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(3-21-91)

MRID No. 416851-01

DATA EVALUATION RECORD

1. **CHEMICAL:** Clethodim
Shaughnessey No. ~~059639~~ 121011

2. **TEST MATERIAL:** Select 2EC Technical Standard; Lot No. SX-1839; 25.6% active ingredient; CAS No. 99129-21-2; a clear, amber liquid.

3. **STUDY TYPE:** Freshwater Invertebrate Static Acute Toxicity Test. Species Tested: Daphnia magna

4. **CITATION:** Burgess, D., J.W. Blasberg, and L. Stuerman. 1990. Acute Toxicity of Select 2EC to Daphnia magna. Final Report No. 38601. Prepared by Analytical Bio-Chemistry Laboratories, Inc., Columbia, MO. Submitted by Valent U.S.A. Corporation, Walnut Creek, CA. EPA MRID No. 416851-01.

5. **REVIEWED BY:**

Richard C. Petrie, Agronomist
EEB/EFED

Signature: Richard C. Petrie
Date: 3/21/91

6. **APPROVED BY:**

Charles Lewis, Acting Head
Section 3,
EEB/EFED

Signature: Charles Lewis
Date: 3/21/91

7. **CONCLUSIONS:** This study is scientifically sound and meets the guideline requirements for an acute static toxicity test for freshwater invertebrates. Based on measured concentrations, the 48-hour LC_{50} was 20.2 mg/L. Therefore, Select 2EC is classified as slightly toxic to Daphnia magna. The NOEC was determined to be 5.5 mg/L.

8. **RECOMMENDATIONS:** See Section 14.D.3.

9. **BACKGROUND:**

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

11. **MATERIALS AND METHODS:**

A. **Test Animals:** Test animals were obtained from in-house cultures. The primary culture was obtained from the Columbia National Fisheries Research Laboratory in Columbia, MO. The cultures were housed in 2-liter glass containers in a temperature controlled area (20°±1°C) on a 16-hour daylight photoperiod (30 minute dawn/dusk simulation). The light intensity was maintained at 50-70 ft-candles. Blended hard water (well water and reverse-osmosis water) was used to culture the daphnids. The characteristics of the culture/test dilution water are given in Table 1 (attached).

At least every three days, adult Daphnia magna were fed algae (Selenastrum capricornutum, Ankistrodesmus falcatus, and/or Chlamydomonas reinhardtii) at a concentration of 2.0×10^8 cells/mL, and 1.5 mL of a supplement (5.0 mg/mL suspended solids) of trout chow and yeast.

First instar Daphnia magna were selected for the test. Less than 24-hour old daphnids were obtained by transferring the adult daphnids to new culture vessels the day before the test. The adults had been cultured for 12 days and were considered free of disease and signs of stress.

B. **Test System:** Vessels used in the test were 250-mL glass beakers containing 200 mL of culture water (controls) or test solution. The depth and surface diameter of the test solution in the test vessels were approximately 6.3 and 6.5 cm, respectively. The beakers were covered with loose-fitting petri dish covers. Lighting and photoperiod during the test were the same as those in culturing (listed above). The vessels were held in a temperature controlled water bath (20°±1°C).

The daphnids were not fed during the test.

C. **Dosage:** Forty-eight-hour static test. Based on a preliminary test, five nominal concentrations (13, 22, 36, 60, and 100 mg/L) and a dilution water control were

used. The concentrations made were based on total product.

D. **Design:** Two beakers were used for each concentration and ten daphnids were used per beaker. All concentrations were observed once at 3, 6, 24, and 48 hours for mortality and abnormal effects such as immobilization, surfacing, clumping together, and lying on the bottom of the chambers. The temperature, dissolved oxygen (D.O.), and pH were measured in all concentrations at the beginning and end of the test.

Select 2EC concentrations were measured by HPLC analysis from samples taken at test initiation and termination.

E. **Statistics:** The 48-hour median lethal concentration (LC_{50}) and associated 95% confidence interval (C.I.) were calculated using a computer program developed by Stephan et al. (1978).

12. **REPORTED RESULTS:** The mean measured concentrations were 5.5, 20, 35, 60, and 100 mg/L. The four highest mean measured concentrations ranged from 91 to 100% of nominal test concentrations. The lowest mean measured concentration, 5.5 mg/L, was 42% of nominal.

The responses of Daphnia magna are given in Table 3 (attached). The 48-hour LC_{50} based on measured concentrations was 20 mg/L (95% C.I. = 15-25 mg/L). The slope of the dose-response curve was given as 4.3. The no-observed-effect concentration (NOEC), based on the lack of mortality and abnormal effects, was 5.5 mg/L after 48 hours.

Oxygen saturation ranged from 101 to 113% and 93 to 94% of saturation at test initiation and termination, respectively. The pH values ranged from 8.1 to 8.5. The temperature remained 21°C throughout the test.

13. **STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:**

The author presented no conclusions.

Quality Assurance and Good Laboratory Practice Regulation Statements were included in the report, indicating that the study was conducted in accordance with FIFRA Good Laboratory Practice Standards set forth in 40 CFR Part 160.

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

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A. **Test Procedure:** The test procedures were generally in accordance with protocols recommended by the guidelines, but deviated from the SEP as follows:

The inert ingredients in the formulation were not tested separately from the product. The SEP states that test designs for formulated products should include a control where the organisms are exposed to the carriers or inert ingredients.

Oxygen saturation was greater than 100% (the recommended range is 60%-100% at initiation) in all solutions at test initiation (101%-113%).

The test temperature was not monitored every six hours as recommended.

The length of time between solution preparation and test initiation was not given and the method used to transfer daphnids to test solutions was not included in the report.

First instar Daphnia magna used in tests should be from the fourth or later broods of a given parent. The author did not indicate which brood was the source of the test animals.

B. **Statistical Analysis:** The reviewer used EPA's Toxanal program to calculate the EC_{50} values and obtained similar results (see attached printout).

C. **Discussion/Results:** Judging from the response of the control organisms, oxygen supersaturation did not modify the response of the daphnids in the test. The controls experienced the highest level of supersaturation but no abnormal effects are noted in Table 3 (attached).

The 48-hour LC_{50} of 20.2 mg/L (based on measured concentrations) classifies Select 2EC as slightly toxic to Daphnia magna. The slope of the probit line was 4.6.

D. **Adequacy of the Study:**

(1) **Classification:** CORE

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

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

15. **COMPLETION OF ONE-LINER FOR STUDY:** Yes, 02-04-91.

DATA EVALUATION RECORD

1. **CHEMICAL:** Clethodim
   Shaughnessey No. 059639

2. **TEST MATERIAL:** Select 2EC Technical Standard; Lot No. SX-1839; 25.6% active ingredient; CAS No. 99129-21-2; a clear, amber liquid.

3. **STUDY TYPE:** Freshwater Invertebrate Static Acute Toxicity Test. Species Tested: Daphnia magna

4. **CITATION:** Burgess, D., J.W. Blasberg, and L. Stuerman. 1990. Acute Toxicity of Select 2EC to Daphnia magna. Final Report No. 38601. Prepared by Analytical Bio-Chemistry Laboratories, Inc., Columbia, MO. Submitted by Valent U.S.A. Corporation, Walnut Creek, CA. EPA MRID No. 416851-01.

5. **REVIEWED BY:**

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

   Signature: Louis M Rifici
   Date: 2/18/91

6. **APPROVED BY:**

   Pim Kosalwat, Ph.D.
   Senior Scientist
   KBN Engineering and
   Applied Sciences, Inc.

   Signature: P. Kosalwat
   Date: 2/18/91

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

   Signature:
   Date:

7. **CONCLUSIONS:** This study is scientifically sound but does not meet the guideline requirements for an acute static toxicity test for freshwater invertebrates. Based on measured concentrations, the 48-hour LC_{50} was 20.2 mg/L. Therefore, Select 2EC is classified as slightly toxic to Daphnia magna. The NOEC was determined to be 5.5 mg/L. The formulation contains over 70% inert ingredients (and/or carriers) which were not tested separately from the product.

8. **RECOMMENDATIONS:** See Section 14.D.3.

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9. **BACKGROUND:**

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

11. **MATERIALS AND METHODS:**

A. **Test Animals:** Test animals were obtained from in-house cultures. The primary culture was obtained from the Columbia National Fisheries Research Laboratory in Columbia, MO. The cultures were housed in 2-liter glass containers in a temperature controlled area (20°±1°C) on a 16-hour daylight photoperiod (30 minute dawn/dusk simulation). The light intensity was maintained at 50-70 ft-candles. Blended hard water (well water and reverse-osmosis water) was used to culture the daphnids. The characteristics of the culture/test dilution water are given in Table 1 (attached).

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First instar Daphnia magna were selected for the test. Less than 24-hour old daphnids were obtained by transferring the adult daphnids to new culture vessels the day before the test. The adults had been cultured for 12 days and were considered free of disease and signs of stress.

B. **Test System:** Vessels used in the test were 250-mL glass beakers containing 200 mL of culture water (controls) or test solution. The depth and surface diameter of the test solution in the test vessels were approximately 6.3 and 6.5 cm, respectively. The beakers were covered with loose-fitting petri dish covers. Lighting and photoperiod during the test were the same as those in culturing (listed above). The vessels were held in a temperature controlled water bath (20°±1°C).

The daphnids were not fed during the test.

C. **Dosage:** Forty-eight-hour static test. Based on a preliminary test, five nominal concentrations (13, 22, 36, 60, and 100 mg/L) and a dilution water control were

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used. The concentrations made were based on total product.

D. **Design:** Two beakers were used for each concentration and ten daphnids were used per beaker. All concentrations were observed once at 3, 6, 24, and 48 hours for mortality and abnormal effects such as immobilization, surfacing, clumping together, and lying on the bottom of the chambers. The temperature, dissolved oxygen (D.O.), and pH were measured in all concentrations at the beginning and end of the test.

Select 2EC concentrations were measured by HPLC analysis from samples taken at test initiation and termination.

E. **Statistics:** The 48-hour median lethal concentration (LC_{50}) and associated 95% confidence interval (C.I.) were calculated using a computer program developed by Stephan et al. (1978).

12. **REPORTED RESULTS:** The mean measured concentrations were 5.5, 20, 35, 60, and 100 mg/L. The four highest mean measured concentrations ranged from 91 to 100% of nominal test concentrations. The lowest mean measured concentration, 5.5 mg/L, was 42% of nominal.

The responses of Daphnia magna are given in Table 3 (attached). The 48-hour LC_{50} based on measured concentrations was 20 mg/L (95% C.I. = 15-25 mg/L). The slope of the dose-response curve was given as 4.3. The no-observed-effect concentration (NOEC), based on the lack of mortality and abnormal effects, was 5.5 mg/L after 48 hours.

Oxygen saturation ranged from 101 to 113% and 93 to 94% of saturation at test initiation and termination, respectively. The pH values ranged from 8.1 to 8.5. The temperature remained 21°C throughout the test.

13. **STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:**

The author presented no conclusions.

Quality Assurance and Good Laboratory Practice Regulation Statements were included in the report, indicating that the study was conducted in accordance with FIFRA Good Laboratory Practice Standards set forth in 40 CFR Part 160.

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

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A. **Test Procedure:** The test procedures were generally in accordance with protocols recommended by the guidelines, but deviated from the SEP as follows:

The inert ingredients in the formulation were not tested separately from the product. The SEP states that test designs for formulated products should include a control where the organisms are exposed to the carriers or inert ingredients.

Oxygen saturation was greater than 100% (the recommended range is 60%-100% at initiation) in all solutions at test initiation (101%-113%).

The test temperature was not monitored every six hours as recommended.

The length of time between solution preparation and test initiation was not given and the method used to transfer daphnids to test solutions was not included in the report.

First instar Daphnia magna used in tests should be from the fourth or later broods of a given parent. The author did not indicate which brood was the source of the test animals.

B. **Statistical Analysis:** The reviewer used EPA's Toxanal program to calculate the EC_{50} values and obtained similar results (see attached printout).

C. **Discussion/Results:** Judging from the response of the control organisms, oxygen supersaturation did not modify the response of the daphnids in the test. The controls experienced the highest level of supersaturation but no abnormal effects are noted in Table 3 (attached).

As stated in Section 14.A., a control solution containing the inert ingredients or carriers in the formulated product should have been part of the test design. Since these ingredients make-up 74.4% of the formulation, their contribution to the overall toxicity is an important part of determining the toxicity of the active ingredient.

The 48-hour LC_{50} of 20.2 mg/L (based on measured concentrations) classifies Select 2EC as slightly toxic

to Daphnia magna. The slope of the probit line was 4.6.

D. **Adequacy of the Study:**

(1) **Classification:** Supplemental

(2) **Rationale:** Inert ingredients in the formulation were not tested.

(3) **Repairability:** Submit data on the toxicity of the inert ingredients to Daphnia magna.

15. **COMPLETION OF ONE-LINER FOR STUDY:** Yes, 02-04-91.

LOUIS M. RIFICI SELECT 2EC DAPHNIA MAGNA 2-1-91

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CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB.(PERCENT)
98	20	20	100	9.536742E-05
60	20	20	100	9.536742E-05
35	20	16	80	.5908966
20	20	11	55	41.19014
5.5	20	0	0	9.536742E-05

THE BINOMIAL TEST SHOWS THAT 5.5 AND 35 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 18.36589

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS	
2	9.753803E-02	18.96878	14.72554	25.13056

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
6	.1833288	1	.7152733

SLOPE = 4.573611
95 PERCENT CONFIDENCE LIMITS = 2.615332 AND 6.53189

LC50 = 20.16439
95 PERCENT CONFIDENCE LIMITS = 14.77694 AND 24.69643

LC10 = 10.63911
95 PERCENT CONFIDENCE LIMITS = 5.22868 AND 14.58136

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CLETHODIM

Page _____ is not included in this copy.

Pages 12 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.

Haughniessey No. 059639 Chemical Name Clethodium Chemical Class ____ Page 1 of 1

Study/Species/Lab/ Accession	Chemical % a.i.	Results	Reviewer/ Date	Validation Status
4-Day Single Dose Oral LD_{50}		LD50 = mg/kg (95% C.L.) Contr. Mort.(%)=		
pecies	____	Slope= # Animals/Level= Age(Days)= Sex =		
ab		14-Day Dose Level mg/kg/(% Mortality) ( ), ( ), ( ), ( ), ( )	____	____
:c.		Comments:		
4-Day Single Dose Oral LD_{50}		LD50 = mg/kg. (95% C.L.) Contr. Mort.(%)=		
ecies	____	Slope= # Animals/Level= Age(Days)= Sex =		
ab		14-Day Dose Level mg/kg/(% Mortality) ( ), ( ), ( ), ( ), ( )	____	____
:c.		Comments:		
-Day Dietary LC_{50}		LC50 = ppm (95% C.L.) Contr. Mort.(%)=		
ecies		Slope= # Animals/Level= Age(Days)= Sex =		
b	____	8-Day Dose Level ppm/(%Mortality) ( ), ( ), ( ), ( ), ( )	____	____
c.		Comments:		
Day Dietary LC_{50}		LC50 = ppm (95% C.L.) Contr. Mort.(%)=		
ecies		Slope= # Animals/Level= Age(Days)= Sex =		
b	____	8-Day Dose Level ppm/(%Mortality) ( ), ( ), ( ), ( ), ( )	____	____
c.		Comments:		
-Hour LC_{50}		LC50 = 20.2 ppm* (95% C.L. 14.8 - 24.7 Probit) Contr. Mort.(%)= 0 Sol. Contr. Mort.(%)= N/A		
ecies Daphnia magna		Slope= 4.6 # Animals/Level= 20 Temperature = 21°C	LR 2/4/91	Supple-mental
ABC Laboratories	25.6	48-Hour Dose Level ppm* (%Mortality) 5.5 (0), 20 (55), 35 (80), 60 (100), 98 (100)		
RID 416851-01		Comments: *Mean measured concentrations		
-Hour LC_{50}		LC50 = pp__ (95% C.L.) Con. Mor(%)= Sol. Con. Mor.(%)=		
ecies		Slope= # Animals/Level= Temp.=		
)	____	96-Hour Dose Level pp /(%Mortality) ( ), ( ), ( ), ( ), ( )	____	____
:.		Comments:		
-Hour LC_{50}		LC50 = pp__ (95% C.L.) Con. Mort.(%)= Sol. Con. Mort.(%)=		
cies		Slope= # Animals/Level= Temp.=		
	____	96-Hour Dose Level pp /(%Mortality) ( ), ( ), ( ), ( ), ( )	____	____
		Comments:		