

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: DE-565 acid Purity: >96%
(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 DE-565
Acid Exposed Freshwater Diatom, *Navicula
pelliculosa*

Study Completion Date: May 20, 1998

Laboratory: The Dow Chemical Company, Midland, MI

Sponsor: Dow AgroSciences, LLC, Indianapolis, IN

Laboratory Report ID: 981028

DP Barcode: D252903

MRID No.: 447445-12

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: 4/15/99

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 EC₅₀ and NOEC for *N. pelliculosa* exposed to DE-565 acid were 59.1 and 47.5 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>Navicula pelliculosa</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 with silica and selenium

B. Test System

Guideline Criteria	Reported Information
<u>Solvent</u>	DMF
<u>Temperature</u> Skeletonema: 20°C Others: 24-25°C	22.8 - 23.4°C
<u>Light Intensity</u> Anabaena: 2.0 KLux (±15%) Others: 4.0-5.0 KLux (±15%)	2.6-5.2 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 5.0-8.3

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 (459 μ L/L) control groups
<u>Replicates per dose</u> 3 or more	3
<u>Duration of test</u> 120 or 96 hours	120 hours
<u>Daily observations were made?</u>	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 89-96%, 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	3.0	3.0
6.3	6.0	5.9
12.5	11.9	11.9
25	24.0	24.0
50	47.5	47.4
100	93.6	88.5

Dose Response

Nominal Concentration (ppm)	Initial measured concentration (ppm)	Day 5 Avg. Cell Density ($\times 10^4$ cells/mL)	% Inhibition*	pH Range
Control	<LOQ	80.8	N/A	7.7-7.8
Sol. Con.	<LOQ	91.5	N/A	7.8-7.9
3.1	3.0	105.4	-15	7.9-8.0
6.3	6.0	89.6	2	7.9-8.0
12.5	11.9	83.1	9	8.1-8.3
25	24.0	95.3	-4	8.2
50	47.5	96.3	-5	8.2-8.3
100	93.6	1.3	99	5.0-5.1

*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: 68.7 ppm
Probit Slope: N/A

95% C.I.: not determined
NOEC: 47.5 ppm

13. VERIFICATION OF STATISTICAL RESULTS:

Statistical Methods: Linear interpolation was used to estimate the EC₅₀ value. Williams' test was used to determine the NOEC.

EC₅₀: 59.1 ppm
Probit Slope: N/A

95% C.I.: not determined
NOEC: 47.5 ppm

- 14. REVIEWER'S COMMENTS:** This study is scientifically sound and fulfills the guideline requirements for an algal toxicity test. The 120-hour EC₅₀ and NOEC for *N. pelliculosa* exposed to DE-565 acid were 59.1 and 47.5 ppm, respectively. This study can be categorized as **Core**.

Navicula 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	915441.000	5.955	5.989
2	3.0 ppm	3	1053615.000	6.023	5.989
3	6.0 ppm	3	895680.000	5.936	5.950
4	11.9 ppm	3	830691.333	5.907	5.950
5	24.0 ppm	3	953341.333	5.977	5.950
6	47.5 ppm	3	963324.667	5.979	5.950
7	93.6 ppm	3	13090.000	4.112	4.112

Navicula 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.	5.989				
3.0 ppm	5.989	0.445		1.76	k= 1, v=14
6.0 ppm	5.950	0.073		1.85	k= 2, v=14
11.9 ppm	5.950	0.073		1.88	k= 3, v=14
24.0 ppm	5.950	0.073		1.89	k= 4, v=14
47.5 ppm	5.950	0.073		1.90	k= 5, v=14
93.6 ppm	4.112	24.477	*	1.91	k= 6, v=14

$\alpha = 0.092$

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

NOEC = 47.5 ppm