UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

March 22, 2006

PC Code: 129121
DP Barcode:D306156

MEMORANDUM

SUBJECT: Review of Aquatic Invertebrate Toxicity Studies for Fipronil

FROM: Nader Elkassabany, Ph.D.,, Acting Branch Chief
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Attached are the Environmental Fate and Effects Division Data Evaluation Records for the following studies of fipronil effects on aquatic invertebrates:

MRID46329905 - Effect of BAS 350 I (Fipronil) on the Caddisfly *Hydropsyche instabilis* in a 48 Hours Static, Acute Toxicity Test

MRID46329904 - Fipronil - Acute Toxicity to Clams (*Corbicula fluminea*) Under Static Renewal Conditions

MRID46329903 - Fipronil - Acute Toxicity to Oligochaetes (*Lumbriculus variegatus*) Under Static Renewal Conditions

MRID46329902 - Fipronil - Acute Toxicity to Mayfly Nymphs (*Hexagenia* sp.) Under Static Renewal Conditions

Of the four studies reviewed: MRID46329905 is classified as acceptable or core, MRID46329904 and MRID46329903 are classified as supplemental as they are scientifically valid but are not recommended test species, and MRID46329902 is classified as invalid owing to a control mortality (20%) that exceeds acceptable limits (10%).

Recall that testing recommendations provided to the Registration Division in the risk assessment for fipronil granular formulations for fire ant control indicated that testing of two of three aquatic insect orders (mayflies, caddisflies, and stoneflies) would provide high value information on the extent of aquatic invertebrate sensitivity to fipronil. Of the above studies, only MRID46329905 provides technically valid data to address this recommendation.

Data Requirement:

PMRA DATA CODE	
EPA DP Barcode	D306156
OECD Data Point	
EPA MRID	46329905
EPA Guideline	nonguideline

Test material: BAS 350 I **Purity:** 99.7%
Common name: Fipronil
Chemical name: IUPAC: 5-amino-1-[2,6-dichloro-4-(troflouromethyl)phenyl]-4-[(triflouromethyl)sulfinyl}-1H-pyrazole-3-carbonitrile
CAS name: Not reported
CAS No.: 120068-37-03
Synonyms: Not reported

Primary Reviewer: Rebecca Bryan
Staff Scientist, Dynamac Corporation
Signature: Rebecca Bryan
Date: 11/01/04

QC Reviewer: John Marton
Staff Scientist, Dynamac Corporation
Signature:
Date: 11/15/04

Primary Reviewer: Ed Odenkirchen, Chemist
OPP/EFED/ERB I
Date:

Secondary Reviewer(s):
{EPA/OECD/PMRA}
Date:

Reference/Submission No.:

Company Code:
Active Code:
EPA PC Code: 129121

Date Evaluation Completed:

CITATION: Funk, M. 2003. Effect of BAS 350 I (Fipronil) to the Caddisfly *Hydropsyche instabilis* in a 48 Hours Static, Acute Toxicity Test. Unpublished study performed and submitted by BASF Aktiengesellschaft, Agricultural Center, Crop Protection Division, Ecology and Environmental Analytics, Limburgerhof, Germany. Laboratory Project Identification No. 174580. Study initiated July 25, 2003 and completed November 14, 2003.

EXECUTIVE SUMMARY:

The 48-hour acute toxicity of Fipronil to the caddisfly, *Hydropsyche instabilis*, was studied under static conditions. Caddisflies were exposed to the test material at nominal concentrations of 0 (negative and solvent controls), 0.2, 0.4, 0.8, 1.6, and 3.2 ppb a.i. Mean-measured concentrations were 0 (controls), 0.15, 0.36, 0.74, 1.47, and 2.98 ppb a.i., respectively.

After 48 hours, the percent mortality was 10, 20, 40, 50, and 80% in the 0.15, 0.36, 0.74, 1.47, and 2.98 ppb a.i. treatment groups, respectively. The negative control had 5% mortality and the solvent control had 20% mortality. During the study, the sublethal effect of partial paralysis was observed in the ≥0.74 ppb a.i. treatment groups.

Since the solvent control mortality was >10%, this study is scientifically unsound and does not fulfill guideline requirements for an acute toxicity study with freshwater invertebrates (§72-2) and is classified INVALID.

Results Synopsis

Test Organism Age (eg. 1st instar): Fifth larval stage; total mean body length (standard deviation) was 12.20 (2.80) mm (from a sample of 119 individuals); mean fresh weight (standard deviation) was 32.43 (16.22) mg (from a sample of 123 individuals)
Test Type (Flow-through, Static, Static Renewal): Static

48-hour; INVALID STUDY (results not reported):
LC/EC_{50}: 95% C.I.: Slope:
NOEC:
LOEC:

I. MATERIALS AND METHODS

GUIDELINES FOLLOWED: The study was based on procedures outlined in the OECD guideline for testing chemicals No. 202: "Daphnia, Acute Immobilization Test"; EC Directive 79/831, Annex V, Part C2 "Acute Toxicity for Daphnia" (1990); U.S. EPA Guideline 72-2; and U.S. EPA OPPTS 850.1010 (1996). Deviations from §72-2 included:

1. Solvent control mortality was 20% in this study, which exceeds the acceptable limit of 10%.
2. The pH range (7.89-8.06) was slightly greater than recommended (7.2-7.6).
3. The temperature range (16.5-20.1°C) fell below the recommended temperature of 20 °C.
4. The TOC, presence of metals, pesticides, particulate matter, and chlorine are not reported.
5. The biomass loading rate was not reported.
6. This test species is not recommended for aquatic invertebrate studies.
7. The age and pretest health of the test organism was not reported.
8. The acclimation period of 72 hours was less than recommended (7 days).
9. The dilution water concentrations of metals, pesticides, particulate matter, and chlorine were not reported.

10. The nominal 0.2 ppb recoveries (59.3-66.3%) were <70% of nominal at test initiation.

The excessive solvent control mortality affects the scientific validity of this study.

COMPLIANCE: Signed and dated GLP, No Data Confidentiality, and Quality Assurance statements were provided. The study followed the OECD Principles of Good Laboratory Practice and the German "Chemicals Act" (p. 3).

A. MATERIALS:

1. Test Material Fipronil

Description: Fine white powder

Lot No./Batch No. : BES1905

Purity: 99.7%

Stability of Compound Under Test Conditions: The stability of the test substance in the dilution water during the course of the study was demonstrated by analytical determination at 0 and 48 hours. Recoveries were 92.0-97.7% of nominal concentrations at 0 hours (except for the lowest concentration which had an average recovery of 62.8%) and 86.3-90.1% of nominal concentrations at 48 hours.

Storage conditions of test chemicals: The test chemical was stored under refrigeration

OECD requires water solubility, stability in water and light, pK_a, P_{ow}, and vapor pressure of the test compound. The following OECD requirements was reported:

Water solubility: 2.4 ppm at pH 5; 1.9 ppm at pH 7; and 2.2 ppm at pH 9.

Log P_{ow}: 4.0

Vapor pressure: 2.0×10^{-6} pa (25°C)

2. Test organism:

Species: Caddisfly larvae, *Hydropsyche instabilis*

Age at test initiation: 5th larval stage at test termination (based on head capsule width measurements).

Source: Collected from stream "Triefenbach" near Edenkoben (Weinstrasse, South-West Germany).

B. STUDY DESIGN:

1. Experimental Conditions

a. Range-finding Study: A range-finding study was not reported.

b. Definitive Study

Table 1: Experimental Parameters

Parameter	Details	Remarks *Criteria*
Acclimation period: Conditions: (same as test or not) Feeding: Health: (any mortality observed)	72 hours Same as test; except caddisfly larvae were acclimated under aerated conditions. Cultures were fed detritus. Not reported.	The acclimation period of 72 hours was less than recommended (7 days). *EPA requires 7 day minimum acclimation period.*
Duration of the test	48 hours	*EPA requires 48 hours*
Test condition - static/flow through Type of dilution system (for flow through method) Renewal rate (for static renewal)	Static N/A N/A	*EPA requires consistent flow rate of 5 - 10 volumes/24 hours, meter systems calibrated before study and checked twice daily during test period*
Aeration, if any	No aeration was used during the study.	
Test vessel Material: *(glass/stainless steel)* Size: Fill volume:	Glass 800 mL 500 mL	*EPA requires: size 250 ml or 3.9 L fill 200 ml*

Parameter	Details	Remarks *Criteria*
Source of dilution water	The dilution water, "M4 synthetic medium", was prepared using ultrapure deionized water (Table 1, p. 13).	*EPA requires soft reconstituted water or water from a natural source, not dechlorinated tap water.*
Water parameters: Hardness pH Dissolved oxygen Temperature Total Organic Carbon Particulate matter Metals Pesticides Chlorine	 2.70 mmol/L 7.89-8.06 8.55-9.5 mg/L (>60% saturation) 16.5-20.1°C Not reported Not reported Not reported Not reported Not reported	The hardness and the pH range was greater than recommended. The temperature range went below the required temperature. *EPA requires:* *hardness: 40 - 48 mg/L as $CaCO_3$* *pH: 7.2 - 7.6* *-Temperature: 20°C (measured continuously or if water baths are used, every 6 hr, may not vary > 1°C* *Dissolved oxygen:* *Static: ≥ 60% during 1st 48 hr and ≥ 40% during 2nd 48 hr* *Flow-through: ≥60%*

Parameter	Details	Remarks *Criteria*
Number of organisms per replicate Solvent control: Negative control: Treatments:	 5 5 5	The loading rate was not reported. *EPA requires 5 treatment levels plus control with a minimum of 20 daphnid per treatment. Biomass loading rate for static ≤ 0.8 g/L at ≤ 17°C, ≤ 0.5 g/L at > 17°C; flow-through: ≤ 1 g/L/day.*
Number of replicates Solvent control: Negative control: Treatments:	 4 4 4	
Treatment concentrations nominal: measured:	 0 (negative and solvent controls), 0.2, 0.4, 0.8, 1.6, and 3.2 ppb 0 (controls), 0.15, 0.36, 0.74, 1.47, and 2.98 ppb	Mean-measured concentrations were determined from 0 and 48 hour data; results are provided in Table 3, p. 17 and Tables 8-9, p. 24. *EPA requires a geometric series with each concentration being at least 60% of the next higher one.*
Solvent (type, percentage, if used)	Acetone, 0.1 mL/L	*EPA requires solvents not to exceed 0.5 mL/L for static tests or 0.1 mL/L for flow-though tests.*
Lighting	16 hours light/8 hours dark	Light intensity was <322 to 573 lux. *EPA requires 16 hours light, 8 hours dark.*
Feeding	Animals were not fed during testing.	*EPA/OECD requires: No feeding during the study*
Stability of chemical in the test system	Recoveries (all test levels) were 59.3-97.7% of nominal concentrations at 0 hours, and 86.3-90.1% of nominal concentrations at 48 hours.	Tables 8-9, p. 24. The nominal 0.2 ppb recoveries (59.3-66.3%) were <70% at test initiation.

Parameter	Details	Remarks *Criteria*
Recovery of chemical Level of Quantitation Level of Detection	98.5-103.1% of nominal Not reported Not reported	Based on matrix spikes (at 0.195 ppb a.i.) analyzed concurrently with the samples (Table 10, p. 23).
Positive control {if used, indicate the chemical and concentrations}	N/A	
Other parameters, if any	None	

2. Observations:

Table 2: Observations

Criteria	Details	Remarks *Criteria*
Parameters measured including the sublethal effects	Mortality (immobility) and sublethal effects	
Observation intervals	After 24 and 48 hours	
Were raw data included?	No	
Other observations, if any	DO and pH were determined in one replicate of each treatment group at 0 and 48 hours. Temperature for each treatment group was determined at test initiation.	

II. RESULTS AND DISCUSSION

A. MORTALITY

After 48 hours, the percent mortality was 10, 20, 40, 50, and 80% in the 0.15, 0.36, 0.74, 1.47, and 2.98 ppb treatment groups, respectively (Table 3, p. 17). The negative control had 5% mortality and the solvent control had 20% mortality. The 96-hour LC_{50} (with 95% C.I.) was 1.54 ppb (1.12-2.12 ppb). The NOEC for mortality was 0.36 ppb.

Table 3: Effects of Fipronil on mortality of *Hydropsyche instabilis*

Treatment, ppb Mean-Measured and (Nominal) Concn.	Observation Period			
	24 Hours		48 Hours	
	Endpoint	% Affected	Endpoint	% Affected
Dilution water control	Mortality	5	Mortality	5
Solvent control	Mortality	5	Mortality	20
0.15 (0.2)	Mortality	0	Mortality	10
0.36 (0.4)	Mortality	10	Mortality	20
0.74 (0.8)	Mortality	20	Mortality	40
1.47 (1.6)	Mortality	20	Mortality	50
2.98 (3.2)	Mortality	30	Mortality	80
NOEC, ppb	Not reported		0.36	
LOEC, ppb	Not reported		0.74	
LC_{50} (with 95% C.I.), ppb	Not reported		1.54 (1.12-2.12)	

B. SUBLETHAL TOXICITY ENDPOINTS:

During the study, the sublethal effect of partial paralysis was observed in the ≥0.74 ppb treatment groups. The NOEC for sublethal effects was 0.36 ppb.

C. REPORTED STATISTICS:

The NOEC was determined using ANOVA followed by Dunnett's-, Williams- or Bonferroni-test. The LC_{50} was calculated by Abbott's correction following probit analysis. The statistical analyses were performed using the computer software, TOXSTAT 3.5. The results were based on mean-measured concentrations.

48-hour
LC/EC_{50}: 1.54 ppb 95% C.I.: 1.12-2.12 ppb Slope: N/A
NOEC: 0.36 ppb
LOEC: 0.74 ppb

D. VERIFICATION OF STATISTICAL RESULTS:

The LC_{50} and NOEC values were estimated using the mean-measured concentrations. The LC_{50} was determined using the moving average method via TOXANAL statistical software and the NOEC based on mortality was determined using Fisher's Exact Test via Toxstat statistical software.

48-hour
LC/EC_{50}: 1.45 ppb 95% C.I.: 1.01-2.45 ppb Slope: N/A
NOEC: 0.74 ppb
LOEC: 1.47 ppb

E. STUDY DEFICIENCIES:

This study is scientifically sound, but does not satisfy the guideline requirements for an acute toxicity study with freshwater invertebrates (§72-2) because the control mortality exceeded 10%.

F. REVIEWER'S COMMENTS:

The reviewer's conclusions differed from those of the study author; the reviewer's LC/EC_{50} estimate was higher than the study author's and was associated with a broader 95% confidence interval. Furthermore, the reviewer's NOEC was one concentration higher than the study authors'. The study author's results are not reported in the Executive Summary and Conclusions sections because this study is classified as INVALID due to excessively high solvent control mortality.

To determine the larval stages of the animals, the headcapsule width, total body length, and fresh weight measurements were taken at the end of the study. Mortality was defined as the absence of movement of the test organism after repeated stimulation with a preparation needle.

The mortality of the solvent control was 20%, which is well-above the allowable control mortality level of 10%, and the study author provided no information as to the possible reason for the high control mortality.

The recovery of the test material in the dilution water at 0 hours was 59.3 and 66.3 ppb a.i. for the nominal 0.2 ppb a.i. treatment level which is below the recommended recovery level (>70%). The study author did not provide an explanation for the low recovery, however at 48 hours the nominal 0.2 ppb a.i. treatment level had a recovery of 89.0 ppb a.i.

G. CONCLUSIONS:

This study is not scientifically sound and does not satisfy the guideline requirements for an acute toxicity study with freshwater invertebrates (§72-2) because the control mortality exceeded 10%. This study is classified as INVALID.

48-hour; INVALID STUDY (results not reported):
LC/EC_{50}: 95% C.I.: Slope:
NOEC:
LOEC:

III. REFERENCES:

Pitsch, T., 1993. Zur Larvaltaxonomie, Faunistik und Okologie mitteleurpaischer FlieBwasser-Kochefliegen. Gerhard Weinert GmbH, 120099 Berlin.

Waringer, J., Graf, W. 1997. Atlas der osterreichischen Kocherfliegenlarven unter Einschluss der angrenzenden Geibiet. Facultas Universitatsverlag, Berggasse 5, A- 1090 Wien.

APPENDIX I. VERIFICATION OF REVIEWER'S STATISTICAL OUTPUT

TOXANAL RESULTS:

AN APPROXIMATE LC50 FOR THIS SET OF DATA IS 1.694058

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS	
3	.2052517	1.447395	1.013689	2.447637

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
3	.1938198	1	.4753537

SLOPE = 1.647713
95 PERCENT CONFIDENCE LIMITS = .9223078 AND 2.373118

LC50 = 1.461527
95 PERCENT CONFIDENCE LIMITS = .9650046 AND 2.782984

LC10 = .2477659
95 PERCENT CONFIDENCE LIMITS = 7.780772E-02 AND .4225476

FISHER'S EXACT TEST

SUMMARY OF FISHERS EXACT TESTS

GROUP	IDENTIFICATION	NUMBER EXPOSED	NUMBER DEAD	SIG (P=.05)
	CONTROL	20	4	
1	0.15	20	2	
2	0.36	20	4	
3	**0.74**	**20**	**8**	
4	1.47	20	10	*
5	2.98	20	16	*

Data Requirement:

PMRA DATA CODE	
EPA DP Barcode	D306156
OECD Data Point	
EPA MRID	46329904
EPA Guideline	nonguideline

Test material: Fipronil **Purity:** 99.7%
Common name: Fipronil
Chemical name: IUPAC: 5-amino-1-(2,6-dichloro-α,α,α-triflouro-p-tolyl)-4-troflouromethylsulfinyl pyrazole-3-carbonitrile
CAS name: Not reported
CAS No.: 120068-37-3
Synonyms: Not reported

Primary Reviewer: Rebecca Bryan
Staff Scientist, Dynamac Corporation
Signature: Rebecca Bryan
Date: 11/01/04

QC Reviewer: John Marton
Staff Scientist, Dynamac Corporation
Signature:
Date: 11/15/04

Primary Reviewer: Ed Odenkirchen, Chemist
OPP/EFED/ERB I
Date:

Secondary Reviewer(s):
{EPA/OECD/PMRA}
Date:

Reference/Submission No.:

Company Code:
Active Code:
EPA PC Code: 129121

Date Evaluation Completed:

CITATION: Putt, A. 2003. Fipronil-Acute Toxicity to Clams (*Corbicula fluminea*), Under Static-Renewal Conditions. Unpublished study performed by Springborn Smithers Laboratories, Wareham, Massachusetts. Laboratory Project Identification No. 986.6161. Study submitted by BASF Corporation, Research Triangle Park, North Carolina. Study initiated October 13, 2003 and completed November 24, 2003.

EXECUTIVE SUMMARY:

The 96-hour acute toxicity of Fipronil to the clam, *Corbicula fluminea*, was studied under static renewal conditions. Clams were exposed to the test material at nominal concentrations of 0 (negative and solvent controls), 0.26, 0.43, 0.72, 1.2, and 2.0 ppm a.i. Mean-measured concentrations were <0.00017 to 0.00053 (controls), 0.25, 0.45, 0.69, 1.1, and 2.0 ppm a.i., respectively.

After 96 hours, there was one mortality in the 1.1 ppm a.i. treatment group. No other mortalities were observed in the controls or treatment groups. The 96-hour LC_{50} was estimated as >2.0 ppm a.i., which categorizes Fipronil as moderately toxic to the clam (*Corbicula fluminea*) on an acute toxicity basis. During the study, one clam in the 0.25 ppm a.i. treatment group and one clam in the 1.1 ppm a.i. treatment group did not bury in the substrate. The NOEC for mortality and sublethal effects was 2.0 ppm a.i.

Since the test organism, *Corbicula flumenia*, is not a recommended test species, this study does not fulfill guideline requirements for an acute toxicity study with freshwater invertebrates (§72-2) and is classified SUPPLEMENTAL.

Results Synopsis

Test Organism Age (eg. 1st instar): Not reported; the mean soft tissue weight was 0.093 g and mean shell width was 12.4 mm (determined from a sample population [N=20] of test organisms)
Test Type (Flow-through, Static, Static Renewal): Static renewal

96-Hour
LC/EC_{50}: >2.0 ppm a.i. 95% C.I.: N/A Slope: N/A
NOEC: 2.0 ppm a.i.
LOEC: >2.0 ppm a.i.

I. MATERIALS AND METHODS

GUIDELINES FOLLOWED: The study was based on procedures outlined in the U.S. EPA Pesticide Assessment Guidelines, §72-2, U.S. EPA Standard Evaluation Procedure (Hazard Evaluation Division, EPA OPP) and ASTM Guideline E-729. Deviations from §72-2 included:

1. The water hardness (36-38 mg $CaCO_3$/L) was slightly less than recommended (40-48 mg/L as $CaCO_3$).
2. The pH range (6.8-7.5) was slightly less than recommended (7.2-7.6).
3. This test species is not recommended for aquatic invertebrate studies.
4. The age and pretest health of the test organism was not reported.
5. The study author did not report whether or not aeration was used.
6. At 72 hours, the temperature ranged from 18-20°C.
7. The TOC, presence of metals, pesticides, chlorine and particulate matter are not reported.

These deviations do not affect the validity of the study, however, the ~~mean~~ test species deviation affects the acceptability of the study

COMPLIANCE: Signed and dated GLP, No Data Confidentiality, and Quality Assurance statements were provided. The study followed the U.S. EPA Good Laboratory Practice Guidelines except for the routine food and water screening analyses (p. 3).

A. MATERIALS:

1. Test Material Fipronil

Description: Fine White Powder

Lot No./Batch No. : BES1905

Purity: 99.7%

Stability of Compound Under Test Conditions: The stability of the test substance in the dilution water during the course of the study was demonstrated by analytical determination of new solutions at 0 and 48 hours, and of aged solutions at 96 hours. Recoveries (all test levels) were 88.5-125% of nominal concentrations at 0 hours, 91.7-109.3% of nominal concentrations at 48 hours, and 80-100% of nominal at 96 hours.

Storage conditions of test chemicals: The test chemical was stored under refrigeration.

OECD requires water solubility, stability in water and light, pK_a, P_{ow}, and vapor pressure of the test compound. The following OECD requirements was reported:

Water solubility: 2.4 ppm

2. Test organism:

Species: *Corbicula fluminea*

Age at test initiation: Not reported (From a representative sample of 20 clams, mean soft tissue weight was 0.093 g and mean shell width was 12.4 mm).

Source: Current in-house (Springborn) cultures; originally from Osage Catfisheries, Osage Beach, Missouri.

B. STUDY DESIGN:

1. Experimental Conditions

a. Range-finding Study: The definitive nominal test concentrations were based on results of a range finding toxicity test and reported water solubility (2.4 ppm). The range-finding test was conducted at concentrations of 0.50, 1.0, and 2.0 ppm a.i., with a solvent control. After 96 hours, there were no mortalities or sublethal effects in the treatment groups or control.

b. Definitive Study

Table 1: Experimental Parameters

Parameter	Details	Remarks *Criteria*
Acclimation period: Conditions: (same as test or not) Feeding: Health: (any mortality observed)	Two weeks. Same as test; except clams were acclimated under static conditions. Cultures were fed *Ankistrodesmus falcatus* algae (approximately 4 x 10^7 cells/mL) at rates of 10-50 mL per week. Not reported.	*EPA requires 7 day minimum acclimation period.*
Duration of the test	96 hours	*EPA requires 48 hours*
Test condition - static/flow through Type of dilution system (for flow through method) Renewal rate (for static renewal)	Static renewal N/A At 48 hours	*EPA requires consistent flow rate of 5 - 10 volumes/24 hours, meter systems calibrated before study and checked twice daily during test period*
Aeration, if any	The study author did not report whether or not aeration was used.	
Test vessel Material: *(glass/stainless steel)* Size: Fill volume:	Glass aquaria 9.5 L 4.5 L	The test vessels contained a 2-cm layer of washed fine silica sand as a substrate. *EPA requires: size 250 ml or 3.9 L fill 200 ml*
Source of dilution water	The dilution water was laboratory well water.	

Parameter	Details	Remarks *Criteria*
		EPA requires soft reconstituted water or water from a natural source, not dechlorinated tap water.
Water parameters: Hardness pH Dissolved oxygen Temperature Total Organic Carbon Particulate matter Metals Pesticides Chlorine	 36-38 mg $CaCO_3$/L 6.8-7.5 8.3-9.3 mg/L (>60% saturation) 20°C Not reported Not reported Not reported Not reported Not reported	The hardness and the pH range was slightly less than recommended. At 72 hours, the temperature ranged from 18-20°C. *EPA requires:* *hardness: 40 - 48 mg/L as $CaCO_3$* *pH: 7.2 - 7.6* *-Temperature: 20°C (measured continuously or if water baths are used, every 6 hr, may not vary > 1°C* *Dissolved oxygen:* *Static: ≥ 60% during 1st 48 hr and ≥ 40% during 2nd 48 hr* *Flow-through: ≥60%*

Parameter	Details	Remarks *Criteria*
Number of organisms per replicate Solvent control: Negative control: Treatments:	 10 10 10	The loading rate was 0.21 g/L. *EPA requires 5 treatment levels plus control with a minimum of 20 daphnid per treatment. Biomass loading rate for static ≤ 0.8 g/L at ≤ 17°C, ≤ 0.5 g/L at > 17°C; flow-through: ≤ 1 g/L/day.*
Number of replicates Solvent control: Negative control: Treatments:	 2 2 2	
Treatment concentrations nominal: measured:	 0 (negative and solvent controls), 0.26, 0.43, 0.72, 1.2, and 2.0 ppm a.i. <0.00017 to 0.00053 (controls), 0.25, 0.45, 0.69, 1.1, and 2.0 ppm a.i.	Mean-measured concentrations were determined from 0, 48, and 96 hour data; results are provided in Table 2, p. 23. *EPA requires a geometric series with each concentration being at least 60% of the next higher one.*
Solvent (type, percentage, if used)	Acetone, 0.10 mL/L	*EPA requires solvents not to exceed 0.5 mL/L for static tests or 0.1 mL/L for flow-though tests.*
Lighting	16 hours light/8 hours dark with a transition period	Light intensity was 690 to 900 lux. *EPA requires 16 hours light, 8 hours dark.*
Feeding	Animals were not fed during testing.	*EPA/OECD requires: No feeding during the study*
Stability of chemical in the test system	Verified. Recoveries (all test levels) were 88.5-125% of nominal concentrations at 0 hours, 91.7-109.3% of nominal concentrations at 48 hours, and 80-100% of nominal at 96 hours.	Samples acquired from new solutions at 0 and 48 hours and aged solutions at 96 hours. Reviewer calculated from Table 2, p. 23.

Parameter	Details	Remarks *Criteria*
Recovery of chemical Level of Quantitation Level of Detection	93.0-117% of nominal 0.00017-0.00044 ppm a.i. Not reported	Based on matrix spikes (at 0.250, 1.20, and 2.00 ppm a.i.) analyzed concurrently with the samples (Table 2, p. 23).
Positive control {if used, indicate the chemical and concentrations}	N/A	
Other parameters, if any	None	

2. Observations:

Table 2: Observations

Criteria	Details	Remarks *Criteria*
Parameters measured including the sublethal effects	Mortality (immobility) and sublethal effects	
Observation intervals	After 24, 48, 72, and 96 hours	
Were raw data included?	No	
Other observations, if any	DO, temperature, and pH were determined daily in each test aquarium (including the aged and new solutions at 48 hours). Temperature was continuously measured in one replicate of the 1.2 ppm a.i. treatment group.	

II. RESULTS AND DISCUSSION

A. MORTALITY

After 96 hours, there was one mortality in the 1.1 ppm a.i. treatment group. No other were mortalities were observed in the controls or treatment groups (Table 3, p. 24). The 96-hour LC_{50} was estimated as >2.0 ppm a.i. The NOEC for mortality was 2.0 ppm a.i.

Table 3: Effects of Fipronil on mortality of *Corbicula fluminea*

Treatment, ppm a.i. Mean Measured and (Nominal) Concn.	Observation Period					
	24 Hours		48 Hours		96 Hours	
	Endpoint	% Affected	Endpoint	% Affected	Endpoint	% Affected
Dilution water control	Mortality	0	Mortality	0	Mortality	0
Solvent control	Mortality	0	Mortality	0	Mortality	0
0.25 (0.26)	Mortality	0	Mortality	0	Mortality	0
0.45 (0.43)	Mortality	0	Mortality	0	Mortality	0
0.69 (0.72)	Mortality	0	Mortality	0	Mortality	0
1.1 (1.2)	Mortality	0	Mortality	0	Mortality	5
2.0 (2.0)	Mortality	0	Mortality	0	Mortality	0
NOEC, ppm a.i.	2.0		2.0		2.0	
LOEC, ppm a.i.	>2.0		>2.0		>2.0	
LC_{50} (with 95% C.I.), ppm a.i.	>2.0		>2.0		>2.0	

B. SUBLETHAL TOXICITY ENDPOINTS:

During the study, one clam in the 0.25 ppm a.i. treatment group and one clam in the 1.1 ppm a.i. treatment group did not bury in the substrate. No other sublethal effects were observed in the controls or treatment groups (Table 3, p. 23). The NOEC for sublethal effects was 2.0 ppm a.i.

C. REPORTED STATISTICS:

The LC_{50} was empirically estimated and the NOEC was estimated from the lethal and sublethal effects data. The results were based on mean-measured concentrations.

96-Hour
LC/EC_{50}: >2.0 ppm a.i. 95% C.I.: N/A Slope: N/A
NOEC: 2.0 ppm a.i.
LOEC: >2.0 ppm a.i.

D. VERIFICATION OF STATISTICAL RESULTS:

The LC_{50} and NOEC values were visually estimated from the lethal and sublethal effects data using mean-measured concentrations.

96-Hour
LC/EC_{50}: >2.0 ppm a.i. 95% C.I.: N/A Slope: N/A
NOEC: 2.0 ppm a.i.
LOEC: >2.0 ppm a.i.

E. STUDY DEFICIENCIES:

This study is scientifically sound, but does not satisfy the guideline requirements for an acute toxicity study with freshwater invertebrates (§72-2) because the test organism, *Corbicula flumenia*, is not a recommended test species.

F. REVIEWER'S COMMENTS:

The reviewer's conclusions were identical to the study author's.

Trace levels of fipronil were detected in the solvent control samples at 0, 48, and 96 hours of exposure. In these types of studies, it is standard practice at Springborn Smithers Labs to target a detection limit for a specific study to be 5 to 10 times lower than the lowest nominal exposure concentration. Given this, the detection limit for the *Corbicula* study would be approximately 0.026 ppm a.i. Establishing the detection limit is ordinarily a function of the concentration range of the analytical standards and the magnitude of the concentration or dilution of the lowest sample. In an effort to utilize the same method for the mayfly exposure (nominal treatment levels of 0.000063 to 0.0010 ppm a.i., Springborn Smithers Study No. 986.6160) and the *Corbicula* test (0.26 to 2.0 ppm a.i.), some concessions were made with the concentration range of the analytical standards and this consequently lowered the detection limit for the *Corbicula* study to approximately 0.0066 ppm a.i. The day 0 dilution water and solvent control samples were inadvertently concentrated approximately 30 times more than the lowest exposure solutions, resulting in a detection limit of approximately 0.00020 ppm a.i. (range 0.00017 to 0.00044 ppm a.i.). To be consistent in the method of sample preparation throughout the study, the concentration of the control samples was continued at 48 and 96 hours.

Fipronil was detected in all of the solvent control samples and none of the dilution water control samples in the *Corbicula* study. Fipronil concentrations in newly prepared solvent control solutions were 0.00045 and 0.00053 ppm a.i. in the 0 and 48 hour solutions, respectively. Fipronil concentrations of 0.011 and 0.00032 ppm a.i. were measured in aged solutions at 48 and 96 hours, respectively.

A review of the test solution preparation procedures did not reveal any potential source for the observed fipronil in the solvent control samples. Sample blanks included during each fipronil analysis consistently exhibited fipronil peaks in their chromatograms. During the 48-hour sampling interval, the peak areas in the sample blanks yielded quantifiable levels and thus, the mean peak area of these samples was subtracted from all samples analyzed during the analysis. Due to the sensitivity of the fipronil method, the adsorptive properties of fipronil and the high sample concentrations relative to the low chromatographic detection limits, trace but quantifiable amounts of fipronil were detected in some of the solvent control samples from this acute study. The exact source of the fipronil in the solvent control samples could not be determined for individual samples, however, it is apparent that instrumental carryover and the unusually low detection limits play a significant role.

Although the identification of the source of the fipronil contamination in the solvent control samples during this study was not determined, the observed levels in the solvent control (0.00032 to 0.011 ppm a.i.) are three to four orders of magnitude below the observed NOEC for this species (i.e., 2.0 ppm a.i.). Therefore, it can be concluded that the observed peaks in the solvent control have no impact on the scientific validity of this study.

The reviewer agrees with the study author.

Testing at concentrations >2.0 ppm a.i. was not considered necessary by the study author as the levels in this study approximate the water solubility of fipronil (2.4 mg/L).

Representative samples of the food source and dilution water were analyzed for the presence of toxic metals, pesticides and PCB's by GeoLabs, Inc, Braintree, Massachusetts. None of the compounds were detected at concentrations that could be considered deleterious to the test organisms.

Clams were considered dead if touching the gaping shell produced no reaction. In addition, biological observations, including observations of stress, abnormal behavior of the exposed clams, and observations of the physical characteristics of the test solution (e.g., presence of undissolved test substance, surface film) were made and recorded at each observation interval.

Method validation was performed in October 2003 using freshwater and fortification levels of 0.0600, 0.500, and 1.00 ppm a.i. The mean procedural recovery was 103 ± 5.61% (Appendix II, p. 35).

G. CONCLUSIONS:

This study is scientifically sound, but does not satisfy the guideline requirements for an acute toxicity study with freshwater invertebrates (§72-2) because the test organism, *Corbicula fluminea*, is not a recommended test species. This study provides useful information, and is classified SUPPLEMENTAL. Based on the results of this study, Fipronil is categorized as moderately toxic to the clam, *Corbicula flumenia*, on an acute toxicity basis.

96-Hour
LC/EC_{50}: >2.0 ppm a.i. 95% C.I.: N/A Slope: N/A
NOEC: 2.0 ppm a.i.
LOEC: >2.0 ppm a.i.

III. REFERENCES:

APHA, AWWA, WPCF. 1995. *Standard Methods for the Examination of Water and Waste Water.* 19th Edition, Washington, D.C.

ASTM. 2002. Standard Guide for Conducting Acute Toxicity Tests with Fishes, Macroinvertebrates and Amphibians. Standard E729-96a. American Society for Testing and Materials, 100 Barr Harbor Drive, West Conshohocken, PA 19428.

U.S. EPA. 40 CFR, Part 160. Federal Insecticide, Fungicide and Rodenticide Act. Good Laboratory Practice Standards; Final Rule. Office of the Federal Register, National Archives and Records Administration. U.S. Government Printing Office, Washington, D.C.

U.S. EPA. 1982. Office of Pesticide Programs. Pesticide Assessment Guidelines. Subdivision E, Hazard Evaluation: Wildlife and Aquatic Organisms. EPA-540/9-85-024. October 1982. U.S. Environmental Protection Agency, Washington, D.C.

U.S. EPA. 1985. Office of Pesticide Programs. Standard Evaluation Procedure for Acute Toxicity Test for Freshwater Invertebrates. EPA-540/9-85-005. June 1985. U.S. Environmental Protection Agency, Washington, D.C.

Data Evaluation Report on the Acute Toxicity of Fipronil to Freshwater Invertebrates - *Lumbriculus variegatus*
PMRA Submission Number{.....} EPA MRID Number 46329903

Data Requirement:

PMRA DATA CODE	
EPA DP Barcode	D306156
OECD Data Point	
EPA MRID	46329903
EPA Guideline	nonguideline

Test material: Fipronil **Purity:** 99.7% w/w
Common name: Fipronil
Chemical name: IUPAC: 5-amino-1-(2,6-dichloro-α,α,α-triflouro-p-tolyl)-4-troflouromethylsulfinyl pyrazole-3-carbonitrile
CAS name: Not reported
CAS No.: 120068-37-3
Synonyms: Not reported

Primary Reviewer: Rebecca Bryan
Staff Scientist, Dynamac Corporation
Signature: Rebecca Bryan
Date: 11/01/04

QC Reviewer: John Marton
Staff Scientist, Dynamac Corporation
Signature:
Date: 11/15/04

Primary Reviewer: Ed Odenkirchen, Chemist
OPP/EFED/ERB I
Date:

Secondary Reviewer(s):
{EPA/OECD/PMRA}
Date:

Reference/Submission No.:

Company Code:
Active Code:
EPA PC Code: 129121

Date Evaluation Completed:

CITATION: Putt, A. 2003. Fipronil-Acute Toxicity to Oligochaetes (*Lumbriculus variegatus*) Under Static-Renewal Conditions. Unpublished study performed by Springborn Smithers Laboratories, Wareham, Massachusetts. Laboratory Project Identification No. 986.6162. Study submitted by BASF Corporation, Research Triangle Park, North Carolina. Study initiated October 13, 2003 and completed November 24, 2003.

EXECUTIVE SUMMARY:

The 96-hour acute toxicity of Fipronil to the oligochaete, *Lumbriculus variegatus*, was studied under static renewal conditions. Oligochaetes were exposed to the test material at nominal concentrations of 0 (negative and solvent controls), 0.26, 0.43, 0.72, 1.2, and 2.0 ppm a.i. Mean-measured concentrations were <0.00017 to 0.00090 (controls), 0.25, 0.41, 0.71, 1.2, and 1.9 ppm a.i., respectively.

After 96 hours, there were no mortalities or sublethal effects in the controls or treatment groups. The 96-hour LC_{50} was estimated as >1.9 ppm a.i., which categorizes Fipronil as moderately toxic to the oligochaete (*Lumbriculus variegatus*) on an acute toxicity basis. The NOEC for mortality and sublethal effects was 1.9 ppm a.i.

Since the test organism, *Lumbriculus variegatus*, is not a recommended test species, this study does not fulfill guideline requirements for an acute toxicity study with freshwater invertebrates (§72-2) and is classified SUPPLEMENTAL.

Results Synopsis

Test Organism Age (eg. 1st instar): Not reported; the mean wet weight was 0.0039 g and mean length was 1.8 cm (range of 1.3- 2.1 cm). Values determined from sample of 20 test organisms.
Test Type (Flow-through, Static, Static Renewal): Static renewal

96-Hour
LC/EC_{50}: >1.9 ppm a.i. 95% C.I.: N/A Slope: N/A
NOEC: 1.9 ppm a.i.
LOEC: >1.9 ppm a.i.

I. MATERIALS AND METHODS

GUIDELINES FOLLOWED: The study was based on procedures outlined in the U.S. EPA Pesticide Assessment Guidelines, §72-2; ASTM Guideline E-729 "Standard Guide for Conducting Acute Toxicity Test on Test Materials with Fishes, Macroinvertebrates, and Amphbians,"; and U.S EPA Office of Pesticide Programs, Standard Evaluation Procedure. Deviations from §72-2 included:

1. The water hardness (36 mg $CaCO_3$/L) was slightly less than recommended (40-48 mg/L as $CaCO_3$).
2. The pH range (6.8-7.3) was slightly less than recommended (7.2-7.6).
3. This test species is not recommended for aquatic invertebrate studies.
4. The age and pretest health of the test organism was not reported.
5. The TOC, presence of particulate matter, presence of metals, presence of pesticides and presence of chlorine were not reported.

These deviations do not affect the validity of the study, however, the ~~mean~~ test species deviation affects the acceptability of the study

COMPLIANCE: Signed and dated GLP, No Confidentiality, and Quality Assurance statements were provided. The study followed the U.S. EPA Good Laboratory Practice Guidelines except for the routine food and water screening analyses (p. 3).

A. MATERIALS:

1. Test Material Fipronil

Description: Fine White Powder

Lot No./Batch No. : BES1905

Purity: 99.7%

Stability of Compound Under Test Conditions: The stability of the test substance in the dilution water during the course of the study was demonstrated by analytical determination of the new solutions at 0 and 48 hours and of the aged solutions at 96 hours. Recoveries (all test levels) were 93-108% of nominal concentrations at 0 hours, 91-100% of nominal concentrations at 48 hours, and 88-104% of nominal at 96 hours.

Storage conditions of test chemicals: The test chemical was stored under refrigeration.

OECD requires water solubility, stability in water and light, pK_a, P_{ow}, and vapor pressure of the test compound. The following OECD requirements was reported:

Water solubility: 2.4 ppm

2. Test organism:

Species: *Lumbriculus variegatus*

Age at test initiation: Not reported (From a representative sample of 20 oligochaetes, the mean wet weight was 0.0039 g and mean length was 1.8 cm).

Source: In-house laboratory (Springborn) cultures

B. STUDY DESIGN:

1. Experimental Conditions

a. Range-finding Study: The definitive nominal test concentrations were based on results of a range finding toxicity test and reported water solubility (2.4 ppm). The range-finding test was conducted at concentrations of 0.50, 1.0, and 2.0 ppm a.i., with a solvent control. After 96 hours, there were no mortalities or sublethal effects in the treatment groups or control.

b. Definitive Study

Table 1: Experimental Parameters

Parameter	Details	Remarks *Criteria*
Acclimation period: Conditions: (same as test or not) Feeding: Health: (any mortality observed)	Two weeks. Same as test; except oligochaetes were acclimated under flow-through conditions, test type was static-renewal Cultures were fed a suspension (100 mg/mL) of flaked fish food at rates of 10-15 mL once per week. Not reported.	*EPA requires 7 day minimum acclimation period.*
Duration of the test	96 hours	*EPA requires 48 hours*
Test condition - static/flow through Type of dilution system (for flow through method) Renewal rate (for static renewal)	Static renewal N/A At 48 hours	*EPA requires consistent flow rate of 5 - 10 volumes/24 hours, meter systems calibrated before study and checked twice daily during test period*
Aeration, if any	Study author did not report whether or not aeration was used during the definitive study.	
Test vessel Material: *(glass/stainless steel)* Size: Fill volume:	Glass beakers 250 mL 200 mL	*EPA requires: size 250 ml or 3.9 L fill 200 ml*
Source of dilution water	The dilution water 100 m bedrock well water supplemented on demand with untreated well water from the town of Wareham.	

Parameter	Details	Remarks *Criteria*
		EPA requires soft reconstituted water or water from a natural source, not dechlorinated tap water.
Water parameters: Hardness pH Dissolved oxygen Temperature Total Organic Carbon Particulate matter Metals Pesticides Chlorine	 36 mg $CaCO_3$/L 6.7-7.3 6.0-9.2 mg/L (>60% saturation) 22-23°C Not reported Not reported Not reported Not reported Not reported	The hardness and the pH range was slightly less than recommended. The TOC, presence of particulate matter, presence of metals, presence of pesticides and presence of chlorine were not reported.
		EPA requires: *hardness: 40 - 48 mg/L as $CaCO_3$* *pH: 7.2 - 7.6* *-Temperature: 20°C (measured continuously or if water baths are used, every 6 hr, may not vary > 1°C* *Dissolved oxygen:* *Static: ≥ 60% during 1st 48 hr and ≥ 40% during 2nd 48 hr* *Flow-through: ≥60%*

Parameter	Details	Remarks *Criteria*
Number of organisms per replicate Solvent control: Negative control: Treatments:	 5 5 5	The loading rate was 0.098 g/L. *EPA requires 5 treatment levels plus control with a minimum of 20 daphnid per treatment. Biomass loading rate for static ≤ 0.8 g/L at ≤ 17°C, ≤ 0.5 g/L at > 17°C; flow-through: ≤ 1 g/L/day.*
Number of replicates Solvent control: Negative control: Treatments:	 4 4 4	
Treatment concentrations nominal: measured:	0 (negative and solvent controls), 0.26, 0.43, 0.72, 1.2, and 2.0 ppm a.i. <0.00017 to 0.00090 (controls), 0.25, 0.41, 0.71, 1.2, and 1.9 ppm a.i.	Mean-measured concentrations were determined from new solutions at 0 and 48 hours and the aged solutions at 96 hours; results are provided in Table 2, p. 22. *EPA requires a geometric series with each concentration being at least 60% of the next higher one.*
Solvent (type, percentage, if used)	Acetone, 0.10 mL/L	*EPA requires solvents not to exceed 0.5 mL/L for static tests or 0.1 mL/L for flow-though tests.*
Lighting	16 hours light/8 hours dark with a transition period	Light intensity was 580 to 770 lux. *EPA requires 16 hours light, 8 hours dark.*
Feeding	Animals were not fed during testing.	*EPA/OECD requires: No feeding during the study*
Stability of chemical in the test system	Verified. Recoveries (all test levels) were 93-108% of nominal concentrations at 0 hours, 91-100% of nominal concentrations at 48 hours, and 88-104% of nominal at 96 hours.	Reviewer calculated from Table 2, p. 22.

Parameter	Details	Remarks *Criteria*
Recovery of chemical Level of Quantitation Level of Detection	88.8-107% of nominal 0.00017-0.00026 ppm a.i. Not reported	Based on matrix spikes (at 0.250, 1.20, and 2.00 ppm a.i.) analyzed concurrently with the samples (Table 2, p. 22).
Positive control {if used, indicate the chemical and concentrations}	N/A	
Other parameters, if any	None	

2. Observations:

Table 2: Observations

Criteria	Details	Remarks *Criteria*
Parameters measured including the sublethal effects	Mortality (immobility) and sublethal effects	
Observation intervals	0, 24, 48, 72, and 96 hours	
Were raw data included?	Yes, sufficient	
Other observations, if any	DO, temperature, and pH were determined daily in each test aquarium (including the aged and new solutions at 48 hours). Temperature was continuously measured in one replicate of the 0.26 ppm a.i. (nominal) treatment group.	

II. RESULTS AND DISCUSSION

A. MORTALITY

After 96 hours, there were no mortalities in the controls or treatment groups (Table 3, p. 23). The 96-hour LC_{50} was estimated as >1.9 ppm a.i. The NOEC for mortality was 1.9 ppm a.i.

Table 3: Effects of Fipronil on mortality of *Lumbriculus variegatus*

Treatment, ppm a.i. Mean Measured and (Nominal) Concn.	Observation Period					
	24 hours		48 hours		96 hours	
	Endpoint	%Affected	Endpoint	% Affected	Endpoint	% Affected
Dilution water control	Mortality	0	Mortality	0	Mortality	0
Solvent control	Mortality	0	Mortality	0	Mortality	0
0.25 (0.26)	Mortality	0	Mortality	0	Mortality	0
0.41 (0.43)	Mortality	0	Mortality	0	Mortality	0
0.71 (0.72)	Mortality	0	Mortality	0	Mortality	0
1.2 (1.2)	Mortality	0	Mortality	0	Mortality	0
1.9 (2.0)	Mortality	0	Mortality	0	Mortality	0
NOEC, ppm a.i.	1.9		1.9		1.9	
LOEC, ppm a.i.	>1.9		>1.9		>1.9	
LC_{50} (with 95% C.I.), ppm a.i.	>1.9		>1.9		>1.9	

B. SUBLETHAL TOXICITY ENDPOINTS:

During the study, no sublethal effects were observed in the controls or treatment groups (Table 3, p. 23). The NOEC for sublethal effects was 1.9 ppm a.i.

C. REPORTED STATISTICS:

The LC_{50} was empirically estimated and the NOEC was estimated from the lethal and sublethal effects data. The results were based on mean-measured concentrations.

96-Hour
LC/EC_{50}: >1.9 ppm a.i. 95% C.I.: N/A Slope: N/A
NOEC: 1.9 ppm a.i.
LOEC: >1.9 ppm a.i.

D. VERIFICATION OF STATISTICAL RESULTS:

The EC_{50} and NOEC values were visually determined using mean-measured concentrations due to the lack of treatment related mortality in the controls and at all treatment levels after 96 hours of exposure.

96-Hour
LC/EC_{50}: >1.9 ppm a.i. 95% C.I.: N/A Slope: N/A
NOEC: 1.9 ppm a.i.
LOEC: >1.9 ppm a.i.

E. STUDY DEFICIENCIES:

This study is scientifically sound, but does not satisfy the guideline requirements for an acute toxicity study with freshwater invertebrates (§72-2) because the test organism, *Lumbriculus variegatus*, is not a recommended test species.

F. REVIEWER'S COMMENTS:

The reviewer's conclusions were identical to the study author's.

Representative samples of the dilution water were analyzed periodically for the presence of pesticides, PCBs and toxic metals by GeoLabs, Inc. None of these compounds were detected at concentrations that are considered toxic in any of the water samples analyzed, in agreement with ASTM (2002) standard procedures. The output from these analyses were not provided in the study.

Mean-measured concentrations were calculated using the new test solutions at 0 and 48 hours and the aged solution at 96 hours. Since the recovery percentages remained stable throughout the study, it does not appear to be deleterious that the aged solution at 48 hours was not measured and utilized when determining the mean-measured concentrations.

While being maintained in culture prior to the test, oligochaetes were fed 10 to 15 mL of flaked food fish food suspension (100 mg/mL) once weekly, however the feeding rates were modified based on the size of each culture population.

The 0.72, 1.2 and 2.0 ppm a.i. nominal test solutions were cloudy upon dosing, however, following approximately two minutes of stirring, these test solutions were observed to be clear and colorless with no undissolved test substance present.

Test organisms were carefully transferred into the freshly-prepared test solutions using a wide-borne pipette. Biological observations, including sublethal effects (e.g. lethargy) of the test organisms, observations of the physical characteristics of the test solutions (e.g., presence of undissolved test substance, surface film) were made and recorded at each observation interval. The endpoint for this study was death, which was defined as the absence of a response by the oligochaete to gentle probing with a glass pipette.

Trace levels of fipronil were detected in the aged control sample at 96 hours. Although the source of the fipronil contamination was not determined, the observed level in the control (0.00090 ppm a.i.) is four orders of magnitude below the observed NOEC, based on mean measured concentrations, (1.9 ppm a.i.) for this species. It is likely however, that the contamination can be attributed to the sensitivity of the method, the adsorptive properties of fipronil and the high sample concentrations relative to the low chromatographic detection limits.

Method validation was performed in October 2003 using freshwater and fortification levels of 0.0600, 0.500, and 1.00 ppm a.i. The mean procedural recovery and the LOQ for the method validation was 103 ± 5.61% and 0.00460 ppb a.i., respectively. (Appendix II, p. 34).

G. CONCLUSIONS:

This study is scientifically sound, but does not satisfy the guideline requirements for an acute toxicity study with freshwater invertebrates (§72-2) because the test organism, *Lumbriculus variegatus*, is not a recommended test species. This study provides useful information, and is classified SUPPLEMENTAL. Based on the results of this study, Fipronil is categorized as moderately toxic to the oligochaete, *Lumbriculus variegatus*, on an acute toxicity basis.

96-Hour
LC/EC_{50}: >1.9 ppm a.i. 95% C.I.: N/A Slope: N/A
NOEC: 1.9 ppm a.i.
LOEC: >1.9 ppm a.i.

III. REFERENCES:

APHA, AWWA, WPCF. 1995. *Standard Methods for the Examination of Water and Waste Water.* 19th Edition, Washington, D.C.

ASTM. 2002. Standard Guide for Conducting Acute Toxicity Tests with Fishes, Macroinvertebrates and Amphibians. Standard E729-96a. American Society for Testing and Materials, 100 Barr Harbor Drive, West Conshohocken, PA 19428.

U.S. EPA. 40 CFR, Part 160. Federal Insecticide, Fungicide and Rodenticide Act. Good Laboratory Practice Standards; Final Rule. Office of the Federal Register, National Archives and Records Administration. U.S. Government Printing Office, Washington, D.C.

U.S. EPA. 1982. Pesticide Assessment Guidelines. Subdivision E, Hazard Evaluation: Wildlife and Aquatic Organisms. EPA-540/9-85-024. October 1982. U.S. Environmental Protection Agency, Washington, D.C.

U.S. EPA. 1985. Office of Pesticide Programs. Standard Evaluation Procedure for Acute Toxicity Test for Freshwater Invertebrates. EPA-540/9-85-005. June 1985. U.S. Environmental Protection Agency, Washington, D.C.

Data Evaluation Report on the Acute Toxicity of Fipronil to Freshwater Invertebrates - *Hexagenia sp.*
PMRA Submission Number{.....} EPA MRID Number 46329902

Data Requirement:

PMRA DATA CODE	
EPA DP Barcode	D306156
OECD Data Point	
EPA MRID	46329902
EPA Guideline	§72-2

Test material: Fipronil **Purity:** 99.7%
Common name: Fipronil
Chemical name: IUPAC: 5-amino-1-(2,6-dichloro-α,α,α-triflouro-p-tolyl)-4-troflouromethylsulfinyl pyrazole-3-carbonitrile
CAS name: Not reported
CAS No.: 120068-37-3
Synonyms: Not reported

Primary Reviewer: Rebecca Bryan
Staff Scientist, Dynamac Corporation
Signature: Rebecca Bryan
Date: 11/12/04

QC Reviewer: John Marton
Staff Scientist, Dynamac Corporation
Signature:
Date: 11/15/04

Primary Reviewer: Ed Odenkirchen, Chemist
OPP/EFED/ERB I
Date:

Secondary Reviewer(s):
{EPA/OECD/PMRA}
Date:

Reference/Submission No.:

Company Code:
Active Code:
EPA PC Code: 129121

Date Evaluation Completed:

CITATION: Putt, A. 2003. Fipronil-Acute Toxicity Mayfly Nymphs (*Hexagenia* sp.), Under Static-Renewal Conditions. Unpublished study performed by Springborn Smithers Laboratories, Wareham, Massachusetts. Laboratory Project Identification No. 986.6160. Study submitted by BASF Corporation, Research Triangle Park, North Carolina. Study initiated October 6, 2003 and completed November 24, 2003.

EXECUTIVE SUMMARY:

The 96-hour acute toxicity of Fipronil to the mayfly nymph, *Hexagenia sp.*, was studied under static renewal conditions. Nymphs were exposed to the test material at nominal concentrations of 0 (negative and solvent controls), 0.063, 0.13, 0.25, 0.50, and 1.0 ppb a.i. Mean-measured concentrations were <0.0046-0.0049 (<LOQ, controls), 0.059, 0.14, 0.24, 0.52, and 1.1 ppb a.i., respectively.

After 96 hours, mortality/immobility was observed in 0, 0, 20, 75, and 100% of mayfly nymphs in the 0.059, 0.14, 0.24, 0.52, and 1.1 ppb a.i. treatment groups, respectively. The dilution water and solvent controls had 10% mortality. The 96-hour LC_{50} (with 95% C.I.) was 0.44 ppb a.i. (0.39-0.49 ppb a.i.), which categorizes Fipronil as very highly toxic to the mayfly nymph (*Hexagenia sp.*) on an acute toxicity basis. During the study, the sublethal effect of lethargy was observed in the 0.24, 0.52, and 1.1 ppb a.i. treatment groups. The NOEC for mortality and sublethal effects was 0.14 ppb a.i.

This study is scientifically sound and satisfies the guideline requirements for an acute toxicity study with freshwater invertebrates (§72-2). This study is classified as CORE.

Results Synopsis

Test Organism Age (eg. 1st instar): Nymphs, 60 days old
Test Type (Flow-through, Static, Static Renewal): Static renewal

96-Hour

LC/EC_{50}: 0.44 ppb a.i.	95% C.I.: 0.39-0.49 ppb a.i.	Slope: N/A
NOEC: 0.14 ppb a.i.		
LOEC: 0.24 ppb a.i.		

I. MATERIALS AND METHODS

GUIDELINES FOLLOWED: The study was based on procedures outlined in the U.S. EPA Pesticide Assessment Guidelines, §72-2, the Standard Evaluation Procedure (Hazard Evaluation Disvios, EPA OPP) and ASTM Guideline E-729. Deviations from §72-2 included:

1. The water hardness (170 mg $CaCO_3$/L) was significantly higher than recommended (40-48 mg/L as $CaCO_3$).
2. The pH range (7.7-8.0) was slightly higher than recommended (7.2-7.6).
3. The pretest health of the test organisms was not reported.
4. The study author did not report whether or not aeration was used.

These deviations did not affect the acceptability or the validity of the study.

COMPLIANCE: Signed and dated GLP, No Data Confidentiality, and Quality Assurance statements were provided. The study followed the U.S. EPA Good Laboratory Practice Guidelines except for the routine food and water screening analyses (p. 3).

A. MATERIALS:

1. Test Material Fipronil

Description: Fine White Powder

Lot No./Batch No. : BES1905

Purity: 99.7%

Stability of Compound Under Test Conditions: The stability of the test substance in the dilution water during the course of the study was demonstrated by analytical determination at 0, 48, and 96 hours. Recoveries (all test levels) were 92-120% of nominal concentrations at 0 hours, 88-100% of nominal concentrations at 48 hours (new solution), and 92-108% of nominal at 96 hours (aged solution, reviewer calculated from Table 2, p. 23).

Storage conditions of test chemicals: The test chemical was stored under refrigeration.

OECD requires water solubility, stability in water and light, pK_a, P_{ow}, and vapor pressure of the test compound. The OECD requirements were not reported

2. Test organism:

Species: *Hexagenia sp.* (*Hexagenia rigida* and *Hexagenia limbata*)

Age at test initiation: Nymphs, 60 days old

Source: University of Windsor, Ontario, Canada

B. STUDY DESIGN:

1. Experimental Conditions

a. Range-finding Study: The definitive nominal test concentrations were based on results of a range finding toxicity test. The range-finding test was conducted at concentrations of 0.060, 0.20, 0.50, 1.3, 4.0, and 10 ppb a.i., with a solvent control. After 96 hours, mortality was 0, 0, 80, 93, 100, and 100% in the 0.060, 0.20, 0.50, 1.3, 4.0, and 10 ppb a.i. treatment groups, respectively. No mortality or sublethal effects were observed in the solvent control. All the surviving mayflies were lethargic in the 0.50 and 1.3 ppb a.i. treatment group, and some of the surviving 0.20 ppb a.i. treatment group mayflies were lethargic.

b. Definitive Study

Table 1: Experimental Parameters

Parameter	Details	Remarks *Criteria*
Acclimation period:	60 days prior to test initiation.	
Conditions: (same as test or not)	Same as test; except for the 2 cm layer of artificial soil substrate with overlying water (p. 12).	
Feeding:	*Hexagenia* cultures were fed a finely-ground suspension of flaked fish food, yeast, and cerophyll at rates of 5-10 mL per aquarium per week.	*EPA requires 7 day minimum acclimation period.*
Health: (any mortality observed)	Not reported.	
Duration of the test	96 hours	*EPA requires 48 hours*
Test condition - static/flow through	Static renewal	
Type of dilution system (for flow through method)	N/A	
Renewal rate (for static renewal)	At 48 hours	*EPA requires consistent flow rate of 5 - 10 volumes/24 hours, meter systems calibrated before study and checked twice daily during test period*
Aeration, if any	The study author did not report whether or not aeration was used.	
Test vessel Material: *(glass/stainless steel)*	Glass beakers	The beakers contained fine pieces of glass tube (approximately 10 mm in length) as an artificial substrate.
Size:	1000 mL	
Fill volume:	800 mL	
		EPA requires: size 250 ml or 3.9 L fill 200 ml
Source of dilution water	The dilution water was fortified laboratory well water.	

Parameter	Details	Remarks *Criteria*
		EPA requires soft reconstituted water or water from a natural source, not dechlorinated tap water.
Water parameters: Hardness pH Dissolved oxygen Temperature Total Organic Carbon Particulate matter Metals Pesticides Chlorine	 170 mg $CaCO_3$/L 7.7-8.0 7.4-9.1 mg/L (>60% saturation) 21-22°C Not reported Not reported Not detected Not detected Not reported	The hardness and pH were higher than recommended. *EPA requires:* *hardness: 40 - 48 mg/L as $CaCO_3$* *pH: 7.2 - 7.6* *-Temperature: 20°C (measured continuously or if water baths are used, every 6 hr, may not vary > 1°C* *Dissolved oxygen:* *Static: ≥ 60% during 1st 48 hr and ≥ 40% during 2nd 48 hr* *Flow-through: ≥60%*

Parameter	Details	Remarks *Criteria*
Number of organisms per replicate Solvent control: Negative control: Treatments:	 5 5 5	The loading rate was 0.019 g/L. *EPA requires 5 treatment levels plus control with a minimum of 20 daphnid per treatment. Biomass loading rate for static ≤ 0.8 g/L at ≤ 17°C, ≤ 0.5 g/L at > 17°C; flow-through: ≤ 1 g/L/day.*
Number of replicates Solvent control: Negative control: Treatments:	 4 4 4	
Treatment concentrations nominal: measured:	0 (negative and solvent controls), 0.063, 0.13, 0.25, 0.50, and 1.0 ppb a.i. <0.0046-0.0049 (<LOQ, controls), 0.059, 0.14, 0.24, 0.52, and 1.1 ppb a.i.	Mean-measured concentrations were determined from 0, 48, and 96 hour data; results are provided in Table 2, p. 23. *EPA requires a geometric series with each concentration being at least 60% of the next higher one.*
Solvent (type, percentage, if used)	Acetone, 0.1 mL/L	 *EPA requires solvents not to exceed 0.5 mL/L for static tests or 0.1 mL/L for flow-though tests.*
Lighting	16 hours light/8 hours dark with a transition period	Light intensity was 650 to 830 lux. *EPA requires 16 hours light, 8 hours dark.*
Feeding	Animals were not fed during testing.	 *EPA/OECD requires: No feeding during the study*
Stability of chemical in the test system	Verified. Recoveries (all test levels) were 92-120% of nominal concentrations at 0 hours, 88-100% of nominal concentrations at 48 hours, and 92-108% of nominal at 96 hours.	The recoveries at 0 and 48 hours were determined from the new solutions and the recovery from 96 hours was determined from the aged solution. Reviewer calculated from Table 2, p. 23.

Parameter	Details	Remarks *Criteria*
Recovery of chemical Level of Quantitation Level of Detection	90.0-113% of nominal 0.046-0.049 ppb a.i. Not reported	Based on matrix spikes (at 0.0600, 0.500, and 1.00 ppb a.i.) analyzed concurrently with the samples (Table 2, p. 23).
Positive control {if used, indicate the chemical and concentrations}	N/A	
Other parameters, if any	None	

2. Observations:

Table 2: Observations

Criteria	Details	Remarks *Criteria*
Parameters measured including the sublethal effects	Mortality (immobility) and sublethal effects	
Observation intervals	After 24, 48, 72, and 96 hours	
Were raw data included?	No	
Other observations, if any	DO, temperature, and pH were determined daily in each test aquarium (including the aged and new solutions at 48 hours). Temperature was continuously measured in one replicate of the 0.13 ppb a.i. treatment group.	

II. RESULTS AND DISCUSSION

A. MORTALITY

After 96 hours, mortality/immobility was observed in 0, 0, 20, 75, and 100% of mayfly nymphs in the 0.059, 0.14, 0.24, 0.52, and 1.1 ppb a.i. treatment groups, respectively (Table 3, p. 24). The dilution water and solvent controls had 10% mortality. The 96-hour LC_{50} (with 95% C.I.) was 0.44 ppb a.i. (0.39-0.49 ppb a.i.). The NOEC for mortality was 0.14 ppb a.i.

Table 3: Effects of Fipronil on mortality of *Hexagenia sp.*

Treatment, ppb a.i. Mean-Measured and (Nominal) Concn.	Observation Period					
	24 Hours		48 Hours		96 Hours	
	Endpoint	% Affected	Endpoint	% Affected	Endpoint	% Affected
Dilution water control	Mortality	0	Mortality	0	Mortality	10
Solvent control	Mortality	0	Mortality	0	Mortality	10
0.059 (0.063)	Mortality	0	Mortality	0	Mortality	0
0.14 (0.13)	Mortality	0	Mortality	0	Mortality	0
0.24 (0.25)	Mortality	0	Mortality	0	Mortality	20
0.52 (0.50)	Mortality	0	Mortality	0	Mortality	75
1.1 (1.0)	Mortality	0	Mortality	0	Mortality	100
NOEC, ppb a.i.	1.1		1.1		0.14	
LOEC, ppb a.i.	>1.1		>1.1		0.24	
LC_{50} (with 95% C.I.), ppb a.i.	>1.1		>1.1		0.44 (0.39-0.49)	

B. SUBLETHAL TOXICITY ENDPOINTS:

During the study, the sublethal effect of lethargy was observed in the 0.24, 0.52, and 1.1 ppb a.i. treatment groups (Table 3, p. 24). The controls and the 0.059 and 0.14 ppb a.i. treatment group mayfly nymphs appeared normal and healthy during the study. The NOEC for sublethal effects was 0.14 ppb a.i.

C. REPORTED STATISTICS:

The LC_{50} and 95% C.I. was calculated using Log-Log analysis of a computer program (Gulley, 1996). The NOEC values were estimated from the lethal and sublethal effects data. The results were based on mean-measured concentrations.

96-Hour
LC/EC_{50}: 0.44 ppb a.i. 95% C.I.: 0.39-0.49 ppb a.i. Slope: N/A
NOEC: 0.14 ppb a.i.
LOEC: 0.24 ppb a.i.

D. VERIFICATION OF STATISTICAL RESULTS:

The EC_{50} and NOEC values were estimated from the lethal and sublethal effects data using mean-measured concentrations. The EC_{50} was determined using the moving average method via TOXANAL statistical software and the NOEC based on mortality was determined using Fisher's Exact Test via Toxstat statistical software.

96-Hour
LC/EC_{50}: 0.377 ppb a.i. 95% C.I.: 0.32-0.45 ppb a.i. Slope: N/A
NOEC: 0.24 ppb a.i.
LOEC: 0.52 ppb a.i.

E. STUDY DEFICIENCIES:

There were no significant deviations from U.S. EPA guideline §72-2 that affected the acceptability of this study.

F. REVIEWER'S COMMENTS:

The reviewer's LC/EC_{50} estimate was slightly lower than the study author's and it was associated with a slightly broader 95% confidence interval than the study author's estimate. Furthermore, the study author's NOEC estimate was one concentration lower than the reviewer's estimate. As a result, the study author's estimates are reported in the Executive Summary and Conclusions sections. Both the study author and the reviewer concluded that, based on the results of this study, Fipronil is categorized as very highly toxic to the mayfly nymph (*Hexagenia sp.*) on an acute toxicity basis.

The test species of *Hexagenia* sp. is acceptable, but not the preferred test species (*Daphnia magna*). The mayfly eggs used in this study were collected from female imagoes of *Hexagenia rigida* and *Hexagenia limbata*. Taxonomic separation of these female imagoes is not possible. Therefore, as these two species are sympatric, it is not possible to identify the specific species or ratio of species used in this test.

To prevent undue stress on the test organisms during the renewal, test vessels were removed from the water bath and 80% (approximately 640 mL) of the aged solution was siphoned from the 1-L vessel, then 640 mL of newly prepared test solution was slowly returned to the exposure vessel.

Effects for this study were based on death, defined as failure of mayfly nymphs to respond by movement to gentle probing with a glass pipette.

The aged solution was not measured during the renewal at 48 hours, however the concentrations remained relatively constant from 48-96 hours.

Method validation was performed in October 2003 using freshwater and fortification levels of 0.0600, 0.500, and 1.00 ppm a.i. The mean procedural recovery was 103 ± 5.61% (Appendix II, p. 36).

G. CONCLUSIONS:

This study is scientifically sound, fulfills U.S. EPA guideline §72-2, and is classified as CORE. Based on the results of this study, Fipronil is categorized as very highly toxic to the mayfly nymph, *Hexagenia sp.*, on an acute toxicity basis.

96-Hour
LC/EC_{50}: 0.44 ppb a.i. 95% C.I.: 0.39-0.49 ppb a.i. Slope: N/A
NOEC: 0.14 ppb a.i.

LOEC: 0.24 ppb a.i.

III. REFERENCES:

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APPENDIX I. OUTPUT OF REVIEWER'S STATISTICAL VERIFICATION

Toxanal Results
RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS	
3	4.850269E-02	.3774243	.3177614	.4508643

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
6	.1300222	1	.8608184

SLOPE = 5.298137
95 PERCENT CONFIDENCE LIMITS = 3.387703 AND 7.208571

LC50 = .3700273
95 PERCENT CONFIDENCE LIMITS = .3058426 AND .4536481

LC10 = .2130722
95 PERCENT CONFIDENCE LIMITS = .1481195 AND .2633542

Fisher's Exact Test Results

SUMMARY OF FISHERS EXACT TESTS

GROUP	IDENTIFICATION	NUMBER EXPOSED	NUMBER DEAD	SIG (P=.05)
	CONTROL	20	2	
1	0.059	20	0	
2	0.14	20	0	
3	**0.24**	**20**	**4**	
4	0.52	20	15	*
5	1.1	20	20	*