DATA EVALUATION RECORD ALGAE OR DIATOM EC50 TEST GUIDELINE 123-2 (TIER II)

CHEMICAL: Cloransulam-methyl (DE-565) PC Code No.: 129116

5-Hydroxy-DE-565 Acid Purity: >97% TEST MATERIAL:

(A metabolite of DE-565)

3. CITATION:

> Authors: H.D. Kirk, M.M. Gilles, D.L. Rick, L.G.

> > McFadden

Phytotoxicological Evaluation of 5-Title:

Hydroxy-DE-565 Acid Exposed Freshwater Green Alga, Selenastrum capricornutum

Study Completion Date: July 28, 1998

Health & Environmental Research Laboratory:

Laboratories, The Dow Chemical Company,

Midland, MI

Dow AgroSciences, Indianapolis, IN Sponsor:

Laboratory Report ID: 981076

D252903 DP Barcode: MRID No.: 447445-09

REVIEWED BY: Max Feken, M.S., Environmental Toxicologist,

Golder Associates Inc.

Signature:

Date: 4/2/99

Pim Kosalwat, Ph.D., Senior Scientist, APPROVED BY:

Golder Associates Inc.

1. Kosalwat Signature:

Date: 4/2/99

Date: 4/5/99

APPROVED BY: 5.

Signature:

STUDY PARAMETERS: 6.

> Definitive Test Duration: 96 hours

Mean measured Type of Concentrations:

7. **CONCLUSIONS:** This study is scientifically sound and fulfills the quideline requirements for an aquatic plant toxicity test.

Results Synopsis

EC₅₀: 32.6 ppm 95% C.I.: 30.1 - 35.3 ppm

NOEC: 23.1 ppm Probit Slope: 15.6

8. ADEQUACY OF THE STUDY:

A. Classification: Core

B. Rationale: N/A

C. Repairability: N/A

9. GUIDELINE DEVIATIONS:

1. The duration of this study was 96 hours; however, the quidelines recommend a study duration of 120 hours.

2. The pH of the two highest test concentrations were lower than recommended as a result of the acidic nature of the test substance. Reduction in algal growth (or no growth) was observed at these two concentrations and was, more than likely, directly related to the low pH of the test solutions.

10. SUBMISSION PURPOSE:

11. MATERIALS AND METHODS:

A. Test Organisms

Guideline Criteria	Reported Information
Species Skeletonema costatum Anabaena flos-aquae Selenastrum capricornutum Navicula pelliculosa	Selenastrum capricornutum
<u>Initial Number of Cells</u> 3,000 - 10,000 cells/mL	6,400 cells/mL
<u>Nutrients</u> Standard formula, e.g. 20XAAP	Algal Assay Medium

B. Test System

Guideline Criteria	Reported Information
<u>Solvent</u>	DMF (0.04 mL/L)
Temperature Skeletonema: 20°C Others: 24-25°C	23.9 - 24.1°C
Light Intensity Anabaena: 2.0 Klux (±15%) Others: 4.0-5.0 Klux (±15%)	3.6 - 5.1 Klux
Photoperiod Skeletonema: 14 h light, 10 h dark or 16 h light, 8 h dark Others: Continuous	Continuous
pH Skeletonema: approx. 8.0 Others: approx. 7.5	Without alga: 3.5 - 7.1 With growth: 3.6 - 8.5

C. Test Design

Guideline Criteria	Reported Information
Dose range 2X or 3X progression	2X
<u>Doses</u> at least 5	Six doses: 3.13, 6.25, 12.5, 25, 50, and 100 mg/L
<u>Controls</u> negative and/or solvent	Negative and solvent control
Replicates per dose 3 or more	3
<u>Duration of test</u> 120 hours	96 hours
Daily observations were made?	Yes
Method of Observations	Cellular counts
Maximum Labeled Rate	Not reported

12. REPORTED RESULTS:

Guideline Criteria	Reported Information
Initial and 120 h cell densities were measured?	Yes
Control cell count at 120 hr >2X initial count?	Yes
Initial chemical concentrations measured? (Optional)	Yes
Raw data included?	Yes

Measured Concentrations

Nominal	Measured Concentration (mg/L)				
Conc. (mg/L)	0-Hour	96-Hour	Mean	% Nominal	
Control	<0.8	<0.8			
Solvent Control	<0.8	<0.8			
3.13	3.57	1.99	2.78	89	
6.25	7.14	4.44	5.79	93	
12.5	14.4	9.51	12.0	96	
25.0	27.9	18.3	23.1	92	
50.0	52.4	39.6	46.0	92	
100	106	87.3	96.7	97	

Dose Response

Mean Measured Concentration (mg/L)	Avg. Cell Density (x 10 ³ cells/ml)	% reduction ^a	pH range	
Control	496		7.5 - 7.7	
Solvent Cont.	527		7.9 - 8.1	
2.78	647	-23 ^b	8.2 - 8.4	
5.79	608	-15	8.3 - 8.4	
12.0	624	-18	8.3 - 8.5	
23.1	521	1	7.9	
46.0	3	99	4.0 - 4.1	
96.7	2	100	3.6	

^aCompared to the solvent control

Other Significant Results: None

Statistical Results

Statistical Method: Least squares linear regression was used

to estimate the EC_{50} and Dunnett's test was used for mean comparisons versus the pooled control. Results based on the

initial measured concentrations.

EC₅₀: 39.3 mg/L 95% C.I.: 3.77 - 408 mg/L

Probit Slope: Not reported NOEC: 27.9 mg/L

13. VERIFICATION OF STATISTICAL RESULTS:

Statistical Method: Probit for EC50 and Williams' test for

mean comparisons versus the solvent

control. Results based on mean measured

concentrations.

EC₅₀: 32.6 ppm 95% C.I.: 30.1 - 35.3 ppm

Probit Slope: 15.6 NOEC: 23.1 ppm

bNegative value indicates stimulation of growth

14. REVIEWER'S COMMENTS: This study is scientifically sound and fulfills the guideline requirements for an aquatic plant toxicity test. Based on mean measured concentrations, the 96-hour EC₅₀ for Selenastrum capricornutum exposed to 5-hydroxy-DE-565 Acid was 32.6 ppm. The NOEC was 23.1 ppm. This study is categorized as Core.

MAX FEKEN 5-OH-DE-565 ACID SELENASTRUM 03-23-99

CONC.	NOMBEK	MONDEK	LEWCENT	DINO
	EXPOSED	DEAD	DEAD	PROB
96.7	100	100	100	0
46	100	99	99	0
23.1	100	1	1	0
12	100	Ó	O ·	.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 32.59755

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

 SPAN
 G
 LC50
 95 PERCENT CONFIDENCE LIMITS

 3
 8.831131E-03
 33.0794
 30.73817
 35.59896

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS G H GOODNESS OF FIT PROBABILITY

11 4.946407E-02 1 .9994584

SLOPE = 15.55304

95 PERCENT CONFIDENCE LIMITS = 12.09397 AND 19.01212

LC50 = 32.59754

95 PERCENT CONFIDENCE LIMITS = 30.13452 AND 35.26187

LC10 = 27.01026

95 PERCENT CONFIDENCE LIMITS = 24.44409 AND 29.26746

5-OH-DE-565 ACID

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WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1 2 3 4 5	SOLVENT 2.78 5.79 12.0 23.1 46.0	3 3 3 3 3	526615.000 647416.667 607899.667 623825.667 520699.000 2767.667	526615.000 647416.667 607899.667 623825.667 520699.000 2767.667	601439.250 601439.250 601439.250 601439.250 520699.000 2767.667
7	96.7	3	2236.000	2236.000	2236.000

5. I-DE-565 ACID

File: 44744509 Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

WINDIAMO IDO	(150001120				
IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
SOLVENT	601439.250				
	601439.250	1.034		1.76	k=1, v=14
5.79	601439.250	1.034		1.85	k= 2, v=14
12.0	601439.250	1.034		1.88	k=3, v=14
23.1	520699.000	0.082		1.89	k=4, v=14
46.0	2767.667	7.241	*	1.90	k=5, v=14
96.7	2236.000	7.248	*	1.91	k = 6, v = 14

s 88604.280

Note: df used for table values are approximate when v > 20.

NOEC = 23.1 mg/L