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

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)* During a 10-Day Sediment Exposure

Study Completion Date: March 10, 2003

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Laboratory Report ID: 1781.6636

MRID No.: 45904002

DP Barcode: D290358

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

Signature: **Date:** 5/10/04

APPROVED BY: Teri Myers, Staff Scientist, Dynamac Corporation

Signature: **Date:** 6/15/04

5. **APPROVED BY:**

Signature: **Date:** 7/27/04

6. STUDY PARAMETERS:

Age of Test Organism:	3rd Instar, 9 days old
Definitive Test Duration:	10 days
Study Method:	Static-renewal
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 static-renewal conditions in sediment-spiked exposures. The nominal sediment concentrations were 0 (negative and solvent controls), 63, 130, 250, 500 and 1000 ppm a.i. The mean-measured sediment concentrations were <LOD (4.7 to 4.8 ppm a.i.; negative and solvent controls), 24, 60, 130, 270 and 620 ppm a.i. and were 38, 46, 52, 54 and 62% of nominal, respectively. Pore water mean-measured concentrations were <LOD (0.51 to 0.57 ppm a.i., solvent and negative controls) 4.0, 21.5, 26, 29 and 30 ppm a.i. and overlying water concentrations were <LOD (0.029 to 0.030 ppm a.i., solvent and negative controls) 0.086, 0.42, 1.26, 1.96 and 2.03 ppm a.i. Endpoints assessed were larval mortality and growth; emergence and development rates were not assessed in this study.

Survival was 99, 94, 96, 97, 95, 86, and 92% in the negative control, solvent control, 24, 60, 130, 270, and 620 ppm a.i. treatment groups, respectively. Average dry weight was 1.74, 1.62, 1.51, 1.27, 1.02, 1.42, and 1.18 mg in the negative control, solvent control, 24, 60, 130, 270, and 620 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: Sediment: 130 ppm a.i.
Pore Water: 26 ppm a.i.
LOEC: Sediment: 270 ppm a.i.
Pore Water: 29 ppm a.i.

Growth

NOEC: Sediment: 24 ppm a.i.
Pore Water: 4.0 ppm a.i.
LOEC: Sediment: 60 ppm a.i.
Pore Water: 21.5 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. Total volatile sulfides were not characterized for the test sediment.

2. It was not reported if the test vessels were aerated or not during the definitive exposure.

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*
Life Stage Second to third instar larvae (about 10 d old larvae with at least 50% at third instar.	3rd Instar, 9 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.	Prior to test initiation, fresh egg masses were removed from the culture aquaria and placed in clean laboratory well water. Following hatching, each egg mass of hatched larvae was transferred to a 19-L aquarium containing 15-L of laboratory well water and a thin layer of silica sand. Midge larvae were reared under test conditions fro nine days and were nine days old at test initiation (third instar).

Guideline Criteria	Reported Information
Feeding Feeding should begin on day 0 and continue through day 9 unless food is not being eaten.	Midges were fed daily during culturing and rearing. Midges were fed a finely-groung suspension of Zeigler Brothers flaked fish food (i.e., 4.0 mg/ml). Feed was periodically analyzed for the presence of toxic metals, pesticides and PCBs. in agreement with ASTM 2000, standard practice.
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 44 to 46 and 34 to 35 mg/L, respectively and a pH of 7.6, and a specific conductivity of 170 to 190 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.

Guideline Criteria	Reported Information
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 22-25°C, and was measured in each exposure vessel at test initiation and termination, in one alternating replicate on days 1 through 9, and continuously in replicate 1 of the nominal 130 ppm a.i. treatment vessel.
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 6.4 to 6.8 by 10-days and was measured in each exposure vessel at test initiation and termination.
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 4.0 to 9.0 mg/L, and was measured daily in each test vessel and was measured in each exposure vessel at test initiation and termination, and in one alternating replicate on days 1 through 9. DO was > 40% saturation (i.e. 3.4 to 3.5 mg/L at 22 to 24°C, respectively).
Total Hardness Prefer 40 - 200 mg/L as $CaCO_3$.	At test initiation, 52 mg/L as $CaCO_3$ and 52 to 60 mg/L as $CaCO_3$ at test termination based on a composite sample from each treatment level collected on Day 0 and 10.
Conductivity Not specified, but should be amenable to the test species.	At test initiation, 200 to 210 µmhos/cm and 220 µmhos/cm at test termination based on a composite sample from each treatment level collected on Day 0 and 10.

Guideline Criteria	Reported Information
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.	Field-collected sediment was used for the test exposure, which was collected from Glen Charlie Pond, Wareham, MA (a freshwater site). Prior to use and characterization, the sediment was wet pressed to remove large particles, and then stored under refrigeration. The sediment was characterized by Agvise Labs, Northwood, ND as having a percent organic carbon content of 2.2%, a particle size distribution of 96% sand, 4% silt and 0% clay, a pH of 6.5, and water holding capacity at 1/3 bar of 6.5% (p. 15). Total volatile sulfides were not characterized.
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.	None reported.
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.	50 ml aliquots of the super stock were coated to their respective 3.79-L glass jars and rolled for several minutes to allow the acetone to evaporate (pp. 16-17). Approx. 1.16 kg (dry weight) of sediment was added to each jar and rolled for four hours at ~15 rpm. The sediment was rolled for an additional 2 hours prior to allocation of the sediment to the test vessels. The solvent control sediment was also rolled in the same manner as the treated sediment.

Guideline Criteria	Reported Information
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.	A 23.2 ppm a.i. super stock solution was prepared by placing 4.7102g of atrazine technical SF (4.6395 as active ingredient) in a 200-ml volumetric flask and diluting with acetone (pp. 16-17). A volume of super stock solution was added to the appropriate amount of dilution water to achieve 100 ml per desired nominal stock concentration (spiking stock conc.). A negative control and solvent (acetone) control were also prepared. Nominal spiking stock conc. were 1.46, 3.02, 5.80 11.6 and 23.2 ppm a.i.
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.	Test concentrations were selected based on toxicity information developed at Springborn Smithers from a range-finding exposure. Nominal sediment concentrations were 63, 130, 250, 500 and 1000 ppm a.i. The definitive test was performed under static-renewal conditions, only the sediment was spiked with test material, renewal was performed to maintain adequate overlying water characteristics, i.e DO, pH etc.
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 vessels that were chemically cleaned and rinsed several times using diluent water. 2. 300 ml containing 100 ml (2-cm layer) of sediment and 175 ml of overlying water. Test vessels had a hole cut on the top edge of the beaker (vessel) that was covered with 4-mesh Nitex® screen for drainage.

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.	Not reported.
Type of Dilution System Must provide reproducible supply of toxicant.	Intermittent delivery system (p. 18) in combination with a calibrated water delivery system that provided 1 L of water per cycle to the distribution system, which provided 50 ml of water per cycle to each replicate test chamber.
Flow Rate Consistent flow rate of 5-10 vol/24 hours, meter systems calibrated before study and checked twice daily during test period.	Approx. 2 volume additions per day sufficient enough to provide consistent and acceptable water quality conditions throughout the ten day exposure.
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 540-860 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.	Acetone, concentration not reported. It was reported (p. 17) that 50 ml of acetone was added to the test vessel as a control in the same manner that the test material was added to the treatment vessels. The solvent, acetone, was allowed to evaporate before the sediment was added.

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	Approx. 1.16 kg (dry weight) of sediment was added to each jar and rolled for four hours at ~15 rpm. The sediment was rolled for an additional 2 hours prior to allocation of the sediment to the test vessels, which occurred one day prior to test initiation. Overlying water was added with the aid of a turbulence minimizing plastic disk which allowed the water to be added without disturbing the underlying sediment.
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. 2 volume additions per day sufficient enough to provide consistent and acceptable water quality conditions throughout the ten day exposure.
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 test vessels one at a time into each individual test vessel. Midges were introduced into the test vessels under the surface of the test solution using a pipette until the test concentrations and control vessels contained a total of eighty midges (10/replicate vessels; 8 reps./level). Four replicates per level and control were maintained for chemical analysis of the sediment, pore water and overlying water at day 0 and 10.

Guideline Criteria	Reported Information
Range Finding Test	Test concentrations were selected based upon toxicity information developed at Springborn Smithers from a range-finding exposure (pp. 16, 23-25). See the Reviewer's Comments section of this DER for details.
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 an solvent controls), 63, 130, 250, 500 and 1000 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 eight (8) replicates/treatment level and 8 replicates for the negative and solvent controls.
Test organisms randomly or impartially assigned to test vessels?	Yes.
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 4.0 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.

Guideline Criteria	Reported Information
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 in the overlying water in each exposure vessel at test initiation and termination. In one alternating replicate, DO and temperature were measured on days 1 through 9. Temperature was continuously monitored in replicate 1 of the nominal 130 ppm a.i. treatment vessel. Conductivity, alkalinity, hardness and ammonia as nitrogen were measured test initiation, and at test termination based on a composite sample from each treatment level collected on Day 0 and 10.
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 were determined in the sediment, pore water and overlying water on test day 0 and 10. See the Reviewer's Comments section of this DER for details.

12. REPORTED RESULTS:

A. General Results

Guideline Criteria	Reported Information
Quality assurance and GLP compliance statements were included in the report?	Yes.

Guideline Criteria	Reported Information
Control Mortality Must be ≤ 30% in the sediment at end of the test.	Negative control: 1% (79/80 survive) Solvent control: 6% (75/80 survive) Reviewer calculated from mean percent survival, Table 6, p. 37. See Excel file 4002_850-1735_Survival and Mortality in Raw Data folder.

Guideline Criteria	Reported Information
Percent Recovery of Chemical: 1) % of nominal;	1. In spiked sediment at the nominal 63, 130, 250, 500 and 1000 ppm a.i. treatment levels recoveries were 51, 68, 80, 77 and 89% of nominal on day-0 and 27, 25, 28, 31 and 36% of nominal on day-10 (Table 3, p. 34). Pore water measured concentrations at the nominal 63, 130, 250, 500 and 1000 ppm a.i. treatment levels were <0.51 (LOD, neg. and solvent controls), 5.1, 26, 27, 29 and 26 ppm a.i. on day-0 and <0.57 (LOD, neg. and solvent controls), 2.9, 17, 25, 29 and 34 ppm a.i. on day-10. Overlying water measured concentrations at the nominal 63, 130, 250, 500 and 1000 ppm a.i. treatment levels were <0.029 (LOD, neg. and solvent controls), 0.13, 0.67, 2.0, 3.1 and 3.1 ppm a.i. on day-0 and <0.030 (LOD, neg. and solvent controls), 0.042, 0.17, 0.52, 0.81 and 0.95 ppm a.i. on day-10.
2) Procedural recovery;	2. Sediment quality control samples were 93.0, 90.3 and 88.6% of the nominal 50.0, 250 and 1000 ppm a.i. QC levels on day-0 and 102, 91.4 and 94.7% of nominal on day-10. Overlying water quality control samples were 119, 91.5 and 113% of the nominal 0.0100, 0.600 and 24.0 ppm a.i. QC levels on day-0 and 118, 91.3 and 99.5% of nominal on day-10.
3) Limit of quantitation (LOQ)	3. Sediment LOD = 4.7 ppm a.i. day 0 and 10.

Guideline Criteria	Reported Information
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 22 hours at 63°C, p. 29)
Raw data included?	No.

Effects Data

Toxicant Concentration				Cumulative Number Dead (% mortality)[4]	Mean Dry Weight per midge, mg (Standard Deviation)
Nominal Sediment, ppm a.i.	Mean-Measured				
	Sediment ppm (% Nominal[1])	Pore Water ppm[2]	Overlying Water ppm[3]		
Neg. Control	<LOD	<LOD	<LOD	1 (1)	1.74 (0.21)
Solvent Control	<LOD	<LOD	<LOD	5 (6)	1.62 (0.16)
63	24 (38)	4.0	0.086	3 (4)	1.51 (0.22)
130	60 (46)	21.5	0.42	2 (3)	1.27 (0.16)*
250	130 (52)	26	1.26	4 (5)	1.02 (0.06)*
500	270 (54)	29	1.96	11 (14)	1.42 (0.21)*
1000	620 (62)	30	2.03	6 (8)	1.18 (0.16)*

ND - Not determined

[1] Reviewer determined based on data provided in Table 3, p. 34, see Excel file 4002_850-1735_Survival and Mortality in Raw Data folder.

[2] Reviewer determined based on data provided in Table 4, p. 35.

[3] Reviewer determined based on data provided in Table 5, p. 36.

[4] The cumulative number dead and (%) mortality were calculated from % survival data by the reviewer

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

Nominal Sediment, 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
Solvent Control	ND	ND	ND	ND	ND	ND
63	ND	ND	ND	ND	ND	ND
130	ND	ND	ND	ND	ND	ND
250	ND	ND	ND	ND	ND	ND
500	ND	ND	ND	ND	ND	ND
1000	ND	ND	ND	ND	ND	ND

ND - Not determined

Other Significant Results: None reported

B. Statistical Results

Method: Survival and growth control data, respectively, were compared using a t-test, which indicated no significant differences. Consequently, the solvent control data was compared to the treatment concentrations in the determination of all toxicity values. After confirming normality and homogeneity of variances using Shapiro-Wilkes test and Bartlett's test, respectively, NOEC and LOEC values based on growth data were determined using William's test. Percent survival (arcsine square-root percentage transformed) did not pass Bartlett's test for normality and was therefore analyzed non-parametrically using Steel's Many-One Rank test. The 10-day LC_{50} value was empirically estimated due to a lack of 50% mortality at any treatment level. The 10-day EC_{50} value was empirically estimated due to a lack of 50% reduction in growth at any treatment level. For both endpoints, biological data were compared to the solvent control group and mean-measured sediment concentrations. The above mentioned statistical methods were conducted via TOXSTAT v.3.5 statistical software.

Mortality
LC_{50}: >620 ppm a.i. 95% C.I.: N/A
NOEC: 620 ppm a.i.
LOEC: >620 ppm a.i.

Growth
EC_{50}: >620 ppm a.i. 95% C.I.: N/A
NOEC: 24 ppm a.i.
LOEC: 60 ppm a.i.

Emergence Rate
EC_{50}: ND* 95% C.I.: ND
NOEC: ND
LOEC: ND

Development Rate
EC_{50}: ND 95% C.I.: ND
NOEC: ND
LOEC: ND

*ND = Not Determined

13. VERIFICATION OF STATISTICAL RESULTS:

Method: Based on the results of the t-test, there were no significant differences between the solvent and negative control data for mortality and growth endpoints. Therefore, the two data sets were pooled prior to comparison with the treatment concentrations. The mortality data failed both tests for normality and homogeneity of variance. 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 from the pooled control data at two highest concentrations (270 and 620 ppm a.i.). These results are different than the study author's because the author compared only the solvent control group (rather than the pooled control data) to treatment concentrations. After confirming normality and homogeneity of variance using the Chi-square test and Bartlett's test, respectively, NOEC and LOEC values based on growth were determined using Bonferroni's t-test. The results of the reviewer's statistical analysis of the growth endpoint were identical to the study author's, although only the NOEC and LOEC values were verified. Based on this analysis, the NOEC and LOEC values for growth endpoint are 24 and 60 ppm a.i., respectively. All statistical methods were conducted via TOXSTAT v3.3 statistical software.

Mortality
NOEC: 130 ppm a.i.
LOEC: 270 ppm a.i.

Growth
NOEC: 24 ppm a.i.
LOEC: 60 ppm a.i.

14. REVIEWER'S COMMENTS:

The study author noted (p. 23-25) that: prior to initiating the definitive study, a preliminary range-finding exposure was conducted at nominal atrazine levels of 1.0, 10, 50, 100, 500 and 1000 ppm a.i. with negative and solvent controls. Forty midge larvae were exposed at each treatment level and control for 10 days. Mean-measured treatment values were not reported. By 10 days, average percent survival was 100 and 95% in the negative and solvent controls, respectively, and 88, 83, 80, 85, 90 and 90% at the nominal 1.0, 10, 50, 100, 500 and 1000 ppm a.i. treatment levels, respectively. Average dry weight per midge larvae was 2.43 and 2.31 in the negative and solvent controls, respectively, and 2.18, 2.47, 2.55, 2.25, 2.14 and 2.20 mg at the nominal 1.0, 10, 50, 100, 500 and 1000 ppm a.i. treatment levels, respectively. Based on these range-finding results, nominal atrazine concentrations of 63, 130, 250, 500 and 1000 ppm a.i. were selected for an initial definitive exposure. Eighty midge larvae (9-days old) were exposed to each treatment level and control for 10 days. Mean-measured treatment values were not reported. By 10 days, average percent survival was 93 and 94% in the negative and solvent controls, respectively, and 91, 100, 93, 91 and 90% at the nominal 63, 130, 250, 500 and 1000 ppm a.i. treatment levels, respectively. Average dry weight per midge larvae was 1.40 and 1.63 in the negative and solvent controls, respectively, and 1.33, 1.21, 1.36, 1.17 and 0.96 mg at the nominal 63, 130, 250, 500 and 1000 ppm a.i. treatment levels, respectively. By day-10, growth was significantly reduced at all treatment levels compared to the solvent control data and was significantly reduced at the 130, 250, 500 and 1000 ppm a.i. treatment levels compared to the negative control. Based on the observed difference in midge growth as well as a lack of an established NOEC, a second definitive exposure (the current study, MRID # 45904002) was conducted at the same nominal treatment levels.

The study author also noted (p. 25-26) that: Prior to sediment spiking, the stock solutions were observed to be cloudy and colorless. Three days prior to definitive study initiation during the mixing and equilibration period, a sample of each treated and control sediment was analyzed. Measured concentrations for the nominal 63, 130, 250, 500 and 1000 ppm a.i. treatment levels were 39, 100, 210, 420 and 820 ppm a.i., respectively. Percent recovery ranged from 62 to 85% of nominal and recovery increased with increasing test concentration. This trend may indicate that a fraction of the test material applied to the

sediment bound and became non-extractable over time. The impact on the percent recovery in the lower test levels is more significant than in the higher treatment levels suggesting that the loss is mass dependent. The QC samples did not reflect the same trend because they were prepared and analyzed immediately at each specific sampling interval.

During the in-life phase of the definitive study, pore water and overlying water samples were removed and analyzed for atrazine technical SF (a.i.) concentration on test days 0 and 10. On day 0, overlying water samples were removed from replicate I of all treatment levels and controls and were analyzed, and on day 10, samples were removed from replicate J of all treatment levels and controls and were analyzed. Samples were taken from the approximate midpoint of each vessel. Replicates I through L did not contain midge larvae (replicate A through H did contain midge larvae) and were established for chemical analysis purposes. Pore water samples were collected by removing the entire sediment sample and centrifuging for 15 minutes at 3,000 rpm (approx. 1000 g). The resulting pore water was decanted and composited. Sediment samples were collected with a steel spatula from the centrifuge tubes, following centrifugation and removal of the pore water sample.

The reviewer did not verify the study author's 10-day LC_{50} and EC_{50} values because the purpose of the chronic test is to derive NOEC and LOEC values, based on hypothesis testing. The reviewer's NOEC and LOEC values based on mortality data were lower than those reported by the study author because the reviewer compared all treatment concentrations to pooled control data, rather than the solvent control data only. The results of the reviewer's statistical analysis of the growth endpoint were identical to the study author's

Development and emergence rates were not assessed within this definitive study.

15. REFERENCES:

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MIDGE 10-DAY SEDIMENT SURVIVAL 459040-02
File: 45904002.txt Transform: NO TRANSFORMATION

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	3.752	13.552	21.392	13.552	3.752
OBSERVED	5	11	16	24	0

Calculated Chi-Square goodness of fit test statistic = 14.0617
Table Chi-Square value (alpha = 0.01) = 13.277

Data FAIL normality test. Try another transformation.

Warning - The two homogeneity tests are sensitive to non-normal data and should not be performed.

MIDGE 10-DAY SEDIMENT SURVIVAL 459040-02
File: 45904002.txt Transform: NO TRANSFORMATION

Bartletts test for homogeneity of variance

Calculated B statistic = 23.36
Table Chi-square value = 16.81 (alpha = 0.01)
Table Chi-square value = 12.59 (alpha = 0.05)

Average df used in calculation ==> df (avg n - 1) = 7.00
Used for Chi-square table value ==> df (#groups-1) = 6

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).

MIDGE 10-DAY SEDIMENT SURVIVAL 459040-02
File: 45904002.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	3.752	13.552	21.392	13.552	3.752
OBSERVED	5	11	16	24	0

```
---------------------------------------------------------------------------
Calculated Chi-Square goodness of fit test statistic =  14.0617
Table Chi-Square value (alpha = 0.01) = 13.277

Data FAIL normality test. Try another transformation.

Warning -  The two homogeneity tests are sensitive to non-normal data and
           should not be performed.


MIDGE 10-DAY SEDIMENT SURVIVAL 459040-02
File: 45904002.txt       Transform: ARC SINE(SQUARE ROOT(Y))

Bartletts test for homogeneity of variance
---------------------------------------------------------------------------

Calculated B statistic =   18.07
Table Chi-square value =   16.81  (alpha = 0.01)
Table Chi-square value =   12.59  (alpha = 0.05)

Average df used in calculation  ==>   df (avg n - 1) =   7.00
Used for Chi-square table value ==>   df (#groups-1) =   6
---------------------------------------------------------------------------

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).


MIDGE 10-DAY SEDIMENT SURVIVAL 459040-02
File: 45904002.txt       Transform: ARC SINE(SQUARE ROOT(Y))

       t-test of Solvent and Blank Controls          Ho:GRP1 MEAN = GRP2 MEAN
---------------------------------------------------------------------------
-
 GRP1 (SOLVENT CRTL) MEAN =     1.3033     CALCULATED t VALUE =    -1.7178
 GRP2 (BLANK CRTL) MEAN   =     1.3751     DEGREES OF FREEDOM =    14
 DIFFERENCE IN MEANS      =    -0.0718
---------------------------------------------------------------------------
-
TABLE t VALUE (0.05 (2),14) =  2.145    NO significant difference at
alpha=0.05
TABLE t VALUE (0.01 (2),14) =  2.977    NO significant difference at
alpha=0.01



TITLE:     MIDGE 10-DAY SEDIMENT SURVIVAL 459040-02
FILE:      45904002.txt
TRANSFORM: ARC SINE(SQUARE ROOT(Y))                NUMBER OF GROUPS: 6
---------------------------------------------------------------------------
```

GRP	IDENTIFICATION	REP	VALUE	TRANS VALUE
1	GRPS 1&2 POOLED	1	1.0000	1.3931
1	GRPS 1&2 POOLED	2	1.0000	1.3931
1	GRPS 1&2 POOLED	3	1.0000	1.3931
1	GRPS 1&2 POOLED	4	0.9000	1.2490
1	GRPS 1&2 POOLED	5	0.9000	1.2490
1	GRPS 1&2 POOLED	6	0.9000	1.2490
1	GRPS 1&2 POOLED	7	0.8000	1.1071
1	GRPS 1&2 POOLED	8	1.0000	1.3931
1	GRPS 1&2 POOLED	9	0.9000	1.2490
1	GRPS 1&2 POOLED	10	1.0000	1.3931
1	GRPS 1&2 POOLED	11	1.0000	1.3931
1	GRPS 1&2 POOLED	12	1.0000	1.3931
1	GRPS 1&2 POOLED	13	1.0000	1.3931
1	GRPS 1&2 POOLED	14	1.0000	1.3931
1	GRPS 1&2 POOLED	15	1.0000	1.3931
1	GRPS 1&2 POOLED	16	1.0000	1.3931
2	24	1	1.0000	1.3931
2	24	2	1.0000	1.3931
2	24	3	0.9000	1.2490
2	24	4	0.9000	1.2490
2	24	5	1.0000	1.3931
2	24	6	1.0000	1.3931
2	24	7	0.9000	1.2490
2	24	8	1.0000	1.3931
3	60	1	1.0000	1.3931
3	60	2	1.0000	1.3931
3	60	3	0.9300	1.3030
3	60	4	0.9000	1.2490
3	60	5	1.0000	1.3931
3	60	6	0.9000	1.2490
3	60	7	1.0000	1.3931
3	60	8	1.0000	1.3931
4	130	1	0.9000	1.2490
4	130	2	1.0000	1.3931
4	130	3	1.0000	1.3931
4	130	4	1.0000	1.3931
4	130	5	0.8000	1.1071
4	130	6	0.9000	1.2490
4	130	7	1.0000	1.3931
4	130	8	1.0000	1.3931
5	270	1	1.0000	1.3931
5	270	2	1.0000	1.3931
5	270	3	0.8000	1.1071
5	270	4	0.5000	0.7854
5	270	5	1.0000	1.3931
5	270	6	0.9000	1.2490
5	270	7	0.8000	1.1071
5	270	8	0.9000	1.2490
6	620	1	0.9000	1.2490
6	620	2	0.9000	1.2490
6	620	3	0.9300	1.3030
6	620	4	1.0000	1.3931
6	620	5	1.0000	1.3931

6	620	6	0.9000	1.2490
6	620	7	0.9000	1.2490
6	620	8	0.8000	1.1071

MIDGE 10-DAY SEDIMENT SURVIVAL 459040-02
File: 45904002.txt Transform: ARC SINE(SQUARE ROOT(Y))

SUMMARY STATISTICS ON TRANSFORMED DATA TABLE 1 of 2

GRP	IDENTIFICATION	N	MIN	MAX	MEAN
1	GRPS 1&2 POOLED	16	1.107	1.393	1.339
2	24	8	1.249	1.393	1.339
3	60	8	1.249	1.393	1.346
4	130	8	1.107	1.393	1.321
5	270	8	0.785	1.393	1.210
6	620	8	1.107	1.393	1.274

MIDGE 10-DAY SEDIMENT SURVIVAL 459040-02
File: 45904002.txt Transform: ARC SINE(SQUARE ROOT(Y))

SUMMARY STATISTICS ON TRANSFORMED DATA TABLE 2 of 2

GRP	IDENTIFICATION	VARIANCE	SD	SEM
1	GRPS 1&2 POOLED	0.008	0.089	0.022
2	24	0.006	0.075	0.026
3	60	0.005	0.067	0.024
4	130	0.012	0.108	0.038
5	270	0.044	0.209	0.074
6	620	0.009	0.092	0.033

MIDGE 10-DAY SEDIMENT SURVIVAL 459040-02
File: 45904002.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	GRPS 1&2 POOLED	16	0.963	1.339	1.341
2	24	8	0.963	1.339	1.341
3	60	8	0.966	1.346	1.341
4	130	8	0.950	1.321	1.321
5	270	8	0.863	1.210	1.242
6	620	8	0.916	1.274	1.242

MIDGE 10-DAY SEDIMENT SURVIVAL 459040-02
File: 45904002.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
GRPS 1&2 POOLED	1.341				
24	1.341	0.033		1.68	k= 1, v=50
60	1.341	0.033		1.76	k= 2, v=50
130	1.321	0.366		1.79	k= 3, v=50
270	1.242	1.994	*	1.80	k= 4, v=50
620	1.242	1.994	*	1.80	k= 5, v=50

s = 0.113
Note: df used for table values are approximate when v > 20.

MIDGE 10-DAY SEDIMENT SURVIVAL 459040-02
File: 45904002.txt Transform: ARC SINE(SQUARE ROOT(Y))

KRUSKAL-WALLIS ANOVA BY RANKS - TABLE 1 OF 2 (p=0.05)

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	RANK SUM
1	GRPS 1&2 POOLED	1.339	0.963	515.000
2	24	1.339	0.963	250.000
3	60	1.346	0.966	259.500
4	130	1.321	0.950	239.000
5	270	1.210	0.863	162.000
6	620	1.274	0.916	170.500

Calculated H Value = 6.446 Critical H Value Table = 11.070
Since Calc H < Crit H FAIL TO REJECT Ho:All groups are equal.

MIDGE 10-DAY SEDIMENT SURVIVAL 459040-02
File: 45904002.txt Transform: ARC SINE(SQUARE ROOT(Y))

DUNNS MULTIPLE COMPARISON - KRUSKAL-WALLIS - TABLE 2 OF 2 (p=0.05)

GROUP	IDENTIFICATION	TRANSFORMED MEAN	ORIGINAL MEAN	GROUP 0 5	0 6	0 4	0 2	0 1	0 3
5	270	1.210	0.863	\					
6	620	1.274	0.916	.	\				

```
  4              130       1.321      0.950  . . \
  2               24       1.339      0.963  . . . \
  1   GRPS 1&2 POOLED      1.339      0.963  . . . . \
  3               60       1.346      0.966  . . . . . \
-------------------------------------------------------------------------
* = significant difference (p=0.05)            . = no significant difference
Table q value (0.05,6) =   2.936          Unequal reps - several SE values
used


MIDGE 10-DAY SEDIMENT BW 459040-02
File: 02BW.TXT       Transform: NO TRANSFORMATION

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     3.752        13.552            21.392          13.552       3.752
OBSERVED     2            15                24              10           5

-------------------------------------------------------------------------
Calculated Chi-Square goodness of fit test statistic =   2.6369
Table Chi-Square value (alpha = 0.01) = 13.277

Data PASS normality test. Continue analysis.


MIDGE 10-DAY SEDIMENT BW 459040-02
File: 02BW.TXT       Transform: NO TRANSFORMATION

Bartletts test for homogeneity of variance
-------------------------------------------------------------------------

Calculated B statistic =   10.96
Table Chi-square value =   16.81  (alpha = 0.01)
Table Chi-square value =   12.59  (alpha = 0.05)

Average df used in calculation  ==>   df (avg n - 1) =   7.00
Used for Chi-square table value ==>   df (#groups-1) =   6
-------------------------------------------------------------------------

Data PASS homogeneity test at 0.01 level. Continue analysis.

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


MIDGE 10-DAY SEDIMENT BW 459040-02
File: 02BW.TXT       Transform: NO TRANSFORMATION

      t-test of Solvent and Blank Controls          Ho:GRP1 MEAN = GRP2 MEAN
```

GRP1 (SOLVENT CRTL) MEAN =	1.6263	CALCULATED t VALUE =	-1.1936
GRP2 (BLANK CRTL) MEAN =	1.7388	DEGREES OF FREEDOM =	14
DIFFERENCE IN MEANS =	-0.1125		

TABLE t VALUE (0.05 (2),14) = 2.145 NO significant difference at alpha=0.05
TABLE t VALUE (0.01 (2),14) = 2.977 NO significant difference at alpha=0.01

TITLE: MIDGE 10-DAY SEDIMENT BW 459040-02
FILE: 02BW.TXT
TRANSFORM: NO TRANSFORMATION NUMBER OF GROUPS: 6

GRP	IDENTIFICATION	REP	VALUE	TRANS VALUE
1	GRPS 1&2 POOLED	1	1.7400	1.7400
1	GRPS 1&2 POOLED	2	1.5800	1.5800
1	GRPS 1&2 POOLED	3	1.5400	1.5400
1	GRPS 1&2 POOLED	4	1.5100	1.5100
1	GRPS 1&2 POOLED	5	1.4000	1.4000
1	GRPS 1&2 POOLED	6	1.8000	1.8000
1	GRPS 1&2 POOLED	7	1.5700	1.5700
1	GRPS 1&2 POOLED	8	1.8700	1.8700
1	GRPS 1&2 POOLED	9	1.6700	1.6700
1	GRPS 1&2 POOLED	10	1.6100	1.6100
1	GRPS 1&2 POOLED	11	1.7000	1.7000
1	GRPS 1&2 POOLED	12	1.6200	1.6200
1	GRPS 1&2 POOLED	13	2.2000	2.2000
1	GRPS 1&2 POOLED	14	1.6600	1.6600
1	GRPS 1&2 POOLED	15	1.5500	1.5500
1	GRPS 1&2 POOLED	16	1.9000	1.9000
2	24	1	1.7100	1.7100
2	24	2	1.4100	1.4100
2	24	3	1.5500	1.5500
2	24	4	1.3500	1.3500
2	24	5	1.5000	1.5000
2	24	6	1.1900	1.1900
2	24	7	1.9000	1.9000
2	24	8	1.4400	1.4400
3	60	1	1.4300	1.4300
3	60	2	1.3100	1.3100
3	60	3	0.9900	0.9900
3	60	4	1.5100	1.5100
3	60	5	1.2500	1.2500
3	60	6	1.1600	1.1600
3	60	7	1.2700	1.2700
3	60	8	1.2500	1.2500
4	130	1	1.0300	1.0300

4	130	2	0.9600	0.9600
4	130	3	1.0300	1.0300
4	130	4	0.9400	0.9400
4	130	5	0.9900	0.9900
4	130	6	1.0800	1.0800
4	130	7	1.0800	1.0800
4	130	8	1.0900	1.0900
5	270	1	1.3800	1.3800
5	270	2	1.4100	1.4100
5	270	3	1.4100	1.4100
5	270	4	1.8500	1.8500
5	270	5	1.1200	1.1200
5	270	6	1.2900	1.2900
5	270	7	1.5000	1.5000
5	270	8	1.3800	1.3800
6	620	1	1.2800	1.2800
6	620	2	1.2200	1.2200
6	620	3	0.8400	0.8400
6	620	4	1.2500	1.2500
6	620	5	1.2300	1.2300
6	620	6	1.3900	1.3900
6	620	7	1.0900	1.0900
6	620	8	1.1200	1.1200

MIDGE 10-DAY SEDIMENT BW 459040-02
File: 02BW.TXT Transform: NO TRANSFORMATION

SUMMARY STATISTICS ON TRANSFORMED DATA TABLE 1 of 2

GRP	IDENTIFICATION	N	MIN	MAX	MEAN
1	GRPS 1&2 POOLED	16	1.400	2.200	1.683
2	24	8	1.190	1.900	1.506
3	60	8	0.990	1.510	1.271
4	130	8	0.940	1.090	1.025
5	270	8	1.120	1.850	1.418
6	620	8	0.840	1.390	1.177

MIDGE 10-DAY SEDIMENT BW 459040-02
File: 02BW.TXT Transform: NO TRANSFORMATION

SUMMARY STATISTICS ON TRANSFORMED DATA TABLE 2 of 2

GRP	IDENTIFICATION	VARIANCE	SD	SEM
1	GRPS 1&2 POOLED	0.037	0.191	0.048
2	24	0.048	0.219	0.078
3	60	0.025	0.159	0.056
4	130	0.003	0.057	0.020

5	270	0.043	0.208	0.073
6	620	0.027	0.165	0.058

MIDGE 10-DAY SEDIMENT BW 459040-02
File: 02BW.TXT Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	3.022	0.604	19.177
Within (Error)	50	1.576	0.032	
Total	55	4.597		

Critical F value = 2.45 (0.05,5,40)
Since F > Critical F REJECT Ho:All groups equal

MIDGE 10-DAY SEDIMENT BW 459040-02
File: 02BW.TXT Transform: NO TRANSFORMATION

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

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	GRPS 1&2 POOLED	1.683	1.683		
2	24	1.506	1.506	2.293	
3	60	1.271	1.271	5.350	*
4	130	1.025	1.025	8.554	*
5	270	1.418	1.418	3.447	*
6	620	1.177	1.177	6.570	*

Bonferroni T table value = 2.40 (1 Tailed Value, P=0.05, df=50,5)

MIDGE 10-DAY SEDIMENT BW 459040-02
File: 02BW.TXT Transform: NO TRANSFORMATION

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

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	GRPS 1&2 POOLED	16			
2	24	8	0.185	11.0	0.176
3	60	8	0.185	11.0	0.411

4	130	8	0.185	11.0	0.657
5	270	8	0.185	11.0	0.265
6	620	8	0.185	11.0	0.505