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Data Evaluation Record

Non-guideline Mesocosm Study

1. **Chemical:** Fipronil **PC Code:** 129121

2. **Test Material:** Chipco TopChoice granules **Purity:** 0.014%
   Active Ingredient (a.i.): Fipronil
   CAS No.: 120068-37-3
   IUPAC name of a.i.: 5-amino-1-[2,6-dichloro-4-(trifluoromethyl)phenyl]-4 (trifluoromethylsulfinyl)pyrazole-3-carbonitrile

3. **Study Title:** Chipco® TopChoiceTM — Effects on Aquatic Fauna in Outdoor Simulate Ponds

4. **Study Identification**
   Study Director: James R. Hoberg
   Laboratory: Springborn Smithers Laboratories
   Study Dates: June 1 to September 24, 2004
   Study Completion Date: October 12, 2005
   Study Identification: Springborn Smithers Study No. 13798.6164
   Sponsor Protocol/Project No. EBFIY001
   Sponsor: Bayer CropScience
   EPA Identification: MRID 467339-01; DP 325893

5. **Reviewed by:** Anita Ullagaddi, EFED/ERB1
   Signature: [signature] **Date:** March 4, 2009

6. **Approved by:** Edward Odenkirchen, Ph.D., EFED
   Signature: [signature] **Date:** 3/12/09

7. **Conclusions:**
   Fipronil added to outdoor mesocosms was associated with reduced abundance in some aquatic invertebrates. Apparent recovery was observed in some species by Day 21 (mean fipronil concentration at Day 21 was 0.019 ug a.i./L). However, marked reductions in abundance of some aquatic invertebrate species remained for the study duration. Significant ($p<0.05$) effects occurred in mayflies for the entire study duration. The average fipronil concentration was lower than the detection limit at Day 28 and thereafter. The mean measured concentration for the study was 0.042 ug a.i./L (42 ng a.i./L).

2083583

8. Adequacy of the Study:

A. Classification: Supplemental

B. Rationale: The study does not satisfy any current guideline requirement.

C. Reparability: N/A

9. Study Purpose: The objective of this study was to evaluate the potential ecological effects of Chipco® TopChoiceTM (active ingredient: fipronil, and its metabolites, MB 45950, MB 46136 and MB 46513) on aquatic communities exposed in complex outdoor simulated shallow freshwater ponds. The major issue addressed was whether prolonged Chipco® TopChoiceTM exposure resulted in ecologically significant long-term effects on a community of zooplankton and macroinvertebrates exposed to simulated runoff from a broadcast application of the test substance. A secondary objective of this study was to assess exposure dynamics (concentration over time) of Chipco® TopChoiceTM (active ingredient: fipronil and its metabolites, MB 45950, MB 46136 and MB 46513) under the environmental conditions in simulated ponds.

11. Study Design and Protocol: The simulated pond guidelines published by the U.S. Environmental Protection Agency, 850.1900, 850.1925, 850.1950 (U.S. EPA, 1996) and the recommendations of international associations (Crossland *et al.,* 1994; Campbell *et al.,* 1999; Giddings *et al.,* 2002) and the European Commission (2002) were considered in the development of the protocol for this study.

<u>Test substance:</u>
Name: Chipco® TopChoiceTM
Batch No.: CO302 1003
CAS No.: 120068-37-3
Purity: 0.014% (fipronil)
Recertification Date: 16 April 2005

<u>Water System</u>
Eight outdoor simulated ponds were used for this study. Square, wooden frames were lined with heavy-duty EPDM rubber liners, which were designed for aquatic horticulture. A 10-cm layer of soil (red clay) from an adjacent field was added to the bottom of each pond. Twelve 250-mL wide-mouth jars were filled with 10-cm of soil and placed in the soil of each pond. Unfiltered water was pumped from an adjacent pond to fill each simulated pond to a height of 53 cm and a volume of 2800 L; water was pumped through a screen to allow zooplankton and phytoplankton but to prevent fish from entering the simulated ponds. A drain was installed in each pond to prevent the water level from exceeding 53 cm due to rainfall events. On site well water was used to replace water loss due to evaporation. Well water was added when water depth decreased by 5 cm in any simulated pond. Make-up water was added to all simulated ponds at that time. Representative samples of the pond water, well water, and soil (sediment) were analyzed for the presence of pesticides, PCBs and toxic metals by GeoLabs, Inc., Braintree, Massachusetts. None

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of these compounds have been detected at concentrations that are considered toxic in any of the samples analyzed, in agreement with ASTM (2002) standard practice.

Flora and Fauna
Detritus, comprised mainly of leaf and pine needle litter, was collected from the bottom of the source water pond. The detritus contained native macroinvertebrates and served as a biological inoculum. Approximately 35 L of detritus was added to the water column of each pond and allowed to settle. Aquatic macrophytes from two additional local ponds; a bladderwort, green filamentous algae, small amounts of water meal and duck weed were added to the ponds. Several gallons of water were collected with the plants from each pond and equally dispersed in each pond. Four pickerel weed and one water lily plant, purchased from a commercial supply were also planted in each pond. The plants and water were divided into 12 equal portions and one portion was added to each pond. Additions of soil, water and detritus into the ponds were completed between May 10 and 15, 2004. Phytoplankton, periphyton and aquatic macrophytes were included in the simulated ponds because they are part of natural shallow freshwater pond ecosystems. Data on these communities were not analyzed for treatment-related effects, since the active ingredient is an insecticide and not expected to affect the plant community. Existing data indicate that zooplankton and macroinvertebrates are likely to be the most sensitive communities and therefore, were the primary focus of the effects investigation.

Stock Preparation
A leachate from Chipco® TopChoice granules was prepared by recirculating pond water through the granules overnight. The concentration of fipronil in leachate was measured and the leachate was added in dilute aqueous solution to the ponds.

The leachate used as the test substance was prepared at Springborn Smithers Laboratories, Wareham, Massachusetts, in duplicate column leaching systems. Each column contained 135 g of Chipco® TopChoiceTM granules with an equivalent amount of 6-mm glass beads. The granules and glass beads were added to a 290-n/L glass column with a stopcock and glass wool in the lower portion, to maintain the mixture. A metering pump circulated 9 L of water collected from Horseshoe Pond located in Wareham, Massachusetts, through the column at a rate of 50 mL/minute. The water was previously filtered through 1.5 micrometer filters to remove the majority of biological organisms. Silicon tubing connected the water reservoir to the pump, the pump to the column, and the column to the reservoir. The water was contained in a covered, 2-L glass vessel which received continuous aeration. The reservoir was covered with a black plastic to minimize photodegradation. After circulating water through the columns for approximately 24 hours, each 9-L aliquot of leachate was composited in a 20-L glass vessel. Two samples were removed from the composite sample for analytical confirmation of fipronil and the metabolite, MB 45950 concentration. The leachate was then siphoned into five, 4-L amber Nalgene bottles which were placed into a freezer until they were shipped on dry ice to the test site in Snow Camp, NC on 28 June 2004.

An equivalent amount of untreated, filtered water was placed in five amber Nalgene bottles, frozen and shipped on dry ice in a second cooler to the test site for addition to the control ponds.

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This untreated water (3 L) was added to the control ponds prior to dosing the treatment ponds. The addition of water to the control ponds was made in the same manner as described below for the treatment ponds, but without the addition of the test substance.

Test Substance Application

On day 0 of the study (June 30), a target concentration of 400 ng/L Chipco TopChoice was applied to four test replicate ponds. The other four ponds were used as controls. The dose was added to the pond water and mixed thoroughly, but care was taken as to not disturb the sediment.

Ecological and Test System Monitoring

Biological sampling began two weeks prior to the test substance application and continued for approximately 12 weeks after the application of the test substance. Sample sizes varied for some parameters during the study due to population changes or the need to increase the biological material required. The biological monitoring schedule, including water and sediment analyses for fipronil, MB 45950, MB 46136, and MB 46513, is summarized in the following table.

Biological and Chemical Monitoring Schedule

Week of Test Day	Phytoplankton Pigments	Zooplankton	Artificial Substrates	Emergence	Benthic Substrates	Water Analysis	Sediment Analysis
-14		X	X	X			
0						X (2 hr)	
7	X	X	X	X	X	X	X
14		X	X	X		X	
21		X					
28	X	X	X	X	X	X	X
42		X					
56	X	X	X	X	X	X	X
84	X	X	X	X	X	X	X

Temperature and dissolved oxygen were measured in each pond once a week. Rainfall was recorded daily. Conductivity and pH were measured in each pond weekly.

Phytoplankton pigments were measured as an indicator of algal productivity; chlorophyll-a, phaeophytin and total pigments were reported as µg pigment/L. Zooplankton and free-swimming macroinvertebrates were sampled and reported as individuals/L. Macrophytes were allowed to grow throughout the study; vegetative cover mapping was conducted at test termination to estimate the approximate density of each species. Macroinvertebrates on artificial substrates and benthic substrates were allowed to colonize for four weeks prior to collection; densities were reported as individuals/sample. Traps to catch emerging insects were placed on the surface of each

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pond 24 hours before the sampling event; densities were reported as individuals/sample. Odonata exuvia were counted on emergent vegetation rather than in insect traps; counts were performed over 48 hours on exposure days 36 to 38, days 56 to 58 and days 85 to 87.

Two weeks prior to test initiation, a composite water sample and a composite sediment sample were collected from the ponds and measured for fipronil and its metabolites to confirm there were no residues of these analytes in the water from local sources. Depth-integrated water samples were collected for analysis of fipronil, MB 45950, MB 46136, and MB 46513. Samples were collected on days 0 (2 hour), 7, 14, 28, 56 and 84 of the exposure. A single sample (composited from four of more depth-integrated samples) was analyzed from each treated pond at each sampling interval. The samples from the four control ponds were composited prior to analysis. Sediment samples were collected on days 7, 28, 56 and 84 by removing two glass jars from each pond per sampling interval. In addition, sediment core samples were collected on day 85 from the treated and control ponds. All aqueous and sediment samples were analyzed for fipronil, MB 45950, MB 46136 and MB 46513 using a liquid chromatography/mass spectrometry (LC/MS/MS) procedure based on methodology validated at Springborn Smithers.

12. Results & Statistical Analysis:

Water Chemistry

The daily minimum and maximum water temperatures were relatively consistent throughout the exposure period, with a seasonal increase and then decrease occurring in the latter portion of the exposure phase. Weekly morning temperatures, recorded in association with the dissolved oxygen measurements, ranged from 21 and 27 °C. Dissolved oxygen concentrations ranged between 3.8 and 10.3 mg/L, with a minimum percent of saturation of 48% (3.8 mg/L at 27°C). Conductivity ranged from 40 to 90 µmhos/cm during the exposure. Morning pH values ranged from 6.7 to 9.0 throughout the exposure. The above water quality parameters were similar between treated and control ponds. Well water was added to each pond on 9 and 16 July 2004, and 27 August 2004 to maintain pond water levels. Rainfall was common during the study. A total of 446 mm of rainfall was received during the exposure.

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Fipronil and Metabolite Levels in Water and Sediment

Measured average fipronil + metabolite (total residues) and fipronil and its metabolites are presented in the following tables.

Analytical Results for Total Fipronil Residues (ng/L)[a]

Target Concentration (ng/L)	Day	-14	0	7	14	28	56	84
	Date	6/18	6/30	7/7	7/14	7/28	8/25	9/22
Pretreat		<4.00	NA[b]	NA	NA	NA	NA	NA
Control[c]		NA	<4.00	<4.00	<4.00	<4.00	<4.00	<4.00
400	Rep 1	NA	420	170	96.4	42.3	8.92	5.36
	Rep 2	NA	375	180	98.3	49.6	26.7	12.4
	Rep 3	NA	398	169	101	40.5	27.1	11.2
	Rep 4	NA	395	171	100	42.9	27.3	4.77
	Mean[d]	NA	397	172	98.8	43.8	22.5	8.43

[a] Total fipronil residue is the sum of fipronil and its metabolites for each sample interval.
[b] NA = Not Applicable.
[c] Composite sample from all four replicate control ponds.
[d] Daily means were calculated from the unrounded values, and not the rounded values presented in this table.

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Analytical Results for Fipronil (ng/L)

Target Concentration (ng/L)	Day	-14	0	7	14	28	56	84
	Date	6/18	6/30	7/7	7/14	7/28	8/25	9/22
Pretreat		<4.00	NA[a]	NA	NA	NA	NA	NA
Control[b]		NA	<4.00	<4.00	<4.00	<4.00	<4.00	<4.00
400	Rep 1	NA	407	81.1	17.8	<4.00	<4.00	<4.00
	Rep 2	NA	366[c]	106	15.8	<4.00	<4.00	<4.00
	Rep 3	NA	388	82.2	24.0	4.84	<4.00	<4.00
	Rep 4	NA	386	84.1	17.8	<4.00	<4.00	<4.00
	Mean[d]	NA	387	88.4	18.8	<4.00	<4.00	<4.00
	SD	NA	16.6	11.9	3.54	NA	NA	NA

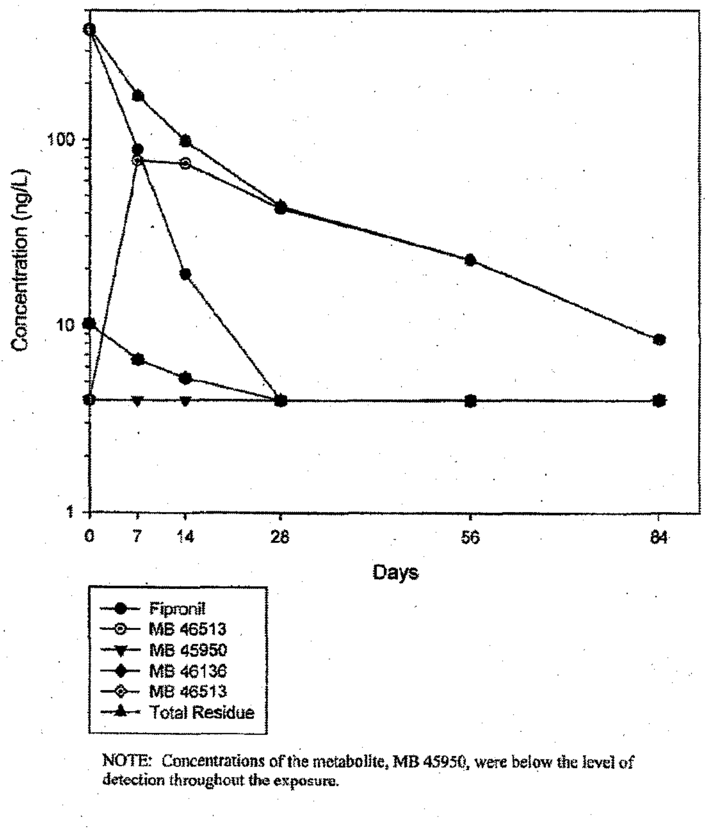
Analytical Results for MB 46513 (ng/L)

Target Concentration (ng/L)	Day	-14	0	7	14	28	56	84
	Date	6/18	6/30	7/7	7/14	7/28	8/25	9/22
Pretreat		<4.00	NA	NA	NA	NA	NA	NA
Control		NA	<4.00	<4.00	<4.00	<4.00	<4.00	<4.00
400	Rep 1	NA	<4.00	81.1	73.4	40.5	27.1	11.2
	Rep 2	NA	<4.00[c]	67.0	77.2	42.3	8.92	5.36
	Rep 3	NA	<4.00	80.5	71.1	44.7	26.7	12.4
	Rep 4	NA	<4.00	81.1	77.2	42.9	27.3	4.77
	Mean	NA	<4.00	77.4	74.7	42.6	22.5	8.43
	SD	NA	NA	6.97	3.05	1.74	9.04	3.92

a NA = Not Applicable.
b Composite sample from all four replicate control ponds.
c The initial result was lower than expected (266 ng/L). Therefore, the frozen archived sample which was removed from ponds on the indicated date was analyzed on 14 July 2004. Result of the archive sample is presented.
d Daily means and standard deviations (SD) were calculated from the unrounded values, and not the rounded values presented in this table.

The mean measured fipronil concentration in the water column was 387 ng/L on day 0. The mean total fipronil residue concentration (sum of fipronil and metabolites) on day 0 was 397 ng/L, closely approximating the desired nominal concentration of 400 ng/L. Dissipation of fipronil from the water was relatively rapid and concentrations progressively decreased to a mean concentration of 18.8 ng/L by day 14. The concentration of fipronil in the water column decreased to below the level of detection (4.00 ng/L) by day 28 in all but one replicate, which measured 4.84 ng/L. Concentrations of MB 46513 were present in the treated pond water on day 7 (77.4 ng/L), decreased slightly on day 14 (74.7 ng/L), and continued to decrease for the remainder of the exposure. The mean measured concentration of MB 46513 in treated pond water was 8.43 ng/L at test termination (day 84). Concentrations of MB 45950 in the water column were below the level of detection (4.00 ng/L) throughout the exposure. Concentrations of MB 46136 were present in the treated pond water on day 0 (10.2 ng/L), decreased on day 7(6.59 ng/L), and remained relatively consistent on day 14 (5.26 ng/L). By day 28, the concentration of MB 46136 present in the pond water was below the level of detection (4.00 ng/L). Total fipronil residues measured on day 0 was 397 ng/L. Total fipronil residue concentrations decreased with time from 397 ng/L to 172 (day 7), 98.8 (day 14), 43.8 (day 28), 22.5 (day 56) and 8.43 ng/L by test termination (day 84). The following figure (Study Figure 2) illustrates measured water concentrations of fipronil and its metabolites in the mesocosms over the study duration.

Figure 2. **Mean analyte concentrations (ng/L) in pond water over time during the simulated pond study.**



NOTE: Concentrations of the metabolite, MB 45950, were below the level of detection throughout the exposure.

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Measured fipronil + metabolites (total residues) and measured concentrations of fipronil and its metabolites (individually) in sediment are summarized in the following tables.

Analytical Results for Fipronil (ng/kg)

Target Water Concentration (ng/L)	Day	-14[a]	7[a]	28[a]	56[a]	84[a]	85[b]			
	Date	6/18	7/7	7/28	8/25	9/22	9/23			
							Core A	Core B	Core C	Core D
Control		<30.0	<30.0	<30.0	<30.0	<30.0	<30.0	<30.0	<30.0	<30.0
400	Rep 1	<30.0	536	30.0	<30.0	<30.0	<30.0	<30.0	<30.0	<30.0
	Rep 2	<30.0	236	<30.0	<30.0	<30.0	<30.0	<30.0	<30.0	<30.0
	Rep 3	<30.0	329	<30.0	<30.0	<30.0	<30.0	<30.0	<30.0	<30.0
	Rep 4	<30.0	593	<30.0	<30.0	<30.0	<30.0	<30.0	<30.0	<30.0
	Mean[c]	NA[d]	424	NA	NA	NA	NA	NA	NA	NA
	SD	NA	169	NA	NA	NA	NA	NA	NA	NA
							Total Mean: NA Total SD: NA			

Analytical Results for MB 46513 (ng/kg)

Target Concentration (ng/L)	Day	-14[a]	7[a]	28[a]	56[a]	84[a]	85[b]			
	Date	6/18	7/7	7/28	8/25	9/22	9/23			
							Core A	Core B	Core C	Core D
Pretreat		<30.0	NA	NA	NA	NA	NA	NA	NA	NA
Control		NA	65	<30.0	<30.0	<30.0	<30.0	<30.0	<30.0	<30.0
400	Rep 1	NA	691	599	331	486	204	114	252	202
	Rep 2	NA	194	856	556	881	105	370	138	266
	Rep 3	NA	312	716	823	434	87	298	191	224
	Rep 4	NA	545	600	704	499	111	158	266	89
	Mean[c]	NA	435	693	604	575	127	235	212	195
	SD	NA	224	122	212	206	52.5	120	59.1	75.6
							Total Mean: 192 Total SD: 83.5			

[a] Results of upper 1 cm of sediment.
[b] Results from an aliquot of a homogenized sample of the upper 5 cm core. Note: not all cores contained a 5-cm column. These samples were proportionally adjusted for actual core depth.
[c] Daily means and standard deviations (SD) were calculated from the unrounded values, and not the rounded values presented in this table.
[d] NA = Not Applicable.

Analytical Results for MB 45950 (ng/kg)

Target Water Concentration (ng/L)	Day	-14[a]	7[a]	28[a]	56[a]	84[a]	85[b]			
	Date	6/18	7/7	7/28	8/25	9/22	9/23			
							Core A	Core B	Core C	Core D
Pretreat		<30.0	NA	NA	NA	NA	NA	NA	NA	NA
Control		NA	114	<30.0	<30.0	40	<30.0	<30.0	<30.0	<30.0
400	Rep 1	NA	1390	420	256	572	225	106	180	160
	Rep 2	NA	332	932	356	970	111	340	90	160
	Rep 3	NA	1180	644	555	307	104	265	147	134
	Rep 4	NA	935	425	391	440	104	100	199	69
	Mean[c]	NA	960	605	390	572	136	203	154	131
	SD	NA	459	242	124	286	59.3	119	47.9	43.1
							Total Mean: 156 Total SD: 72.3			

Analytical Results for MB 46136 (ng/kg)

Target Water Concentration (ng/L)	Day	-14[a]	7[a]	28[a]	56[a]	84[a]	85[b]			
	Date	6/18	7/7	7/28	8/25	9/22	9/23			
							Core A	Core B	Core C	Core D
Pretreat		<30.0	NA	NA	NA	NA	NA	NA	NA	NA
Control		NA	35	<30.0	<30.0	<30.0	<30.0	<30.0	<30.0	<30.0
400	Rep 1	NA	192	119	86	125	41	28	56	52
	Rep 2	NA	59	433	104	221	28	80	34	66
	Rep 3	NA	104	130	155	103	<30.0	68	42	47
	Rep 4	NA	204	108	125	108	39	47	56	<30.0
	Mean[c]	NA	140	198	118	139	36.1	55.8	47.0	54.8
	SD	NA	70	157	29	55	7.4	22.9	10.8	9.7
							Total Mean: 48.8 Total SD: 15.3			

[a] Results of upper 1 cm of sediment.
[b] Results from an aliquot of a homogenized sample of the upper 5 cm core. Note: not all cores contained a 5-cm column. These samples were proportionally adjusted for actual core depth.
[c] Daily means and standard deviations (SD) were calculated from the unrounded values, and not the rounded values presented in this table.
[d] NA = Not Applicable.

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Analytical Results for Total Fipronil Residues (ng/kg)

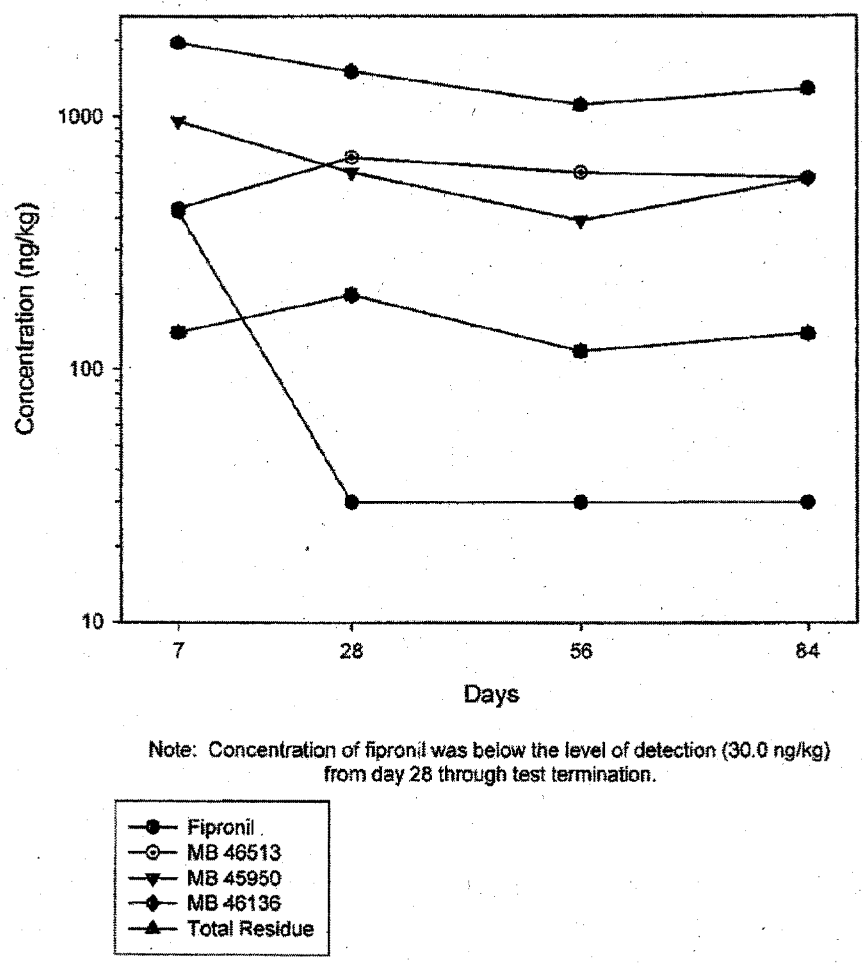
Target Water Concentration (ng/L)	Day	7[a]	28[a]	56[a]	84[a]	85[b] (cores)
	Date	7/7	7/28	8/25	9/22	9/23
Pretreat		NA[c]	NA	NA	NA	NA
Control		<30.0	<30.0	<30.0	<30.0	<30.0
400	Rep 1	2810	1170	673	1180	299
	Rep 2	821	2220	1020	2070	494
	Rep 3	1930	1490	1530	844	413
	Rep 4	2280	1130	1220	1050	381
	Mean[d]	**1960**	**1500**	**1110**	**1290**	**397**
	SD	**842**	**505**	**361**	**542**	**81**

[a] The total residue concentrations were calculated from rounded (whole numbers) measured concentrations of each analyte.
[b] The total residue concentrations were calculated from replicate mean measured concentrations for each analyte.
[c] NA = Not Applicable.
[d] Mean and standard deviation (SD) were calculated from the replicate values presented in this table.

Fipronil was present in the sediment (upper 1 cm) of the treated ponds on day 7 with a mean measured concentration of 424 ng/kg. Fipronil concentrations decreased to ≤ 30 ng/kg by day 28 and remained below the limit of detection (30 ng/kg) for the remainder of the study (day 84). Concentrations of fipronil in the upper 5-cm core samples collected on day 85 were also below the limit of detection. Measurable concentrations of MB 46513 were present in the sediment (upper 1 cm) at each sample interval, day 7, 28, 56 and 84 and resulted in mean measured concentrations of 435, 693, 604 and 575 ng/kg, respectively. Day 85 measured concentrations in the upper 5-cm core samples resulted in a mean concentration of 192 ng/kg. Measurable concentrations of MB 45950 were present in the sediment (upper 1 cm) at each sample interval, day 7, 28, 56 and 84 and resulted in mean measured concentrations of 960, 605, 390 and 572 ng/kg, respectively. In general, the concentrations of MB 45950 declined over the 84-day period. Day 85 measured concentrations in the upper 5-cm core samples resulted in a mean concentration of 156 ng/kg.

Measurable concentrations of MB 46136 were present in the sediment (upper 1 cm) at each sample interval, day 7, 28, 56 and 84 and resulted in mean measured concentrations of 140, 198, 118 and 139 ng/kg, respectively. Day 85 measured concentrations in the upper 5-cm core samples resulted in a mean concentration of 48.8 ng/kg. The total mean measured fipronil residues in the upper 1 cm of sediment on days 7, 28, 56 and 84 were 1960, 1500, 1110 and 1290 ng/kg, respectively. The total mean measured concentration of fipronil residues in the upper 5-cm sediment core samples on day 85 was 397 ng/kg. The following figure (Study Figure 3) illustrates measured sediment concentrations of fipronil and its metabolites in the mesocosms over the study duration.

Figure 3. **Mean analyte concentrations (ng/kg, based on dry weight) in the upper 1 cm of pond sediment over time during the simulated pond study.**



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Biological Results.
Taxa were selected for statistical analysis using the following criteria: dominant taxa and major taxonomic groups. If an effect was noted in a major group, subgroups within that group were investigated. The biological data were imported into SYSTAT software (SPSS, Inc., 1999) and analysis of variance (ANOVA) was performed to determine if the treatment data were significantly different ($p \leq 0.05$) from the control data. Abundance tables are included in Attachment 1.

A) Phytoplankton
Measured quantities of each pigment increased during the study. Since the test substance is an insecticide and not expected to affect plant growth, these data were not statistically analyzed. Pigment concentrations indicate that the ponds continued to mature during the study and provide sufficient primary production for primary consumers.

B) Macrophytes

Table 13. Macrophytes stocked in the simulated ponds during the pretreatment phase and those observed at test termination.

Macrophytes stocked in all ponds during pretreatment phase		Macrophytes observed at test termination							
		Control				400 ng/L			
	Rep	1	2	3	4	1	2	3	4
Rooted									
Pickerel weed (*Pontederia cordata*)		X	X	X	X	X	X	X	X
Water lily (*Nymphae odorata*)		X	X	X	X	X	X	X	X
Bushy pondweed (*Najas* sp.)			X	X	X	X	X	X	X
Pondweed (*Potamogeton* sp.)				X		X			
Rush (*Juncus* sp.)		X	X		X	X	X	X	X
Muskgrass (*Chara* sp.)		X	X	X	X		X	X	X
Cattail (*Typha* sp.)		X							
Free-floating									
Duckweed (*Lemna* sp.)									
Watermeal (*Wolfia* sp.)									
Green filamentous algae		X	X	X	X	X	X		X
Bladder wort (*Utricula* sp.)		X	X		X	X	X	X	

C) Zooplankton
Seventy-nine taxa were distinguished in the zooplankton samples. In summary, the only significant differences noted in zooplankton samples between major taxonomic groups were as follows: 1) Nematoda in the treated ponds were significantly less than the control on day 42, and 2) Total zooplankton was significantly increased in the treated ponds relative to the control on day 56.

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Zooplankton[a]

Major Group	Days					
	7	14	21	28	42	56
Annelida						
Arthropoda						
Insecta						
Ostracoda						
Cladocera						
Copepoda						
Cyclopoida sp. nauplius						
Diaptomus pallidus adult						
Diaptomus pallidus copepodite			I			
Diaptomus sp. nauplius						
Mesocyclops edax adult	R	R				
Mesocyclops edax copepodite	R					
Tropocyclops prasinus adult	R	R				
Tropocyclops prasinus copepodite						
Nematoda					R	
Protozoa						
Rotifera						
Total Zooplankton						I

[a] An "R" indicates a significant reduction in abundance relative to the control based on ANOVA, while an "I" indicates a significant increase in abundance relative to the control based on ANOVA.

Annelida - There was no significant difference between control and treated populations.

Arthropoda - Mean density of arthropods ranged from 30.9 to 124.5 individuals/L in the control and 37.1 to 142.5 individuals/L in the treated ponds. As a group, arthropods were not affected in the treated ponds relative to the control population throughout the study.

Insecta - Mean density of insects ranged from 0.0 to 0.6 individuals/L in the control and 0.1 to 0.6 individuals/L in the treated ponds. No statistical differences were detected between control and treatment ponds.

Ostracoda - Mean density of ostracods ranged from 2.2 to 7.9 individuals/L in the control and 2.9 to 16.0 individuals/L in the treated ponds. Ostracods were not affected in the treated ponds relative to the control population at any sample interval.

Cladocera - Mean density of cladocerans ranged from 0.8 to 24.4 individuals/L in the control and 0.2 to 35.5 individuals/L in the treated ponds. Cladocerans were not significantly affected in the treated ponds relative to the control population at any sample interval.

Copepoda - Mean density of copepods ranged from 22.7 to 92.8 individuals/L in the control and 33.7 to 92.2 individuals/L in the treated ponds. As a group, Copepoda were not significantly affected in the treated ponds relative to the control population at any sample interval. On day 7, copepodite and adult *M. edax* and adult *T. prasinus* in the treated ponds were significantly reduced relative to the control population. On day 14, only adults of *M. edax* and *T. prasinus* were significantly reduced in the treatment. On days 21, 28, 42, and 56 copepod density was greater in the treated ponds than the control ponds.

Nematoda* - Mean density of nematodes ranged from 0.0 to 5.3 individuals/L in the control and 0.1 to 3.9 individuals/L in the treated ponds. Nematodes were significantly reduced in the treated ponds relative to the control population on day 42 only.

Protozoa - Mean density of protozoa ranged from 1.9 to 19.1 individuals/L in the control and 2.1 to 51.2 individuals/L in the treated ponds. Protozoa were not significantly affected in the treated ponds relative to the control throughout the study.

Rotifera - Mean density of rotifers ranged from 0.4 to 4.2 individuals/L in the control and 0.5 to 17.9 individuals/L in the treated ponds. Rotifers were not significantly affected in the treated ponds relative to the control at any sample interval.

Total Zooplankton* - Mean density of total zooplankton ranged from 34.1 to 127.8 individuals/L in the control and 40.9 to 206.0 individuals/L in the treated ponds. Total zooplankton was significantly increased in the treated ponds relative to the control population on day 56 only.

***Significant difference observed.**

D) Macroinvertebrates – Artificial Substrates

Fifty taxa were distinguished in the Hester-Dendy samples. Although significant differences were observed in some groups, the macroinvertebrate (artificial substrates) group as a whole did not have significant differences between the treatment and the control.

Macroinvertebrates (Hester-Dendy samples)[a]

Major Group	Days			
	8	14	28	56
Clitellora	I	I		I
Naididae	I	I		I
Tubificidae				
Lumbriculide				
Gastropoda				
Insecta				
Coleoptera				
Diptera	R			
Ceratopogonidae				
Chaoboridae				
Chironomidae	R			
Culicidae				
Ephemeroptera	R	R	R	R
Hemiptera				
Heteroptera				
Odonata				
Aeshnidae				
Coenagrionidae	R			
Libellulidae				
Trichoptera				R
Turbellaria				I
Total Macroinvertebrates				

[a] An "R" indicates a significant reduction in abundance relative to the control based on ANOVA, while an "I" indicates a significant increase in abundance relative to the control based on ANOVA.

Clitellora* - Mean density of Clitellora ranged from 17 to 30 individuals per sample from the control ponds and 51 to 90 individuals per sample from the treated ponds. On days 8, 14 and 56,

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members of Clitellora, primarily of the family Naididae, were significantly more abundant in the 400 ng/L treatment level than the controls.

Gastropoda - Mean density of gastropods ranged from 17 to 24 individuals per sample in the control ponds and 26 to 46 individuals per sample in the treated ponds. No significant differences were found between the control and treated populations.

Insecta - Mean density of total insects ranged from 75 to 122 individuals per sample in the control ponds and 61 to 100 individuals per sample in the treated ponds. No significant differences were found in total insect abundance between the control and treated populations.

Diptera - On day 8, dipterans and chironomids were significantly reduced in the treated ponds relative to the control populations. Densities of dipterans and chironomids in the treated ponds were similar to the control for the remainder of the study. Statistical analyses were not conducted on the densities of ceratopogonids and culicids since they rarely occurred in the samples.

Ephemeroptera* - At each sample interval, day 8, 14, 28, and 56, ephemeropterans were significantly reduced in the treated ponds relative to the control populations.

Hemiptera and Heteroptera - No more than three organisms were found in a sample, consequently, statistical analyses were not performed on these two groups.

Odonata - The densities of odonates in the treated ponds were statistically similar to the control ponds at each sampling interval.

Trichoptera* - Trichopteran (caddisflies) were only present in control samples on day 56, with a mean density of 2 individuals per sample. Statistical analysis of this interval indicated a significant reduction of Trichopterans in the treated ponds (density 0) relative to the density in the control ponds on day 56. However, due to the low density of organisms on day 56, the noted effect may not be related to the treatment.

Turbellaria* - Mean density of Turbellaria ranged from 1 to 17 individuals per sample in the control ponds and 20 to 32 individuals per sample in the treated ponds. The density of Turbellaria in the treated ponds was significantly increased relative to the control ponds on day 56.

Total Macroinvertebrates (Artificial Substrate) - Mean density of total macroinvertebrates ranged from 116 to 176 individuals per sample in the control ponds and 193 to 259 individuals per sample in the treated ponds. The densities of total macroinvertebrates in the treated ponds were statistically similar to the control ponds at each sampling interval.

***Significant difference observed.**

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E) Macroinvertebrates – Benthic Substrates

Fifty taxa were distinguished in the benthic samples. There was a significant increase in total macroinvertebrates as a group on day 28. A significant increase was seen in Clitellora (Naididae) on day 28. On day 8, a significant reduction was seen in Insecta (day 8 and 28 significant reductions in dipteran and chironomids; day 8, 28, and 56 significant reduction in ephemeroptera).

Macroinvertebrates (benthic samples)[a]

Major Group	Days		
	8	28	56
Clitellora		I	
Naididae		I	
Tubificidae			
Lumbriculide			
Gastropoda			
Insecta	R		
Coleoptera			
Diptera	R	R	
Ceratopogonidae			
Chaoboridae			
Chironomidae	R	R	
Culicidae			
Ephemeroptera	R	R	R
Hemiptera			
Heteroptera			
Odonata			
Aeshnidae			
Coenagrionidae			
Libellulidae			
Trichoptera			
Turbellaria			
Total Macroinvertebrates		I	

[a] An "R" indicates a significant reduction in abundance relative to the control based on ANOVA, while an "I" indicates a significant increase in abundance relative to the control based on ANOVA.

Clitellora* - Mean density of total Clitellora ranged from 81 to 246 individuals per sample from the control ponds and 266 to 408 individuals per sample from the treated ponds. On day 28, members of Clitellora were significantly more abundant in the 400 ng/L treatment level than the controls.

Naididae* - On days 28, Naididae were significantly more abundant in the 400 ng/L treatment level than the controls.

Tubificidae - No significant differences were detected between the control and treated populations.

Lumbriculidae - Although the mean density of Lumbriculids was consistently reduced in the treated ponds, no significant differences were detected between the control and treated populations. Variation between replicate ponds lessened the sensitivity of the analyses.

Gastropoda - Mean density of gastropods ranged from 9 to 77 individuals per sample in the control ponds and 19 to 52 individuals per sample in the treated ponds. No significant differences were found between the control and treated populations.

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Insecta* - Mean density of total insects ranged from 197 to 370 individuals per sample in the control ponds and 73 to 294 individuals per sample in the treated ponds. A significant reduction was detected in total insect density in the treated ponds relative to the control on day 8 only.

Coleoptera - No significant differences were found between the control and treated populations.

Diptera* - On days 8 and 28, dipterans and chironomids were significantly reduced in the treated ponds relative to the control ponds. No significant differences in density for ceratopogonids and culicids were found between the treated and control ponds.

Ephemeroptera* - At each sample interval, days 8, 28 and 56, ephemeropterans were significantly reduced in the treated ponds relative to the control populations.

Hemiptera and Heteroptera - No statistical differences were detected between the treated and control populations.

Odonata - The densities of total odonates in the treated ponds were statistically similar to the control ponds at each sampling interval.

Trichoptera - Statistical analysis of this interval did not detect a significant reduction of density in the treated ponds relative to the density in the control ponds.

Turbellaria - Mean density of Turbellaria ranged from 8 to 133 individuals per sample in the control ponds and 9 to 79 individuals per sample in the treated ponds. No significant differences were detected in the populations from the treated ponds relative to the control ponds.

Total Macroinvertebrates (Benthic Substrates)* - Mean density of total macroinvertebrates ranged from 419 to 592 individuals per sample in the control ponds and 437 to 804 individuals per sample in the treated ponds. On day 28, the density of total macroinvertebrates in the treated ponds was significantly increased relative to the control ponds.

***Significant difference observed.**

<u>F) Emergent Insects</u>
Seventeen taxa were distinguished in the emergent insect samples. No significant differences were observed

Diptera - Mean density of dipterans ranged from 2 to 27 individuals per sample in the control ponds and 0 to 6 individuals per sample in the treated ponds. No significant differences in dipterans were detected in the treated ponds relative to the control ponds. No significant

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differences in density for ceratopogonids, chaoborids, and culicids were found between the treated and control ponds. Mean density of chironomids ranged from 2 to 26 individuals per sample in the control ponds and 0 to 6 individuals per sample in the treated ponds. No significant differences in chironomid numbers from the treated ponds relative to the control populations were detected.

Ephemeroptera - Ephemeropterans (mayflies) were rarely noted and only observed in the control ponds. One ephemeropteran was observed on days 28 and 56.

Total Emergent Insects - Mean density of total emergent insects ranged from 2 to 28 individuals per sample in the control ponds and 0 to 6 individuals per sample in the treated ponds. No significant differences of treated ponds insect density relative to the control ponds were detected. Since odonates emerge on vegetation which was not covered by the emergent insect traps, exuvia, shed larval skins left by emerging adults, were counted over three, 48 hour periods during the study. No significant differences were detected between the treated and control data.

13. Study Author's Discussion and Conclusions

The analytical measurements of simulated pond water for fipronil and its metabolites confirmed the desired nominal concentration, 400 ng/L, was achieved in each treated pond. Aqueous concentrations of fipronil readily declined and dropped to or below measurable concentrations (approximately 4.00 ng/L) by day 28. Additionally, the analytical measurements clearly characterized the generation and decline of two major metabolites of fipronil. The mean total fipronil residues, the sum of fipronil and its three metabolites, on days 0, 7, 14,28, 56 and 84 were 397, 172, 98.8, 43.8, 22.5 and 8.43 ng/L, respectively, declining over the study period.

Fipronil was measured in sediment samples seven days after dosing occurred, but was equal to or below detectable limits (≤ 30 ng/kg) by day 28. All three major metabolites, MB 46513, MB 45950, and MB 46136, were at measurable concentrations in the upper centimeter of sediment from day 7 through test termination (day 84). Five one-centimeter sediment core samples collected on day 85 confirmed that fipronil was below quantifiable concentrations and that all three metabolites were present in these samples. The mean total fipronil residues, the sum of fipronil and its three metabolites, on days 7, 28, 56 and 84, in the upper 1 cm of sediment, were 1960, 1500, 1110, and 1290 ng/kg, respectively, declining slightly over the study period.

The water quality data collected during this study confirmed that conditions within the simulated ponds were representative of natural ponds in the North Carolina area. The aquatic vegetation stocked in the ponds survived and matured over the exposure period and provided habitat and food for developing populations of aquatic organisms. Phytoplankton pigment counts confirmed continuous primary production in all ponds, critical to maintaining the energy cycle within these systems. In conclusion, the water quality and biological observations mentioned above, confirmed that the conditions within the simulated ponds were acceptable for the survival and growth of the organisms of interest in this study.

The following conclusions were drawn from these results by the study authors:

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- The initial mean measured water concentration of total fipronil residues, 397 ng/L, confirmed that the desired nominal concentration of 400 ng/L was achieved in the treated ponds.
- Fipronil was sensitive to degradation and/or dissipation in pond water and sediment, and mean measured concentrations dropped to below detectable limits in both media within 28 days.
- MB 46513 was the primary fipronil metabolite in water and was present in sediment at each sampling interval from day 7 until test termination (day 84).
- Metabolite MB 45950 was below detectable limits in pond water samples during the exposure, but was present in the sediment throughout the exposure phase.
- Metabolite MB 46136 was present in pond water at low concentrations for the initial 14 days of the exposure and present in sediment throughout the exposure.
- Measured water quality parameters indicated that the pond water was representative of local, natural ponds.
- The diversity and growth of aquatic plants in the ponds were sufficient to provide habitat for growth and reproduction of macroinvertebrates.
- The zooplankton community was diverse containing seventy-nine taxa. Of the 81 statistical comparisons within this group of organisms, only 7% represented adverse effects and recovery of the affected species was evident within three weeks of application.
- Macroinvertebrates collected from artificial substrates (Hester-Dendy samplers) represented 17 major taxonomic groups. Of 66 statistical comparisons made, seven positive effects (11%) and eight negative effects (12%) were observed. Ephemeroptera (mayflies) was the only group that indicated a reduction in numbers in the treated ponds for more than one consecutive interval. Recovery was evident in all other affected groups within one week (days 8 to 14).
- Macroinvertebrates collected from benthic samplers represented 17 major taxonomy groups. Of 54 statistical comparisons, three positive effects (6%) and eight negative effects (15%) were observed. Ephemeroptera was again the only group that indicated a significant reduction in the treated ponds at all sampling intervals. Chironomidae (midge), a family within Diptera, was reduced during the initial two intervals (days 8 and 28) and not on day 56, indicating recovery over time.
- Emergent insects trapped on each pond represented three Orders, Diptera, Ephemeroptera, and Odonata. Although significant differences were detected for two of these three groups within macroinvertebrate samples mentioned above, no significant effects were noted for insects emerging from the ponds. This may be due to the small sample size for these analyses.
- Life-cycle length appeared to be an important factor in the ability of affected organisms to recover from treatment effects. In addition, it is not clear whether the mid-summer (last day of June) start of exposure contributed to a lack of observed recovery for some species, especially the species with longer life cycles.

14. Reviewer's Discussion:

Nominal fipronil concentration was 400 ug/L. At Day 0, measured concentrations were similar to nominal; however, fipronil rapidly dissipated. Water concentrations were 387, 88, 19 ng/L at Days 0, 7, and 14 and were lower than the detection limit of 4 ng/L at Day 28 and thereafter. The time-weighted average concentration of fipronil in treated mesocosms from initiation of biological exposure out to 84 days (holding non-detects at half detection limit) was 0.042 ug/L.

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Similarly, fipronil levels in the sediment were below the level of detection (30 ng/kg) at all time periods except Day 7. Therefore, at time points when recovery reportedly occurred, fipronil levels were markedly lower than nominal values.

Mean arthropod abundance was lower in the treatment than control at day 7 (52% of control), and Day 14 (61% of control) This trend reversed for days 21, 28, 42, and 56, where treatments showed increases in abundance relative to controls ranging from 0.5% to 183 %. None of the overall arthropod effects, though marked were statistically significant (P>0.05). Within the arthropods, certain copepod species (Mesocyclops edax and Tropocyclops prasinus) showed statistically significant ($p \leq 0.05$) reductions in abundance in treatment relative to control. Treatment abundances ranged from 65 to 365% of control values. By day 21 (the last day of analysis at species resolution) these reductions were not statistically significant, but were still markedly reduced 14% to 60% of controls. The average fipronil concentration in the water at Day 21 was 0.019 ug/L.

The study authors concluded that reduction in macroinvertebrate abundance collected from artificial substrates (Hester-Dendy samplers) occurred in some species, and that Ephemeroptera (mayflies) was the only group that indicated a reduction in numbers in the treated ponds for more than one consecutive interval. Recovery was evident in other affected groups between Days 8 and 14. The reviewers note, however, the Diptera abundance remained at approximately ½ the control levels until the 56-Day sampling, when fipronil levels were below the detection limit. Mayfly abundance was statistically reduced until the end of the study (Day 56).

Hester-Dendy sampling results for clitellora (worms and leeches) showed statistically significant increase in treatments relative to control for sampling days 8, 14, and 56 (increases greater than 200%). Gastropods (snails) showed similar increases in abundance, though not statistically significant, in treatments relative to control.

Evaluation of total macroinvertebrate insect abundance showed mixed results as the study progressed. Reductions in total abundance were statistically significant ($P<0.5$) for fipronil treatment at day 8, with reductions still evident at day 28 and 56, though not statistically significant. Benthic sampling for mayfly juveniles showed significant reductions in the presence of fipronil ($p<0.05$) at days 8, 14, 28, and 56 of the study. Juvenile chironomids were also reduced significantly ($p<0.05$) by fipronil at day 8, though these effects were largely reversed by day 56 of the study. Numbers of emergent insects were too small in the study to make definitive statistically supported statements about individual taxonomic groups. However, total emergent insects were lower in the fipronil treatment than in control for all sampling days of the study.

Adequacy of Study: Supplemental
Rationale: The study does not satisfy any current guideline requirement.
Repairable: N/A

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Attachment 1. Abundance Data

Table 15. Zooplankton abundance (individuals/L) in simulated ponds dosed with Chipco® TopChoice™.

Day 7

Taxon		Mean	
Phylum	**Class**	**Control**	**400 ng/L**
Annelia		0.0	0.0
Arthropoda		124.5	64.7
	Insecta	0.0	0.2
	Ostracoda	7.3	16.0
	Cladocera	24.4	14.6
	Copepoda	92.8	33.7
Nematoda		0.0	0.1
Protozoa		2.9	3.8
Rotifera		0.4	2.0
Total Zooplankton		**127.8**	**70.6**

Day 14

Taxon		Mean	
Phylum	**Class**	**Control**	**400 ng/L**
Annelia		0.0	0.0
Arthropoda		67.1	40.7
	Insecta	0.1	0.2
	Ostracoda	7.1	5.6
	Cladocera	0.8	0.5
	Copepoda	58.9	34.4
Nematoda		0.0	0.1
Protozoa		1.9	2.5
Rotifera		0.6	0.5
Total Zooplankton		**69.7**	**43.8**

[a] Value represents a significant reduction relative to the control based on ANOVA.
[b] Value represents a significant increase relative to the control based on ANOVA.
Arthropods = sum of insects, ostracods, cladocera and copepods.
Zooplankton = sum of all organisms.
Note: Enumeration and identification at more specific levels are presented in Appendix 5.

Day 21

Taxon		Mean	
Phylum	**Class**	**Control**	**400 ng/L**
Annelia		0.0	0.1
Arthropoda		30.9	51.6
	Insecta	0.1	0.4
	Ostracoda	6.6	6.5
	Cladocera	1.7	1.3
	Copepoda	22.7	43.3
Nematoda		0.1	0.2
Protozoa		2.5	3.1
Rotifera		0.5	2.5
Total Zooplankton		**34.1**	**57.6**

Day 28

Taxon		Mean	
Phylum	**Class**	**Control**	**400 ng/L**
Annelia		0.0	0.0
Arthropoda		36.9	37.1
	Insecta	0.1	0.1
	Ostracoda	2.2	2.9
	Cladocera	1.4	0.2
	Copepoda	33.2	33.9
Nematoda		0.1	0.2
Protozoa		4.9	2.1
Rotifera		2.6	1.6
Total Zooplankton		**44.6**	**40.9**

[a] Value represents a significant reduction relative to the control based on ANOVA.
[b] Value represents a significant increase relative to the control based on ANOVA.
Arthropods = sum of insects, ostracods, cladocera and copepods.
Zooplankton = sum of all organisms.
Note: Enumeration and identification at more specific levels are presented in Appendix 5.

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Day 42

Taxon		Mean	
Phylum	**Class**	**Control**	**400 ng/L**
Annelia		0.7	0.4
Arthropoda		67.4	115.0
	Insecta	0.5	0.6
	Ostracoda	5.5	9.8
	Cladocera	16.1	12.3
	Copepoda	45.1	92.2
Nematoda		5.3	0.2[a]
Protozoa		19.1	33.3
Rotifera		4.2	17.9
Total Zooplankton		**96.6**	**166.9**

Day 56

Taxon		Mean	
Phylum	**Class**	**Control**	**400 ng/L**
Annelia		0.0	0.3
Arthropoda		77.6	142.5
	Insecta	0.6	0.4
	Ostracoda	7.9	15.9
	Cladocera	19.4	35.5
	Copepoda	49.6	90.7
Nematoda		3.6	3.9
Protozoa		14.6	51.2
Rotifera		2.9	8.1
Total Zooplankton		**98.7**	**206.0**[b]

[a] Value represents a significant reduction relative to the control based on ANOVA.
[b] Value represents a significant increase relative to the control based on ANOVA.
Arthropods = sum of insects, ostracods, cladocera and copepods.
Zooplankton = sum of all organisms.
Note: Enumeration and identification at more specific levels are presented in Appendix 5.

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Table 16. Statistical analysis of individual copepod species for days 7, 14 and 21.

Day 7

	Mean density	
Species	Control	400 ng/L
Cyclopoida sp. nauplius	20.7	8.0
Diaptomus pallidus adult	6.0	6.3
Diaptomus pallidus copepodite	2.7	6.9
Diaptomus sp. nauplius	4.0	3.4
Mesocyclops edax adult	9.8	3.5[a]
Mesocyclops edax copepodite	25.1	3.9[a]
Tropocyclops prasinus adult	21.7	1.6[a]
Tropocyclops prasinus copepodite	2.9	0.2

Day 14

	Mean density	
Species	Control	400 ng/L
Cyclopoida sp. nauplius	2.7	1.1
Diaptomus pallidus adult	6.8	9.1
Diaptomus pallidus copepodite	1.6	9.7
Diaptomus sp. nauplius	3.9	4.1
Mesocyclops edax adult	11.2	0.7[a]
Mesocyclops edax copepodite	15.9	8.1
Tropocyclops prasinus adult	10.7	1.0[a]
Tropocyclops prasinus copepodite	6.2	0.6

Day 21

	Mean density	
Species	Control	400 ng/L
Cyclopoida sp. nauplius	2.6	2.5
Diaptomus pallidus adult	6.2	15.4
Diaptomus pallidus copepodite	1.0	17.5[b]
Diaptomus sp. nauplius	1.8	4.8
Mesocyclops edax adult	5.6	1.2
Mesocyclops edax copepodite	2.0	1.2
Tropocyclops prasinus adult	2.1	0.3
Tropocyclops prasinus copepodite	1.6	0.4

[a] Value indicates negative effect when compared to the control data (natural log transformed data), based on ANOVA.

[b] Positive effect when compared to the control data (natural log transformed data), based on ANOVA.

NOTE: No further days were analyzed since no negative effects were observed after day 21 in the copepod populations.

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Table 17. Macroinvertebrate abundance (Hester-Dendy samples) in the simulated pond study with Chipco® TopChoice™.

Day 8

Taxon			Mean	
Class	Order	Family	Control	400 ng/L
Clitellora (worms and leeches)			30	75[a]
		Naididae	30	75[a]
		Tubificidae	0	0
		Lumbriculidae	0	0
Gastropoda (snails)			23	46
Insecta (insects)			75	61
	Coleoptera (beetles)		0	0
	Diptera (gnats, flies, midge)		21	2[b]
		Ceratopogonidae	0	0
		Chaoboridae	0	0
		Chironomidae	21	2[b]
		Culicidae	0	0
	Ephemeroptera (mayflies)		5	1[b]
	Hemiptera (true bugs)		0	0
	Heteroptera		0	0
	Odonata (dragonflies, damselflies)		50	58
		Aeshnidae	0	0
		Coenagrionidae	3	1[b]
		Libellulidae	46	58
	Trichoptera (caddisflies)		0	0
Turbellaria (flatworms)			11	24
Total			**139**	**205**

[a] Significant increase relative to the control based on ANOVA.
[b] Significant reduction relative to the control based on ANOVA.

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Day 14

Taxon			Mean	
Class	Order	Family	Control	400 ng/L
Clitellora (worms and leeches)			23	90[a]
		Naididae	23	88[a]
		Tubificidae	0	0
		Lumbriculidae	0	0
Gastropoda (snails)			17	37
Insecta (insects)			97	100
	Coleoptera (beetles)		0	0
	Diptera (gnats, flies, midge)		21	9
		Ceratopogonidae	1	2
		Chaoboridae	0	0
		Chironomidae	20	7
		Culicidae	0	1
	Ephemeroptera (mayflies)		7	0[b]
	Hemiptera (true bugs)		0	0
	Heteroptera		1	0
	Odonata (dragonflies, damselflies)		68	90
		Aeshnidae	0	0
		Coenagrionidae	3	2
		Libellulidae	65	88
	Trichoptera (caddisflies)		0	0
Turbellaria (flatworms)			17	32
Total			155	259

[a] Significant increase relative to the control based on ANOVA.
[b] Significant reduction relative to the control based on ANOVA.

Table 17. **Continued. Macroinvertebrate abundance (Hester-Dendy samples) in the simulated pond study with Chipco® TopChoice™.**

Day 28

Taxon			Mean	
Class	Order	Family	Control	400 ng/L
Clitellora (worms and leeches)			23	51
		Naididae	23	50
		Tubificidae	0	0
		Lumbriculidae	0	0
Gastropoda (snails)			24	37
Insecta (insects)			122	85
	Coleoptera (beetles)		0	0
	Diptera (gnats, flies, midge)		15	8
		Ceratopogonidae	0	0
		Chaoboridae	0	0
		Chironomidae	14	5
		Culicidae	1	2
	Ephemeroptera (mayflies)		15	1[b]
	Hemiptera (true bugs)		0	0
	Heteroptera		3	0
	Odonata (dragonflies, damselflies)		88	76
		Aeshnidae	3	6
		Coenagrionidae	1	3
		Libellulidae	84	67
	Trichoptera (caddisflies)		0	0
Turbellaria (flatworms)			7	21
Total			**176**	**193**

a Significant increase relative to the control based on ANOVA.
b Significant reduction relative to the control based on ANOVA.

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Table 17. Continued. Macroinvertebrate abundance (Hester-Dendy samples) in the simulated pond study with Chipco® TopChoice™.

Day 56

Taxon			Mean	
Class	Order	Family	Control	400 ng/L
Clitellora (worms and leeches)			17	89[a]
		Naididae	16	86[a]
		Tubificidae	1	0
		Lumbriculidae	0	0
Gastropoda (snails)			20	26
Insecta (insects)			77	91
	Coleoptera (beetles)		0	0
	Diptera (gnats, flies, midge)		12	26
		Ceratopogonidae	0	1
		Chaoboridae	0	0
		Chironomidae	11	24
		Culicidae	1	0
	Ephemeroptera (mayflies)		9	1[b]
	Hemiptera (true bugs)		0	0
	Heteroptera		1	1
	Odonata (dragonflies, damselflies)		53	63
		Aeshnidae	2	0
		Coenagrionidae	5	4
		Libellulidae	47	59
	Trichoptera (caddisflies)		2	0[b]
Turbellaria (flatworms)			1	20[a]
Total			**116**	**226**

[a] Significant increase relative to the control based on ANOVA.
[b] Significant reduction relative to the control based on ANOVA.

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Table 18. Macroinvertebrate abundance (benthic samples) in the simulated pond study with Chipco® TopChoice™.

Day 8

Taxon			Mean	
Class	Order	Family	Control	400 ng/L
Clitellora (worms and leeches)			246	266
		Naididae	212	260
		Tubificidae	9	5
		Lumbriculidae	24	1
Gastropoda (snails)			9	19
Insecta (insects)			204	73[b]
	Coleoptera (beetles)		7	4
	Diptera (gnats, flies, midge)		104	20[b]
		Ceratopogonidae	3	1
		Chaoboridae	0	0
		Chironomidae	101	19[b]
		Culicidae	0	0
	Ephemeroptera (mayflies)		42	6[b]
	Hemiptera (true bugs)		0	0
	Heteroptera		3	3
	Odonata (dragonflies, damselflies)		47	41
		Aeshnidae	0	0
		Coenagrionidae	1	0
		Libellulidae	45	41
	Trichoptera (caddisflies)		0	0
Turbellaria (flatworms)			133	79
Total			**592**	**437**

[a] Significant increase relative to the control based on ANOVA.
[b] Significant reduction relative to the control based on ANOVA.

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Table 18. Continued. Macroinvertebrate abundance (benthic samples) in the simulated pond study with Chipco® TopChoice™.

Day 28

Taxon			Mean	
Class	Order	Family	Control	400 ng/L
Clitellora (worms and leeches)			81	408[a]
		Naididae	76	376[a]
		Tubificidae	1	32
		Lumbriculidae	4	0
Gastropoda (snails)			27	47
Insecta (insects)			370	294
	Coleoptera (beetles)		0	0
	Diptera (gnats, flies, midge)		59	27[b]
		Ceratopogonidae	2	1
		Chaoboridae	0	0
		Chironomidae	55	23[b]
		Culicidae	2	2
	Ephemeroptera (mayflies)		122	8[b]
	Hemiptera (true bugs)		0	0
	Heteroptera		4	0
	Odonata (dragonflies, damselflies)		186	259
		Aeshnidae	0	2
		Coenagrionidae	3	0
		Libellulidae	182	253
	Trichoptera (caddisflies)		0	0
Turbellaria (flatworms)			24	55
Total			**502**	**804[a]**

[a] Significant increase relative to the control based on ANOVA.
[b] Significant reduction relative to the control based on ANOVA.

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Table 18. **Continued. Macroinvertebrate abundance (benthic samples) in the simulated pond study with Chipco® TopChoice™.**

Day 56

Taxon			Mean	
Class	Order	Family	Control	400 ng/L
Clitellora (worms and leeches)			137	287
		Naididae	62	201
		Tubificidae	41	83
		Lumbriculidae	34	2
Gastropoda (snails)			77	52
Insecta (insects)			197	121
	Coleoptera (beetles)		0	2
	Diptera (gnats, flies, midge)		123	49
		Ceratopogonidae	2	0
		Chaoboridae	0	0
		Chironomidae	121	47
		Culicidae	0	2
	Ephemeroptera (mayflies)		8	0[b]
	Hemiptera (true bugs)		0	0
	Heteroptera		4	0
	Odonata (dragonflies, damselflies)		57	70
		Aeshnidae	0	0
		Coenagrionidae	1	1
		Libellulidae	55	69
	Trichoptera (caddisflies)		6	0
Turbellaria (flatworms)			8	9
Total			419	469

[a] Significant increase relative to the control based on ANOVA.
[b] Significant reduction relative to the control based on ANOVA.

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Table 19. Emergent insect abundance in the simulated pond study with Chipco® TopChoice™.

Day 8

Taxon			**Mean**	
Class	Order	Family	Control	400 ng/L
Insecta (insects)			2	1
	Coleoptera (beetles)		0	0
	Diptera (gnats, flies, midge)		2	1
		Ceratopogonidae	0	0
		Chaoboridae	0	0
		Chironomidae	2	1
		Culicidae	0	0
	Ephemeroptera (mayflies)		0	0
	Hemiptera (true bugs)		0	0
	Heteroptera		0	0
	Odonata (dragonflies, damselflies)		0	0
		Aeshnidae	0	0
		Coenagrionidae	0	0
		Libellulidae	0	0
	Trichoptera (caddisflies)		0	0
Total			2	1

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Table 19. **Continued. Emergent insect abundance in the simulated pond study with Chipco® TopChoice™.**

Day 14

Taxon			Mean	
Class	Order	Family	Control	400 ng/L
Insecta (insects)			7	0
	Coleoptera (beetles)		0	0
	Diptera (gnats, flies, midge)		7	0
		Ceratopogonidae	0	0
		Chaoboridae	0	0
		Chironomidae	7	0
		Culicidae	0	0
	Ephemeroptera (mayflies)		0	0
	Hemiptera (true bugs)		0	0
	Heteroptera		0	0
	Odonata (dragonflies, damselflies)		0	0
		Aeshnidae	0	0
		Coenagrionidae	0	0
		Libellulidae	0	0
	Trichoptera (caddisflies)		0	0
Total			**7**	**0**

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Table 19. Continued. Emergent insect abundance in the simulated pond study with Chipco® TopChoice™.

Day 28

Taxon			Mean	
Class	Order	Family	Control	400 ng/L
Insecta (insects)			4	3
	Coleoptera (beetles)		0	0
	Diptera (gnats, flies, midge)		3	3
		Ceratopogonidae	1	2
		Chaoboridae	0	0
		Chironomidae	2	1
		Culicidae	0	0
	Ephemeroptera (mayflies)		1	0
	Hemiptera (true bugs)		0	0
	Heteroptera		0	0
	Odonata (dragonflies, damselflies)		0	0
		Aeshnidae	0	0
		Coenagrionidae	0	0
		Libellulidae	0	0
	Trichoptera (caddisflies)		0	0
Total			**4**	**3**

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Table 19. **Continued. Emergent insect abundance in the simulated pond study with Chipco® TopChoice™.**

Day 56

Taxon			Mean	
Class	Order	Family	Control	400 ng/L
Insecta (insects)			28	6
	Coleoptera (beetles)		0	0
	Diptera (gnats, flies, midge)		27	6
		Ceratopogonidae	0	0
		Chaoboridae	1	0
		Chironomidae	26	6
		Culicidae	0	0
	Ephemeroptera (mayflies)		1	0
	Hemiptera (true bugs)		0	0
	Heteroptera		0	0
	Odonata (dragonflies, damselflies)		0	0
		Aeshnidae	0	0
		Coenagrionidae	0	0
		Libellulidae	0	0
	Trichoptera (caddisflies)		0	0
Total			**28**	**6**

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Table 20. Number of Odonate exuvia counted over 48 hours within each simulated pond to represent emergence.

Target Concentration (ng/L)	Replicate	Day		
		37-38	57-58	85-86
Control	1	1	14	0
	2	1	9	0
	3	3	8	0
	4	8	4	0
	Total	**13**	**35**	**0**
400	1	9	2	0
	2	2	5	0
	3	3	4	0
	4	8	4	0
	Total	**22**	**15**	**0**

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