(8-17-92) 121011

MRID No. 420297-05

DATA EVALUATION RECORD

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

2. **TEST MATERIAL:** Select 2.0 EC; SX-1839; 10894-05; 25.6% active ingredient.

3. **STUDY TYPE:** Growth and Reproduction of Aquatic Plants -- Tier 2. Species Tested: *Anabaena flos-aquae*.

4. **CITATION:** Thompson, S.G., C.M. Holmes, and G.T. Peters. 1991. Select 2.0 EC: A 5-Day Toxicity Test with the Freshwater Alga (*Anabaena flos-aquae*). Laboratory Project ID 162A-119A. Conducted by Wildlife International Ltd., Easton, MD. Submitted by Valent U.S.A. Corporation, Walnut Creek, CA. EPA MRID No. 420297-05.

5. **REVIEWED BY:**

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

   Signature: [signature]

   Date: 11/27/91

6. **APPROVED BY:**

   Louis 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 8/8/92 Richard Petrie 7/28/92

   Date: [signature] 8-7-92

7. **CONCLUSIONS:** This study is scientifically sound and meets the guideline requirements for a Tier 2 aquatic plant growth and reproduction test. The 5-day EC_{50} and NOEC for clethodim were 16.7 and 5.6 mg ai/l, respectively. These values translate to an EC_{50} and NOEC for Select 2.0 EC of 58 and 22 mg/l of formulated product, respectively.

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

9. **BACKGROUND:**

Core Study

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

11. **MATERIALS AND METHODS:**

A. **Test Species:** *Anabaena flos-aquae* cultures used in the test came from laboratory stock cultures originally obtained from University of Texas, Austin, Texas. Cultures that were exponentially growing were used for test inoculum.

B. **Test System:** Test vessels used were 250-ml flasks. The test medium (Table 2, attached) was the same as that used for culturing with the pH adjusted to 7.5 ±0.1 and steam sterilized.

One-hundred milliliters of the appropriate test or control solution were placed into each flask which were kept at 24 ±2°C in an environmental chamber under continuous illumination (1,605-2,140 lux).

C. **Dosage:** Five-day growth and reproduction test. Based on the results of a preliminary test, seven nominal concentrations of 1.6, 3.1, 6.3, 12.5, 25, 50, and 100 mg ai/l, a solvent control (0.513 ml acetone/l), a formulation control, and a medium control were selected for the definitive test. An initial stock solution of the test material was prepare in acetone. The test concentrations were prepared by diluting appropriate volumes of the stock solution in 1000 ml of medium.

D. **Test Design:** An inoculum of *Anabaena flos-aquae* designed to provide 7,050 cells/ml was added to each flask (3 containers per treatment). Algal growth was monitored daily by conducting cell counts on a microscope using a hemocytometer.

The pH was measured at the beginning and end of the study. Temperature within the growth chamber was monitored continuously.

At the beginning and end of the test, samples were removed from exposure and control solutions, frozen, and sent to Chevron Chemical Company, Richmond, CA, for analysis by reverse phase high-pressure liquid chromatography (HPLC).

E. **Statistics:** All calculations were made using mean measured concentrations. The growth rate was computed from cell density data. The 5-day EC values and associated 95% confidence intervals were calculated

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using the binomial method on growth rate versus mean measured concentration data. The no-observed-effects concentration (NOEC) was estimated using a Kruskal-Wallis test.

12. **REPORTED RESULTS:** The mean measured concentrations were 1.5, 3.1, 5.6, 11.2, 25.7, 47.8, and 89.9 mg ai/l (Table 1, attached).

Cell counts for each concentration after five days are given in Table 4 (attached). Percent inhibition increased with increasing toxicant concentration for the four highest test concentrations. Growth in the formulation blank was significantly lower than that of the negative control.

The 5-day EC_{50} was calculated to be 14.8 mg ai/l with a 95% confidence limit of 11.2-25.7 mg ai/l based on growth rate inhibition. The NOEC was 5.6 mg ai/l.

The pH ranged from 6.9 to 7.6 in all test solutions and the controls at test initiation and from 7.0 to 7.6 at test termination. The temperature ranged from 23.5 to 25.5°C.

13. **STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:** The authors concluded that if Select 2.0 EC were applied at 0.25 lb ai/A to a water depth of 6 inches, the estimated environmental concentration of clethodim would be 0.184 mg/l, or 3.28% of the NOEC.

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 cell inoculum (7,050 cells/ml) was greater than recommended (3,000 cells/ml).

The conductivity of the test solutions was not measured.

B. **Statistical Analysis:** The reviewer used EPA's Toxanal program and ANOVA (coupled with Dunnett's test) analyses on the 5-day percent inhibition and cell count

data to determine the EC and NOEC values, respectively. The reviewer obtained slightly less conservative values for the EC_{50} and NOEC than the authors. The EC_{50} and NOEC for Select 2.0 EC are 58 and 22 mg/l of formulated product, respectively, based on cell density.

C. **Discussion/Results:** Since the title of this study indicates that the formulated product Select 2.0 EC is the subject of the test, the reviewer calculated the EC and NOEC values in terms of mg/l of formulated product. The reviewer used the solvent control to compare the treatments against since the formulation was the focus of the study.

Based on mean measured concentrations transformed to mg/l formulated product, the 5-day EC_{50} was calculated to be 58 mg/l with a 95% confidence limit of 44-100 mg/l. The NOEC was 22 mg/l. These values are equivalent to EC_{50} and NOEC values of 15 and 5.6 mg ai/l, respectively, for clethodim.

This study is scientifically sound and meets the guideline requirements for a Tier 2 toxicity study using non-target aquatic plants.

D. **Adequacy of the Study:**

(1) **Classification:** Core for Select 2.0 EC only.

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

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

15. **COMPLETION OF ONE-LINER:** Yes, 11-14-91.

CLETHODIM

Page _____ is not included in this copy.

Pages 5 through 8 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.

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MOSSLER CLETHODIM ANABAENA FLOS-AQUAE 11-14-91

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CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
346	100	99	99	0
184	100	99	99	0
98.9	100	98	98	0
43.1	100	5	5	0
21.6	100	0	0	0
11.9	100	0	0	0
5.8	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 63.51599

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS	
5	9.743826E-03	65.02201	58.8249	71.89094

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
8	288.7708	5558.684	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 = 6.897039

95 PERCENT CONFIDENCE LIMITS =-110.3061 AND 124.1002

LC50 = 66.14347

95 PERCENT CONFIDENCE LIMITS = 0 AND +INFINITY

LC10 = 43.28625

95 PERCENT CONFIDENCE LIMITS = 0 AND +INFINITY

****

% inhibition calculated from cell densities compared to solvent control - Table 4 (attached).

Anabaena cell density

Summary Statistics and ANOVA

Transformation = None

Group	n	Mean	s.d.	cv%
1 = control	3	154666.6667	31085.9025	20.1
2 5.5	3	259666.6667	127032.8042	48.9
3 11.9	3	285333.3333	62740.2051	22.0
4 21.6	3	389666.6667	117627.9445	30.2
5 43.1	3	147333.3333	41956.3265	28.5
6* 98.7	3	3666.6667	3214.5503	87.7
7* 184	3	333.3333	577.3503	173.2
8* 346	3	666.6667	577.3503	86.6

Concentration (mg/l)*

1 = solvent control

NOEC = 43.1 mg/l of Select 2.0 EC.

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

* Concentrations based on measured concentrations of ai converted to mg/l of formulated product. Raw data from Table 4 (attached).

Minumum detectable difference for Dunnett's test = -141472.655858
This difference corresponds to -91.47 percent of control

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

Error mean square = ********** with 16 degrees of freedom.

Bartlett's test p-value for equality of variances = .001

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*                                              *
* Warning - the test for equality of variances *
* is significant (p less than 0.01).  The      *
* results of this analysis should be inter-    *
* preted with caution.                         *
*                                              *
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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}	_____	EC_{50} = pp (95% C.L.) # plants/vessel = Slope = Species: Temperature = Lab: 14-Day Dose Level pp /(% Effect) ( ), ( ), ( ), ( ), ( ) MRID # Comments:	_____	_____

5-Day EC_{50}	25.6	EC_{50} = ~~6558~~ 58 ~~pp~~ mg/l* for select 2.0 EC (95% C.L. ~~52-72~~ 44-100) 7 moving average
		 EC_{50} = 14.8 mg a.i./l for clethodim (11.2-25.7)
		 # Cells/ml = 7050
		 Slope = N/A
		 Species: Anabaena flos-aquae
		 Temperature = 24°C
		 Lab: W. Wildl. Intern.
		 5-Day Dose Level ~~pp~~ mg/l* /(% Effect)
		 5.8 (0), 11.9 (0), 21.6 (0), 43.1 (5), 98.9 (98)
		 184 (99), 346 (99)
		 MRID # 420297-05
		 Comments:
		 NOEC = 22 mg/l* for select 2.0 EC
		 NOEC = 5.6 mg a.i./l for clethodim
		 * - Based on mean measured concentrations of active ingredient converted to mg/l of formulated product.
			M. Mossler 11/14/91	Core.