# 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: *Anabaena flos-aquae*.

4. **CITATION:** Smyth, D.V., S.A. Sankey, and S.K. Cornish. 1992. Paraquat Dichloride: Toxicity to the Blue-Green Alga *Anabaena flos-aquae*. Laboratory ID No. T168/B. Conducted by Imperial Chemical Industries PLC, Devon, UK. Submitted by ICI Americas, Inc. EPA MRID No. 426010-05.

5. **REVIEWED BY:**

Renee Lamb
Biologist
EFED/EEB

**Signature:** Renée Lamb
**Date:** 7/7/93

6. **APPROVED BY:**

for Ann Stavola
Head, Section 5
EFED/EEB

**Signature:** Allen W Vaughan
**Date:** 3 9.95

7. **CONCLUSIONS:** This study is scientifically sound and meets the guideline requirements for a Tier 2 non-target plant growth and reproduction test for a formulated product. ~~The technical material was of less than 80% purity.~~ Based on mean measured concentrations, the 5-day NOEC, LOEC, and $EC_{50}$ for *A. flos-aquae* exposed to paraquat dichloride were 3.2, 6.3, and 15 µg/l, respectively.

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

9. **BACKGROUND:** N/A

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 5 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 5.

12. **REPORTED RESULTS:** Measured concentrations on day 0 were ~~from~~ 70 to 95% of nominal while day 5 measured concentrations were ~~from~~ 52 to 78% of nominal (Table 1, attached). The measured concentrations for the nominal 4 and 8 µg/l concentrations at 0 and 120 hours and for the nominal 16 µg/l concentration at 120 hours were below the limit of detection. Since the mean percentage recoveries of the detectable test concentrations were reasonably uniform (between 72 and 86%), a "mean" factor of 79% was applied to the nominal concentrations that were below the limit of detection. The means of the measured concentrations were 3.2, 6.3, 13, 25, 48, 110, 210, and 370 µg/l. The control and exposure solutions were clear and colorless.

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

By day 5, 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 3.2, 6.3, and 15 µg/l, respectively. The 95% confidence interval was 5.7-28 µg/l.

By day 5, the effect of the test material on the growth rate, relative to the control, ranged between 0 and 100% inhibition (Table 4, attached). The NOEC, LOEC, and $EC_{50}$ were 13, 25, and 24 µg/l, respectively. The 95% confidence interval was 16-37 µg/l.

The pH in the control and the exposure concentrations was 7.3 at the beginning of the study and 7.2-7.8 at the conclusion. Temperature ranged from 24.0 to 25.0°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 inoculum level (20,000 cells/ml) was much greater than recommended (3000 cells/ml).

The light intensity (3.6 klux) was greater than recommended (2 klux).

The $EC_{50}$ 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 absorbance 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. The authors' NOEC and LOEC values based on area under the growth curve were more conservative than those determined by the reviewer. A narrower confidence interval (C.I.) was determined using the moving average angle method. Combining the most conservative values, the 5-day NOEC, LOEC, and $EC_{50}$ were determined to be 3.2, 6.3, and 15 µg/l (95% C.I.= 14-16 µg/l), respectively.

C. **Discussion/Results:** Although the light intensity was almost double the recommended intensity and the cellular inoculum was 7 times greater than recommended, the growth of the control algae proceeded in an exponential fashion. This indicated that the two exceedances might have actually offset one another (i.e., more algae resulted in less light penetration). Therefore, the reviewer does not feel that these exceedances negatively affected the outcome of the study.

This study is scientifically sound and meets the guideline requirements for a Tier 2 non-target plant growth and reproduction test for a formulated product. Based on mean measured concentrations, the 5-day NOEC, LOEC, and $EC_{50}$ for *A. flos-aquae* exposed to paraquat dichloride were 3.2, 6.3, and 15 µg/l, respectively.

D. **Adequacy of the Study:**

(1) **Classification:** Core for a formulated product.

(2) **Rationale:** N/A

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

15. **COMPLETION OF ONE-LINER:** Yes, 2-9-93.

DER# 42601006

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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.

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anabaena cell absorbance
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ANOVA TABLE

| SOURCE | DF | SS | MS | F |
|---|---|---|---|---|
| Between | 4 | 2.479 | 0.620 | 455.491 |
| Within (Error) | 13 | 0.018 | 0.001 | |
| Total | 17 | 2.496 | | |

Critical F value = 3.18 (0.05,4,13)
Since F > Critical F REJECT Ho:All groups equal

NOEC = 6.3 mg/l
LOEC = 13 mg/l

anabaena cell absorbance
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BONFERRONI T-TEST - TABLE 1 OF 2 Ho:Control<Treatment

| GROUP | IDENTIFICATION (conc (mg/l)) | TRANSFORMED MEAN | MEAN CALCULATED IN ORIGINAL UNITS | T STAT | SIG |
|---|---|---|---|---|---|
| 1 | control | 1.042 | 1.042 | | |
| 2 | 3.2 | 1.035 | 1.035 | 0.268 | |
| 3 | 6.3 | 1.031 | 1.031 | 0.422 | |
| 4 | 13 | 0.798 | 0.798 | 9.368 | * |
| 5 | 25 | 0.022 | 0.022 | 39.109 | * |

Bonferroni T table value = 2.53 (1 Tailed Value, P=0.05, df=13,4)

anabaena cell absorbance
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BONFERRONI T-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 | control | 6 | | | |
| 2 | 3.2 | 3 | 0.066 | 6.3 | 0.007 |
| 3 | 6.3 | 3 | 0.066 | 6.3 | 0.011 |
| 4 | 13 | 3 | 0.066 | 6.3 | 0.244 |
| 5 | 25 | 3 | 0.066 | 6.3 | 1.020 |

MOSSLER PARAQUAT ANABAENA FLOS AQUAE 2-9-93

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| CONC. | NUMBER EXPOSED | NUMBER DEAD | PERCENT DEAD | BINOMIAL PROB.(PERCENT) |
|---|---|---|---|---|
| 25 | 100 | 98 | 98 | 0 |
| 13 | 100 | 23 | 23 | 0 |
| 6.3 | 100 | 1 | 1 | 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 15.92014

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

| SPAN | G | LC50 | 95 PERCENT CONFIDENCE LIMITS | |
|---|---|---|---|---|
| 2 | 1.142894E-02 | 14.99103 | 14.05298 | 16.051 |

RESULTS CALCULATED USING THE PROBIT METHOD

| ITERATIONS | G | H | GOODNESS OF FIT PROBABILITY |
|---|---|---|---|
| 5 | 34.14106 | 18.17161 | 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 = 8.458932
95 PERCENT CONFIDENCE LIMITS =-40.9669 AND 57.88477

LC50 = 15.33407
95 PERCENT CONFIDENCE LIMITS = 0 AND +INFINITY

LC10 = 10.85226
95 PERCENT CONFIDENCE LIMITS = 0 AND +INFINITY

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