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: *Navicula pelliculosa*.

4. **CITATION:** Smyth, D.V., S.A. Sankey, and S.K. Cornish. 1992. Paraquat Dichloride: Toxicity to the Freshwater Diatom *Navicula pelliculosa*. Laboratory ID No. T168/D. Conducted by Imperial Chemical Industries PLC, Devon, UK. Submitted by ICI Americas, Inc. EPA MRID No. 426010-06.

5. **REVIEWED BY:**

Renée Costello
Biologist
EFED/EEB

Signature: Renée Costello
Date: 4/12/93

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 adjusted nominal concentrations, the 4-day NOEC, LOEC, and EC_{50} for *N. pelliculosa* exposed to paraquat dichloride were 0.22, 0.45, and 0.55 µg/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, *Navicula pelliculosa*, came from laboratory stock cultures kept under axenic conditions. Stock cultures were maintained in synthetic nutrient medium at a temperature of 24 ±1°C, with orbital shaking at 140 rpm. Cool white illumination provided a light intensity of 4010 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 8.2-8.6.

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 0.04, 0.08, 0.16, 0.32, 0.64, 1.28, 2.56, and 5.12 µg/l, and a medium control were used for the definitive test.

A stock solution of 51,200 µg/l was prepared by direct addition of the test material to sterile culture medium. An intermediate stock solution of 102.4 µg/l was prepared in sterile medium from the primary stock. Aliquots of the intermediate stock or the 5.12 µg/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 added diatoms) was also incubated concurrently.

An inoculum volume of 1.6 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.

Since the concentrations of the exposure solutions were below the limit of detection for the chromatographic procedure, only the primary and intermediate stocks were sampled. Samples were analyzed at test initiation and 100 mls of each of these solutions were incubated under test conditions. Samples were taken of each stock at test termination and analyzed.

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:** Nominal concentrations were used as the basis for 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 of the primary and intermediate stocks were 90 and 68% of nominal at test initiation, respectively. After 4 days, measured concentrations of these two stocks were 90 and 70% of nominal, respectively. The control and exposure solutions were clear and colorless.

Diatom densities for the control and the exposure concentrations throughout the test are given in Table 1 (attached).

By day 4, the effect of the test material on the area under the growth curve, relative to the control, ranged between 55% stimulation and 100% inhibition (Table 2, attached). The no-observed-effect concentration (NOEC), lowest-observed-effect concentration (LOEC), and EC_{50} were 0.64, 1.28, and 0.69 µg/l, respectively. The 95% confidence interval was 0.51-0.96 µg/l.

By day 4, the effect of the test material on the growth rate, relative to the control, ranged between 17% stimulation and 100% inhibition (Table 3, attached). The NOEC, LOEC, and EC_{50} were 0.64, 1.28, and 1.03 µg/l, respectively. The 95% confidence interval was 0.72-1.47 µg/l.

The pH in the control and the exposure concentrations was 8.2-8.6 at the beginning of the study and 7.4-7.8 at the conclusion. Temperature ranged from 24.0 to 25.1°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 light intensity (4.0 klux) was less than recommended (4.3 klux).

The initial pH (8.2-8.6) was higher than recommended (7.5).

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:** Since the intermediate stock was used to prepare the test solutions, the reviewer multiplied the nominal concentrations by 0.7 (adjusted for 70% of nominal according to analytical measurements). Adjusted nominal concentrations were 0.03, 0.06, 0.11, 0.22, 0.45, 0.90, 1.79, and 3.58 mg/l.

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 more conservative EC_{50} and narrower confidence interval (C.I.) were calculated using the moving average angle method. The 4-day NOEC, LOEC, and EC_{50} were determined to be 0.22, 0.45, and 0.55 µg/l (95% C.I.= 0.50-0.59 µg/l), respectively.

C. **Discussion/Results:** Although not stated in this report, studies conducted with this same material (MRID No.'s 426010-02, -04, and -06) indicated that the test solutions were not corrected for the percent purity of the test material.

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 adjusted nominal concentrations, the 4-day NOEC, LOEC, and EC_{50} for *N. pelliculosa* exposed to paraquat dichloride were 0.22, 0.45, and 0.55 µg/l, respectively.

D. **Adequacy of the Study:**

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

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

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

DER 426010-06

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Pages 6 through 9 are not included in this copy.

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.

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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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navicula cell density
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ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	8	1082.217	135.277	9.962
Within (Error)	21	285.153	13.579	
Total	29	1367.370		

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

NOEC - 0.45 µg/l - however 54% inhibition at this level, therefore -
NOEC = 0.22 µg/l
LOEC = 0.45 µg/l

navicula cell density
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BONFERRONI T-TEST - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	control	10.147	10.147		
2	0.03	12.160	12.160	-0.773	
3	0.06	14.667	14.667	-1.735	
4	0.11	13.233	13.233	-1.185	
5	0.22	18.100	18.100	-3.052	
6	0.45	4.633	4.633	2.116	
7	0.90	2.127	2.127	3.078	*
8	1.79	0.267	0.267	3.792	*
9	3.58	0.373	0.373	3.751	*

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

navicula 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	0.03	3	7.119	70.2	-2.013
3	0.06	3	7.119	70.2	-4.520
4	0.11	3	7.119	70.2	-3.087
5	0.22	3	7.119	70.2	-7.953
6	0.45	3	7.119	70.2	5.513
7	0.90	3	7.119	70.2	8.020
8	1.79	3	7.119	70.2	9.880
9	3.58	3	7.119	70.2	9.773

MOSSLER PARAQUAT NAVICULA PELLICULOSA 2-10-93

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CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
1.79	100	97	97	0
.9	100	79	79	0
.45	100	54	54	0
.22	100	0	0	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 .4338279

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS	
3	1.073705E-02	.5478721	.5035424	.5942298

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
3	1.098707	9.027512	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 = 4.030658

95 PERCENT CONFIDENCE LIMITS =-.1942468 AND 8.255562

LC50 = .525097

95 PERCENT CONFIDENCE LIMITS = 0 AND +INFINITY

LC10 = .2541877

95 PERCENT CONFIDENCE LIMITS = 0 AND .4772417

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