8-17-92 EEB FILE

121011

MRID No. 420297-01

DATA EVALUATION RECORD

1. **CHEMICAL:** Clethodim.
Shaughnessey No. 121011.

2. **TEST MATERIAL:** Clethodim technical (RE-45601); Lot No. SX-1688; CAS No. 99129-21-2; 82.4% active ingredient; a clear, amber liquid.

3. **STUDY TYPE:** Growth and Reproduction of Aquatic Plants -- Tier 2. Species Tested: Duckweed (*Lemna gibba*).

4. **CITATION:** Rhodes, J.E. and J.S. Hughes. 1991. The Phytotoxicity of RE-45601 Technical with Duckweed (*Lemna gibba* G3) in a Static System. Laboratory Project ID B765-01-1. Conducted by Malcolm Pirnie, Inc., Tarrytown, NY. Submitted by Valent U.S.A. Corporation, Walnut Creek, CA. EPA MRID No. 420297-01.

5. **REVIEWED BY:**

Richard C. Petrie
Agronomist
EEB/EFED/OPP

Signature: Richard C. Petrie

Date: 7/23/92

6. **APPROVED BY:**

Daniel Rieder, Section 3 Head,
EEB/EFED/OPP

Signature: Daniel Rieder

Date: 8-17-92

Henry T. Craven

7. **CONCLUSIONS:** This study is scientifically sound and meets the guideline requirements for a Tier 2 aquatic plant growth and reproduction test. Based on mean measured concentrations, the 14-day EC_{50} was calculated to be 1.34 mg ai/L; the NOEC value is 0.37 mg ai/L and the LOEC value is 0.79 mg ai/L. - CHANGED - ADDED

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

9. **BACKGROUND:**

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

Upgraded to Core

11. **MATERIALS AND METHODS:**

concluded that the pH probably did not affect the results of the test.

Based on mean measured concentrations, the 14-day EC_{25} was calculated to be 0.47 mg ai/l with a 95% confidence interval of 0.29-0.77 mg ai/l. The 14-day EC_{50} was calculated to be 1.34 mg ai/l with a 95% confidence limit of 0.98-1.83 mg ai/l. The NOEC was 0.37 mg ai/l.

D. **Adequacy of the Study:**

(1) **Classification:** Core - Changed

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

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

15. **COMPLETION OF ONE-LINER:** Yes, 11-12-91.

V

DATA EVALUATION RECORD

1. **CHEMICAL:** Clethodim.
Shaughnessey No. 121011.

2. **TEST MATERIAL:** Clethodim technical (RE-45601); Lot No. SX-1688; CAS No. 99129-21-2; 82.4% active ingredient; a clear, amber liquid.

3. **STUDY TYPE:** Growth and Reproduction of Aquatic Plants -- Tier 2. Species Tested: Duckweed (*Lemna gibba*).

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5. **REVIEWED BY:**

Mark A. Mossler, M.S.
Agronomist
KBN Engineering and
Applied Sciences, Inc.

Signature: Mark A. Mossler
Date: 11/26/91

6. **APPROVED BY:**

Louis M. Rifici, M.S.
Associate Scientist
KBN Engineering and
Applied Sciences, Inc.

Signature: Louis M Rifici
Date: 12/2/91

Henry T. Craven, M.S.
Supervisor, EEB/EFED
USEPA

Signature: Henry T. Craven 8/17-92
Date: 8/18/92

7. **CONCLUSIONS:** This study is not scientifically sound and does not meet the guideline requirements for a Tier 2 aquatic plant growth and reproduction test. The concentrations of test substance that the plants were exposed to were largely unknown. Based on mean measured concentrations, the 14-day EC_{25} was calculated to be 0.47 mg ai/l with a 95% confidence interval of 0.29-0.77 mg ai/l. The 14-day EC_{50} was calculated to be 1.34 mg ai/l with a 95% confidence limit of 0.98-1.83 mg ai/l. The NOEC was 0.37 mg ai/l. — changed

5.15

B

The pH of the medium was much higher than the recommended pH. Based on the frond count data, growth in all test concentrations gradually increased over time and a clear exposure-related inhibition could be seen as early as five days into the test. It can be concluded that the pH probably did not affect the results of the test.

Based on mean measured concentrations, the 14-day EC_{25} was calculated to be 0.47 mg ai/l with a 95% confidence interval of 0.29-0.77 mg ai/l. The 14-day EC_{50} was calculated to be 1.34 mg ai/l with a 95% confidence limit of 0.98-1.83 mg ai/l. The NOEC was 0.37 mg ai/l.

This study is not scientifically sound and does not meet the guideline requirements for a Tier 2 toxicity study using non-target aquatic plants. Deleted

D. **Adequacy of the Study:**

(1) **Classification:** Invalid. - changed

(2) **Rationale:** The concentration of test material in the exposure solutions was unknown for a majority of the experimental period.

(3) **Repairability:** No.

15. **COMPLETION OF ONE-LINER:** Yes, 11-12-91.

4

RPETRIE CLETHODIM LEMNA 07/22/92

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CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
8.4	100	78	78	0
4	100	77	77	0
1.7	100	53	53	0
.79	100	41	41	0
.37	100	7	7	0
.053	100	1	1	0
.022	100	7	7	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 1.404561

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS	
5	2.777367E-02	1.743381	1.380407	2.252307

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
3	.3189084	8.910339	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 = 1.18816

95 PERCENT CONFIDENCE LIMITS = .5171827 AND 1.859138

LC50 = 1.570195

95 PERCENT CONFIDENCE LIMITS = .6250406 AND 5.029541

LC10 = .1339906

95 PERCENT CONFIDENCE LIMITS = 5.903873E-03 AND .3868791

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-10031-01

Solvent Control

14 day

RPETRIE CLETHODIM LEMNA 07/22/92

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CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
8.4	100	86	86	0
4	100	83	83	0
1.7	100	54	54	0
.79	100	43	43	0
.37	100	6	6	0
.053	100	3	3	0
.022	100	0	0	0

THE BINOMIAL TEST SHOWS THAT .79 AND 1.7 CAN BE USED AS STATISTICALLY SOUND CONSERVATIVE 95 PERCENT CONFIDENCE LIMITS, BECAUSE THE ACTUAL CONFIDENCE LEVEL ASSOCIATED WITH THESE LIMITS IS GREATER THAN 95 PERCENT.

AN APPROXIMATE LC50 FOR THIS SET OF DATA IS 1.287035

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS	
5	2.532347E-02	1.431108	1.146866	1.804415

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
4	.1395366	4.013493	1.213722E-03

SINCE THE PROBABILITY IS LESS THAN 0.05, RESULTS CALCULATED USING THE PROBIT METHOD PROBABLY SHOULD NOT BE USED.

SLOPE = 1.633631

95 PERCENT CONFIDENCE LIMITS = 1.023395 AND 2.243868

LC50 = 1.406097

95 PERCENT CONFIDENCE LIMITS = .8678359 AND 2.271414

LC10 = .2347502

95 PERCENT CONFIDENCE LIMITS = 7.006312E-02 AND .4388232

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

9. **BACKGROUND:**

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

11. **MATERIALS AND METHODS:**

A. **Test Species:** *Lemna gibba* G3 used in the test came from laboratory stock cultures originally obtained from Dr. C.F. Cleland, Smithsonian Institution, Rockville, MD. Stock cultures were maintained in synthetic twenty-strength algal assay procedure nutrient medium (20X-AAP) under 4198-5813 lux warm-white illumination, and a temperature of 25 ±2°C. Transfers were made to provide 7 to 11 day old cultures. The culture used as inoculum had been transferred to fresh medium seven days before test initiation.

B. **Test System:** All glassware was cleaned according to EPA methods and autoclaved before use. Test vessels used were 500-ml Erlenmeyer flasks fitted with foam stoppers which permitted gas exchange. The test medium was the same as that used for culturing with the pH adjusted to 7.5 ±0.1 and filtered (0.22 µm).

Two-hundred milliliters of the appropriate test or control solution were placed into each flask. The test vessels were kept in an incubator under continuous illumination and environmental conditions like those employed in culturing.

C. **Dosage:** Fourteen-day growth and reproduction test. Based on the results of a preliminary test, seven nominal concentrations of 0.05, 0.1, 0.625, 1.25, 2.5, 5.0, and 10 mg ai/l, a solvent control [0.25 ml N,N-dimethylformamide (DMF)/l], and a medium control were selected for the definitive test. Stock solutions of 40, 20, 5, 2.5, 0.4 and 0.2 mg ai/ml were prepared by adding 0.4854 g of clethodim technical to 10 ml of DMF and serially diluting accordingly. The material went into solution after inverting the flask several times. The test concentrations were prepared by diluting appropriate volumes of the stock solutions in 1000 ml of medium.

D. **Test Design:** An inoculum of *Lemna gibba* consisted of three plants, each with four fronds, in each test container (3 containers per treatment). The flasks were randomly repositioned each working day to minimize

1

spacial differences in the incubator. Frond counts were made on test days 3, 5, 7, 10, 12, and 14 at approximately the same time each day. Every frond visibly projecting beyond the edge of the parent was counted.

The pH was measured at test initiation and termination. The temperature in the incubator was recorded continuously.

At test initiation and termination (Day 0 and Day 14), samples were removed from test and control solutions, frozen, and sent to ABC Laboratories, Columbia, MO, for analysis by reverse phase high-pressure liquid chromatography (HPLC).

E. **Statistics:** All calculations were made using mean measured concentrations. The 14-day EC values and associated 95% confidence intervals were computed using weighted least squares non-linear regression of the log of test concentration against the day-14 frond counts. The no-observed-effects concentration (NOEC) was estimated using analysis of variance (ANOVA) and Dunnett's Test.

12. **REPORTED RESULTS:** The mean measured concentrations were 0.022, 0.053, 0.37, 0.79, 1.7, 4.0, and 8.4 mg ai/l. Measured concentrations averaged 83-104% of nominal at test initiation and 6-66% of nominal on day 14 (Table 3, attached).

Mean frond count and percent inhibition for each concentration after fourteen days are given in Table 5 (attached). Percent inhibition increased with increasing toxicant concentration.

The 14-day EC_{25} was calculated to be 0.47 mg ai/l with a 95% confidence interval of 0.29-0.77 mg ai/l. The 14-day EC_{50} was calculated to be 1.34 mg ai/l with a 95% confidence limit of 0.98-1.83 mg ai/l. The NOEC was 0.37 mg ai/l.

The pH ranged from 7.54 to 7.74 in all test solutions and the controls at test initiation and from 9.13 to 9.48 at test termination.

13. **STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:** No conclusions were made by the authors.

8

Good Laboratory Practice and Quality Assurance statements were included in the report indicating compliance with EPA Good Laboratory Practice Standards, 40 CFR Part 160, under the Federal Insecticide, Fungicide, and Rodenticide Act.

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

A. **Test Procedure:** The test procedure and the report were generally in accordance with the SEP and Subdivision J guidelines, except for the following deviations:

The medium (20X-AAP) used in the study had a pH of 7.5 ±0.1. The guidelines state that the pH of the medium should be 5.0 ±0.1.

Three plants (four fronds each) were used to inoculate each test flask. Five plants per flask are recommended.

The conductivity of the test solutions was not measured.

The light intensity during the test (4196-5813 lux) was occasionally lower or higher than recommended (5 klux).

B. **Statistical Analysis:** The reviewer performed probit and ANOVA (Dunnett's) analyses on the 14-day data to determine the EC and NOEC values, respectively. The results obtained by the reviewer are in agreement or are slightly less conservative than those obtained by the authors.

C. **Discussion/Results:** The authors stated that the measured concentration on day 14 for the lowest concentration was 0.00276 mg ai/l. However, the limit of detection was stated to be 0.00568 mg ai/l. The reviewer is unclear as to how the test concentration was derived.

The amount of recovery in the 14-day solutions ranged between 6 and 66%. These results were indicative of a chemical that is fairly unstable over a two-week period. Previous reports from the performing laboratory have used 3-day renewals for chemicals that are unstable over the test period. The reviewer questions why this study was not performed in this manner. Because the variability of the percent recoveries was so great, the concentrations that the duckweeds were exposed to are largely unknown.

9

CLETHODIM

Page _____ is not included in this copy.

Pages 10 through 13 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.

X FIFRA registration data.

_____ The document is a duplicate of page(s) _____.

_____ 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.

lemna frond number

Summary Statistics and ANOVA

Transformation = None

Group	n	Mean	s.d.	cv%
1 = control	3	654.6667	32.7159	5.0
2 0.022	3	664.3333	21.1975	3.2
3 0.053	3	642.0000	24.5561	3.8
4 0.37	3	614.6667	36.5285	5.9
5* 0.79	3	375.6667	71.3466	19.0
6* 1.7	3	306.3333	69.6946	22.8
7* 4.0	3	122.6667	17.4738	14.2
8* 8.4	3	102.0000	31.1929	30.6

rate (mg ai/l)*

1 = solvent control.

*) the mean for this group is significantly less than the control mean at alpha = 0.05 (1-sided) by Dunnett's test

Minumum detectable difference for Dunnett's test = -89.548175
This difference corresponds to -13.68 percent of control

Between groups sum of squares = 1213476.958333 with 7 degrees of freedom.

Error mean square = 1835.375000 with 16 degrees of freedom.

Bartlett's test p-value for equality of variances = .477

*- based on mean measured concentrations.

NOEC = 0.37 mg ai/l

Based on data from Table 4 (attached).

lemna frond number

Estimated EC Values and Confidence Limits

Point	Conc.	Lower 95% Confidence Limits	Upper 95% Confidence Limits
EC 1.00	0.0599	0.0097	0.1482
EC 5.00	0.1517	0.0399	0.3024
EC10.00	0.2489	0.0838	0.4467
EC15.00	0.3477	0.1373	0.5855
EC50.00	1.4278	0.9184	2.2098
EC85.00	5.8633	3.5016	14.6361
EC90.00	8.1899	4.5923	23.9571
EC95.00	13.4377	6.7878	50.2700
EC99.00	34.0144	13.8618	205.6818

$Y = 4.74 + 1.69(x)$

Y = probit % inhibition

x = log (rate)

$EC_{25} = 0.57$ mg ai/l

Raw data from Table 5 (attached)

Shaughnessey # 121011 Chemical Name Clethodim Chemical Class _____ Page 1 of 1

Study/Species/Lab/ MRID #	Chemical % a.i.	Results	Reviewer/ Date	Validation Status

14-Day EC_{50}	82.4	EC_{50} = 1.34 ~~pp~~ mg ai/l * 95% C.L. (0.98 - 1.83) - non-linear regression
		# plants/vessel = 3 plants / 4 fronds each
		Slope = N/C **
		Temperature = 25 ± 2 °C
		Species: Lemna gibba
		Lab: Malcolm Pirnie
		14-Day Dose Level ~~pp~~ mg ai/l * /(% Effect)
		0.022 (0), 0.053 (2), 0.37 (6), 0.79 (43), 1.7 (54), 4.0 (83), 8.4 (86).
		MRID # 420297-01
		Comments: NOEC = 0.37 mg ai/l *
		* - based on mean measured concentrations
		** - not given
			M. Mossler 11/12/91	CORE Invalid RCP 7/22/92

5-Day EC_{50}	_____	EC_{50} = pp 95% C.L. ( ) # Cells/ml = Slope = Species: Temperature = Lab: 5-Day Dose Level pp /(% Effect) ( ), ( ), ( ), ( ), ( ) MRID # Comments:	_____	_____