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

OCT 23 1995

OFFICE OF
PREVENTION, PESTICIDES, AND
TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Data review for Isoxaflutole (D219146, Chemical #123000, Case 286745)

FROM: Elizabeth M.K. Leovey, Chief
Environmental Risk Characterization Branch
Environmental Fate and Effects Division (7507C)

TO: Joanne Miller, PM 23
Registration Division (7505C)

The Environmental Risk Characterization Branch (ERCB) has completed the review of the data submitted in support of registration of Isoxaflutole, chemical number 123000. The following is a brief summary of the data reviewed:

**Citation:** RPA 201772 Technical - Acute toxicity to mysid shrimp (*Mysidopsis bahia*) under flow-through conditions **EPA MRID No. 435732-40.**

**Conclusions:** This study is scientifically sound and meets the guideline requirements for a mysid acute toxicity test. Based on mean measured concentrations, the 96 hour $LC_{50}$ of isoxaflutole technical is 17.8 $\mu$g ai/L. Technical isoxaflutole is classified as very highly toxic to mysid shrimp. The NOEC is 5.1 $\mu$g ai/L.

If there are any questions regarding this data review contact Renée Costello of my staff at 305-5294.

**DATA EVALUATION RECORD**
**ACUTE $LC_{50}$ TEST WITH AN ESTUARINE/MARINE SHRIMP**
**§ 72-3(C)**

1. **CHEMICAL:** Isoxaflutole PC Code No.: 123000

2. **TEST MATERIAL:** Isoxaflutole Purity: 96.8%

3. **CITATION**

Authors: Michael J. Bettencourt
Title: RPA 201772 Technical - Acute toxicity to mysid shrimp ~~mysid shrimp~~ (*Mysidopsis bahia*) under flow-through conditions
Study Completion Date: April 20, 1994
Laboratory: Springborn Laboratories
Sponsor: Rhone-Poulenc
Laboratory Report ID: 10566.0194.6319.515
MRID No.: 435732-40
DP Barcode: D219146

4. **REVIEWED BY:** Renée Costello, Biologist, ERCB, EFED

**Signature:** Renée Costello **Date:** 10/19/95

5. **REVIEWED BY:** Andrew Bryceland, Fishery Biologist, ERCB, EFED

**Signature:** **Date:** 10/19/95

6. **STUDY PARAMETERS**

**Age or Size of Test Organism:** ≤ 24 hours old
**Definitive Test Duration:** 96 hours
**Study Method:** Flow-through
**Type of Concentrations:** Mean measured and Nominal

7. **CONCLUSIONS:**

**Results Synopsis**

| Parameter | Result |
|---|---|
| Probit $LC_{50}$ (95% C.I.) | 17.8 (13.7-23.1) µg ai/L |
| Probit Slope | 2.9 |
| NOEC | 5.1 µg ai/L |

8. **ADEQUACY OF THE STUDY**

**A. Classification:** Core

**B. Rationale:** N/A

**C. Repairability:** N/A

**9. BACKGROUND**

**10. GUIDELINE DEVIATIONS**

1. Biological observation of the test species (for general health and diseases) was not reported. This deviation did not effect the results of the study.

**11. SUBMISSION PURPOSE:** Product registration.

**12. MATERIALS AND METHODS**

**A. Test Organisms**

| Guideline Criteria | Reported Information |
|---|---|
| **Species** Preferred species are *Mysidopsis bahia*, *Penaeus setiferus*, *P. duorarun*, *P. aztecus* and *Palaemonetes sp.* | *Mysidopsis bahia* |
| **Age** Juvenile, mysids should be ≤ 24 hours old | ≤ 24 hours old |
| **Supplier** | Aquatic Biosystems, Inc. |
| **All shrimp are from same source?** | Yes |
| **All shrimp are from the same year class?** | ~~Not reported~~ yes |

**B. Source/Acclimation**

| Guideline Criteria | Reported Information |
|---|---|
| **Acclimation Period** minimum 10 days | 14 days |
| **Wild caught organisms were quarantined for 7 days?** | N/A |
| **Were there signs of disease or injury?** | Not reported |

| Guideline Criteria | Reported Information |
|---|---|
| **If treated for disease, was there no sign of the disease remaining during the 48 hours prior to testing?** | N/A |
| **Feeding**<br>No feeding during the study and no feeding for 24 hour before the beginning of the test if organisms are over 0.5 g each. | Shrimp were fed live brine shrimp twice daily during the exposure period. |
| **Pretest Mortality**<br><3% mortality 48 hours prior to testing | 0 % mortality prior to testing. |

C. **Test System**

| Guideline Criteria | Reported Information |
|---|---|
| **Source of dilution water**<br>Soft reconstituted water or water from a natural source, **not** dechlorinated tap water | Seawater from Cape Cod Canal, Bourne, Massachusetts |
| **Does water support test animals without observable signs of stress?** | Yes |
| **Salinity**<br>30-34 ‰ for marine (stenohaline) shrimp and 10-17 ‰ for estuarine (euryhaline) shrimp, weekly range < 6 ‰ | 30 to 31 ‰ |
| **Water Temperature**<br>Approx. 22 ± 1 °C | 24 to 31°C |
| **pH**<br>8.0-8.3 for marine (stenohaline) shrimp, 7.7-8.0 for estuarine (euryhaline) shrimp, monthly range < 0.8 | 7.8 |
| **Dissolved Oxygen**<br>Static: ≥ 60% during 1st 48 hrs and ≥ 40% during 2nd 48 hrs, Flow-through: ≥ 60% | ≥ 60% -- lowest DO 94% at 48 hour |
| **Total Organic Carbon** | 1.4 mg/L |

| Guideline Criteria | Reported Information |
|---|---|
| **Test Aquaria**<br>1. Material:<br>Glass or stainless steel<br>2. Size:<br>19.6 L is acceptable for organisms ≥ 0.5 g (e.g. pink shrimp, white shrimp, and brown shrimp), 3.9 L is acceptable for smaller organisms (e.g. mysids and grass shrimp).<br>3. Fill volume:<br>15 L is acceptable for organisms ≥ 0.5 g, 2-3 L is acceptable for smaller organisms. | 1. Glass<br>2. 19.5 L (39 x 20 x 25 cm)<br>3. 11 L |
| **Type of Dilution System**<br>Must provide reproducible supply of toxicant | Constant flow serial diluter |
| **Flow Rate**<br>Consistent flow rate of 5-10 vol/24 hours, meter systems calibrated before study and checked twice daily during test period | 6.5 vol/24 hours, calibrated before study and checked during the study |
| **Biomass Loading Rate**<br>Static: ≤ 0.8 g/L at ≤ 17°C, ≤ 0.5 g/L at > 17°C; flow-through: ≤ 1 g/L/day | 0.0001 g/L/day |
| **Photoperiod**<br>16 hours light, 8 hours dark | 16 h light, 8 h dark. |
| **Solvents**<br>Not to exceed 0.5 ml/L for static tests or 0.1 ml/L for flow-through tests | Solvent: acetone<br>Maximum conc.: 0.1 ml/L. |



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**D. Test Design**

| Guideline Criteria | Reported Information |
|---|---|
| **Range Finding Test**<br>If $LC_{50}$ >100 mg/L with 30 shrimp, then no definitive test is required. | 9.7, 16, 27, 45, and 75 µg ai/L -- 60, 30, and 80% mortality at 3 highest concentrations |
| **Nominal Concentrations of Definitive Test**<br>Control & 5 treatment levels; a geometric series in which each concentration is at least 60% of the next higher one. | Control, solvent control and 4.7, 9.3, 19, 37, and 75 µg ai/L. |
| **Number of Test Organisms**<br>Minimum 20/level, may be divided among containers | 20/level |
| **Test organisms randomly or impartially assigned to test vessels?** | Yes |
| **Biological observations made every 24 hours?** | Yes |
| **Water Parameter Measurements**<br>1. Temperature<br>Measured constantly or, if water baths are used, every 6 hrs, may not vary > 1°C<br>2. DO and pH<br>Measured at beginning of test and ever 48 h in the high, medium, and low doses and in the control | 1. Continuous of surrounding water. Once daily in test containers.<br>2. Once daily in each treatment level. |
| **Chemical Analysis**<br>needed if solutions were aerated, 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 | High, middle and low sampled prior to test. During the test, samples taken at 0 and 96 hours for analysis of parent compound and degradate. |



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## 13. REPORTED RESULTS

### A. General Results

| Guideline Criteria | Reported Information |
|---|---|
| **Quality assurance and GLP compliance statements were included in the report?** | Yes |
| **Recovery of Chemical** | 80.4 - 94.9 % |
| **Control Mortality** Not more than 10% of control organisms may die or show abnormal behavior. | 0% |
| **Raw data included?** | No |
| **Signs of toxicity (if any) were described?** | Yes |

Mortality

| Concentration (µg ai/L) | | Number of Shrimp | Cumulative Number Dead | | | |
|---|---|---|---|---|---|---|
| | | | Hour of Study | | | |
| Nominal | Mean Measured | | 24 | 48 | 72 | 96 |
| Control | Control | 20 | 0 | 0 | 0 | 0 |
| Solvent Control | Solvent Control | 20 | 0 | 0 | 0 | 0 |
| 4.7 | 5.1 | 20 | 0 | 0 | 0 | 0 |
| 9.3 | 9.8 | 20 | 0 | 0 | 1 | 5 |
| 19 | 18 | 20 | 0 | 0 | 5 | 13 |
| 37 | 36 | 20 | 0 | 0 | 7 | 15 |
| 75 | 77 | 20 | 1 | 1 | 11 | 19 |

Other Significant Results:

Sublethal effects (lethargy, erratic swimming behavior) were observed among all of the surviving mysids exposed to the 36 and 77 µg ai/L treatment levels and among several of the surviving mysids exposed to the 18 µg ai/L treatment level. Mortality of 25% was observed in the 9.8 µg ai/L treatment level. No

mortality or sublethal effects were noted in the 5.1 µg ai/L treatment level.

**B. Statistical Results**

Method: Moving average angle analysis

96-hr $LC_{50}$: 18 µg ai/L 95% C.I.: 14 - 23 µg ai/L
NOEC: 5.1 µg ai/L

## 14. VERIFICATION OF STATISTICAL RESULTS

| Parameter | Result |
|---|---|
| Probit $LC_{50}$ (95% C.I.) | 17.8 (13.7-23.1) µg ai/L |
| Probit Slope | 2.9 |
| NOEC | 5.1 µg ai/L |

costello isoxaflutole mysid flow-through

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| CONC. | NUMBER EXPOSED | NUMBER DEAD | PERCENT DEAD | BINOMIAL PROB. (PERCENT) |
|---|---|---|---|---|
| 77 | 20 | 19 | 95 | 2.002716E-03 |
| 36 | 20 | 15 | 75 | 2.069473 |
| 18 | 20 | 13 | 65 | 13.1588 |
| 9.8 | 20 | 5 | 25 | 2.069473 |
| 5.1 | 20 | 0 | 0 | 9.536742E-05 |

THE BINOMIAL TEST SHOWS THAT 9.8 AND 36 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 14.38676

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

| SPAN | G | LC50 | 95 PERCENT CONFIDENCE LIMITS | |
|---|---|---|---|---|
| 4 | .0647261 | 18.03722 | 14.32504 | 22.77781 |

RESULTS CALCULATED USING THE PROBIT METHOD

| ITERATIONS | G | H | GOODNESS OF FIT PROBABILITY |
|---|---|---|---|
| 5 | .1041094 | 1 | .3023034 |

SLOPE = 2.889133
95 PERCENT CONFIDENCE LIMITS = 1.956926 AND 3.821341

LC50 = 17.81151
95 PERCENT CONFIDENCE LIMITS = 13.78344 AND 23.08655

LC10 = 6.473253
95 PERCENT CONFIDENCE LIMITS = 3.702033 AND 8.973364

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