3-30-93

MRID No. 404892-04

DATA EVALUATION RECORD

1. **CHEMICAL:** Iprodione.
Shaughnessey No. 109801.

2. **TEST MATERIAL:** Iprodione technical; an off-white powder.

3. **STUDY TYPE:** 72-3. Marine Invertebrate Acute Flow-Through Toxicity Test. Species Tested: Mysid shrimp (Mysidopsis bahia).

4. **CITATION:** Surprenant, D.C. 1987. Acute Toxicity of Iprodione Technical to Mysid Shrimp (Mysidopsis bahia) Under Flow-Through Conditions. SLS Report No. 87-12-2580. Prepared by Springborn Life Sciences, Inc., Wareham, MA. Submitted by Rhone-Poulenc Ag Company, Research Triangle Park, NC. EPA MRID No. 404892-04.

5. **REVIEWED BY:**

Mark A. Mossler, M.S.
Associate Scientist
KBN Engineering and
Applied Sciences, Inc.

Signature:

Date:

6. **APPROVED BY:**

Pim Kosalwat, Ph.D.
Senior Scientist
KBN Engineering and
Applied Sciences, Inc.

Signature:

Date: 3.30.93

Henry T. Craven, M.S.
Supervisor, EEB/EFED
USEPA

Signature:

Date: 3-30-93

7. **CONCLUSIONS:** This study is not scientifically sound and does not meet the requirements for an acute toxicity study using marine invertebrates due to excessive control mortality. A 96-hour LC_{50} value of 0.72 mg/l classifies iprodione as highly toxic to the mysid shrimp. A precise NOEC could not be determined.

8. **RECOMMENDATIONS:** N/A.

9. **BACKGROUND:**

10. **DISCUSSION OF INDIVIDUAL TESTS:** N/A.

11. **MATERIALS AND METHODS:**

A. **Test Animals:** Mysid shrimp (Mysidopsis bahia) were obtained from in-house cultures. The shrimp were ≤24 hours old. The water used for culturing was collected from the Cape Cod Canal, Bourne, MA, filtered (5- and 20-micron and activated carbon), and held in a 950-l fiberglass tanks. A photoperiod of 16 hours light/8 hours dark at an intensity of 70-110 footcandles was provided and the temperature was maintained at 25 ±1°C. The shrimp were fed brine shrimp nauplii twice daily and a commercial food three times weekly.

B. **Test System:** The system consisted of 14 glass aquaria (39 x 20 x 25 cm) with self-starting siphons which allowed the solution volume to fluctuate from 3.1 to 7.0 l of test solution. Each aquarium contained 2 retention chambers constructed of petri dishes with 15-cm Nitex® collars. A proportional diluter with a dilution factor of 65% delivered test solution or control water to the individual aquaria at a rate of 12 volume replacements per day. The aquaria were impartially placed in a circulating water bath set to maintain 25 ±1°C. The dilution water was from the same source as that used in holding/culturing and the photoperiod in the test area was identical to that used for culturing with a light intensity of 30-80 footcandles.

A 37.7 mg/ml diluter stock solution was prepared by diluting 7.54 g of test material to 200 ml with acetone. The stock was delivered to the diluter using a continuous injector system. Twenty-one microliters of stock solution were delivered per minute to the mixing cell, which also received 317 ml of seawater per minute.

C. **Dosage:** Ninety-six-hour flow-through acute toxicity test. Based on preliminary tests, five nominal concentrations (0.45, 0.69, 1.1, 1.6, and 2.5 mg/l), a solvent control (0.066 ml acetone/l of solution), and a dilution water control were selected for the definitive test.

D. **Design:** Twenty shrimp were impartially distributed to each treatment and control aquarium (ten per aquarium). Within each aquarium, five mysids were contained in

each of two retention chambers. The maximum biomass loading was <3 mg/l at any given time.

Observations of mortality, sublethal effects, and test solution characteristics were made every 24 hours and dead organisms were removed when observed. Criteria for death were lack of mobility or response from gentle prodding. The shrimp were fed live brine shrimp nauplii twice daily which were supplied to each retention chamber.

The temperature, dissolved oxygen concentration, salinity, and pH were measured once daily in each replicate of the exposure concentrations and the controls. The temperature was measured continuously in one replicate of the solvent control.

Water samples were taken from the approximate mid-point of each treatment and control aquarium at test initiation and termination and analyzed for the test material using high pressure liquid chromatography.

E. **Statistics:** The median lethal concentration (LC_{50}) and associated 95% confidence interval (C.I.) for each 24-hour interval were calculated using a computer program that employed three methods of analysis. The probit, moving average angle, and binomial probability methods were examined to determine the best-fitting model. The no-observed-effect concentration (NOEC) was defined as the highest concentration tested at and below which there were no toxicant-related mortalities or sublethal effects.

12. **REPORTED RESULTS:** Mean measured concentrations ranged from 32 to 55% of nominal (Table 2, attached). The concentrations were determined to be 0.25, 0.27, 0.36, 0.57, and 0.97 mg/l. All test solutions contained undissolved material (white crystals, generally in proportion to the test concentrations) but none of this material was observed in the screened chambers.

The responses of the shrimp are presented in Table 3 (attached). Mortality in the control and solvent control was 10 and 5%, respectively. The 96-hour LC_{50} was determined to be 0.68 mg/l with a 95% confidence interval of 0.54-1.0 mg/l. The NOEC was determined to be <0.25 mg/l, the lowest concentration tested.

Dissolved oxygen ranged from 5.9 to 7.2 mg/l or 65 to 79% of saturation. The pH ranged from 7.7 to 7.8 and salinity was 30 parts per thousand (ppt). The temperature was 24-25°C during the test.

13. **STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:** "Based on criteria established by the U.S. EPA (1985), IPRODIONE Technical would be classified as highly toxic to mysid shrimp."

Quality Assurance and Good Laboratory Practice Statements were included in the report, indicating that the study was conducted in accordance with all pertinent EPA Good Laboratory Practice Regulations. However, the stability, characterization, and verification of the test substance is the responsibility of the study sponsor.

14. **REVIEWER'S DISCUSSION AND INTERPRETATION OF STUDY RESULTS:**

A. **Test Procedure:** The test procedures were not in accordance with protocols recommended by the SEP. The following are deviations:

The test material was not properly identified. The purity, lot number, and batch number should be reported.

Ten percent mortality occurred in the control group; a flow-through mysid test is not acceptable if >5% of the control organisms die during the test.

The test was conducted at a higher temperature (25°C) than recommended. The guidelines recommend that mysid shrimp be tested at 22°C.

Dilution water with a salinity of 30 ppt was used; a salinity of 10-17 ppt is recommended for a euryhaline species.

No photoperiod transition was listed. A 30-minute transition period is recommended.

No parental stock acclimation period was given.

B. **Statistical Analysis:** The reviewer used EPA's Toxanal program to calculate the LC_{50} value and obtained similar results using probit analysis. Since the author did not report the slope of the probit curve, the reviewer's values will be used (see attached

printout). The 96-hour LC_{50} for mysid shrimp exposed to iprodione was 0.72 mg/l (95% C.I.= 0.58-1.07 mg/l). The slope of the curve was 3.3.

C. **Discussion/Results:** After review of the response data, the reviewer concurs that the NOEC could not be determined in this study due to effects observed at all test levels.

This study is not scientifically sound and does not meet the requirements for an acute toxicity study using marine invertebrates. A 96-hour LC_{50} value of 0.72 mg/l classifies iprodione as highly toxic to the mysid shrimp. The NOEC could not be determined due to mortality and sublethal effects observed at all test concentrations.

D. **Adequacy of the Study:**

(1) **Classification:** Invalid.

(2) **Rationale:** Ten percent mortality occurred in the control group.

(3) **Repairability:** No.

15. **COMPLETION OF ONE-LINER FOR STUDY:** Yes, 12-15-92.