EEB FILE

(8-17-92) 121011

MRID No. 420297-04

DATA EVALUATION RECORD

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

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

3. **STUDY TYPE:** Growth and Reproduction of Aquatic Plants -- Tier 2. Species Tested: *Skeletonema costatum*.

4. **CITATION:** Grimes, J., S.G. Thompson, C.M. Holmes, and G.T. Peters. 1991. Select 2.0 EC: A 5-Day Toxicity Test with the Marine Diatom (*Skeletonema costatum*). Laboratory Project ID 162A-120. Conducted by Wildlife International Ltd., Easton, MD. Submitted by Valent U.S.A. Corporation, Walnut Creek, CA. EPA MRID No. 420297-04.

5. **REVIEWED BY:**

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

Signature: Mark A. Mossler
Date: 11/21/91

6. **APPROVED BY:**

Louis M. Rifici, M.S.
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Signature: Louis M. Rifici
Date: 12/2/91

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

Signature: Henry T. Craven 8/18/92 Richard C. Petersen 7/23/92
Date: Thomas Bailey 8-17-92

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 8.6 and 5.4 mg ai/l, respectively. These values translate to an EC_{50} and NOEC for Select 2.0 EC of 33 and 21 mg/l of formulated product, respectively.

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

9. **BACKGROUND:** Core Study

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

11. **MATERIALS AND METHODS:**

A. **Test Species:** *Skeletonema costatum* 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 7.8-8.1 and filter sterilized (0.2 µm).

One-hundred milliliters of the appropriate test or control solution were placed into each flask which were kept at 20 ±2°C in an environmental chamber under a 16-hour photoperiod (3,745-4,548 lux). The test vessels were shaken at 100 rpm.

C. **Dosage:** Five-day growth and reproduction test. Based on the results of a preliminary test, five nominal concentrations of 3.9, 6.5, 10.8, 18, and 30 mg ai/l, a solvent control (0.15 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 prepared 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 *Skeletonema costatum* designed to provide 40,000 cells/ml was added to each flask (3 containers per treatment). Diatom growth was monitored daily by obtaining a small sample of test solution (1-2 ml) and 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

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from cell density data. The 5-day EC values and associated 95% confidence intervals were calculated using the binomial method on growth rate versus mean measured concentration data. The no-observed-effects concentration (NOEC) was estimated using the Kruskal-Wallis test.

12. **REPORTED RESULTS:** The mean measured concentrations were 3.0, 5.4, 9.7, 15.6, and 27.4 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 three highest test concentrations. Cell growth in the formulation blank was significantly reduced compared to the negative control.

The 5-day EC_{50} was calculated to be 8.55 mg ai/l with a 95% confidence limit of 5.36-9.72 mg ai/l based on growth rate inhibition. The NOEC was 5.36 mg ai/l.

The pH ranged from 7.9 to 8.1 in all test solutions and the controls at test initiation and from 8.4 to 8.9 at test termination. The temperature ranged from 18.7 to 21.3°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 3.4% 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 (40,000 cells/ml) was greater than recommended (10,000 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 EC_{50} and NOEC for Select 2.0 EC are 33 and 21 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 33 mg/l with a 95% confidence limit of 30-37 mg/l. The NOEC was 21 mg/l. These values are equivalent to EC_{50} and NOEC values of 8.6 and 5.4 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 7 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.

MOSSLER CLETHODIM SKELETONEMA COSTATUM 11-14-91

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CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
105	100	94	94	0
60.1	100	92	92	0
37.4	100	87	87	0
20.6	100	0	0	0
11.4	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 30.19969

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS	
4	1.802498E-02	33.40384	30.33289	36.60883

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
6	2.595804	42.88126	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 = 5.350573

95 PERCENT CONFIDENCE LIMITS =-3.270002 AND 13.97115

LC50 = 33.27504

95 PERCENT CONFIDENCE LIMITS = 0 AND +INFINITY

LC10 = 19.26464

95 PERCENT CONFIDENCE LIMITS = 0 AND +INFINITY

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% inhibition computed from comparison to solvent control - Table 4 (attached).

Skeletonema cell density

Summary Statistics and ANOVA

Transformation = None

Group	n	Mean	s.d.	cv%
1 = control	3	486666.6667	68879.1212	14.2
2 11.4	3	1112166.6667	298719.0709	26.9
3 20.6	2	987000.0000	84852.8137	8.6
4*37.4	3	63000.0000	11135.5287	17.7
5*60.1	3	39000.0000	6244.9980	16.0
6*105	3	26666.6667	11590.2258	43.5

Concentrations (mg/l)*

1 = solvent control

NOEC = 20.6 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 a t - test with Bonferroni adjustment of alpha level

* Based on measured concentrations of ai transformed to mg/l of Select 2.0 EC

Raw data from Table 4 (attached)

Minumum detectable difference for
t-tests with Bonferroni adjustment = -296122.338193
This difference corresponds to -60.85 percent of control

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*                                                *
* Note - the above value for the minimum         *
* detectable difference is approximate as        *
* the sample sizes are not the same for all of   *
* the groups.                                    *
*                                                *
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Between groups sum of squares =********** with 5 degrees of freedom.

Error mean square = ********** with 11 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} _____ Species: Lab: MRID # -	_____	EC_{50} = pp (95% C.L.) ; Slope = ; # plants/vessel = ; Temperature = ; 14-Day Dose Level pp /(% Effect): ( ), ( ), ( ), ( ), ( ); Comments:	_____	_____
5-Day EC_{50} Species: Skeletonema costatum Lab: Wildlife Intern. MRID # 420297-04	25.6	EC_{50} = 33 mg/l* (95% C.L. 30 - 37) for Select 2.0 EC, 7 moving average; EC_{50} = 8.6 mg ai/l (7.7 - 9.5) for clethodim; # Cells/ml = 40,000; Slope = N/A; Temperature = 20 °C; 5-Day Dose Level mg/l* /(% Effect): 11.4 (0), 20.6 (0), 37.4 (87), 60.1 (92), 105 (94); Comments: NOEC = 21 mg/l* for Select 2.0 EC; NOEC = 5.4 mg ai/l for clethodim; *- Based on mean measured concentrations of active ingredient converted to mg/l of formulated product.	M. Mossler 11/14/91	Core

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