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121011

MRID No. 420297-03

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: *Selenastrum capricornutum*.

4. **CITATION:** Grimstead, S.R., C.M. Holmes, and G.T. Peters. 1991. Select 2.0 EC: A 5-Day Toxicity Test with the Freshwater Alga (*Selenastrum capricornutum*). Laboratory Project ID 162A-121. Conducted by Wildlife International Ltd., Easton, MD. Submitted by Valent U.S.A. Corporation, Walnut Creek, CA. EPA MRID No. 420297-03.

5. **REVIEWED BY:**

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Signature: Mark A. Mossler
Date: 11/20/92

6. **APPROVED BY:**

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Henry T. Craven, M.S.
Supervisor, EEB/EFED
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Signature: Henry T. Craven 8/18/92 Richard C. Petersen 7/22/92
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 19.5 and 11.1 mg ai/l, respectively. These values translate to an EC_{50} and NOEC for Select 2.0 EC of 76 and 43.5 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:** *Selenastrum capricornutum* cultures used in the test came from laboratory stock cultures originally obtained from University of Texas, Austin, Texas. Cultures that were actively growing (exponential growth phase) 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 filter sterilized (0.2 µm).

One-hundred milliliters of the appropriate test or control solution were placed into each flask. The test vessels were kept at 24 ±2°C in an environmental chamber under continuous illumination (4,280-4,815 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, eight nominal concentrations of 2.8, 4.7, 7.8, 13, 22, 36, 60, and 100 mg ai/l, a solvent control, a formulation control, and a medium control were selected for the definitive test. Stock solutions were prepared by dissolving the test material in acetone. The test concentrations were prepared by diluting appropriate volumes of the stock solutions in 1000 ml of medium.

D. **Test Design:** An inoculum of *Selenastrum capricornutum* designed to provide 10,000 cells/ml was added to each flask (3 containers per treatment). Algal growth was monitored daily using an electronic particle counter. Each solution replicate was counted three times.

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

12. **REPORTED RESULTS:** The mean measured concentrations were 2.1, 3.6, 6.5, 11.3, 16.2, 33.7, 53.0, and 75.5 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. Mean cell density in the formulation blank was significantly lower than that of the negative control.

The 5-day EC_{50} was calculated to be 22.4 mg ai/l with a 95% confidence limit of 16.2-33.7 mg ai/l based on growth rate inhibition. The NOEC was 11.3 mg ai/l.

The pH ranged from 6.8 to 7.1 in all test solutions and the controls at test initiation and from 7.7 to 9.7 at test termination. The temperature ranged from 23 to 26°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 1.6% 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 (10,000 cells/ml) was greater than recommended (3,000 cells/ml).

The conductivity of the test solutions was not measured.

The light intensity during the test was slightly higher than recommended.

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 76 and 62.4 mg/l of formulated product, respectively, based on cell density. However, 62.4 mg/l of Select 2.0 EC decreased the cell number by 35%. Therefore, the NOEC should be taken to be 43.5 mg/l rather than 62.4 mg/l. These values translate to an EC_{50} and NOEC of 19.5 and 11.1 mg ai/l, respectively, for the active ingredient clethodim.

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 76 mg/l with a 95% confidence limit of 70-83 mg/l. The NOEC was 43.5 mg/l. These values are equivalent to 19.5 and 11.1 mg ai/l for the EC_{50} and NOEC, 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, 11-13-91.

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.

_____ 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 SELENASTRUM CAPRICORNUTUM 11-13-91

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CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB.(PERCENT)
291	100	97	97	0
204	100	97	97	0
130	100	96	96	0
62.4	100	35	35	0
43.5	100	0	0	0
25.1	100	0	0	0
13.7	100	0	0	0
7.9	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 72.7097

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS	
7	7.920951E-03	75.94914	69.69961	82.88994

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
6	.3833924	13.43834	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 = 5.374596
95 PERCENT CONFIDENCE LIMITS = 2.046717 AND 8.702474

LC50 = 80.54263
95 PERCENT CONFIDENCE LIMITS = 54.42425 AND 125.3526

LC10 = 46.74436
95 PERCENT CONFIDENCE LIMITS = 17.76589 AND 65.75031

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Raw data from Table 4 (attached)
% inhibition calculated by comparison to solvent control

Selenastrum cell density
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ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	8	16996108748992.000	2124513593624.000	20.407
Within (Error)	18	1873901418304.000	104105634350.250	
Total	26	18870010167296.000		

Critical F value = 2.51 (0.05,8,18)
Since F > Critical F REJECT Ho:All groups equal

Selenastrum cell density
File: sel Transform: NO TRANSFORM

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

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	solvent control	727515.667	727515.667		
2	7.9 mg/l	2008413.667	2008413.667	-4.862	
3	13.7 mg/l	2111853.333	2111853.333	-5.255	
4	25.1 mg/l	1481080.000	1481080.000	-2.860	
5	43.5 mg/l	1230600.000	1230600.000	-1.910	
6	62.4 mg/l	469982.333	469982.333	0.978	
7	130 mg/l	31331.000	31331.000	2.643	*
8	204 mg/l	18222.333	18222.333	2.692	*
9	291 mg/l	19962.333	19962.333	2.686	*

Dunnett table value = 2.58 (1 Tailed Value, P=0.05, df=18,8)

NOEC = 62.4 mg/l of Select 2.0EC,
Based on mean measured concentrations of ai converted to formulated product.

Selenastrum cell density
File: sel Transform: NO TRANSFORM Raw data from Table 4 (attached).

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	solvent control	3			
2	7.9 mg/l	3	679690.491	93.4	-1280898.000
3	13.7 mg/l	3	679690.491	93.4	-1384337.667
4	25.1 mg/l	3	679690.491	93.4	-753564.333
5	43.5 mg/l	3	679690.491	93.4	-503084.333
6	62.4 mg/l	3	679690.491	93.4	257533.333
7	130 mg/l	3	679690.491	93.4	696184.667
8	204 mg/l	3	679690.491	93.4	709293.333
9	291 mg/l	3	679690.491	93.4	707553.333

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} for Select 2.0 EC = 76 mg/l* pp (95% C.L. 70-83) moving average EC_{50} for clethodim = 19.5 mg ai/l (17.9 - 21.3) Slope = N/A # Cells/ml = 10,000 Species: Selenastrum capricornutum Temperature = 24°C Lab: Wildlife Intern. 5-Day Dose Level mg/l* pp /(% Effect) 7.9 (0), 13.7 (0), 25.1 (0), 43.5 (0), 62.4 (35) 130 (96), 204 (97), 291 (97) MRID # 420297-03 Comments: *Based on mean measured concentrations of active ingredient converted to mg/l of formulated product NOEC for Select 2.0 EC = 43.5 mg/l* NOEC for clethodim = 11.1 mg ai/l	11/13/91	Core