

(3-15-95)

MRID No. 426010-02

DATA EVALUATION RECORD

1. **CHEMICAL:** Paraquat dichloride.
Shaughnessey No. 061601.
2. **TEST MATERIAL:** Paraquat dichloride technical; 1,1'-dimethyl-4,4'-bipyridylium dichloride; CAS No. 1910-42-5; RS No. RS151/B; purity of 32.7% w/w; a dark brown liquid.
3. **STUDY TYPE:** 123-2. Growth and Reproduction of Aquatic Plants - Tier 2. Species Tested: *Selenastrum capricornutum*.
4. **CITATION:** Smyth, D.V., S.A. Sankey, and A.J. Penwell. 1992. Paraquat Dichloride: Toxicity to the Green Alga *Selenastrum capricornutum*. Laboratory ID No. T168/G. Conducted by Imperial Chemical Industries PLC, Devon, UK. Submitted by ICI Americas, Inc. EPA MRID No. 426010-02.
5. **REVIEWED BY:**

Renée Costello
Biologist
EFED/EEB

Signature: *Renée Costello*
Date: 9/12/93
6. **APPROVED BY:**

Ann Stavola
Head, Section 5
EEB/EFED

Signature: *Ann Stavola*
Date: 3/15/95
7. **CONCLUSIONS:** This study is scientifically sound and meets the guideline requirements for a Tier 2 non-target plant growth and reproduction test with a formulated product. Based on mean measured concentrations, the 4-day NOEC, LOEC, and EC₅₀ for *S. capricornutum* exposed to paraquat dichloride were 0.08, 0.20, and 0.32 mg/l, respectively.
8. **RECOMMENDATIONS:** N/A.

9. **BACKGROUND:**

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

11. **MATERIALS AND METHODS:**

A. **Test Species:** The alga used in the test, *Selenastrum capricornutum* Printz, came from laboratory stock cultures kept under axenic conditions. Stock cultures were maintained in synthetic nutrient medium at a temperature of $24 \pm 1^\circ\text{C}$, with orbital shaking at 100 rpm. Cool white illumination provided a light intensity of 3970 lux continuously. Cultures that were in a logarithmic growth phase were used as inoculum for the test.

B. **Test System:** Test vessels used were 250-ml glass conical flasks fitted with foam stoppers. The test medium was the same as that used for culturing, with a pH of 7.3-7.4.

The test vessels were kept in an incubator with environmental conditions like those employed in culturing.

C. **Dosage:** Four-day growth and reproduction study. Nominal rates of 0.056, 0.10, 0.18, 0.32, 0.56, 1.0, 1.8, and 3.2 mg/l, and a medium control were used for the definitive test. The solutions were not corrected for percent purity of the test material.

A stock solution of 64 mg/l was prepared by direct addition of the test material to sterile culture medium. Aliquots of the stock or the 3.2 mg/l test solution were added to sterile culture medium to obtain the nominal test concentrations.

D. **Test Design:** One-hundred milliliters of the test solution were placed in each of three replicate flasks (3 per treatment level). The control flasks were replicated six times. A blank set of solutions (extra set of control and test solutions without algae) was also incubated concurrently.

An inoculum volume of 0.47 ml per flask was used to provide 3000 cells/ml. Cell counts were performed every 24 hours using an electronic particle counter. The flasks were randomized daily by rows within the incubator.

At the start of the test, samples taken from each test solution and control were analyzed for the concentration of the test substance by high performance liquid chromatography. At the end of the test, each blank solution was sampled and analyzed in the same manner.

The pH of the test solutions were measured at test initiation and termination. Light intensity was measured once during the experiment. Temperature was monitored continuously electronically as well as manually daily.

- E. **Statistics:** For each nominal concentration, the mean of the measured concentration of the day 0 and 4 samples was calculated. The mean measured concentrations were then used as the basis for the data analysis. The area under the growth curve and growth rate were examined as a function of time. Probit and Dunnett's analyses ($p \leq 0.05$) were conducted on both of these parameters at day 4.

12. **REPORTED RESULTS:** Measured concentrations on day 0 were from 44 to 78% of nominal while day 4 measured concentrations were from 31 to 47% of nominal (Table 1, attached). The means of the measured concentrations were 0.029, 0.052, 0.082, 0.20, 0.28, 0.41, 0.81, and 1.60 mg/l. The control and exposure solutions were clear and colorless.

Algal cell densities for the control and the exposure concentrations throughout the test are given in Table 2 (attached).

By day 4, the effect of the test material on the area under the growth curve, relative to the control, ranged between 0 and 100% inhibition (Table 3, attached). The no-observed-effect concentration (NOEC), lowest-observed-effect concentration (LOEC), and EC_{50} were 0.20, 0.28, and 0.29 mg/l, respectively. The 95% confidence interval was 0.17-0.55 mg/l.

By day 4, the effect of the test material on the growth rate, relative to the control, ranged between 0 and 89% inhibition (Table 4, attached). The NOEC, LOEC, and EC_{50} were 0.20, 0.28, and 0.71 mg/l, respectively. The 95% confidence interval was 0.23->1.60 mg/l.

The pH in the control and the exposure concentrations was 7.3-7.4 at the beginning of the study and 7.1-7.8 at the conclusion. Temperature ranged from 24.1 to 24.5°C.

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

No conclusions were made by the authors.

Good Laboratory Practice and Quality Assurance Unit statements were included in the report indicating compliance with EPA Good Laboratory Practice Standards as set forth in 40 CFR Part 160.

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

- A. Test Procedure:** The test procedures and the report were generally in accordance with SEP and Subdivision J guidelines, but deviated as follows:

The study was conducted for 4 days rather than the recommended 5 days.

The EC₅₀ was computed based on growth rate and area under the growth curve, rather than cell density.

An inert ingredients control was not incorporated into the study design. This type of control should be included for any technical test material of less than 80% purity.

- B. Statistical Analysis:** Using cell density data, the reviewer used EPA's Toxanal program to determine the EC value. Analysis of variance and Bonferroni's test were used to determine LOEC and NOEC values. More conservative values were determined for the NOEC and LOEC. A narrower confidence interval (C.I.) was determined using the moving average angle method. The 4-day NOEC, LOEC, and EC₅₀ were determined to be 0.08, 0.20, and 0.32 mg/l (95% C.I. = 0.29-0.34 mg/l), respectively.

- C. Discussion/Results:** This study is scientifically sound and meets the guideline requirements for a Tier 2 non-target plant growth and reproduction test with a formulated product. Based on mean measured concentrations, the 4-day NOEC, LOEC, and EC₅₀ for *S. capricornutum* exposed to paraquat dichloride were 0.08, 0.20, and 0.32 mg/l, respectively.

D. Adequacy of the Study:

- (1) **Classification:** Core for a formulated product.
- (2) **Rationale:** N/A
- (3) **Repairability:** N/A

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