10/14/92 EEB files

MRID NO. 416041-11

DATA EVALUATION RECORD

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

2. **TEST MATERIAL:** Iprodione Technical; Lot No. 8906201; 96.2% active ingredient; an off-white granular powder.

3. **STUDY TYPE:** Growth and Reproduction of Aquatic Plants -- Tier 2. Species Tested: Navicula Pelliculosa.

4. **CITATION:** Giddings, J. M. 1990. Iprodione Technical - Toxicity to the Freshwater Diatom Navicula pelliculosa. SLI Report No. 90-6-3340. Prepared by Springborn Laboratories, Inc., Wareham, MA. Submitted by Rhone-Poulenc Ag Company, Research Triangle Park, NC. EPA MRID No. 416041-11.

5. **REVIEWED BY:**

Dennis J. McLane **Signature:** Dennis J. McLane
Wildlife Biologist
Ecological Effects Branch **Date:** 10-7-92
Environmental Fate and Effects Division

6. **APPROVED BY:**

Les Touart, Section Chief **Signature:** Les Touart
Section 1
Ecological Effects Branch **Date:** 10-14-92
Environmental Fate and Effects Division

7. **CONCLUSIONS:** This study is not scientifically sound. This study did not establish a dose-response relationship. The cells adhered to the side of the flasks.Differences in the effects of the treatment, the effectiveness of sonication in moving the cells off the side of the flasks, and the cell damage done by sonication, cannot be separated.

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

9. **BACKGROUND:** Part of a package of data submitted for reregistration.

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

11. **MATERIALS AND METHODS:**

A. **Test Species:** The alga used in the test, Navicula pelliculosa, came from laboratory stock cultures originally obtained from Carolina Biological Supply

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5. **REVIEWED BY:**

Louis M. Rifici, M.S.
Associate Scientist II
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Signature: Louis M. Rifici
Date: 4/30/91

6. **APPROVED BY:**

Pim Kosalwat, Ph.D.
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Henry T. Craven, M.S.
Supervisor, EEB/HED
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Signature:
Date:

7. **CONCLUSIONS:** This study is not scientifically sound. The concentration of active ingredient in the exposure concentrations greatly decreased during the exposure period indicating the actual concentrations the algae were exposed to are unknown. Under the conditions of the test, the 120-hour EC_{50} of Iprodione Technical for Navicula pelliculosa was 0.051 mg a.i./L (mean measured concentration).

8. **RECOMMENDATIONS:** Repeat the test and measure the concentration of the test material present in solution daily.

9. **BACKGROUND:**

(- 1hrs

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Company, Burlington, NC. Stock cultures were maintained in Marine Biological Laboratory medium (MBL Medium; Nichols, 1973) under test conditions. Transfers to fresh medium were approximately once or twice a week. The culture used as inoculum was transferred 6 days before test initiation.

B. **Test System:** Test vessels used were sterile 125-mL Erlenmeyer flasks fitted with stainless steel caps which permitted gas exchange. The test medium was the same as that used for culturing (excluding EDTA) with the pH adjusted to 7.5 Test vessels were maintained on an orbital shaker (100 rpm) under continuous illumination (approximately 4-5 klux at the surface of the media) in a growth chamber. Lighting was provided by Vita-Lite and Cool-White fluorescent lights. The temperature in the growth chamber was maintained at 22°-27°C.

A 5 mg/mL stock was prepared with 0.2599 g of Iprodione Technical diluted to 50 mL with acetone. Appropriate volumes of primary stock were diluted to 10 mL with acetone to create secondary stocks. Equal volumes (0.05 mL) of the secondary stocks were diluted to 500 mL in sterile MBL Medium. Solvent and media controls were also prepared. The solvent control contained 0.1 mL/L of acetone in medium which was equivalent to the concentration of solvent present in all test solutions.

C. **Dosage:** Five-day growth reproduction test. Based on the results of preliminary tests , six nominal Iprodione Technical concentrations of 0.016, 0.033, 0.065, 0.13, 0.25, and 0.50 mg a.i./L were selected for the definitive test.

D. **Design:** Three replicates 125-mL flasks (3 per treatment level and the controls) were conditioned by rinsing with the appropriate test solution. Fifty mL of the appropriate test solution were placed into each flask.

An inoculum of Navicula pelliculosa cells calculated to provide 0.3 x 10^4 cells/mL was cells/mL was aseptically introduced into each flask. The inoculum volume was 760 µL per flask. The flasks were impartially placed on the shaker in the growth chamber. At each 24-hour interval, cell counts were conducted on each replicate vessel using a hemacytometer and compound microscope. Upon test termination, the culture flasks were sonicated for

3 minutes to separate the cells from the flask walls and break up clumps.

Water quality (pH) was measured at test initiation and termination. Temperature was recorded continuously with a minimum.maximum thermometer. The shaking rate of the orbit shaker was recorded daily. The light intensity was measured at the beginning of the test and every 24-hour interval of the exposure period.

At test initiation and termination, samples were removed form each test solution and the controls for analysis by high-performance liquid chromatography (HPLC).

E. **Statistics:** For each observation period, the EC_{50} value and its 95% confidence limits were determined by linear regression of response (percent reduction of cell density as compared with controls) vs. mean measured exposure concentration over the range of test concentration excluding controls. 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).

A t-test (Sokal and Rohlf, 1981) was used to compare controls with solvent controls. The no-observed-effects concentration (NOEC) was determined using one-way analysis of variance (Sokal and Rohlf, 1981) and Bonferroni's Test (Weber et al., 1989).

12. **REPORTED RESULTS:** An initial definitive test was performed using concentration ranging from 0.0322 to 0.92 mg a.i./L. The 120-hour EC_{50} value (0.021 mg a.i./L; 95% C.I. = 0.0052-0.067 mg a.i./L was below the lowest concentration tested, so the test was repeated.

Mean measured concentrations for the present test are given in Table 2 (attached). Measured concentrations averaged 100% and 37% of nominal at test initiation and termination, respectively.

Cell densities determined at each observation time are presented in Table 3 (attached). Cell densities observed at 24, 48, 72, and 96 hours were very low (due to adherence of cells to the walls of the culture flask). Sonication greatly increased the number of cells counted at test termination. Some cell walls in the higher concentrations were observed

to be thin (concentrations ≥ 0.13 mg a.i./L (mean measured) with a 95% confidence interval of 0.0059-0.36 mg a.i./L. The 120-hour NOEC was determined as 0.013 mg a.i./L using Bonferroni's Test.

The pH was between 7.4 and 7.5. The temperature ranged from 22 to 27°C during the study. The conductivity of the solutions were not measured.

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

No conclusions were made by the study author.

Quality Assurance and GLP Compliance Statements were included in the report indicating adherence to USEPA GLP Regulations.

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

A. Test Procedure: The following test procedures deviated from guideline procedure:

The maximum label application rate was not given in the report. The rate used by the reviewer in this report was taken from another report using the same chemical and Anabaena flos-aquae (MRID # 416041-10); p.12).

The temperature during the study ranged from 22°C. to 27°C. The recommend test temperature is 24°±2°C.

The conductivity, dissolved oxygen, hardness, and alkalinity of the test solutions were not measured.

Sonication may fragment the cells and reduce the number of cells.

However a large number of the cells stuck to the sides of the flasks in this study. Differences in the effects of the treatment, the effectiveness of sonication in moving the cells off the side of the flasks, and the cell damage done by sonication, can not be separated. This may explain the lack of a dose-response relationship (toxanal printout).

The concentration of active ingredient in the exposure concentrations greatly decreased during the exposure period indicating the actual concentrations of algae were exposed to are unknown.

B. Statistical Analysis: The reviewer used a computer

program (Toxstat Version 3.0) and methods similar to those cited in the report and obtained the same NOEC (see attached printout 1 and 2). The probit method can not be used due to a poor goodness of fit. Hence, the slope value cannot be used to estimate EC values such as the EC_{25}, or EC_{10}.

The control and solvent control should not be pooled. Using the solvent control the moving average method provides an EC_{50} is 0.052 mg a.i./L. (see attached printout 3)

C. **Discussion/Results:** This study is not scientifically sound. The cells were adhered to the side of the flask. Differences in the effects of the treatment, the effectiveness of sonication in moving the cells off the side of the flasks, and the cell damage done by sonication, cannot be separated interpretation of the cell counts. This may explain the lack of a dose-response relationship (toxanal printout). This study cannot be used to calculate the EC_{10} or EC_{25}. The concentration decrease jeopardizes the validity of the test concentrations.

D. **Adequacy of the Study:**

(1) **Classification:** Invalid.

(2) **Rationale:** Because a large number of cells adhered to the sides of the flasks, sonication was used to move the cells. This jeopardized the results of the study, because of the difference in effectiveness of the sonication between treatment level is unknown, and the damage to cells due to sonication.

(3) **Repairability:** No.

15. **COMPLETION OF ONE-LINER FOR STUDY:** yes, 9-24-92

DENNIS MCLANE - IPRODIONE PLANT AQUATIC SOLVENT CONTROL

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CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
.26	100	88	88	0
.19	100	75	75	0
9.399999E-02		100	67	67
0				
.047	100	55	55	0
.02	100	60	60.00001	0
.013	100	0	0	0

THE BINOMIAL TEST SHOWS THAT .013 AND .02 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 1.899783E-02

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS	
5	2.085549E-02		5.227614E-02	
4.428821E-02		6.100944E-02		

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
4	1.018082	17.45852	
0			

A PROBABILITY OF 0 MEANS THAT IT IS LESS THAN 0.001.

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

SLOPE = 1.397311

95 PERCENT CONFIDENCE LIMITS =-1.257634E-02 AND 2.807198

LC50 = 4.305395E-02

95 PERCENT CONFIDENCE LIMITS = 0 AND .4681956

LC10 = 5.310364E-03

95 PERCENT CONFIDENCE LIMITS = 0 AND .022078

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IPRODIONE

Page_____ is not included in this copy.

Pages 8 through 10 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.

Shaughnessey No. 109801 Chemical Name IPRODIONE Chemical Class ____ Page 1 of 1

Study/Species/Lab/ Accession	Chemical % a.i.	Results	Reviewer/ Date	Validatio Status
14-Day Single Dose Oral LD_{50}		LD50 = mg/kg (95% C.L.) Contr. Mort.(%)=		
Species ____		Slope= # Animals/Level= Age(Days)= Sex =	____	____
Lab		14-Day Dose Level mg/kg/(% Mortality) ( ), ( ), ( ), ( ), ( )		
Acc.		Comments:		
14-Day Single Dose Oral LD_{50}		LD50 = mg/kg. (95% C.L) Contr. Mort.(%)=		
Species ____		Slope= # Animals/Level= Age(Days)= Sex =	____	____
Lab		14-Day Dose Level mg/kg/(% Mortality) ( ), ( ), ( ), ( ), ( )		
Acc.		Comments:		
8-Day Dietary LC_{50}		LC50 = ppm (95% C.L.) Contr. Mort.(%)=		
Species		Slope= # Animals/Level= Age(Days)= Sex =		
Lab ____		8-Day Dose Level ppm/(%Mortality) ( ), ( ), ( ), ( ), ( )	____	____
Acc.		Comments:		
8-Day Dietary LC_{50}		LC50 = ppm (95% C.L) Contr. Mort.(%)=		
Species		Slope= # Animals/Level= Age(Days)= Sex =		
Lab ____		8-Day Dose Level ppm/(%Mortality) ( ), ( ), ( ), ( ), ( )	____	____
Acc.		Comments:		
~~48-Hour~~ 120-hour LC_{50}		LC50 = 0.052 ppm (0.04-0.06) 95% C.L. Moving Average Inhibition Contr. Mort.(%)=0		
Species *Navicula pelliculosa*		Slope= N/A # Animals/Level= 0.3x10⁴ cells/mL Sol. Contr. Mort.(%)=0	DSM ~~LR~~ Invalid	
Lab Springborn Labs,	96.2%	120 ~~48~~-Hour Dose Level ppm /(%Mortality) Temperature = 24°C % inhibition	3/16/94	
Acc. 41604-11		0.013 (0), 0.026 (60), 0.047 (55), 0.094 (67), 0.19 (75), 0.26 (85)		9-24-92
		Comments: * Mean measured concentrations ** Worse the solvent control		
96-Hour LC_{50}		LC50 = ppm (95% C.L.) Con. Mort(%)= Sol. Con. Mort.(%)=		
Species		Slope= # Animals/Level= Temp.=		
Lab ____		96-Hour Dose Level ppm /(%Mortality) ( ), ( ), ( ), ( ), ( )	____	____
Acc.		Comments:		
96-Hour LC_{50}		LC50 = ppm (95% C.L) Con. Mort.(%)= Sol. Con. Mort.(%)=		
Species		Slope= # Animals/Level= Temp.=		
Lab ____		96-Hour Dose Level ppm /(%Mortality) ( ), ( ), ( ), ( ), ( )	____	____
Acc.		Comments:		