

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, J.M. Hugo, and  
L.G. McFadden  
Title: Phytotoxicological Evaluation of 5-  
Hydroxy-DE-565 Exposed Bluegreen Alga,  
*Anabaena flos-aquae*

Study Completion Date: September 17, 1998  
Laboratory: The Dow Chemical Company, Midland, MI  
Sponsor: Dow AgroSciences, LLC, Indianapolis, IN  
Laboratory Report ID: 981109  
DP Barcode: D252903  
MRID No.: 447445-01

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

Signature:  Date: 3/26/99

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

Signature: P. Kosalwat Date: 3/26/99

5. **APPROVED BY:**

Signature:  Date: 4/14/99

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 *A. flos-aquae* exposed to 5-hydroxy-DE-565 were 48.3 and 30.0 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
<u>Species</u> <i>Skeletonema costatum</i> <i>Anabaena flos-aquae</i> <i>Selenastrum capricornutum</i> <i>Navicula pelliculosa</i>	<i>Anabaena flos-aquae</i>
<u>Initial Number of Cells</u> 3,000 - 10,000 cells/mL	11,000 cells/mL
<u>Nutrients</u> Standard formula, e.g. 20XAAP	Algal assay medium

B. Test System

Guideline Criteria	Reported Information
<u>Solvent</u>	DMF
<u>Temperature</u> Skeletonema: 20°C Others: 24-25°C	23.4 - 23.6°C
<u>Light Intensity</u> Anabaena: 2.0 KLux (±15%) Others: 4.0-5.0 KLux (±15%)	1.7-2.3 KLux
<u>Photoperiod</u> Skeletonema: 14 h light, 10 h dark or 16 h light, 8 h dark Others: Continuous	Continuous
<u>pH</u> Skeletonema: approx. 8.0 Others: approx. 7.5	Range of 3.7-8.8

## 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 (412 $\mu$ L/L) control groups
<u>Replicates per dose</u> 3 or more	3
<u>Duration of test</u> 120 or 96 hours	120 hours
Daily observations were made?	Counts on days 3, 4, and 5
<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 120 h cell densities were measured?	Yes
Control cell count at 120 hr $\geq$ 2X initial count?	Yes
Initial chemical concentrations measured? (Optional) Percent of nominal, Procedural recovery, Limit of quantitation (LOQ)	Samples analyzed by HPLC  100-124%, Procedural recovery not reported, LOQ = 0.8 ppm
Raw data included?	Yes

Analytical Results

Concentration (ppm)	Measured concentrations (ppm)	
Nominal	Hour of Study	
	0	120
Control	<LOQ	<LOQ
Solvent Control	<LOQ	<LOQ
3.1	3.1	3.7
6.3	7.7	7.5
12.5	15.4	14.9
25	30.0	29.8
50	58.9	60.9
100	114	114

Dose Response

Nominal Concentration (ppm)	Initial measured concentration (ppm)	Day 5 Avg. Cell Density ( $\times 10^4$ cells/mL)	% Inhibition*	pH Range
Control	<LOQ	107.6	N/A	8.5-8.6
Sol. Con.	<LOQ	91.5	N/A	8.2-8.3
3.1	3.1	109.5	-20	8.7-8.8
6.3	7.7	104.0	-14	8.6-8.7
12.5	15.4	100.7	-10	8.6
25	30.0	95.2	-4	8.5-8.6
50	58.9	8.9	90	5.8-5.9
100	114	3.2	97	3.7-3.8

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

Other Significant Results: It was stated that the metabolite 5-hydroxy-DE-565 was less toxic than the parent material, for which the 120-hour EC<sub>50</sub> was reported to be 12.4 ppb.

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Statistical Methods: linear regression and Dunnett's test

EC<sub>50</sub>: 55.9 ppm  
Probit Slope: N/A

95% C.I.: 12.7 - 99.0 ppm  
NOEC: 30 ppm

**13. VERIFICATION OF STATISTICAL RESULTS:**

Statistical Methods: The moving average method was used to estimate the EC<sub>50</sub> value. Williams' test was used to determine the NOEC.

EC<sub>50</sub>: 48.3 ppm  
Probit Slope: N/A

95% C.I.: 45.2 - 51.4 ppm  
NOEC: 30.0 ppm

- 14. REVIEWER'S COMMENTS:** 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 *A. flos-aquae* exposed to 5-hydroxy-DE-565 were 48.3 and 30.0 ppm, respectively. This study can be categorized as **Core**.

Anabaena cell density

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WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	Sol. Con.	3	915056.667	5.949	6.001
2	3.1 ppm	3	1095448.333	6.037	6.001
3	7.7 ppm	3	1039636.000	6.015	6.001
4	15.4 ppm	3	1007127.333	6.002	6.001
5	30.0 ppm	3	952379.667	5.977	5.977
6	58.9 ppm	3	89062.333	4.949	4.949
7	114 ppm	3	32128.333	4.499	4.499

Anabaena cell density

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WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
Sol. Con.	6.001				
3.1 ppm	6.001	0.896		1.76	k= 1, v=14
7.7 ppm	6.001	0.896		1.85	k= 2, v=14
15.4 ppm	6.001	0.896		1.88	k= 3, v=14
30.0 ppm	5.977	0.494		1.89	k= 4, v=14
58.9 ppm	4.949	17.146	*	1.90	k= 5, v=14
114 ppm	4.499	24.855	*	1.91	k= 6, v=14

s = 0.071

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

NOEC = 30.0 ppm

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Mossler 5-hydroxy DE565 Anabaena flos-aquae 3-17-99

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CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
114	100	97	97	0
58.9	100	90	90	0
30	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 45.47879

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS
2	1.073705E-02	48.31739	45.24559 51.38038

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
7	229.7102	140.8475	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 = 8.449064  
95 PERCENT CONFIDENCE LIMITS = -119.6066 AND 136.5047

LC50 = 47.57737  
95 PERCENT CONFIDENCE LIMITS = 0 AND +INFINITY

LC10 = 33.65796  
95 PERCENT CONFIDENCE LIMITS = 0 AND +INFINITY

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