

8-29-95

DATA EVALUATION RECORD

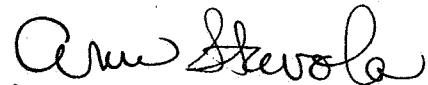
1. CHEMICAL: MB46030 Fipronil
2. TEST MATERIAL: MB 46030 (Fipronil): 5-amino-1-(2,6-dichloro- α,α,α -trifluoro-p-tolyl)-4-trifluoromethylsulfinylpyrazole-3-carbonitrile; 96.1% TGAI, grey powder, Batch Number 6ADM93.
3. STUDY TYPE: §72-3 Acute Toxicity Test for Estuarine and Marine Organisms (Shrimp 96-Hour Acute Toxicity Test).
Species Tested: *Mysidopsis bahia*
4. CITATION:

Author: Mark W. Machado
Title: MB 46030 - Acute Toxicity To Mysids
(*Mysidopsis bahia*) Under Static Conditions
Date: 29 March 1994
Laboratory Report #: 94-4-5224
Any Other Study #: 10566.0394.6340.510
Sponsor: Rhone-Poulenc Ag Company
Laboratory: Springborn Laboratories, Inc. Wareham, MA
MRID No.: 432797-01

5. REVIEWED BY:

N.E. Federoff, Wildlife Biologist Signature: 
Ecological Effects Branch
Environmental Fate and Effects Division (7507C) Date: 8/29/95

6. APPROVED BY:

Ann Stavola, Chief, Section 5 Signature: 
Ecological Effects Branch
Environmental Fate and Effects Division (7507C) Date: 8/29/95

7. CONCLUSION

This study is scientifically sound and fulfills the guideline requirements for an acute toxicity test on mysid shrimp. The EEB has reviewed the rebuttal by Rhone-Poulenc regarding the EPA evaluation of the Mysid Acute Study (MRID# 432797-01) prepared by Springborn Laboratories for the registration of the chemical Fipronil. After review of the comments, the EEB concludes that the study should be upgraded to the designation of CORE. EEB's main concern was chemical contamination in the negative control group. This in itself will normally invalidate a study. Although the chemical was reported in the negative control solution at test

termination, the chemical was absent at test initiation. The reported concentration (approximately 15.5 pptr) was 4X less than the lowest reported exposure level (62 pptr). Although the NOEC was less than 62 pptr, no mortality was reported in the contaminated negative control group. The 96-hour EC50 was 140 pptr which classifies Fipronil as very highly toxic to mysid shrimp. The EEB concludes, that by repeating the study, no new information would be gained.

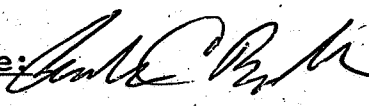
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MRID No.: 432979-01

5. REVIEWED BY:

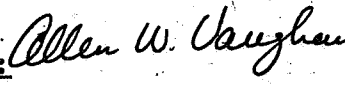
Andrew C. Bryceland, Fishery Biologist
Ecological Effects Branch
Environmental Fate and Effects Division (7507C)

Signature: 

Date: 1/4/95

6. APPROVED BY:

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

Signature: 

Date: 1.4.95

7. CONCLUSION

This study is scientifically sound but does not fulfill the guideline requirements for an acute toxicity test on mysid shrimp. The reasons for this conclusion are the following; negative control contamination, test temperature was too high (25 to 26°C), and the study chambers were too small with an insufficient amount of test solution (1 liter glass beakers with 1 liter test solution). The 96-hour EC_{50} was 140 ng ai/L (95% c.i.; 120 to 160 ng ai/L), which classifies Fipronil (MB 46030) as being very highly toxic to mysid shrimp. The NOEC is less than 62 ng ai/L.

8. RECOMMENDATIONS

9. BACKGROUND

10. MATERIALS AND METHODS

A. Test Organisms: Mysid Shrimp

Guideline Criteria	Reported Information
Species (Scientific Name)	<i>Mysidopsis bahia</i>
Mean Weight (> 0.5 grams)	≤ 24 hours old
Supplier	Aquatic Biosystems, Ft. Collins, Colorado
All shrimp from same source (yes or no)	yes
All shrimp from the same year class (yes or no)	yes
Other Comments	

B. Source/Acclimation

Guideline Criteria	Reported Information
Acclimation Period (minimum 10 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)	Information not available
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)	Fed once daily throughout the study
<3% mortality 48 hours prior to testing (% mortality, if any)	Information not available

C. Test System:

Guideline Criteria	Reported Information
Describe source of dilution water	Seawater collected from Cape Cod Canal, Bourne, MA
Does water support test animals without observable signs of stress?	yes
What was the salinity of the water used? (30-34% ppt for marine (stenohaline) shrimp and 10-17% ppt for estuarine (euryhaline) shrimp.	30-32%
Water Temperature (22°C)	25 ± 1°C Waterbath 25 - 26°C Daily test chamber
pH 8.0-8.3 marine (stenohaline) shrimp 7.7-8.0 estuarine (euryhaline) shrimp	7.7-7.8
Dissolved Oxygen (Static 1 st 48 hrs 40%; 2 nd 48 hrs 60%; Flow-through 60%) (% of lowest conc. & hour)	78-100% saturation
Total Organic Carbon	<2.0 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 liter glass beakers with 1 liter of test solution. 2.7 cm depth.
Type of Dilution System (Reproducible supply of toxicant)	yes
Flow rate Consistent flow rate-meter systems calibrated before study and checked 2*24 hours - 5 to 10 vol/24 hours	N/A Study under static conditions.

Biomass Loading Rate (Static no > 0.8 g/L ≤ 17°C; >17°C 0.5 g/L; Flow-through 1 g/L/24)	0.0033 g biomass/L
Photoperiod (16 L & 8 D)	16 light and 8 dark
Solvents 1. (Do not exceed 0.5 ml/L for static tests) 2. (Do not exceed 0.1 ml/L for flow-through)	0.10 ml/L
Other Comments	

D. Test Design:

Guideline Criteria	Reported Information
<u>Range Finding Test</u> (LC ₅₀ >100 mg/L with 30 shrimp, no definitive test required.)	13, 22, 36, 60, 100 ng ai/L 15% mortality 100 @ ng ai/L
<u>Definitive Test</u>	
Nominal Concentrations (control+5 treatment levels; dosage should be 60% of the next highest concentration; concentrations should be geometric series)	61, 100, 170, 280, 470 ng ai/L
Controls (Minimum control mortality; static 10%; flow-through 5%)	0% in negative control 5% in solvent control
Number of Test Organisms; (Minimum 20/level can be divided among containers)	20 /test concentration and controls
All organisms must be randomly assigned to test vessels. (yes or no, describe if no)	yes
Biological Observations (yes or 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.	Temp. continuously measured in the surrounding waterbath. Temp. measured in all other test and control vessels daily. For DO and pH see Table 1
Chemical Analysis (needed if aeration, volatile, insoluble, precipitate, not steel or glass, known to adsorb, and flow-through) (yes or no)	No visible signs of undissolved test material
Other Comments	

11. REPORTED RESULTS:

Guideline Criteria	Reported Information
Mean Measured Concentrations (report conc.)	62, 97, 140, 240, 390 ng ai/L
Recovery of Chemical (% recovery)	
Mortality & Observations (Describe observations & attach mortality tables)	See Table 4
Author's Comments	Repl. A of the solvent control showed 10% mortality. Negative control showed 16 ng ai/l of test substance.

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

No conclusions were made.

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 except for the following:

Routine water and food contaminant screening analyses for pesticides, PCBs, and metals were conducted using standard U.S. EPA procedures by Lancaster Laboratories, Lancaster, PA. These data were not collected in accordance with GLP procedures (i.e., no distinct protocol, Study director,

etc.). Stability, characterization, and verification are the responsibility of the Study Sponsor. Total organic carbon analyses for filtered seawater conducted by Galbraith Laboratories, Knoxville, Tennessee, utilized standard U.S. EPA procedures, but were not conducted in accordance GLP procedures.

13. REVIEWER'S DISCUSSION AND INTERPRETATION

A. Test Procedure:

The following items did not meet the guideline criteria:

1. Negative control contamination.
2. No notation of acclimation observations prior to testing for disease or mortality.
3. Test temperature was too high (25 to 26°C). SEP states a test temperature of 22°± 1°C.
4. Study chambers were 1 liter glass beakers with 1 liter test solution. Smaller than SEP recommendation. SEP states; "For static tests larger organisms (0.5 g each or larger) should be exposed in 19.6 liter containers with 15 liters of solution. Smaller organisms may be exposed in 3.9 liter containers with two or three liters of solution."

B. Statistical Analysis

Guideline Criteria	Reported Information
Binomial (yes, no, or not reported)	
Moving Average Angle (yes, no, or not reported)	
Probit (yes, no, or not reported)	yes, 140 ng a.i./L 95% CI 120 to 160 ng a.i./L
Other Comments -- study used nonlinear interpolation	

C. Discussion/Results:

This study is scientifically sound but does not fulfill the guideline requirements for an acute toxicity test on mysid shrimp. The 96-hour EC₅₀ was 140 ng ai/L (95% c.i.; 120 to 160 ng ai/L), which classifies Fipronil (MB 46030) as being very highly toxic to mysid shrimp. The NOEC is less than 62

ng ai/L.

D. Adequacy of the Study:

1. Classification: Supplemental

2. Rational: Negative control contamination. Test temperature was too high (25 to 26°C). Study chambers too small, 1 liter glass beakers with 1 liter test solution.

3. Reparability: Irreparable.

14. COMPLETION DATE OF ONE-LINER FOR STUDY:

DP Barcode : D217900
PC Code No : 129121
EEB Out :

AUG 29 1995

To: Richard Keigwin
Product Manager, PM 10
Insecticides Branch, Registration Division (7505C)

From: Anthony F. Maciorowski, Branch Chief
Ecological Effects Branch/EFED (7507C)

Attached, please find the EEB review of...

Reg./File # : 264-LLU
Chemical Name : Fipronil
Type Product : Insecticide
Product Name : See chemical name
Company Name : Rhone-Poulenc
Purpose : Company rebuttal to EEB evaluation of Mysid
Acute study (MRID# 432797-01).

Action Code : 101 Date Due : 0/00/95
Reviewer : N.E. Federoff

EEB Guideline/MRID Summary Table: The review in this package contains an evaluation of the following:

GDLN NO	MRID NO	CAT	GDLN NO	MRID NO	CAT	GDLN NO	MRID NO	CAT
71-1(A)			72-2(A)			72-7(A)		
71-1(B)			72-2(B)			72-7(B)		
71-2(A)			72-3(A)			122-1(A)		
71-2(B)			72-3(B)			122-1(B)		
71-3			72-3(C)			122-2		
71-4(A)			72-3(D)			123-1(A)		
71-4(B)			72-3(E)			123-1(B)		
71-5(A)			72-3(F)			123-2		
71-5(B)			72-4(A)			124-1		
72-1(A)			72-4(B)			124-2		
72-1(B)			72-5			141-1		
72-1(C)			72-6			141-2		
72-1(D)						141-5		

Y=Acceptable (Study satisfied Guideline)/Concur

P=Partial (Study partially fulfilled Guideline but additional information is needed)

S=Supplemental (Study provided useful information but Guideline was not satisfied)

N=Unacceptable (Study was rejected)/Nonconcur

DP BARCODE: D217900

CASE: 031271
SUBMISSION: S490525

DATA PACKAGE RECORD
BEAN SHEET

DATE: 08/02/95
Page 1 of 1

* * * CASE/SUBMISSION INFORMATION * * *

CASE TYPE: REGISTRATION ACTION: 101 RESB NC-FOOD/FEED USE
RANKING : 35 POINTS (KO)
CHEMICALS: 129121 Fipronil

96.5000%

ID#: 000264-LLU FIPRONIL TECHNICAL
COMPANY: 000264 RHONE-POULENC AG COMPANY
PRODUCT MANAGER: 10 RICK KEIGWIN
PM TEAM REVIEWER: ANN SIBOLD
RECEIVED DATE: 07/18/95 DUE OUT DATE: 01/24/96

703-305-6788 ROOM: CM2 210
703-305-6502 ROOM: CM2 201

* * * DATA PACKAGE INFORMATION * * *

DP BARCODE: 217900 EXPEDITE: Y DATE SENT: 08/02/95 DATE RET.: / /
CHEMICAL: 129121 Fipronil
DP TYPE: 001 Submission Related Data Package

CSF: Y LABEL: Y

ASSIGNED TO	DATE IN	DATE OUT	ADMIN DUE DATE: 11/30/95
DIV : EFED	8/2/95	/ /	NEGOT DATE: / /
BRAN: EEB	8/13/95	/ /	PROJ DATE: / /
SECT: RS1	/ /	/ /	
REVR :	/ /	/ /	
CONTR:	/ /	/ /	

* * * DATA REVIEW INSTRUCTIONS * * *

Note to Ann Stavola, Here is a rebuttal from Rhone Poulenc on a mysid shrimp study MRID 432797-01 which EEB graded supplemental and irreparable. Please let me know if you have questions or need anything else to complete your review. Thanks, Ann Sibold, 305-6502

* * * DATA PACKAGE EVALUATION * * *

No evaluation is written for this data package

* * * ADDITIONAL DATA PACKAGES FOR THIS SUBMISSION * * *

DP BC	BRANCH/SECTION	DATE OUT	DUE BACK	INS	CSF	LABEL
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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF
PREVENTION, PESTICIDES, AND
TOXIC SUBSTANCES

AUG 29 1995

SUBJECT: EEB Response to Rebuttal by Rhone-Poulenc Regarding
Evaluation of Mysid Acute Study for Fipronil (Chemical
No. 129121) (D217900)

FROM: Anthony F. Maciorowski, Branch Chief *APCwk for 08-29-95*
Ecological Effects Branch
Environmental Fate and Effects Division (7507 C)

TO: Richard Keigwin, PM 10
Insecticide Branch
Registration Division (7505 C)

The EEB has reviewed the rebuttal by Rhone-Poulenc regarding the EPA evaluation of the Mysid Acute Study (MRID# 432797-01) prepared by Springborn Laboratories for the registration of the chemical Fipronil. After review of the comments, the EEB concludes that the study should be upgraded to the designation of CORE. EEB's main concern was chemical contamination in the negative control group. This in itself will normally invalidate a study. Although the chemical was reported in the negative control solution at test termination, the chemical was absent at test initiation. The reported concentration (approximately 15.5 pptr) was 4X less than the lowest reported exposure level (62 pptr). Although the NOEC was less than 62 pptr, no mortality was reported in the contaminated negative control group. The 96-hour EC50 was 140 pptr which classifies Fipronil as very highly toxic to mysid shrimp. The EEB concludes, that by repeating the study, no new information would be gained.

If any questions should arise from this review please contact Nicholas Federoff of my staff at 703-305-5064.

RHÔNE-POULENC AG COMPANY

EPA Correspondence No. 95-24FR
July 17, 1995

Ann.

264-LLU

~~101~~ (101)

Mr. Richard P. Keigwin, PM 10
Office of Pesticide Programs (H7505C)
Document Processing Desk
U.S. Environmental Protection Agency
Room 266A, Crystal Mall 2
1921 Jefferson Davis Highway
Arlington, Virginia 22202

Re: Application for Registration of REGENT® 1.5G Insecticide on Corn
(EPA Reg. No. 264-LLL)

EPA's Review (Dated January 10, 1995) of the Study Titled, "MB 46030 -
Acute Toxicity to Mysids (*Mysidopsis bahi*) Under Static Conditions".
MRID Number 43279701.

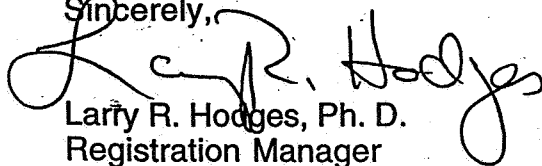
Dear Mr. Keigwin:

In reference to the above cited study I would first like to clarify the MRID Number which was incorrectly cited in EPA's review as 432979-01. The correct MRID Number for this study is 432797-01.

Appended to this letter is a detailed response from Springborn Laboratories, Inc., dated April 6, 1995. As the response from Springborn has thoroughly addressed and resolved each of EPA's rejection points we request that this study be accepted by EPA as core.

You may contact me at (919) 549-2870 if you have any questions concerning this response.

Sincerely,


Larry R. Hodges, Ph. D.
Registration Manager

6 April 1995

Ellen Mihaich, Ph.D., D.A.B.T.
Rhone Poulenc
PO Box 12014
2 T.W. Alexander Drive
Research Triangle Park, NC 27709

RE: Rebuttal - EPA's Data Evaluation Record for the Acute Exposure of Mysid Shrimp to Fipronil

Dear Ellen:

Springborn Laboratories has completed the review of the EPA's Data Evaluation record for the acute exposure of mysid shrimp (*Mysidopsis bahia*) to MB 46030 (Fipronil). Based on this evaluation, EPA has concluded that the submitted study is scientifically sound but does not fulfill the guideline requirements for an acute toxicity test on mysid shrimp. The following reasons for this conclusion were provided.

1. Test temperature was too high.
2. The study chambers were too small with an insufficient amount of test solution.
3. Negative control contamination.

Springborn has prepared the following response to each of the Agency's concerns.

EPA Comment #1. Test temperature was too high.

SLI Response: The EPA published guidelines (SEP's) do not provide specific guidance regarding the most appropriate test temperature for individual species. The SEP statement regarding test temperature is generic and provides no guidance to acceptable and non-acceptable temperatures.

EPA SEP for Acute Toxicity Tests for Estuarine and Marine Organisms (Shrimp 96-Hour Acute Toxicity Test) states "Most shrimp are to be test at or around 22°C. The actual measured temperature should not deviate more than 1°C or so during the test."

ASTM Standard Guideline for Conducting Acute Toxicity Tests with Fishes, Macroinvertebrates and Amphibians recommends at test temperature of 27°C for mysid shrimp.

The Agency reviewer reports that the SEP's state an acceptable range of 22±1°C. Based on the above quote from the SEP regarding test temperature, Springborn would conclude that the reviewer implied a strict criterion which was extracted from a very generic and broad statement

 **Springborn**
Laboratories

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intended to cover a multiple of shrimp species. The test temperature maintained during this test averaged 25°C which is considered an optimal temperature for survival and reproduction for this species. The recommended temperature of 27°C by ASTM clearly demonstrates that the temperature maintained during this study was not too high.

2. The study chambers were too small with an insufficient amount of test solution.

SLI Response: The Agency reviewer reports that the SEP's state " For static tests, larger organisms (0.5 grams each or larger) should be exposed in 19.6 liter containers with 15 liters of solution. Smaller organisms may be exposed in 3.9 liter containers with two or three liters of solution."

SLI Response: In addition to the general guidance for test container size (as reported by the Agency reviewer), the SEP provides more specific guidelines for biomass loading which is the reason for the selection of the test container size. The SEP states that the test container should be such that the loading factor (test organism mass per volume of test solution) for tests conducted at temperatures higher than 17°C should be ≤ 0.5 grams per liter.

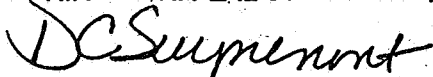
An adult mysid shrimp (wet weight) weighs approximately 5 mg. If ten adult mysids are placed in a vessel containing 1 liter of solution, the biomass loading rate is 50 mg/L or 0.05 g/L, which is considerably less than the maximum loading rate recommended in the SEP. It also should be recognized that juvenile (<24 hour old) mysids were used during the acute test, therefore, the average organism weight and loading biomass were significantly less than 0.05 g/L for the submitted study.

3. Negative control contamination.

SLI Response: During the acute study, a single measurement of Fipronil was reported in the negative control solution. The measurement was approximately 2X the minimum detection limit established for this study and was 4X less than the concentration of test article measured in the lowest exposure level. The single measurement occurred at test termination from a solution which did not contain test article at 0-hour. Since the solutions were not renewed between 0 and 96 hours, it was concluded that there was not a vehicle to transfer test article to the control solutions. That is, contamination from a pipet or measuring probe, even from the highest treatment level, would not be sufficient to significantly alter measurements in the control. Therefore, it was concluded that the single measurement of Fipronil recorded for the negative control solution was due to a condition during the analytical process and not representative of the exposure conditions. In addition, the level of the isolated contamination was minimal and no mortality or abnormal behavior or appearance was observed among the negative control population. Detectable concentrations were not observed in the solvent control solutions, which also demonstrated acceptable organism survival.

Following your review of the above draft responses, please call me to discuss how to proceed.

Sincerely,
SPRINGBORN LABORATORIES, INC.



Donald C. Surprenant
Director, Environmental Toxicology