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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY  
WASHINGTON, DC 20460

OFFICE OF PREVENTION,  
PESTICIDES AND  
TOXIC SUBSTANCES

MEMORANDUM

PC Code: 129121  
DP Barcode: 289903

**Date:** October 2, 2009

**Subject:** DER Transmittal Memo for One Study on a Fipronil Degradate (MB 45950)

**To:** Richard Gebken, RM  
Ann Sibold, PM  
Registration Division (7505P)

**From:** Anita Ullagaddi, EPS  
Nancy Andrews, Branch Chief  
Environmental Risk Branch I  
Environmental Fate and Effects Division (7507P)

*Nancy Andrews* 10/11/2009

Please find the attached DER summary for the following submitted ecotoxicity study:

- MRID 458510-01. Chronic Toxicity Test with Midge Larvae (*Chironomus riparius*) in a Water/Sediment System.

The data from this study are summarized below. Additional discussion of the data is included in the associated DER.

Study Type	Organism	MRID	Study Classification	Summary
28-Day Chronic Toxicity Test	<i>Chironomus riparius</i> (larvae)	458510-01	Supplemental (non-guideline)	EC <sub>50</sub> = 3.8 µg/kg dw sediment (nominal concentration based on emergence rate) NOAEC = 1.9 µg/kg dw sediment (day 0 concentration based on emergence rate) NOAEC = 1.9 µg/kg dw sediment (day 0 concentration based on development rate) NOAEC = 1.1 µg/kg dw sediment (day -10 concentration based on lethargy; may be an overestimated NOAEC as day 0 concentration was not measured at this treatment level)

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**DATA EVALUATION RECORD**  
**FRESHWATER SEDIMENT *Chironomus riparius* EMERGENCE TEST**

1. **CHEMICAL:** Fipronil degradate PC Code: 129121
2. **TEST MATERIAL:** [<sup>14</sup>C]MB 45950 Radiochemical Purity: 99.5%

3. **CITATION:**

Authors: Kolk, J.  
Title: Chronic Toxicity Test with Midge Larvae (*Chironomus riparius*) in a Water/Sediment System.

Study Completion Date: November 29, 2002

Laboratory: Springborn Smithers Laboratories (Europe) AG  
 Seestrasse, Horn, Switzerland

Sponsor: Bayer CropScience  
 2 T.W. Alexander Drive  
 Research Triangle Park, NC 27709

Laboratory Report ID: 1067.006.173

MRID No.: 458510-01

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

Signature: *Christie E. Padova* **Date:** 08/27/09

- APPROVED BY:** John Marton, Staff Scientist, Cambridge Environmental Inc.

Signature: *John Marton* **Date:** 08/31/09

5. **APPROVED BY:** Anita Ullagaddi, OPP/EFED/ERB-I

Signature: *Anita Ullagaddi* **Date:** 10/02/09

6. **STUDY PARAMETERS**

Scientific Name of Test Organism: *Chironomus riparius*  
 Age of Test Organism: 1<sup>st</sup> instar larvae, 2 to 3 days post-hatch  
 Definitive Test Duration: 28 days  
 Study Method: Static, with aeration  
 Type of Concentrations: Measured sediment concentrations on Day -10 (and Day 0 when reported)

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DP Barcode: 289903

MRID No.: 458510-01

File: 1001dr Transform: NO TRANSFORMATION

Bartlett's test for homogeneity of variance

Calculated B statistic = 3.12  
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).

Development rate (days<sup>-1</sup>), Days 0-28; ug/kg  
File: 1001dr Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	0.0025	0.0005	1.667
Within (Error)	18	0.0051	0.0003	
Total	23	0.0076		

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

Development rate (days<sup>-1</sup>), Days 0-28; ug/kg  
File: 1001dr 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	0.728	0.728		
2	0.16	0.717	0.717	0.816	
3	0.31	0.722	0.722	-0.408	
4	0.63	0.743	0.743	-1.225	
5	1.3	0.730	0.730	-0.204	
6	2.5	0.710	0.710	1.429	

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

Development rate (days<sup>-1</sup>), Days 0-28; ug/kg  
File: 1001dr Transform: NO TRANSFORMATION

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

## 11. MATERIALS AND METHODS

**Stability of Compound Under Test Conditions:** Freshly-spiked test sediment (nominal concentrations of 0.16, 0.31, 0.63, 1.3, 2.5, 5.0, and 10  $\mu\text{g}/\text{kg}$ ) was analyzed for total radioactive residues (TRR) of fipronil degradate using LSC following combustion. The analytical LOQ was 0.7  $\mu\text{g TRR}/\text{kg}$ . Excluding the 0.16, 0.31, and 0.63  $\mu\text{g}/\text{kg}$  levels, which were below the LOQ, recoveries averaged 83, 95, 101, and 92% of nominal concentrations, respectively. Recoveries indicated that accuracy was obtained in the dosing method employed for those treatment levels above the LOQ. The established test systems were then equilibrated under aerated test conditions for 10 days.

The majority of radioactivity remained associated with the sediment during the 10-day equilibration phase and subsequent 28-day study. On Day 0, analysis of the test systems (sediment, overlying water, and pore water) prepared at 2.5 and 10  $\mu\text{g}/\text{kg}$  indicated that the radioactivity associated with the sediment decreased 21 and 13% from the initial analysis, respectively (reviewer-calculated); radioactivity was not measured for other treatment levels. By Day 28, radioactivity associated with the sediment decreased another 5 and 4%, respectively (reviewer-calculated). In sediment, recoveries ranged from 76 to 81% of nominal levels at Day 0 and 72 to 78% of nominal on Day 28. In overlying water,  $\leq 0.09 \mu\text{g TRR}/\text{L}$  was measured. Due to the limited quantity of pore water available, an accurate determination of the radioactivity in pore water could not be determined. The LOQ for water samples was 0.01  $\mu\text{g TRR}/\text{L}$ .

As the radioactivity was not further characterized, the stability of the fipronil degradate was not assessed.

### **Physicochemical properties of fipronil degradate.**

Parameter	Values	Comments
Water solubility at 20°C	Not reported	
Vapor pressure	Not reported	
UV absorption	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.*

Data FAIL to meet homogeneity of variance assumption.  
 Additional transformations are useless.

Emergence rate, days 0-28; ug/kg  
 File: 1001er Transform: NO TRANSFORMATION

KRUSKAL-WALLIS ANOVA BY RANKS - TABLE 1 OF 2

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	RANK SUM
1	neg control	0.955	0.955	107.000
2	0.16	0.877	0.877	87.500
3	0.31	0.797	0.797	65.000
4	0.63	0.797	0.797	66.500
5	1.3	0.880	0.880	84.000
6	2.5	0.875	0.875	82.000
7	5.0	0.033	0.033	20.000
8	10.0	0.000	0.000	16.000

Calculated H Value = 21.468      Critical H Value Table = 14.070  
 Since Calc H > Crit H REJECT Ho: All groups are equal.

Emergence rate, days 0-28; ug/kg  
 File: 1001er Transform: NO TRANSFORMATION

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

GROUP	IDENTIFICATION	TRANSFORMED MEAN	ORIGINAL MEAN	GROUP													
				0	0	0	0	0	0	0	0						
8	10.0	0.000	0.000	8	7	3	4	6	2	5	1						
7	5.0	0.033	0.033	.	.	.	.	.	.	.	.	.	.	.	.	.	.
3	0.31	0.797	0.797	.	.	.	.	.	.	.	.	.	.	.	.	.	.
4	0.63	0.797	0.797	.	.	.	.	.	.	.	.	.	.	.	.	.	.
6	2.5	0.875	0.875	.	.	.	.	.	.	.	.	.	.	.	.	.	.
2	0.16	0.877	0.877	.	.	.	.	.	.	.	.	.	.	.	.	.	.
5	1.3	0.880	0.880	.	.	.	.	.	.	.	.	.	.	.	.	.	.
1	neg control	0.955	0.955	*	*	.	.	.	.	.	.	.	.	.	.	.	.

\* = significant difference (p=0.05)      . = no significant difference  
 Table q value (0.05,8) = 3.124      SE = 6.538

Development rate (days<sup>-1</sup>), Days 0-28; ug/kg  
 File: 1001dr Transform: NO TRANSFORM

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

GRP1 (SOLVENT CTRL) MEAN =	0.0728	CALCULATED t VALUE =	0.1448
GRP2 (BLANK CTRL) MEAN =	0.0725	DEGREES OF FREEDOM =	6
DIFFERENCE IN MEANS =	0.0003		

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

Guideline Criteria	Reported Information
<p><b><u>Health of parent culture stock</u></b>                      Were parent chironomids in good health during the culture period?</p>	<p>N/A</p>

**B. Test System**

Guideline Criteria	Reported Information
<p><b><u>Type of Test System</u></b>                      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.</p>	<p>Static, with aeration. Replenishment of overlying water, if any, was not described.</p> <p>Additional samples were prepared at 2.5 and 10 µg/kg and served exclusively for analytical measurement. Therefore, the sampling method did not affect the biological load or concentration level of the test substance.</p>
<p><b><u>Test Materials</u></b></p>	<p>Identity: [<sup>14</sup>C]MB 45950                      Common name: fipronil degradate                      Physical description: white solid                      Lot No.: PJS 1028/1                      Radiochemical Purity: 99.5%                      Specific Activity: 1.4467 Gbq/mmol                      Label position: U-phenyl-ring                      Storage: ca. -20°C</p>
<p><b><u>Test Water</u></b>                      Soft reconstituted water or water from a natural source is preferred. De-chlorinated 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.</p>	<p>Elendt M4 medium (Batch No. 32.02)                      Hardness: 164 mg/L as CaCO<sub>3</sub>                      Alkalinity: 28 mg/L as CaCO<sub>3</sub>                      pH: 7.86                      Specific conductivity: 490 µSiemens/cm</p>

**15. REFERENCES:**

APHA, AWWA, WPCF. 1989. Standard Methods for the Examination of Water and Wastewater. 17<sup>th</sup> Edition, Washington, DC.

Dunnett, C.W. 1955. A multiple comparison procedure for comparing several treatments with a control. *J. Amer. Stat. Assoc.* 50:1096-1121.

Dunnett, C.W. 1964. New tables for multiple comparisons with a control. *Biometrics* 20:482-491.

Eidgenössisches Departement des Innern, Switzerland. March 2000. Swiss Ordinance relating to Good Laboratory Practice adopted February 2<sup>nd</sup>, 2000 [RS813.016.5].

OECD. 1998. OECD Principles of Good Laboratory Practice and Monitoring. Number 1. OECD Principles of Good Laboratory Practice (as revised in 1997). Environment Directorate OECD. Paris. France. 41 pp.

OECD. 2001. OECD Guidelines for the Testing of Chemicals. Proposal for a New Guideline 218. Sediment – Water Chironomid Toxicity Test Using Spiked Sediment. Draft Document. February 2001.

Schneider-Orelli, O. 1947. Entomologisches Praktikum. Einführung in die Landwirtschaftliche Insektenkunde. Verlag H.R. Sauerländer & Co. Aarau (zweite, erweiterte Auflage).



Guideline Criteria	Reported Information
<p><b><u>Introduction of Test Organisms</u></b>  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.</p>	<p>At test initiation (day 0), midge larvae were impartially added to each replicate test vessel. Aeration was stopped while the animals were added and was resumed 1 day later to allow the midges to settle into the sediment.</p>
<p><b><u>Solvents</u></b>  If used, minimal (i.e., <math>\leq 0.1</math> ml/l) and same concentration in all treatments. Suitable solvents are acetone, ethanol, methanol, ethylene 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)</p>	<p>Acetone; volume not reported.</p> <p>The acetone was allowed to completely evaporate prior to the addition of the sediment, and a solvent control level was included.</p>
<p><b><u>Water Temperature</u></b>  <math>20^{\circ}\text{C} \pm 2^{\circ}\text{C}</math> (Should not deviate between vessels by more than <math>1^{\circ}\text{C}</math>.)</p>	<p>19.0 to <math>21.1^{\circ}\text{C}</math></p> <p>Room temperature ranged from 19.5 to <math>20.5^{\circ}\text{C}</math></p>
<p><b><u>pH</u></b>  <u>Sediment:</u> <math>7.0 \pm 0.5</math>  <u>Interstitial Water:</u>  <u>Overlying Water:</u> 6.0 to 9.0  (Should not vary by more than 1 unit during test)</p>	<p><u>Sediment:</u> 6.70 (at preparation)  <u>Interstitial Water:</u> Not reported  <u>Overlying Water:</u> 7.56 to 8.34</p>
<p><b><u>TOC</u></b>  <u>Sediment:</u> <math>2 \pm 0.5\%</math>  <u>Overlying Water:</u> 2 mg/L</p>	<p><u>Sediment:</u> 2.18% (at preparation)  <u>Overlying Water:</u> Not reported</p>
<p><b><u>Ammonia</u></b>  <u>Interstitial Water:</u>  <u>Overlying Water:</u></p>	<p><u>Interstitial Water:</u> Not reported  <u>Overlying Water:</u> 0.25 to 0.4 mg/L (negative control and 10 <math>\mu\text{g}/\text{kg}</math> levels at 0 and 28 Days)</p>

**Verification Statistical Endpoint Values<sup>(a)</sup>**

Statistical Endpoint	28-day Emergence	28-day Development Rate	10-d Survival	10-d Dry Weight
NOAEC	2.5 µg/kg	2.5 µg/kg	--	--
LOAEC	5.0 µg/kg	>2.5 µg/kg	--	--
IC <sub>50</sub> (95% C.I.)	Not Determined	>2.5 µg/kg	--	--
Slope (Standard Error)	Not Determined	N/A	--	--

<sup>(a)</sup> Results are based on mean nominal sediment test concentrations.

**14. REVIEWER'S COMMENTS:**

The reviewer's NOAEC values agreed with those reported by the study author; however, the reviewer was unable to calculate an EC<sub>50</sub> value due to a near-singular matrix in the probit analyses. Therefore, the study author's results are reported in the Conclusions section of this DER. The primary objectives of a chironomid study test and to determine the toxicological effects (if any) on emergence rate and development rate. In this study, the NOAEC for both endpoints was nominally 2.5 µg/kg. However, at the 2.5 µg/kg level, almost all fully-emerged midges were on the surface of the water and lethargic. Thus, the NOAEC for this study was nominally 1.3 µg/kg based on lethargy (the most sensitive endpoint), which corresponded to an initial measured concentration (Day -10) of 1.1 µg/kg. This value may be a slight overestimation as Day 0 sediment concentrations were not measured at this treatment level.

Time-weighted averages could not be calculated for this study as analytical concentrations were only performed at Days 0 and 28. Additionally, three treatment levels were below the LOQ, which caused poor analytical recoveries at the three lowest treatment levels (<LOQ of 0.7 µg/kg). The higher treatment levels, however, had acceptable recoveries and the corresponding NOAEC and LOAEC values could be determined. The NOAEC and LOAEC values for emergence rate were 2.5 and 5.0 µg/kg, respectively, based on nominal concentrations, which corresponded to initial measured concentrations (Day -10) of 2.4 and 5.1 µg/kg, respectively.

The reviewer did not detect any significant differences between the negative and solvent controls for either endpoint. However, the reviewer felt that the 21% inhibition in emergence rate in the solvent control relative to the negative control was biologically significant.

To obtain actual initial treatment levels, LSC (following combustion) analysis was performed on

Guideline Criteria	Reported Information
<p><b><u>Food Concentration and Frequency</u></b>                      Preferably feed daily but at least 3 times per week.  <u>day 1 to 10:</u> 0.25-0.5 mg per larvae per day  <u>remainder of test:</u> 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)</p>	<p>Generally 0.3 mL per vessel per day depending on the amount of midges emerged and the state of the overlying water.</p>

**C. Test Design**

Guideline Criteria	Reported Information
<p><b><u>Duration</u></b>  <i>Chironomus riparius</i>: 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).</p>	<p>28 days</p>

Toxicity Observations: For the control through (nominal) 2.5 µg/kg levels, emergence generally occurred from Days 13 to 18, with an additional single male chironomid emerging from the 0.16 µg/kg level on Day 12. At the 5.0 µg/kg level, only two females successfully emerged: one on Day 14 and one on Day 16. No emergence was observed at the 10.0 µg/kg level. Statistical analysis of the ratio of number of male and number of female midges emerged indicated no differences between the test groups; therefore, male and female results were pooled for subsequent analysis.

The calculated average emergence rates were 0.95, 0.75, 0.88, 0.80, 0.80, 0.88, 0.88, 0.03, and 0 for the negative control, solvent control, 0.16, 0.31, 0.63, 1.3, 2.5, 5.0, and 10.0 µg/kg test levels, respectively. Differences in emergence rate were statistically-significant ( $p \leq 0.001$ ) compared to the pooled control at the 5.0 and 10.0 µg/kg levels. The resultant NOAEC was (nominally) 2.5 µg/kg. The 28-day  $EC_{50}$  (with 95% C.I.), based upon the number of midges that did not hatch, was 3.8 (2.5 to 5.0) µg/kg.

Mean development rates were 0.073, 0.072, 0.072, 0.072, 0.074, 0.073, and 0.071 days<sup>-1</sup> for the negative control, solvent control, 0.16, 0.31, 0.63, 1.3, and 2.5 µg/kg test levels, respectively, with no statistically-significant differences from the pooled control detected. Since emergence was only observed in one replicate (C) of the 5.0 µg/kg level and in no replicates from the 10.0 µg/kg level, no development rates were calculated for these levels. The resultant NOAEC was (nominally) 2.5 µg/kg.

At the 2.5 µg/kg level, almost all midges (which had fully emerged) were on the surface of the water and lethargic. At the 5.0 and 10.0 µg/kg levels from Day 12 onwards, the larvae were noted to be on the surface of the sediment instead of burrowed into the sediment. The observed larvae were alive and moving at the 5.0 µg/kg level. At the 10.0 µg/kg level, only a portion of the larvae were alive and moving. At test termination, all midge larvae observed on the sediment surface were not moving. Based on lethargy, the NOAEC was (nominally) 1.3 µg/kg.

## **B. Statistical Results (From Study Report)**

The NOAEC and LOAEC for emergence rate and development rate were calculated using Dunnett's multiple t-test ( $p=0.05$ ).

The number of emerged midges per test level was used to calculate the number of midges which did not emerge (per level). This "mortality" was corrected for control mortality according to Schneider-Orelli (1947). The  $EC_{50}$  (with 95% confidence intervals) was then calculated using the binomial probability method.

Results were provided in terms of nominal sediment concentrations.

Guideline Criteria	Reported Information
<p><b><u>Overlying Water Parameter Measurements</u></b></p> <ol style="list-style-type: none"> <li>1. Dissolved oxygen should be measured daily in all test chambers.</li> <li>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.</li> <li>3. Temperature should be monitored at least hourly throughout the test in one test chamber.</li> <li>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.</li> </ol>	<p>1. – 3. Dissolved oxygen, temperature, and pH were measured in all test vessels on Days 0, 7, 14, 21, and 28.</p> <p>4. Hardness and ammonium concentrations were determined at the negative control and 10 µg/kg levels on Days 0 and 28.</p>
<p><b><u>Chemical Analysis-Overlying Water</u></b> 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.</p>	<p>Overlying water was isolated on Days 0 and 28 from supplemental test vessels prepared at 2.5 and 10 µg/kg. Samples were analyzed for total radioactive residues (TRR) of fipronil degradate using LSC.</p>
<p><b><u>Interstitial Water and Sediment Isolation Method</u></b> Centrifugation (e.g., 10,000 g and 4°C for 30 min) is recommended. If test substance is demonstrated not to adsorb to filters, filtration may be acceptable.</p>	<p>Centrifugation for 30 minutes at 10,000 g</p>
<p><b><u>Chemical Analysis-Interstitial Water</u></b> At a minimum must be analyzed at the end of the test in at least the highest concentration and one lower concentration.</p>	<p>Pore water was isolated on Days 0 and 28 from supplemental test vessels prepared at 2.5 and 10 µg/kg. Samples were analyzed for total radioactive residues (TRR) of fipronil degradate using LSC.</p>

Guideline Criteria	Reported Information
<p><b><u>Data Endpoints</u></b></p> <p><u>Emergence Test (28 day)</u></p> <ul style="list-style-type: none"> <li>- Number alive</li> <li>- Time to emergence</li> <li>- Number of emerged male and female midges</li> <li>- Number of visible pupae that have failed to emerge</li> <li>- Number of egg masses deposited</li> <li>- Observations of other effects, abnormal behavior, or appearance or clinical signs (e.g., leaving sediment, unusual swimming)</li> </ul> <p><u>Growth and Survival (10-day) (Optional)</u></p> <ul style="list-style-type: none"> <li>- Number alive</li> <li>- Instar level of surviving larvae</li> <li>- Dry weight (ash free) per test chamber of surviving larvae by instar level</li> </ul>	<p><u>Emergence Test (28 days)</u></p> <ul style="list-style-type: none"> <li>- Number alive</li> <li>- Time to emergence</li> <li>- Number of emerged male and female midges</li> <li>- Number of visible pupae that failed to emerge</li> <li>- Number of dead visible larvae</li> <li>- Number of dead emerged midges</li> </ul> <p><u>Growth and Survival (10-day) (Optional)</u></p> <p>N/A</p>
<p><b>Raw data included?</b></p>	<p>Yes</p>