DATA EVALUATION RECORD
FRESHWATER SEDIMENT *Chironomus riparius* EMERGENCE TEST

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

2. **TEST MATERIAL:** [^{14}C]Flubendiamide-desiodo Purity: 99%

3. **CITATION:**

Authors: Thomas, S., *et al.*
Title: [^{14}C]NNI-0001-desiodo: A Prolonged Sediment Toxicity Test with *Chironomus riparius* Using Spiked Sediment.
Study Completion Date: July 28, 2010
Laboratory: Wildlife International Ltd.
8598 Commerce Drive
Easton, MD 21601
Sponsor: Bayer CropScience
P.O. Box 12014, 2T.W. Alexander Drive
Research Triangle Park, NC 27709
Laboratory Report ID: 149A-235
MRID No.: 48175605

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

Signature: Christie E. Padova **Date:** 01/31/11

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

Signature: Teri S Myers **Date:** 02/16/11

5. **APPROVED BY:** Robin Sternberg, EPA

Signature: Robin Sternberg **Date:** 7/19/11

6. **STUDY PARAMETERS**

Scientific Name of Test Organism: *Chironomus riparius*
Age of Test Organism: 1st instar larvae, 1 to 4 days post-hatch
Definitive Test Duration: 28 days
Study Method: Static, with aeration
Type of Concentrations: TWA sediment, pore water, and overlying water

M-425247-01-1

7. CONCLUSIONS:

Results Synopsis:

Time-Weighted Average (TWA) Sediment Concentrations:
28-day LC_{50}: >52.6 µg TRR/kg
28-day NOAEC: 52.6 µg TRR/kg
28-day LOAEC: >52.6 µg TRR/kg

Time-Weighted Average (TWA) Pore Water Concentrations:
28-day LC_{50}: >19.5 µg TRR/L
28-day NOAEC: 19.5 µg TRR/L
28-day LOAEC: >19.5 µg TRR/L

Time-Weighted Average (TWA) Overlying Water Concentrations:
28-day LC_{50}: >7.18 µg TRR/L
28-day NOAEC: 7.18 µg TRR/L
28-day LOAEC: >7.18 µg TRR/L

Assessment endpoints: percent emergence (survival), emergence ratio, development rate, and development time
Most sensitive endpoints: none

8. ADEQUACY OF THE STUDY:

A. Classification: Supplemental

B. Rationale: This study was conducted according to OECD Guideline 218: *Sediment-Water Chironomid Toxicity Test Using Spiked Sediment* (April 2004), and does not fulfill any current U.S. EPA data requirement.

C. Reparability: N/A

9. MAJOR GUIDELINE DEVIATIONS (from OECD Guideline 218):

It was not reported if aeration of the overlying water was stopped for a 24-hour period during and immediately following the insertion of the larvae.

10. SUBMISSION PURPOSE: RS Non-PRIA 575 data

11. MATERIALS AND METHODS

Stability of Compound Under Test Conditions: The stability of flubendiamide-desiodo was not specifically assessed. However, overlying water, pore water, and sediment samples were analyzed for total radioactive residues (TRR) of the test substance using LSC analyses on Days 0, 7, and 28. In general, the concentrations of TRR were variable but showed an overall decrease in sediment, while concentrations of TRR decreased in pore water and increased in overlying water. The majority of radioactivity remained associated with the sediment.

In the treated sediment, recoveries of TRR ranged from 55.2 to 71.3% of nominal concentrations at 0 Days, 40.5 to 83.2% of nominal at 7 Days, and 43.4 to 57.7% of nominal at 28 Days. In overlying water samples, concentrations of TRR increased 71 to 146% of initial measured levels from Days 0 to 28 at all levels (reviewer-calculated). In pore water, concentrations of TRR decreased 43 to 52% of initial measured levels from Days 0 to 28 at all levels. For all matrices, time-weighted averaged (TWA) concentrations were reviewer-calculated (using Excel software; copy provided in Appendix II).

Mass balance approximations were provided by the study authors. The TRR recovered ranged from 71.7 to 112% of the applied for all levels and intervals.

Physicochemical properties of flubendiamide-desiodo.

Parameter	Values	Comments
Water solubility at 20°C	Not reported	
Vapor 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*, identity verified by supplier
Source	Egg masses were supplied by Environmental Consulting and Testing, Superior, Wisconsin
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%	N/A
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.	The organisms were held for 5 days prior to the start of the test at approximately the same temperature used during testing and in water from the same source as used during testing. During the 5-day holding period preceding the test, water temperatures ranged from 19.9 to 20.4°C, the pH ranged from 8.3 to 8.5, and the dissolved oxygen ranged from 7.8 to 8.9 mg/L (≥86% saturation).
Age of Test Larvae First instar (1 to 4 days post-hatch with confirmation)	1st instar, 1 to 4 days post-hatch The hatched midges from at least three separate egg masses were used to initiate the test.

Guideline Criteria	Reported Information
Food Green algae (e.g., *Scenedesmus subspicatus, Chlorella vulgaris*) or flaked fish food as a ground powder, suspension, or filtrate	Ground Hartz® pet rabbit food
Health of parent culture stock Were parent chironomids in good health during the culture period?	N/A

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. Additional vessels were prepared at each level for analytical sampling; thus, the method for analytical sampling did not affect volume, biological load, or test concentration.
Test Material	Identity: [^{14}C]flubendiamide-desiodo Batch No.: Not reported Description: solid Radiochemical purity: 99% Specific activity: 79.26 mCi/mmol Label position: uniformly on the phthalic acid ring Storage: frozen conditions

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.	Moderately-hard freshwater obtained from an on-site well *ca.* 40-m deep was sand-filtered, aerated, and filtered again (0.45 µm) and UV-sterilized prior to use. During the 4-week period immediately preceding the study, the specific conductance of the well water ranged from 338 to 366 µS/cm, the hardness ranged from 140 to 144 mg/L as $CaCO_3$, the alkalinity ranged from 180 to 182 mg/L as $CaCO_3$, and the pH ranged from 8.1 to 8.2.
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 consisted of 75% industrial quartz sand, 20% kaolin clay, and 5% sphagnum peat moss. The dry ingredients were mixed in a PK Twinshell® mixer for 40 minutes and stored under ambient conditions until use. The amount of peat added to the batch sediment was adjusted for the moisture content in the peat suspension (70%). The laboratory-determined pH of the sediment was 7.2. The soil was characterized by Agvise Laboratories (Northwood, ND). The following characteristics were provided: Composition: 77% sand, 9% silt, and 14% clay USDA textural class: sandy loam Bulk density: 1.24 g/cm^3 CEC: 9.3 meq/100 g Moisture at 1/3 bar: 11.5% Organic carbon: 1.9% Organic matter: 3.2% pH (1:1 soil:water ratio): 7.5

Guideline Criteria	Reported Information
Sediment Spiking	A 0.140 mg/mL primary stock solution was prepared by dissolving the radio-labeled test material in acetone. Secondary stocks (10.0, 5.00, 2.50, 1.30, 0.63, and 0.31 µg/mL) were prepared by proportional dilution and mixed by inversion. The primary and secondary stock solutions appeared clear and colorless. A 15-mL aliquot of the appropriate stock solution was added to 150 g of formulated sediment and mixed by hand, and the acetone was allowed to partially evaporate. The 150-g premix was added to 600 g of untreated sediment and mixed for an unspecified period of time, and then 750 g of untreated sediment was added and the final batches (1500 g final weight) mixed using a rotary mixer for *ca.* 40 hours. Batches of negative and solvent control sediment were also prepared. No adjustments were made for the purity of the test material.
Sediment Conditioning Artificial sediment: 7 days in flowing dilution water prior to test initiation, chambers may be aerated	Test systems (spiked-sediment:overlying water) were prepared and acclimated for *ca.* 50 hours prior to the introduction of the test organisms. The systems were gently aerated and maintained in an environmental chamber.
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.	At test initiation, midge larvae were impartially added one and two at a time to the test chambers. It was not reported if aeration was discontinued during and 24 hour immediately following the insertion of larvae.

Guideline Criteria	Reported Information
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)	Acetone, 15 mL/1500 g sediment The reviewer-calculated maximum possible concentration of acetone in the sediment (assuming no evaporation occurred) was equivalent to 0.8% (where ρ of acetone = 0.79 g/mL).
Water Temperature 20°C ± 2°C (Should not deviate between vessels by more than 1°C.)	Daily: 19.8 to 20.8°C Continuous: 19 to 20°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: 7.2 to 7.5 (initial analysis) Interstitial Water: Not determined Overlying Water: 8.0 to 8.6
TOC Sediment: 2 ± 0.5% Overlying Water: 2 mg/L	Sediment: 1.9% (initial analysis) Overlying Water: Not determined
Ammonia Interstitial Water: Overlying Water:	Interstitial Water: Not determined Overlying Water: Day 0: <0.17 mg/L Day 28: ≤1.57 mg/L
Total Water Hardness 200 mg/L as $CaCO_3$ (prefer 160 to 180 mg/L as $CaCO_3$)	156 to 164 mg/L as $CaCO_3$
Dissolved Oxygen 60% air saturation value throughout test	≥5.6 mg/L (≥62% of saturation)

Guideline Criteria	Reported Information
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.	Gentle aeration (>1 bubble/sec) was provided to each vessel through a glass pipette that did not extend to a depth closer than 2 cm from the sediment's surface. It was not reported if aeration was stopped during the addition of larvae.
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	Test vessels were 1-quart glass jars containing 2 cm of sediment and 600 mL of overlying water. The measured depth in sediment and overlying water from one representative chamber was 2.1 and 8.3 cm, respectively. Thus, the sediment:water ratio was ≈1:4.
Covers Test vessels should be covered with a glass plate.	Vessels were loosely covered with plastic dishes.
Photoperiod 16 hours light, 8 hours dark (Light intensity 500 to 1000 lux)	16 hours light:8 hours dark, with 30-minute low light transition periods Light intensity was 446 lux at the surface of one representative test chamber.
Food Green algae (e.g., *Scenedesmus subspicatus, Chlorella vulgaris*) or flaked fish food as a ground powder, suspension, or filtrate	Ground Hartz® pet rabbit food
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)	Three times per week 10 to 30 mg per vessel per feeding

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
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) ECx endpoint: test concentrations should bracket ECx and span the environmental concentration range. Dilution factor should not be greater than two between exposure concentrations. NOEC/LOEC endpoint: factor between concentrations must not be greater than 3.	Negative control, solvent control, 3.1, 6.3, 13, 25, 50, and 100 µg/kg dw sediment (not corrected for purity) ECx endpoint: N/A NOAEC/LOAEC endpoint: A nominal factor rate of 2 was used.
Number of Test Organisms** ECx endpoint: 60 larvae per treatment level; 3 replicates per treatment level 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%) *(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..	ECx endpoint: N/A NOAEC/LOAEC endpoint: 80 larvae per treatment level divided evenly into four replicates (each containing 20 organisms). **(Optional) 10-day growth data were not collected.

Guideline Criteria	Reported Information
Test organisms randomly or impartially assigned to test vessels?	Yes
Overlying Water Parameter Measurements 1. Dissolved oxygen should be measured daily in all test chambers. 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. 3. Temperature should be monitored at least hourly throughout the test in one test chamber. 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.	1. – 3. DO and temperature were measured daily in one alternating replicate chamber for each level. Temperature was also continuously monitored in a beaker of water adjacent to the test chambers. The pH was measured at test initiation, weekly during the test, and at test termination in one alternating replicate chamber for each level. 4. Hardness, ammonia, specific conductance, and alkalinity were measured in a composite sample of overlying water from the control groups and from the highest treatment level (i.e., 100 µg/kg) 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.	Surrogate samples (three per level) were collected for analysis on Days 0, 7, and 28. Overlying water was decanted and 10-mL aliquots analyzed for total radioactive residues of [^{14}C]flubendiamide-desiodo using LSC. The limit of quantitation (LOQ) was 0.0133 µg/L.
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.	Not reported

Guideline Criteria	Reported Information
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.	Surrogate samples (three per level) were collected for analysis on Days 0, 7, and 28; 10-mL aliquots were analyzed for total radioactive residues of [^{14}C]flubendiamide-desiodo using LSC. The limit of quantitation (LOQ) was 0.0133 µg/L.
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.	Surrogate samples (three per level) were collected for analysis on Days 0, 7, and 28. Isolated sediment was dried overnight and analyzed for total radioactive residues of [^{14}C]flubendiamide-desiodo using LSC following combustion. The limit of quantitation (LOQ) was 0.293 µg/kg.

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 U.S. EPA 40 CFR, Parts 160 and 792, with the following exceptions: periodic analysis of well water and sediment for potential contaminants, and the stability of the test substance under conditions of storage at the testing facility. It was reported that the periodic analysis (of water and sediment) was performed using a certified laboratory and standard U.S. EPA analytical methods.
Control Mortality <30%	Negative control – 29% Solvent control – 30%
Did chironomids emerge in controls between day 12 and 23?	Negative controls – Days 15 to 28 Solvent control – Days 15 to 28

Guideline Criteria	Reported Information
Control Emergence Mean emergence between 50-70%	Negative control – 71% emergence Solvent control – 73% emergence
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 days) - Mortality - Time to emergence - Number of emerged male and female midges - Emergence rate - Development rate - Development time Growth and Survival (10-day) (Optional) N/A
Raw data included?	Yes

Effects Data

Table 1. Summary of [^{14}C]Flubendiamide-desiodo effects on *Chironomus riparius* emergence success and sex ratio

Toxicant Concentration				Initial No.	Mean Number Emerged			Mean Sex Ratio[b] (%)		% Emergence (Day 28)
Mean-measured (and Nominal) Sediment (µg/kg dw)	TWA Measured[a]				♂	♀	Total	♂	♀	
	Sediment (µg TRR/ kg dw)	Overlying Water (µg TRR/L)	Pore Water (µg TRR/L)							
Negative control	<LOQ	<LOQ	<LOQ	80	34	23	57	60	40	71
Solvent control	<LOQ	<LOQ	<LOQ	80	36	22	58	62	38	73
1.7 (3.1)	1.75	0.195	0.551	80	27	22	49	55	45	61
4.3 (6.3)	4.44	0.453	1.10	80	27	24	51	53	47	64
7.8 (13)	7.31	0.895	2.44	80	25	32	57	44	56	71
13 (25)	12.2	1.72	4.28	80	32	27	59	54	46	74
30 (50)	28.5	3.50	9.06	80	23	32	55	42	58	69
55 (100)	52.6	7.18	19.5	80	35	29	64	55	45	80

[a] TWA concentrations were determined by the reviewer using Excel software (copy of worksheet in Appendix II). The limit of quantitation (LOQ) was 0.293 µg TRR/kg for sediment and 0.0133 µg TRR/L for overlying and pore water. TRR = Total Radioactive Residues of [^{14}C]flubendiamide-desiodo.

[b] Equivalent to the number of emerged males (or females)/number of emerged larvae x 100; reviewer-calculated.

Table 2. Summary of [^{14}C]Flubendiamide-desiodo effects on *Chironomus riparius* development time and rate.

Toxicant Concentration				Mean Emergence Ratio	Mean Development Rate[b]	Mean Development Time
Mean-measured (and Nominal) Sediment (µg/kg dw)	TWA Measured[a]					
	Sediment (µg TRR/ kg dw)	Overlying Water (µg TRR/L)	Pore Water (µg TRR/L)		(1/days)	(days)
Negative control	<LOQ	<LOQ	<LOQ	0.71	0.0471	22.5
Solvent control	<LOQ	<LOQ	<LOQ	0.73	0.0482	21.9
1.7 (3.1)	1.75	0.195	0.551	0.61	0.0466	22.6
4.3 (6.3)	4.44	0.453	1.10	0.64	0.0490	21.7
7.8 (13)	7.31	0.895	2.44	0.71	0.0494	21.1
13 (25)	12.2	1.72	4.28	0.74	0.0495	21.4
30 (50)	28.5	3.50	9.06	0.69	0.0469	22.4
55 (100)	52.6	7.18	19.5	0.80	0.0480	21.9

[a] TWA concentrations were determined by the reviewer using Excel software (copy of worksheet in Appendix II). The limit of quantitation (LOQ) was 0.293 µg TRR/kg for sediment and 0.0133 µg TRR/L for overlying and pore water. TRR = Total Radioactive Residues of [^{14}C]flubendiamide-desiodo.

[b] 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).

Toxicity Observations: Emergence was first noted on Day 15, and adults that emerged appeared normal. There were a few observations of organisms climbing the walls of the test chamber, on the surface of the sediment, and/or swimming in the water column prior to adult maturation; occasional partial emergence; and adults that emerged and subsequently died during the maturation period. The observations were few in incidence and occurred in the controls as well as the treatment levels, and were thus not considered to be related to treatment.

Mean mortality at Day 28 was 29, 30, 39, 39, 29, 29, 31, and 20% for the negative control, solvent control, and mean-measured 1.7, 4.3, 7.8, 13, 30, and 55 µg TRR/kg test levels, respectively (TRR = total radioactive residues of [^{14}C]flubendiamide-desiodo). The observed EC_{50} for mortality of midges was >55 µg TRR/kg based on mean-measured sediment concentrations. Conversely, percent emergence averaged 71, 73, 61, 64, 71, 74, 69, and 80% for the negative control, solvent control, and mean-measured 1.7, 4.3, 7.8, 13, 30, and 55 µg TRR/kg test levels, respectively. No statistically-significant differences were indicated at any treatment level compared to the pooled control, and the NOAEC for percent emergence was 55 µg TRR/kg.

Mean development time was 22.5 and 21.9 days in the negative and solvent control groups, respectively, compared to 22.6, 21.7, 21.1, 21.4, 22.4, and 21.9 days for the mean-measured 1.7, 4.3, 7.8, 13, 30, and 55 µg TRR/kg test levels, respectively. There were no statistically-significant differences indicated for any treatment level compared to the pooled control. Thus, the NOAEC for development time was 55 µg TRR/kg, based on mean-measured sediment concentrations.

Based upon an ANOVA procedure looking at the interaction between sexes, no significant interaction was found between sex and treatments for development rates, and therefore the data for each sex were pooled for this endpoint. Mean development rates were 0.0471, 0.0482, 0.0466, 0.0490, 0.0494, 0.0495, 0.0469, and 0.0480 days^{-1} for the negative control, solvent control, and mean-measured 1.7, 4.3, 7.8, 13, 30, and 55 µg TRR/kg test levels, respectively; no statistically-significant differences were indicated for any treatment level compared to the pooled control. Thus, the NOAEC for development rate was 55 µg TRR/kg based on mean-measured sediment concentrations.

As previously described for development rates, the interaction between sexes was evaluated for emergence ratios (although it was noted that evaluations of the sensitivity for this endpoint are not meaningful as it is impossible to know the initial number of male and female 1- to 4-day old larvae). No significant interaction was found between sex and treatment. Emergence ratios averaged 0.71, 0.73, 0.61, 0.64, 0.71, 0.74, 0.69, and 0.80 for the negative control, solvent control, and mean-measured 1.7, 4.3, 7.8, 13, 30, and 55 µg TRR/kg test levels, respectively. No statistically-significant differences were indicated for any treatment

level compared to the pooled control. Thus, the NOAEC for emergence ratio was 55 µg TRR/kg based on mean-measured sediment concentrations.

B. Statistical Results (From Study Report)

Endpoints that were statistically evaluated included percent emergence (i.e., survival data), development time, emergence ratio, and development rate. The emergence ratio data were arcsine transformed prior to analysis. NOAEC and LOAEC values were determined by visual interpretation of the dose-response pattern and statistical significance of the data.

The data were analyzed using an appropriate t-test to determine any statistical differences between the negative and solvent control groups. No significant differences were indicated for any endpoint, and the control data were pooled for all subsequent comparisons. Data were analyzed using Dunnett's test, at the $p<0.05$ level of sensitivity. ANOVA was used to evaluate sensitivity between sexes.

The 28-day EC_{50} was determined by visual interpretation of the mortality data collected at study termination.

All statistical procedures were performed using SAS statistical software and were reported in terms of mean-measured sediment concentrations.

Most sensitive endpoint: none

Endpoint	Methods	LC_{50}/EC_{50} (95% CI) (µg TRR/kg)	NOAEC (µg TRR/kg)	LOAEC (µg TRR/kg)
Percent Emergence	Dunnett's t-test	>55	55	>55
Emergence Ratio	Dunnett's t-test	---	55	>55
Development Rate	Dunnett's t-test	---	55	>55
Development Time	Dunnett's t-test	---	55	>55

13. VERIFICATION OF STATISTICAL RESULTS

Summary of Statistical Methods used for NOAEC/LOAEC Analyses.

Endpoint	Solvent vs Dilution Control		NOAEC/LOAEC	
	Method	Diff [1] (%)	Method	Diff [2] (%)
28-d Emergence Rate	Student's t-test	-1.8	ANOVA, Dunnett's test	-12.7
28-d Survival	Student's t-test	1.4	ANOVA, Dunnett's test	-12.7
Development time	Student's t-test	3.1	ANOVA, Dunnett's test	2.9
28-d Development Rate	Student's t-test	-2.3	ANOVA, Dunnett's test	-2.0
10-d Survival (Optional)	---	---	---	---
10-day Dry Weight (Optional)	---	---	---	---

[1] Difference between the mean dilution water and solvent control responses; a negative number indicates a promoted response in the solvent control, relative to the negative control.
[2] Difference between the dilution water and NOAEC concentration treatment; a negative number indicates a promoted response in the NOAEC, relative to the negative control.

Most sensitive endpoint: none

Verification Statistical Endpoint Values[a].

Statistical Endpoint	28-day Emergence	28-day Survival	Development time	28-day Development Rate
NOAEC Sediment: Overlying Water: Pore Water:	52.6 µg TRR/kg 7.18 µg TRR/L 19.5 µg TRR/L	52.6 µg TRR/kg 7.18 µg TRR/L 19.5 µg TRR/L	52.6 µg TRR/kg 7.18 µg TRR/L 19.5 µg TRR/L	52.6 µg TRR/kg 7.18 µg TRR/L 19.5 µg TRR/L
LOAEC Sediment: Overlying Water: Pore Water:	>52.6 µg TRR/kg >7.18 µg TRR/L >19.5 µg TRR/L	>52.6 µg TRR/kg >7.18 µg TRR/L >19.5 µg TRR/L	>52.6 µg TRR/kg >7.18 µg TRR/L >19.5 µg TRR/L	>52.6 µg TRR/kg >7.18 µg TRR/L >19.5 µg TRR/L
IC_{50} (95% C.I.) Sediment: Overlying Water: Pore Water:	>52.6 µg TRR/kg >7.18 µg TRR/L >19.5 µg TRR/L	>52.6 µg TRR/kg >7.18 µg TRR/L >19.5 µg TRR/L	>52.6 µg TRR/kg >7.18 µg TRR/L >19.5 µg TRR/L	>52.6 µg TRR/kg >7.18 µg TRR/L >19.5 µg TRR/L
Slope (Standard Error)	N/A	N/A	N/A	N/A

[a] Results are based on TWA test concentrations.

14. REVIEWER'S COMMENTS:

The reviewer's conclusions agreed with the study authors'. There was no treatment-related toxicity in this study.

The study was designed to fulfill OECD Guideline 218 *Sediment-Water Chironomid Toxicity Test Using Spiked Sediment* (2004). Although this study does not fulfill any current U.S. EPA guideline requirement, there were no significant deviations from OECD Guideline 218 that would affect the scientific soundness of this study.

In order for the test to be valid, OECD 218 Guidance requires the following conditions: emergence in the controls must be at least 70% at the end of the test; *C. riparius* emergence to adults should occur between 12 and 23 days after their insertion into the vessels; at the end of the test, pH and dissolved oxygen should be measured in each vessel (the oxygen concentration should be at least 60% of the air saturation value at the temperature used, the pH of overlying water should be in the 6-9 range in all test vessels); and the water temperature should not differ by more than ±1.0°C. In this study, all validity requirements were considered to be fulfilled. Although emergence in controls occurred between Days 15 and 28 (both groups), this deviation did not have any effect on the scientific soundness of this study.

Although OECD 218 prefers that results are provided in terms of (initial) nominal sediment concentrations, TWA concentrations were reviewer-calculated (refer to associated Excel worksheet in Appendix II). As TWA concentrations are more indicative of exposure levels throughout the study, they were reported in the Statistical Verification and Conclusions sections of the DER. TWA concentrations were calculated 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).

At test initiation, the overlying water appeared slightly cloudy and light tan in all test chambers. At termination, it appeared cloudy and tan in all test chambers.

The mean recovery from LSC analysis of the primary stock solution (nominal 150 µg/mL) was 93.3% of nominal. Recoveries from LSC analyses of the working stock solutions (nominal 0.31, 0.63, 1.30, 2.50, 5.00, and 10.0 µg/mL) ranged from 106 to 108% of nominal concentrations.

Experimental test dates were November 17 to December 16, 2009.

15. REFERENCES:

OECD Guideline 218. 2004. *Sediment-Water Chironomid Toxicity Test Using Spiked Sediment.* Adopted April 2004.

APHA, AWWA, WPCF. 1998. *Standard Methods for the Examination of Water and Wastewater*. 20th Edition. American Public Health Association. American Waterworks Association. Water Pollution Control Federation, New York.

The SAS Sytem for Windows. 1999-2001. Release 8.2 (TS2M0). SAS Institute, Inc., Cary, North Carolina.

APPENDIX I. OUTPUT OF REVIEWER'S STATISTICAL ANALYSIS:

```
Title:  Percent Emergence
File:        5605e          Transform:          NO TRANSFORMATION

     t-Test of Solvent and Blank Controls          Ho: GRP1 Mean = GRP2 Mean
==========================================================================
GRP1 (Solvent cntl) Mean =      0.7125     Calculated t value =     -0.1777
GRP2 (Blank cntl) Mean   =      0.7250     Degrees of freedom =      6
Difference in means      =     -0.0125
==========================================================================
2-sided t value (0.05, 6) = 2.4469  No significant difference at alpha=0.05
2-sided t value (0.01, 6) = 3.7074  No significant difference at alpha=0.01

   WARNING:  This procedure assumes normality and equal variances!
Title:  Percent Emergence
File:              5605e              Transform:      ARC SINE(SQUARE ROOT(Y))

                  Shapiro - Wilk's Test for Normality
--------------------------------------------------------------------------

     D =   0.4953
     W =   0.9269

     Critical W  = 0.8960 (alpha = 0.01 , N = 28)
              W  = 0.9240 (alpha = 0.05 , N = 28)

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

Data PASS normality test (alpha = 0.01). Continue analysis.


Title:  Percent Emergence
File:              5605e              Transform:      ARC SINE(SQUARE ROOT(Y))

                  Levene's Test for Homogeneity of Variance

                              ANOVA Table
--------------------------------------------------------------------------

   SOURCE             DF              SS              MS          F
--------------------------------------------------------------------------
   Between             6            0.0451          0.0075      0.6245

   Within (Error)     21            0.2528          0.0120
--------------------------------------------------------------------------
   Total              27            0.2979
--------------------------------------------------------------------------
                                                   (p-value = 0.7088)

   Critical F  = 3.8117  (alpha = 0.01, df = 6,21)
               = 2.5727  (alpha = 0.05, df = 6,21)

   Since  F < Critical F  FAIL TO REJECT  Ho: All equal (alpha = 0.01)
```

Title: Percent Emergence
File: 5605e Transform: ARC SINE(SQUARE ROOT(Y))

ANOVA Table

SOURCE	DF	SS	MS	F
Between	6	0.1251	0.0208	0.8841
Within (Error)	21	0.4953	0.0236	
Total	27	0.6204		

(p-value = 0.5237)

Critical F = 3.8117 (alpha = 0.01, df = 6,21)
= 2.5727 (alpha = 0.05, df = 6,21)

Since F < Critical F FAIL TO REJECT Ho: All equal (alpha = 0.05)

Title: Percent Emergence
File: 5605e Transform: ARC SINE(SQUARE ROOT(Y))

Dunnett's Test - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	TRANS T STAT	SIG 0.05
1	Neg Control	1.0110	0.7125		
2	1.75	0.9079	0.6125	0.9498	
3	4.44	0.9266	0.6375	0.7773	
4	7.31	1.0068	0.7125	0.0389	
5	12.2	1.0684	0.7375	-0.5286	
6	28.5	0.9844	0.6875	0.2455	
7	52.6	1.1111	0.8000	-0.9218	

Dunnett critical value = 2.4600 (1 Tailed, alpha = 0.05, df [used] = 6,20)
(Actual df = 6,21)

Title: Percent Emergence
File: 5605e Transform: ARC SINE(SQUARE ROOT(Y))

Dunnett's Test - TABLE 2 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	MIN SIG DIFF (IN ORIG. UNITS)	% OF CONTROL	DIFFERENCE FROM CONTROL
1	Neg Control	4			
2	1.75	4	0.2595	36.1	0.1000
3	4.44	4	0.2595	36.1	0.0750

4	7.31	4	0.2595	36.1	0.0000
5	12.2	4	0.2595	36.1	-0.0250
6	28.5	4	0.2595	36.1	0.0250
7	52.6	4	0.2595	36.1	-0.0875

Title: Percent Emergence
File: 5605e Transform: ARC SINE(SQUARE ROOT(Y))

William's Test - TABLE 1 OF 2 Ho: Control<Treatment

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSPORMED MEAN	ISOTONIZED MEAN
1	Neg Control	4	0.7125	1.0110	1.0110
2	1.75	4	0.6125	0.9079	1.0009
3	4.44	4	0.6375	0.9266	1.0009
4	7.31	4	0.7125	1.0068	1.0009
5	12.2	4	0.7375	1.0684	1.0009
6	28.5	4	0.6875	0.9844	1.0009
7	52.6	4	0.8000	1.1111	1.0009

Title: Percent Emergence
File: 5605e Transform: ARC SINE(SQUARE ROOT(Y))

William's Test - TABLE 2 OF 2 Ho: Control<Treatment

IDENTIFICATION	COMPARED MEANS	CALC. WILLIAMS	SIG 0.05	TABLE WILLIAMS	DEGREES OF FREEDOM USED
Neg Control	1.0110				
1.75	1.0009	0.0935		1.7200	k= 1, v=21
4.44	1.0009	0.0935		1.8000	k= 2, v=21
7.31	1.0009	0.0935		1.8300	k= 3, v=21
12.2	1.0009	0.0935		1.8400	k= 4, v=21
28.5	1.0009	0.0935		1.8500	k= 5, v=21
52.6	1.0009	0.0935		1.8500	k= 6, v=21

s = 0.1536

WARNING: Procedure has used isotonized means which differ from original (transformed) means.

Title: Percent Survival
File: 5605s Transform: NO TRANSFORMATION

t-Test of Solvent and Blank Controls Ho: GRP1 Mean = GRP2 Mean

GRP1 (Solvent cntl) Mean = 0.7125 Calculated t value = 0.1901

```
GRP2 (Blank cntl) Mean     =      0.7000      Degrees of freedom =      6
Difference in means        =      0.0125
=============================================================================
2-sided t value (0.05, 6) = 2.4469  No significant difference at alpha=0.05
2-sided t value (0.01, 6) = 3.7074  No significant difference at alpha=0.01

   WARNING:  This procedure assumes normality and equal variances!

 Title:  Percent Survival
 File:             5605s              Transform:      ARC SINE(SQUARE ROOT(Y))

                   Shapiro - Wilk's Test for Normality
-----------------------------------------------------------------------------

      D =   0.4496
      W =   0.9478

      Critical W  = 0.8960 (alpha = 0.01 , N = 28)
                  W  = 0.9240 (alpha = 0.05 , N = 28)

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

Data PASS normality test (alpha = 0.01). Continue analysis.


Title:  Percent Survival
File:             5605s              Transform:      ARC SINE(SQUARE ROOT(Y))

                   Levene's Test for Homogeneity of Variance

                                ANOVA Table
-----------------------------------------------------------------------------

   SOURCE              DF                SS                MS          F
-----------------------------------------------------------------------------
   Between              6                0.0349            0.0058      0.6688

   Within (Error)      21                0.1827            0.0087
-----------------------------------------------------------------------------
   Total               27                0.2176
-----------------------------------------------------------------------------
                                                        (p-value = 0.6758)

   Critical F  = 3.8117  (alpha = 0.01, df = 6,21)
               = 2.5727  (alpha = 0.05, df = 6,21)

   Since  F < Critical F  FAIL TO REJECT  Ho: All equal (alpha = 0.01)

 Title:  Percent Survival
 File:             5605s              Transform:      ARC SINE(SQUARE ROOT(Y))

                                ANOVA Table
-----------------------------------------------------------------------------

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

Between	6	0.1254	0.0209	0.9763
Within (Error)	21	0.4496	0.0214	
Total	27	0.5750		

(p-value = 0.4654)

Critical F = 3.8117 (alpha = 0.01, df = 6,21)
= 2.5727 (alpha = 0.05, df = 6,21)

Since F < Critical F FAIL TO REJECT Ho: All equal (alpha = 0.05)

Title: Percent Survival
File: 5605s Transform: ARC SINE(SQUARE ROOT(Y))

Dunnett's Test - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	TRANS T STAT	SIG 0.05
1	Neg Control	1.0110	0.7125		
2	1.75	0.9079	0.6125	0.9969	
3	4.44	0.9015	0.6125	1.0590	
4	7.31	1.0068	0.7125	0.0409	
5	12.2	1.0274	0.7125	-0.1584	
6	28.5	0.9844	0.6875	0.2577	
7	52.6	1.1111	0.8000	-0.9674	

Dunnett critical value = 2.4600 (1 Tailed, alpha = 0.05, df [used] = 6,20)
(Actual df = 6,21)

Title: Percent Survival
File: 5605s Transform: ARC SINE(SQUARE ROOT(Y))

Dunnett's Test - TABLE 2 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	MIN SIG DIFF (IN ORIG. UNITS)	% OF CONTROL	DIFFERENCE FROM CONTROL
1	Neg Control	4			
2	1.75	4	0.2469	34.4	0.1000
3	4.44	4	0.2469	34.4	0.1000
4	7.31	4	0.2469	34.4	0.0000
5	12.2	4	0.2469	34.4	0.0000
6	28.5	4	0.2469	34.4	0.0250
7	52.6	4	0.2469	34.4	-0.0875

Title: Percent Survival
File: 5605s Transform: ARC SINE(SQUARE ROOT(Y))

William's Test - TABLE 1 OF 2 Ho: Control<Treatment

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	Neg Control	4	0.7125	1.0110	1.0110
2	1.75	4	0.6125	0.9079	0.9898
3	4.44	4	0.6125	0.9015	0.9898
4	7.31	4	0.7125	1.0063	0.9898
5	12.2	4	0.7125	1.0274	0.9898
6	28.5	4	0.6875	0.9844	0.9898
7	52.6	4	0.8000	1.1111	0.9898

Title: Percent Survival
File: 5605s Transform: ARC SINE(SQUARE ROOT(Y))

William's Test - TABLE 2 OF 2 Ho: Control<Treatment

IDENTIFICATION	COMPARED MEANS	CALC. WILLIAMS	SIG 0.05	TABLE WILLIAMS	DEGREES OF FREEDOM USED
Neg Control	1.0110				
1.75	0.9898	0.2048		1.7200	k= 1, v=21
4.44	0.9898	0.2048		1.8000	k= 2, v=21
7.31	0.9898	0.2048		1.8300	k= 3, v=21
12.2	0.9898	0.2048		1.8400	k= 4, v=21
28.5	0.9898	0.2048		1.8500	k= 5, v=21
52.6	0.9898	0.2048		1.8500	k= 6, v=21

s = 0.1463

WARNING: Procedure has used isotonized means which differ from original (transformed) means.

Title: Development time
File: 5605t Transform: NO TRANSFORMATION

t-Test of Solvent and Blank Controls Ho: GRP1 Mean = GRP2 Mean

GRP1 (Solvent cntl) Mean = 22.5000 Calculated t value = 0.5932
GRP2 (Blank cntl) Mean = 21.8500 Degrees of freedom = 6
Difference in means = 0.6500

2-sided t value (0.05, 6) = 2.4469 No significant difference at alpha=0.05
2-sided t value (0.01, 6) = 3.7074 No significant difference at alpha=0.01

WARNING: This procedure assumes normality and equal variances!

Title: Development time
File: 5605t Transform: NO TRANSFORMATION

Shapiro - Wilk's Test for Normality

D = 36.8450
W = 0.9852

Critical W = 0.8960 (alpha = 0.01 , N = 28)
W = 0.9240 (alpha = 0.05 , N = 28)

Data PASS normality test (alpha = 0.01). Continue analysis.

Title: Development time
File: 5605t Transform: NO TRANSFORMATION

Levene's Test for Homogeneity of Variance

ANOVA Table

SOURCE	DF	SS	MS	F
Between	6	6.2521	1.0420	2.7268
Within (Error)	21	8.0250	0.3821	
Total	27	14.2771		

(p-value = 0.0405)

Critical F = 3.8117 (alpha = 0.01, df = 6,21)
= 2.5727 (alpha = 0.05, df = 6,21)

Since F < Critical F FAIL TO REJECT Ho: All equal (alpha = 0.01)

Title: Development time
File: 5605t Transform: NO TRANSFORMATION

ANOVA Table

SOURCE	DF	SS	MS	F
Between	6	8.6821	1.4470	0.8247
Within (Error)	21	36.8450	1.7545	
Total	27	45.5271		

(p-value = 0.5636)

Critical F = 3.8117 (alpha = 0.01, df = 6,21)
= 2.5727 (alpha = 0.05, df = 6,21)

Since F < Critical F FAIL TO REJECT Ho: All equal (alpha = 0.05)

Title: Development time
File: 5605t Transform: NO TRANSFORMATION

Dunnett's Test - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG 0.05
1	Neg Control	22.5000	22.5000		
2	1.75	22.6000	22.6000	-0.1068	
3	4.44	21.6500	21.6500	0.9075	
4	7.31	21.0750	21.0750	1.5214	
5	12.2	21.3500	21.3500	1.2278	
6	28.5	22.4250	22.4250	0.0801	
7	52.6	21.8500	21.8500	0.6940	

Dunnett critical value = 2.4600 (1 Tailed, alpha = 0.05, df [used] = 6,20)
(Actual df = 6,21)

Title: Development time
File: 5605t Transform: NO TRANSFORMATION

Dunnett's Test - TABLE 2 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	MIN SIG DIFF (IN ORIG. UNITS)	% OF CONTROL	DIFFERENCE FROM CONTROL
1	Neg Control	4			
2	1.75	4	2.3041	10.2	-0.1000
3	4.44	4	2.3041	10.2	0.8500
4	7.31	4	2.3041	10.2	1.4250
5	12.2	4	2.3041	10.2	1.1500
6	28.5	4	2.3041	10.2	0.0750
7	52.6	4	2.3041	10.2	0.6500

Title: Development time
File: 5605t Transform: NO TRANSFORMATION

William's Test - TABLE 1 OF 2 Ho: Control<Treatment

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	Neg Control	4	22.5000	22.5000	22.5500
2	1.75	4	22.6000	22.6000	22.5500
3	4.44	4	21.6500	21.6500	21.6700
4	7.31	4	21.0750	21.0750	21.6700
5	12.2	4	21.3500	21.3500	21.6700
6	28.5	4	22.4250	22.4250	21.6700
7	52.6	4	21.8500	21.8500	21.6700

Title: Development time
File: 5605t Transform: NO TRANSFORMATION

William's Test - TABLE 2 OF 2 Ho: Control<Treatment

IDENTIFICATION	COMPARED MEANS	CALC. WILLIAMS	SIG 0.05	TABLE WILLIAMS	DEGREES OF FREEDOM USED
Neg Control	22.5000				
1.75	22.5500	-0.0534		1.7200	k= 1, v=21
4.44	21.6700	0.8862		1.8000	k= 2, v=21
7.31	21.6700	0.8862		1.8300	k= 3, v=21
12.2	21.6700	0.8862		1.8400	k= 4, v=21
28.5	21.6700	0.8862		1.8500	k= 5, v=21
52.6	21.6700	0.8862		1.8500	k= 6, v=21

s = 1.3246

WARNING: Procedure has used isotonized means which differ from original (transformed) means.

Title: Development rate
File: 5605d Transform: NO TRANSFORMATION

t-Test of Solvent and Blank Controls Ho: GRP1 Mean = GRP2 Mean

GRP1 (Solvent cntl) Mean = 4.7075 Calculated t value = -0.4155
GRP2 (Blank cntl) Mean = 4.8200 Degrees of freedom = 6
Difference in means = -0.1125

2-sided t value (0.05, 6) = 2.4469 No significant difference at alpha=0.05
2-sided t value (0.01, 6) = 3.7074 No significant difference at alpha=0.01

WARNING: This procedure assumes normality and equal variances!

Title: Development rate
File: 5605d Transform: NO TRANSFORMATION

Shapiro - Wilk's Test for Normality

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

     D =   2.0659
     W =   0.9843

     Critical W  = 0.8960 (alpha = 0.01 , N = 28)
              W  = 0.9240 (alpha = 0.05 , N = 28)

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

Data PASS normality test (alpha = 0.01). Continue analysis.


Title:  Development rate
File:             5605d              Transform:              NO TRANSFORMATION

                 Levene's Test for Homogeneity of Variance

                                ANOVA Table
-------------------------------------------------------------------------------

   SOURCE            DF              SS              MS            F
-------------------------------------------------------------------------------
   Between            6              0.3531          0.0588        3.2532

   Within (Error)    21              0.3799          0.0181
-------------------------------------------------------------------------------
   Total             27              0.7330
-------------------------------------------------------------------------------
                                                              (p-value = 0.0202)

   Critical F  = 3.8117  (alpha = 0.01, df = 6,21)
               = 2.5727  (alpha = 0.05, df = 6,21)

   Since  F < Critical F  FAIL TO REJECT  Ho: All equal (alpha = 0.01)

 Title:  Development rate
File:             5605d              Transform:              NO TRANSFORMATION

                                ANOVA Table
-------------------------------------------------------------------------------

   SOURCE            DF              SS              MS            F
-------------------------------------------------------------------------------
   Between            6              0.3711          0.0619        0.6288

   Within (Error)    21              2.0659          0.0984
-------------------------------------------------------------------------------
   Total             27              2.4370
-------------------------------------------------------------------------------
                                                              (p-value = 0.7056)

   Critical F  = 3.8117  (alpha = 0.01, df = 6,21)
               = 2.5727  (alpha = 0.05, df = 6,21)
```

Since F < Critical F FAIL TO REJECT Ho: All equal (alpha = 0.05)

Title: Development rate
File: 5605d Transform: NO TRANSFORMATION

Dunnett's Test - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG 0.05
1	Neg Control	4.7075	4.7075		
2	1.75	4.6600	4.6600	0.2142	
3	4.44	4.8975	4.8975	-0.8567	
4	7.31	4.9450	4.9450	-1.0709	
5	12.2	4.9475	4.9475	-1.0821	
6	28.5	4.6875	4.6875	0.0902	
7	52.6	4.8025	4.8025	-0.4283	

Dunnett critical value = 2.4600 (1 Tailed, alpha = 0.05, df [used] = 6,20)
(Actual df = 6,21)

Title: Development rate
File: 5605d Transform: NO TRANSFORMATION

Dunnett's Test - TABLE 2 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	MIN SIG DIFF (IN ORIG. UNITS)	% OF CONTROL	DIFFERENCE FROM CONTROL
1	Neg Control	4			
2	1.75	4	0.5456	11.6	0.0475
3	4.44	4	0.5456	11.6	-0.1900
4	7.31	4	0.5456	11.6	-0.2375
5	12.2	4	0.5456	11.6	-0.2400
6	28.5	4	0.5456	11.6	0.0200
7	52.6	4	0.5456	11.6	-0.0950

Title: Development rate
File: 5605d Transform: NO TRANSFORMATION

Williams's Test - TABLE 1 OF 2 Ho: Control<Treatment

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	Neg Control	4	4.7075	4.7075	4.8315

2	1.75	4	4.6600	4.6600	4.8315
3	4.44	4	4.8975	4.8975	4.8315
4	7.31	4	4.9450	4.9450	4.8315
5	12.2	4	4.9475	4.9475	4.8315
6	28.5	4	4.6875	4.6875	4.7450
7	52.6	4	4.8025	4.8025	4.7450

Title: Development rate
File: 5605d Transform: NO TRANSFORMATION

William's Test - TABLE 2 OF 2 Ho: Control<Treatment

IDENTIFICATION	COMPARED MEANS	CALC. WILLIAMS	SIG 0.05	TABLE WILLIAMS	DEGREES OF FREEDOM USED
Neg Control	4.7075				
1.75	4.8315	-0.5591		1.7200	k= 1, v=21
4.44	4.8315	-0.5591		1.8000	k= 2, v=21
7.31	4.8315	-0.5591		1.8300	k= 3, v=21
12.2	4.8315	-0.5591		1.8400	k= 4, v=21
28.5	4.7450	-0.1691		1.8500	k= 5, v=21
52.6	4.7450	-0.1691		1.8500	k= 6, v=21

s = 0.3136

WARNING: Procedure has used isotonized means which differ from original (transformed) means.

APPENDIX II. COPY OF REVIEWER'S TIME-WEIGHTED AVERAGE (TWA) CALCULATIONS USING EXCEL SOFTWARE:

SEDIMENT

Nominal Concentration (ug/kg)	Time (Day)	14C-Novaluron Equivalents Measured Concentration (ug/kg)	TWA (ug/kg)
3.1	0	1.73	
	7	2.06	
	28	1.35	
			1.75
6.3	0	3.98	
	7	5.24	
	28	3.53	
			4.44
13	0	9.26	
	7	6.9	
	28	7.21	
			7.31
25	0	13.8	
	7	10.1	
	28	14.4	
			12.2
50	0	35.2	
	7	29.3	
	28	25.2	
			28.5
100	0	60.1	
	7	50.6	
	28	52.8	
			52.6