US EPA ARCHIVE DOCUMENT

Text Searchable Document

DATA EVALUATION RECORD
AQUATIC INVERTEBRATE LIFE CYCLE TEST
GUIDELINE 72-4(C)

1. **CHEMICAL:** Atrazine PC Code No.: 080803

2. **TEST MATERIAL:** Atrazine Technical Purity: 97.1%

3. **CITATION**

Authors:	Cafarella, M.A.
Title:	Atrazine (G-30027) – Life-Cycle Toxicity Test with Mysids (*Americamysis bahia*)
Study Completion Date:	September 14, 2005
Laboratory:	Springborn Smithers Laboratories 720 Main Street Wareham, MA 02571-1037
Sponsor:	Syngenta Crop Protection, Inc. 410 Swing Road Post Office Box 18300 Greensboro, NC 27419
Laboratory Report ID:	1781.6641
MRID No.:	466482-02
DP Barcode:	D322057

4. **REVIEWED BY:** Christie E. Padova, Staff Scientist, Dynamac Corporation

Signature: Christie E. Padova **Date:** 1/20/06

APPROVED BY: Teri S. Myers, Senior Scientist, Cambridge Environmental Inc.

Signature: Teri S Myers **Date:** 2/2/06

5. **APPROVED BY:** Anita Pease, OPP/EFED/ERB-III

Signature: Anita Pease **Date:** 3/27/06

US EPA ARCHIVE DOCUMENT

6. DISCLAIMER: This document provides guidance for EPA and PMRA reviewers on how to complete a data evaluation record after reviewing a scientific study concerning the chronic toxicity of a pesticide to mysids. It is not intended to prescribe conditions to any external party for conducting this study nor to establish absolute criteria regarding the assessment of whether the study is scientifically sound and whether the study satisfies any applicable data requirements. Reviewers are expected to review and to determine for each study, on a case-by-case basis, whether it is scientifically sound and provides sufficient information to satisfy applicable data requirements. Studies that fail to meet any of the conditions may be accepted, if appropriate; similarly, studies that meet all of the conditions may be rejected, if appropriate. In sum, the reviewer is to take into account the totality of factors related to the test methodology and results in determining the acceptability of the study.

7. STUDY PARAMETERS

Age of Test Organism:	Neonates, <24 hours old
Definitive Test Duration:	28 days
Study Method:	Flow-through
Type of Concentrations:	Mean measured

8. CONCLUSIONS:

Results Synopsis
NOEC: 0.26 mg ai/L LOEC: 0.50 mg ai/L

NOEC's for specific effects
Young/Female/Repro. Day: 1.1 mg ai/L
Larvae Survival: 1.1 mg ai/L
Growth 1) length: Male 0.26 mg ai/L
Female 0.50 mg ai/L
2) weight: Male 0.26 mg ai/L
Female 0.26 mg ai/L

9. ADEQUACY OF THE STUDY

A. Classification: Supplemental

B. Rationale: Raw data were not provided to generate gender-specific conclusions about first-generation survivors and to support the replicate means for non-gender-specific percent survival at test termination. Gender-specific replicate data were provided once organisms were paired, but the replacement of males between days 14 and 28 precludes the ability to attribute changes in male mortality to treatment.

US EPA ARCHIVE DOCUMENT

C. Repairability: This study may be upgraded if appropriate raw data are provided for review.

10. MAJOR GUIDELINE DEVIATIONS:

1. Pretest health and/or mortality of the adult culture were not reported. Furthermore, it was not reported if the brood stock was isolated from the main culture.

2. The quantity of live brine shrimp fed to the mysids was not specified.

3. Mortality of the male and female mysids was not assessed individually at study termination, and raw mortality data were not provided for reviewer assessment or verification. Although raw mortality data were provided for the paired mysids, males that died during the study were replaced, and therefore percent survival cannot be calculated accurately from this population only. Raw mortality data for the entire population need to be provided.

4. As raw mortality data for the entire population were not provided, survival replicate means provided in Table 3 could not be verified. The reported results were presumably calculated with respect to the initial number of mysids, i.e., 60/treatment level.

5. Raw data pertaining to offspring production were not provided.

11. MATERIALS AND METHODS:

A. Biological System

Guideline Criteria	Reported Information
Species: An estuarine shrimp species, preferably *Americamysis bahia*.	*Americamysis bahia*.

US EPA ARCHIVE DOCUMENT

Guideline Criteria	Reported Information
Duration of the Test: A mysid test must not be terminated before 7 days past the median time of 1st brood release in the control treatment.	The study duration was adequate, as the first control brood release occurred on Day 14.
Source (or supplier)	In-house cultures maintained by Springborn (originally from Aquatic BioSystems, Inc., Ft. Collins, Colorado).
Parental Acclimation 1) Parental stock must be maintained separately from the brood culture in dilution water and under test conditions. 2) Mysids should be in good health.	1) The culture stock was maintained in artificial seawater formulated in the same manner as used in the definitive test. Isolation of the parental culture was not described. 2) Health of the mysid population was not reported.
Parental Acclimation Period At least 14 days	14 days

US EPA ARCHIVE DOCUMENT

Guideline Criteria	Reported Information
Chamber Location: Treatments should be randomly assigned to test chamber locations.	Organisms were impartially selected and distributed to the retention chambers.
Brood Stock: Test started with mysids: 1) from only one brood stock or 2) from brood stock which has not obtained sexual maturity or had been maintained for > 14 days in a laboratory with same food, water, temperature, and salinity used in the test.	At test initiation, juvenile mysids were collected from the laboratory culture stock. The in-house culture was maintained with the same food, water, temperature, salinity, and pH as used in the definitive test.
Distribution: **No. of mysids before pairing**: Minimum of 15 mysids per compartment, 2 compartments per chamber, 2 chambers per concentration for a total of 60/treatment level. **No. of mysids after pairing**: $\geq$ 20 randomly selected pairs/treatment (excess males should be held in separate compartment in same treatment to replace paired males).	15 mysids per retention chamber, 2 chambers per aquarium, and 2 aquaria per treatment level (60 mysids/treatment level). 10 mature pair per aquarium (20 pair/treatment level); excess organisms were pooled and retained in one initial retention chamber; males from the pool were used to replace dead males from the paired groups.
Pairing: 1) Should be conducted when most of the mysids are sexually mature (usu. 10-14 days after test initiation). 2) Should be paired on the same day	1) When the mysids reached sexual maturity (based on appearance of gravid females), they were redistributed (paired) within the test aquaria. 2) All pairing occurred on Day 14.

US EPA ARCHIVE DOCUMENT

Guideline Criteria	Reported Information
Feeding: 1) Mysids should be fed live brine shrimp nauplii at least once daily. 2) 150 live brine shrimp nauplii per mysid per day or 75 twice a day is recommended.	1) Mysids were fed live brine shrimp (*Artemia salina*) nauplii, <48 hours old (post-hydration), twice daily. Prior to pairing, at least one of these feedings was with brine shrimp nauplii enriched with Selco®, a substance high in saturated fatty acids. Following pairing, the mysids were fed brine shrimp nauplii daily, enriched with Selco® every other day. 2) Quantity not reported.
Counts: Live adult mysids should be counted 1) at initiation, 2) at pairing, 3) and daily after pairing. 4) Live young must be counted and removed daily. 5) Missing or impinged animals should be recorded.	Live adult mysids were counted 1) at initiation 2) at pairing 3) and daily after pairing. 4) Live young were counted and removed daily. 5) Missing or impinged animals were recorded.
Controls: Negative control and carrier control (when applicable) are required.	A negative control group was included.

Comments: The maximum organism loading concentration (based on a maximum average wet weight of 4.5 mg per mature adult mysid) was 2.7 mg of biomass/L of flowing test solution per day.

US EPA ARCHIVE DOCUMENT

B. Physical System:

Guideline Criteria	Reported Information
Test Water: 1) May be natural (sterilized and filtered) or a commercial mixture;	1) Artificial seawater was prepared using freshwater (soft) and a commercially prepared salt formula (hw-MARINEMIX®).
2) Water must be free of pollutants.	2) Periodic analyses for pesticides, PCB's, and toxic metals in the dilution water indicated that none of these compounds were detected at concentrations that are considered toxic.
3) During the test, difference between highest and lowest measured salinities must be less than 10 ‰ (parts per thousand). Should be measured daily.	3) Difference of 2 ‰. Salinity was measured daily in each replicate aquarium.
4) Salinity should be between 15 and 30 ‰.	4) Salinity ranged from 19-21 ‰.
5) pH should be measured at the beginning, end of test and weekly.	5) pH was measured daily in each replicate aquarium.
6) DO must be measured @ each conc. @ least once a wk.	6) DO was measured daily in each replicate aquarium. DO was maintained at 85-106% of saturation.
7) See details in ASTM E-1191.	

US EPA ARCHIVE DOCUMENT

Guideline Criteria	Reported Information
Test Temperature: 1) Measured daily in one chamber and at least 3 times in all chambers. 2) Mean measured temperature for each chamber at test termination should be within 1 °C of selected test temperature. 3) Each individual measured temperature must be within 3 °C of the mean of the time-weighted averages. 4) For mysid shrimp, 27 °C is recommended. 5) Whenever temp. is measured concurrently in more than one test chamber the highest & lowest temp. must not differ by more than 2 °C.	1) Temperature was measured daily in each replicate aquarium, and continuously in one control vessel. 2) Target: 26 ± 2°C. Actual range: 25-27°C from daily measurements and 23-28°C for continuously monitoring. Neither daily temperatures nor mean temperatures were provided. 3) Criteria met, based on temp. range data. 4) Actual temp. was appropriate for species. 5) Criteria met, based on temp. range data.
Photoperiod: Recommend 16L/8D. 14L/10D also acceptable.	16 hours light, 8 hours dark, with an intensity of 32-65 footcandles (340-700 lux).
Dosing Apparatus: 1) Intermittent flow proportional diluters or continuous flow serial diluters should be used. 2) A minimum of 5 toxicant concentrations 3) A dilution factor not greater than 0.5 and controls should be used.	1) An intermittent-flow proportional diluter. 2) 5 toxicant concentrations 3) A dilution factor of 0.5 and a dilution water control were used.
Toxicant Mixing: 1) Mixing chamber is recommended but not required; 2) Aeration should not be used for mixing; 3) It must be demonstrated that the test solution is completely mixed before intro. into the test system; 4) Flow splitting accuracy must be within 10%.	1) A mixing chamber was used. 2) Aeration was not used for mixing. 3) Verified by analytical measurements. 4) Within 5% of the targeted delivery.

US EPA ARCHIVE DOCUMENT

Guideline Criteria	Reported Information
Test Vessels: 1) Material: all glass, No. 316 stainless steel, or perflorocarbon plastic 2) Size: most common - 300x450x150 mm deep with solution depth of 100 mm. 3) Should be covered. **Test Compartments (within chambers):** 1) Size: 250 ml beaker with side cutouts covered with nylon mesh or stainless steel screen. or 90 or 140 mm i.d. glass Petri dish bottoms with collars made of 200 - 250 um mesh screen.	1) Glass 2) 390 x 200 x 250 mm 3) Not reported 1) Prior to pairing: glass petri dishes (10-cm diameter, 2-cm depth) with 15-cm high collars of nylon mesh (350 µm). The solution volume fluctuated from 390 to 710 ml (due to siphon drains). Following pairing: cylindrical glass jars (5.1-cm diameter, 10-cm height) with two holes covered with nylon mesh (350 µm) screen. The solution volume fluctuated from 100 to 180 ml.
Flow Rate: 1) Flow rates should provide 5 to 10 volume additions per 24 hr. 2) Flow rate must maintain DO at or above 60% of saturation and maintain the toxicant level. 3) Meter systems calibrated before study and checked twice daily during test period.	1) 7.5 volume additions/24 hours 2) DO was maintained at ≥85% saturation. 3) Meter systems were calibrated before the study and visually checked twice daily during the test period.
Aeration: 1) Dilution water should be aerated to insure DO concentration at or near 100% saturation. 2) Test tanks may be aerated.	1) The dilution water was aerated prior to use. 2) The test chambers were not aerated.

Comments: The TOC concentration of the dilution water source was 0.58 and 0.45 mg/L for July and August 2005, respectively.

US EPA ARCHIVE DOCUMENT

C. Chemical System:

Guideline Criteria	Reported Information
Concentrations: 1) Minimum of 5 concentrations and a control, all replicated, plus solvent control if appropriate. 2) Toxicant conc. must be measured in one tank at each treatment level every week. 3) One concentration must adversely affect a life stage and one concentration must not affect any life stage. 4) The measured conc. of the test material of any treatment should be at least 50% of the time-weighted average measured conc. for >10% of the duration of the test. 5) The measured conc. for any treatment level should not be more than 30% higher than the time-weighted average measured conc. for more than 5% of the duration of the test.	1) Five concentrations plus a dilution water control. All levels were maintained in duplicate. 2) Measured in alternating replicate aquaria from all levels on Days 0, 7, 14, 21, and 28. 3) Criteria met. 4) High-low ratios were <1.5 for all treatment groups, indicating consistency in atrazine recoveries. 5) High-low ratios were <1.5 for all treatment groups, indicating consistency in atrazine recoveries.
Solvents: 1) Should not exceed 0.1 ml/L in a flow-through system. 2) Following solvents are acceptable: triethylene glycol, methanol, acetone, ethanol.	N/A

Comments: Three quality control (QC) samples were prepared at each sampling interval and remained with the exposure solution samples throughout the analytical process. The QC samples were prepared in dilution water at nominal concentrations similar to the exposure concentration range, and results were used to judge the precision and quality control maintained during the analysis of test samples. Recoveries of atrazine from the QC samples ranged from 88.9 to 103% of nominal concentrations.

Throughout the exposure period, no visible sign of undissolved test substance (e.g., precipitate) was observed in the mixing chamber, the chemical cells of the diluter system, or in any of the exposure solutions.

US EPA ARCHIVE DOCUMENT

12. REPORTED RESULTS:

Guideline Criteria	Reported Information
Quality assurance and GLP compliance statements were included in the report?	Yes. This study was performed according to U.S. EPA (FIFRA) Good Laboratory Practice Standards (40 CFR, Part 160) with the exception of the collection for the water and food contaminant screening analyses.
Controls: 1) Survival of the first-generation controls (between pairing and test termination) must not be less than 70%. 2) At least 75% of the paired 1st generation females in the controls produced young or 3) The average number of young produced by the 1st generation females in the control(s) was at least 3.	1) Mean control survival was 72% (of initial population). 2) 95% of paired control females (19/20) produced young. 3) An average of approximately 6 young per reproducing female were produced in each of the control replicates.

US EPA ARCHIVE DOCUMENT

Guideline Criteria	Reported Information
Data Endpoints must include: 1) Survival of first-generation mysids Female Male	1) Mortality for male and female mysids was not assessed individually. The percent survival results reported (Day 28; Table 3) were for combined sexes, and were presumably calculated with respect to the initial population; however raw data were not provided to verify.
2) Number of live young produced per female	2) Yes, an acceptable reproductive endpoint was assessed. Specifically, offspring/female/-reproductive day.
3) Dry weight of each first-generation mysid alive at the end of the test Female Male	3) Yes, of surviving paired organisms.
4) Length of each first-generation mysid alive at the end of the study Female Male	4) Yes, of surviving paired organisms.
5) Incidence of pathological or histological effects;	5) None reported
6) Observations of other effects or clinical signs.	6) None reported
Raw data included? (Y/N) At a minimum, individual data should be included for: 1) Surviving 1st generation ♂ and ♀ mysids.	1) No, only partial survival data were provided, i.e., for the paired organisms. Raw data regarding survival of the overall population were not provided. Summarized results (Table 3) could not be verified.
2) Number of live young produced per female.	2) No raw data provided.
3) Individual length measurements of ♂ and ♀ mysids.	3) Yes, for the paired organisms.
4) Individual dry weight measurements for ♂ and ♀ mysids at the end of the test.	4) Yes, for the paired organisms.

US EPA ARCHIVE DOCUMENT

Effects Data:

Toxicant Conc. (mg/L)		Mean # of Young	Mean # Young/fem./ Repro. day	%Mortality (28 days)			Mean Total Length (mm)			Mean Dry Weight (mg)		
Nom.	Meas.			♂	♀	♂ & ♀ (# dead)[1]	♂	♀	♂ & ♀[2]	♂	♀	♂ & ♀[3]
Ctrl	<0.015	NR	0.469	NR	NR	28 (17)	7.1	7.3	7.2	0.90	1.29	1.10
0.063	0.068	NR	0.572	NR	NR	55 (33)	6.8	7.0	7.0	0.89	1.25	1.06
0.13	0.14	NR	0.738	NR	NR	40 (24)	6.8	7.1	7.0	0.89	1.13	1.00
0.25	0.26	NR	0.759	NR	NR	40 (24)	6.8	7.1	6.9	0.84	1.27	1.06
0.50	0.50	NR	0.697	NR	NR	32 (19)	6.4*	7.1	6.7	0.80*	1.18*	0.99
1.0	1.1	NR	0.431	NR	NR	23 (14)	5.9*	6.3*	6.1	0.78*	1.08*	0.93

NR – Not reported.
[1] Reviewer-calculated from summarized percent survival data provided in Table 3. Values were back-calculated with respect to the initial population of 60 mysid/treatment level.
[2] Reviewer-calculated from mean replicate data provided in Table 4. Data for combined sexes were not statistically evaluated by the study author.
[3] Reviewer-calculated from mean replicate data provided in Table 5. Data for combined sexes were not statistically evaluated by the study author.

Toxicity Observations: First-generation mysids were reportedly observed daily for abnormal appearance and behavior. No discussion of findings (if any) was provided.

Overall survival of first-generation mysids (combined sexes) was not affected by treatment, and ranged from 45% at the 0.068 mg ai/L level to 77% at the 1.1 mg ai/L level (control survival averaged 72%). The study author reported that the reduced survival at the 0.068 mg ai/L level was not dose-related and that the other endpoints assessed (i.e., reproduction and growth) were comparable to control organism performance.

Brood appearance was observed in the controls and all treatment levels by Day 14. The mean number of offspring per female per reproductive day was 0.469 for the control group, and ranged from 0.431-0.759 for all treatment levels, with no statistical significance observed at any treatment level.

US EPA ARCHIVE DOCUMENT

Growth was adversely affected in both sexes by exposure to atrazine. In males, statistically-significant differences in average total body length and dry weight were observed at the 0.50 and 1.1 mg ai/L levels compared to the controls. In females, statistically-significant differences in average total body length were observed at the 1.1 mg ai/L level and in dry weight at the 0.50 and 1.1 mg ai/L levels compared to the controls. Total body length averaged 7.1 mm in control males, compared to 6.8, 6.8, 6.8, 6.4, and 5.9 mm in males for the 0.068, 0.14, 0.26, 0.50, and 1.1 mg ai/L level males, respectively. Total body length averaged 7.3 mm for the control females, compared to 7.0, 7.1, 7.1, 7.1, and 6.3 mm in females for the 0.068, 0.14, 0.26, 0.50, and 1.1 mg ai/L level females, respectively. Dry body weight averaged 0.90 mg for control males, compared to 0.89, 0.89, 0.84, 0.80, and 0.78 mg for the 0.068, 0.14, 0.26, 0.50, and 1.1 mg ai/L level males, respectively. Dry body weight averaged 1.29 mg for the control females, compared to 1.25, 1.13, 1.27, 1.18, and 1.08 mg, for the 0.068, 0.14, 0.26, 0.50, and 1.1 mg ai/L level females respectively. Growth was the most sensitive endpoint; the subsequent NOEC, LOEC, and MATC values were 0.26 mg ai/L, 0.50 mg ai/L, and 0.36 mg ai/L, respectively.

Statistical Results:

Statistical Method: Statistical analyses were performed on terminal (Day 28) survival of the first-generation mysids, the number of young released per female per reproductive day, and the terminal length and dry weight of each surviving first-generation mysid (gender specific). Data were analyzed by standard statistical techniques using a computer program (West, Inc., and Gulley, 1996).

The Shapiro-Wilk's Test was used to determine that data were normally distributed, and Bartlett's Test was used to determine that variances were homogeneous. All data passed the tests for normality and homogeneity of variance. For each endpoint, the performance of organisms exposed to each treatment level of the test substance was compared with the performance of the control data using Williams' Test. Analyses were conducted at the 95% level of certainty, except for the Bartlett's and Shapiro-Wilk's Tests, in which the 99% level of certainty was applied. The MATC was calculated as the geometric mean of the NOAEC and LOAEC. Mean-measured values were used in all estimations.

Most sensitive endpoint: Growth (total length and dry weight)

Endpoint	Method	NOAEC	LOAEC
Survival	Williams' Test	1.1 mg ai/L	>1.1 mg ai/L

US EPA ARCHIVE DOCUMENT

Endpoint	Method	NOAEC	LOAEC
Reproduction (offspring/-female/repro. day)	Williams' Test	1.1 mg ai/L	>1.1 mg ai/L
Male length	Williams' Test	0.26 mg ai/L	0.50 mg ai/L
Female length	Williams' Test	0.50 mg ai/L	1.1 mg ai/L
Male dry weight	Williams' Test	0.26 mg ai/L	0.50 mg ai/L
Female dry weight	Williams' Test	0.26 mg ai/L	0.50 mg ai/L

Comments: None.

13. REVIEWER'S STATISTICAL RESULTS:

Statistical Method: Statistical analyses were performed on terminal (Day 28) survival of the first-generation mysids (not gender-specific), the number of young released per female per reproductive day, and the terminal length and dry weight of each surviving first-generation mysid (gender-specific).

Data were analyzed using the Chi-square and Shapiro-Wilks tests for normality and the Hartley and Bartlett's tests for homogeneity of variances. Data which did not meet these assumptions were analyzed using the non-parametric Kruskal-Wallis test. Data which did satisfy these assumptions were analyzed using ANOVA, followed by William's test via TOXSTAT statistical software. Mean-measured values were used in all estimations.

US EPA ARCHIVE DOCUMENT

Most sensitive endpoint: Growth (male length and female dry weight)

Endpoint	Method	NOAEC	LOAEC
Survival	Kruskal-Wallis (males); ANOVA (females)	1.1 mg ai/L	>1.1 mg ai/L
Reproduction (offspring/-female/repro. day)	ANOVA	1.1 mg ai/L	>1.1 mg ai/L
Male length	Williams' Test	0.26 mg ai/L	0.50 mg ai/L
Female length	Kruskal-Wallis	1.1 mg ai/L	>1.1 mg ai/L
Male dry weight	ANOVA	1.1 mg ai/L	>1.1 mg ai/L
Female dry weight	Williams' Test	0.26 mg ai/L	0.50 mg ai/L

Comments:

The reviewer's conclusions were similar to the study author's. Different statistical tests were used to derive the NOAEC and LOAEC values for female length and male dry weight, so these endpoint results differed; however, the reviewer defers to the study author's more conservative conclusions (i.e., that growth of all gender-specific endpoints was adversely affected at the 0.5 mg ai/L treatment level).

The reviewer could not derive the replicate data averages for percent survival of first generation mysids in Table 3 (p. 26 of 74) using the daily mysid survival data provided in Appendix 3 (pp. 65-70 of 74). If data could be provided to support the percent survival replicate means in Table 3, this study could be upgraded to ACCEPTABLE.

A 28-day range-finding study was conducted under flow-through conditions with a single replicate of 30 mysids/level (<24 hours old) at nominal concentrations of 0 (negative control), 0.11, 0.21, 0.43, 0.85, and 1.7 mg ai/L. This study was scientifically invalid, however, as control survival was only 57%.

US EPA ARCHIVE DOCUMENT

14. REFERENCES:

ASTM. 2002. Standard practice for conducting acute toxicity test with fishes, macroinvertebrates and amphibians. Standard E729-96. American Society for Testing and Materials, 100 Barr Harbor Drive, West Conshohocken, PA 19428.

Dunnett, C.W. 1955. A multiple comparison procedure for comparing several treatments with a control. *J. Amer. Stat. Assoc.* 50:1096-1121.

Dunnett, C.W. 1964. New tables for multiple comparisons with a control. *Biometrics* 20:482-491.

Horning, W.B. and C.I. Weber. 1985. Short-term methods for estimating the chronic toxicity of effluents and receiving waters to freshwater organisms. Environmental Monitoring and Support Laboratory, U.S. Environmental Protection Agency, Cincinnati, Ohio. EPA/600/4-85/014.

Mount, D.I. and W.A. Brungs. 1967. A simplified dosing apparatus for fish toxicological studies. *Water Research* 1:21-29.

Rand, G.M. and S.R. Petrocelli. 1985. Fundamentals of Aquatic Toxicology. Hemisphere Publishing Co., New York.

Sokal, R.R. and F.J. Rohlf. 1981. *Biometry*. 2nd Edition. W.H. Freeman and Company, New York. 859 pp.

Sprague, J.B. 1969. Measurement of pollutant toxicity to fish. 1. Bioassay methods for acute toxicity. *Water Research* 3:793-821.

U.S. EPA. 1985. *Toxic Substances Control Act Test Guidelines.* Federal Register 50(188): 39252-39516, September 27, 1985.

U.S. EPA. Federal Insecticide, Fungicide and Rodenticide Act (FIFRA): Good Laboratory Practice Standards: Final Rule (40 CFR, Part 160). U.S. Environmental Protection Agency, Washington, D.C.

US EPA ARCHIVE DOCUMENT

Weber, C.I. *et al.* 1989. Short-Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms. 2nd Edition. EPA/600/4/89/001. Environmental Monitoring Systems Laboratory, U.S. Environmental Protection Agency, Cincinnati, OH.

West, Inc. and D.D. Gulley. 1996 Toxstat®, Release 3.5. West, Inc., Cheyenne, WY.

Williams, D.A. 1971. 1972. A comparison of several dose levels with a zero control. *Biometrics* 27: 103-117.

Zar, J.H. 1985. Biostatistical Analysis. Prentice-Hall, Englewood Cliffs, N.J.

US EPA ARCHIVE DOCUMENT

15. RESULTS OF STATISTICAL VERIFICATION:

percent survival
File: 8202s Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	1249.417	249.883	2.090
Within (Error)	6	717.500	119.583	
Total	11	1966.917		

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

percent survival
File: 8202s Transform: NO TRANSFORMATION

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

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	control	71.500	71.500		
2	0.068	45.000	45.000	2.423	
3	0.14	60.000	60.000	1.052	
4	0.26	60.000	60.000	1.052	
5	0.50	68.500	68.500	0.274	
6	1.1	76.500	76.500	-0.457	

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

percent survival
File: 8202s Transform: NO TRANSFORMATION

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

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	control	2			
2	0.068	2	30.947	43.3	26.500
3	0.14	2	30.947	43.3	11.500
4	0.26	2	30.947	43.3	11.500
5	0.50	2	30.947	43.3	3.000
6	1.1	2	30.947	43.3	-5.000

US EPA ARCHIVE DOCUMENT

percent survival
File: 8202s Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	control	2	71.500	71.500	58.250
2	0.068	2	45.000	45.000	58.250
3	0.14	2	60.000	60.000	60.000
4	0.26	2	60.000	60.000	60.000
5	0.50	2	68.500	68.500	68.500
6	1.1	2	76.500	76.500	76.500

percent survival
File: 8202s Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
control	58.250				
0.068	58.250	1.212		1.94	k= 1, v= 6
0.14	60.000	1.052		2.06	k= 2, v= 6
0.26	60.000	1.052		2.10	k= 3, v= 6
0.50	68.500	0.274		2.12	k= 4, v= 6
1.1	76.500	0.457		2.13	k= 5, v= 6

s = 10.935
Note: df used for table values are approximate when v > 20.

reproductive success
File: 8202r Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	0.172	0.034	1.214
Within (Error)	6	0.167	0.028	
Total	11	0.340		

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

US EPA ARCHIVE DOCUMENT

reproductive success
File: 8202r Transform: NO TRANSFORMATION

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

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	control	0.469	0.469		
2	0.068	0.578	0.578	-0.654	
3	0.14	0.740	0.740	-1.623	
4	0.26	0.752	0.752	-1.694	
5	0.50	0.700	0.700	-1.383	
6	1.1	0.468	0.468	0.006	

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

reproductive success
File: 8202r Transform: NO TRANSFORMATION

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

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	control	2			
2	0.068	2	0.474	101.1	-0.110
3	0.14	2	0.474	101.1	-0.271
4	0.26	2	0.474	101.1	-0.284
5	0.50	2	0.474	101.1	-0.231
6	1.1	2	0.474	101.1	0.001

reproductive success
File: 8202r Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	control	2	0.469	0.469	0.648
2	0.068	2	0.578	0.578	0.648
3	0.14	2	0.740	0.740	0.648
4	0.26	2	0.752	0.752	0.648
5	0.50	2	0.700	0.700	0.648
6	1.1	2	0.468	0.468	0.468

US EPA ARCHIVE DOCUMENT

reproductive success
File: 8202r Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
control	0.648				
0.068	0.648	1.073		1.94	k= 1, v= 6
0.14	0.648	1.073		2.06	k= 2, v= 6
0.26	0.648	1.073		2.10	k= 3, v= 6
0.50	0.648	1.073		2.12	k= 4, v= 6
1.1	0.468	0.006		2.13	k= 5, v= 6

s = 0.167
Note: df used for table values are approximate when v > 20.

male body length
File: 8202m Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	1.887	0.377	10.189
Within (Error)	6	0.220	0.037	
Total	11	2.107		

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

male body length
File: 8202m Transform: NO TRANSFORMATION

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

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	control	7.100	7.100		
2	0.068	6.850	6.850	1.300	
3	0.14	6.850	6.850	1.300	
4	0.26	6.750	6.750	1.820	
5	0.50	6.350	6.350	3.899	*
6	1.1	5.900	5.900	6.239	*

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

US EPA ARCHIVE DOCUMENT

male body length
File: 8202m Transform: NO TRANSFORMATION

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

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	control	2			
2	0.068	2	0.544	7.7	0.250
3	0.14	2	0.544	7.7	0.250
4	0.26	2	0.544	7.7	0.350
5	0.50	2	0.544	7.7	0.750
6	1.1	2	0.544	7.7	1.200

male body length
File: 8202m Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	control	2	7.100	7.100	7.100
2	0.068	2	6.850	6.850	6.850
3	0.14	2	6.850	6.850	6.850
4	0.26	2	6.750	6.750	6.750
5	0.50	2	6.350	6.350	6.350
6	1.1	2	5.900	5.900	5.900

male body length
File: 8202m Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
control	7.100				
0.068	6.850	1.306		1.94	k= 1, v= 6
0.14	6.850	1.306		2.06	k= 2, v= 6
0.26	**6.750**	**1.828**		**2.10**	**k= 3, v= 6**
0.50	6.350	3.917	*	2.12	k= 4, v= 6
1.1	5.900	6.267	*	2.13	k= 5, v= 6

s = 0.191
Note: df used for table values are approximate when v > 20.

US EPA ARCHIVE DOCUMENT

female body length
File: 8202f Transform: NO TRANSFORM

KRUSKAL-WALLIS ANOVA BY RANKS - TABLE 1 OF 2

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	RANK SUM
1	control	7.300	7.300	23.000
2	0.068	7.050	7.050	13.000
3	0.14	7.100	7.100	15.000
4	0.26	7.050	7.050	12.000
5	0.50	7.050	7.050	12.000
6	1.1	6.300	6.300	3.000

Calculated H Value = 8.270 Critical H Value Table = 11.070
Since Calc H < Crit H FAIL TO REJECT Ho:All groups are equal.

female body length
File: 8202f Transform: NO TRANSFORM

DUNNS MULTIPLE COMPARISON - KRUSKAL-WALLIS - TABLE 2 OF 2

GROUP	IDENTIFICATION	TRANSFORMED MEAN	ORIGINAL MEAN	GROUP 0 0 0 0 0 0 6 4 5 2 3 1
6	1.1	6.300	6.300	\
4	0.26	7.050	7.050	. \
5	0.50	7.050	7.050	. . \
2	0.068	7.050	7.050	. . . \
3	0.14	7.100	7.100	. . . . \
1	**control**	**7.300**	**7.300**	**. . . . . **

* = significant difference (p=0.05) . = no significant difference
Table q value (0.05,6) = 2.936 SE = 3.529

male body weight
File: 8202md Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	0.030	0.006	3.000
Within (Error)	6	0.013	0.002	
Total	11	0.043		

US EPA ARCHIVE DOCUMENT

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

male body weight
File: 8202md Transform: NO TRANSFORMATION

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

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	control	0.910	0.910		
2	0.068	0.875	0.875	0.783	
3	0.14	0.895	0.895	0.335	
4	0.26	0.840	0.840	1.565	
5	0.50	0.795	0.795	2.571	
6	1.1	0.775	0.775	3.019	*

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

male body weight
File: 8202md Transform: NO TRANSFORMATION

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

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	control	2			
2	0.068	2	0.127	13.9	0.035
3	0.14	2	0.127	13.9	0.015
4	0.26	2	0.127	13.9	0.070
5	0.50	2	0.127	13.9	0.115
6	1.1	2	0.127	13.9	0.135

male body weight
File: 8202md Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	control	2	0.910	0.910	0.910
2	0.068	2	0.875	0.875	0.885
3	0.14	2	0.895	0.895	0.885
4	0.26	2	0.840	0.840	0.840
5	0.50	2	0.795	0.795	0.795
6	1.1	2	0.775	0.775	0.775

US EPA ARCHIVE DOCUMENT

male body weight
File: 8202md Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
control	0.910				
0.068	0.885	0.533		1.94	k= 1, v= 6
0.14	0.885	0.533		2.06	k= 2, v= 6
0.26	0.840	1.492		2.10	k= 3, v= 6
0.50	0.795	2.452	*	2.12	k= 4, v= 6
1.1	0.775	2.878	*	2.13	k= 5, v= 6

s = 0.047
Note: df used for table values are approximate when v > 20.

female body weight
File: 8202fd Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	0.076	0.015	5.000
Within (Error)	6	0.016	0.003	
Total	11	0.092		

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

female body weight
File: 8202fd Transform: NO TRANSFORMATION

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

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	control	1.295	1.295		
2	0.068	1.245	1.245	0.913	
3	0.14	1.115	1.115	3.286	*
4	0.26	1.280	1.280	0.274	
5	0.50	1.185	1.185	2.008	

US EPA ARCHIVE DOCUMENT

6	1.1	1.085	1.085	3.834 *

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

female body weight
File: 8202fd Transform: NO TRANSFORMATION

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

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	control	2			
2	0.068	2	0.155	12.0	0.050
3	0.14	2	0.155	12.0	0.180
4	0.26	2	0.155	12.0	0.015
5	0.50	2	0.155	12.0	0.110
6	1.1	2	0.155	12.0	0.210

female body weight
File: 8202fd Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	control	2	1.295	1.295	1.295
2	0.068	2	1.245	1.245	1.245
3	0.14	2	1.115	1.115	1.198
4	0.26	2	1.280	1.280	1.198
5	0.50	2	1.185	1.185	1.185
6	1.1	2	1.085	1.085	1.085

female body weight
File: 8202fd Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
control	1.295				
0.068	1.245	0.968		1.94	k= 1, v= 6
0.14	1.198	1.887		2.06	k= 2, v= 6
0.26	**1.198**	**1.887**		**2.10**	**k= 3, v= 6**
0.50	1.185	2.129	*	2.12	k= 4, v= 6
1.1	1.085	4.064	*	2.13	k= 5, v= 6

US EPA ARCHIVE DOCUMENT

---

s = 0.052
Note: df used for table values are approximate when v > 20.

US EPA ARCHIVE DOCUMENT