

**DATA EVALUATION RECORD
MIDGE CHRONIC TOXICITY STUDY
OPPTS Draft Guideline 850.1735**

1. **CHEMICAL**: Penoxsulam

PC Code No.: 119031
199031

2. **TEST MATERIAL**: XDE-638

Purity: 97.7%

3. **CITATION**:

Author: Putt, A.E.

Title: XDE-638 - The Full Life-Cycle Toxicity To Midge
(*Chironomus riparius*) Under Static Conditions Using
Spiked Sediment and Spiked Water.

Study Completion Date: September 26, 2002

Laboratory: Springborn Laboratories, Inc.
790 Main Street
Wareham, MA 02571-1075

Sponsor: The Dow Chemical Company
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Laboratory Report ID: 12550.6254/Dow Study No. 021160

MRID No.: 45831102


DP Barcode: D288160

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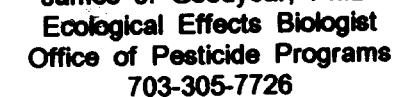
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James J. Goodyear, Ph.D.

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Date: 



6. STUDY PARAMETERS:

Age of Test Organism:	1 st Instar, 3 days old
Definitive Test Duration:	28 days
Study Method:	Static
Type of Concentrations:	Mean-measured

7. RESULTS and CONCLUSIONS:

The 28-day chronic toxicity of XDE-638 Technical (penoxsulam) to the midge, *Chironomus riparius*, was studied under static conditions in sediment-spiked and water-spiked exposures. Endpoints assessed were the percent emerged and development rate (male, female, and combined sexes). Survival and ash-free dry weights were not assessed in this study.

Sediment-spiked exposure: The nominal test concentrations were 0 (negative and solvent controls), 31, 63, 130, 250, 500, and 1000 mg a.i./kg; initial (0 hour) mean-measured concentrations measured in the sediment were <0.77 (<LOQ; controls), 10, 29, 76, 170, 400, and 810 mg a.i./kg dry weight. After 28 days, recoveries averaged 10-24% of nominal in the sediment, 0.4-10% of nominal in the overlying water, and 0.4-5% in the pore water.

No treatment-related effects on percent emergence or development rates were observed. Mean percent emergence ranged from 76-91% and mean development rates (combined sexes) ranged from 0.0627 to 0.0661 for all test and control groups. The NOAEC was the highest concentration tested, 810 mg a.i./kg dry weight (nominal 1000 mg a.i./kg dry weight).

Water-spiked exposure: The nominal test concentrations were 0 (negative control), 13, 25, 50, 100, and 200 mg a.i./L; initial (1 hour) mean-measured concentrations were <0.33 (<LOQ; control), 7.1, 15, 31, 61, and 140 mg a.i./L. After 28 days, recoveries averaged 49-70% of nominal in the overlying water, 24-34% of nominal in the sediment, and 48-70% of nominal in the pore water.

Statistically-significant reductions in the development rate (combined sexes) were observed at the 15 and 140 mg a.i./L levels (nominal 25 and 200 mg a.i./L levels, respectively). The development rates were 0.0660 for the control group, compared to 0.0639, 0.0604, 0.0636, 0.0629, and 0.0615 for 7.1, 15, 31, 61, and 140 mg a.i./L test levels, respectively. No treatment-related effects on percent emergence were observed; mean percent emergence ranged from 90-96% for all test and control groups. Based on reductions in the development rate, the NOAEC and LOAEC were 7.1 and 15 mg a.i./L, respectively.

This study was designed to fulfill OECD Draft Guidelines 218 and 219, and does not fulfill any current U.S. EPA guideline. This study is scientifically sound, and provides useful information on the 28-day toxicity of XDE-638 Technical to the midge, *Chironomus riparius*, under static conditions. This study is classified as SUPPLEMENTAL. It deviates from proposed guidelines requirements of OPPTS Guidelines 218 and 219. If OECD Guidelines 217 and 218 are required in the future, and if the registrant requests registrations in the future, this study may have to be repeated.

Results Synopsis:**Sediment-spiked exposure** (initial sediment concentrations):LC₅₀ (mortality): Not reportedEC₅₀ (growth): Not reportedEC₅₀ (emergence): >810 mg a.i./kgEC₅₀ (development rate): >810 mg a.i./kg

NOAEC: 810 mg a.i./kg

LOAEC: >810 mg a.i./kg

Endpoints affected: None

Water-spiked exposure:LC₅₀ (mortality): Not reportedEC₅₀ (growth): Not reportedEC₅₀ (emergence): >140 mg a.i./LEC₅₀ (development rate): >140 mg a.i./L

NOAEC: 7.1 mg a.i./L

LOAEC: 13 mg a.i./L

Endpoints affected: Development rate for combined sexes

8. ADEQUACY OF THE STUDY:**A. Classification:** Supplemental**B. Rationale:** This study was not designed to fulfill any current U.S. EPA guideline.**C. Repairability:** N/A**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 (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. The recommended species for use is *Chironomus tentans*.
2. The study was initiated with 3-day old larvae (first instar), whereas 10-day old larvae (second to third instar) are recommended.
3. Initial measurements of length and weight should have been provided for a sub-set,

and terminal ash-free dry weights should have been determined.

4. The pH of the overlying water during the sediment-spiked exposure ranged from 7.0 to 8.2 and of the overlying water during the water-spiked exposure ranged from 6.7 to 7.8; guidance states that the pH should not deviate more than 0.4 units.
 5. Sediments were not analyzed for BOD, cation exchange capacity, COD, Eh, total inorganic carbon, total volatile solids, acid volatile sulfides, metals, oil and grease, and petroleum hydrocarbons. These are suggested for additional sediment analyses.
 6. The test vessels were covered by clear plastic plates instead of glass covers as recommended for static tests.
 7. Although eight replicate vessels were maintained, only four of the eight vessels were used to collect biological data. The remaining four were used to measure test concentrations.
 8. Overlying and pore water concentrations were not analyzed at every nominal level in the sediment-spiked exposure, and pore water and sediment concentrations were not analyzed at every nominal level tested in the water-spiked exposure.
 9. Survival of larvae was not measured.
10. **SUBMISSION PURPOSE:** This study was submitted to provide information on the toxicity of XDE-638 Technical (penoxsulam) to sediment-dwelling chironomids, for the purpose of pesticide registration.

11. **MATERIALS AND METHODS:**

A. Test Organisms

Guideline Criteria	Reported Information
Species	<i>Chironomus riparius</i>
Life Stage Second to third instar larvae (about 10 d old larvae with at least 50% at third instar).	1 st instar, 3 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.)	In house culture. Originally from U.S. Geological Survey, Columbia Environmental Research Center, Columbia, Missouri.

Guideline Criteria	Reported Information
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 h; Avoid unnecessary stress, crowding and rapid temperature and water quality changes.	The brood stock were reared in same dilution water as used in testing. The culture water was soft, laboratory well water and maintained at a temperature of 21°C in culture bowls.
Feeding Feeding should begin on day 0 and continue through day 9 unless food is not being eaten.	During rearing, hatched larvae were fed daily with a finely-ground suspension of flaked fish food (10 mg/mL).
Pretest Mortality A group of organisms should not be used if they appear unhealthy, discolored (e.g. <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 dechlorinated tap water. [Unpolluted well or spring that has been tested for contaminants, or appropriate reconstituted water (see ASTM for details)].	Overlying water was from the same source as the culture water (laboratory well water). The artificial sediment was prepared in the laboratory and consisted of 8.3% sphagnum peat, 20% kaolin clay, and 71.7% industrial sand (based on dry weight).
Does water support test animals without observable signs of stress?	Midges have successfully survived and reproduces over several generations in the dilution water.
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 observed.
Water Temperature 23°C ± 1°C. Temperature should be monitored at least hourly throughout the test in one test chamber, and near the beginning, Daily mean test	Sediment Spiked Exposure: Test water temperature was maintained at 19-22°C and measured daily in each replicate vessel and continuously in one replicate test vessel (31 mg a.i./kg treatment group).

Guideline Criteria	Reported Information
temperature Must not deviate more than $\pm 1^{\circ}\text{C}$ and instantaneous temperature must be within \pm middle and end of the test in all test chambers.	Water Spiked Exposure: Test water temperature was maintained at $20\text{--}22^{\circ}\text{C}$ and measured daily in each replicate vessel and continuously in one replicate test vessel (50 mg a.i./kg treatment group).
pH Not specified, but should be appropriate to the test species and should not deviate more than 0.4 pH units.	Sediment Spiked Exposure: 7.0-8.2 Water Spiked Exposure: 6.7-7.8
Dissolved Oxygen Should be measured at the beginning and end of short term tests. DO should be >40 percent and <100 percent saturation.	Sediment Spiked Exposure: 6.3-9.6 mg/L Water Spiked Exposure: 6.0-9.5 mg/L DO was measured daily in each replicate test vessel (60% of the air saturation value at $20^{\circ}\text{C}=5.4$ mg/L).
Total Hardness Prefer 40 - 200 mg/L as CaCO_3 .	Sediment Spiked Exposure: 68-124 mg/L as CaCO_3 Water Spiked Exposure: 48-108 mg/L as CaCO_3
Conductivity Not specified, but should be amenable to the test species.	Sediment Spiked Exposure: 230-430 $\mu\text{mhos/cm}$ Water Spiked Exposure: 180-430 $\mu\text{mhos/cm}$
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.	pH: 7.1 TOC: 2.2% Total volatile sulfides: Not reported Particle size distribution: 76% sand, 6% silt, 18% clay Water holding capacity: 15.5% (percent moisture at 1/3 bar)
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.	Not 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.	The test substance, XDE-638 Technical, was described (p. 15). Purity: 97.7% Water Solubility: <500 mg a.i./L (functional, determined in range-finding experiment) Kd (partition coefficient): 0.13 to 10.4 DT ₅₀ (biodegradation potential): 15 days in a sediment/water system
Stock Solutions	Sediment Spiked Exposure: Stock solutions were

Guideline Criteria	Reported Information
<p>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.</p>	<p>prepared by dissolving the test material in acetone; 10-mL aliquots of these stock solutions were mixed with 20 g fine sand. Negative (dilution water) control and solvent (acetone) control were used in the test.</p> <p>Water Spiked Exposure: Test material was added directly to the dilution water. Negative (dilution water) control was used in the test.</p>
<p>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.</p>	<p>31, 63, 130, 250, 500, and 1000 mg a.i./kg dry weight</p> <p>Test solution/sand mixtures were added to the sediment using a Hobart mixer.</p>
<p>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.</p>	<p>1. Glass beakers</p> <p>2. 600 ml: 75 ml (1.5 cm layer) of sediment and 300 ml of overlying water.</p>
<p>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.</p>	<p>Test vessels were covered with clear plastic plates.</p>
<p>Type of Dilution System Must provide reproducible supply of toxicant.</p>	<p>N/A - Static system.</p>
<p>Flow Rate Consistent flow rate of 5-10 vol/24 hours, meter systems calibrated before study and checked twice daily during test period.</p>	<p>N/A - Static system.</p>
<p>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.</p>	<p>Aerated at 1 to 3 bubbles per second starting on day 1 (after water and sediment was added).</p>
<p>Photoperiod 16 hours light, 8 hours dark with a 15-30 min transition period and illuminance of about 100 to 1000 lux.</p>	<p>16 hours light, 8 hours dark. The light intensity range was 750-1300 lux.</p>

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.	The acetone was allowed to completely evaporate (under a hood) from the sand/test substance mixture prior to adding to the sediment (p. 21).

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 thoroughly homogenized and added to test chambers; Overlying water is added to chambers in a manner that minimizes suspension of sediment	<p>Sediment Spiked Exposure: The sediment was treated, and the treated sediment and overlying water were distributed to replicate test vessels 1 day prior to organism introduction (Day -1).</p> <p>Water Spiked Exposure: Untreated sediment and overlying water were distributed to replicate test vessels 3 days prior to treatment and organism introduction.</p> <p>In both cases, a turbulence reducer (modified plastic disk) was used to minimize the disruption of the sediment layer during the introduction of the water phase. The vessels were placed into a water bath with circulating water.</p>
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.	N/A
Placing Organisms in Test Chambers: Should be handled as little as possible and introduced into overlying water below the air-water interface.	Twenty midge larvae were impartially added to each of eight replicate test vessels (not further described). At the time of addition of the midge larvae, aeration of the water was suspended overnight.
Range Finding Test	Sediment Spiked Exposure: A 27-day range-finding test was conducted with three replicates of 20 midges (2 days old) per replicate at nominal sediment concentrations of 0 (solvent control), 3.3, 33, 330, 1700, and 3300 mg a.i./kg. The mean percent emergence was 95% in the solvent control group, and 87, 90, 87, 92, and 90% for the 3.3, 33, 330, 1700, and 3300 mg a.i./kg treatment groups, respectively. The mean development rate (combined male/female) was 0.0615, 0.0608,

Guideline Criteria	Reported Information
<p>Water Spiked Exposure: A 28-day range-finding test was conducted with three replicates of 20 midges (2 days old) per replicate at nominal water concentrations of 0 (negative control), 1.0, 10, 50, 100, and 500 mg a.i./kg. The mean percent emergence was 93% in the solvent control group, and 88, 78, 85, 78, and 48% for the 1.0, 10, 50, 100, and 500 mg a.i./kg treatment groups, respectively. The mean development rate (combined male/female) was 0.0631, 0.0616, 0.0603, 0.0594, and 0.0494 for the 1.0, 10, 50, 100, and 500 mg a.i./kg treatment groups, respectively, compared to a 0.0605 control development rate.</p>	<p>0.0609, 0.0602, and 0.600 for the 3.3, 33, 330, 1700, and 3300 mg a.i./kg treatment groups, respectively, compared to a 0.0632 solvent control development rate.</p>
<p>Monitoring the test All test chambers should be checked daily and observations made to assess organism behavior such as sediment avoidance.</p>	<p>Test vessels were observed daily for emergence and abnormal behavior.</p>
<p>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.</p>	<p>Sediment Spiked Exposure: 0 (negative and solvent controls), 31, 63, 130, 250, 500, and 1000 mg a.i./kg</p> <p>Water Spiked Exposure: 0 (negative control), 13, 25, 50, 100, and 200 mg a.i./kg</p>
<p>Number of Test Organisms 10 organisms per test chamber are recommended. 8 replicates per treatment should be used.</p>	<p>20 larvae/container; 4 replicates were prepared for biological response and 4 replicates were maintained for chemical analysis for each treatment.</p>
<p>Test organisms randomly or impartially assigned to test vessels?</p>	<p>Yes</p>
<p>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.</p>	<p>During the study, 0.50 ml of finely-ground suspension of flaked fish food (10 mg/ml) was provided from Day 0 to Day 10, and 1.0 ml of this suspension was provided from Day 11 to termination.</p>
<p>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.</p>	<p>The hardness, alkalinity, conductivity, ammonia concentration were measured at test initiation and termination and varied less than 50% during the test. The temperature, pH, and dissolved oxygen content were measured at test initiation and termination. Dissolved oxygen and temperature was also measured daily in each replicate test vessel.</p>
<p>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.</p>	<p>Sediment Spiked Exposure: Sediment was sampled from alternating replicates of each test concentration on Days 0, 7, and 28. Pore water and overlying water were sampled from alternating replicates of the control, 31, 250, and</p>

Guideline Criteria	Reported Information
Concentrations should be measured in bulk sediment, interstitial water, overlying water, and stock solution.	<p>1000 mg a.i./kg treatment groups on Days 0, 7, and 28.</p> <p>Water Spiked Exposure: Overlying water samples were collected from alternating replicates of each test concentration on Days 0, 7, and 28. Sediment and pore water were sampled from alternating replicates of the control, 13, 50, and 200 mg a.i./kg treatment groups on Days 0, 7, and 28.</p>

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 $\leq 30\%$ in the sediment at end of the test.	There was 82% emergence of inserted larvae in the pooled controls at test termination; mortality was not reported.
Percent Recovery of Chemical: 1) % of nominal; 2) Procedural recovery; 3) Limit of quantitation (LOQ)	Sediment Spiked Exposure: 1) In sediment: 33-81% on Day 0, 19-74% on Day 7, and 10-24% on Day 28. In overlying water: 2-9% on Day 0, 7-21% on Day 7, and 0.4-10% on Day 28. In pore water: 13-45% on Day 0, 13-31% on Day 7, and 0.4-5% on Day 28. 2) QC recoveries were 72.2-107% of nominal from sediment and 88.6-102% of nominal from water. 3) <0.79 mg/kg dry weight (sediment) and <0.13 mg a.i./L (water)
1) % of nominal; . 2) Procedural recovery; 3) Limit of quantitation (LOQ)	Water Spiked Exposure: 1) In overlying water: 55-71% on Day 0, 52-60% on Day 7, and 49-70% on Day 28. In pore water: 41-44% on Day 0, 50-65% on Day 7, and 48-70% on Day 28. In sediment: 10-30% on Day 0, 7-42% on Day 7, and 24-34% on Day 28. 2) QC recoveries were generally 75.1-94.2% (two outliers) of nominal from sediment and 88.6-101% of nominal from water. 3) <0.66 mg/kg dry weight (sediment) and <0.35 mg a.i./L (water)
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)	-emergence (time, number, and sex) -development rate
Raw data included?	Yes

Effects Data:

Sediment-spiked exposure

Toxicant Concentration				Cumulative Number Dead	Mean Dry Weight per midge (mg)
Nominal (mg a.i./kg)	Measured (0 Hour)				
	Sediment (mg a.i./kg)	Pore Water (mg a.i./L)	Surface Water (mg a.i./L)		
Control	<0.77	<0.11	<0.11	NR	NR
Solvent Control	<0.77	NR	NR	NR	NR
31	10	14	2.8	NR	NR
63	29	NR	NR	NR	NR
130	76	NR	NR	NR	NR
250	170	70	14	NR	NR
500	400	NR	NR	NR	NR
1000	810	130	23	NR	NR

NR=Not Reported.

Nominal Concentration (mg a.i./kg)	Percent Emerged	Mean Development Rate (1/day) ¹
Control	84	0.0636
Solvent Control	79	0.0657
Pooled Control	82	0.0647
31	91	0.0628
63	89	0.0627
130	91	0.0649
250	76	0.0661
500	90	0.0654
1000	86	0.0627

¹ Male and Female development rate.
Data obtained from Table 13, p. 50.

Water-spiked exposure

Toxicant Concentration				Cumulative Number Dead	Mean Dry Weight per midge (mg)
Nominal (mg a.i./L)	Measured (1 Hour)				
	Sediment (mg a.i./kg)	Pore Water (mg a.i./L)	Surface Water (mg a.i./L)		
Control	<0.66	<0.35	<0.33	NR	NR
13	1.3	5.3	7.1	NR	NR
25	NR	NR	15	NR	NR
50	13	22	31	NR	NR
100	NR	NR	61	NR	NR
200	59	82	140	NR	NR

NR=Not Reported.

Nominal Concentration (mg a.i./L)	Percent Emerged	Mean Development Rate (1/day) ¹
Control	94	0.0660
13	90	0.0639
25	90	0.0604*
50	96	0.0636
100	95	0.0629
200	94	0.0615*

¹ Male and Female development rate.* Significantly reduced compared to control ($p \leq 0.05$).

Data obtained from Table 14, p. 51.

Other Significant Results: Development rates were determined for individual sexes, and the rate was statistically-reduced in females exposed at 25 mg a.i./L during the water-spiked exposure (Table 14, p. 51).

B. Statistical Results

Method: At the termination of the study, data obtained on midges emergence and development rate (as male, female, and combined) were statistically analyzed to identify significant treatment-related effects. The NOAEC and LOAEC were based on significance data. A computer program (Gulley, *et al.* 1996) was used to perform the statistical analyses. Analyses were performed using the mean replicate organism response, generally at the 95% level of certainty. Percent emergence data were arcsine square-root transformed prior to analyses. Initial mean-measured concentrations of the respectively-spiked matrix were used in reporting the results.

The EC₅₀ was empirically estimated to be greater than the highest concentration tested, as none of the treatment levels tested reduced emergence by 50% or more. Survival and growth endpoints were not

assessed.

For the sediment-spiked exposure, a t-test was used to compare the negative and solvent control groups. For percent emergence, there was a significant difference between the two, and therefore treatment data were compared to the solvent control group. For the development rate, treatment data were compared to pooled controls. Data were assessed for normality using the Shapiro-Wilks Test and for homogeneity of variance using Bartlett's Test. The percent emergence and development rate data for males and combined sexes met the assumptions for normal distribution and homogeneity, and Williams' Test was used to establish treatment-related effects. Female development rate data failed the test for homogeneity, and therefore Wilcoxon's Rank Sum test was used to establish treatment-related effects.

LC₅₀ (mortality): Not reported
EC₅₀ (growth): Not reported
EC₅₀ (emergence): >810 mg a.i./kg
EC₅₀ (development rate): >810 mg a.i./kg

NOAEC: 810 mg a.i./kg
LOAEC: >810 mg a.i./kg
Endpoints affected: None

For the water-spiked exposure, data were assessed for normality using the Shapiro-Wilks Test and for homogeneity of variance using Bartlett's Test. The percent emergence and development rate (male, female, and combined) met the assumptions for normal distribution and homogeneity, and Dunnett's Test was used to establish treatment-related effects.

LC₅₀ (mortality): Not reported
EC₅₀ (growth): Not reported
EC₅₀ (emergence): >140 mg a.i./L
EC₅₀ (development rate): >140 mg a.i./L

NOAEC: 7.1 mg a.i./L
LOAEC: 13 mg a.i./L
Endpoints affected: Development rate for combined sexes

13. VERIFICATION OF STATISTICAL RESULTS:

Method: The NOAEC and LOAEC values were determined for 28-day percent emerged and development rate (males, females, and male:female) for both the sediment-spiked and water-spiked exposure tests. Data were analyzed to determine if they satisfied the assumptions of ANOVA (i.e., normal distribution and variance homogeneity) and, if so, toxicity values were determined using ANOVA, followed by Dunnett's test (for development rate male:female water-spiked exposure only). Data for development rate female (sediment-spiked exposure) did not satisfy the assumptions of ANOVA, so toxicity values were determined using the non-parametric Kruskal-Wallis test. For the sediment-spiked exposure test, the solvent control group was compared to the negative control group using a Student's t-test; if no difference was found, the two groups were pooled for comparison to the treatment groups. If a difference was found between the two control groups (% emerged), the treatment groups were compared to the solvent control group. These analyses were conducted using TOXSTAT statistical software. For all calculations, the initial measured concentrations (0-hours for sediment-spiked exposure and 1-hour for water-spiked exposure) were used.

Sediment-spiked exposure (initial measured concentrations in the sediment):

PARAMETER	RESULT
Binomial Test LC ₅₀ mortality (95% C.I.) EC ₅₀ growth (95% C.I.) EC ₅₀ emergence (95% C.I.) EC ₅₀ development rate (95% C.I.)	N/A, as no endpoint was affected at >50%.
Moving Average Angle Test LC ₅₀ mortality (95% C.I.) EC ₅₀ growth (95% C.I.) EC ₅₀ emergence (95% C.I.) EC ₅₀ development rate (95% C.I.)	N/A, as no endpoint was affected at >50%.
Probit Test LC ₅₀ mortality (95% C.I.) EC ₅₀ growth (95% C.I.) EC ₅₀ emergence (95% C.I.) EC ₅₀ development rate (95% C.I.)	N/A, as no endpoint was affected at >50%.
Probit Slope: Mortality Growth Emergence Development rate	N/A, as no endpoint was affected at >50%.
NOAEC: Mortality Growth Emergence Development rate	810 mg a.i./kg dry weight Not determined 810 mg a.i./kg dry weight 810 mg a.i./kg dry weight

Water-spiked exposure (initial measured concentrations in the overlying water):

PARAMETER	RESULT
Binomial Test LC ₅₀ mortality (95% C.I.) EC ₅₀ growth (95% C.I.) EC ₅₀ emergence (95% C.I.) EC ₅₀ development rate (95% C.I.)	N/A, as no endpoint was affected at >50%.
Moving Average Angle Test LC ₅₀ mortality (95% C.I.) EC ₅₀ growth (95% C.I.) EC ₅₀ emergence (95% C.I.) EC ₅₀ development rate (95% C.I.)	N/A, as no endpoint was affected at >50%.
Probit Test LC ₅₀ mortality (95% C.I.) EC ₅₀ growth (95% C.I.) EC ₅₀ emergence (95% C.I.) EC ₅₀ development rate (95% C.I.)	N/A, as no endpoint was affected at >50%.
Probit Slope: Mortality Growth Emergence Development rate	N/A, as no endpoint was affected at >50%.
NOAEC: Mortality Growth Emergence Development rate	140 mg a.i./L Not determined 140 mg a.i./L 7.1 mg a.i./L

14. REVIEWER'S COMMENTS:

The reviewer's conclusions were identical to the study author's; water-spiked exposure to XDE-638 Technical caused significant reductions in midge development rate (combined male:female) at the 15 and 140 mg a.i./L levels (nominal concentrations of 50 and 100 mg a.i./L, respectively). Sediment-spiked exposure resulted in no effect on midge emergence or development. As a result, the NOAEC and LOAEC for water-spiked and sediment-spiked exposures were 7.1 and 15 mg a.i./L (water-spiked) and 810 and >810 mg a.i./L (sediment-spiked).

This study is scientifically sound, and provides useful information on the 28-day toxicity of XDE-638 Technical to the midge, *Chironomus riparius*. This study was not designed to fulfill any current U.S. EPA FIFRA guideline.

In method validation experiments conducted prior to the definitive study, the average recovery of XDE-638 Technical from freshwater was $98.1 \pm 3.28\%$ (Table 1A of Appendix II, p. 83), and $88.6 \pm 3.52\%$ from artificial sediment (Table 1B of Appendix III, p. 95).

This study was conducted in accordance with OECD GLP regulations, with the following exceptions: routine water and food contaminant screening analyses for pesticides, PCBs and toxic metals were conducted/collected using standard U.S. EPA procedures (p. 3). A Quality Assurance Statement was included.

15. REFERENCES:

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- Sokal, R.R. and F.J. Rohlf. 1981. *Biometry*. 2nd Edition. W.H. Freeman and Co. New York. 859 pp.
- Weber, C.I. *et al.* (eds). 1989. Short-Term Methods for Estimating the Full Life-Cycle Toxicity of Effluents and Receiving Waters to Freshwater Organisms. 2nd ed. EPA/600/4/89/001. Environmental Monitoring Systems Laboratory, U.S. Environmental Protection Agency, Cincinnati, OH.

16. OUTPUT OF REVIEWER'S STATISTICAL VERIFICATION:

% emerged (sediment)

File: 1102e Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	6	905.357	150.893	1.536
Within (Error)	21	2062.500	98.214	
Total	27	2967.857		

Critical F value = 2.57 (0.05,6,21)

Since $F < \text{Critical } F$ FAIL TO REJECT H_0 : All groups equal

% emerged (sediment)

File: 1102e Transform: NO TRANSFORMATION

DUNNETTS TEST - TABLE 1 OF 2 H_0 : Control < Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	CALCULATED IN ORIGINAL UNITS	T STAT	SIG
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1	solvent control	78.750	78.750	
2	10	91.250	91.250	-1.784
3	29	88.750	88.750	-1.427
4	76	91.250	91.250	-1.784
5	170	76.250	76.250	0.357
6	400	90.000	90.000	-1.605
7	810	86.250	86.250	-1.070

Dunnett table value = 2.46 (1 Tailed Value, P=0.05, df=20,6)

% emerged (sediment)

File: 1102e Transform: NO TRANSFORMATION

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

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of DIFFERENCE CONTROL FROM CONTROL
1	solvent control	4		
2	10	4	17.239	21.9 -12.500
3	29	4	17.239	21.9 -10.000
4	76	4	17.239	21.9 -12.500
5	170	4	17.239	21.9 2.500
6	400	4	17.239	21.9 -11.250
7	810	4	17.239	21.9 -7.500

% emerged (sediment)

File: 1102e Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	ORIGINAL N	MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	solvent control	4	78.750	78.750	78.750
2	10	4	91.250	91.250	86.875
3	29	4	88.750	88.750	86.875
4	76	4	91.250	91.250	86.875
5	170	4	76.250	76.250	86.875
6	400	4	90.000	90.000	88.125
7	810	4	86.250	86.250	88.125

% emerged (sediment)

File: 1102e Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

 ISOTONIZED CALC. SIG TABLE DEGREES OF
 IDENTIFICATION MEAN WILLIAMS P=.05 WILLIAMS FREEDOM

solvent control	78.750			
10	86.875	1.159	1.72	k= 1, v=21
29	86.875	1.159	1.80	k= 2, v=21
76	86.875	1.159	1.83	k= 3, v=21
170	86.875	1.159	1.84	k= 4, v=21
400	88.125	1.338	1.85	k= 5, v=21
810	88.125	1.338	1.85	k= 6, v=21

s = 9.910

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

development rate male (sediment)

File: 1102dm Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	6	0.452	0.075	1.154
Within (Error)	25	1.633	0.065	
Total	31	2.085		

Critical F value = 2.49 (0.05,6,25)

Since F < Critical F FAIL TO REJECT Ho:All groups equal

development rate male (sediment)

File: 1102dm 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	6.940	6.940		
2	10	6.665	6.665	1.761	
3	29	6.757	6.757	1.169	
4	76	6.820	6.820	0.769	
5	170	7.010	7.010	-0.448	
6	400	6.970	6.970	-0.192	
7	810	6.735	6.735	1.313	

Bonferroni T table value = 2.57 (1 Tailed Value, P=0.05, df=25,6)

development rate male (sediment)

File: 1102dm 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 DIFFERENCE CONTROL FROM CONTROL
1	GRPS 1&2 POOLED	8		
2	10	4	0.401	5.8 0.275
3	29	4	0.401	5.8 0.183
4	76	4	0.401	5.8 0.120
5	170	4	0.401	5.8 -0.070
6	400	4	0.401	5.8 -0.030
7	810	4	0.401	5.8 0.205

development rate male (sediment)

File: 1102dm Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	ORIGINAL N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	GRPS 1&2 POOLED	8	6.940	6.940	6.940
2	10	4	6.665	6.665	6.845
3	29	4	6.757	6.757	6.845
4	76	4	6.820	6.820	6.845
5	170	4	7.010	7.010	6.845
6	400	4	6.970	6.970	6.845
7	810	4	6.735	6.735	6.735

development rate male (sediment)

File: 1102dm 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
GRPS 1&2 POOLED	6.940				
10	6.845	0.610	1.71	k= 1, v=25	
29	6.845	0.610	1.79	k= 2, v=25	
76	6.845	0.610	1.82	k= 3, v=25	
170	6.845	0.610	1.83	k= 4, v=25	
400	6.845	0.610	1.84	k= 5, v=25	
810	6.735	1.310	1.84	k= 6, v=25	

s = 0.256

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

development rate female (sediment)

File: 1102df Transform: NO TRANSFORMATION

KRUSKAL-WALLIS ANOVA BY RANKS - TABLE 1 OF 2

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	RANK SUM
1	GRPS 1&2 POOLED	6.051	6.051	152.000
2	10	5.918	5.918	55.000
3	29	5.663	5.663	20.000
4	76	6.140	6.140	94.000
5	170	6.258	6.258	79.500
6	400	6.035	6.035	74.500
7	810	5.868	5.868	53.000

Calculated H Value = 10.365 Critical H Value Table = 12.590
 Since Calc H < Crit H FAIL TO REJECT Ho: All groups are equal.

development rate female (sediment)
 File: 1102df Transform: NO TRANSFORMATION

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

GROUP	IDENTIFICATION	TRANSFORMED MEAN	GROUP ORIGINAL MEAN	0 0 0 0 0 0
3	29	5.663	5.663 \	
7	810	5.868	5.868 . \	
2	10	5.918	5.918 .. \	
6	400	6.035	6.035 ... \	
1	GRPS 1&2 POOLED	6.051	6.051 \	
4	76	6.140	6.140 \	
5	170	6.258	6.258 \	

* = significant difference (p=0.05) . = no significant difference
 Table q value (0.05,7) = 3.038 Unequal reps - multiple SE values

development rate mf (sediment)
 File: 1102dr Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	6	0.494	0.082	1.464
Within (Error)	25	1.401	0.056	
Total	31	1.895		

Critical F value = 2.49 (0.05,6,25)
 Since F < Critical F FAIL TO REJECT Ho: All groups equal

development rate mf (sediment)

File: 1102dr 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	6.465	6.465		
2	10	6.278	6.278	1.294	
3	29	6.270	6.270	1.346	
4	76	6.495	6.495	-0.207	
5	170	6.610	6.610	-1.001	
6	400	6.535	6.535	-0.483	
7	810	6.273	6.273	1.328	

Bonferroni T table value = 2.57 (1 Tailed Value, P=0.05, df=25,6)

development rate mf (sediment)

File: 1102dr 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 DIFFERENCE CONTROL FROM CONTROL
1	GRPS 1&2 POOLED	8		
2	10	4	0.372	5.8 0.188
3	29	4	0.372	5.8 0.195
4	76	4	0.372	5.8 -0.030
5	170	4	0.372	5.8 -0.145
6	400	4	0.372	5.8 -0.070
7	810	4	0.372	5.8 0.192

development rate mf (sediment)

File: 1102dr Transform: NO TRANSFORMATION

WILLIAMS TEST - (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	ORIGINAL N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	GRPS 1&2 POOLED	8	6.465	6.465	6.465
2	10	4	6.278	6.278	6.438
3	29	4	6.270	6.270	6.438
4	76	4	6.495	6.495	6.438
5	170	4	6.610	6.610	6.438
6	400	4	6.535	6.535	6.438
7	810	4	6.273	6.273	6.273

development rate mf (sediment)

File: 1102dr Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE DEGREES OF WILLIAMS FREEDOM
GRPS 1&2 POOLED	6.465			
10	6.438	0.190	1.71	k= 1, v=25
29	6.438	0.190	1.79	k= 2, v=25
76	6.438	0.190	1.82	k= 3, v=25
170	6.438	0.190	1.83	k= 4, v=25
400	6.438	0.190	1.84	k= 5, v=25
810	6.273	1.328	1.84	k= 6, v=25

s = 0.237

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

% emergence (h20)

File: 1102ew Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	134.375	26.875	0.737
Within (Error)	18	656.250	36.458	
Total	23	790.625		

Critical F value = 2.77 (0.05,5,18)

Since F < Critical F FAIL TO REJECT Ho:All groups equal

% emergence (h20)

File: 1102ew 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	93.750	93.750		
2	7.1	90.000	90.000	0.878	
3	15	90.000	90.000	0.878	
4	31	96.250	96.250	-0.586	
5	61	95.000	95.000	-0.293	
6	140	93.750	93.750	0.000	

Dunnett table value = 2.41 (1 Tailed Value, P=0.05, df=18,5)

% emergence (h20)

File: 1102ew Transform: NO TRANSFORMATION

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

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of DIFFERENCE CONTROL FROM CONTROL
1	control	4		
2	7.1	4	10.290	11.0 3.750
3	15	4	10.290	11.0 3.750
4	31	4	10.290	11.0 -2.500
5	61	4	10.290	11.0 -1.250
6	140	4	10.290	11.0 0.000

% emergence (h20)

File: 1102ew Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	ORIGINAL N	MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	control	4	93.750	93.750	91.250
2	7.1	4	90.000	90.000	91.250
3	15	4	90.000	90.000	91.250
4	31	4	96.250	96.250	95.000
5	61	4	95.000	95.000	95.000
6	140	4	93.750	93.750	95.000

% emergence (h20)

File: 1102ew 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	91.250				
7.1	91.250	0.586	1.73	k= 1, v=18	
15	91.250	0.586	1.82	k= 2, v=18	
31	95.000	0.293	1.85	k= 3, v=18	
61	95.000	0.293	1.86	k= 4, v=18	
140	95.000	0.293	1.87	k= 5, v=18	

s = 6.038

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

development rate male (H2O)

File: 1102mw Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	0.386	0.077	1.100
Within (Error)	18	1.264	0.070	
Total	23	1.650		

Critical F value = 2.77 (0.05,5,18)

Since $F < \text{Critical } F$ FAIL TO REJECT H_0 : All groups equal

development rate male (H2O)

File: 1102mw Transform: NO TRANSFORMATION

DUNNETTS TEST - TABLE 1 OF 2 H_0 : Control < Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	control	6.883	6.883		
2	7.1	6.723	6.723	0.855	
3	15	6.633	6.633	1.336	
4	31	6.870	6.870	0.067	
5	61	6.690	6.690	1.029	
6	140	6.522	6.522	1.924	

Dunnett table value = 2.41 (1 Tailed Value, $P=0.05$, $df=18,5$)

development rate male (H2O)

File: 1102mw Transform: NO TRANSFORMATION

DUNNETTS TEST - TABLE 2 OF 2 H_0 : Control < Treatment

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of DIFFERENCE CONTROL FROM CONTROL
1	control	4		
2	7.1	4	0.451	6.6 0.160
3	15	4	0.451	6.6 0.250
4	31	4	0.451	6.6 0.013
5	61	4	0.451	6.6 0.193
6	140	4	0.451	6.6 0.360

development rate male (H2O)

File: 1102mw Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	ORIGINAL N	MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	control	4	6.883	6.883	6.883
2	7.1	4	6.723	6.723	6.742
3	15	4	6.633	6.633	6.742
4	31	4	6.870	6.870	6.742
5	61	4	6.690	6.690	6.690
6	140	4	6.522	6.522	6.522

development rate male (H2O)

File: 1102mw Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. SIG WILLIAMS	TABLE P=0.05	DEGREES OF WILLIAMS FREEDOM
control	6.883			
7.1	6.742	0.752	1.73	k= 1, v=18
15	6.742	0.752	1.82	k= 2, v=18
31	6.742	0.752	1.85	k= 3, v=18
61	6.690	1.027	1.86	k= 4, v=18
140	6.522	1.921	* 1.87	k= 5, v=18

s = 0.265

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

development rate female (H2O)

File: 1102fw Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	0.503	0.101	2.405
Within (Error)	18	0.752	0.042	
Total	23	1.255		

Critical F value = 2.77 (0.05,5,18)

Since F < Critical F FAIL TO REJECT Ho: All groups equal

development rate female (H2O)

File: 1102fw 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	6.185	6.185		
2	7.1	6.058	6.058	0.880	
3	15	5.753	5.753	2.985	*
4	31	5.902	5.902	1.949	
5	61	6.030	6.030	1.070	
6	140	5.840	5.840	2.381	

Dunnett table value = 2.41 (1 Tailed Value, P=0.05, df=18,5)

development rate female (H2O)

File: 1102fw Transform: NO TRANSFORMATION

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

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of DIFFERENCE CONTROL FROM CONTROL
1	control	4		
2	7.1	4	0.349	5.6
3	15	4	0.349	5.6
4	31	4	0.349	5.6
5	61	4	0.349	5.6
6	140	4	0.349	5.6

development rate female (H2O)

File: 1102fw Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION		ORIGINAL N	MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	control	4	6.185	6.185	6.185	
2	7.1	4	6.058	6.058	6.058	
3	15	4	5.753	5.753	5.895	
4	31	4	5.902	5.902	5.895	
5	61	4	6.030	6.030	5.895	
6	140	4	5.840	5.840	5.840	

development rate female (H2O)

File: 1102fw Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

ISOTONIZED CALC. SIG TABLE DEGREES OF
IDENTIFICATION MEAN WILLIAMS P=.05 WILLIAMS FREEDOM

control	6.185				
7.1	6.058	0.882	1.73	k= 1, v=18	
15	5.895	2.006	*	1.82	k= 2, v=18
31	5.895	2.006	*	1.85	k= 3, v=18
61	5.895	2.006	*	1.86	k= 4, v=18
140	5.840	2.386	*	1.87	k= 5, v=18

s = 0.204

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

development rate m:f (H2O)

File: 1102rw Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	0.767	0.153	4.250
Within (Error)	18	0.644	0.036	
Total	23	1.411		

Critical F value = 2.77 (0.05,5,18)

Since F > Critical F REJECT Ho:All groups equal

development rate m:f (H2O)

File: 1102rw 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	6.598	6.598		
2	7.1	6.390	6.390	1.547	
3	15	6.040	6.040	4.155	*
4	31	6.357	6.357	1.789	
5	61	6.283	6.283	2.348	
6	140	6.145	6.145	3.373	*

Dunnett table value = 2.41 (1 Tailed Value, P=0.05, df=18,5)

development rate m:f (H2O)

File: 1102rw Transform: NO TRANSFORMATION

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

NUM OF	Minimum Sig Diff	% of	DIFFERENCE
--------	------------------	------	------------

GROUP IDENTIFICATION REPS (IN ORIG. UNITS) CONTROL FROM CONTROL

1	control	4			
2	7.1	4	0.323	4.9	0.208
3	15	4	0.323	4.9	0.558
4	31	4	0.323	4.9	0.240
5	61	4	0.323	4.9	0.315
6	140	4	0.323	4.9	0.452

development rate m:f (H2O)

File: 1102rw Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	control	4	6.598	6.598	6.598
2	7.1	4	6.390	6.390	6.390
3	15	4	6.040	6.040	6.227
4	31	4	6.357	6.357	6.227
5	61	4	6.283	6.283	6.227
6	140	4	6.145	6.145	6.145

development rate m:f (H2O)

File: 1102rw Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. MEAN	SIG WILLIAMS	TABLE P=.05	DEGREES OF WILLIAMS	FREEDOM
control	6.598					
7.1	6.390	1.552		1.73	k= 1, v=18	
15	6.227	2.774	*	1.82	k= 2, v=18	
31	6.227	2.774	*	1.85	k= 3, v=18	
61	6.227	2.774	*	1.86	k= 4, v=18	
140	6.145	3.385	*	1.87	k= 5, v=18	

s = 0.189

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