JAN 10 1995

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

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OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM

Subject: Review of data for Fipronil submitted by Rhone-Poulenc, Inc. (Shaughnessy #: 129121) (D209888)

From: Anthony F. Maciorowski, Chief
Ecological Effects Branch
Environmental Fate and Effects Division (7507C)
1/6/95

To: Marion Johnson, PM 10
Registration Division (7505C)

The Ecological Effects Branch has completed the review of the data submitted by Springborn Laboratories, Inc. for Fipronil. The following is a brief summary of the data reviewed:

§72-3 (b) - 96 Hour LC_{50} Acute Toxicity Test for Eastern Oyster (*Crassostrea virginica*)

CITATION: Dionne, E. 1993. MB 46030 - Acute Toxicity to the Eastern Oyster (*Crassostrea virginica*) Under Flow-Through Conditions. SLI Report No. 93-5-4774. Prepared by Springborn Laboratories, Inc., Wareham, MA. Submitted by Rhone-Poulenc Ag Company; 2 T.W. Alexander Drive Research Triangle Park; North Carolina 27709. EPA MRID No. 432917-01.

CONCLUSIONS: This study is scientifically sound and fulfills the guideline requirements for an acute toxicity test using the Eastern oyster. Under the conditions of the test, the 96-hour EC_{50} was 0.77mg ai/L (95% c.i. 0.18 to 1.7 mg ai/L) which classifies MB 46030 (Fipronil) as highly toxic to Eastern oysters.

§72-3 (a) - 96 Hour LC_{50} Acute Toxicity Test for Sheepshead Minnow (*Cyprinodon variegatus*)

CITATION: Machado, M.W. 1993. MB 46030 - Acute toxicity to Sheepshead minnow (*Cyprinodon variegatus*) Under Flow-Through Conditions. SLI Report No. 93-6-4822. Prepared by Springborn Laboratories, Inc., Wareham, MA. Submitted by Rhone-Poulenc Ag

Company; 2 T.W. Alexander Drive Research Triangle Park; North Carolina 27709. EPA MRID No. 432917-02.

CONCLUSIONS: This study is scientifically sound and fulfills the guideline requirements for an acute toxicity test using the sheepshead minnow (*Cyprinodon variegatus*). Under the conditions of the test, the 96-hour LC_{50} was 0.13 (0.11-0.15) mg ai/L, which classifies MB 46030 (Fipronil) as highly toxic to sheepshead minnows.

§72-1 (c) - 96 Hour LC_{50} Acute Toxicity Test for Rainbow Trout (*Oncorhynchus mykiss*)

CITATION: Collins M.K. 1993. RPA 104615 - Acute Toxicity to Rainbow Trout (*Oncorhynchus mykiss*) Under Static Renewal Conditions. SLI Report No. 93-6-4822. Prepared by Springborn Laboratories, Inc., Wareham, MA. Submitted by Rhone-Poulenc Ag Company; 2 T.W. Alexander Drive Research Triangle Park; North Carolina 27709. EPA MRID No. 432917-18.

CONCLUSIONS: This study is scientifically sound and fulfills the guideline requirements for a static acute toxicity test for freshwater fish (*Oncorhynchus mykiss*) with a metabolite. Based on the test nominal concentrations the 96-hour LC_{50} is greater than 100 mg/l. This classifies RPA 104615 as practically non-toxic to rainbow trout. The NOEC is 36 mg/l.

§72-2 (a) - 96 Hour LC_{50} Acute Toxicity Test for Dapnids (*Daphnia magna*)

CITATION: Collins M.K. 1993. RPA 104615 - Acute Toxicity to Daphnids (*Daphnia magna*) Under Static Conditions. SLI Report No. 93-6-4822. Prepared by Springborn Laboratories, Inc., Wareham, MA. Submitted by Rhone-Poulenc Ag Company; 2 T.W. Alexander Drive Research Triangle Park; North Carolina 27709. EPA MRID No. 432917-19.

CONCLUSIONS: This study is scientifically sound and fulfills the guideline requirements for a static acute toxicity test for freshwater invertebrates (*Daphnia magna*) with a metabolite. Based on the test nominal concentrations the 48-hour EC_{50} is greater than 100 mg/l. This classifies RPA 104615 as practically non-toxic to *Daphnia magna*. The NOEC is 22 mg/l.

If there are any questions contact Andrew Bryceland 305-5746.

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JAN 10 1995

MRID 432917-01

DATA EVALUATION RECORD

1. CHEMICAL: MB 46030 (Fipronil)

2. TEST MATERIAL: MB 46030 (Fipronil): 5-amino-1-(2,6-dichloro-α,α,α-trifluoro-p-tolyl)-4-trifluoromethylsulfinylpyrazole-3-carbonitrile; 96.1% active ingredient. grey powder. CAS Number: 120068-37-3. Lot Number: 6ADM93.

3. STUDY TYPE: §72-3(b) Acute Toxicity Test for Estuarine and Marine Organisms (Mollusc 96-Hour Flow-Through Deposition Study). Species Tested: *Crassostrea virginica*

4. CITATION:

Author:	Emily Dionne
Title:	MB 46030 - Acute Toxicity to the Eastern Oyster (*Crassostrea virginica*) Under Flow-Through Conditions.
Date:	July 1993
Laboratory Report #:	93-5-4774
Any Other Study #:	10566.0393.6269.504
Sponsor:	Rhone-Poulenc Ag Company; 2 T.W. Alexander Drive Research Triangle Park; North Carolina 27709
Laboratory:	Springborn Laboratories, Inc.; Environmental Sciences Division; 790 Main Street; Wareham, Massachusetts 02571
MRID No.:	432917-01

5. REVIEWED BY:

Andrew C. Bryceland, Fishery Biologist
Ecological Effects Branch
Environmental Fate and Effects Division (7507C)

Signature: Andrew C. Bryceland Date: 1/4/95

6. APPROVED BY:

for Ann Stavola, Chief, Section 5
Ecological Effects Branch
Environmental Fate and Effects Division (7507C)

Signature: Allen W. Vaughan Date: 1.4.95

7. CONCLUSION

This study is scientifically sound and fulfills the guideline requirements for an acute toxicity test using the Eastern oyster. Under the conditions of the test, the 96-hour EC_{50} was 0.77mg ai/L (95% c.i. 0.18 to 1.7 mg ai/L) which classifies MB 46030 (Fipronil) as highly toxic to Eastern oysters.

8. RECOMMENDATIONS

9. BACKGROUND

10. MATERIALS AND METHODS

A. Test Organisms: Eastern Oysters

Guideline Criteria	Reported Information
Species (Scientific Name)	*Crassostrea virginica*
Mean valve height (25 - 50 mm -- the long axis)	30 mm (sd ±3 mm)
Supplier	P. Cummins Oyster Company, Pasedena, MD
All oysters from same source (yes or no)	yes
All oysters from the same year class (yes or no)	yes
Other Comments	

B. Source/Acclimation

Guideline Criteria	Reported Information
Acclimation Period (minimum 10 days)	10 days
Wild caught 7 day quarantine (yes or no)	no
Check for signs of disease or injury (yes or no, if yes describe)	yes,test organisms were held approximately 24-hours after grinding and the carefully examined for any signs of stress.
If diseased it can be treated in 48-hr pretest no sign of the disease remains (Report hours prior to test in which no sign of disease or N/A)	N/A
Was peripheral shell growth removed prior to testing? If so how much.	yes, 3-5 mm

Feeding during the acclimation	Fed a supplemental algal diet of *Isochrysis galbana* and *Tetraselmis maculata* (180 mls of 10^7 cells/ml each aquarium 3 times daily during test).
<3% mortality 48 hours prior to testing (% mortality, if any)	no mortality

C. Test System:

Guideline Criteria	Reported Information
Describe source of dilution water (natural unfiltered seawater)	Natural unfiltered seawater from Cape Cod Canal, Bourne, MA
Does water support test animals without observable signs of stress?	yes
What was the salinity of the test water?	31 to 32% ppt
Water Temperature (between 15°C and 30°C -- but must be consistent)	21°C
pH	7.6 to 7.8
Dissolved Oxygen (Static 1st 48 hrs 40%; 2nd 48 hrs 60%; Flow-through 60%) (% of lowest conc. & hour)	See Table 1, attached
Total Organic Carbon	0.45 to 4.9 mg/L from Sept. 92 to Mar. 93
Test Aquaria 1. Material (glass or stainless steel) 2. a. Static volume (18.9 L (5 gal or 19000 cc) with 15 L solution) b. Static or flow-through volume (300x600x300 = 54000 cc.)	 glass 18 L 49.5 X 25.5 X 29 cm
Type of Dilution System (Reproducible supply of toxicant)	Harvard Apparatus peristaltic pump was calibrated to deliver 0.150 ml/min of the 3.0 mg ai/ml stock solution.

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Flow rate Consistent flow rate-meter systems calibrated before study and checked 2*24 hours - 5 to 10 vol/24 hours	6 solution volume replacements per 24 hours
Biomass Loading Rate (all oysters should be able to sit on the bottom with water flowing freely around them)	yes; oysters were spaced equidistant from one another with their valve in-flow openings facing toward the flow of water.
Photoperiod (16 L & 8 D)	16 hours light and 8 hours dark
Solvents (Do not exceed 0.5 ml/L for flow-through)	0.5 ml/L acetone
Other Comments	

D. Test Design:

Guideline Criteria	Reported Information
Range Finding Test (LC_{50} >100 mg/L with 30 shrimp, no definitive test required.)	nominal concentrations tested were 0.012 to 1.5 mg/L. After 96-hours, growth reduction of 1.5 mg/L was 98%, and 11, 29 and 24% in 0.041, 0.14 and 0.45 mg/L treatments respectively. No growth reduction was observed in 0.012 mg/L.
Definitive Test	
Nominal Concentrations (control+5 treatment levels; dosage should be 60% of the next highest concentration; concentrations should be geometric series)	0.094, 0.19, 0.38, 0.75, 1.5 mg ai/L, solvent control, negative control
Controls (Minimum control mortality; static 10%; flow-through 5%	no mortality observed
Number of Test Organisms; (Minimum 10/level can be divided among containers)	20 oysters in each test aquarium (40 per treatment level)

All organisms must be randomly assigned to test vessels. (yes or no, describe if no)	yes
Biological Observations (yes or no)	yes, every 24 hours
Water Parameter Measurements 1. Temperature - record every 6 hrs;>1°C. 2. D.O. beginning,48 hrs,end for control high, medium, and low dose. 3. pH beginning,48 hrs, end for control, high, medium, and low dose.	20-21°C See Table 1 See Table 1
Chemical Analysis (needed if aeration, volatile, insoluble, precipitate, not steel or glass, known to adsorb, and flow-through) (yes or no)	Throughout the test period, a small amount of undissolved test material was present at the end of the syringe tubing; a slight foam was present in the first chemical cell.
Other Comments	

11. REPORTED RESULTS:

Guideline Criteria	Reported Information
Mean Measured Concentrations (report conc.)	0.088, 0.16, 0.35, 0.59, 1.2 mg ai/L
Recovery of Chemical (% recovery)	
Mortality & Observations (Describe observations & attach mortality tables)	No mortality at any treatment level; however sublethal effects (e.g., reduced fecal and psuedofecal production) at 1.2mg ai/l
Measurements of shell increments per control and test concentration.	See Table 3
Ratio of mean growth of test concentration to mean growth of controls. (provides percentage index of the response of the molluscs to toxicant)	See Table 4

EC_{50} = reduced shell deposition by 50% compared to the controls	EC_{50} = 0.77 mg ai/L (c.i. 0.18 to 1.7) using the mean measured concentrations
Author's Comments	See tables 2 & 3, attached

12. STUDY AUTHOR'S CONCLUSIONS / QUALITY ASSURANCE MEASURES:

Based on the test data, the 96-hour EC_{50} was calculated by linear regression to be 0.77 mg ai/L (c.i. 0.18 to 1.7). Based on these results and criteria established be the U.S. Environmental Protection Agency (1985), MB 46030 (Fipronil) would be classified as highly toxic to *Crassostrea virginica*.

Quality assurance and good laboratory practice statements were included in the report, indicating that the study was conducted in accordance with U.S. EPA Good Laboratory Practices Regulations set forth in FIFRA 40 CFR Part 160.

13. REVIEWER'S DISCUSSION AND INTERPRETATION

A. Test Procedure:

This study meets the guideline requirements for an acute aquatic marine mollusk study with eastern oyster.

B. Statistical Analysis

Guideline Criteria	Reported Information
Binomial (yes, no, or not reported)	no; (EPA) Binom. unreliable; EC50 = 0.819 mg ai/L
Moving Average Angle (yes, no, or not reported)	no; (EPA) EC50 = 0.814 mg ai/L (ci: 0.75 - 0.89 mg ai/L)
Probit (yes, no, or not reported)	no; (EPA) EC50 = 0.801 mg ai/L (ci: 0 to infinity)
Williams Test	no
Linear Regression Analysis	EC50 = 0.77 mg ai/L (95% ci: 0.18 - 1.7 mg ai/L)

C. Discussion/Results:

This study is scientifically sound and fulfills the guideline requirements for an acute toxicity test using the Eastern oyster. Under the conditions of the test, the 96-hour EC_{50} was 0.77mg ai/L (95% c.i. 0.18 to 1.7 mg ai/L) which classifies MB 46030 (Fipronil) as highly toxic to Eastern oysters.

D. Adequacy of the Study:

1. Classification: Core

2. Rational: N/A

3. Reparability: N/A

14. COMPLETION DATE OF ONE-LINER FOR STUDY: 10-5-94

Final Review

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_____ Sales or other commercial/financial information.

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JAN 10 1995

MRID 432917-02

DATA EVALUATION RECORD

1. CHEMICAL: MB 46030 (Fipronil)

2. TEST MATERIAL: MB 46030 (Fipronil): 5-amino-1-(2,6-dichloro-α,α,α-trifluoro-p-tolyl)-4-trifluoromethylsulfinylpyrazole-3-carbonitrile; 96.1% active ingredient. fine white powder. CAS Number: 120068-37-3. Lot Number: 6ADM93.

3. STUDY TYPE: §72-3 Estuarine Fish 96-hour Acute Toxicity Test. Species Tested: *Cyprinodon variegatus*

4. CITATION:

Author:	Mark W. Machado
Title:	MB 46030 - Acute toxicity to Sheepshead minnow (*Cyprinodon variegatus*) under flow-through conditions.
Date:	23 November 1993
Laboratory Report #:	93-6-4822
Any Other Study #:	10566.0393.6267.505
Sponsor:	Rhone-Poulenc Ag Company; 2 T.W. Alexander Drive Research Triangle Park; North Carolina 27709
Laboratory:	Springborn Laboratories, Inc.; Environmental Sciences Division; 790 Main Street; Wareham, Massachusetts 02571
MRID No.:	432917-02

5. REVIEWED BY:

Andrew C. Bryceland, Fishery Biologist
Ecological Effects Branch
Environmental Fate and Effects Division (7507C)

Signature: [signature]
Date: 1/4/95

6. APPROVED BY:

Ann Stavola, Chief, Section 5
Ecological Effects Branch
Environmental Fate and Effects Division (7507C)

Signature: Allen W. Vaughan
Date: 1.4.95

7. CONCLUSION

This study is scientifically sound and fulfills the guideline requirements for an acute toxicity test using the sheepshead minnow (*Cyprinodon variegatus*). Under the conditions of the test, the 96-hour LC_{50} was 0.13 (0.11-0.15) mg ai/L, which classifies MB 46030 (Fipronil) as highly toxic to sheepshead

minnows.

8. RECOMMENDATIONS

9. BACKGROUND

10. MATERIALS AND METHODS

A. Test Organisms: Sheepshead minnow

Guideline Criteria	Reported Information
Species (Scientific Name)	*Cyprinodon variegatus*
Mean Weight (0.5-5 grams)	0.29 (0.21 to 0.38) grams
Mean Length(S.L. longest not > 2x shortest	26 (21 to 32) mm
Supplier	Aquatic Biosystems
All fish from same source (yes or no)	yes
All fish from the same year class (yes or no)	yes
Other Comments	

B. Source/Acclimation

Guideline Criteria	Reported Information
Acclimation Period (minimum 14 days)	14 days
Wild caught 7 day quarantine (yes or no)	no
Check for signs of disease or injury (yes or no, if yes describe)	no, only checked for mortality
If diseased it can be treated in 48-hr pretest no sign of the disease remains (Report hours prior to test in which no sign of disease or N/A)	no mortality in fish 48 hours prior to start of the test.

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No feeding during the study (When last fed)	48 hours prior to testing.
<3% mortality 48 hours prior to testing (% mortality, if any)	0% mortality prior to testing.

C. Test System:

Guideline Criteria	Reported Information
Describe source of dilution water (prefer soft reconstituted water)	Collected from Cape Cod Canal, Bourne, MA. Seawater was then passed through a series of polypropylene core filters & then recirculated within an epoxy-lined concrete reservoir prior to use.
Does water support test animals without observable signs of stress?	yes
Salinity of water used. (reconstituted seawater of 30-34% salinity) (weekly range of salinity is less than 6%)	31 to 32%
Water Temperature (22 ± 1)	22 ± 1
pH (8.0-8.3 for marine-stenohaline fish and 7.7-8.0 for estuarine-euryhaline fish species) (monthly range is less than 0.8 of a pH unit)	7.9-8.0
Dissolved Oxygen (Static 1st 48 hrs 40%; 2nd 48 hrs 60%; Flow-through 60%) (% of lowest conc. & hour)	75 to 96% in all test and controls vessels over 96 hours of testing.

Test Aquaria 1. Material (glass or stainless steel) 2. a. Static volume (18.9 L (5 gal or 19000 cc) with 15 L solution) b. Static or flow-through volume (300x600x300 = 54000 cc.)	each glass test aquaria measured 39X20X25 cm.
Type of Dilution System (Reproducible supply of toxicant)	flow-through, reproducible. Tested prior to and after testing.
Flow rate Consistent flow rate-meter systems calibrated before study and checked 2*24 hours - 5 to 10 vol/24 hours	Flow of exposure to each test aquarium was approx. 50 ml/min., which equaled approx. 6.5 volume replacements per 24 hours per aquarium.
Biomass Loading Rate (Static no > 0.8 g/L ≤ 17°C; >17°C 0.5g/L; Flow-through 1 g/L/24	0.040 g/L of flowing test solution per day.
Photoperiod (16 L & 8 D)	16 hours light, 8 hours dark.
Solvents 1. (Do not exceed 0.5 ml/L for static tests) 2. (Do not exceed 0.1 ml/L for flow-through)	0.40 ml/ml
Other Comments	

D. Test Design:

Guideline Criteria	Reported Information
Range Finding Test (LC_{50} >100 mg/L with 30 fish, no definitive test required.)	0.097, 0.16, 0.27, 0.45, & 0.75 mg ai/L plus controls
Definitive Test	

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Nominal Concentrations (control+5 treatment levels; dosage should be 60% of the next highest concentration; concentrations should be geometric series)	0.097, 0.16, 0.27, 0.45, & 0.75 mg/l
Controls (Minimum control mortality; static 10%; flow-through 5%	0% mortality observed in the solvent control, 0% mortality in non-solvent control.
Number of Test Organisms; (Minimum 10/level can be divided among containers)	20 fish (10 per test aquaria) per concentration and controls were used.
All organisms must be randomly assigned to test vessels. (yes or no, describe if no)	impartially selected.
Biological Observations (yes or no)	yes, at test initiation and every 24 hours.
Water Parameter Measurements 1. Temperature - record every 6 hrs;>1°C. 2. D.O. beginning,48 hrs,end for control high, medium, and low dose. 3. pH beginning,48 hrs, end for control, high, medium, and low dose.	recorded continuously in replicate A aquaria. pH, temperature, and DO were measured in each replicate vessel daily throughout exposure period.
Chemical Analysis (needed if aeration, volatile, insoluble, precipitate, not steel or glass, known to adsorb, and flow-through) (yes or no)	yes, sample from each replicate solution of high, medium, and low treatment levels and dilution water control analyzed twice prior to definitive test. In addition, water samples were taken both replicate test solutions of each treatment level and the controls at 0-hour and 96 -hours of exposure for analysis.
Other Comments	

11. REPORTED RESULTS:

Guideline Criteria	Reported Information

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Mean Measured Concentrations (report conc.)	0.11, 0.15, 0.24, 0.34, & 0.66 mg ai/L (Table 2, attached)
Recovery of Chemical (% recovery)	92% of nominal concentrations
Mortality & Observations (Describe observations & attach mortality tables)	At 96 hours 100% mortality was observed at 0.15, 0.24, 0.34, & 0.66 mg/L. 0.11 mg/l all fish showed complete loss of equilibrium. (Table 3, attached)
Author's Comments	

12. STUDY AUTHOR'S CONCLUSIONS / QUALITY ASSURANCE MEASURES:

The 96-hour LC_{50} for MB 46030 (Fipronil) was determined to be 0.13 (0.11-0.15) mg ai/L. The NOEC (no observable effect concentration) was less than 0.11 mg/l.

Quality Assurance and Good Laboratory Practice statements were included in the report, indicating that the study was conducted in accordance with U.S. EPA Good Laboratory Practice Standards, 40 CFR Part 160.

13. REVIEWER'S DISCUSSION AND INTERPRETATION

A. Test Procedure:
This study meets the guideline requirements for and acute aquatic marine study with the sheepshead minnow.

B. Statistical Analysis

Guideline Criteria	Reported Information
Binomial (yes, no, or not reported)	yes, 96-hour LC_{50} = 0.13 (C.I. 0.11 - 0.15) mg ai/L
Moving Average Angle (yes, no, or not reported)	no
Probit (yes, no, or not reported)	no
Other Comments	

C. Discussion/Results:

This study is scientifically sound and fulfills the guideline requirements for an acute toxicity test using the sheepshead minnow (*Cyprinodon variegatus*). Under the conditions of the test, the 96-hour LC_{50} was 0.13 (0.11-0.15) mg ai/L, which classifies MB 46030 (Fipronil) as highly toxic to sheepshead minnows.

D. Adequacy of the Study:

1. Classification: Core

2. Rational: N/A

3. Reparability: N/A

14. COMPLETION DATE OF ONE-LINER FOR STUDY: 9-29-94

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Pages 11 through 13 are not included in this copy.

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BRYCELAND MB46030 (FIPRONIL) SHEEPSHEAD. FLOWTHROUGH ACUTE LC50

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CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB.(PERCENT)
.66	20	20	100	9.536742E-05
.34	20	20	100	9.536742E-05
.24	20	20	100	9.536742E-05
.15	20	20	100	9.536742E-05
.11	20	0	0	9.536742E-05

THE BINOMIAL TEST SHOWS THAT .11 AND .15 CAN BE USED AS STATISTICALLY SOUND CONSERVATIVE 95 PERCENT CONFIDENCE LIMITS, BECAUSE THE ACTUAL CONFIDENCE LEVEL ASSOCIATED WITH THESE LIMITS IS GREATER THAN 95 PERCENT.

AN APPROXIMATE LC50 FOR THIS SET OF DATA IS .1284523

WHEN THERE ARE LESS THAN TWO CONCENTRATIONS AT WHICH THE PERCENT DEAD IS BETWEEN 0 AND 100, NEITHER THE MOVING AVERAGE NOR THE PROBIT METHOD CAN GIVE ANY STATISTICALLY SOUND RESULTS.

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JAN 10 1995

MRID 432917-18

DATA EVALUATION RECORD

1. CHEMICAL: RPA 104615 (Fipronil) photometabolite

2. TEST MATERIAL: 5-amino-3-cyano-1-(2,6-dichloro-4-trifluoromethylphenyl) pyrazole-4-sulfonic acid, potassium salt, Lot# EA3133RF1, 94.7% active ingredient (a.i.), a white powder

3. STUDY TYPE: §72-1 (c). Acute Toxicity for Freshwater Fish. Species Tested: *Oncorhynchus mykiss*.

4. CITATION:

Author:	Maura K. Collins
Title:	RPA 104615 - Acute Toxicity to Rainbow Trout (*Oncorhynchus mykiss*) Under Static Renewal Conditions
Date:	April 1993
Laboratory Report #:	92-9-4428
Any Other Study #:	10566.0792.6246.103
Sponsor:	Rhone-Poulenc Ag, 2 T.W. Alexander Dr., Research Triangle Park, North Carolina 27709
Laboratory:	Springborn Laboratories, Inc. Environmental Sciences Division, 790 Main Street, Wareham, Massachusetts, 02571.
MRID No.:	432917-18

5. REVIEWED BY:

Andrew C. Bryceland, Fishery Biologist
Ecological Effects Branch
Environmental Fate and Effects Division (7507C)

Signature: [signature]
Date: 1/4/95

6. APPROVED BY:

Ann Stavola, Chief, Section 5
Ecological Effects Branch
Environmental Fate and Effects Division (7507C)

Signature: Allen W. Vaughan
Date: 1-4-95

7. CONCLUSION: This study is scientifically sound and fulfills the guideline requirements for a static acute toxicity test for freshwater fish (*Oncorhynchus mykiss*). Based on the test nominal concentrations the 96-hour LC_{50} is greater than 100 mg/l. This classifies RPA 104615 as practically non-toxic to rainbow trout. The NOEC is 36 mg/l.

8. RECOMMENDATIONS: N/A

9. BACKGROUND: N/A

10. MATERIALS AND METHODS: N/A

A. Test Organisms:

Guideline Criteria	Reported Information
Species (Scientific Name)	*Oncorhynchus mykiss*
Mean Weight (0.5-5 grams)	0.85 g (range 0.4 - 1.1g)
Mean Length(S.L. longest not > 2x shortest	45 mm (range 36 - 52mm)
Supplier	Mount Lassen Trout Farm
All fish from same source (yes or no)	yes
All fish from the same year class (yes or no)	yes
Other Comments	N/A

B. Source/Acclimation

Guideline Criteria	Reported Information
Acclimation Period (minimum 14 days)	14 days
Wild caught 7 day quarantine (yes or no)	no
Check for signs of disease or injury (yes or no, if yes describe)	no
If diseased it can be treated in 48-hr pretest no sign of the disease remains (Report hours prior to test in which no sign of disease or N/A)	N/A
No feeding during the study (When last fed)	fed daily except 24 hrs. prior to testing
<3% mortality 48 hours prior to testing (% mortality, if any)	0.2% mortality was observed

C. Test System:

Guideline Criteria	Reported Information
Describe source of dilution water (prefer soft reconstituted water)	soft water, reconstituted from deionized water.
Does water support test animals without observable signs of stress?	yes
Was dechlorinated water used (not recommended)	no
Water Temperature (Warm water-17°C or 22°C) (Cold water-12°C)	Daily 11-13°C Continuous 10-13°C (Waterbath)
pH	7.1 - 7.4
Dissolved Oxygen (Static 1st 48 hrs 60%; 2nd 48 hrs 40%; Flow-through 60%) (% of lowest conc. & hour)	33 - 104% (33% was just before renewal at 48 hrs.)
Total hardness (40 to 48 mg/L as $CaCO_3$ well water)	38 mg/l
Total Alkalinity	23 mg/l
Specific Conductance	110 µS/cm
Total Organic Carbon	2.2 mg/l
Test Aquaria 1. Material (glass or stainless steel) 2. a. Static volume (18.9 L (5 gal or 19000 cc) with 15 L solution) b. Static or flow-through volume (300x600x300 = 54000 cc.)	1. 18.9 liter glass aquaria 2. a. Static vol. 15 liters of test solution, 18.4 cm depth, 819 cm^2 surface area, 15069.6 cm. Following 48 hrs; 12 liters, depth 15.2 liters
Type of Dilution System (Reproducible supply of toxicant)	Static renewal (test solutions renewed at 48 hrs.)

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Flow rate Consistent flow rate-meter systems calibrated before study and checked 2*24 hours - 5 to 10 vol/24 hours	N/A
Biomass Loading Rate (Static no > 0.8 g/L ≤ 17°C; >17°C 0.5g/L; Flow-through 1 g/L/24	0.71 g biomass/l
Photoperiod (16 L & 8 D)	16 hrs light/8 hrs dark (970 lux) Sudden transitions from light to dark were avoided.
Solvents 1. (Do not exceed 0.5 ml/L for static tests) 2. (Do not exceed 0.1 ml/L for flow-through)	N/A
Other Comments	

D. Test Design:

Guideline Criteria	Reported Information
Range Finding Test (LC_{50} >100 mg/L with 30 fish, no definitive test required.)	Treatment 100, 10, 1.0, 0.4, and 0.1 mg a.i./l; no mortality or sublethal effects
Definitive Test	
Nominal Concentrations (control+5 treatment levels; dosage should be 60% of the next highest concentration; concentrations should be geometric series)	Control, 13, 22, 36, 60, & 100 mg a.i./l
Controls (Minimum control mortality; static 10%; flow-through 5%	zero control mortality
Number of Test Organisms; (Minimum 10/level can be divided among containers)	10 organisms per replicate; one replicate per treatment level and control
All organisms must be randomly assigned to test vessels. (yes or no, describe if no)	yes

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Biological Observations (yes or no)	yes (Table 2, attached)
Water Parameter Measurements 1. Temperature - record every 6 hrs;>1°C. 2. D.O. beginning,48 hrs,end for control high, medium, and low dose. 3. pH beginning,48 hrs, end for control, high, medium, and low dose.	1. Temp. - every 24 hrs (all test aquaria); continuous for waterbath; 11-13°C. 2. D.O. and pH see Table 1, attached
Chemical Analysis (needed if aeration, volatile, insoluble, precipitate, not steel or glass, known to adsorb, and flow-through) (yes or no)	No chemical analysis; No undissolved test material was observed
Other Comments	

11. REPORTED RESULTS:

Guideline Criteria	Reported Information
Mean Measured Concentrations (report conc.)	N/A (static renewal)
Recovery of Chemical (% recovery)	N/A
Mortality & Observations (Describe observations & attach mortality tables)	10% mortality in the 60 mg a.i./l treatment; 1 of the surviving fish of this treatment exhibited darkened pigmentation.
Author's Comments	

12. STUDY AUTHOR'S CONCLUSIONS / QUALITY ASSURANCE MEASURES: The LC_{50} for RPA 104615 (Fipronil) was determined to be greater than 100 mg a.i./l. The NOEC (no observable effect) was 36 mg a.i./l.

Quality Assurance and Good Laboratory Practice statements were included in the report, indicating that the study was conducted in accordance with U.S. EPA Good Laboratory Practice Regulations set forth under FIFRA.

13. REVIEWER'S DISCUSSION AND INTERPRETATION

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A. Test Procedure:

The following items did not meet the guideline criteria:

1. Dissolved oxygen dropped below 40% at the end of 48hrs just prior to the renewal. The SEP states that "The DO level during the first 48 hours of testing should remain between 60% and 100% of saturation and 40% and 100% saturation after 48 hours". (Table 1, attached)

2. Due to the limited availability of the test material, exposure vessel contained 12 liters of test solution after the 48 hour renewal. The SEP states that test vessels frequently contain 15 liters of test solution. The study chambers did not contain the recommended volume of test solution after the 48 hour renewal.

B. Statistical Analysis

Guideline Criteria	Reported Information
Binomial (yes, no, or not reported)	no
Moving Average Angle (yes, no, or not reported)	no
Probit (yes, no, or not reported)	no
Other Comments	The LC50 was greater than the highest test concentration.

C. Discussion/Results: This study is scientifically sound and fulfills the guideline requirements for a static acute toxicity test for freshwater fish (*Oncorhynchus mykiss*). Based on the test nominal concentrations the 96-hour LC_{50} is greater than 100 mg/l. This classifies RPA 104615 as practically non-toxic to rainbow trout. The NOEC is 36 mg/l.

D. Adequacy of the Study:

1. Classification: Core.

2. Rational: N/A

3. Reparability: N/A

14. COMPLETION DATE OF ONE-LINER FOR STUDY: 9/13/94

Final Review

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Pages 32 through 33 are not included in this copy.

The material not included contains the following type of information:

_____ Identity of product inert ingredients.

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_____ Sales or other commercial/financial information.

_____ A draft product label.

_____ The product confidential statement of formula.

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JAN 10 1995

DATA EVALUATION RECORD

432917-19

1. CHEMICAL: RPA 104615 (Fipronil) Photometabolite

2. TEST MATERIAL: 5-amino-3-cyano-1-(2,6-dichloro-4-trifluoromethylphenyl) pyrazole-4-sulfonic acid, potassium salt. Lot# EA3133RF1. 94.7% active ingredient (a.i.). white powdery solid.

3. STUDY TYPE: §72-2 (b). Acute Toxicity for Freshwater Invertebrates. Species Tested: *Daphnia magna*.

4. CITATION:

Author:	Maura K. Collins
Title:	RPA 104615 - Acute Toxicity to Daphnids (*Daphnia magna*) Under Static Conditions.
Laboratory Report #:	92-9-4430
Any Other Study #:	10566.0792.6245.110
Test Date:	August 1992
Sponsor:	Rhone-Polenc Ag Company; 2 T.W. Alexander Drive; Research Triangle Park; North Carolina 27709
Laboratory:	Springborn Laboratories, Inc.; Environmental Sciences Division; 790 Main Street; Wareham, Massachusetts 02571
MRID No.:	432917-19

5. REVIEWED BY:

Andrew C. Bryceland, Fishery Biologist
Ecological Effects Branch
Environmental Fate and Effects Division (7507C)

Signature: [signature]
Date: 1/4/95

6. APPROVED BY:

for Ann Stavola, Chief, Section 5
Ecological Effects Branch
Environmental Fate and Effects Division (7507C)

Signature: Allen W. Vaughan
Date: 1.4.95

7. CONCLUSION: This study is scientifically sound and fulfills the guideline requirements for a static acute toxicity test for freshwater invertebrates (*Daphnia magna*). Based on the test nominal concentrations the 48-hour EC_{50} is greater than 100 mg/l. This classifies RPA 104615 as practically non-toxic to *Daphnia magna*. The NOEC is 22 mg/l.

8. RECOMMENDATIONS: N/A

9. BACKGROUND: N/A

10. MATERIALS AND METHODS: N/A

A. Test Organisms:

Guideline Criteria	Reported Information
Species (Scientific Name)	*Daphnia magna*
All organisms should be approximately the same size and weight.	No notation of organism weight or length.
Immature organism should be used. Daphnids 1st (<24hrs). Amphipods, stoneflies, and mayflies in 2nd instar; midges 2nd & 3th instar	≤24 hours
Supplier	Laboratory cultures from Springborn Laboratories
All organisms from same source (yes or no)	yes
Other Comments	

B. Source/Acclimation

Guideline Criteria	Reported Information
Acclimation Period (minimum 7 days)	Water and lighting were the same for both culturing and testing.
Wild caught 7 day quarantine (yes or no)	N/A
Check for signs of disease or injury (yes or no, if yes describe)	No notation made for checking for disease.
If diseased it can be treated in 48-hr pretest no sign of the disease remains (Report hours prior to test in which no sign of disease or N/A)	
No feeding during the study (When last fed)	Daphnids were not fed during the test. Report does not indicate when the organisms were last fed before the test.

<3% mortality 48 hours prior to testing (% mortality, if any)	

C. Test System:

Guideline Criteria	Reported Information
Describe source of dilution water (prefer soft reconstituted water)	Fortifying well water based on the formula for hard water (ASTM 1980) and filtering it through an Amberlite XAD-7 resin column to remove organic contaminants.
Does water support test animals without observable signs of stress?	yes
Was dechlorinated water used (not recommended)	no
Water Temperature (Daphnia-20°C) (Amphipods and mayflies-17°C) (Midges and mayflies-22°C) (Stoneflies-12°C)	21-23°C (Table 1,attached)
pH	8.3-8.6 (Table 1,attached)
Dissolved Oxygen (Static 1st 48 hrs 60%; 2nd 48 hrs 40%; Flow-through 60%) (% of lowest conc. & hour)	8.0-8.6 (Table 1,attached)
Total hardness (40 to 48 mg/L as $CaCO_3$ well water)	Control: 170 mg/l, 100 mg/l: 160 mg/l
Test Aquaria 1. Material (glass or stainless steel) 2. a. Small organisms (3.9 L (1 gal) with 2 to 3 L solution) b. Daphnids and midges (250 ml glass beakers 200mls of test solution)	1. glass beakers 2. 250 ml beakers with 200 mls of test solution

Type of Dilution System (Reproducible supply of toxicant)	N/A
Flow rate Consistent flow rate-meter systems calibrated before study and checked 2*24 hours - 5 to 10 vol/24 hours	N/A static test
Biomass Loading Rate (Static no > 0.8 g/L ≤ 17°C; >17°C 0.5g/L; Flow-through 1 g/L/24 & must not be >10 g/L at any time at or below 17°C or 5 g/L at higher temperatures.	N/A
Photoperiod (16 L & 8 D)	16 light/8 dark (750 lux)
Solvents 1. (Do not exceed 0.5 ml/L for static tests) 2. (Do not exceed 0.1 ml/L for flow-through)	N/A
Other Comments	

D. Test Design:

Guideline Criteria	Reported Information
Range Finding Test (LC_{50} >100 mg/L with 30 individuals, no definitive test required.)	100, 10, 1.0, 0.40, 0.10 mg/l, control; EC_{50}>100mg/l
Definitive Test	
Nominal Concentrations (control+5 treatment levels; dosage should be 60% of the next highest concentration; concentrations should be geometric series)	100, 60, 36, 22, 13 mg/l, and control
Controls (Minimum control mortality; static 10%; flow-through 5%	zero mortality

Number of Test Organisms; (Minimum 20/level can be divided among containers)	4 daphnids per replicate; 4 replicates per concentration
All organisms must be randomly assigned to test vessels. (yes or no, describe if no)	yes
Water Parameter Measurements 1. Temperature - record every 6 hrs;>1°C. 2. D.O. beginning,48 hrs,end for control high, medium, and low dose. 3. pH beginning,48 hrs, end for control, high, medium, and low dose.	1. Temperature continuously monitored in waterbath. 2. D.O. was measured daily in replicate A in each test concentration and control. 3. pH was measured daily in replicate A in each test concentration and control.
Chemical Analysis (needed if aeration, volatile, insoluble, precipitate, not steel or glass, known to adsorb, and flow-through) (yes or no)	No aeration, undissolved material, and chemical analyses were performed Static test
Other Comments	

11. REPORTED RESULTS:

Guideline Criteria	Reported Information
Mean Measured Concentrations (report conc.)	N/A (no chemical analyses)
Recovery of Chemical (% recovery)	N/A
Mortality & Observations (Describe observations & attach mortality tables)	No Mortality; 100 mg/l - all daphnids were lethargic. 60 and 36 mg/l - several daphnids were lethargic and on bottom of test vessel. (see Table 2, attached)
Author's Comments	

12. STUDY AUTHOR'S CONCLUSIONS / QUALITY ASSURANCE MEASURES: The LC_{50} for RPA 104615 (Fipronil) was determined to be greater than 100 mg/l. The NOEC (no observable effect concentration) was 22 mg/l.

Quality Assurance and Good Laboratory Practice statements were included in the report, indicating that the study was conducted in accordance with U.S. EPA Good Laboratory Practice Standards, 40 CFR Part 160.

13. REVIEWER'S DISCUSSION AND INTERPRETATION

A. Test Procedure:

The following items did not meet the guideline criteria:

1. Test temperature ranged from 21 to 23°C. The recommended test temperature for daphnids is 20 ± 1°C.

2. Dissolve oxygen was only monitored in replicate A for each test concentration and control

B. Statistical Analysis

Guideline Criteria	Reported Information
Binomial (yes, no, or not reported)	no
Moving Average Angle (yes, no, or not reported)	no
Probit (yes, no, or not reported)	no
Other Comments	No mortality occurred in any concentration or control.

C. Discussion/Results: This study is scientifically sound and fulfills the guideline requirements for a static acute toxicity test for freshwater invertebrates (*Daphnia magna*). Based on the test nominal concentrations the 48-hour EC_{50} is greater than 100 mg/l. This classifies RPA 104615 as practically non-toxic to *Daphnia magna*. The NOEC is 22 mg/l.

D. Adequacy of the Study:

1. Classification: Core

2. Rational: N/A

3. Reparability: N/A

14. COMPLETION DATE OF ONE-LINER FOR STUDY: 9/26/94

Fipanil derived

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