DATA EVALUATION RECORD
MIDGE CHRONIC TOXICITY STUDY
Non Guideline (OPPTS 850.1735)

7/27/2004

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

2. **TEST MATERIAL:** Atrazine Technical SF (a.i.) — Purity: 98.5%

3. **CITATION:**

Author: Arthur E. Putt

Title: Atrazine Technical SF - Toxicity to Midge (*Chironomus tetans)* Under Flow-Through Conditions

Study Completion Date: August 30, 2002

Laboratory: Springborn Smithers Laboratories
790 Main Street
Wareham, MA 02571

Sponsor: Syngenta Crop Protection, Inc.
410 Swing Road
Post Office Box 18300
Greensboro, NC 27419

Laboratory Report ID: 1781.6635

MRID No.: 45904001

DP Barcode: D290358

4. **REVIEWED BY:** Gregory Hess, Staff Scientist, Dynamac Corporation

Signature: [signature] **Date:** 5/10/04

APPROVED BY: Teri Myers, Staff Scientist, Dynamac Corporation

Signature: [signature] **Date:** 6/15/04

5. **APPROVED BY:**

Signature: [signature] **Date:** 7/27/04

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6. STUDY PARAMETERS:

Age of Test Organism:	3rd Instar, 10 days old
Definitive Test Duration:	10 days
Study Method:	Flow-through
Type of Concentrations:	Nominal and mean measured

7. CONCLUSIONS:

The 10-day acute toxicity of Atrazine Technical SF (a.i.; Atrazine) to the midge, *Chironomus tentans*, was studied under flow-through conditions in water-spiked exposures (sediment was not introduced into this test system). The nominal test concentrations were 0 (negative control), 4.3, 7.1, 12, 20 and 33 ppm a.i. The mean-measured concentrations <LOQ (0.48 to 0.49 ppm a.i.; negative control), 3.2, 5.3, 8.4, 16 and 24 ppm a.i. were 74, 75, 70, 81 and 73% of nominal, respectively. Endpoints assessed were larval mortality and growth; emergence and development rates were not assessed in this study.

Survival was 100, 98, 98, 100, 90, and 73% in the control, 3.2, 5.3, 8.4, 16, and 24 ppm a.i. treatment groups, respectively. Average dry weight per larvae was 4.09, 3.20, 3.14, 1.98, 0.31, and 0.12 mg in the control, 3.2, 5.3, 8.4, 16, and 24 ppm a.i. treatment groups, respectively.

This study was designed to follow guideline OPPTS 850.1735 (Public Draft), EPA-712-C-96-354 (April 1996), and does not fulfill any currently-approved U.S. EPA SEP guideline. This study is scientifically sound and is classified as SUPPLEMENTAL.

Results Synopsis:

Mortality
NOEC: 16 ppm a.i.
LOEC: 24 ppm a.i.

Growth
NOEC: <3.2 ppm a.i.
LOEC: 3.2 ppm a.i.

Endpoints affected: Survival and dry weight

8. ADEQUACY OF THE STUDY:

A. Classification: SUPPLEMENTAL

B. Rationale: The study does not fulfill any currently-approved U.S. EPA SEP guideline.

C. Repairability: None.

9. GUIDELINE DEVIATIONS:

The following sources were used as guidance in evaluating this study, and deviations from these guidance documents are listed below:

U.S. EPA. 1996. Ecological Effects Test Guidelines, OPPTS 850.1735 & 850.1790 (Public Draft), EPA-712-C-96-354. April 1996.

U.S. EPA. 2000. Methods for Measuring the Toxicity and Bioaccumulation of Sediment Associated Contaminants with Freshwater Invertebrates. Office of Research and Development and Office of Water, Washington, D.C. EPA/600/R-99/064. March 2000.

1. Particulate, TOC, COD and residual chlorine concentrations in the dilution water were not reported.

10. SUBMISSION PURPOSE: This study was submitted to provide information on the toxicity of Atrazine Technical SF (a.i.; Atrazine) to sediment-dwelling chironomids for the purpose of pesticide re-registration.

11. MATERIALS AND METHODS:

A. Test Organisms

Guideline Criteria	Reported Information
Species Chironomus tentans *Other species which can be used are Hyalella azteca, Chironomus riparius, Daphnia sp., Ceriodaphnia sp.* (Specific criteria for these species are not listed in this report)	*Chironomus tetans*

Guideline Criteria	Reported Information
Life Stage Second to third instar larvae (about 10 d old larvae with at least 50% at third instar.	3rd Instar, 10 days old
Supplier Brood stock can be obtained from laboratory, commercial, or government sources.(Sources obtained from the wild should be avoided unless cultured through several generations in the laboratory.)	From in house culture
All organisms from the same source?	Yes.

B. Source/Acclimation

Guideline Criteria	Reported Information
Acclimation Period Brood stock must be acclimated to culture water gradually from transport water to 100% culture water; water temperature exchange rate not to exceed 2°C within 24 hr; Avoid unnecessary stress, crowding and rapid temperature and water quality changes.	Twelve days prior to test initiation, fresh egg masses were removed from the culture aquaria and each individual egg mass was placed in a 250-ml beaker with 150 ml of culture water until hatching ended (May 10, 2002). Hatched larvae were transferred to a shallow plastic tub containing 1 L of culture water and a thin layer of silica sand as a substrate. Midge larvae were reared under static conditions at 23 to 25 °C with 8.2 to 9.6 mg/L dissolved oxygen under 18:6 light:dark photoperiod until the initiation of the definitive test.
Feeding Feeding should begin on day 0 and continue through day 9 unless food is not being eaten.	Larvae were fed 1.5 ml of a 10 mg/ml finely-ground flaked fish food suspension per replicate vessel per day. Feed was periodically analyzed for the presence of toxic metals, pesticides and PCBs in agreement with ASTM 2000, standard practice.

Guideline Criteria	Reported Information
Pretest Mortality A group of organisms should not be used if they appear unhealthy, discolored (eg <20% mortality 48 h before the beginning of a test).	No mortality was observed 48-hours prior to test initiation.

C. Test System

Guideline Criteria	Reported Information
Source of dilution water (Overlying water) and sediment Soft reconstituted water or water from a natural source, **not** de-chlorinated tap water. [Unpolluted well or spring that has been tested for contaminants, or appropriate reconstituted water (see ASTM for details)].	Dilution water was same as culture water, laboratory well-water with a total hardness and total alkalinity ranges as calcium carbonate of 40 to 52 and 30 to 36 mg/L, respectively and a pH of 7.6-7.7, and a specific conductivity of 160 to 170 µmhos/cm.
Does water support test animals without observable signs of stress?	Yes.
Quality Of Water If problems are observed in culturing or testing of organisms, it is desirable to test water quality. Particulate, TOC, COD should be <5 mg/L and residual chlorine <11 µg/L	No problems were reported. Midges have survived and reproduced several generations in the dilution water with no signs of disease or stress. Particulate, TOC, COD and residual chlorine concentrations were not reported.
Water Temperature 23°C ± 1°C. Daily mean test temperature Must not deviate more than ±1°C and instantaneous temperature must be within ±. Temperature should be monitored at least hourly throughout the test in one test chamber, and near the beginning, middle and end of the test in all test chambers.	Water temperature was maintained within the range of 22-24°C, and was measured daily in each exposure vessel and continuously in replicate C of the nominal 33 ppm a.i. treatment vessel.

Guideline Criteria	Reported Information
pH Not specified, but should be appropriate to the test species and should not deviate more than 0.4 pH units.	Treatment water ranged from 7.3 to 7.6 by 10-days and was measured daily in each test vessel.
Dissolved Oxygen Should be measured at the beginning and end of short term tests. DO should be >40 percent and <100 percent saturation.	DO ranged from 7.5-9.4 mg/L, and was measured daily in each test vessel.
Total Hardness Prefer 40 - 200 mg/L as $CaCO_3$.	At test initiation, 52 mg/L as $CaCO_3$ in the negative control and 56 mg/L as $CaCO_3$ at the nominal 33 ppm a.i. treatment level.
Conductivity Not specified, but should be amenable to the test species.	At test initiation, 200 µmhos/cm in the negative control and 210 µmhos/cm at nominal 33 ppm a.i. treatment level.
Sediment Characterization All sediment must be characterized for: pH, organic carbon content (TOC), total volatile sulfides, particle size distribution (% sand, silt, clay), and percent water content.	N/A; a thin layer (5 to 7 mm) of fine silica sand was added to each test vessel to reduce stress of the exposure system to the benthic organisms.
Additional Sediment Analysis BOD, COD, cation exchange capacity, Eh, pE, total inorganic carbon, total volatile solids, acid volatile sulfides, total ammonia, metals, organosilicones, synthetic organic compounds, oil and grease, petroleum hydrocarbons, and interstitial water analysis.	N/A

Guideline Criteria	Reported Information
Laboratory Spiked Sediment Material should be reagent grade unless prior evaluations dictate formulated materials, etc.; Must know the test material's identity, quantity of major ingredients and impurities, water solubility, estimated toxicity, precision and bias of analytical method, handling and disposal procedures.	N/A
Stock Solutions Test material should be dissolved in a solvent prior to mixing into test sediment; If solvent is used, both solvent control and negative control are required.	The test material was dissolved in laboratory well-water and mixed. A negative (dilution water) control was used in the test.
Test Concentrations For Spiked Sediment For LC50 calculation, test concentrations should bracket the predicted LC50; Sediment concentrations may be normalized to factors other than dry weight (e.g. organic content, acid volatile sulfides); Sediment may be mixed using rolling mill, feed mixer or hand mixer.	N/A, as the sediment was not spiked. Test concentrations were selected based upon the water solubility of the test material and information provided by the Study Sponsor (p. 14). Nominal test concentrations were 4.3, 7.1, 12, 20 and 33 ppm a.i. The definitive test was performed under flow-through conditions via an intermittent-proportional flow diluter with approx. 6 solution volume replacements per day in order to provide a 90% test solution replacement rate of approx. 9.0 hours.
Test Aquaria 1. Material: Glass or stainless steel or perfluorocarbon plastics. 2. Size: 300 ml high-form lipless beakers containing 100ml of sediment and 175 ml of overlying water.	1. Glass battery jars 2. 1.6 L jars containing two 2-cm holes (approximately 15 cm from the bottom) with a 5-7 mm layer of silica sand and 1.4 L of overlying water.

Guideline Criteria	Reported Information
Covers Static: Test vessels should be covered with a glass plate. Flow-through: openings in test compartments should be covered with mesh nylon or stainless steel screen.	Openings in test compartments were covered with 40-mesh Nitex® screen for drainage.
Type of Dilution System Must provide reproducible supply of toxicant.	Intermittent-flow proportional diluter.
Flow Rate Consistent flow rate of 5-10 vol/24 hours, meter systems calibrated before study and checked twice daily during test period.	Approx. 6 solution volume replacements per day in order to provide a 90% test solution replacement rate of approx. 9.0 hours.
Aeration Dilution water should be vigorously aerated so that dissolved oxygen in the overlying water remains above 40% saturation. In static systems, overlying water may be gently aerated through a 1-mL pipet located not closer than 2 cm from the sediment surface; Test organisms should not added 12 to 24h; Water quality characteristics should be measured before test organisms are added.	Not reported.
Photoperiod 16 hours light, 8 hours dark with a 15-30 min transition period and illuminance of about 100 to 1000 lux.	16 hours light, 8 hours dark. Light intensity averaged 750-970 lux.

Guideline Criteria	Reported Information
Solvents Use of a solvent should be avoided since they may influence the concentration in pore water. If used,it should not exceed 0.5 mL/L for static tests or 0.1 mL/L for flow-through tests. Acceptable solvents include triethylene glycol, methanol, ethanol, or acetone. Surfactants should not be used.	N/A.

D. Test Design

Guideline Criteria	Reported Information
Sediment Into Test Chambers One day prior (Day -1) to start of test: test sediment, reference sediment, and negative control sediment should be throughly homogenized and added to test chambers; Overlying water is added to chambers in a manner that minimizes suspension of sediment	Not reported, although it was reported that the exposure system was in operation 6 days prior to test initiation to allow equilibration of the test substance in the diluter apparatus and exposure vessels.
Renewal of Overlying Water: Renewal is required and flow rates should not differ by more than 10% in any two test chambers and should begin on day -1.	Approx. 6 solution volume replacements per day in order to provide a 90% test solution replacement rate of approx. 9.0 hours.

Guideline Criteria	Reported Information
Placing Organisms in Test Chambers: Should be handled as little as possible and introduced into overlying water below the air-water interface.	At test initiation, 3rd instar larvae were added from intermediate unlabeled vessels to the individual test vessels, one at a time. Midges were introduced into the test vessels under the surface of the test solution using a pipette until the test concentrations and negative control vessels contained a total of forty midges (10/replicate vessel; 4 reps./level).
Range Finding Test	Test concentrations were selected based upon the water solubility of the test material and information provided by the Study Sponsor (p. 14).
Monitoring the test All test chambers should be checked daily and observations made to assess organism behavior such as sediment avoidance.	Monitoring (behavioral and mortalities and physical characteristics) was performed daily.
Nominal Concentrations of Definitive Test Control(s) and at least 5 test concentrations; dilution factor not greater than 50%. Concentrations above aqueous solubility may be used.	0 (negative control), 4.3, 7.1, 12, 20 and 33 ppm a.i.
Number of Test Organisms 10 organisms per test chamber are recommended. 8 replicates per treatment should be used.	10 larvae/replicate with four (4) replicates/treatment level and 4 replicates for the negative control.
Test organisms randomly or impartially assigned to test vessels?	Yes.

Guideline Criteria	Reported Information
Feeding Midges in each test chamber are fed 1.5 ml of a 4 g/L Tetrafin® suspension daily. A drop in d.o. level below 2.5 mg/L may indicate over-feeding and feeding should be suspended in all treatments until d.o. levels increase.	Larvae were fed 1.5 ml of a 10.0 mg/ml finely-ground flaked fish food suspension per replicate vessel per day. Feed was periodically analyzed for the presence of toxic metals, pesticides and PCBs in agreement with ASTM 2000, standard practice.
Water Parameter Measurements Overlying Water Quality should measure conductivity, hardness, pH, alkalinity, and ammonia in all treatments at beginning and end of a test and should not vary by more than 50% within a treatment during the test.	Temperature, DO and pH were measured daily in each replicate vessel, temperature was also measured continuously in replicate c of the nominal 33 ppm a.i. treatment group. Conductivity, hardness and alkalinity of the dilution water were measured at test initiation.
Chemical Analysis Needed if solutions were aerated, if chemical was volatile, insoluble, or known to absorb, if precipitate formed, if containers were not steel or glass, or if flow-through system was used. Concentrations should be measured in bulk sediment, interstitial water, overlying water, and stock solution.	Test material concentrations within the exposure system were measured twice prior to test initiation in one replicate of the high, middle and low treatment level and the dilution water control. Test material concentrations were also measured at test initiation and termination in each replicate test and control vessel. The sediment was not assessed for treatment material.

12. REPORTED RESULTS:

A. General Results

Guideline Criteria	Reported Information
Quality assurance and GLP compliance statements were included in the report?	Yes.
Control Mortality Must be ≤ 30% in the sediment at end of the test.	Negative control: 0% (40/40) Solvent control: N/A (Table 4, p. 27).
Percent Recovery of Chemical: 1) % of nominal; 2) Procedural recovery; 3) Limit of quantitation (LOQ)	1. In overlying water at the nominal 4.3, 7.1, 12, 20 and 33 ppm a.i. treatment levels were 74, 75, 70, 81 and 73% of nominal by 10 days (Table 3, p. 26). 2. Quality control samples ranged from 84.0 to 90.5% of the nominal 4.0, 10.0 and 35.0 ppm a.i. QC levels by 10 days. 3. LOQ = 0.49 ppm a.i. day-0, 0.48 ppm a.i. day-10
Data Endpoints - Survival of Larvae - Ash-free dry weight (AFDW) should be determined by pooling all living organisms from a replicate and drying to a constant weight (e.g. 60°C for 24 h)	- Survival of larvae - Dry weight (dried for 24 hours at 60°C, p. 16)
Raw data included?	No.

Effects Data

Toxicant Concentration				Cumulative Number Dead (% mortality)[1]	Mean Dry Weight per midge, mg (Standard Deviation)
Nominal Overlying Water (ppm a.i.)	Mean-Measured				
	Sediment ppm	Pore Water ppm	Overlying Water ppm (% nominal)		
Neg. Control	ND	ND	<0.49 (N/A)	0 (0)	4.09 (0.29)
4.3	ND	ND	3.2 (74)	1 (2)	3.20 (0.15)*
7.1	ND	ND	5.3 (75)	1 (2)	3.14 (0.19)*
12	ND	ND	8.4 (70)	0 (0)	1.98 (0.41)*
20	ND	ND	16 (81)	4 (10)	0.31 (0.14)*
33	ND	ND	24 (73)	11 (27)	0.12 (0.01)*

ND - Not determined

[1] The cumulative number dead and (%) mortality were calculated from % survival data by the reviewer, see Excel file 4001_850-1735_Survival and Mortality in Raw Data folder.

* Significantly different ($p \leq 0.05$) from the control data according to the study author.

Nominal Overlying Water Concentrations (ppm a.i.)	No. of emerged midges	Mean Emergence Rate (%)			Mean Development Time (days)	Mean Development Rate (1/days)
		Total	Male	Female		
Neg. Control	ND	ND	ND	ND	ND	ND
4.3	ND	ND	ND	ND	ND	ND
7.1	ND	ND	ND	ND	ND	ND
12	ND	ND	ND	ND	ND	ND
20	ND	ND	ND	ND	ND	ND
33	ND	ND	ND	ND	ND	ND

ND - Not determined

Other Significant Results: None reported

B. Statistical Results

Method: After confirming normality and homogeneity of variances using Shapiro-Wilkes test and Bartlett's test, respectively, NOEC and LOEC values based on percent survival (arcsine square-root percentage transformed) data were determined using William's test. Growth data did not pass Bartlett's test for homogeneity of variances and was therefore analyzed non-parametrically using Steel's Many-One Rank test. The 10-day LC50 value was empirically estimated due to a lack of 50% mortality at any treatment level. EC_x values and associated 95% C.I. were calculated based on growth data. The study authors noted (pp. 9-10 and 21) that the NOEC values based on growth data were estimated based on the EC_{10} and EC_{25} values. For both endpoints, treatment groups were compared to the negative control group and mean-measured treatment concentrations. The above mentioned statistical methods were conducted via TOXSTAT v.3.5 statistical software.

Mortality
LC_{50}: >24 ppm a.i.
NOEC: 16 ppm a.i.
LOEC: 24 ppm a.i.

Growth

EC_{50}: 8.3 ppm a.i.	95% C.I.: 7.4 to 9.4 ppm a.i.
EC_{10}/NOEC: 1.3 ppm a.i.	95% C.I.: NR
EC_{25}/NOEC: 5.4 ppm a.i.	95% C.I.: NR

13. **VERIFICATION OF STATISTICAL RESULTS:**

Method: The mortality data passed the normality test, based on the Chi-Square statistic, but failed the test for homogeneity of variance, based on the Hartley and Bartlett's tests. NOEC and LOEC values based on percent survival (arcsine square-root percentage transformed) data were determined using William's test. Based on the reviewer's analysis, mortality was significantly different than the control group at the highest concentration tested (24 ppm a.i.). Growth data did not pass Bartlett's test for homogeneity of variances and was therefore analyzed non-parametrically using Steel's Many-One Rank test. The reviewer's statistical analysis of the growth endpoint was identical to study authors, although only the NOEC and LOEC values were verified. Based on this analysis, the NOEC and LOEC values for the growth endpoint are <3.2 and 3.2 ppm a.i., respectively. All statistical methods were conducted via TOXSTAT v3.3 statistical software.

Mortality
NOEC: 16 ppm a.i.
LOEC: 24 ppm a.i.

Growth
NOEC: <3.2 ppm a.i.
LOEC: 3.2 ppm a.i.

14. REVIEWER'S COMMENTS:

The study author's NOEC/LOEC values for mortality were identical to that of the reviewer. NOEC and LOEC values for the growth endpoint were derived based on hypothesis testing; therefore inclusion of EC_{50} and estimates of NOEC values based on EC_{10} and EC_{25} are not considered appropriate.

Development and emergence rates were not directly assessed within the definitive study because the study was only conducted for 10 days. However, the study author noted (p. 21) that :

> "Sibley, *et al.* (1997) demonstrated that *C. tentans* larvae need to reach a minimum dry weight (per larvae) of 0.5 to 0.8 mg in order to emerge successfully. In this feeding study, the midge larvae that reached a mean dry weight of 1.0 to 2.0 mg per larvae showed similar emergence patters and total midge emergence numbers. The results of this feeding study also established that the midge larvae that reached a mean dry weight of 1.5 mg per larvae all produced a similar mean number of eggs per female. Midge larvae that did not reach the 1.5 mg dry weight per larvae had reductions in eggs per female." "Based on Sibley, et al. (1997) and the dry weight observed in the three lowest treatment levels of this study (3.2, 5.3 and 8.4 ppm a.i.), the numbers of emerged midge and number of eggs per female should be similar to the control group if the organisms were allowed to continue their life-cycle under these conditions."

The reviewer concluded that the above statements may be valid inferences based on the actual collected data, but these statements are not a substitute for actual assessed reproductive endpoints that should be determined under the OPPTS guideline number 850.1790 and U.S. EPA. 2000. Methods for Measuring the Toxicity and Bioaccumulation of Sediment Associated Contaminants with Freshwater Invertebrates. Office of Research and Development and Office of Water, Washington, D.C. EPA/600/R-99/064. March 2000.

This study was conducted with a sediment-dwelling midge, and sediment concentrations were not measured at any treatment level in this study. However, sediment concentrations rather than overlying water concentrations were assessed in study MRID #: 45904002 (Atrazine

Technical SF; a.i.).

15. REFERENCES:

APHA, AWWA, WPCF. 1992. Standard Methods for the Examination of Water and Wastewater. 18th Edition, Washington, DC.

ASTM. 2000. Standard Paractice for Conducting Acute Toxicity Tests with Fishes, MacroInvertebrates and Amphibians. Standard E729-88a. American Society for Testing and Substances, 1916 Race Street, Philadelphia, PA. 19103.

Gulley, D.D., Boetler, A.M. and Bergman, H.L. 1996. Toxstat release 3.5. University of Wyoming, Laramie, Wyoming.

Oliver, D.R. 1971. Life History of the chironomide. Annual Review of Entomolgy. Volume 16, pp. 211-280.

Sibley, P.K., D.A. Benoit and G.T. Ankley. 1997. Significance of Growth in *Chironomus tentans* Sediment Toxicity Tests: Relationship to Reproduction and Demographic Endpoints. Environmental Toxicology and Chemistry, Vol. 16, No. 2, pp. 336-345.

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

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

U.S. EPA. 1982. Office of Pesticide Programs. Pesticide Guidelines, Subdivision E, Hazard Evaluation: Wildlife and Aquatic Organisms. EPA 540/9-82-024. October 1982. U.S. Environmental Protection Agency, Washington, DC.

U.S. EPA. 1985. Office of Pesticide Programs. Standard Evaluation Procedue for Acute Toxicity Test for Freshwater Invertebrates. EPA 540/9-85-005. June 1985. U.S. Environmental Protection Agency, Washington, DC.

U.S. EPA. 1996. Office of Prevention, Pesticides and Toxic Substances. Ecological Effects Test Guideline, OPPTS 850.1010. Aquatic Invertebrate Acute Toxicity Test, Freshwater Daphnids, "Public Draft" EPA 712-C-96-114 April 1996. U.S. Environmental Protection Agency. Washington, D.C.

Weber, C.I. *et al.* 1989. *Short-term methods for estimating the full life-cycle toxicity of effluents*

and recieving waters to freshwater organisms. 2nd edition. EPA/600/4/89/001. Environmental Monitoring Systems Laboratory, U.S. Environmental Protection Agency, Cincinnati, OH.

Williams, D.A. 1971. A test for differences between treatment means when several dose levels are compared with a zero dose control *Biometrics*, 27: 103-117.

Williams, D.A. 1972. A comparison of several dose levels iwth a zero dose control *Biometrics*, 28: 519-531.

MIDGE 10-DAY WATER SURVIVAL 459040-01
File: 001S.TXT Transform: ARC SINE(SQUARE ROOT(Y))

Chi-square test for normality: actual and expected frequencies

INTERVAL	<-1.5	-1.5 to <-0.5	-0.5 to 0.5	>0.5 to 1.5	>1.5
EXPECTED	1.608	5.808	9.168	5.808	1.608
OBSERVED	0	4	16	4	0

Calculated Chi-Square goodness of fit test statistic = 9.4329
Table Chi-Square value (alpha = 0.01) = 13.277

Data PASS normality test. Continue analysis.

MIDGE 10-DAY WATER SURVIVAL 459040-01
File: 001S.TXT Transform: ARC SINE(SQUARE ROOT(Y))

Hartley test for homogeneity of variance
Bartletts test for homogeneity of variance

These two tests can not be performed because at least one group has zero variance.

Data FAIL to meet homogeneity of variance assumption.
Additional transformations are useless.

TITLE: MIDGE 10-DAY WATER SURVIVAL 459040-01
FILE: 001S.TXT
TRANSFORM: ARC SINE(SQUARE ROOT(Y)) NUMBER OF GROUPS: 6

GRP	IDENTIFICATION	REP	VALUE	TRANS VALUE
1	1CONTROL	1	1.0000	1.3181
1	1CONTROL	2	1.0000	1.3181
1	1CONTROL	3	1.0000	1.3181
1	1CONTROL	4	1.0000	1.3181
2	3.2	1	1.0000	1.3181
2	3.2	2	1.0000	1.3181
2	3.2	3	0.9000	1.2490
2	3.2	4	1.0000	1.3181
3	5.3	1	1.0000	1.3181

3	5.3	2	1.0000	1.3181
3	5.3	3	0.9000	1.2490
3	5.3	4	1.0000	1.3181
4	8.4	1	1.0000	1.3181
4	8.4	2	1.0000	1.3181
4	8.4	3	1.0000	1.3181
4	8.4	4	1.0000	1.3181
5	16	1	1.0000	1.3181
5	16	2	0.7000	0.9912
5	16	3	1.0000	1.3181
5	16	4	0.9000	1.2490
6	24	1	0.6000	0.8861
6	24	2	0.4000	0.6847
6	24	3	1.0000	1.3181
6	24	4	0.9000	1.2490

MIDGE 10-DAY WATER SURVIVAL 459040-01
File: 001S.TXT Transform: ARC SINE(SQUARE ROOT(Y))

SUMMARY STATISTICS ON TRANSFORMED DATA TABLE 1 of 2

GRP	IDENTIFICATION	N	MIN	MAX	MEAN
1	1CONTROL	4	1.318	1.318	1.318
2	3.2	4	1.249	1.318	1.301
3	5.3	4	1.249	1.318	1.301
4	8.4	4	1.318	1.318	1.318
5	16	4	0.991	1.318	1.219
6	24	4	0.685	1.318	1.034

MIDGE 10-DAY WATER SURVIVAL 459040-01
File: 001S.TXT Transform: ARC SINE(SQUARE ROOT(Y))

SUMMARY STATISTICS ON TRANSFORMED DATA TABLE 2 of 2

GRP	IDENTIFICATION	VARIANCE	SD	SEM
1	1CONTROL	0.000	0.000	0.000
2	3.2	0.001	0.035	0.017
3	5.3	0.001	0.035	0.017
4	8.4	0.000	0.000	0.000
5	16	0.024	0.155	0.078
6	24	0.090	0.300	0.150

MIDGE 10-DAY WATER SURVIVAL 459040-01
File: 001S.TXT Transform: ARC SINE(SQUARE ROOT(Y))

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	1CONTROL	4	1.000	1.318	1.318
2	3.2	4	0.975	1.301	1.307
3	5.3	4	0.975	1.301	1.307
4	8.4	4	1.000	1.318	1.307
5	16	4	0.900	1.219	1.219
6	24	4	0.725	1.034	1.034

MIDGE 10-DAY WATER SURVIVAL 459040-01
File: 001S.TXT Transform: ARC SINE(SQUARE ROOT(Y))

WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
1CONTROL	1.318				
3.2	1.307	0.117		1.73	k= 1, v=18
5.3	1.307	0.117		1.82	k= 2, v=18
8.4	1.307	0.117		1.85	k= 3, v=18
16	1.219	1.003		1.86	k= 4, v=18
24	1.034	2.875	*	1.87	k= 5, v=18

s = 0.140
Note: df used for table values are approximate when v > 20.

MIDGE 10-DAY WATER SURVIVAL 459040-01
File: 001S.TXT Transform: ARC SINE(SQUARE ROOT(Y))

STEELS MANY-ONE RANK TEST - Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	RANK SUM	CRIT. VALUE	df	SIG
1	1CONTROL	1.318				
2	3.2	1.301	16.00	10.00	4.00	
3	5.3	1.301	16.00	10.00	4.00	
4	8.4	1.318	18.00	10.00	4.00	
5	16	1.219	14.00	10.00	4.00	
6	24	1.034	12.00	10.00	4.00	

Critical values use k = 5, are 1 tailed, and alpha = 0.05

MIDGE 10-DAY WATER BODY WEIGHT 459040-01
File: 001W.TXT Transform: NO TRANSFORMATION

Shapiro Wilks test for normality

D = 0.979

W = 0.978

Critical W (P = 0.05) (n = 24) = 0.916
Critical W (P = 0.01) (n = 24) = 0.884

Data PASS normality test at P=0.01 level. Continue analysis.

MIDGE 10-DAY WATER BODY WEIGHT 459040-01
File: 001W.TXT Transform: NO TRANSFORMATION

Bartletts test for homogeneity of variance

Calculated B statistic = 17.80
Table Chi-square value = 15.09 (alpha = 0.01)
Table Chi-square value = 11.07 (alpha = 0.05)

Average df used in calculation ==> df (avg n - 1) = 3.00
Used for Chi-square table value ==> df (#groups-1) = 5

Data FAIL homogeneity test at 0.01 level. Try another transformation.

NOTE: If groups have unequal replicate sizes the average replicate size is used to calculate the B statistic (see above).

TITLE: MIDGE 10-DAY WATER BODY WEIGHT 459040-01
FILE: 001W.TXT
TRANSFORM: NO TRANSFORMATION NUMBER OF GROUPS: 6

GRP	IDENTIFICATION	REP	VALUE	TRANS VALUE
1	1CONTROL	1	4.4700	4.4700
1	1CONTROL	2	3.8300	3.8300
1	1CONTROL	3	3.9000	3.9000
1	1CONTROL	4	4.1400	4.1400
2	3.2	1	3.3600	3.3600
2	3.2	2	3.3100	3.3100
2	3.2	3	3.0600	3.0600
2	3.2	4	3.0900	3.0900

3	5.3	1	2.9300	2.9300
3	5.3	2	3.2600	3.2600
3	5.3	3	3.0600	3.0600
3	5.3	4	3.3400	3.3400
4	8.4	1	2.2700	2.2700
4	8.4	2	1.5200	1.5200
4	8.4	3	1.7500	1.7500
4	8.4	4	2.3700	2.3700
5	16	1	0.3600	0.3600
5	16	2	0.3400	0.3400
5	16	3	0.4300	0.4300
5	16	4	0.1200	0.1200
6	24	1	0.1400	0.1400
6	24	2	0.1300	0.1300
6	24	3	0.1300	0.1300
6	24	4	0.1100	0.1100

MIDGE 10-DAY WATER BODY WEIGHT 459040-01
File: 001W.TXT Transform: NO TRANSFORMATION

SUMMARY STATISTICS ON TRANSFORMED DATA TABLE 1 of 2

GRP	IDENTIFICATION	N	MIN	MAX	MEAN
1	1CONTROL	4	3.830	4.470	4.085
2	3.2	4	3.060	3.360	3.205
3	5.3	4	2.930	3.340	3.147
4	8.4	4	1.520	2.370	1.978
5	16	4	0.120	0.430	0.313
6	24	4	0.110	0.140	0.128

MIDGE 10-DAY WATER BODY WEIGHT 459040-01
File: 001W.TXT Transform: NO TRANSFORMATION

SUMMARY STATISTICS ON TRANSFORMED DATA TABLE 2 of 2

GRP	IDENTIFICATION	VARIANCE	SD	SEM
1	1CONTROL	0.083	0.289	0.144
2	3.2	0.023	0.152	0.076
3	5.3	0.035	0.187	0.093
4	8.4	0.167	0.409	0.204
5	16	0.018	0.134	0.067
6	24	0.000	0.013	0.006

MIDGE 10-DAY WATER BODY WEIGHT 459040-01

File: 001W.TXT Transform: NO TRANSFORMATION

STEELS MANY-ONE RANK TEST - Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	RANK SUM	CRIT. VALUE	df	SIG
1	1CONTROL	4.085				
2	3.2	3.205	10.00	10.00	4.00	*
3	5.3	3.147	10.00	10.00	4.00	*
4	8.4	1.978	10.00	10.00	4.00	*
5	16	0.313	10.00	10.00	4.00	*
6	24	0.128	10.00	10.00	4.00	*

Critical values use k = 5, are 1 tailed, and alpha = 0.05