EEB files

10-14-92

MRID NO. 416041-10

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:Anabaena flos-aquae

4. **CITATION:** Giddings, J. M. 1990. Iprodione Technical - Toxicity to the Freshwater Bluegreen Alga Anabaena flos-aquae. SLI Report No. 90-05-3338. Prepared by Springborn Laboratories, Inc., Wareham, MA. Submitted by Rhone-Poulenc Ag Company, Research Triangle Park, NC. EPA MRID No. 416041-10.

5. **REVIEWED BY:**

Dennis J. McLane **Signature:** Dennis J. McLane
Wildlife Biologist
Ecological Effects Branch **Date:** 10-5-92
Environmental Fate and Effects Division

6. **APPROVED BY:**

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

7. **CONCLUSIONS:** The study does not fulfill the guideline requirements. The cell counts were erratic. It appears that sonication or some other factor has affected the cell count.

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, Anabaena flos-aquae, came from laboratory stock cultures originally obtained from Carolina Biological Supply Company,

INVALID

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Add highlight area

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   Wildlife Biologist
   Ecological Effects Branch
   Environmental Fate and Effects Division

   Signature: Dennis J. McLane

   Date: 10-5-92

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   Les Touart, Section Chief
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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, *Anabaena flos-aquae*, came from laboratory stock cultures originally obtained from Carolina Biological Supply Company,

Burlington, NC. Stock cultures were maintained in Algal Assay Procedure growth medium (AAP Medium; Miller et al., 1987) under test conditions. Transfers to fresh medium were made approximately once or twice a week. The culture used as inoculum had been transferred six 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± 0.1. Test vessels were maintained on an orbital shaker (100 rpm) under continuous illumination (approximately 0.9-2 klux at the surface of the media) in a growth chamber. Lighting was supplied by Vita-Lite fluorescent lights. The temperature in the growth chamber was maintained at 19°-22°C.

A 20-mg/mL stock was prepared by diluting 1.0393 g of Iprodione Technical 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 AAP medium. Solvent and medium 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 and reproduction test. Based on the results of preliminary tests, seven nominal concentrations of 0.03, 0.064, 0.13, 0.26, 0.50, 1.0, and 2.0 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 Anabaena flos-aquae cells calculated to provide 0.3×10^4 cells/mL was aseptically introduced into each flask. The inoculum volume was 660 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. At test termination, the cultures were sonicated for 60 seconds

to break the algal filaments into individual cells. One sample was taken from each flask.

Water quality (pH and conductivity) 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:** A t-test (Sokal and Rohlf, 1981) was used to compare controls with solvent controls. The no-observed-effects concentration (NOEC) 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:** The measured concentrations are given in Table 2 (attached). Measured concentration averaged 97% and 29% of nomial at test initiation and termination, respectively.

Cell densities determined at each observation time are precented in Table 3 (attached). Ce,, counts on the first four days were irregular dur to the filamentous growth form of Anabaena Flos-aquae. Sonication of samples at test termination broke the algal filaments into single cells. The pooled before determination of the NOEC. None of the Iprodione treatment groups had significantly fewer cells than the pooled controls (α=0.05). Because of the loss of Iprodione during the test, initial measured concentrations were used in the determination of the NOEC.

The percent effect based on day 5 cell densities ranged from 17.25 inhibition to 32.5% stimulation and a concentration-response relationship was not apparent. No EC_{50} values could be calculated from the data.

conductivity ranged from 270 to 350 μmhos/cm. The pH was between 7.4 and 7.6 in all test solutions and the controls at the test initiation anf between 7.5 and 8.3 at termination. The temperature ranged from 24 to 26°C during

the study.

13. **STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:** No conclusions were made by the author.

Good laboratory practice and Quality Assurance Unit statements were included in the report indicating compliance with EPA Good Laboratory Practice Standards.

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

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

The light intensity during the test (0.9 to 2 klux) was lower than recommended (2 klux).

The temperature during the test was 24°-26°C. The recommended test temperature is 24°C.

The concentration of active ingredient in the exposure concentration greatly decreased during the exposure period. Because the test solutions were not monitored thereafter, the actual concentrations the duckweed exposed to are unknown.

Sonication to move the cell off the sides of the flasks can fragment cells, may move cells at different rates according to how many are adhering to the flask wall.

B. **Statistical Analysis:** The following items concerning the statistical approach do not meet guideline requirements:

1) Statistical analysis should not be performed on the with pooled control in this test.
2) The Bonferroni's Test should not be used over the Dunnett's when the equal number of replicates are available.
3) Pooled solvent control and control data should not be used in statistical analysis.
4) The effect of sonication on the cell count cannot be determined.
5)Notice the erratic results for following dosage level:

Measured Conc.	24 hour			72 hour			120 hour		
	A	B	C	A	B	C	A	B	C
0.45	0.0	0.0	0.0	4.5	0.0	15.25	50.75	19.25	36.5
0.25	0.0	6.75	13.5	8.5	7.25	13.0	23.75	43.75	36.25
0.13	13.25	0.0	0.0	9.75	20.5	9.0	26.25	40.	30.75
0.069	0.0	0.0	0.0	16.25	16.5	6.5	17.0	38.5	17.5
0.029	3.75	6.75	14.25	23.25	9.5	15.75	30.75	44.0	35.5

C. **Discussion/Results:** The study does not fulfill the guideline requirements. The cell counts were erratic. It appears that sonication or some other factor has affect the cell count. The EC_{50} is greater than 0.88 mg/L and the NOEC could not be determined.

D. **Adequacy of the Study:**

(1) **Classification:** Invalid

(2) **Rationale:** Erratic cell counts indicates that the integrity of the test has been sacrificed.

(3) **Repairability:** N/A

15. **COMPLETION OF ONE-LINER FOR STUDY:** yes, 9-29-92

IPRODIONE

Page_____ is not included in this copy.

Pages 7 through 9 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.

TITLE: ANABAENA FLOS-AQUAE TIER II
FILE: B:ANABAENA.DAT
TRANSFORM: NO TRANSFORM NUMBER OF GROUPS: 8

GRP	IDENTIFICATION	REP	VALUE	TRANS VALUE
1	SOLVENT CONTROL	1	24.0000	24.0000
1	SOLVENT CONTROL	2	22.2500	22.2500
1	SOLVENT CONTROL	3	57.0000	57.0000
2	.0187	1	30.7500	30.7500
2	.0187	2	44.0000	44.0000
2	.0187	3	35.5000	35.5000
3	.0455	1	17.0000	17.0000
3	.0455	2	38.5000	38.5000
3	.0455	3	17.5000	17.5000
4	0.0855	1	26.2500	26.2500
4	0.0855	2	40.0000	40.0000
4	0.0855	3	30.7500	30.7500
5	.1625	1	23.7500	23.7500
5	.1625	2	43.7500	43.7500
5	.1625	3	36.2500	36.2500
6	.3	1	50.7500	50.7500
6	.3	2	19.2500	19.2500
6	.3	3	36.5000	36.5000
7	.545	1	52.7500	52.7500
7	.545	2	28.5000	28.5000
7	.545	3	27.0000	27.0000
8	.86	1	37.7500	37.7500
8	.86	2	42.7500	42.7500
8	.86	3	36.2500	36.2500

ANABAENA FLOS-AQUAE TIER II
File: B:ANABAENA.DAT Transform: NO TRANSFORM

SUMMARY STATISTICS ON TRANSFORMED DATA TABLE 1 of 2

GRP	IDENTIFICATION	N	MIN	MAX	MEAN
1	SOLVENT CONTROL	3	22.250	57.000	34.417
2	.0187	3	30.750	44.000	36.750
3	.0455	3	17.000	38.500	24.333
4	0.0855	3	26.250	40.000	32.333
5	.1625	3	23.750	43.750	34.583
6	.3	3	19.250	50.750	35.500
7	.545	3	27.000	52.750	36.083
8	.86	3	36.250	42.750	38.917

ANABAENA FLOS-AQUAE TIER II
File: B:ANABAENA.DAT Transform: NO TRANSFORM

SUMMARY STATISTICS ON TRANSFORMED DATA TABLE 2 of 2

GRP	IDENTIFICATION	VARIANCE	SD	SEM
1	SOLVENT CONTROL	383.271	19.577	11.303
2	.0187	45.063	6.713	3.876
3	.0455	150.583	12.271	7.085
4	0.0855	49.146	7.010	4.047
5	.1625	102.083	10.104	5.833
6	.3	248.813	15.774	9.107
7	.545	208.896	14.453	8.345
8	.86	11.583	3.403	1.965

ANABAENA FLOS-AQUAE TIER II
File: B:ANABAENA.DAT Transform: NO TRANSFORM

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	7	404.872	57.839	0.386
Within (Error)	16	2398.875	149.930	
Total	23	2803.747		

Critical F value = 2.66 (0.05,7,16)
Since F < Critical F FAIL TO REJECT Ho:All groups equal

ANABAENA FLOS-AQUAE TIER II
File: B:ANABAENA.DAT Transform: NO TRANSFORM

DUNNETTS TEST - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	SOLVENT CONTROL	34.417	34.417		
2	.0187	36.750	36.750	-0.233	
3	.0455	24.333	24.333	1.009	
4	0.0855	32.333	32.333	0.208	
5	.1625	34.583	34.583	-0.017	
6	.3	35.500	35.500	-0.108	
7	.545	36.083	36.083	-0.167	
8	.86	38.917	38.917	-0.450	

Dunnett table value = 2.56 (1 Tailed Value, P=0.05, df=16,7)

ANABAENA FLOS-AQUAE TIER II
File: B:ANABAENA.DAT Transform: NO TRANSFORM

DUNNETTS TEST - TABLE 2 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	SOLVENT CONTROL	3			
2	.0187	3	25.594	74.4	-2.333
3	.0455	3	25.594	74.4	10.083
4	0.0855	3	25.594	74.4	2.083
5	.1625	3	25.594	74.4	-0.167
6	.3	3	25.594	74.4	-1.083
7	.545	3	25.594	74.4	-1.667
8	.86	3	25.594	74.4	-4.500

ANABAENA FLOS-AQUAE TIER II
File: B:ANABAENA.DAT Transform: NO TRANSFORM

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	SOLVENT CONTROL	3	34.417	34.417	31.833
2	.0187	3	36.750	36.750	31.833
3	.0455	3	24.333	24.333	31.833
4	0.0855	3	32.333	32.333	32.333
5	.1625	3	34.583	34.583	34.583
6	.3	3	35.500	35.500	35.500
7	.545	3	36.083	36.083	36.083
8	.86	3	38.917	38.917	38.917

ANABAENA FLOS-AQUAE TIER II
File: B:ANABAENA.DAT Transform: NO TRANSFORM

WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
SOLVENT CONTROL	31.833				
.0187	31.833	0.258		1.75	k= 1, v=16
.0455	31.833	0.258		1.83	k= 2, v=16
0.0855	32.333	0.208		1.86	k= 3, v=16
.1625	34.583	0.017		1.87	k= 4, v=16
.3	35.500	0.108		1.88	k= 5, v=16
.545	36.083	0.167		1.89	k= 6, v=16
.86	38.917	0.450		1.89	k= 7, v=16

s = 12.245
Note: df used for table values are approximate when v > 20.

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

Study/Species/Lab/ Accession	Chemical % a.i.	Results	Reviewer/ Date	Validation 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~~ 12-hour LC_{50}		LC50 = N/A ppm* (95% C.L. N/A) Contr. Mort.(%)= 0 difference		
Species Anabaena flos-aquae		Slope= N/A # Animals/Level= 0.3x10^4 cells/mL Sol. Contr. Mort.(%)= 0		
Lab Springborne Laboratories 92.6		~~48-Hour~~ 12-hour Dose Level ppm/(%Mortality) Temperature = 24°C % difference	LR 3/18/91	Inv
Acc. MRID 41604-12		0.029(26.1), 0.069(17.2), 0.13(10.0), 0.25(17.7), 0.45(20.8)		0.82(22.5), 1.3(32.5
		Comments: * Initial Measured Concentrations		
96-Hour LC_{50}		LC50 = pp__ (95% C.L.) Con. Mor(%)= Sol. Con. Mor.(%)=		
Species		Slope= # Animals/Level= Temp.=		
Lab	____	96-Hour Dose Level pp /(%Mortality) ( ), ( ), ( ), ( ), ( )		
Acc.		Comments:		
96-Hour LC_{50}		LC50 = pp__ (95% C.L.) Con. Mort.(%)= Sol. Con. Mort.(%)=		
Species		Slope= # Animals/Level= Temp.=		
Lab	____	96-Hour Dose Level pp /(%Mortality) ( ), ( ), ( ), ( ), ( )		
Acc.		Comments:		