

DATA EVALUATION RECORD  
ALGAE OR DIATOM EC<sub>50</sub> TEST  
GUIDELINE 123-2 (TIER II)

1. CHEMICAL: Cloransulam-methyl PC Code No.: 129116

2. TEST MATERIAL: DE-565 Acid Purity: >96%  
(A metabolite of DE-565 herbicide)

3. CITATION

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

Title: Phytotoxicological Evaluation of DE-565  
Acid Exposed Bluegreen Alga, *Anabaena*  
*flos-aquae*

Study Completion Date: June 4, 1998

Laboratory: Health & Environmental Research  
Laboratories, The Dow Chemical Company,  
Midland, MI

Sponsor: Dow AgroSciences, LLC, Indianapolis, IN

Laboratory Report ID: 981031

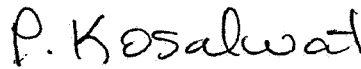
DP Barcode: D252903

MRID No.: 447445-11

4. REVIEWED BY: Karl Bullock, M.S., Environmental Scientist,  
Golder Associates Inc.

Signature:  Date: 3/24/99

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

Signature:  Date: 3/24/99

5. APPROVED BY:

Signature:  Date: 4/13/99

6. STUDY PARAMETERS

Definitive Test Duration: 120 hours

Type of Concentrations: Mean measured

7. CONCLUSIONS: This study is scientifically sound and fulfills  
the guideline requirements for an algal toxicity test.

Results Synopsis

EC<sub>50</sub>: 7.42 ppm 95% C.I.: Could not be determined

NOEC: 3.65 ppm Probit Slope: N/A

8. ADEQUACY OF THE STUDY

- A. **Classification:** Core.
- B. **Rationale:** Fulfills guideline requirements.
- C. **Repairability:** N/A

9. GUIDELINE DEVIATIONS: The number of cells at test initiation (13800/ml) was greater than recommended (3000 - 10000 cells/mL).

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	13,800 cells/mL
<u>Nutrients</u> Standard formula, e.g. 20XAAP	Algal assay medium (AAM)

B. **Test System**

Guideline Criteria	Reported Information
<u>Solvent</u>	DMF
<u>Temperature</u> Skeletonema: 20°C Others: 24-25°C	23.4 - 23.5 °C
<u>Light Intensity</u> Anabaena: 2.0 KLux (±15%) Others: 4.0-5.0 KLux (±15%)	1.7 - 2.3 KLux

Guideline Criteria	Reported Information
<b>Photoperiod</b> Skeletonema: 14 h light, 10 h dark or 16 h light, 8 h dark Others: Continuous	Continuous
<b>pH</b> Skeletonema: approx. 8.0 Others: approx. 7.5	Initial: 7.1 - 7.6 Final: 8.6 - 9.2

### C. Test Design

Guideline Criteria	Reported Information
<b>Dose range</b> 2X or 3X progression	2X
<b>Doses</b> at least 5	Seven (0.125, 0.25, 0.50, 1.0, 2.0, 4.0, and 8.0 mg/L, not corrected for percent active ingredient).
<b>Controls</b> negative and/or solvent	Negative and solvent control (300 µl/L DMF)
<b>Replicates per dose</b> 3 or more	3
<b>Duration of test</b> 120 hours	120 hours
<b>Daily observations were made?</b>	Yes
<b>Method of Observations</b>	Algal cell count
<b>Maximum Labeled Rate</b>	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

Guideline Criteria	Reported Information
Initial chemical concentrations measured? (Optional)	Yes, solutions were collected for analysis from each treatment level at test initiation and termination.
Raw data included?	Yes

### Dose Response

Nominal	Concentration (mg/L)			Day 5 Mean Cell Count ( $\times 10^4$ cells/mL)	% Reduction <sup>a</sup>	Final pH
	Initial	Final	Mean			
Control	<0.05	<0.05	<0.05	65.39	--	8.7
Solvent Control	<0.05	<0.05	<0.05	68.62	--	8.8
0.125	0.112	0.0959	0.104	78.18	0 (-13.9)	8.9
0.25	0.240	0.207	0.224	79.06	0 (-15.2)	9.0
0.50	0.492	0.406	0.449	87.93	0 (-28.1)	9.1
1.0	0.956	0.794	0.875	95.12	0 (-38.6)	9.2
2.0	1.91	1.62	1.77	87.17	0 (-27)	9.1
4.0	3.86	3.44	3.65	78.18	0 (-13.9)	8.9
8.0	7.83	7.58	7.71	31.90	53.5 <sup>b</sup>	8.7

<sup>a</sup> Compared to the solvent control. Negative sign indicates stimulation.

<sup>b</sup> Significantly reduced when compared to the solvent control.

Other Significant Results: No signs of test material toxicity were reported.

Statistical Results for Cell Counts

Statistical Method: Least squares linear regression for EC<sub>50</sub> and Dunnett's test for NOEC. Treatments were compared to the negative control based on initial measured concentrations.

EC<sub>50</sub>: >7.83 mg/L

95% C.I.: N/A

Probit Slope: N/A

NOEC: 3.86 mg/L

**13. VERIFICATION OF STATISTICAL RESULTS**

Statistical Method: Binomial test for EC<sub>50</sub>; Williams' test for NOEC. Treatments were compared to the solvent control based on mean measured concentrations.

EC<sub>50</sub>: 7.42 ppm

95% C.I.: Could not be determined

Probit Slope: N/A

NOEC: 3.65 ppm

- 14. REVIEWER'S COMMENTS:** This study is scientifically sound and fulfills the guideline requirements for an algal toxicity test. There appeared to be a growth stimulation in algae exposed up to 3.65 ppm. The 120-hour EC<sub>50</sub> for *A. flos-aquae* exposed to DE-565 Acid was 7.42 ppm. The NOEC was determined to be 3.65 ppm. This study is categorized as **Core**.

Karl Bullock Cloransulam-methyl Anabaena 3-22-99

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CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
7.71	100	54	54	0
3.65	100	0	0	0
1.77	100	0	0	0
.875	100	0	0	0
.449	100	0	0	0
.224	100	0	0	0
.104	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 7.420697

WHEN THERE ARE LESS THAN TWO CONCENTRATIONS AT WHICH THE PERCENT DEAD IS BETWEEN 0 AND 100, NEITHER THE MOVING AVERAGE NOR THE PROBIT METHOD CAN GIVE ANY STATISTICALLY SOUND RESULTS.

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DE-565 Acid: Toxicity to Anabaena  
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WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	Solvent Control	3	686158.000	686158.000	826796.333
2	.104	3	781809.000	781809.000	826796.333
3	.224	3	790582.667	790582.667	826796.333
4	.449	3	879289.000	879289.000	826796.333
5	.875	3	951249.667	951249.667	826796.333
6	1.77	3	871689.667	871689.667	826796.333
7	3.65	3	781780.333	781780.333	781780.333
8	7.71	3	318991.333	318991.333	318991.333

DE-565 Acid: Toxicity to Anabaena  
 File: 44744511 Transform: NO TRANSFORM

WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
Solvent Control	826796.333				
.104	826796.333	1.026		1.75	k= 1, v=16
.224	826796.333	1.026		1.83	k= 2, v=16
.449	826796.333	1.026		1.86	k= 3, v=16
.875	826796.333	1.026		1.87	k= 4, v=16
1.77	826796.333	1.026		1.88	k= 5, v=16
3.65	781780.333	0.697		1.89	k= 6, v=16
7.71	318991.333	2.678	*	1.89	k= 7, v=16

s = 167944.502

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