2	20	0	0	9.536742E-05
1.4	20	0	0	9.536742E-05

THE BINOMIAL TEST SHOWS THAT 3.8 AND 6.3 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 4.785323

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS	
4	4.557268E-02	4.573244	3.968218	5.360389

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
6	.1649973	1	.9999995

SLOPE = 13.33154
95 PERCENT CONFIDENCE LIMITS = 7.91629 AND 18.7468

LC50 = 4.74165
95 PERCENT CONFIDENCE LIMITS = 4.263238 AND 5.318471

LC10 = 3.807736
95 PERCENT CONFIDENCE LIMITS = 3.163379 AND 4.238397

****

MRID No.: 43212702

DATA EVALUATION RECORD

1. CHEMICAL: Triphenyltin Hydroxide (TPTH)

2. TEST MATERIAL: 97.23% TGAI, white powder

3. STUDY TYPE: §72-3 Estuarine Fish 96-hour Acute Toxicity Test.

4. CITATION:

Author: Mark W. Machado
Title: Triphenyltin Hydroxide (TPTH)-Acute Toxicity to Sheephead Minnow (*Cyprinodon variegatus*) under Flow-Through Conditions
Date: March 15, 1994
Laboratory Report #: 93-10-4952
Any Other Study #: 11117.0593.6102.505
Sponsor: Griffin Corp.
Sponsor #: N/A
Laboratory: Springborn Laboratories, Inc.
MRID No.: 43212702

5. REVIEWED BY:

Dennis J. McLane, Wildlife Biologist Signature: Dennis McLane
Ecological Effects Branch
Environmental Fate and Effects Division (7507 C) Date: 8-26-94

6. APPROVED BY:

Les Touart, Chief, Section 1 Signature: L. Touart
Ecological Effects Branch
Environmental Fate and Effects Division (7507C) Date: 9-12-94

7. CONCLUSION
This study fulfills the guideline requirements for an acute toxicity test using sheepshead minnows. Under the conditions of the test, the 96-hour LC_{50} was 25.5 (21-33) µg ai/L, which classifies TPTH as very highly toxic to sheepshead minnows.

8. RECOMMENDATIONS N/A

9. BACKGROUND Submitted in response to list A process.

10. MATERIALS AND METHODS

A. Test Organisms: Sheepshead minnow

Guideline Criteria	Reported Information
Species (Scientific Name)	*Cyprinodon variegatus*
Mean Weight (0.5-5 grams)	0.28 (0.20-0.34) grams
Mean Length(S.L. longest not > 2x shortest	26 (22-32) mm
Supplier	Aquatic Biosystems
All fish from same source (yes or no)	yes
All fish from the same year class (yes or no)	Not reported
Other Comments	

B. Source/Acclimation

Guideline Criteria	Reported Information
Acclimation Period (minimum 14 days)	14 days
Wild caught 7 day quarantine (yes or no)	no
Check for signs of disease or injury (yes or no, if yes describe)	no, only checked for mortality
If diseased it can be treated in 48-hr pretest no sign of the disease remains (Report hours prior to test in which no sign of disease or N/A)	no mortality in fish 48 hours prior to start of the test.
No feeding during the study (When last fed)	48 hours prior to testing.
<3% mortality 48 hours prior to testing (% mortality, if any)	0% mortality prior to testing.

C. Test System:

Guideline Criteria	Reported Information
Describe source of dilution water (prefer soft reconstituted water)	Collected from Cape Cod Canal, Bourne, MA. Seawater was then passed through a series of polypropylene core filters & then recirculated within an epoxy-lined concrete reservoir prior to use.
Does water support test animals without observable signs of stress?	yes
Salinity of water used. (reconstituted seawater of 30-34% salinity) (weekly range of salinity is less than 6%)	31-32‰
Water Temperature (22 ± 1)	22 ± 1
pH (8.0-8.3 for marine-stenohaline fish and 7.7-8.0 for estuarine-euryhaline fish species) (monthly range is less than 0.8 of a pH unit)	7.7-8.0
Dissolved Oxygen (Static 1st 48 hrs 40%; 2nd 48 hrs 60%; Flow-through 60%) (% of lowest conc. & hour)	>71% in all test and controls vessels over 96 hours of testing.
Test Aquaria 1. Material (glass or stainless steel) 2. a. Static volume (18.9 L (5 gal or 19000 cc) with 15 L solution) b. Static or flow-through volume (300x600x300 = 54000 cc.)	 1. each glass test aquaria and silicone sealant a. N/A b. measured 39X20X25 cm with a 14.5 cm standpipe to maintain a volume of 11 L.
Type of Dilution System (Reproducible supply of toxicant)	flow-through, reproducible.

102

Flow rate 1. Consistent flow rate-meter systems calibrated before study 2. Checked 2*24 hours - 5 to 10 vol/24 hours	1. Tested and verified 6 days and 2 days prior to start of the test. 2. Monitored daily and checked twice visually each day. Flow of exposure to each test aquarium was approx. 50 ml/min, which equaled approx. 6.5 volume replacements per 24 hours per aquarium.
Biomass Loading Rate (Static no > 0.8 g/L ≤ 17°C; >17°C 0.5g/L; Flow-through 1 g/L/24	0.039 g of biomass/L of flowing test solution per day.
Photoperiod (16 L & 8 D)	16 hours light, 8 hours dark.
Solvents 1. (Do not exceed 0.5 ml/L for static tests) 2. (Do not exceed 0.1 ml/L for flow-through)	1. N/A 2. 0.092 ml/L
Other Comments	

D. Test Design:

Guideline Criteria	Reported Information
Range Finding Test (LC_{50} >100 mg/L with 30 fish, no definitive test required.)	13, 36, 100µg a.i./L 100% mortality @ 36 and 100 µg/L levels and no effects seen @ the 13 µg/L level.
Definitive Test	
Nominal Concentrations (control+5 treatment levels; dosage should be 60% of the next highest concentration; concentrations should be geometric series)	13, 22, 36, 60, 100 µg ai/L + control and solvent control. Dosage levels were 60% of the next highest level.
Controls (Minimum control mortality; static 10%; flow-through 5%	0% mortality observed in the solvent control and control.

10

Number of Test Organisms; (Minimum 10/level can be divided among containers)	20 fish (10 per test aquaria) per concentration and controls were used. 140 fish total.
All organisms must be randomly assigned to test vessels. (yes or no, describe if no)	impartially selected.
Biological Observations (yes or no)	yes, at test initiation and every 24 hours.
Water Parameter Measurements 1. Temperature - record every 6 hrs;>1°C. 2. D.O. beginning,48 hrs,end for control high, medium, and low dose. 3. pH beginning,48 hrs, end for control, high, medium, and low dose.	recorded continuously in one control aquaria. pH, temperature, and DO were measured in each replicate vessel once daily throughout exposure period.
Chemical Analysis (needed if aeration, volatile, insoluble, precipitate, not steel or glass, known to adsorb, and flow-through) (yes or no)	yes, sample from each replicate solution of high, medium, and low treatment levels and dilution water control were taken twice prior to definitive test. In addition, water samples were taken both replicate test solutions of each treatment level and the controls at 0-hour and 96 -hours of exposure for analysis.
Other Comments	

11. REPORTED RESULTS:

Guideline Criteria	Reported Information
Mean Measured Concentrations (report conc.)	16, 21, 33, 61, 110 µg ai/L
Recovery of Chemical (% recovery)	104% of nominal concentrations
Mortality & Observations (Describe observations & attach mortality tables)	See attached Table 3.

loy

Author's Comments	

12. STUDY AUTHOR'S CONCLUSIONS / QUALITY ASSURANCE MEASURES:

Author provided no conclusions. Quality assurance unit statement was provided and signed by the Patricia D. Royal the Regulatory Affairs and Quality Assurance Manager. Also a good laboratory practice compliance statement was include with signature from the Study director, Sponsor study monitor, and Applicant/Submitter.

13. REVIEWER'S DISCUSSION AND INTERPRETATION

A. Test Procedure:

The following items did not meet the guideline criteria:

1. Study fish weighed less than what is generally recommended (0.12-0.56), should be between 0.5-5.0 grams.

2. Small amount of precipitate was observed in the mixing chamber of diluter system, however analyticl data indicates the test concentrations were maintained to be 81% on nominal.

B. Statistical Analysis

Guideline Criteria	Reported Information
Binomial (yes, no, or not reported)	yes, 96-hour LC_{50} = 26 (C.I. 21 - 33) µg ai/L
Moving Average Angle (yes, no, or not reported)	no
Probit (yes, no, or not reported)	no
Other Comments	The EEB Toxanal program indicates that the binomial is correct 25.55 (21-33) µg/L. (See the attached printout)

C. Discussion/Results:

This study is scientifically sound and fulfills the guideline requirements for an acute toxicity test using sheepshead

minnows. Under the conditions of the test, the 96-hour LC_{50} was 25.5 µg ai/L, which classifies TPTH as very highly toxic to sheepshead minnows.

D. Adequacy of the Study:

1. Classification: Core

2. Rational: Fulfills guideline requirements.

3. Reparability: N/A

14. COMPLETION DATE OF ONE-LINER FOR STUDY:

106