

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: DE-565 acid Purity: >96%  
(A metabolite of DE-565)

3. CITATION:  
Authors: H.D. Kirk, M.M. Gilles, and J.M. Hugo  
Title: Phytotoxicological Evaluation of DE-565  
Acid Exposed Saltwater Diatom,  
*Skeletonema costatum*

Study Completion Date: May 20, 1998  
Laboratory: The Dow Chemical Company, Midland, MI  
Sponsor: Dow AgroSciences, LLC, Indianapolis, IN  
Laboratory Report ID: 981029  
DP Barcode: D252903  
MRID No.: 447445-13

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

Signature:  Date: 4/5/99

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

Signature: P. Kosalwat Date: 4/5/99

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 *S. costatum* exposed to DE-565 acid were >90.8 and 18.0 ppm, respectively.

8. ADEQUACY OF THE STUDY:

A. Classification: Core.

B. Rationale: N/A.

C. Repairability: N/A.

9. GUIDELINE DEVIATIONS: The inoculum density was higher 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	51,000 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	19.8 - 20.6°C
<u>Light Intensity</u> Anabaena: 2.0 KLux (±15%) Others: 4.0-5.0 KLux (±15%)	3.2-5.5 KLux
<u>Photoperiod</u> Skeletonema: 14 h light, 10 h dark or 16 h light, 8 h dark Others: Continuous	14 h of light/day
<u>pH</u> Skeletonema: approx. 8.0 Others: approx. 7.5	Range of 8.7-8.9

## 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 (246 $\mu$ 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 $>2X$ initial count?	Yes
Initial chemical concentrations measured? (Optional) Percent of nominal, Procedural recovery, Limit of quantitation (LOQ)	Samples analyzed by HPLC 41-91%, 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	2.0	1.3
6.3	4.2	2.5
12.5	8.5	5.1
25	18.0	10.5
50	35.9	23.5
100	90.8	48.2

Dose Response

Nominal Concentration (ppm)	Initial measured concentration (ppm)	Day 5 Avg. Cell Density ( $\times 10^4$ cells/mL)	% Inhibition*	pH Range
Control	<LOQ	92.0	N/A	8.7
Sol. Con.	<LOQ	98.8	N/A	8.7-8.8
3.1	2.0	84.7	14	8.8
6.3	4.2	89.1	10	8.7-8.8
12.5	8.5	94.0	5	8.8-8.9
25	18.0	88.2	11	8.8-8.9
50	35.9	79.9	19	8.8
100	90.8	85.9	13	8.7-8.8

\*Comparison to the solvent control.

Other Significant Results: No other results were reported.

Statistical Methods: no statistical analyses were conducted

EC<sub>50</sub>: >90.8 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>: >90.8 ppm  
Probit Slope: N/A

95% C.I.: N/A  
NOEC: 18.0 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 *S. costatum* exposed to DE-565 acid were >90.8 and 18.0 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	988103.000	988103.000	988103.000
2	2.0 ppm	3	847071.000	847071.000	892613.444
3	4.2 ppm	3	890524.333	890524.333	892613.444
4	8.5 ppm	3	940245.000	940245.000	892613.444
5	18.0 ppm	3	882461.000	882461.000	882461.000
6	35.9 ppm	3	799106.000	799106.000	828949.833
7	90.8 ppm	3	858793.667	858793.667	828949.833

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.	988103.000				
2.0 ppm	892613.444	1.551		1.76	k= 1, v=14
4.2 ppm	892613.444	1.551		1.85	k= 2, v=14
8.5 ppm	892613.444	1.551		1.88	k= 3, v=14
18.0 ppm	882461.000	1.716		1.89	k= 4, v=14
35.9 ppm	828949.833	2.585	*	1.90	k= 5, v=14
90.8 ppm	828949.833	2.585	*	1.91	k= 6, v=14

75407.849

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

*NOEC = 18.0 ppm*

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