

DATA EVALUATION RECORD  
ALGAE OR DIATOM EC<sub>50</sub> 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, and J.M. Hugo  
Title: Phytotoxicological Evaluation of 5-Hydroxy-DE-565 Exposed Saltwater Diatom, *Skeletonema costatum*

Study Completion Date: August 18, 1998

Laboratory: The Dow Chemical Company, Midland, MI


Sponsor: Dow AgroSciences, LLC, Indianapolis, IN

Laboratory Report ID: 981105

DP Barcode: D252903

MRID No.: 447445-03

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: 4/13/99

6. STUDY PARAMETERS:

Definitive Test Duration: 120 hours

Type of Concentrations: Initial measured

7. CONCLUSIONS: This study is scientifically sound and fulfills the guideline requirements for an algal toxicity test. The 120-hour EC<sub>50</sub> and NOEC for *S. costatum* exposed to 5-hydroxy-DE-565 were >93.3 and 93.3 ppm, respectively.

8. ADEQUACY OF THE STUDY:

A. Classification: Core.

B. Rationale: N/A.

C. Repairability: N/A.

9. GUIDELINE DEVIATIONS:

1. The test was conducted under continuous lighting; a photoperiod of 14-16 hours of light/day is recommended.
2. Cell inoculum was four times greater than recommended.

10. SUBMISSION PURPOSE:

11. MATERIALS AND METHODS:

A. Test Organisms

Guideline Criteria	Reported Information
<u>Species</u> <i>Skeletonema costatum</i> <i>Anabaena flos-aquae</i> <i>Selenastrum capricornutum</i> <i>Navicula pelliculosa</i>	<i>Skeletonema costatum</i>
<u>Initial Number of Cells</u> 3,000 - 10,000 cells/mL	42,000 cells/mL
<u>Nutrients</u> Standard formula, e.g. 20XAAP	Algal assay medium with a salinity of 26-30 ppt

B. Test System

Guideline Criteria	Reported Information
<u>Solvent</u>	DMF
<u>Temperature</u> Skeletonema: 20°C Others: 24-25°C	20.3 - 21.0°C
<u>Light Intensity</u> Anabaena: 2.0 KLux (±15%) Others: 4.0-5.0 KLux (±15%)	3.5-5.0 KLux
<u>Photoperiod</u> Skeletonema: 14 h light, 10 h dark or 16 h light, 8 h dark Others: Continuous	Continuous

## C. Test Design

Guideline Criteria	Reported Information
<b><u>Dose range</u></b> 2X or 3X progression	2X
<b><u>Doses</u></b> Must be tested at the maximum label rate	3.1, 6.3, 12.5, 25, 50, and 100 ppm
<b><u>Controls</u></b> Negative and/or solvent	Negative and solvent (365 $\mu$ L/L) control groups
<b><u>Replicates per dose</u></b> 3 or more	3
<b><u>Duration of test</u></b> 120 or 96 hours	120 hours
<b>Daily observations were made?</b>	Counts on days 3, 4, and 5
<b><u>Method of Observations</u></b>	Cellular counts
<b><u>Maximum Labeled Rate</u></b>	Test material is a metabolite

## 12. REPORTED RESULTS:

Guideline Criteria	Reported Information
<b>Initial and 120 h cell densities were measured?</b>	Yes
<b>Control cell count at 120 hr <math>\geq</math>2X initial count?</b>	Yes
<b>Initial chemical concentrations measured?</b> (Optional) Percent of nominal, Procedural recovery, Limit of quantitation (LOQ)	Samples analyzed by HPLC  35-96%, Procedural recovery not reported, LOQ = 0.6 ppm
<b>Raw data included?</b>	Yes

**Analytical Results**

Concentration (ppm)	Measured concentrations (ppm)	
Nominal	Hour of Study	
	0	120
Control	<LOQ	<LOQ
Solvent Control	<LOQ	<LOQ
3.1	2.8	1.1
6.3	5.7	2.6
12.5	11.7	5.7
25	23.5	12.2
50	47.8	28.3
100	93.3	61.3

**Dose Response**

Nominal Concentration (ppm)	Initial measured concentration (ppm)	Day 5 Avg. Cell Density ( $\times 10^6$ cells/mL)	% Inhibition*	pH Range
Control	<LOQ	74.2	N/A	8.6
Sol. Con.	<LOQ	74.7	N/A	8.6
3.1	2.8	79.1	-6	8.6-8.7
6.3	5.7	76.2	-2	8.6
12.5	11.7	71.5	4	8.7
25	23.5	72.7	3	8.7
50	47.8	69.0	8	8.6-8.7
100	93.3	71.6	4	8.7

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

Other Significant Results: No other results were reported.

Statistical Methods: no methods were employed

EC<sub>50</sub>: >93.3 ppm  
Probit Slope: N/A

95% C.I.: N/A  
NOEC: not reported

**13. VERIFICATION OF STATISTICAL RESULTS:**

Statistical Methods: Williams' test was used to determine the NOEC.

EC<sub>50</sub>: >93.3 ppm  
Probit Slope: N/A

95% C.I.: N/A  
NOEC: 93.3 ppm

- 14. REVIEWER'S COMMENTS:** Since the test material is a metabolite, a maximum label rate equivalent testing concentration is not relevant. However, the maximum concentration of 93.3 ppm translates to a parent application rate of 127 lb ai/A. The reviewer believes that the maximum label rate for the parent compound is much less than this rate. Consequently, this study is scientifically sound and fulfills the guideline requirements for an algal toxicity test. The 120-hour EC<sub>50</sub> and NOEC for *S. costatum* exposed to 5-hydroxy-DE-565 were >93.3 and 93.3 ppm, respectively. This study can be categorized as **Core**.

Skeletonema cell density  
 File: skl Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	Sol. Con.	3	746668.000	746668.000	768914.000
2	2.8 ppm	3	791160.000	791160.000	768914.000
3	5.7 ppm	3	761827.333	761827.333	761827.333
4	11.7 ppm	3	715011.333	715011.333	721118.667
5	23.5 ppm	3	727226.000	727226.000	721118.667
6	47.8 ppm	3	690339.333	690339.333	703081.167
7	93.3 ppm	3	715823.000	715823.000	703081.167

Skeletonema cell density  
 File: skl Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
Sol. Con.	768914.000				
2.8 ppm	768914.000	0.618		1.76	k= 1, v=14
5.7 ppm	761827.333	0.421		1.85	k= 2, v=14
11.7 ppm	721118.667	0.710		1.88	k= 3, v=14
23.5 ppm	721118.667	0.710		1.89	k= 4, v=14
47.8 ppm	703081.167	1.211		1.90	k= 5, v=14
93.3 ppm	703081.167	1.211		1.91	k= 6, v=14

s 44069.906

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

*NOEC = 93.3 ppm*