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TEXT SEARCHABLE DOCUMENT 2009

DP Barcode: D321165 MRID No.: 466191-03

DATA EVALUATION RECORD
AQUATIC INVERTEBRATE LIFE CYCLE TEST
GUIDELINE 72-4(B)

1. **CHEMICAL:** Fipronil PC Code No.: 129121

2. **TEST MATERIAL:** Fipronil Purity: 99.7%

3. **CITATION**

Authors:	Cafarella, Mark A.
Title:	Fipronil - Life-Cycle Toxicity Test with Mysids (*Americamysis bahia*) Under Static Conditions in a Water-Sediment System
Study Completion Date:	February 24, 2005
Laboratory:	Springborn Smithers Laboratories
Sponsor:	BASF Corporation
Laboratory Report ID:	Springborn Smithers Study No. 986.6163 Sponsor Protocol/Project No. 198247 BASF Reg. Doc. No. 2005/5000047
MRID No.:	466191-03
DP Barcode:	

4. **REVIEWED BY:** Anita Ullagaddi, EPS, EFED/ERB1

Signature: Date: 3/12/09

5. **APPROVED BY:** Edward Odenkirchen, Ph.D., EFED/ERB1

Signature: Date: 3/12/09

6. **STUDY PARAMETERS**

Age of Test Organism:	Parental mysids: sexually mature, 21 days old Juvenile mysids: ≤24 hours old
Definitive Test Duration:	28 days
Study Method:	Static, water-sediment system
Type of Concentrations:	Measured

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7. CONCLUSIONS:

In a mysid life cycle study (MRID 46619103), fipronil concentrations in the water decreased over the study duration. Measured concentrations were below the level of detection (4 ng/L) on and after Day 14 (15 ng/L nominal group), Day 21 (30 ng/L nominal group), and 28 (60 ng/L nominal group). Therefore, measured concentrations are used to represent exposure levels in this study. Time-weighted average mean measured concentrations were calculated to estimate the concentration to which the mysids were exposed.

No statistically significant effects were observed in this study. However, there was a trend of decreasing population over time. The apparent population effect did not reach statistical significance when compared to the solvent control; however, these results appear to be biologically relevant because there was a dose-response trend and the total number of mysids was approximately 25% lower than controls at the highest mean measured concentration of 0.014 ug a.i./L (14 ng a.i./L). Therefore, based on a reduction in the number of free-ranging mysids, the NOAEC appears to be between 0.004 and 0.014 ug a.i./L.

Results Synopsis

A clear NOAEC was not established. There was a concentration-response relationship between mysid population and fipronil. The total number of mysids was approximately 25% lower than controls at the highest mean measured concentration of 0.014 ug/L. Therefore, based on a reduction in the number of free-ranging mysids, the NOAEC appears to be between 0.004 and 0.014 ug/L.

8. ADEQUACY OF THE STUDY

A. Classification: Supplemental

B. Rationale: The study does not satisfy a data requirement.

C. Repairability: N/A

9. MAJOR GUIDELINE DEVIATIONS:

1) Three toxicant concentrations were used instead of five.
2) Individual numbers of offspring per female was not reported.
3) A negative control was not used. Therefore, potential solvent effects could not evaluated.

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10. MATERIALS AND METHODS:

A. Biological System:

Guideline Criteria	Reported Information
Species: An estuarine shrimp species, preferably Americamysis bahia.	Test species is Americamysis bahia. Juvenile mysids (≤ 24 hours old) and sexually mature (21 days old) mysids, *Americamysis bahia*, formerly *Mysidopsis bahia,* were used to initiate the life-cycle test.
Duration of the Test: A mysid test must not be terminated before 7 days past the median time of 1st brood release in the control treatment.	28 days
Source (or supplier)	Aquatic BioSystems, Inc., Springborn Smithers culture
Parental Acclimation 1) Parental stock must be maintained separately from the brood culture in dilution water and under test conditions. 2) Mysids should be in good health.	1) Protocol does not state whether parental stock was maintained separately from brood culture. 2) Health of mysids not reported.
Parental Acclimation Period At least 14 days	14 days
Chamber Location: Treatments should be randomly assigned to test chamber locations.	Organisms were impartially placed in test vessels.
Brood Stock: Test started with mysids: 1) from only one brood stock or 2) from brood stock which has not obtained sexual maturity or had been maintained for >	Mysids were cultured in one of several 76-L glass aquaria with a closed-loop recirculating filtration system providing seawater to the aquaria. The seawater in the

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Guideline Criteria	Reported Information
14 days in a laboratory with same food, water, temperature, and salinity used in the test.	aquaria was characterized as having a salinity of 19 to 21‰, and a pH range of 8.2 to 8.3 during the 14-day period prior to test initiation. Sediment and overlying water were added to each replicate exposure vessel and allowed to equilibrate for three days prior to test substance application. The test substance was introduced into the aquaria one hour before test initiation. The test was initiated when the test organisms were impartially placed in the test vessels.
Distribution: **No. of mysids before pairing**: Minimum of 15 mysids per compartment, 2 compartments per chamber, 2 chambers per concentration for a total of 60/treatment level.	200 mysids per treatment level and control (50 per aquarium)
No. of mysids after pairing: ≥ 20 randomly selected pairs/treatment (excess males should be held in separate compartment in same treatment to replace paired males).	20 pairs/treatment (excess males were held in separate compartment in same treatment to replace paired males). **Note:** Each exposure aquarium contained five mysid pairing chambers used to house sexually mature male and female organisms. At test initiation (day 0), and the time of sexual maturity (approximately day 14 of the test), adult mysids were transferred to the pairing chambers. The first group of mysids (21 days old, designated group 1) were placed into the retention chambers on day 0. These mysids were selected from the holding tank in the culture facility. The second group of sexually mature mysids were selected from the initial group of juvenile mysids exposed

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Guideline Criteria	Reported Information
	in the aquarium on test day 0 (designated group 2). One male and one female were kept in each pairing chamber. A maximum of five pairing chambers were impartially suspended so as to not disturb the sediment layer in each test chamber. At initiation, each aquarium was stocked with 50 mysids (200 mysids per treatment level and control). Mysids, 24 hours old, were divided among 16 beakers. The beakers contained culture water and were held in a water bath maintained at 25 °C. The organisms were impartially selected and distributed to the beakers by adding five organisms at a time to each beaker until each beaker contained 50 mysids. Each group of 50 mysids was then transferred to one of the 16 labeled aquaria. In addition, forty sexually mature mysids were placed in the twenty retention chambers (five per aquarium) of each treatment level and solvent control. The test was initiated when the mysids were placed in their respective test aquaria. Each group of 50 mysids was then transferred to one of the 16 labeled aquaria. In addition, forty sexually mature mysids were placed in the twenty retention chambers (five per aquarium) of each treatment level and solvent control.
Pairing: 1) Should be conducted when most of the mysids are sexually mature (usu. 10-14 days after test initiation). 2) Should be paired on the same day	1) At test initiation, 21-day old mature male/female pairs were transferred from cultures into each of five individual glass pairing chambers. These organisms were used to

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Guideline Criteria	Reported Information
	evaluate mysid reproduction during the initial 14-day exposure (designated group 1). 2) Juveniles (designated group 2) were exposed for 14 days, then paired.
Feeding: 1) Mysids should be fed live brine shrimp nauplii at least once daily. 2) 150 live brine shrimp nauplii per mysid per day or 75 twice a day is recommended.	During culture and testing, mysids were fed live brine shrimp (Anemia sauna) nauplii <48 hours old (post-hydration), twice daily, with one feeding supplemented with Selco®.
Counts: Live adult mysids should be counted 1) at initiation, 2) at pairing, 3) and daily after pairing. 4) Live young must be counted and removed daily. 5) Missing or impinged animals should be recorded.	Live adult mysids were counted daily. 4) Live young in each pairing chamber were counted, recorded, and removed daily up until test day 14. At test day 14, all mysids were removed and counted from the retention chambers in order to initiate the mysid pairing of group 2. 5) Dead and missing mysids were counted and removed daily. Female survival was adjusted to account for impinged mysids.
Controls: Negative control and carrier control (when applicable) are required.	Four replicates of a solvent control were studied. One replicate was found to have been inadvertently dosed and was excluded from the pooled solvent control data. A negative control was not used.

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B. Physical System:

Guideline Criteria	Reported Information
Test Water: 1) May be natural (sterilized and filtered) or a commercial mixture; 2) Water must be free of pollutants. 3) During the test, difference between highest and lowest measured salinities must be less than 10 ‰ (parts per thousand). Should be measured daily. 4) Salinity should be between 15 and 30 ‰. 5) pH should be measured at the beginning, end of test and weekly. 6) DO must be measured @ each conc. @ least once a wk. 7) See details in ASTM E-1191.	1) Natural filtered seawater was diluted with laboratory well water. 2) Pesticides, PCBs, and metals were not detected. 4) Salinity was 20 ‰ at test preparation. Maintained at 20 ± 3 ‰. 5) pH was 7.9 at test preparation. 6) DO was 9.7 mg/L at test preparation. Dissolved oxygen concentration, pH and salinity were measured daily in each replicate of each treatment level and the solvent control solutions throughout the exposure period.
Test Temperature: 1) Measured daily in one chamber and at least 3 times in all chambers. 2) Mean measured temperature for each chamber at test termination should be within 1°C of selected test temperature. 3) Each individual measured temperature must be within 3°C of the mean of the time-weighted averages. 4) For mysid shrimp, 27°C is recommended. 5) Whenever temp. is measured concurrently in more than one test chamber the highest &	A Visitherm submersible heater was used in each tank to maintain test solution temperature at 25 ± 2 °C. Temperature was measured daily in each replicate of each treatment level and solvent control.

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Guideline Criteria	Reported Information
lowest temp. must not differ by more than 2°C.	
Photoperiod: Recommend 16L/8D. 14L/10D also acceptable.	16L/8D
Dosing Apparatus: 1) Intermittent flow proportional diluters or continuous flow serial diluters should be used. 2) A minimum of 5 toxicant concentrations 3) with a dilution factor not greater than 0.5 and controls should be used.	 Three toxicant concentrations were studies. A dilution factor of 0.5 was used.
Toxicant Mixing: 1) Mixing chamber is recommended but not required; 2) Aeration should not be used for mixing; 3) It must be demonstrated that the test solution is completely mixed before intro. into the test system; 4) Flow splitting accuracy must be within 10%.	 2) Due to the anaerobic nature of marine sediments, aeration with oil-free air was utilized throughout the test.
Test Vessels: 1) Material: all glass, No. 316 stainless steel, or perflorocarbon plastic **Test Chambers:** 1)Most common: 300x450x150 mm deep with solution depth of 100 mm. 2) Should be covered. **Test Compartments (within chambers):** 1) Size: 250 ml beaker with side cutouts covered with nylon mesh or stainless steel screen.	 Glass aquaria and pairing chambers were used. 39 x 20 x 25 cm Cylindrical glass jars with 5.1 cm diameter

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Guideline Criteria	Reported Information
or 3) 90 or 140 mm inside dia. glass Petri dish bottoms with collars made of 200 - 250 um mesh screen.	and 10 cm height
Flow Rate: 1) Flow rates should provide 5 to 10 volume additions per 24 hr. 2) Flow rate must maintain DO at or above 60% of saturation and maintain the toxicant level. 3) Meter systems calibrated before study and checked twice daily during test period.	Not applicable for a static study.
Aeration: 1) Dilution water should be aerated to insure DO concentration at or near 100% saturation. 2) Test tanks may be aerated.	Aeration with oil-free air was utilized throughout the test.

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C. **Chemical System:**

Guideline Criteria	Reported Information
Concentrations: 1) Minimum of 5 concentrations and a control, all replicated, plus solvent control if appropriate.	1) 3 concentrations (15, 30, and 60 ng a.i./L) plus a solvent control were tested in replicates of 4.
2) Toxicant conc. must be measured in one tank at each treatment level every week.	2) For the first two replicates of each treatment level, concentrations were measured on day 0, day 7, and day 21. For the second two replicates, concentrations were measured on day 0, day 4, day 14, and day 28. In the 30 ng a.i./L treatment group, the fourth replicate was not measured for concentration because it was lost due to a broken heater. On day 0, the pooled solvent control had a measured concentration of the test chemical. On day 1, it was determined that the second replicate had been inadvertently dosed.
3) One concentration must adversely affect a life stage and one concentration must not affect any life stage.	3) Based on the author's reported LOEC of >60 ng a.i./L, no adverse effects were observed in any life stage. Statistically significant effects were observed; however, since there was no dose-response relationship, effects were determined not to be biologically significant.
4) The measured conc. of the test material of any treatment should be at least 50% of the time-weighted average measured conc. for >10% of the duration of the test.	4) Measured concentrations were 2pproximately 20% to 40% of nominal at Day 4.

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Guideline Criteria	Reported Information
5) The measured conc. for any treatment level should not be more than 30% higher than the time-weighted average measured conc. for more than 5% of the duration of the test.	5)
Solvents: 1) Should not exceed 0.1 ml/L in a flow-through system. 2) Following solvents are acceptable: triethylene glycol, methanol, acetone, ethanol.	1) Solvent prepared at a concentration of 0.1 mL/L for this static system. 2) Acetone was used as a solvent.

Comments:

The test system also included sediments. The study report included the following description of the sediment. "The sediment used during this study was collected from Little Harbor Beach, Wareham, Massachusetts. Prior to use and characterization, the sediment was wet pressed through a 2.0-mm sieve to remove large particles. The sediment used in this study was characterized by Agvise Laboratories, Northwood, North Dakota, as having a percent organic carbon of 2.7%, a particle size distribution of 77% sand, 14% silt and 9% clay, a pH of 7.7 and a percent moisture at 1/3 bar (water holding capacity) of 19.2%. Prior to allocation to the test vessels, a sample of the sediment pore water was collected by centrifugation and measured for total ammonia. The concentration of ammonia in the pore water was 18.6 mg/L as nitrogen, and was below levels that would affect the performance of the test. A representative sample of the sediment source was analyzed for the presence of pesticides, PCBs and toxic metals by GeoLabs, Inc., Braintree, Massachusetts. None of these compounds were detected at concentrations that would be considered to have an adverse impact on the results of the test."

11. REPORTED RESULTS:

Guideline Criteria	Reported Information
Quality assurance and GLP compliance statements were included in the report?	Yes.
Controls: 1) Survival of the first-generation controls	1) Survival of parental females was 92 ±

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Guideline Criteria	Reported Information
(between pairing and test termination) must not be less than 70%.	14% (second replicate of solvent control excluded from analysis since it was inadvertently dosed). Survival of juvenile females was 100%. Survival was adjusted for impingement.
2) At least 75% of the paired 1st generation females in the controls produced young	2) On day 14, 87% of females in the three pooled solvent control replicates had produced young, although not all parental females that produced young were still alive.
3) The average number of young produced by the 1st generation females in the control(s) was at least 3.	Average number of young produced in the solvent control was >3 for all replicates. However, the average number of young in the inadvertently dosed control replicate was 1.8.
Data Endpoints must include: 1) Survival of first-generation mysids Female Male	Survival of first generation male and female mysids was evaluated.
2) Number of live young produced per female	2) The ratio of the total number of offspring produced to the total number of females per chamber per reproductive day was evaluated; individual data not reported.
3) Dry weight of each first-generation mysid alive at the end of the test Female Male 4) Length of each first-generation mysid alive at the end of the study Female Male	Individual lengths and weights of all surviving males and females were recorded for each replicate of each concentration and the solvent control.
5) Incidence of pathological or histological effects; 6) Observations of other effects or clinical signs.	5) Data not reported. 6) Observations were inadvertently not conducted.

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Guideline Criteria	Reported Information
Raw data included? (Y/N) At a minimum, individual data should be included for: 1) surviving 1st generation _ and _ mysids. 2) Number of live young produced per female. 3) Individual length measurements of _ and _ mysids. 4) Individual dry weight measurements for _ and _ mysids at the end of the test.	Yes 1) Survivors reported. 2) Total number of live young reported per group of females per reproductive day. Individual lengths and dry weights reported.

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Results tables from the study report are presented below. Water and sediment measurements are presented in study tables 2 and 3, and effects data summaries are presented in Tables 4 and 5 of the study report. These tables are included below.

Table 2. Concentrations of fipronil measured in overlying water samples during the 28-day life-cycle exposure of mysids (*Americamysis bahia*) in a water-sediment system.

Nominal Concentration	Overlying Water Measured Concentration (ng a.i./L)													
(ng a.i./L)	Replicate	Day 0	Mean	Day 1	Day 4	Mean	Day 7	Mean	Day 14	Mean	Day 21	Mean	Day 28	Mean
Solvent Control	A	14 [a]		<4.0	<4.0	<4.0	<4.0	<4.0	NA	<4.0	<4.0	<4.0	NA	<4.0
	B			53[b]	48		53		NA		34		NA	
	C			<4.0	<4.0		NA		<4.0		NA		<4.0	
	D			<4.0	<4.0		NA		<4.0		NA		<4.0	
15	A	14	17	NS[c]	NA[d]	6.4	6.2	5.6	NA	<4.0	<4.0	<4.0	NA	<4.0
	B	15		NS	NA		4.9		NA		<4.0		NA	
	C	20		NS	6.4		NA		<4.0		NA		<4.0	
	D	19		NS	<4.0		NA		<4.0		NA		<4.0	
30	A	31	31	NS	NA	8.2	8.1	8.7	NA	6.9	<4.0	<4.0	NA	4.2
	B	34		NS	NA		9.2		NA		<4.0		NA	
	C	27		NS	8.2		NA		6.9		NA		4.2	
	D[e]	30		NS	NS		NS		NS		NS		NS	
60	A	54	55	NS	NA	12	19	17	NA	16	6.6	5.3	NA	<4.0
	B	53		NS	NA		15		NA		4.0		NA	
	C	58		NS	11		NA		14		NA		<4.0	
	D	53		NS	13		NA		18		NA		<4.0	
QC #1 [f] 15.0		16.6 (111)		13.9 (92.8)	<4.0[g] (NA)		14.8 (98.8)		13.1 (87.1)		13.4 (89.0)		12.2 (81.4)	
QC #2 30.0		33.6 (112)		--- (NS)	23.9 (79.6)		25.4 (84.5)		25.4 (84.7)		23.4 (77.9)		21.9 (72.9)	
QC #3 70.0		65.7 (93.9)		--- (NS)	54.4 (77.7)		60.6 (86.6)		56.2 (80.3)		51.6 (73.8)		56.9 (81.3)	

[a] On test day 0, the control sample consisted of a composite sample of replicates A-D.
[b] The mean solvent control value on day 0 resulted in a measured concentration of 14 ng a.i./L, suggesting one of the solvent controls were inadvertently dose with fipronil. Therefore on day 1, all replicate solvent control samples were reanalyzed separately. For the remainder of the study, solvent control samples were removed similarly to the treatment level samples (i.e., alternating between intervals).
[c] NS = Not Sampled.
[d] NA = Not Analyzed. Sample taken from replicate tank and archived.
[e] Aquarium heater failed in exposure tank and this replicate was not included in the analysis after day 0.
[f] QC = Quality Control sample. Percent recovery of each sample is presented in the table.
[g] QC sample #1 on test day 4 is below detection limit due to matrix difficulties with this sample.

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Based on the measured concentrations, the mean measured concentration was 14 ng/L, 7.5 ng/L, and 4.3 ng/L at the nominal 60 ng/L, 30 ng/L, and 15 ng/L exposure groups, respectively.

Table 3. Concentrations of fipronil measured in sediment samples during the 28-day life-cycle exposure of mysids (*Americamysis bahia*) in a water-sediment system.

Nominal Concentration (ng a.i./L)	Sediment Measured Concentration (ng a.i./kg)					
	Day 0	Day 4	Day 7	Day 14	Day 21	Day 28
60	<30	140	115	78	98	<30
QC #1[a]	98.9	157	149	161	154	157
200	(49.5)[b]	(78.3)	(74.7)	(80.6)	(77.0)	(78.3)

[a] QC = Quality Control sample. Percent recovery of each sample is presented in the table.
[b] Percent recovery for this QC sample was outside of the established range (i.e., 70.0 to 120%, Appendix 3).

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Table 4. Summary of endpoints for the water-sediment life-cycle exposure of mysids (*Americamysis bahia*) to fipronil.

	Group 1 Means (Standard Deviation)		Group 2 Means (Standard Deviation)						Free-ranging Mysids, Means (Standard Deviation)		
Nominal Concentration (ng a.i./L)	Female % Survival	# of Offspring per Reprod. Day	Female % Survival	# of Offspring per Reprod. Day	Male Length (mm)	Male Weight (mg)	Female Length (mm)	Female Weight (mg)	# of Immature Mysids	# of Mature Mysids	Population (free-ranging)
Solvent Control[a]	92 (14)	0.59 (0.1 4)	100 (0)	2.2 (0.31)	7.7 (0.36)	0.94 (0.14)	7.9 (0.26)	1.2 (0.14)	489 (82)	46 (10)	535 (83)
15	90 (12)	0.40 (0.15)	85 (10)	1.6 (0.22)[a]	7.6 (0.32)	0.82 (0.21)	7.9 (0.28)	1.3 (0.08)	480 (104)	31 (3)	511 (106)
30	93 (12)	0.31 (0.11)[b]	100 (0)	1.8 (0.36)	7.7 (0.42)	0.93 (0.15)	7.9 (0.40)	1.2 (0.30)	397 (143)	41 (10)	438 (151)
60	89 (13)	0.44 (0.010)	95 (10)	2.0 (0.33)	7.6 (0.26)	0.88 (0.07)	8.1 (0.20)	1.3 (0.18)	362 (71)	42 (10)	404 (61)

Note: Female survival was adjusted for organisms lost due to impingement.
All replicate data are presented in Appendix 4.

[a] Replicate B of the solvent control was excluded from analysis due to contamination.
[b] Statistically different compared to the solvent control, using Bonferroni T-test, however was not considered biologically relevant.

Toxicity Observations:

In addition to the endpoints presented in the preceding table, the total number of free-ranging mysids were evaluated. The study report indicates that "the total number of immature mysids in the 15, 30 and 60 ng a.i./L treatment levels was 480, 397 and 362, respectively, and was not statistically different from the solvent control (489), based on Williams' test. The total number of mature mysids in the 15, 30 and 60 ng a.i./L treatment levels were 31, 41 and 42, respectively, and was not statistically different from the solvent control (46), based on Williams' test. The total free-ranging mysids in the 15, 30 and 60 ng a.i./L treatment levels were 511, 438 and 404, respectively, and was not statistically different from the solvent control (535), based on Williams' test."

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Reported Statistical Results:

Most sensitive endpoint:

Endpoint	NOEC (nominal)	LOEC (nominal)
Survival	60 ng/L	>60 ng/L
Reproduction	60 ng/L	>60 ng/L
Weight	60 ng/L	>60 ng/L
Length	60 ng/L	>60 ng/L

Comments:

The time weighted average concentrations were as follows:

Nominal Concentration: 60 ng/L
Mean Measured Concentration: 14 ng/L

Nominal Concentration: 30 ng/L
Mean Measured Concentration: 7.5 ng/L

Nominal Concentration: 15 ng/L
Mean Measured Concentration: 4.3 ng/L

Mean measured concentrations assumed that levels that were below the level of detection of 4 ng/L were equal to ½ the detection limit (2 ng/L).

Given that fipronil concentrations decreased dramatically over the study duration, mean measured values were used to define toxicity endpoints from this study.

12. Reviewer's Statistical Results:

The reviewer performed statistical analyses using Toxstat statistical software. The reviewer's conclusions with respect to statistical significance are equivalent to the study authors' analyses. However, there was a trend of decreasing population over time (study table 4). The apparent population effect did not reach statistical significance when compared to the solvent control;

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however, these results appear to be biologically relevant because there was a concentration-response trend and the total number of mysids was approximately 25% lower than controls at the highest mean measured concentration of 0.014 ug/L. Therefore, based on a reduction in the number of free-ranging mysids, the NOAEC appears to be between 0.004 and 0.014 ug a.i./L (4 and 14 ng a.i./L).

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Attachment 1. Effects Data Tables

Daily Summary of Reproduction Data for a Mysid-Sediment Life Cycle Definitive Study (Group #1)
Test Material: Fipronil
Study Number: 486.6163
Test Day: 14
Date: 01/05/05

Nominal Conc. (ng A.I./L)	Rep	Paired males	Paired females	# males dead	# impinged	%Female Survival per rep.	%Female Survival per conc.	# females dead	# impinged	% impinged	% female survival (adjusted)	mean % female survival (adjusted)	St. Dev.	Females Prod. Young	Cum. # of Offsp. to date	Actual Repro.days	# of Ave.#F1 per Repro.day (Rep.)	Ave.#F1 per Repr.day (Conc.	St. Dev.
60	1A	5	4	6	4	80	80	1	1	100	100	89	13	4	34	61	0.5574	0.4365	0.09959
	1B	4	3	3	1	60		2	1	50	75			5	25	57	0.4386		
	1C	4	4	6	4	80		1	0	0	80			5	29	64	0.4531		
	1D	5	5	4	2	100		0	0		100			5	22	70	0.3143		
30	2A	5	4	0	0	80	87	1	1	100	100	93	12	5	18	66	0.2727	0.3131	0.1061466
	2B	5	5	9	4	100		0	0		100			4	30	70	0.4286		
	2C	4	4	2	2	80		1	0	0	80			4	14	62	0.2258		
	2D	TERMINATED																	
15	3A	4	5	4	2	100	80	0	0		80	90	12	4	21	70	0.3000	0.4000	0.1501004
	3B	2	2	4	2	40		3	3	100	100			5	32	55	0.5818		
	3C	5	5	5	3	100		0	0		100			3	19	70	0.2714		
	3D	4	4	5	3	80		1	0	0	80			5	32	65	0.4923		
Sol.Cont.	4A	3	3	5	3	60	60	2	1	50	75	92	14	4	32	54	0.5926	0.5896	0.136592
	4B[1]	1	2	7	3	40		3	0	0	40			5	9	56	0.1607		
	4C	4	3	3	1	60		2	2	100	100			4	26	58	0.4483		
	4D	4	4	3	2	80		1	1	100	100			5	44	61	0.7213		

Total Young To Date 387

[1] Contamination present (51 ng/L measured) excluded from endpoint calculations.

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Daily Summary of Reproduction Data for a Mysid-Sediment Life Cycle Definitive Study (Group #2)--Paired mysids only
Test Material: Fipronil
Study Number: 986.6163
Test Day: 28
Date: 01/19/05

Nominal Conc. (ng A.I./L)	Rep	Paired males	females	# males dead	# impinged	%Female Survival per rep.	%Female Survival per conc	# females dead	# impinged	% impinged	% female survival (adjusted)	mean % female survival (adjusted)	St Dev.	Females Prod. Young	Cum. # of Off. to date	Actual # of Repr. days	Ave.#F1 per Repr.day (Rep.)	Ave.#F1 per Repr.day (Conc.)	Standard Deviation
60	1A	3	3	7	6	60	75	2	1	50	80	95	10	5	127	53	2.3962	1.9593	0.3316273
	1B	4	4	4	4	80		1	1	100	100			5	108	68	1.5882		
	1C	4	4	5	3	80		1	1	100	100			4	127	63	2.0159		
	1D	4	4	5	3	80		1	1	100	100			5	120	62	1.9355		
30	2A	5	5	0	0	100	73	0	0		100	100	0	5	146	70	2.0857	1.8238	0.3619854
	2B	3	3	7	5	60		2	2	100	100			5	108	54	2.0000		
	2C	2	3	8	8	60		2	2	100	100			5	98	69	1.4203		
	2D	TERMINATED																	
15	3A	2	2	8	7	40	55	3	3	100	100	85	10	5	83	45	1.8444	1.6205	0.2212197
	3B	3	3	6	6	60		2	1	50	80			5	88	58	1.5172		
	3C	3	4	6	4	80		1	0	0	80			4	98	57	1.7193		
	3D	2	2	7	7	40		3	2	67	80			3	47	35	1.3429		
Sol.Cont.	4A	3	1	4	3	20	55	4	4	100	100	100	0	5	126	50	2.5200	2.1823	0.3078626
	4B[1]	5	3	0	0	60		2	1	50	80			4	95	47	2.0213		
	4C	4	3	4	3	60		2	2	100	100			5	120	63	1.9048		
	4D	3	4	4	4	80		1	1	100	100			5	149	68	2.1912		

[1] Contamination present (51 ng/L measured) excluded from endpoint calculations.

Total Young To Date 1640

Fipronil- 28 Day Mysid Life Cycle (Water-Sediment) - Male Length (mm) 986.6163

	Solvent Control				15 ng a.I./L				30 ng a.I./L				60 ng a.I./L			
	A	B[1]	C	D	A	B	C	D	A	B	C	D	A	B	C	D
	7.3	7.4	7.5	8.0	7.0	7.9	7.7	7.6	7.7	7.5	7.6		7.7	7.9	7.4	7.5
	7.8	7.1	7.1	7.7	7.9	7.3	7.6	7.8	8.0	6.9	7.9		7.9	7.3	7.1	7.9
	7.3	7.0	8.2	7.8		7.9	7.2		8.4	7.6			7.7	7.2	7.3	7.7
		7.5	8.0						8.1					7.7	7.4	7.6
		7.4							7.4							
mean	7.5	7.3	7.7	7.8	7.5	7.7	7.5	7.7	7.9	7.3	7.8		7.8	7.5	7.3	7.7
std.dev.	0.29	0.22	0.50	0.15	0.64	0.35	0.26	0.14	0.38	0.38	0.21		0.12	0.33	0.14	0.17
min	7.3	7.0	7.1	7.7	7.0	7.3	7.2	7.6	7.4	6.9	7.6		7.7	7.2	7.1	7.5
max	7.8	7.5	8.2	8.0	7.9	7.9	7.7	7.8	8.4	7.6	7.9		7.9	7.9	7.4	7.9
n	3	5	4	3	2	3	3	2	5	3	2		3	4	4	4
cov	4%	3%	6%	2%	9%	4%	4%	2%	5%	5%	3%		1%	4%	2%	2%
mean				7.7				7.6				7.7				7.6
std.dev.				0.36				0.32				0.42				0.26
min				7.1				7.0				6.9				7.1
max				8.2				7.9				8.4				7.9
n				10				10				10				15
cov				5%				4%				5%				3%

[1] Excluded

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Fipronil- 28 Day Mysid Life Cycle (Water-Sediment) - Female Length (mm) 986.6165

	Solvent Control				15 ng a.i./L				30 ng a.i./L				60 ng a.i./L			
	A	B[1]	C	D	A	B	C	D	A	B	C	D	A	B	C	D
	7.9	7.9	8.1	8.2	8.3	8.0	7.6	7.5	7.8	7.6	7.8		8.4	8.2	8.1	8.0
		8.0	7.7	8.0	7.7	7.6	8.2	8.0	8.4	7.9	7.6		8.1	8.3	8.0	7.9
		8.2	7.4	7.8		8.2	8.0		8.6	7.3	8.1		7.9	7.9	7.9	8.4
				8.1			7.8		7.5					7.8	8.1	8.3
									8.2							
mean	7.9	8.0	7.7	8.0	8.0	7.9	7.9	7.8	8.1	7.6	7.8		8.1	8.1	8.0	8.2
std.dev.		0.15	0.35	0.17	0.42	0.31	0.26	0.35	0.45	0.30	0.25		0.25	0.24	0.10	0.24
min	7.9	7.9	7.4	7.8	7.7	7.6	7.6	7.5	7.5	7.3	7.6		7.9	7.8	7.9	7.9
max	7.9	8.2	8.1	8.2	8.3	8.2	8.2	8.0	8.6	7.9	8.1		8.4	8.3	8.1	8.4
n	1	3	3	4	2	3	4	2	5	3	3		3	4	4	4
cov	0%	2%	5%	2%	5%	4%	3%	5%	6%	4%	3%		3%	3%	1%	3%
mean				7.9				7.9				7.9				8.1
std.dev.				0.26				0.28				0.40				0.20
min				7.4				7.5				7.3				7.8
max				8.2				8.3				8.6				8.4
n				8				11				11				15
cov				3%				3%				5%				2%

[1] Excluded

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Fipronil- 28 Day Mysid Life Cycle (Water-Sediment) - Male Dry Weight (mg) 986.6163

	Solvent Control				15 ng a.I./L				30 ng a.I./L				60 ng a.I./L			
	A	B[1]	C	D	A	B	C	D	A	B	C	D	A	B	C	D
	0.92	0.92	1.22	1.05	0.96	0.49	1.15	0.81	0.77	1.13	0.99		0.98	0.89	0.86	0.86
	0.85	0.72	1.01	0.97	0.92	0.54	0.60	0.91	1.16	0.87	0.67		0.88	0.86	0.91	0.94
	0.83	0.93	0.79	1.02		0.99	0.83		0.91	0.97			0.83	0.69	0.82	0.95
		0.94	0.75						0.99					0.95	0.87	0.89
		0.89							0.82							
mean	0.87	0.88	0.94	1.01	0.94	0.67	0.86	0.86	0.93	0.99	0.83		0.90	0.85	0.87	0.91
std.dev.	0.05	0.09	0.22	0.04	0.03	0.28	0.28	0.07	0.15	0.13	0.23		0.08	0.11	0.04	0.04
min	0.83	0.72	0.75	0.97	0.92	0.49	0.60	0.81	0.77	0.87	0.67		0.83	0.69	0.82	0.86
max	0.92	0.94	1.22	1.05	0.96	0.99	1.15	0.91	1.16	1.13	0.99		0.98	0.95	0.91	0.95
n	3	5	4	3	2	3	3	2	5	3	2		3	4	4	4
cov	5%	10%	23%	4%	3%	41%	32%	8%	17%	13%	27%		9%	13%	4%	5%
mean				0.94				0.82				0.93				0.88
std.dev.				0.14				0.21				0.15				0.07
min				0.75				0.49				0.67				0.69
max				1.22				1.15				1.16				0.98
n				10				10				10				15
cov				15%				26%				16%				8%

[1] Excluded

Fipronil- 28 Day Mysid Life Cycle (Water-Sediment) - Female Dry Weight (mg) 986.6163

	Solvent Control				15 ng a.I./L				30 ng a.I./L				60 ng a.I./L			
	A	B[1]	C	D	A	B	C	D	A	B	C	D	A	B	C	D
	1.29	1.65	0.94	0.96	1.22	1.28	1.38	1.25	1.03	1.36	1.49		1.18	1.26	1.23	1.41
		1.08	1.19	1.27	1.29	1.08	1.28	1.27	1.48	0.70	1.13		1.26	1.51	1.42	0.80
		0.96	1.03	1.26		1.38	1.22		1.61	1.31	0.73		1.47	1.31	1.12	1.21
				1.22			1.31		1.16					1.23	1.54	1.35
									1.23							
mean	1.29	1.23	1.05	1.18	1.26	1.25	1.30	1.26	1.30	1.12	1.12		1.30	1.33	1.33	1.19
std.dev.		0.37	0.13	0.15	0.05	0.15	0.07	0.01	0.24	0.37	0.38		0.15	0.13	0.19	0.27
min	1.29	0.96	0.94	0.96	1.22	1.08	1.22	1.25	1.03	0.70	0.73		1.18	1.23	1.12	0.80
max	1.29	1.65	1.19	1.27	1.29	1.38	1.38	1.27	1.61	1.36	1.49		1.47	1.51	1.54	1.41
n	1	3	3	4	2	3	4	2	5	3	3		3	4	4	4
cov	0%	30%	12%	12%	4%	12%	5%	1%	18%	33%	34%		11%	9%	14%	23%
mean				1.15				1.27				1.20				1.29
std.dev.				0.14				0.08				0.30				0.18
min				0.94				1.08				0.70				0.80
max				1.29				1.38				1.61				1.54
n				8				11				11				15
cov				13%				6%				25%				14%

[1] Excluded

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Population census of free-ranging mysids at termination of the 28-day sediment water life-cycle exposure to Fipronil.

Concentration (ng a.i./L)	rep	Total Immature	# of Mature Males	# of Mature Females	Total Mature	Population Total
Solvent Control	A	568	18	35	53	621
	B*	269	20	35	55	324
	C	494	9	25	34	528
	D	405	17	33	50	455
	Mean	489			46	535
	Std Dev	82			10	83
15	A	492	5	25	30	522
	B	344	10	19	29	373
	C	597	11	24	35	632
	D	486	6	23	29	515
	Mean	480			31	511
	Std Dev	104			3	106
30	A	375	12	21	33	408
	B	550	18	34	52	602
	C	266	15	24	39	305
	D**					
	Mean	397			41	438
	Std Dev	143			10	151
60	A	410	7	29	36	446
	B	256	19	38	57	313
	C	388	13	23	36	424
	D	395	12	25	37	432
	Mean	362			42	404
	Std Dev	71			10	61

* Note: Solvent control replicate B was excluded from statistical analyses due to contamination present on day 0.

** Replicate D of the 30 ng/L treatment level was lost due to a broken heater.

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