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: *Lemna gibba*.

4. **CITATION:** Smyth, D.V., S.A. Sankey, S.K. Cornish, and A.J. Penwell. 1992. Paraquat Dichloride: Toxicity to the Duckweed *Lemna gibba*. Laboratory ID No. T168/E. Conducted by Imperial Chemical Industries PLC, Devon, UK. Submitted by ICI Americas, Inc. EPA MRID No. 426010-03.

5. **REVIEWED BY:**

Renee Lamb
Biologist
EFED/EEB

Signature: Renée Lamb
Date: 9/17/93

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 aquatic plant study for a formulated product. The technical material was of less than 80% purity. Based on nominal concentrations, the 14-day NOEC, LOEC, and EC_{50} for *L. gibba* exposed to paraquat dichloride were 16, 32, and 98 µg/l, respectively.

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

9. **BACKGROUND:** N/A

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

11. **MATERIALS AND METHODS:**

A. **Test Species:** The plants used in the test, *Lemna gibba* G3, came from the University of Waterloo, Canada. Plants were maintained in M-type Hoagland's medium under 5000 lux illumination, and a temperature of 25 ±1°C. Warm-white fluorescent tubes and a continuous photoperiod were used. Plants that were growing actively were used as inoculum for the test.

B. **Test System:** Test vessels used were glass 400 ml cylindrical dishes with loose-fitting lids. The test medium was the same as that used for culturing, with a pH of 4.5 to 4.9.

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

C. **Dosage:** Fourteen-day growth and reproduction study. Nominal rates of 4, 8, 16, 32, 64, 128, 256, and 512 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. Aliquots of the stock or the 512 µg/l test solution were added to sterile culture medium to obtain the nominal test concentrations.

D. **Test Design:** One-hundred and sixty ml of the test or control solution were placed in each of three replicate dishes (3 per treatment level and control). Test solutions were renewed on days 4, 8, and 11. The dishes were randomized by rows within the incubator and were re-randomized after 7 days.

Three plants with four fronds each were randomly placed in each replicate dish. Frond counts were performed on test days 1, 4, 6, 8, 11, and 14. All fronds which visibly projected beyond the edge of the parent frond were counted. Toxicity symptoms were recorded. At the end of the test (14 days), the plants from each dish were rinsed with distilled water and dried to a constant weight at 60°C.

Samples were taken from the freshly-prepared solutions of ≥64 µg/l on day 8 and the old test solutions of

these same concentrations on day 14. These samples were analyzed for the test material by spectrophotometric methods.

The pH of the freshly-prepared test solutions was measured on days 0, 4, 8, and 11 and the pH of two replicates of the old test solutions was measured on days 4, 8, 11, and 14. The temperature of the incubator was measured daily by thermometer and hourly by a data logger. The light intensity was measured once during each week of the study.

E. **Statistics:** Due to the limited amount of chemical analyses, nominal concentrations were used as the basis for the data analysis. The increase in frond number over the 14 day test period was calculated by subtracting 12 (the number of fronds inoculated on day 0) from the 14 day counts. Mean increase in frond number was used to determine the percent inhibition. Percent inhibition data were analyzed using the moving average angle method to estimate the 14-day EC_{50} and its associated 95% confidence interval (C.I.). Increase in frond number was examined by one-way analysis of variance, and Dunnett's test ($p \leq 0.05$) was used to identify significant differences from the control.

Increase in dry weight was calculated by subtraction of the estimated initial weight (12 fronds = 1.4 mg dry weight) from the 14 day dry weight. The mean increase for each treatment and mean percent inhibition were calculated. These data were analyzed as previously described.

12. **REPORTED RESULTS:** Mean measured concentrations ranged from 75 to 94% of nominal (Table 1, attached). After solution preparation, a visual assessment showed the solutions to be clear and colorless with some small particles in stirred suspension.

The number of fronds and the number of plants in each vessel at each time period are presented in Table 2 (attached). Increase in frond number and percent inhibition are listed in Table 3 (attached). Plant dry weights and percent inhibition are given in Table 4 (attached).

The reported no-observed-effect concentration (NOEC) for increase in frond number was 64 µg/l. The EC_{50} based on frond number was 113 µg/l (95% C.I.= 101-127 µg/l). The

NOEC and EC_{50} based on dry weight were 64 µg/l and 139 (95% C.I.= 127-153 µg/l), respectively.

From day 4 onwards, plants exposed to solutions of ≥128 µg/l exhibited frond chlorosis, smallness of size, reduced root growth, and abnormal colony formation and floatation. A similar pattern was apparent on plants exposed to the 64 µg/l solutions from day 6 onwards. Plants in the 32 µg/l solutions were noted to be slightly chlorotic with reduced root growth at test termination. There were no effects noted on plants in the ≤16 µg/l solutions except for some transient small colony size and reduced root formation exhibited by plants in one replicate of the 8 µg/l solution on day 11.

The pH in the freshly prepared solutions ranged between 4.5 and 4.9 and between 4.8 and 5.7 in the old test solutions. Temperature ranged between 24.4 and 25.4°C. Light intensity was 5.3 klux on both day 1 and 7 of the test.

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 procedure and the report deviated from the SEP and Subdivision J guidelines in the following areas:

Three plants with 4 fronds each were used as inoculum rather than the recommended 5 plants with 3 fronds each.

The light intensity was 5.3 klux. The recommended intensity is 5 klux.

The pH of the culture medium (4.5-4.9) was lower than the recommended 5.0 ±0.1.

An inert ingredients control was not included in the study design. This type of control should be included for studies in which the technical material is of less than 80% purity.

B. **Statistical Analysis:** The reviewer used EPA's Toxanal program to determine the EC value and Dunnett's test to determine the NOEC and lowest-observed-effect concentration (LOEC). A slightly more conservative estimate of the EC_{50} was obtained by the reviewer. The 14-day EC_{50} based on nominal concentrations was 98 µg/l (95% C.I.= 87-109 µg/l).

C. **Discussion/Results:** The solutions were clear and colorless with some small particles in stirred suspension. Since the measured concentrations were close to nominal concentrations, it is apparent that the slight amount of undissolved material was probably a precipitate. The samples were extracted on a cation exchange column that should have separated this impurity from the analyte. Due to problems with analytical methodology, only test solutions of ≥64 mg/l nominal concentration could be analyzed for the test material. Therefore, the nominal concentrations listed by the authors are considered valid by the reviewer, and were used to report the results.

Although not stated in this report, the algal 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.

Since phytotoxic effects were noted at the 32 µg/l level, the NOEC and LOEC will be reported as 16 and 32 µg/l, respectively.

This study is scientifically sound and meets the guideline requirements for a Tier 2 non-target aquatic plant study with a formulated product. Based on nominal concentrations, the 14-day NOEC, LOEC, and EC_{50} for *L. gibba* exposed to paraquat dichloride were 16, 32, and 98 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, 2-8-93.

DER # 426010-03

Page _____ is not included in this copy.

Pages 6 through 10 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 document is not responsive to the request.

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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lemna frond number
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ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	8	439080.389	54885.049	161.440
Within (Error)	27	9179.250	339.972	
Total	35	448259.639		

Critical F value = 2.31 (0.05,8,27)
Since F > Critical F REJECT Ho:All groups equal

NOEC = 32 mg/l, however, chlorosis and reduced root growth noted so
NOEC = 16 mg/l
LOEC = 32 mg/l

lemna frond number
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DUNNETTS TEST - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	control	260.250	260.250		
2	4	271.000	271.000	-0.825	
3	8	246.750	246.750	1.035	
4	16	305.250	305.250	-3.451	
5	32	311.250	311.250	-3.912	
6	64	216.250	216.250	3.375	*
7	128	75.750	75.750	14.151	*
8	256	31.250	31.250	17.564	*
9	512	22.000	22.000	18.274	*

Dunnett table value = 2.53 (1 Tailed Value, P=0.05, df=24,8)

lemna frond number
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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	control	4			
2	4	4	32.986	12.7	-10.750
3	8	4	32.986	12.7	13.500
4	16	4	32.986	12.7	-45.000
5	32	4	32.986	12.7	-51.000
6	64	4	32.986	12.7	44.000
7	128	4	32.986	12.7	184.500
8	256	4	32.986	12.7	229.000
9	512	4	32.986	12.7	238.25

MOSSLER PARAQUAT LEMNA GIBBA 2-8-93

CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
512	100	96	96	0
256	100	92	92	0
128	100	74	74	0
64	100	18	18	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 95.72669

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS	
2	2.745435E-02	97.98572	87.06219	108.6285

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
5	1.351903	8.321178	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 = 3.273414
95 PERCENT CONFIDENCE LIMITS =-.5326283 AND 7.079457

LC50 = 101.4658
95 PERCENT CONFIDENCE LIMITS = 0 AND +INFINITY

LC10 = 41.52841
95 PERCENT CONFIDENCE LIMITS = 0 AND 91.21439