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8-10-95

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

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

2. **TEST MATERIAL:** 000264-LLU Fipronil technical Purity: 97.7 %

3. **CITATION:** **Fipronil: Chronic toxicity to Mysids (Americamysis bahia) under flow-through conditions.**

Authors:	M.W. Machado
Title:	see citation
Study Completion Date:	4/5/95
Laboratory:	Springborn Labs
Sponsor:	Rhone-Poulenc Ag Company
Laboratory Report ID:	95-4-5820
MRID No.:	436812-01
DP Barcode:	D216635

4. **REVIEWED BY:** N.E. Federoff (Wildlife Biologist) EEB, EFED

Signature: N.E. Federoff **Date: 8/10/95**

5. **APPROVED BY:** Ann Stavola, Head of Section (5), EEB, EFED

Signature: **Date:**

6. **STUDY PARAMETERS**

Age of Test Organism:	<24 hrs
Definitive Test Duration:	28 days
Study Method:	Flow-through
Type of Concentrations:	Mean measured

7. **CONCLUSIONS:** This study is scientifically sound and fulfills guideline requirements for a chronic life-cycle toxicity study. The most sensitive endpoints were reduction in male mysid weight and length.

Results Synopsis
NOEC:<5 ng ai/L (pptr) LOEC:5 ng ai/L (pptr) MATC:<5 ng ai/L
LOEC's for specific effects
Reproduction: 57 ng ai/L (pptr)
F1 Survival: 57 ng ai/L (pptr)
Growth: (length): Male= 15 ng ai/L (pptr)
Female= 28 ng ai/L (pptr)
(Dry weight): Male= 5 ng ai/L (pptr)
Female= 57 ng ai/L (pptr)

8. **ADEQUACY OF THE STUDY**
   - **A. Classification:** Core
   - **B. Rationale:** Fulfills requirements
   - **C. Repairability:** N/A

9. **GUIDELINE DEVIATIONS**

1. None

10 **SUBMISSION PURPOSE**: Registration of a new chemical.

11. **MATERIALS AND METHODS**

A. Test Organisms/Acclimation

Guideline Criteria	Reported Information
Species *Americamysis bahia*	Americamysis bahia
Source	Springborn Lab cultures
Parental Acclimation Conditions Parental stock must be maintained separately from the brood culture in dilution water and under test conditions.	Maintained separately under test conditions.
Parental Acclimation Period At least 14 days.	14 days
Fed at least once per day?	Yes
Food Live Brine Shrimp Nauplii?	Yes
Food Concentration 150 live brine shrimp nauplii per mysid per day is recommended.	Fed ad libitum 2X daily with one feeding supplemented with SELCO (a substance high in saturated fatty acids).
Were mysids in good health during acclimation period?	Yes

B. Test System

Guideline Criteria	Reported Information
Test Water Natural or reconstituted saltwater that has been tested for contaminants (see ASTM for details).	Reconstituted saltwater (i.e., MARINEMIX) was used and tested for contaminants. No contaminants were found at toxic levels.
Water Temperature 27°C ± 2°C. Must not deviate from 27°C by more than 3°C for more than 12 hours.	Target:27°C Range: 26 to 27°C
pH Must not deviate by more than one unit for more than 48 hours.	8.1 to 8.3
Salinity	25-27 parts/thousand
Dissolved Oxygen Renewal: must not drop below 50% for more than 48 hours. Flow-through: ≥ 60% throughout test.	>60% throughout test (86%-104%)
Test Vessels or Compartments 1. Material: Glass, No. 316 stainless steel, or perfluorocarbon plastics 2. Size: 250 ml with 200 ml fill volume is preferred; 100 ml with 80 ml fill volume is acceptable.	39x20x25cm glass test aquaria.
Covers Renewal: Test vessels should be covered with a glass plate. Flow-through: openings in test compartments should be covered with mesh nylon or stainless steel screen.	Nitex screens were used.

Guideline Criteria	Reported Information
Type of Dilution System Must provide reproducible supply of toxicant. Intermittent flow proportional diluters or continuous flow serial diluters should be used.	Intermittent flow proportional diluter was used.
Flow Rate Consistent flow rate of 5-10 vol/24 hours, meter systems calibrated before study and checked twice daily during test period.	10 vol/24 hours
Aeration Dilution water should be vigorously aerated, but the test tanks should not be aerated.,	Aerated vigorously for 24hrs, then aerated for an additional 24hrs.
Photoperiod 16 hours light, 8 hours dark.	16L/8D
Solvents Not to exceed 0.5 ml/L for static tests or 0.1 ml/L for flow-through tests. Acceptable solvents are dimethylformamide, triethylene glycol, methanol, acetone and ethanol.	Solvent: Acetone Maximum conc.: 0.0065 ml/L.

C. Test Design

Guideline Criteria	Reported Information
Duration 28 days/one generation	28 days
Nominal Concentrations Control(s) and at least 5 test concentrations; dilution factor not greater than 50%.	5 treatment concentrations: 4.4, 8.8, 18, 35, and 70 pptr were used as were 2 control groups (dilution and solvent).
Number of Test Organisms At least 2 test chambers containing at least 2 compartments containing at least 15 mysids per treatment/control groups. When sexually mature, pair mysids within each chamber. 20 pairs per concentration.	60 mysids per treatment level. Mysids were paired at sexual maturity (day 15).
Test organisms randomly or impartially assigned to test vessels?	Yes
Specific conductivity measured?	Yes
Water Parameter Measurements 1. Dissolved oxygen must be measured at each concentration at least once a week. 2. pH, alkalinity, hardness, and conductance must be measured once a week in one test concentration and in one control. 3. Temperature should be monitored at least hourly throughout the test in one test chamber, and near the beginning, middle and end of the test in all test chambers.	DO,pH,salinity,and temperature were measured daily in each replicate of each treatment level and the control solutions throughout the exposure period. One dilution control replicate had continuous monitoring for temperature.

Guideline Criteria	Reported Information
Chemical Analysis Needed if chemical was volatile, insoluble, or known to absorb, if precipitate formed, if containers were not steel or glass, or if flow-through system was used.	Analysis reported: 5, 7.7, 15, 28, and 57 ng ai/L (pptr). Concentrations ranged from 81% to 114% of nominal and were measured weekly.

12. REPORTED RESULTS

A. General Results

Guideline Criteria	Reported Information
Quality assurance and GLP compliance statements were included in the report?	Yes
Control Mortality ≤ 30%	85% survival was reported.
Did mysids in each control produce at least 3 young per female by test termination?	Yes
Reported MATC:	11ng ai/L
Data Endpoints - Survival of first-generation mysids, - Number of live young produced per female, - Dry weight and length of each first generation mysid alive at the end of the test, - Observations of other effects or clinical signs.	F1 survival reported. # young/female reported. Dry weight/length reported. Observations reported when noticed.
Raw data included?	Yes

Reported Effects Data

Toxicant Concentration (ng ai/L)		#exposed/ #survived (28 Days)	Mean # young per Female per Repro. Day	M/F Mean Total Length (mm)	Mean M/F Dry Weight (mg)
Nominal	Measured				
Control	0	60/51	0.46	7.4/7.0	.84/.93
Solvent Control	0	60/51	0.24	7.2/7.1	.78/.94
4.4ng/L	5.0ng/L	60/49	0.36	7.0/7.0	0.7/.92
8.8ng/L	7.7ng/L	60/50	0.32	7.2/7.1	.73/.86
18ng/L	15ng/L	60/53	0.24	6.9/6.9	.68/.85
35ng/L	28ng/L	60/43	0.16	6.8/6.8	.69/.89
70ng/L	57ng/L	60/32	0.029	6.6/6.7	.73/.82

Reported Toxicity Observations:

B. Reported Statistical Results

Most sensitive endpoint: Dry weight and length in male Mysids

Endpoint	Method	NOEC	LOEC
Survival	Williams	<57ng/L	>28ng/L
Reproduction	Williams	<57ng/L	>28ng/L
Weight	Williams	Male ≤5ng/L Female<57ng/L	Male ≤5ng/L Female>28ng/L
Length	Williams	Male <15ng/L Female<28ng/L	Male>7.7ng/L Female>15ng/L

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13. VERIFICATION OF STATISTICAL RESULTS

Most sensitive endpoint: Dry weight and length in male Mysids

Endpoint	Method	NOEC	LOEC
F1 Survival	Dunnetts/Williams	28ng/L	57ng/L
Reproduction	Dunnetts/Williams Bonferroni	28ng/L	57ng/L
Dry Weight	Williams	Male <5ng/L Female28ng/L	Male 5ng/L Female57ng/L
Length	Bonferroni Williams	Male 7.7ng/L Female15ng/L	Male 15ng/L Female28ng/L

14. REVIEWER'S COMMENTS: Reported NOEC and LOEC values on pgs. 24-25 of the study are inconsistent with results. Reported values seem to have been mis-marked.

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