DATA EVALUATION RECORD
FRESHWATER SEDIMENT *Chironomus riparius* EMERGENCE TEST

1. **CHEMICAL:** Flubendiamide — PC Code: 027602

2. **TEST MATERIAL:** NNI-0001-des-iodo — Purity: 99.3%

3. **CITATION:**

Authors: Dorgerloh, M.
Title: *Chironomus riparius* 28-day Chronic Toxicity Test with NNI-0001-des-iodo in a Water-Sediment System using Spiked Water
Study Completion Date: November 22, 2004
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Laboratory Report ID: DOM 23069; Project ID E 416 2518-7
MRID No.: 468170-23

4. **REVIEWED BY:** John Marton, Staff Scientist, Cambridge Environmental, Inc.

Signature: John Marton **Date:** 07/30/07

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

Signature: Teri S Myers **Date:** 08/02/07

5. **APPROVED BY:** Holly Galavotti, Biologist, ERB1

Signature: Holly Galavotti **Date:** 5/21/08

6. **STUDY PARAMETERS**

Scientific Name of Test Organism: *Chironomus riparius*
Age of Test Organism: 1st instar (L1) larvae, 2 to 3 days post-hatch
Definitive Test Duration: 28 days
Study Method: Static with aeration
Type of Concentrations: Initial nominal overlying water and TWA (pore and overlying water)

7. CONCLUSIONS:

Results Synopsis:

Percent Emergence:
28-day NOAEC: 4.00 µg ai/L
28-day LOAEC: 8.00 µg ai/L
EC_{50}: 20 µg ai/L 95% C.I.: 18-24 µg ai/L
Probit slope: 4.07±0.664

Male Development Rate:
28-day NOAEC: 16.00 µg ai/L
28-day LOAEC: 32.00 µg ai/L
EC_{50}: >32.00 µg ai/L

Female Development Rate:
28-day NOAEC: 16.00 µg ai/L
28-day LOAEC: 32.00 µg ai/L
EC_{50}: >32.00 µg ai/L

Assessment endpoints: percent emergence and development rate
Endpoints affected: percent emergence and development rate

8. ADEQUACY OF THE STUDY:

A. Classification: Supplemental

B. Rationale: This study followed methods described in the proposal for a new OECD Guideline 219: "Sediment-Water Chironomid Toxicity Test Using Spiked Water" (December 2002), and was not submitted to fulfill any current U.S. EPA data requirement.

C. Reparability: N/A

9. MAJOR GUIDELINE DEVIATIONS:

1. Overlying water was spiked, prefer that the sediment is spiked.

2. Sediment was not analyzed for degradate NNI-0001 des-iodo levels.

10. SUBMISSION PURPOSE: To assess the toxicity of the degradate NNI-0001 des-iodo to the chironomid in a water spiked water-sediment system for the purpose of new chemical

registration (PRIA).

11. MATERIALS AND METHODS

Stability of Compound Under Test Conditions: Overlying water and pore water samples from the surrogate vessels prepared at 0.25, 4.00 and 32.0 µg ai/L (one vessel per interval) were analyzed at 0 (1 hour), 7, and 28 Days and analyzed for NNI-0001-des-iodo. Residues associated with the overlying water were decreased from 82% of nominal concentrations at Day 0 to 32% of nominal concentrations by Day 28. Concentrations in the pore water did not exceed 0.7% of nominal concentrations. No test material was detected in the negative or solvent controls.

Nominal Initial Conc. Applied to Water (µg ai/L)	Day	Analytical Results of NNI-0001-des-iodo In Overlying Water and Pore Water Sample			
		Pore Water		Overlying Water	
		µg ai/L	% of Nominal	µg ai/L	% of Nominal
Control	0, 7, 28	<0.05	N/A	<0.05	N/A
Solvent Control	0, 7, 28	<0.05	N/A	<0.05	N/A
0.25	0	<0.05	N/A	0.199	80
	7	<0.05	N/A	0.156	62
	28	<0.05	N/A	0.119	48
4.00	0	0.135	0.2	3.20	80
	7	0.363	0.7	2.31	58
	28	0.217	0.4	0.914	23
32.0	0	1.18	0.3	27.6	86
	7	5.98	1.4	18.7	58
	28	2.07	0.5	8.52	27

Storage conditions of test chemical: <5°C, dark

Physicochemical properties of NNI-0001-des-iodo.

Parameter	Values	Comments
Water solubility at 20°C	~0.42 mg/L	
Vapour pressure	Not reported	
UV adsorption	Not reported	
pKa	Not reported	
Kow	Not reported	

OECD requires water solubility, stability in water and light, pK_a, P_{ow}, and vapor pressure of the test compound.

A. Test Organisms/Acclimation

Guideline Criteria	Reported Information
Species *Chironomus riparius*	*Chironomus riparius*
Source	In-house laboratory culture originally obtained from the University of Sheffield (UK) in autumn 1991.
Culture Conditions A reproduction and oviposit chamber should consist of an adult area, sufficiently large to allow swarming (minimum 30 x 30 x 30 cm), and an oviposit area. Crystallizing dishes or larger containers with a thin layer of quartz sand (5 to 10 mm) or Kieselgur (thin layer to a few mm) spread over the bottom and containing suitable water to a depth of several cm are suitable as an oviposit area. Environmental conditions: temperature 20±2°C; 16:8 hours light:dark (intensity ca. 1000 lux); air humidity ca. 60%	For breeding, the midges were kept in cages (60 x 60 x 55 cm) with gauze on each side of the cage. A glass basin (45 cm x 55 cm x 10 cm), made of inert plastic, was set on the bottom of each cage, and the bottom of the basin was covered with a thin layer of silica and 2- to3-cm of reconstituted water (Elendt M7). The water was gently aerated. To begin each culture, two to four egg masses are placed into the prepared basin. The cultures were maintained at 20 ± 2°C and a 16:8 hour light:dark photoperiod.
Egg Mass Acclimation Period Four to five days before test initiation freshly laid egg masses should be taken from cultures and maintained separately in culture medium, temperature change should not exceed 2°C per day.	Fresh egg masses were incubated in small dishes with test medium. The temperature was not reported.
Age of Test Larvae First instar (1 to 4 days post-hatch with confirmation)	1st instar (L1), 2-3 days post-hatch
Food Green algae (e.g., *Scenedesmus subspicatus, Chlorella vulgaris*) or flaked fish food as a ground powder, suspension, or filtrate	Hatched chironomus larvae were fed green algae and an aqueous suspension of a vegetable fish food (Tetra Phyll®).

Guideline Criteria	Reported Information
Health of parent culture stock Were parent chironomids in good health during the culture period?	Not reported.

B. Test System

Guideline Criteria	Reported Information
Type of Test System Static (static-renewal or flow-through of overlying water is evaluated on a chemical-specific basis). Distilled or deionized water may be added to overlying water once daily as needed to maintain volume.	Static with aeration. Once a week, test beakers were refilled with deionized water up to the mark (indicating 380 mL of overlying water) to replicate water lost by evaporation. Additional test vessels (with chironomids) were used for chemical analysis of the test item on days 0 and 7 (single additional replicate for the negative and solvent controls, and two additional replicates for the 0.25, 4.00 and 32.0 µg ai/L levels). For chemical analysis on day 28, one beaker of the four beakers for biological evaluations was used. Therefore, the method for analytical sampling did not affect volume, biological load, or test concentration. A further replicate of each test concentration was prepared (with chironomids) to measure the temperature, pH, and oxygen content in the overlying water during the study.

Guideline Criteria	Reported Information
Test Water Soft reconstituted water or water from a natural source is preferred. Dechlorinated tap water may be used if the test organism will survive in it for the duration of the culturing and testing without showing signs of stress.	Elendt M7 medium was prepared with deionized water 7 days prior to test initiation; a detailed chemical composition was provided. Alkalinity, hardness and ammonium were measured in the negative control and 32.0 µg ai/L treatment levels on Days 0 and 28. Alkalinity ranged from 213.6-284.8 mg $CaCO_3$/L, total hardness ranged from 302.6-338.2 mg $CaCO_3$/L and ammonium ranged from 1.3-16.2 mg/L.
Test Sediment Formulated (reconstituted, artificial, or synthetic) sediment is recommended. Content of sediment by dry weight: 5% peat (dry) (pH 5.5-6.0) or alpha-cellulose, 75% quartz sand (>50% in size range of 50-200 microns), 20% kaolinite clay (kaolinite content ca. 30%), $CaCO_3$ 0.05-0.1%). Moisture content 30-50%, TOC 2% (±0.5%) and pH 6.5 - 7.5. Natural sediment can be used if it is fully characterized, unpolluted, and free of organisms that might compete with or consume chironomids. (If solvent other than water will be used, sand content of artificial sediment is adjusted accordingly.)	Formulated (artificial) sediment was prepared on a dry weight basis 7 days before the start of the exposure period: 74% fine quartz sand (68.2% with a particle size of 0.05-0.2 mm) 5.0% dried, finely-ground peat (sphagnum peat, pH 2-4) 20% kaolin (kaolinite content of about 36%, pH 7) *ca.* 1% calcium carbonate to adjust the pH value to 7 ± 0.5. Sediment characterization: TOC: 2.5% Moisture content: 31.4% pH: 6.6 CEC (meq/100 g sediment): 7.4
Sediment Conditioning Artificial sediment: 7 days in flowing dilution water prior to test initiation, chambers may be aerated	Prepared sediment was equilibrated for 7 days prior to test initiation.

Guideline Criteria	Reported Information
Introduction of Test Organisms Twenty-four hours prior to test initiation aeration of chambers is stopped and organisms are added to the chambers. Aeration should not resume for at least 24 hours. At test initiation, the test substance is spiked into the overlying water column.	On day -1, test organisms were introduced into the equilibrated test vessels five at a time, until each replicate test vessel contained 20 larvae. On Day 0 the test substance was applied just below the water surface with a pipette. The bottom of the test vessels were covered with a 1.5-cm layer of sediment. Gentle mixing of the water ensured homogenous distribution without disturbing the sediment. Dilution water (0.38 L) was added over the sediment layer with the aid of a sheet to avoid disturbance of the sediment. The sheet was removed following flooding. The final water height was 6.0 cm. Vessels were gently aerated throughout the study.
Solvents If used, minimal (i.e., #0.1 ml/l) and same concentration in all treatments. Suitable solvents are acetone, ethanol, methanol, elthylene glycol monoethyl ether, ethylene glycol dimethyl ether, dimethylformamide or triethylene glycol. (OECD guidelines also allows use of dispersants: Cremophor RH40, Tween 80, methycellulose 0.01%, and HCO-40)	DMF, 16.3 mg test substance was dissolved in 50 mL DMF to obtain the stock solution. The stock solution was stirred on a magnetic stirrer for 2 minutes. To obtain the application solution, 1.180 mL of the stock solution was made up to 1 L with M7 medium and was stirred on a magnetic stirrer for 2 minutes.
Water Temperature 20°C ± 2°C (Should not deviate between vessels by more than 1°C.)	19.2-19.5°C
pH Sediment: 7.0 ± 0.5 Interstitial Water: Overlying Water: 6.0 to 9.0 (Should not vary by more than 1 unit during test)	Sediment: Not determined Interstitial Water: Not determined Overlying Water: 8.4-8.7

Guideline Criteria	Reported Information
TOC Sediment: 2 ± 0.5% Overlying Water: 2 mg/L	Sediment: 2.5% (determined prior to introduction into vessels) Overlying Water: Not determined
Ammonia Interstitial Water: Overlying Water:	Interstitial Water: Not determined Overlying Water: 1.3-1.7 mg/L on day 0 and 15.7-16.2 mg/L on day 28 (as measured in the control and highest treatment level)
Total Water Hardness 200 mg/L as $CaCO_3$ (prefer 160 to 180 mg/L as $CaCO_3$)	302.6-338.2 mg/L as $CaCO_3$ on days 0 and 28 (as measured in the control and highest treatment level)
Dissolved Oxygen 60% air saturation value throughout test	≥8.2 mg/L (≥91% saturation)
Aeration Aeration (ca. one bubble/sec) is allowed except for when larvae are being added and for at least 24 hours after introduction of test organisms to a test chamber. If one test chamber is aerated all test chambers must be treated the same.	Continuously at a rate of *ca.* 2 bubbles/sec through Pasteur pipettes.
Test Vessels or Compartments 1. Material: Glass, No. 316 stainless steel, teflon or perfluorocarbon plastics 2. Size: Sediment depth of 1.5- 3 cm and the depth ratio of sediment to water should be ca. 1:4, must not be >1:4; 600 ml beaker with 8 cm diameter	Material: glass Size: 600 mL; 1.5-cm layer of sediment and 6-cm laboratory dilution water depth (380 mL). The height ratio was 1:4 sediment to overlying water.
Covers Test vessels should be covered with a glass plate.	Test vessels were covered with clear plastic plates to prevent evaporation
Photoperiod 16 hours light, 8 hours dark (Light intensity 500 to 1000 lux)	16 hours light, 8 hours dark Light intensity ~800 lux

Guideline Criteria	Reported Information
Food Green algae (e.g., *Scenedesmus subspicatus, Chlorella vulgaris*) or flaked fish food as a ground powder, suspension, or filtrate	Tetra Phyll® ornamental fish food suspension (1 g Tetra Phyll® per 20 mL water)
Food Concentration and Frequency Preferably feed daily but at least 3 times per week. day 1 to 10: 0.25-0.5 mg per larvae per day remainder of test: 0.5-1 mg per larvae per day (keep to a minimum, should not accumulate on sediment surface, cause overlying water to be cloudy or cause drop in DO)	At least 3 times per week 1 mg Tetra Phyll® per larvae day every 1 to 3 days

C. Test Design

Guideline Criteria	Reported Information
Duration *Chironomus riparius*: 28 days (if midges emerge early the test can be terminated after a minimum of 5 days after emergence of the last adult in the control).	28 days

Guideline Criteria	Reported Information
Nominal Concentrations Negative control, solvent control (if a solvent was used) and at least 5 test concentrations. (Note exception to dilution factors described below can be made for shallow slope responses but minimum number of test concentrations may need to be increased)	Negative control, solvent control, 0.25, 0.50, 1.00, 2.00, 4.00, 8.00, 16.0 and 32.0 µg ai/L
ECx endpoint: test concentrations should bracket ECx and span the environmental concentration range. Dilution factor should not be greater than two between exposure concentrations.	ECx endpoint: N/A.
NOAEC/LOAEC endpoint: factor between concentrations must not be greater than 3.	NOAEC/LOAEC endpoint: A nominal factor rate of 2.0 was used.
Number of Test Organisms** ECx endpoint: 60 larvae per treatment level; 3 replicates per treatment level	ECx endpoint: N/A
NOAEC/LOAEC endpoint: at least 80 larvae per treatment level with at least 4 replicates per treatment level (adequate power to detect a 20% difference, Type I error rate 5%)	NOAEC/LOAEC endpoint: 80 larvae per treatment level with 4 replicates per treatment level.
**(Optional) If data on 10-day growth and survival are needed additional replicates (number based on ECx or NOEC/LOEC endpoint determination) should be included at test initiation..	**(Optional) 10-day growth data were not collected.
Test organisms randomly or impartially assigned to test vessels?	Organisms were randomly assigned to test containers.

Guideline Criteria	Reported Information
Overlying Water Parameter Measurements 1. Dissolved oxygen should be measured daily in all test chambers.	1. Dissolved oxygen was measured twice weekly in the supplemental replicate vessels prepared for each treatment level.
2. Temperature and pH should be measured in all test chambers at the start and end of the test and at least once a week during the test.	2. Temperature and pH were measured once per week in the supplemental replicate vessels prepared for each treatment level.
3. Temperature should be monitored at least hourly throughout the test in one test chamber.	3. Criteria not required in OECD 219 guidance.
4. Hardness and ammonia should be measured in the controls and one test chamber at the highest concentration at the start and end of the test.	4. Hardness and ammonia levels were measured in one control and one 32.0-mg ai/kg vessel at study initiation and termination.
Chemical Analysis-Overlying Water At a minimum must be analyzed at test initiation (i.e., one hour after introduction of test substance into the test chamber) and at the end of the test in at least the highest concentration and one lower concentration.	The overlying water of the three surrogate vessels prepared at the control, solvent control 0.25, 4.00 and 32.0 µg ai/L levels were analyzed at 0 (before addition of larvae), 7, and 28 days.
Interstitial Water and Sediment Isolation Method Centrifugation (e.g., 10,000 g and 4 EC for 30 min) is recommended. If test substance is demonstrated not to adsorb to filters, filtration may be acceptable.	Overlying water was decanted carefully. The wet sediment of each beaker was filtered by vacuum (glass micro fiber filter, mesh size 1.0 µm) and the filtrate (pore water) was analyzed.
Chemical Analysis-Interstitial Water At a minimum must be analyzed at the end of the test in at least the highest concentration and one lower concentration.	The isolated pore water of the three surrogate vessels prepared at the control, solvent control, 0.25, 4.00 and 32.0 levels were analyzed at 0 (before addition of larvae), 7, and 28 days.

Guideline Criteria	Reported Information
Chemical Analysis-Bulk Sediment At a minimum must be analyzed at the end of the test in at least the highest concentration and one lower concentration.	Analysis of the sediment was not conducted. OECD guidance states that sediment analysis may not be necessary if the partitioning of the test substance between water and sediment has been demonstrated in a separate water/sediment study under similar conditions. No such study was reported.

12. REPORTED RESULTS

A. General Results

Guideline Criteria	Reported Information
Quality assurance and GLP compliance statements were included in the report?	Yes. This study was conducted in compliance with the GLP standards of the OECD and German Chemical Law (ChemG). It also meets the USEPA-FIFRA Good Laboratory Standards (40 CFR Part 160) as well as the GLP standards of the Japanese Ministry of Agriculture, Forestry and Fishereies (JMAFF, 11 Nohsan No. 6283 from Oct. 1999) with the exception that recognized differences exist between the GLP principles/standards of OECD and the GLP principles/standards of FIFRA and JMAFF.
Control Mortality <30%	Yes
Did chironomids emerge in controls between day 12 and 23?	Yes. Emergence was first observed on Day 14 for both controls and was done by Days 22 and 23 in the negative and solvent controls, respectively.
Control Emergence Mean emergence between 50-70%	Negative control – 82.5% emergence (66/80) Solvent control – 83.8% emergence (67/80)

Guideline Criteria	Reported Information
Data Endpoints Emergence Test (28 day) - Number alive - Time to emergence - Number of emerged male and female midges - Number of visible pupae that have failed to emerge - Number of egg masses deposited - Observations of other effects, abnormal behavior, or appearance or clinical signs (e.g., leaving sediment, unusual swimming) Growth and Survival (10-day) (Optional) - Number alive - Instar level of surviving larvae - Dry weight (ash free) per test chamber of surviving larvae by instar level	Emergence Test (28 day) - Number emerged; differentiated by sex - Development rate - Time to emergence - Number of dead larvae, pupae and midges which failed to emerge (visible) - Observations of other effects (i.e., sediment avoidance) Growth and Survival (10-day) (Optional) N/A
Raw data included?	Yes

Effects Data

Table 1. Summary of NNI-0001-des-iodo effects on *Chironomus riparius* emergence success and sex ratio

Toxicant Concentration				Initial No.	Total Number Emerged [c]			Mean Sex Ratio[d] (%)		% Inhibition in Emergence[e]
Nominal Overlying Water (µg ai/L)	TWA Concentrations [a]				♂	♀	Total	♂	♀	
	Overlying Water (µg ai/L)	Sediment[b]	Pore Water (µg ai/L)							
Negative control	<LOQ	N/A	<LOQ	80	38	28	66	57.6	42.4	N/A
Solvent control	<LOQ	N/A	<LOQ	80	35	32	67	52.2	47.8	-1.5
0.25	<LOQ	N/A	<LOQ	80	31	31	62	50.0	50.0	6.1
0.50	Not analyzed			80	33	34	67	49.3	50.7	-1.5
1.00	Not analyzed			80	27	33	60	45.0	55.0	9.1
2.00	Not analyzed			80	29	34	62	46.0	54.0	4.5
4.00	1.90	N/A	0.280	80	30	35	65	46.2	53.8	1.5
8.00	Not analyzed			80	29	26	55	52.7	47.3	16.7*
16.00	Not analyzed				25	19	44	56.8	43.2	33.3*
32.00	16.0	N/A	3.91	80	5	8	13	38.5	61.5	80.3*

[a] Reviewer-calculated time-weighted average for NNI-0001-des-iodo residues (from both overlying and pore water samples; refer to associated Excel spreadsheet). The LOQ for aqueous samples was 0.05 µg/L; when test material was <LOQ, ½ of the LOQ (0.0250 µg ai/L) was used in the TWA calculations.

[b] Samples were not collected from the sediment for analytical verification.

[c] Reviewer-calculated from the raw data

[d] $ER_{♂}$ = number of emerged males/number of emerged larvae; $ER_{♀}$ = number of emerged females/number of emerged larvae; reviewer-calculated.

[e] Reviewer-calculated relative to the negative control

N/A- Not Applicable

* Significant difference in percent emergence (% not emerged as calculated by the study author) from the pooled control (α=0.05).

Table 2. Summary of NNI-0001-des-iodo effects on *Chironomus riparius* development time and rate.

Toxicant Concentration				Days to First Emergence[b]	Mean Development Rate[c] (1/day)	% Inhibition in Mean Development Rate
Nominal Overlying Water (µg ai/L)	TWA Concentrations [a]					
	Overlying Water (µg ai/L)	Sediment[b]	Pore Water (µg ai/L)			
Negative control	<LOQ	N/A	<LOQ	14	0.057	N/A
Solvent control	<LOQ	N/A	<LOQ	14		
0.25	<LOQ	N/A	<LOQ	14	0.057	0.0
0.50	Not analyzed			14	0.056	1.8
1.00	Not analyzed			15	0.058	-1.8
2.00	Not analyzed			15	0.058	-1.8
4.00	1.90	N/A	0.280	15	0.057	0.0
8.00	Not analyzed			14	0.058	-1.8
16.00	Not analyzed			15	0.059	-3.5
32.00	16.0	N/A	3.91	16	0.053	7.0

[a] Reviewer-calculated time-weighted average for NNI-0001-des-iodo residues (from both overlying and pore water samples; refer to associated Excel spreadsheet). The LOQ for aqueous samples was 0.05 µg/L; when test material was <LOQ, ½ of the LOQ (0.0250 µg ai/L) was used in the TWA calculations.

[b] Reviewer-determined from summarized data tables. Does not represent mean days to first emergence.

[c] Mean development rate $= \sum_{i=1}^{m} \frac{f_i x_i}{n_e}$ where: i = index of inspection interval; m = maximum number of inspection intervals; f_i = number of midges emerged in the inspection interval i; n_e = total number of midges emerged; and

$x_i = 1 / \left(day_i - \frac{l_i}{2} \right)$ which is the development rate of the midges emerged in interval i; day_i = inspection day (days since application); and l_i = length of inspection interval i (days, 1 day in this study)

N/A- Not Applicable

Toxicity Observations: The Chi-square test indicated no statistically-different distribution (in number emerged) between sexes compared to the assumption of 50% females and 50%

males. Therefore, males and females were pooled for all further endpoint calculations to increase statistical power. Statistically-significant reductions in emergence were observed compared to the pooled controls at the 8.00, 16.00 and 32.00 µg ai/L treatment levels. No statistically-significant effects were observed in development rate.

No abnormal observations (dead larvae, pupae or midges) were observed in the controls or in the 0.25-16.00 µg ai/L treatment levels. At the highest treatment level, 32.00 µg ai/L, dead midges were observed on Days 17, 20, 21 and 23 and dead larvae/pupae were observed on Days 18, 19, 20 and 22. As these findings were observed in test concentrations clearly above the NOAEC (for emergence rates), they did not affect the outcome of the study.

B. Statistical Results (From Study Report)

Midge emergence, sex ratio, and development rate were statistically analyzed.

Midge emergence was evaluated as the percentage of midges that failed to emerge for each test level. Negative and solvent control emergence and development rate data were compared using a two-sided Chi-square 2 x 2 Table test (alpha = 0.05); no significant differences were observed, and the data were pooled for subsequent comparisons. Threshold concentrations (NOAEC) for emergence were determined using the Williams Multiple Sequential t-test Procedure (alpha = 0.05, one-sided).

The statistical distribution between sexes compared to the assumption of 50% males and 50% females were judged by a Chi-square 2 x 2 Contingency Table test. No significant effects were observed in sex distribution, and therefore, development rate data were reported using combined sexes.

For both endpoints, a range-to-standard-deviation-ratio test on Normal Distribution was tested (α=0.05) to test correspondence with normal distribution and Cochran's Test was conducted to test homogeneity of variance. Both the normality and homogeneity tests were passed, hence the use of a parametric multiple test.

For all endpoints, effective concentrations (ECx) were calculated using probit analysis.

Results were reported in terms of nominal initial overlying water concentrations.

Most sensitive endpoint: percent emergence

Endpoint	Methods	EC_{50} (95% CI) (µg ai/L)	NOAEC (µg ai/L)	LOAEC (µg ai/L)
28-d Percent Emergence (Pooled Sex)	Williams	18.6 (15.7-22.1)	4.00	8.00
28-d Development Rate (Pooled Sex)	Williams	>32.0	16.0	32.0
10-d Survival (Optional)	---	---	---	---
10-d Growth (Optional)	---	---	---	---

13. VERIFICATION OF STATISTICAL RESULTS

Statistical Method(s): Analyzed endpoints included percent emergence of the combined sexes, male development rate and female development rate. First, data from the negative and solvent control groups for all endpoints were compared using a Student's t-test to determine if a significant difference existed between the two controls; no differences were detected between the controls for any of the analyzed endpoints. Next, treatment data were tested for normality using Chi-square and Shapiro-Wilks tests and for homogeneity of variance using Hartley and Bartlett tests. As all data sets met these assumptions of ANOVA, NOAEC and LOAEC values were determined using the parametric Dunnetts' t-test (or Bonferroni's t-test for unequal replicates) and Williams' test via Toxstat Statistical software. The ECx values (with 95% C.I.) and probit slopes were determined using the probit analysis via Nuthatch Statistical software. All analyses were conducted using the nominal overlying water concentrations (µg ai/L). The mean replicate growth rate values for both males and females were multiplied by 10 by the reviewer in order to avoid treatment means of 0 within Toxstat.

Summary of Statistical Methods used for NOAEC/LOAEC Analyses.

Endpoint	Solvent vs Dilution Control		NOAEC/LOAEC	
	Method	Diff [1] (%)	Method	Diff [2] (%)
28-d Percent Emergence (Pooled sexes)	Student's t-test	-1.5%	Williams	1.5
28-d Development Rate- Male	Student's t-test	1.6%	Bonferroni	-0.8
28-d Development Rate- Female	Student's t-test	3.3%	Bonferroni	-2.8
10-d Survival (Optional)	N.D.	N.D.	N.D.	N.D.
10-day Dry Weight (Optional)	N.D.	N.D.	N.D.	N.D.

[1] Difference between the mean dilution water and solvent control responses.
[2] Difference between the dilution water and NOAEC concentration treatment.
N.D.- Not Determined

Most sensitive endpoint: Percent Emergence (combined sexes)

Verification Statistical Endpoint Values[a].

Statistical Endpoint	28-Day Emergence	28-Day Dev. Rate- Male	28-Day Dev. Rate- Female	10-D Survival	10-D Dry Weight
NOAEC	4.00	16.00	16.00	ND	ND
LOAEC	8.00	32.00	32.00	ND	ND
IC_{50} (95% C.I.)	20 (18-24)	>32.00	>32.00	ND	ND
Slope (Standard Error)	4.07±0.664	N/A	N/A	ND	ND

[a] Results are based on nominal initial overlying water concentrations (µg ai/L).
The 10-Day Survival and Dry Weight were not assessed

14. REVIEWER'S COMMENTS:

The reviewer's conclusions were more conservative than those of the study author as the reviewer detected significant inhibitions in male and female development rate at the highest treatment level relative to the negative control. Therefore, the reviewer's results are reported in the Conclusions section of this DER.

Overlying water and pore water samples from the surrogate vessels (one vessel per interval) were analyzed at 0, 7, and 28 days and analyzed for residues of NNI-0001-des-iodo for TWA calculations. However, as actual concentrations were not determined for each treatment level, results were reported in terms of initial nominal overlying water levels.

A detailed statistical report was provided in the study. Development rate data were assessed not only for combined sexes, but also for individual sexes. As assessment of the sex ratio percentages were not statistically different, only combined-sex data were reported within the study. However, the reviewer analyzed both data sets separately as both were readily available.

Overlying and pore water samples were analyzed by direct injection of the samples into an HPLC-MS/MS instrument. The mass spectrometric detector showed linear response in the concentration range of 0.042 µg/L to 12.5 µg/L for NNI-0001 in surface water with a correlation coefficient of 0.9995 and in the concentration range of 0.041 µg/L to 12.3 µg/L for NNI-0001-des-iodo in surface water with a correlation coefficient of 0.9997.

The MS/MS detection of NNI-0001 and NNI-0001-des-iodo were slightly affected by the matrix. The peak area of NNI-0001 in a surface water sample containing 0.5 µg/L was reduced to approximately 82% of the corresponding peak area in milli-Q-water. The peak area of NNI-0001-des-iodo in a surface water sample containing 0.5 µg/L was reduced to approximately 83% of the corresponding peak area in milli-Q-water.

The reviewer calculated the time-weighted average concentrations for the nominal 0.25, 4.00 and 32.00 µg ai/L treatment levels using the following equation:

$$C_{TWA} = \frac{\left(\frac{C_1 + C_0}{2}\right)(t_1 - t_0) + \left(\frac{C_2 + C_1}{2}\right)(t_2 - t_1) + \left(\frac{C_{n-1} + C_2}{2}\right)(t_{n-1} - t_2) + \left(\frac{C_n + C_{n-1}}{2}\right)(t_n - t_{n-1})}{t_n}$$

where:

C_{TWA} is the time-weighted average concentration,

C_j is the concentration measured at time interval j (j = 0, 1, 2,...n)

t_j is the number of hours (or days or weeks, units used just need to be consistent in the equation) of the test at time interval j

(e.g., t_0 = 0 hours (test initiation), t_1 =24 hours, t_2 =96 hours)

The experimental work began on March 5, 2004. The biological and analytical portions of the study were completed on April 9, 2004 and April 26, 2004. An initial definitive test was initiated on October 31, 2003; however, the chosen test concentrations did now show enough dose-related effects. The results from this test were not included in the study report.

APPENDIX I. OUTPUT OF REVIEWER'S STATISTICAL ANALYSIS:

```
% Emergence (pooled sex), Day 28; ug ai/L
File: 7023pe        Transform: NO TRANSFORM

       t-test of Solvent and Blank Controls          Ho:GRP1 MEAN = GRP2 MEAN
-------------------------------------------------------------------------------
 GRP1 (SOLVENT CRTL) MEAN =    82.5000     CALCULATED t VALUE =    -0.3612
 GRP2 (BLANK CRTL) MEAN   =    83.7500     DEGREES OF FREEDOM =     6
 DIFFERENCE IN MEANS      =    -1.2500
-------------------------------------------------------------------------------
TABLE t VALUE (0.05 (2), 6) =  2.447    NO significant difference at alpha=0.05
TABLE t VALUE (0.01 (2), 6) =  3.707    NO significant difference at alpha=0.01

% Emergence (pooled sex), Day 28; ug ai/L
File: 7023pe       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     2.412         8.712           13.752          8.712        2.412
OBSERVED     0            13               12             11           0

-------------------------------------------------------------------------------
Calculated Chi-Square goodness of fit test statistic =   7.7586
Table Chi-Square value (alpha = 0.01) = 13.277

Data PASS normality test. Continue analysis.


% Emergence (pooled sex), Day 28; ug ai/L
File: 7023pe       Transform: NO TRANSFORMATION

Shapiro Wilks test for normality
-------------------------------------------------------------------------------

D = 2693.750

W =    0.964

Critical W (P = 0.05) (n = 36) = 0.935
Critical W (P = 0.01) (n = 36) = 0.912
-------------------------------------------------------------------------------

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


% Emergence (pooled sex), Day 28; ug ai/L
File: 7023pe        Transform: NO TRANSFORMATION

Hartley test for homogeneity of variance
-------------------------------------------------------------------------------

Calculated H statistic (max Var/min Var) =   22.75
Closest, conservative, Table H statistic =  281.0  (alpha = 0.01)

Used for Table H ==>     R (# groups) =  9,    df (# reps-1) =       3
Actual values    ==>     R (# groups) =  9,    df (# avg reps-1) =   3.00

-------------------------------------------------------------------------------
```

Data PASS homogeneity test. Continue analysis.

NOTE: This test requires equal replicate sizes. If they are unequal
but do not differ greatly, the Hartley test may still be used
as an approximate test (average df are used).

% Emergence (pooled sex), Day 28; ug ai/L
File: 7023pe Transform: NO TRANSFORMATION

Bartletts test for homogeneity of variance

Calculated B statistic = 8.58
Table Chi-square value = 20.09 (alpha = 0.01)
Table Chi-square value = 15.51 (alpha = 0.05)

Average df used in calculation ==> df (avg n - 1) = 3.00
Used for Chi-square table value ==> df (#groups-1) = 8

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).

% Emergence (pooled sex), Day 28; ug ai/L
File: 7023pe Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	8	14925.000	1865.625	18.699
Within (Error)	27	2693.750	99.769	
Total	35	17618.750		

Critical F value = 2.31 (0.05,8,27)
Since F > Critical F REJECT Ho:All groups equal

% Emergence (pooled sex), Day 28; ug ai/L
File: 7023pe 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	neg control	82.500	82.500		
2	0.25	77.500	77.500	0.708	
3	0.50	83.750	83.750	-0.177	
4	1.00	75.000	75.000	1.062	
5	2.00	78.750	78.750	0.531	
6	4.00	81.250	81.250	0.177	
7	8.00	68.750	68.750	1.947	
8	16.00	55.000	55.000	3.894	*
9	32.00	16.250	16.250	9.380	*

Dunnett table value = 2.53 (1 Tailed Value, P=0.05, df=24,8)

% Emergence (pooled sex), Day 28; ug ai/L
File: 7023pe Transform: NO TRANSFORMATION

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

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	neg control	4			
2	0.25	4	17.869	21.7	5.000
3	0.50	4	17.869	21.7	-1.250
4	1.00	4	17.869	21.7	7.500
5	2.00	4	17.869	21.7	3.750
6	4.00	4	17.869	21.7	1.250
7	8.00	4	17.869	21.7	13.750
8	16.00	4	17.869	21.7	27.500
9	32.00	4	17.869	21.7	66.250

% Emergence (pooled sex), Day 28; ug ai/L
File: 7023pe Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	neg control	4	82.500	82.500	82.500
2	0.25	4	77.500	77.500	80.625
3	0.50	4	83.750	83.750	80.625
4	1.00	4	75.000	75.000	78.333
5	2.00	4	78.750	78.750	78.333
6	4.00	4	81.250	81.250	78.333
7	8.00	4	68.750	68.750	68.750
8	16.00	4	55.000	55.000	55.000
9	32.00	4	16.250	16.250	16.250

% Emergence (pooled sex), Day 28; ug ai/L
File: 7023pe 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
neg control	82.500				
0.25	80.625	0.265		1.71	k= 1, v=27
0.50	80.625	0.265		1.79	k= 2, v=27
1.00	78.333	0.590		1.81	k= 3, v=27
2.00	78.333	0.590		1.82	k= 4, v=27
4.00	78.333	0.590		1.83	k= 5, v=27
8.00	68.750	1.947	*	1.84	k= 6, v=27
16.00	55.000	3.894	*	1.84	k= 7, v=27
32.00	16.250	9.380	*	1.84	k= 8, v=27

s = 9.988
Note: df used for table values are approximate when v > 20.

Estimates of EC%

Parameter	Estimate	95% Bounds Lower	95% Bounds Upper	Std.Err.	Lower Bound /Estimate
EC5	8.0	5.4	12.	0.087	0.67
EC10	9.9	7.0	14.	0.073	0.71
EC25	14.	11.	18.	0.051	0.79
EC50	20.	18.	24.	0.031	0.87

Slope = 4.07 Std.Err. = 0.664

Goodness of fit: p = 0.84 based on DF= 6.0 27.

7023PE : % Emergence (pooled sex), Day 28; ug ai/L

Observed vs. Predicted Treatment Group Means

Dose	#Reps.	Obs. Mean	Pred. Mean	Obs. -Pred.	Pred. %Control	%Change
0.00	4.00	82.5	79.1	3.43	100.	0.00
0.250	4.00	77.5	79.1	-1.57	100.	3.59e-13
0.500	4.00	83.8	79.1	4.68	100.	2.73e-09
1.00	4.00	75.0	79.1	-4.07	100.	4.88e-06
2.00	4.00	78.8	79.1	-0.321	100.	0.00202
4.00	4.00	81.3	78.9	2.34	99.8	0.200
8.00	4.00	68.8	75.2	-6.43	95.1	4.93
16.0	4.00	55.0	52.6	2.43	66.5	33.5
32.0	4.00	16.3	16.7	-0.482	21.2	78.8

Male development rate, Day 28; ug ai/L
File: 7023md Transform: NO TRANSFORM

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

GRP1 (SOLVENT CRTL) MEAN =	0.6150	CALCULATED t VALUE =	0.7746
GRP2 (BLANK CRTL) MEAN =	0.6050	DEGREES OF FREEDOM =	6
DIFFERENCE IN MEANS =	0.0100		

TABLE t VALUE (0.05 (2), 6) = 2.447 NO significant difference at alpha=0.05
TABLE t VALUE (0.01 (2), 6) = 3.707 NO significant difference at alpha=0.01

Male development rate, Day 28; ug ai/L
File: 7023md 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	2.345	8.470	13.370	8.470	2.345
OBSERVED	0	12	13	10	0

Calculated Chi-Square goodness of fit test statistic = 6.4478
Table Chi-Square value (alpha = 0.01) = 13.277

Data PASS normality test. Continue analysis.

Male development rate, Day 28; ug ai/L
File: 7023md Transform: NO TRANSFORMATION

```
Shapiro Wilks test for normality
--------------------------------------------------------------------------------

D =    0.019

W =    0.949

Critical W (P = 0.05) (n = 35) = 0.934
Critical W (P = 0.01) (n = 35) = 0.910
--------------------------------------------------------------------------------

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


Male development rate, Day 28; ug ai/L
File: 7023md        Transform: NO TRANSFORMATION

Hartley test for homogeneity of variance
--------------------------------------------------------------------------------

Calculated H statistic (max Var/min Var) =   73.50
Closest, conservative, Table H statistic =  281.0  (alpha = 0.01)

Used for Table H ==>     R (# groups) =   9,    df (# reps-1) =       3
Actual values    ==>     R (# groups) =   9,    df (# avg reps-1) =   2.89
                                                (average df used)

--------------------------------------------------------------------------------

Data PASS homogeneity test. Continue analysis.

NOTE: This test requires equal replicate sizes. If they are unequal
      but do not differ greatly, the Hartley test may still be used
      as an approximate test (average df are used).


Male development rate, Day 28; ug ai/L
File: 7023md       Transform: NO TRANSFORMATION

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

Calculated B statistic =   15.82
Table Chi-square value =   20.09  (alpha = 0.01)
Table Chi-square value =   15.51  (alpha = 0.05)

Average df used in calculation  ==>   df (avg n - 1) =   2.89
Used for Chi-square table value ==>   df (#groups-1) =   8
--------------------------------------------------------------------------------

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).

Male development rate, Day 28; ug ai/L
File: 7023md        Transform: NO TRANSFORMATION

                                ANOVA TABLE
--------------------------------------------------------------------------------

SOURCE            DF              SS                MS            F
--------------------------------------------------------------------------------
```

Between	8	0.0068	0.0008	1.143
Within (Error)	26	0.0193	0.0007	
Total	34	0.0261		

Critical F value = 2.32 (0.05,8,26)
Since F < Critical F FAIL TO REJECT Ho:All groups equal

Male development rate, Day 28; ug ai/L
File: 7023md 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	neg control	0.615	0.615		
2	0.25	0.600	0.600	0.802	
3	0.50	0.608	0.608	0.401	
4	1.00	0.620	0.620	-0.267	
5	2.00	0.623	0.623	-0.401	
6	4.00	0.610	0.610	0.267	
7	8.00	0.613	0.613	0.134	
8	16.00	0.620	0.620	-0.267	
9	32.00	0.570	0.570	2.227	

Bonferroni T table value = 2.68 (1 Tailed Value, P=0.05, df=26,8)

Male development rate, Day 28; ug ai/L
File: 7023md 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	neg control	4			
2	0.25	4	0.050	8.2	0.015
3	0.50	4	0.050	8.2	0.007
4	1.00	4	0.050	8.2	-0.005
5	2.00	4	0.050	8.2	-0.007
6	4.00	4	0.050	8.2	0.005
7	8.00	4	0.050	8.2	0.003
8	16.00	4	0.050	8.2	-0.005
9	32.00	3	0.054	8.8	0.045

Male development rate, Day 28; ug ai/L
File: 7023md Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	neg control	4	0.615	0.615	0.615
2	0.25	4	0.600	0.600	0.613
3	0.50	4	0.608	0.608	0.613

4	1.00	4	0.620	0.620	0.613
5	2.00	4	0.623	0.623	0.613
6	4.00	4	0.610	0.610	0.613
7	8.00	4	0.613	0.613	0.613
8	16.00	4	0.620	0.620	0.613
9	32.00	3	0.570	0.570	0.570

Male development rate, Day 28; ug ai/L
File: 7023md 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
neg control	0.615				
0.25	0.613	0.093		1.71	k= 1, v=26
0.50	0.613	0.093		1.79	k= 2, v=26
1.00	0.613	0.093		1.81	k= 3, v=26
2.00	0.613	0.093		1.82	k= 4, v=26
4.00	0.613	0.093		1.83	k= 5, v=26
8.00	0.613	0.093		1.84	k= 6, v=26
16.00	0.613	0.093		1.84	k= 7, v=26
32.00	0.570	2.166	*	1.84	k= 8, v=26

s = 0.027
Note: df used for table values are approximate when v > 20.

Female development rate, Day 28; ug ai/L
File: 7023fd Transform: NO TRANSFORM

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

GRP1 (SOLVENT CRTL) MEAN =	0.5350	CALCULATED t VALUE =	1.5275
GRP2 (BLANK CRTL) MEAN =	0.5175	DEGREES OF FREEDOM =	6
DIFFERENCE IN MEANS =	0.0175		

TABLE t VALUE (0.05 (2), 6) = 2.447 NO significant difference at alpha=0.05
TABLE t VALUE (0.01 (2), 6) = 3.707 NO significant difference at alpha=0.01

Female development rate, Day 28; ug ai/L
File: 7023fd 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	2.345	8.470	13.370	8.470	2.345
OBSERVED	0	11	13	11	0

Calculated Chi-Square goodness of fit test statistic = 6.2117
Table Chi-Square value (alpha = 0.01) = 13.277

Data PASS normality test. Continue analysis.

Female development rate, Day 28; ug ai/L
File: 7023fd Transform: NO TRANSFORMATION

Shapiro Wilks test for normality

D = 0.006

W = 0.969

Critical W (P = 0.05) (n = 35) = 0.934
Critical W (P = 0.01) (n = 35) = 0.910

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

Female development rate, Day 28; ug ai/L
File: 7023fd Transform: NO TRANSFORMATION

Hartley test for homogeneity of variance

Calculated H statistic (max Var/min Var) = 14.75
Closest, conservative, Table H statistic = 281.0 (alpha = 0.01)

Used for Table H ==> R (# groups) = 9, df (# reps-1) = 3
Actual values ==> R (# groups) = 9, df (# avg reps-1) = 2.89
(average df used)

Data PASS homogeneity test. Continue analysis.

NOTE: This test requires equal replicate sizes. If they are unequal but do not differ greatly, the Hartley test may still be used as an approximate test (average df are used).

Female development rate, Day 28; ug ai/L
File: 7023fd Transform: NO TRANSFORMATION

Bartletts test for homogeneity of variance

Calculated B statistic = 6.40
Table Chi-square value = 20.09 (alpha = 0.01)
Table Chi-square value = 15.51 (alpha = 0.05)

Average df used in calculation ==> df (avg n - 1) = 2.89
Used for Chi-square table value ==> df (#groups-1) = 8

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).

Female development rate, Day 28; ug ai/L
File: 7023fd Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	8	0.0043	0.0005	2.500

Within (Error)	26	0.0060	0.0002
Total	34	0.0103	

Critical F value = 2.32 (0.05,8,26)
Since F > Critical F REJECT Ho:All groups equal

Female development rate, Day 28; ug ai/L
File: 7023fd 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	neg control	0.535	0.535		
2	0.25	0.528	0.528	0.750	
3	0.50	0.525	0.525	1.000	
4	1.00	0.540	0.540	-0.500	
5	2.00	0.540	0.540	-0.500	
6	4.00	0.543	0.543	-0.750	
7	8.00	0.545	0.545	-1.000	
8	16.00	0.545	0.545	-1.000	
9	32.00	0.507	0.507	2.623	

Bonferroni T table value = 2.68 (1 Tailed Value, P=0.05, df=26,8)

Female development rate, Day 28; ug ai/L
File: 7023fd 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	neg control	4			
2	0.25	4	0.027	5.0	0.007
3	0.50	4	0.027	5.0	0.010
4	1.00	4	0.027	5.0	-0.005
5	2.00	4	0.027	5.0	-0.005
6	4.00	4	0.027	5.0	-0.008
7	8.00	4	0.027	5.0	-0.010
8	16.00	4	0.027	5.0	-0.010
9	32.00	3	0.029	5.4	0.028

Female development rate, Day 28; ug ai/L
File: 7023fd Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	neg control	4	0.535	0.535	0.538
2	0.25	4	0.528	0.528	0.538
3	0.50	4	0.525	0.525	0.538
4	1.00	4	0.540	0.540	0.538

5	2.00	4	0.540	0.540	0.538
6	4.00	4	0.543	0.543	0.538
7	8.00	4	0.545	0.545	0.538
8	16.00	4	0.545	0.545	0.538
9	32.00	3	0.507	0.507	0.507

Female development rate, Day 28; ug ai/L
File: 7023fd 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
neg control	0.538				
0.25	0.538	0.233		1.71	k= 1, v=26
0.50	0.538	0.233		1.79	k= 2, v=26
1.00	0.538	0.233		1.81	k= 3, v=26
2.00	0.538	0.233		1.82	k= 4, v=26
4.00	0.538	0.233		1.83	k= 5, v=26
8.00	0.538	0.233		1.84	k= 6, v=26
16.00	0.538	0.233		1.84	k= 7, v=26
32.00	0.507	2.446	*	1.84	k= 8, v=26

s = 0.015
Note: df used for table values are approximate when v > 20.