

US EPA ARCHIVE DOCUMENT

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
Wildlife Biologist
Ecological Effects Branch
Environmental Fate and Effects Division

Signature:
Date:

Dennis J. McLane
10-5-92

6. **APPROVED BY:**

Les Touart, Section Chief
Section 1
Ecological Effects Branch
Environmental Fate and Effects Division

Signature:
Date:

LT
10-14-92

- 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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5. **REVIEWED BY:**

| | |
|--|---|
| Dennis J. McLane Wildlife Biologist Ecological Effects Branch Environmental Fate and Effects Division | Signature: <i>Dennis J. McLane</i> Date: 10-5-92 |
|--|---|
6. **APPROVED BY:**

| | |
|--|-----------------------------------|
| Les Touart, Section Chief Section 1 Ecological Effects Branch Environmental Fate and Effects Division | Signature: Date: |
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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,

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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 μ L 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 from 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 nominal at test initiation and termination, respectively.

Cell densities determined at each observation time are presented in Table 3 (attached). Cell counts on the first four days were irregular due to the filamentous growth form of *Anabaena Flos-aquae*. Sonication of samples at test termination broke the algal filaments into single cells. The samples were pooled before determination of the NOEC. None of the Iprodione treatment groups had significantly fewer cells than the pooled controls ($\alpha=0.05$). 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 $\mu\text{mhos/cm}$. The pH was between 7.4 and 7.6 in all test solutions and the controls at the test initiation and 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 | 6.7 5 | 13. 5 | 8.5 | 7.2 5 | 13. 0 | 23. 75 | 43. 75 | 36. 25 |
| 0.13 | 13. 25 | 0.0 | 0.0 | 9.7 5 | 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 | 3.7 5 | 6.7 5 | 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 EC₅₀ 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

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Page ___ is not included in this copy.

Pages 2 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.
 - FIFRA registration data.
 - The document is a duplicate of page(s) _____.
 - The document is not responsive to the request.
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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 $F < \text{Critical } F$ FAIL TO REJECT H_0 :All groups equal

ANABAENA FLOS-AQUAE TIER II

File: B:ANABAENA.DAT

Transform: NO TRANSFORM

DUNNETTS TEST - TABLE 1 OF 2

H_0 :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 | |

Dunnnett 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

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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.

Shannonsey No. 109801

Chemical Name IPRODIONE Chemical Class _____ Page 1 of 1

Study/Species/Lab/ Accession 14-Day Single Dose Oral LD50 Chemical Z a.i. Results Reviewer/Date _____ Validati Status _____

LD50 = mg/kg (95% C.L.) Contr. Mort. (X) = _____

Species _____ Slope = _____ # Animals/Level = _____ Age (Days) = _____ Sex = _____

Lab _____ 14-Day Dose Level mg/kg/(X Mortality) _____

Acc. _____ Comments: _____

14-Day Single Dose Oral LD50 LD50 = mg/kg. (95% C.L.) Contr. Mort. (X) = _____

Species _____ Slope = _____ # Animals/Level = _____ Age (Days) = _____ Sex = _____

Lab _____ 14-Day Dose Level mg/kg/(X Mortality) _____

Acc. _____ Comments: _____

8-Day Dietary LC50 LC50 = ppm (95% C.L.) Contr. Mort. (X) = _____

Species _____ Slope = _____ # Animals/Level = _____ Age (Days) = _____ Sex = _____

Lab _____ 8-Day Dose Level ppm/(X Mortality) _____

Acc. _____ Comments: _____

8-Day Dietary LC50 LC50 = ppm (95% C.L.) Contr. Mort. (X) = _____

Species _____ Slope = _____ # Animals/Level = _____ Age (Days) = _____ Sex = _____

Lab _____ 8-Day Dose Level ppm/(X Mortality) _____

Acc. _____ Comments: _____

~~48-Hour~~ ^{120-hour} LC50 LC50 = N/A ^{ppm} (95% C.L.) Contr. Mort. (X) = 0 ^{difference}

Species Anabreana flus-a-queae Slope = N/A ^{ep 115 mL} # Animals/Level = 0.3 x 10⁴ Sol. Contr. Mort. (X) = 0

Lab Springburne Laboratories 92.6 ^{20-hour} 48-hour Dose Level ppm/(X mortality) ^{% difference} Temperature = 24°C LR Irvin

Acc. MRID 41604-10 0.029 (26.1) 0.069 (17.2) 0.13 (10.0) 0.25 (17.7) 0.45 (20.8) 3/18/91 0.52 (22.5), 1.3 (32.5)

Comments: * initial measured concentrations

96-Hour LC50 LC50 = pp (95% C.L.) Con. Mort. (X) = _____

Species _____ Slope = _____ # Animals/Level = _____ Sol. Con. Mort. (X) = _____

Lab _____ 96-Hour Dose Level pp / (X mortality) Temp. = _____

Acc. _____ Comments: _____

96-Hour LC50 LC50 = pp (95% C.L.) Con. Mort. (X) = _____

Species _____ Slope = _____ # Animals/Level = _____ Sol. Con. Mort. (X) = _____

Lab _____ 96-Hour Dose Level pp / (X mortality) Temp. = _____

Acc. _____ Comments: _____