1-6-93

MRID No. 404892-02

DATA EVALUATION RECORD

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

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

3. **STUDY TYPE:** 72-3. Mollusc 96-Hour Flow-Through Shell Deposition Study. Species Tested: Eastern oyster (*Crassostrea virginica*).

4. **CITATION:** Surprenant, D.C. 1987. Acute Toxicity of Iprodione Technical to Eastern Oysters (*Crassostrea virginica*) Under Flow-Through Conditions. SLS Report No. 87-12-2584. Performed by Springborn Life Sciences, Inc., Wareham, MA. Submitted by Rhone-Poulenc Ag Company, Research Triangle Park, NC. EPA MRID No. 404892-02.

5. **REVIEWED BY:**

Mark A. Mossler, M.S.
Associate Scientist
KBN Engineering and
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Signature: Mark A. Mossler
Date: 12/22/92

6. **APPROVED BY:**

Pim Kosalwat, Ph.D.
Senior Scientist
KBN Engineering and
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Signature: P. Kosalwat
Date: 12/22/92

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

Signature: Henry T. Craven 1/6/93
Date: Dennis McLane 1-11-93

7. **CONCLUSIONS:** This study is scientifically sound but does not meet the guideline requirements for a mollusc shell deposition test. The purity of the test material was not reported and individual measurements of the new shell growth were not submitted. Based on mean measured concentrations, the 96-hour EC_{50} of iprodione for eastern oysters was 2.3 mg ai/l. Therefore, iprodione is classified as moderately toxic to eastern oysters. The NOEC was 1.0 mg ai/l.

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

9. **BACKGROUND:**

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

11. **MATERIALS AND METHODS:**

A. **Test Animals:** Eastern oysters (*Crassostrea virginica*) were obtained from a commercial supplier in Dennis, MA. During the 7 days before test initiation, the oysters were held in flowing seawater with a temperature of 17-20.5°C, a salinity of 29-32 parts per thousand (ppt), a pH of 7.7-7.9, and a dissolved oxygen concentration (DO) of 82-96% of saturation. No mortalities occurred during this time. The oysters were inspected for parasites, were similar in age, and had a mean valve height of 39 ±5 mm.

During the 48-hour acclimation period and testing, the oysters were fed a supplemental diet of *Isochrysis galbana* at a concentration of 10^5 cells/ml.

Twenty-four hours prior to testing, 2-5 mm of new peripheral shell growth was removed by grinding the shell with a grinding wheel. The oysters were held overnight and examined for signs of stress. Any oysters which appeared less than optimal were discarded. Immediately prior to test initiation, the outer edge of the shell was buffed by hand with an emery board to remove any new shell growth.

B. **Test System:** A continuous-flow serial diluter with a dilution factor of 60% was used to deliver test solutions, solvent control solution, and control seawater to the test aquaria. Aquaria were randomly assigned to treatment and positioned in a temperature-controlled water bath set to maintain 20 ±2°C. Each glass aquarium (60 x 30 x 30 cm) was equipped with a 10 cm standpipe and had a total test solution volume of approximately 18 l. The flow to each aquarium (75 ml/minute) provided six volume replacements every 24 hours. Recirculation of the test solution was provided in each individual aquarium to give a flow rate of about 5 l/oyster/hour.

During the exposure, the oysters received supplemental feedings of 180 ml of algal suspension (*I. galbana*, 10^7 cells/ml) per aquarium three times daily. Fluorescent lighting was maintained on a 16-hour light photoperiod.

Natural, unfiltered seawater was used as dilution water. The seawater was pumped from Cape Cod Canal,

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Bourne, MA, into a large fiberglass holding tank before distribution to the diluter. The salinity and pH of the seawater were 29-31 ppt and 7.7-7.9, respectively.

A syringe pump delivered 0.0326 ml/minute of a stock solution [92.02 mg active ingredient (ai)/ml] directly into the sonicating/mixing chamber which also received 375 ml/minute of seawater. This resulted in a solution which was equivalent to the highest nominal test concentration of 8 mg ai/l. A portion of this solution was serially diluted to produce the lower concentrations.

C. **Dosage:** Ninety-six-hour flow-through toxicity test. Based on preliminary testing information, five nominal concentrations (1.0, 1.8, 2.9, 4.8, and 8.0 mg ai/l) and a solvent and dilution water control were selected for the definitive test.

D. **Design:** Twenty oysters were impartially distributed to each aquarium (2 aquaria per concentration) for a total of 40 oysters per concentration or control. Oysters were placed equidistant from each other with their valves facing towards the flow of water from the recirculator.

Every 24 hours, the oysters were observed for visible abnormalities or mortalities. After 96 hours, new shell growth was measured microscopically to the nearest 0.1 mm using a calibrated micrometer.

The pH, temperature, salinity, and DO of the test solutions were measured in each replicate aquarium every 24 hours. Temperature was also monitored continuously in one aquarium.

Water samples were removed from each replicate of all solutions on day 0 and day 4 for analysis of iprodione by high pressure liquid chromatography. The samples were collected from the approximate mid-point of the aquaria.

E. **Statistics:** The 96-hour EC_{50} value was determined by linear regression of response (percent reduction of shell growth as compared with the pooled control) vs. mean measured exposure concentration over the range of test concentrations. Various mathematical manipulations (logarithm and probit transformations) were used on the concentration and response data to get

the linear regression with the highest coefficient of determination (R^2). The 95% confidence interval (C.I.) was determined using the method of inverse prediction. The growth data were subjected to analysis of variance (ANOVA) and the no-observed-effect concentration (NOEC) was determined using William's test ($p \leq 0.05$).

12. **REPORTED RESULTS:** The mean measured concentrations were 1.0, 1.6, 2.3, 3.7, and 5.2 mg ai/l (Table 2, attached). Undissolved test material was observed and removed daily from the pre-mixing chamber and the chemical cells. However, the material was not observed in the test solutions.

No mortality occurred during the test. Oyster shell deposition decreased with increasing iprodione concentration (Table 3, attached). The mean shell growth of the pooled control oysters was 2.3 mm. The 96-hour EC_{50} was 2.3 mg ai/l (95% C.I.= 1.0-5.3 mg ai/l) which classifies iprodione as moderately toxic to eastern oysters. The NOEC was 1.0 mg ai/l.

The temperature during the test was 18-19.5°C. The pH ranged from 7.7 to 7.9, and the DO from 6.4 to 8.2 mg/l or 64 to 82% of saturation. The salinity was 27-31 ppt.

13. **STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:** "Based on EPA (1985) criteria, the test material would be classified as moderately toxic to oysters."

Good laboratory practice and Quality Assurance Unit statements were included in the report indicating compliance to EPA Good Laboratory Practice Standards (GLPs) with the following exception: stability, characterization and verification of the test substance identity and maintenance of records on the test substance are the responsibility of the study sponsor.

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

A. **Test Procedure:** The test procedures were generally in accordance with protocols recommended by the SEP with the following deviations:

The purity of the test material was not reported.

Dusk/dawn lighting transitions were not used.

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The shell deposition raw data (individual measurements) were not submitted.

The flow rate of the "recirculating" test solution was about 5 l/oyster/hour. According to the protocols recommended by the SEP, each oyster should receive a minimum of 5 l of "once-through" flow-through test solution per hour. However, the above method is considered acceptable because a supplemental algal diet was provided.

B. **Statistical Analysis:** The reviewer used EPA's Toxanal program to determine the EC_{50} for oyster shell deposition and obtained results similar to the author's (see attached printouts). However, the 95% C.I. (2.0-2.5 mg ai/l) obtained using the moving average angle method was narrower than the author's. The NOEC could not be verified due to the lack of raw data.

C. **Discussion/Results:** This study is scientifically sound but does not meet the guideline requirements for a mollusc shell deposition test. Based on mean measured concentrations, the 96-hour EC_{50} of iprodione for eastern oysters was 2.3 mg ai/l. Therefore, iprodione is classified as moderately toxic to eastern oysters. The NOEC was 1.0 mg ai/l.

D. **Adequacy of the Study:**

(1) **Classification:** Supplemental.

(2) **Rationale:** The purity of the test material was not reported and individual measurements of the new shell growth were not submitted.

(3) **Repairability:** Yes, submit the above information.

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

IPRODIONE

Page_____ is not included in this copy.

Pages 6 through 7 are not included.

The material not included contains the following type of information:

____ Identity of product inert ingredients.

____ Identity of product impurities.

____ Description of the product manufacturing process.

____ Description of quality control procedures.

____ Identity of the source of product ingredients.

____ Sales or other commercial/financial information.

____ A draft product label.

____ The product confidential statement of formula.

____ Information about a pending registration action.

X FIFRA registration data.

____ The document is a duplicate of page(s) _____.

____ The document is not responsive to the request.

The information not included is generally considered confidential by product registrants. If you have any questions, please contact the individual who prepared the response to your request.

MOSSLER IPRODIONE CRASSOSTREA VIRGINICA 12-15-92

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CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
5.2	100	78	78	0
3.7	100	83	83	0
2.3	100	52	52	0
1.6	100	26	26	0
1	100	13	13	0

BECAUSE THE NUMBER OF ORGANISMS USED WAS SO LARGE, THE 95 PERCENT CONFIDENCE INTERVALS CALCULATED FROM THE BINOMIAL PROBABILITY ARE UNRELIABLE. USE THE INTERVALS CALCULATED BY THE OTHER TESTS.

AN APPROXIMATE LC50 FOR THIS SET OF DATA IS 2.239003

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS	
4	3.844761E-02	2.266389	2.028338	2.524148

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
3	.2858934	3.714605	1.097322E-02

SINCE THE PROBABILITY IS LESS THAN 0.05, RESULTS CALCULATED USING THE PROBIT METHOD PROBABLY SHOULD NOT BE USED.

SLOPE = 3.041889

95 PERCENT CONFIDENCE LIMITS = 1.415421 AND 4.668357

LC50 = 2.338908

95 PERCENT CONFIDENCE LIMITS = 1.656166 AND 3.280499

LC10 = .8943538

95 PERCENT CONFIDENCE LIMITS = .2718371 AND 1.353416

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Ecological Effects Branch One-Liner Data Entry Form

Chemical Iprodione **Shaughnessy No.** 109801 **Pesticide Use** Fungicide

INVERTEBRATE ACUTE TOXICITY	% AI	EC_{50} (95%CL) SLOPE	HRS/ TYPE	NOEC	STUDY/REVIEW DATES	MRID/ CATEGORY	LAB	RC
1. Eastern Oyster Crassostrea Virginica	NR	2.3mg ai/l (2.0-2.5mg ai/l) N/A	96 hr flow-through	1.0mg ai/l	1987/1992	404892-02 Supplemental	SLI	KM
2.								
3.								
4.								
5.								
6.								
7.								

CHRONIC TOX.	% AI	MATC LC_{50}	DAYS	AFFECTED PARA.	STUDY/REVIEW DATES	MRID/ CATEGORY	LAB	RC
1.								
2.								
3.								

COMMENTS: NR = Not reported

SLI = Springborn Life Sciences, Inc.