

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

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

2. TEST MATERIAL: 5-hydroxy-DE-565 Purity: >95%
(A metabolite of DE-565)

3. CITATION:

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

Title: Phytotoxicological Evaluation of 5-
Hydroxy-DE-565 Exposed Freshwater Green
Alga, *Selenastrum capricornutum* Printz

Study Completion Date: September 16, 1998

Laboratory: The Dow Chemical Company, Midland, MI

Sponsor: Dow AgroSciences, LLC, Indianapolis, IN

Laboratory Report ID: 981110

DP Barcode: D252903

MRID No.: 447445-04

4. REVIEWED BY: Mark A. Mossler, M.S., Toxicologist,
Golder Associates Inc.

Signature: 

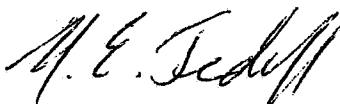
Date: 3/30/99

APPROVED BY: Pim Kosalwat, Ph.D., Senior Scientist,
Golder Associates Inc.

Signature: P. Kosalwat

Date: 3/30/99

5. APPROVED BY:

Signature: 

Date: 4/15/99

6. STUDY PARAMETERS:

Definitive Test Duration: 96 hours

Type of Concentrations: Initial measured

7. CONCLUSIONS: This study is scientifically sound and fulfills the guideline requirements for an algal toxicity test. The 96-hour EC₅₀ and NOEC for *S. capricornutum* exposed to 5-hydroxy-DE-565 were 41.5 and 26.3 ppm, respectively.

8. ADEQUACY OF THE STUDY:

A. Classification: Core.

B. Rationale: N/A.

C. Repairability: N/A.

9. **GUIDELINE DEVIATIONS:** No guideline deviations of consequence were noted.

10. **SUBMISSION PURPOSE:**

11. **MATERIALS AND METHODS:**

A. Test Organisms

Guideline Criteria	Reported Information
Species <i>Skeletonema costatum</i> <i>Anabaena flos-aquae</i> <i>Selenastrum capricornutum</i> <i>Navicula pelliculosa</i>	<i>Selenastrum capricornutum</i>
Initial Number of Cells 3,000 - 10,000 cells/mL	11,000 cells/mL
Nutrients Standard formula, e.g. 20XAAP	Algal assay medium

B. Test System

Guideline Criteria	Reported Information
Solvent	DMF
Temperature Skeletonema: 20°C Others: 24-25°C	24.1 - 24.9°C
Light Intensity Anabaena: 2.0 KLux (±15%) Others: 4.0-5.0 KLux (±15%)	3.6-5.0 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	Range of 3.7-7.4

C. Test Design

Guideline Criteria	Reported Information
<u>Dose range</u> 2X or 3X progression	2X
<u>Doses</u> Must be tested at the maximum label rate	3.1, 6.3, 12.5, 25, 50, and 100 ppm
<u>Controls</u> Negative and/or solvent	Negative and solvent (352 μ L/L) control groups
<u>Replicates per dose</u> 3 or more	3
<u>Duration of test</u> 120 or 96 hours	96 hours
<u>Daily observations were made?</u>	Yes
<u>Method of Observations</u>	Cellular counts
<u>Maximum Labeled Rate</u>	Test material is a metabolite

12. REPORTED RESULTS:

Guideline Criteria	Reported Information
Initial and 96 h cell densities were measured?	Yes
Control cell count at 96 hr \geq2X initial count?	Yes
Initial chemical concentrations measured? (Optional) Percent of nominal, Procedural recovery, Limit of quantitation (LOQ)	Samples analyzed by HPLC 22-108%, Procedural recovery not reported, LOQ = 0.5 ppm
Raw data included?	Yes

Analytical Results

Concentration (ppm)	Measured concentrations (ppm)	
Nominal	Hour of Study	
	0	96
Control	<LOQ	<LOQ
Solvent Control	<LOQ	<LOQ
3.1	3.1	0.68
6.3	6.6	2.0
12.5	13.5	4.7
25	26.3	11.4
50	52.7	36.7
100	103	84.3

Dose Response

Nominal Concentration (ppm)	Initial measured concentration (ppm)	Day 4 Avg. Cell Density ($\times 10^4$ cells/mL)	% Inhibition*	pH Range
Control	<LOQ	41.1	N/A	7.0-7.2
Sol. Con.	<LOQ	35.1	N/A	7.3-7.4
3.1	3.1	43.1	-23	7.3
6.3	6.6	39.3	-12	7.3-7.4
12.5	13.5	45.1	-28	7.3
25	26.3	48.1	-37	7.2-7.3
50	52.7	2.1	94	5.0-5.1
100	103	1.5	96	3.7

*Comparison to the solvent control and negative values indicate growth stimulation

Other Significant Results: No other results were reported.

Statistical Methods: linear regression and Dunnett's test

EC₅₀: 54.6 ppm
Probit Slope: N/A

95% C.I.: 0 - 103 ppm
NOEC: 26.3 ppm

13. VERIFICATION OF STATISTICAL RESULTS:

Statistical Methods: The moving average method was used to estimate the EC₅₀ value. Williams' test was used to determine the NOEC.

EC₅₀: 41.5 ppm
Probit Slope: N/A

95% C.I.: 38.7 - 44.2 ppm
NOEC: 26.3 ppm

- 14. REVIEWER'S COMMENTS:** This study is scientifically sound and fulfills the guideline requirements for an algal toxicity test. The 96-hour EC₅₀ and NOEC for *S. capricornutum* exposed to 5-hydroxy-DE-565 were 41.5 and 26.3 ppm, respectively. This study can be categorized as **Core**.

Selenastrum cell density
 File: sel Transform: LOG BASE 10(Y)

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	Sol. Con.	3	351493.333	5.544	5.619
2	3.1 ppm	3	431065.667	5.627	5.619
3	6.6 ppm	3	392835.667	5.590	5.619
4	13.5 ppm	3	451092.333	5.653	5.619
5	26.3 ppm	3	481305.000	5.681	5.619
6	52.7 ppm	3	20763.000	4.313	4.313
7	103 ppm	3	14880.667	4.170	4.170

Selenastrum cell density
 File: sel Transform: LOG BASE 10(Y)

WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
Sol. Con.	5.619				
3.1 ppm	5.619	1.402		1.76	k= 1, v=14
6.6 ppm	5.619	1.402		1.85	k= 2, v=14
13.5 ppm	5.619	1.402		1.88	k= 3, v=14
26.3 ppm	5.619	1.402		1.89	k= 4, v=14
52.7 ppm	4.313	22.873	*	1.90	k= 5, v=14
103 ppm	4.170	25.543	*	1.91	k= 6, v=14

s 0.066

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

NOEC = 26.3 ppm

Mossler 5-hydroxy DE565 Selenastrum capricornutum 3-17-99

CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
103	100	96	96	0
52.7	100	94	94	0
26.3	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 39.40254

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS	
2	1.116095E-02	41.46255	38.6511	44.24458

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT	PROBABILITY
8	268.2765	173.0915	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 = 7.837363
95 PERCENT CONFIDENCE LIMITS = -120.532 AND 136.2067

LC50 = 41.52227
95 PERCENT CONFIDENCE LIMITS = 0 AND +INFINITY

LC10 = 28.59149
95 PERCENT CONFIDENCE LIMITS = 0 AND +INFINITY
