**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: *Skeletonema costatum.*

4. **CITATION:** Smyth, D.V., S.A. Sankey, and A.J. Penwell. 1992. Paraquat Dichloride: Toxicity to the Marine Alga *Skeletonema costatum.* Laboratory ID No. T168/C. Conducted by Imperial Chemical Industries PLC, Devon, UK. Submitted by ICI Americas, Inc. EPA MRID No. 426010-04.

5. **REVIEWED BY:**

Renée Costello
Biologist
EEB/EFED

**Signature:** ReneéCostello
**Date:** 4/12/93

6. **APPROVED BY:**

Ann Stavola
Head, Section 5
EEB/EFED

**Signature:** Ann Stavola
**Date:** 5/15/93

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 $EC_{50}$ for *S. costatum* exposed to paraquat dichloride were 0.22, 0.47, and 2.84 mg/l, respectively.

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

9. **BACKGROUND:**

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

11. **MATERIALS AND METHODS:**

A. **Test Species:** The diatom used in the test, *Skeletonema costatum*, originally came from the Culture Centre of Algae and Protozoa, Freshwater Biological Association, The Ferry House, Ambleside, Cumbria, UK. The culture had been kept under axenic conditions since April, 1986. Stock cultures were maintained in synthetic nutrient medium at a temperature of 20 ±1°C with orbital shaking at 100 rpm. Cool-white light provided a 16-hour photoperiod of 4800 lux. Cultures that were growing logarithmically were used as inoculum for the test.

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

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 0.25, 0.50, 1, 2, 4, 8, 16, and 32 mg/l, and a medium control were used for the definitive test. The solutions were not adjusted for percent purity of the test material.

A stock solution of the highest test concentration (32 mg/l) was prepared by direct addition of the test material to sterile culture medium. Aliquots of the stock were added to sterile culture medium to obtain the lower 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 added diatoms) was also incubated concurrently.

An inoculum volume of 1.20 ml per flask was used to provide 10,000 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 using spectrophotometric procedures. At the end of the test, each blank solution was sampled and analyzed in the same manner.

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

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 68 to 105% of nominal while day 4 measured concentrations were from 82 to 108% of nominal (Table 1, attached). The means of the measured concentrations were 0.22, 0.47, 0.85, 2.1, 4.3, 7.7, 16, and 32 mg/l. The control and exposure solutions were clear and colorless.

Diatom 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 8 and 97% inhibition (Table 3, attached). The no-observed-effect concentration (NOEC), lowest-observed-effect concentration (LOEC), and $EC_{50}$ were 0.22, 0.47, and 2.2 mg/l, respectively. The 95% confidence interval was 0.41-8.6 mg/l.

By day 4, the effect of the test material on the growth rate, relative to the control, ranged between 1 and 74% inhibition (Table 4, attached). The NOEC, LOEC, and $EC_{50}$ were 0.47, 0.85, and 18 mg/l, respectively. The 95% confidence interval was 0.27->32 mg/l.

The pH in the control and the exposure concentrations was 8.3-8.4 at the beginning of the study and 8.2-8.9 at the conclusion. Temperature ranged from 18.9 to 20.7°C. The salinity was 30.9 parts per thousand.

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 $EC_{50}$ was computed based on growth rate and area under the growth curve, rather than cell density.

The light intensity (4.8 klux) was greater than recommended (4 klux).

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. The same NOEC and LOEC values were determined using cell density as were determined using area under the growth curve. A similar $EC_{50}$ was calculated, but a narrower confidence interval (C.I.) was determined. The 4-day NOEC, LOEC, and $EC_{50}$ were determined to be 0.22, 0.47, and 2.84 mg/l (95% C.I.= 2.42-3.33 mg/l), respectively. The slope of the probit curve was 1.53.

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 the formulated product. Based on mean measured

concentrations, the 4-day NOEC, LOEC, and $EC_{50}$ for *S. costatum* exposed to paraquat dichloride were 0.22, 0.47, and 2.84 mg/l, respectively.

**D. Adequacy of the Study:**

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

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

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

DER # 426010-04

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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.

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_____ A draft product label.

_____ The product confidential statement of formula.

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skeletonema cell density
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ANOVA TABLE

| SOURCE | DF | SS | MS | F |
|---|---|---|---|---|
| Between | 8 | 42975.033 | 5371.879 | 134.484 |
| Within (Error) | 21 | 838.833 | 39.944 | |
| Total | 29 | 43813.867 | | |

Critical F value = 2.42 (0.05,8,21)
Since F > Critical F REJECT Ho:All groups equal

NOEC = 0.22 mg/l
LOEC = 0.47 mg/l

skeletonema cell density
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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 | | 107.500 | 107.500 | | |
| 2 | | 0.22 | 102.667 | 102.667 | 1.082 | |
| 3 | | 0.47 | 91.000 | 91.000 | 3.692 | * |
| 4 | | 0.85 | 80.333 | 80.333 | 6.079 | * |
| 5 | | 2.1 | 70.000 | 70.000 | 8.391 | * |
| 6 | | 4.3 | 45.667 | 45.667 | 13.836 | * |
| 7 | | 7.7 | 25.333 | 25.333 | 18.386 | * |
| 8 | | 16 | 14.333 | 14.333 | 20.847 | * |
| 9 | | 32 | 3.000 | 3.000 | 23.383 | * |

Bonferroni T table value = 2.73 (1 Tailed Value, P=0.05, df=21,8)

skeletonema cell density
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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 | 0.22 | 3 | 12.209 | 11.4 | 4.833 |
| 3 | 0.47 | 3 | 12.209 | 11.4 | 16.500 |
| 4 | 0.85 | 3 | 12.209 | 11.4 | 27.167 |
| 5 | 2.1 | 3 | 12.209 | 11.4 | 37.500 |
| 6 | 4.3 | 3 | 12.209 | 11.4 | 61.833 |
| 7 | 7.7 | 3 | 12.209 | 11.4 | 82.167 |
| 8 | 16 | 3 | 12.209 | 11.4 | 93.167 |
| 9 | 32 | 3 | 12.209 | 11.4 | 104.500 |

MOSSLER PARAQUAT SKELETONEMA COSTATUM 2-9-93

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| CONC. | NUMBER EXPOSED | NUMBER DEAD | PERCENT DEAD | BINOMIAL PROB.(PERCENT) |
|---|---|---|---|---|
| 32 | 100 | 97 | 97 | 0 |
| 16 | 100 | 87 | 87 | 0 |
| 7.7 | 100 | 77 | 77 | 0 |
| 4.3 | 100 | 57 | 57 | 0 |
| 2.1 | 100 | 34 | 34 | 0 |
| .85 | 100 | 24 | 24 | 0 |
| .47 | 100 | 15 | 15 | 0 |
| .22 | 100 | 4 | 4 | 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 3.464744

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

| SPAN | G | LC50 | 95 PERCENT CONFIDENCE LIMITS | |
|---|---|---|---|---|
| 7 | .0139224 | 2.812413 | 2.415626 | 3.276565 |

RESULTS CALCULATED USING THE PROBIT METHOD

| ITERATIONS | G | H | GOODNESS OF FIT PROBABILITY |
|---|---|---|---|
| 3 | 1.329965E-02 | 1 | .3802964 |

SLOPE = 1.534003

95 PERCENT CONFIDENCE LIMITS = 1.357096 AND 1.710911

LC50 = 2.845681

95 PERCENT CONFIDENCE LIMITS = 2.427932 AND 3.334309

LC10 = .4229481

95 PERCENT CONFIDENCE LIMITS = .3132032 AND .542375

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