DATA EVALUATION RECORD
FRESHWATER SEDIMENT *Chironomus riparius* EMERGENCE TEST

1. **CHEMICAL:** Trifluralin — PC Code: 036101

2. **TEST MATERIAL:** Trifluralin TR-4 metabolite — Purity: 100%
   [^{14}C]Trifluralin TR-4 metabolite — Rad. Purity: 100%

3. **CITATION:**

Authors:	Henry, K.S., *et al.*
Title:	Revised Report for Trifluralin TR-4 Metabolite: Chronic Toxicity Study with the Midge, *Chironomus riparius*, Using Spiked Water in a Sediment-Water Exposure System.
Study Completion Date:	May 17, 2004 (revised)
Laboratory:	Toxicology & Environmental Research and Consulting The Dow Chemical Company Midland, MI 48674
Sponsor:	Dow Agrosciences LLC 9330 Zionsville Road Indianapolis, IN 46268
Laboratory Report ID:	031072
MRID No.:	478070-12

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

   Signature: Christie E. Padova **Date:** 11/02/09

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

   Signature: Teri S Myers **Date:** 12/07/09

5. **APPROVED BY:** Christine Hartless, OPP/EFED/ERB 1

   Signature: 5-6-10 **Date:** 5/6/10

6. **STUDY PARAMETERS**

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

7. CONCLUSIONS:

Results Synopsis: (reported as nominal concentrations in overlying water)

Percent Emergence
EC_{50}: 3.8 mg ai/L 95% C.I.: 3.5 to 4.2 mg ai/L
Slope: 5.70±0.612
NOAEC: 0.8305 mg ai/L
LOAEC: 2.077 mg ai/L (=TWA of 0.336 mg TRR/L in overlying water; 0.0812 mg TRR/L in pore water; 2.833 mg TRR/kg in sediment)*

Development Rate (♂ & ♀)
IC_{50}: >5.195 mg ai/L 95% C.I.: N/A
Slope: N/A
NOAEC: 2.077 mg ai/L (=TWA of 0.336 mg TRR/L in overlying water; 0.0812 mg TRR/L in pore water; 2.833 mg TRR/kg in sediment)
LOAEC: 5.195 mg ai/L

*except for the level indicated, TR-4 concentrations were not measured for pore water, overlying water, or sediment concentrations

Assessment endpoints: percent emergence, male development rate, and female development rate
Most sensitive endpoint: percent emergence

8. ADEQUACY OF THE STUDY:

A. Classification: Supplemental

B. Rationale: This study followed OECD Draft Guideline 219 "Sediment-Water Chironomid Toxicity Test Using Spiked Water" (2000), and does not fulfill any current U.S. EPA data requirement.

C. Reparability: N/A

9. MAJOR GUIDELINE DEVIATIONS (from OECD Guideline 219):

1. Aeration should have been stopped for 24 hours following the addition of test organisms.
2. The sediment to overlying water depth ratio was not reported.
3. Raw analytical data were not provided.

10. SUBMISSION PURPOSE: Litigation/Endangered Species

11. MATERIALS AND METHODS

Stability of Compound Under Test Conditions: Overlying water, pore water, and sediment samples from the surrogate vessels (one vessel per interval) were collected at 0, 7, and 28 days and analyzed for total radioactive residues (TRR) of trifluralin TR-4 metabolite. Radioactive residues were not further characterized for stability of the test material.

TRR of trifluralin TR-4 metabolite declined in the overlying water during the 28-day study, with average recoveries of 50.6, 10.1, and 8.9% of target concentrations on Days 0, 7, and 28, respectively. The average recoveries of TRR in pore water remained relatively constant during the study at <5% of the target overlying water concentrations at all intervals. Variability was observed in sediment samples. At the 0.1330 and 2.077 mg/L nominal levels, concentrations of TRR on Day 28 increased 291 and 220% of the Day-0 levels, respectively, indicating a partitioning of the test material from the overlying water to the sediment. At the 12.98 mg/L nominal level, however, the concentration of TRR at Day 28 declined 24% from the Day-0 level (see Reviewer's Comments section). Material balances were not reported.

Physicochemical properties of trifluralin.

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*
Source	Egg masses were obtained from a commercial supplier (Environmental Consulting and Testing, Superior, WI).
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.	Newly-hatched larvae were cultured in glass bowls in an incubator at 20°C with a 16-hour light:8-hour dark photoperiod.
Age of Test Larvae First instar (1 to 4 days post-hatch with confirmation)	1st instar, 2 to 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 5 mL of *Ankistrodesmus falcatus* green algae suspension (4 x 10^7 cells/mL) per L of laboratory dilution water.

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. As needed, water levels were brought back to original levels using laboratory dilution water. Three surrogate test vessels were prepared at the solvent control and low (0.1330 mg/L), middle (2.077 mg/L), and high (12.98 mg/L) levels and were used for analytical verification. Therefore, the method for analytical sampling did not affect volume, biological load, or test concentration.
Test Materials	Identity: [^{14}C]trifluralin TR-4 metabolite Common name: [^{14}C]TR-4 Parent compound: trifluralin Physical description: not reported Inventory No.: 1908 Radiochemical purity: 100% (stated) Specific activity: 23.0 mCi/mmol [^{14}C] position: uniformly phenyl-ring Storage: not reported Identity: trifluralin TR-4 metabolite Common name: TR-4 Parent compound: trifluralin Physical description: not reported Lot No.: F-941-99 Purity: 100% Storage: not reported

Guideline Criteria	Reported Information
Stock Solutions	Working stock solutions of radiolabeled and unlabeled TR-4 were prepared in acetone at 1330 µg/mL and 263,910 µg/mL, respectively. Serial dilutions of the unlabeled stock were also prepared in acetone, and dosing stock solutions were made by combining 150 µL of the [^{14}C]TR-4 working stock and 150 µL of the appropriate non-radiolabeled stock solution. Dosing stock solutions were applied to test vessels using a Hamilton syringe, and were gently stirred with disposable glass pipettes after addition.
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.	Water originated from the upper Saginaw Bay of Lake Huron. The water was limed and flocculated with ferric chloride, and supplied to the laboratory by the City of Midland Water Treatment Plant. Prior to use at the laboratory, the water is sand-filtered, pH-adjusted with gaseous carbon dioxide, carbon-filtered, and UV-irradiated. Results from (the most recent) periodic analysis of the laboratory dilution water (sampled on 4/01/03) for selected inorganic and organic compounds were provided.

Guideline Criteria	Reported Information
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 by combining (on a dry weight basis) 72% sand, 20% kaolinite clay, and 8% sphagnum peat moss. These materials were blended together with deionized water. TOC: 2.3% Moisture content: 38% pH: 7.2
Sediment Conditioning Artificial sediment: 7 days in flowing dilution water prior to test initiation, chambers may be aerated	Test vessels (sediment:water) were prepared 7 days prior to study initiation (Day 0) and acclimated under test conditions.
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.	One day prior to test initiation (i.e., Day -1), midge larvae were impartially added to each replicate test vessel. Aeration was stopped while the animals were added and for the following 3 hours.
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 0.1 mL/L test solution

Guideline Criteria	Reported Information
Water Temperature 20°C ± 2°C (Should not deviate between vessels by more than 1°C.)	19.9 to 20.6°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 (at preparation) Interstitial Water: Not determined Overlying Water: 7.6 to 8.4
TOC Sediment: 2 ± 0.5% Overlying Water: 2 mg/L	Sediment: Not determined Overlying Water: Not determined
Ammonia Interstitial Water: Overlying Water:	Interstitial Water: Not determined Overlying Water: 1.0 to 1.5 mg/L as N
Total Water Hardness 200 mg/L as $CaCO_3$ (prefer 160 to 180 mg/L as $CaCO_3$)	136 to 194 mg/L as $CaCO_3$
Dissolved Oxygen 60% air saturation value throughout test	7.4 to 9.4 mg/L (≥83% 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 1-3 bubbles/sec through Pasteur pipettes, except for approximately 3 hours during and following the addition of the larvae.

Guideline Criteria	Reported Information
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; 75 mL sediment and 350 mL of laboratory dilution water. The sediment:water height ratio was not reported.
Covers Test vessels should be covered with a glass plate.	Test vessels were loosely covered.
Photoperiod 16 hours light, 8 hours dark (Light intensity 500 to 1000 lux)	16 hours light, 8 hours dark Light intensity 613 to 877 lux
Food Green algae (e.g., *Scenedesmus subspicatus, Chlorella vulgaris*) or flaked fish food as a ground powder, suspension, or filtrate	Tetra Min® fish food (Tetra Holdings Inc., Blacksburg, VA)
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)	Daily Days -1 to 10: 0.5 mL of a 10 g fish food/L distilled water suspension Days 11 to 28: 1.0 mL of a 10 g fish food/L distilled water suspension

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) 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, 0.1330, 0.3324, 0.8305, 2.077, 5.195, and 12.98 mg ai/L ECx endpoint: N/A. NOAEC/LOAEC endpoint: A nominal factor rate of 2.5 was used, based on the results of the range-finding work, which indicated effects on emergence between 0.8435 and 6.885 mg ai/L (see Reviewer's Comments section).
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 with 4 replicates per treatment level. *(Optional) 10-day growth data were not collected.
Test organisms randomly or impartially assigned to test vessels?	Yes

Guideline Criteria	Reported Information
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. Dissolved oxygen was measured daily in all test vessels. 2. Temperature and pH were measured at study initiation and weekly thereafter in all test vessels. 3. Criteria not required in OECD 218 guidance. 4. Hardness, ammonia, alkalinity, and conductivity were measured in a negative control vessel and in a vessel from the highest concentration treatment (i.e., 12.98 mg/L) 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 solvent control and low (0.1330 mg/L), middle (2.077 mg/L), and high (12.98 mg/L) levels were analyzed for total radioactivity using LSC at 0 (1 hour), 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.	Sediment and pore water were isolated using centrifugation for 30 minutes at 8600 x *g*.
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 interstitial water of the three surrogate vessels prepared at the solvent control and low (0.1330 mg/L), middle (2.077 mg/L), and high (12.98 mg/L) levels were analyzed for total radioactivity using LSC at 0 (1 hour), 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.	The sediment of the three surrogate vessels prepared at the solvent control and low (0.1330 mg/L), middle (2.077 mg/L), and high (12.98 mg/L) levels were analyzed for total radioactivity using LSC following combustion at 0 (1 hour), 7, and 28 days.

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, EC, and U.S. EPA.
Control Mortality <30%	Yes
Did chironomids emerge in controls between day 12 and 23?	Negative control – days 15 to 24 Solvent control – days 14 to 24
Control Emergence Mean emergence between 50-70%	Negative control – 95.0% emergence Solvent control – 95.0% emergence

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 days) - Number alive - Time to emergence - Number of emerged male and female midges Growth and Survival (10-day) (Optional) N/A
Raw data included?	Yes

Effects Data

Table 1. Summary of trifluralin TR-4 metabolite effects on *Chironomus riparius* emergence success and sex ratio

Toxicant Concentration				Initial No.	Mean Number Emerged			Mean Sex Ratio[c] (%)	
Nominal Overlying Water (mg ai/L)	Mean Measured TWA[a][b]				♂	♀	Total	$ER_{♂}$	$ER_{♀}$
	Overlying Water (mg TRR/L)	Sediment (mg TRR/kg)	Pore Water (mg TRR/L)						
Negative control	Not analyzed	Not analyzed	Not analyzed	80	36	40	76	47	53
Solvent control	<LLQ[d]	<LLQ	<LLQ	80	44	32	76	58	42
0.1330	0.0249	0.200	0.00519	80	39	34	73	53	47
0.3324	Not analyzed	Not analyzed	Not analyzed	81[e]	29	46	75	39	61
0.8305	Not analyzed	Not analyzed	Not analyzed	80	36	38	74	49	51
2.077	0.336	2.83	0.0812	80	32	35	67	48	52
5.195	Not analyzed	Not analyzed	Not analyzed	80	9	8	17	53	47
12.98	1.20	25.2	0.479	80	0	0	0	N/A	N/A

[a] Reviewer-calculated time-weighted averages (refer to associated Excel spreadsheet); results were rounded to three significant figures. The LOQ for aqueous samples was 0.043 µg/L and for sediment samples was 0.115 µg/kg (dw).

[b] TRR – total radioactive residues of trifluralin TR-4 metabolite (from LSC analyses).

[c] $ER_{♂}$ = number of emerged males/number of emerged larvae x 100; $ER_{♀}$ = number of emerged females/number of emerged larvae x 100; reviewer-calculated.

[d] The Day-0 measured value was 0.00011 mg/L, above the LLQ.

[e] There were apparently 21 midges added to replicate D of this treatment level on Day -1.

Table 2. Summary of trifluralin TR-4 metabolite effects on *Chironomus riparius* development time and rate.

Toxicant Concentration				Emergence (%)	Mean Development Rate[c] (1/day)	
Nominal Overlying Water (mg ai/L)	Mean Measured TWA[a][b]				♂	♀
	Overlying Water (mg TRR/L)	Sediment (mg TRR/kg)	Pore Water (mg TRR/L)			
Negative control	Not analyzed	Not analyzed	Not analyzed	95.0	0.0612	0.0524
Solvent control	<LLQ[d]	<LLQ	<LLQ	95.0	0.0603	0.0566
0.1330	0.0249	0.200	0.00519	91.3	0.0639	0.0544
0.3324	Not analyzed	Not analyzed	Not analyzed	93.8	0.0655	0.0541
0.8305	Not analyzed	Not analyzed	Not analyzed	92.5	0.0607	0.0530*
2.077	0.336	2.83	0.0812	83.8*	0.0599	0.0516*
5.195	Not analyzed	Not analyzed	Not analyzed	21.3*	0.0481*	0.0446*
12.98	1.20	25.2	0.479	0.00*	N/A	N/A

* Significantly lower ($p<0.05$) than the pooled controls (emergence and male development rate) or solvent control (female development rate) group.

[a] Reviewer-calculated time-weighted averages (refer to associated Excel spreadsheet); results were rounded to three significant figures. The LOQ for aqueous samples was 0.043 µg/L and for sediment samples was 0.115 µg/kg (dw).

[b] TRR – total radioactive residues of trifluralin TR-4 metabolite (from LSC analyses).

[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).

Toxicity Observations (in terms of nominal concentrations): Mean percent emergence was 95.0% for both the negative and solvent control groups, and 91.3, 93.8, 92.5, 83.8, 21.3, and 0% for the nominal 0.1330, 0.3324, 0.8305, 2.077, 5.195, and 12.98 mg/L treatment levels, respectively. Differences were statistically-reduced ($p<0.05$) compared to the pooled controls (95.0%) at the 2.077, 5.195, and 12.98 mg/L levels. No emergence occurred at the 12.98 mg/L level. The NOAEC for emergence was 0.8305 mg/L.

As males typically develop and emerge faster than females, the average development rates for male and females were significantly different from each other in the controls ($p<0.05$), and thus the development rates for each sex were assessed separately. Mean male development rates were 0.0612, 0.0603, 0.0639, 0.0655, 0.0607, 0.0599, and 0.0481 $days^{-1}$ for the negative control, solvent control, 0.1330, 0.3324, 0.8305, 2.077, and 5.195 mg/L levels, respectively. The difference was statistically-different ($p<0.05$) from the pooled controls (0.0608 $days^{-1}$) at the 5.195 mg/L level, and the subsequent NOAEC for male development rate was 2.077 mg/L.

Female development rates were the most sensitive endpoint, as determined by the study authors. Mean rates were 0.0524, 0.0566, 0.0544, 0.0541, 0.0530, 0.0516, and 0.0446 days-1 for the negative control, solvent control, 0.1330, 0.3324, 0.8305, 2.077, and 5.195 mg/L levels, respectively. Differences were statistically-different ($p<0.05$) from the solvent control group at the 0.8305, 2.077 and 5.195 mg/L levels. The NOAEC for female development rate was reported to be 0.3324 mg/L.

B. Statistical Results (From Study Report)

Analyses were performed using TOXSTAT statistical software and nominal overlying water concentrations. Endpoints that were statistically analyzed included emergence percentages and development rates. The mean development rates for male and female midges were significantly different from each other in the controls (t-test, alpha = 0.05), and were thus analyzed separately.

Negative and solvent control data were compared using a t-test (alpha = 0.05); for emergence and male development rates, no significant differences were observed, and the data were pooled for subsequent comparisons. For female development rates, a statistical difference was indicated, and treatment data were compared to the performance of the solvent control group.

Data were tested for normality using the Shapiro-Wilk's test, and for homogeneity of variance using Bartlett's test. If the data were both normal and homogenous, a parametric analysis was conducted using Dunnett's test (alpha = 0.05). Data that were not normally distributed and/or not homogenous were analyzed using the non-parametric Steel's Many-One Rank Test (equal number of replicates) or the Kruskal-Wallis test (unequal number of

replicates).
The EC_{50} (with 95% confidence intervals) for emergence was calculated using probit analysis.

Most sensitive endpoint: female development rate

Endpoint	Methods	EC_{50} (95% CI) (mg/L)	NOAEC (mg/L)	LOAEC (mg/L)
28-d Emergence Rate	Dunnett	3.682 (3.209 to 4.150)	0.8305	2.077
28-d ♂ Development Rate	Dunnett	---	2.077	5.195
28-d ♀ Development Rate	Dunnett	---	0.3324	0.8305
10-d Survival (Optional)	---	---	---	---
10-d Growth (Optional)	---	---	---	---

13. VERIFICATION OF STATISTICAL RESULTS

Analyses were performed using TOXSTAT 3.5 statistical software and nominal overlying water concentrations. Endpoints that were statistically analyzed included emergence percentages and development rates. The mean development rates for male and female midges were significantly different from each other in the controls (t-test, alpha = 0.05) and were analyzed separately.

Negative and solvent control data were compared using a t-test (alpha = 0.05); for emergence and male development rates, no significant differences were observed. For female development rates, a statistical difference was indicated (solvent control had an 8% higher emergence percentage. All comparisons of treatment groups were made to the negative control.

Data were tested for normality using the Shapiro-Wilk's test, and for homogeneity of variance using Bartlett's test. Since all data were both normal and homogenous, a parametric analysis was conducted using Williams' test (alpha = 0.05).

The EC_{50} for emergence percentage was calculated using the Bruce and Versteeg approach in the Nuthatch software. IC_{50}'s for development rates were not calculated as a 50% decrease from the control was not observed with the exception of the highest treatment group (no

emergence observed). IC_{50}s for male and female development were visually determined at > 5.195 mg/L.

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	0	ANOVA, William's test	2.6
28-d ♂ Development Rate	Student's t-test	1	ANOVA, William's test	2.1
28-d ♀ Development Rate	Student's t-test	-8*	ANOVA, William's test	1.5
10-d Survival (Optional)	---	---	---	---
10-day Dry Weight (Optional)	---	---	---	---

[1] Difference between the mean dilution water and solvent control responses; a negative value indicates promoted solvent control response, * indicates statistically-significant ($p<0.05$).
[2] Difference between the dilution water and NOAEC concentration treatment.

Most sensitive endpoint: 28-day emergence

Verification Statistical Endpoint Values[a].

Statistical Endpoint	28-day Emergence	28-day ♂ & ♀ Development Rate	10-d Survival	10-d Dry Weight
NOAEC	0.8305	2.077	---	---
LOAEC	2.077	5.195	---	---
EC_{50} (95% C.I.)	3.8 (3.5-4.2)	>5.195	---	---
Slope (Standard Error)	5.70±0.612	N/A	---	---

[a] Results are based on nominal overlying water concentrations.

14. **REVIEWER'S COMMENTS:**

The reviewer's conclusions differed from the study authors' because the reviewer compared treated groups to the negative control, while the study authors compared treated responses to the pooled or solvent control group (i.e., for female development rate when a significant difference was detected between the solvent and negative control groups). Current US EPA guidance recommends statistical comparison to only the negative control group in aquatic toxicity tests. Despite the fact that a significant difference ($p<0.05$) was detected between the solvent and negative control groups, the reviewer did not suspect solvent interference as a confounding factor in this study given that the response was a slight (8%) promotion of female development rate and no other endpoints showed significant differences between the solvent and negative control groups. The reviewer's conclusions are reported in the Conclusions section of this DER.

This study does not fulfill any current U.S. EPA guideline. However, it followed methods provided in OECD Guideline 219 (April 2004), "Sediment-Water Chironomid Toxicity Test Using Spiked Water". In order for the test to be valid, OECD Guidance requires the following conditions: The 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 the dissolved oxygen concentration 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 fulfilled.

A 28-day range-finding study was performed at nominal concentrations of 0 (negative control), 0 (acetone control), 0.1033, 0.2952, 0.8435, 2.410, 6.885, and 19.67 mg TR-4/L. Results from this study indicated effects on emergence between 0.8435 and 6.885 mg/L. No other details were provided.

When possible, TWA concentrations were calculated by the reviewer (refer to associated Excel worksheet in Appendix II). Because concentrations were only measured for a select few levels (i.e., high, middle, and low), the reviewer expressed toxicity values in this study using the nominal overlying water concentrations only. When TWA concentrations could be calculated, the following equation was used:

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

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 study authors reported in the Results and Discussion section of the study report that the concentration of test material in the sediment samples increased over the course of the study at all three dose levels quantified, indicating a partitioning into the sediment. This was true for the two lower concentration levels (i.e., 0.1330 and 2.077 mg/L); however, at the 12.98 mg/L level (high-dose level), total radioactive residues (TRR) of trifluralin TR-4 metabolite declined slowly during the 28-day study. On Days 7 and 28, TRRs were -13 and -24% of the Day-0 value, respectively. Raw analytical data were not provided to verify the summarized analytical results.

The variability associated with the analytical method and solution homogeneity were assessed on Day 0 by calculating relative standard deviations (RSD) for overlying water and sediment matrices at the 0.1330 mg/L level (surrogate vessel). The mean RSD values from repeated measurements (five overlying water and three sediment) were 0.292 and 3.07%, respectively.

The definitive study was initiated on August 20, 2003.

15. REFERENCES:

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APPENDIX I. OUTPUT OF REVIEWER'S STATISTICAL ANALYSIS:

% emergence
File: 7012s Transform: NO TRANSFORM

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

GRP1 (SOLVENT CRTL) MEAN =	0.9500	CALCULATED t VALUE =	0.0000
GRP2 (BLANK CRTL) MEAN =	0.9500	DEGREES OF FREEDOM =	6
DIFFERENCE IN MEANS =	0.0000		

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

Title: emergence rate
File: 012-EM~1.TXT Transform: NO TRANSFORMATION

Shapiro - Wilk's Test for Normality

D = 800.0000
W = 0.9578

Critical W = 0.8840 (alpha = 0.01 , N = 24)
W = 0.9160 (alpha = 0.05 , N = 24)

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

Title: emergence rate
File: 012-EM~1.TXT Transform: NO TRANSFORMATION

Levene's Test for Homogeneity of Variance

ANOVA Table

SOURCE	DF	SS	MS	F
Between	5	45.8333	9.1667	0.6000
Within (Error)	18	275.0000	15.2778	
Total	23	320.8333		

(p-value = 0.7006)

Critical F = 4.2479 (alpha = 0.01, df = 5,18)
= 2.7729 (alpha = 0.05, df = 5,18)

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

Title: emergence rate
File: 012-EM~1.TXT Transform: NO TRANSFORMATION

ANOVA Table

SOURCE	DF	SS	MS	F
Between	5	16645.8333	3329.1667	74.9063
Within (Error)	18	800.0000	44.4444	
Total	23	17445.8333		

(p-value = 0.0000)

Critical F = 4.2479 (alpha = 0.01, df = 5,18)
= 2.7729 (alpha = 0.05, df = 5,18)

Since F > Critical F REJECT Ho: All equal (alpha = 0.05)

Title: emergence rate
File: 012-EM~1.TXT 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	95.0000	95.0000		
2	0.1330	91.2500	91.2500	0.7955	
3	0.3324	93.7500	93.7500	0.2652	
4	0.8305	92.5000	92.5000	0.5303	
5	2.077	83.7500	83.7500	2.3865	
6	5.195	21.2500	21.2500	15.6447	*

Dunnett critical value = 2.4100 (1 Tailed, alpha = 0.05, df = 5,18)

Title: emergence rate
File: 012-EM~1.TXT 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	0.1330	4	11.3608	12.0	3.7500
3	0.3324	4	11.3608	12.0	1.2500
4	0.8305	4	11.3608	12.0	2.5000
5	2.077	4	11.3608	12.0	11.2500
6	5.195	4	11.3608	12.0	73.7500

Title: emergence rate
File: 012-EM~1.TXT 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	95.0000	95.0000	95.0000
2	0.1330	4	91.2500	91.2500	92.5000
3	0.3324	4	93.7500	93.7500	92.5000
4	0.8305	4	92.5000	92.5000	92.5000
5	2.077	4	83.7500	83.7500	83.7500
6	5.195	4	21.2500	21.2500	21.2500

Title: emergence rate
File: 012-EM~1.TXT 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	95.0000				
0.1330	92.5000	0.5303		1.7300	k= 1, v=18
0.3324	92.5000	0.5303		1.8200	k= 2, v=18
0.8305	92.5000	0.5303		1.8500	k= 3, v=18
2.077	83.7500	2.3865	*	1.8600	k= 4, v=18
5.195	21.2500	15.6447	*	1.8700	k= 5, v=18

s = 6.6667

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

7012S : % emergence

Williams Test

[One-Sided Test for Decrease, alpha = 0.050000]

Dose	Isotone Means	T-bar	P-value	Significance
0	0.95	.		
0.133	0.921	0.7113	N.S.	
0.3324	0.921	0.7113	N.S.	
0.8305	0.921	0.7113	N.S.	
2.077	0.838	2.743	0.0072	*
5.195	0.213	17.98	<0.005	*
12.98	0	23.17	<0.005	*

"*"=Significant; "N.S."=Not Significant.

Estimates of EC%

Parameter	Estimate	95% Bounds Lower	95% Bounds Upper	Std.Err.	Lower Bound /Estimate
EC5	2.0	1.6	2.5	0.047	0.80
EC10	2.3	1.9	2.8	0.041	0.82
EC25	2.9	2.5	3.4	0.030	0.87
EC50	3.8	3.5	4.2	0.019	0.91

Slope = 5.70 Std.Err. = 0.612

Goodness of fit: p = 0.78 based on DF= 4.0 21.

7012S : % emergence

Observed vs. Predicted Treatment Group Means

Dose	#Reps.	Obs. Mean	Pred. Mean	Obs. -Pred.	Pred. %Control	%Change
0.00	4.00	0.950	0.923	0.0271	100.	0.00
0.133	4.00	0.912	0.923	-0.0104	100.	2.41e-14
0.332	4.00	0.925	0.923	0.00207	100.	7.30e-08
0.831	4.00	0.925	0.923	0.00215	100.	0.00779
2.08	4.00	0.838	0.863	-0.0252	93.5	6.53
5.20	4.00	0.213	0.207	0.00552	22.4	77.6
13.0	4.00	0.00	0.00115	-0.00115	0.124	99.9

male development rate
File: 7012md Transform: NO TRANSFORM

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

GRP1 (SOLVENT CRTL) MEAN = 0.0612 CALCULATED t VALUE = 0.3390
GRP2 (BLANK CRTL) MEAN = 0.0604 DEGREES OF FREEDOM = 6
DIFFERENCE IN MEANS = 0.0009

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
File: 7012md 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     1.608          5.808          9.168          5.808          1.608
OBSERVED     0              9              6              9              0

-------------------------------------------------------------------------------
Calculated Chi-Square goodness of fit test statistic =   7.8193
Table Chi-Square value (alpha = 0.01) = 13.277

Data PASS normality test. Continue analysis.


male development rate
File: 7012md       Transform: NO TRANSFORMATION

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

D =    0.000

W =    0.981

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

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


male development rate
File: 7012md       Transform: NO TRANSFORMATION

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

Calculated H statistic (max Var/min Var) =    7.24
Closest, conservative, Table H statistic =  184.0  (alpha = 0.01)

Used for Table H ==>     R (# groups) =   6,    df (# reps-1) =       3
Actual values    ==>     R (# groups) =   6,    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).


male development rate
File: 7012md       Transform: NO TRANSFORMATION

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

```
Calculated B statistic =    5.84
Table Chi-square value =   15.09  (alpha = 0.01)
Table Chi-square value =   11.07  (alpha = 0.05)

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

Data 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
File: 7012mdm Transform: NO TRANSFORM

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	7.574	1.515	35.233
Within (Error)	18	0.767	0.043	
Total	23	8.341		

Critical F value = 2.77 (0.05,5,18)
Since F > Critical F REJECT Ho:All groups equal

male development rate
File: 7012mdm Transform: NO TRANSFORM

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

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	neg control	6.123	6.123		
2	0.1330	6.388	6.388	-1.807	
3	0.3324	6.550	6.550	-2.916	
4	0.8305	6.067	6.067	0.375	
5	2.077	5.985	5.985	0.938	
6	5.195	4.808	4.808	8.968	*

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

male development rate

File: 7012mdm Transform: NO TRANSFORM

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	0.1330	4	0.353	5.8	-0.265
3	0.3324	4	0.353	5.8	-0.427
4	0.8305	4	0.353	5.8	0.055
5	2.077	4	0.353	5.8	0.137
6	5.195	4	0.353	5.8	1.315

male development rate
File: 7012mdm Transform: NO TRANSFORM

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	neg control	4	6.123	6.123	6.353
2	0.1330	4	6.388	6.388	6.353
3	0.3324	4	6.550	6.550	6.353
4	0.8305	4	6.067	6.067	6.067
5	2.077	4	5.985	5.985	5.985
6	5.195	4	4.808	4.808	4.808

male development rate
File: 7012mdm Transform: NO TRANSFORM

WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
neg control	6.353				
0.1330	6.353	1.581		1.73	k= 1, v=18
0.3324	6.353	1.581		1.82	k= 2, v=18
0.8305	6.067	0.377		1.85	k= 3, v=18
2.077	5.985	0.942		1.86	k= 4, v=18
5.195	4.808	9.009	*	1.87	k= 5, v=18

s = 0.206
Note: df used for table values are approximate when v > 20.

female development rate
File: 7012fd Transform: NO TRANSFORM

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

GRP1 (SOLVENT CRTL) MEAN	= 5.2400	CALCULATED t VALUE	= -3.1855
GRP2 (BLANK CRTL) MEAN	= 5.6600	DEGREES OF FREEDOM	= 6
DIFFERENCE IN MEANS	= -0.4200		

TABLE t VALUE (0.05 (2), 6) = 2.447** 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
File: 7012fd 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	1.608	5.808	9.168	5.808	1.608
OBSERVED	0	7	11	6	0

Calculated Chi-Square goodness of fit test statistic = 3.8331
Table Chi-Square value (alpha = 0.01) = 13.277

Data PASS normality test. Continue analysis.

female development rate
File: 7012fd Transform: NO TRANSFORMATION

Shapiro Wilks test for normality

D = 0.429

W = 0.976

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

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

female development rate

File: 7012fd Transform: NO TRANSFORMATION

Hartley test for homogeneity of variance

Calculated H statistic (max Var/min Var) = 5.28
Closest, conservative, Table H statistic = 184.0 (alpha = 0.01)

Used for Table H ==> R (# groups) = 6, df (# reps-1) = 3
Actual values ==> R (# groups) = 6, 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).

female development rate
File: 7012fd Transform: NO TRANSFORMATION

Bartletts test for homogeneity of variance

Calculated B statistic = 3.04
Table Chi-square value = 15.09 (alpha = 0.01)
Table Chi-square value = 11.07 (alpha = 0.05)

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

Data 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
File: 7012fd Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	2.651	0.530	22.083
Within (Error)	18	0.429	0.024	
Total	23	3.080		

Critical F value = 2.77 (0.05,5,18)
Since F > Critical F REJECT Ho:All groups equal

female development rate
File: 7012fd 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	5.240	5.240		
2	0.1330	5.445	5.445	-1.871	
3	0.3324	5.412	5.412	-1.575	
4	0.8305	5.295	5.295	-0.502	
5	2.077	5.160	5.160	0.730	
6	5.195	4.458	4.458	7.143	*

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

female development rate
File: 7012fd 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	0.1330	4	0.264	5.0	-0.205
3	0.3324	4	0.264	5.0	-0.172
4	0.8305	4	0.264	5.0	-0.055
5	2.077	4	0.264	5.0	0.080
6	5.195	4	0.264	5.0	0.783

female development rate
File: 7012fd 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	5.240	5.240	5.366
2	0.1330	4	5.445	5.445	5.366
3	0.3324	4	5.412	5.412	5.366
4	0.8305	4	5.295	5.295	5.295
5	2.077	4	5.160	5.160	5.160

6	5.195	4	4.458	4.458	4.458

female development rate
File: 7012fd 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	5.366				
0.1330	5.366	1.153		1.73	k= 1, v=18
0.3324	5.366	1.153		1.82	k= 2, v=18
0.8305	5.295	0.504		1.85	k= 3, v=18
2.077	5.160	0.733		1.86	k= 4, v=18
5.195	4.458	7.169	*	1.87	k= 5, v=18

s = 0.154
Note: df used for table values are approximate when v > 20.

APPENDIX II: COPY OF REVIEWER'S TWA CALCULATIONS (USING EXCEL):

SEDIMENT

Nominal Concentration (mg/L)	Time (Day)	14C-Trifluralin TR-4 Metabolite Equivalents Measured Concentration (mg/kg)	TWA (mg/kg)
0.133	0	0.0548	
	7	0.226	
	28	0.214	
			0.20010
2.077	0	1.226	
	7	2.421	
	28	3.919	
			2.83338
12.98	0	29.82	
	7	25.99	
	28	22.76	
			25.25750

OVERLYING WATER

Nominal Concentration (mg/L)	Time (Day)	14C-Trifluralin TR-4 Metabolite Equivalents Measured Concentration (mg/L)	TWA (mg/L)
0.133	0	0.09482	
	7	0.01658	
	28	0.0127	
			0.02491
2.077	0	1.4021	
	7	0.15472	
	28	0.22227	
			0.33597
12.98	0	1.6946	
	7	1.3435	
	28	0.84728	
			1.20131

PORE WATER

14C-Trifluralin TR-4 Metabolite Equivalents

Nominal Concentration (mg/L)	Time (Day)	Measured Concentration (mg/L)	TWA (mg/L)
0.133	0	0.0012	
	7	0.00434	
	28	0.00764	
			0.00519
2.077	0	0.03247	
	7	0.04153	
	28	0.1502	
			0.08115
12.98	0	0.66417	
	7	0.43524	
	28	0.4758	
			0.47907