

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: 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 Freshwater Diatom,
Navicula pelliculosa

Study Completion Date: August 17, 1998

Laboratory: The Dow Chemical Company, Midland, MI

Sponsor: Dow AgroSciences, Indianapolis, IN

Laboratory Report ID: 981104

DP Barcode: D252903

MRID No.: 447445-02

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:

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 EC₅₀ and NOEC for *N. pelliculosa* exposed to 5-hydroxy-DE-565 were 42.7 and 36.3 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	9,100 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	23.8 - 24.7°C
<u>Light Intensity</u> Anabaena: 2.0 KLux (±15%) Others: 4.0-5.0 KLux (±15%)	3.7-5.4 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 3.4-8.5

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 (410 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
<u>Initial and 120 h cell densities were measured?</u>	Yes
<u>Control cell count at 120 hr \geq2X initial count?</u>	Yes
<u>Initial chemical concentrations measured?</u> (Optional) Percent of nominal, Procedural recovery, Limit of quantitation (LOQ)	Samples analyzed by HPLC 26-145%, Procedural recovery not reported, LOQ = 0.7 ppm
<u>Raw data included?</u>	Yes

Analytical Results

Concentration (ppm)	Measured concentrations (ppm)	
Nominal	Hour of Study	
	0	120
Control	<LOQ	<LOQ
Solvent Control	<LOQ	<LOQ
3.1	4.1	0.9
6.3	8.7	1.6
12.5	18.0	4.9
25	36.3	13.4
50	70.4	45.8
100	133	102

Dose Response

Nominal Concentration (ppm)	Initial measured concentration (ppm)	Day 5 Avg. Cell Density ($\times 10^4$ cells/mL)	% Inhibition*	pH Range
Control	<LOQ	70.0	N/A	7.8-8.2
Sol. Con.	<LOQ	127.6	N/A	8.3-8.5
3.1	4.1	154.0	-21	8.4-8.5
6.3	8.7	176.7	-38	8.5
12.5	18.0	171.0	-34	8.5
25	36.3	102.2	20	8.0-8.3
50	70.4	1.4	99	4.1-4.2
100	133	1.1	99	3.4-3.5

*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: 48.2 ppm
Probit Slope: N/A

95% C.I.: 9.5 - >133 ppm
NOEC: 36.3 ppm

13. VERIFICATION OF STATISTICAL RESULTS:

Statistical Methods: The moving average method was used to estimate the EC₅₀ value. Williams' test was used to determine the NOEC.

EC₅₀: 42.7 ppm
Probit Slope: N/A

95% C.I.: 40.2 - 45.5 ppm
NOEC: 36.3 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 5-hydroxy-DE-565 were 42.7 and 36.3 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	1276094.000	6.101	6.191
2	4.1 ppm	3	1539707.000	6.187	6.191
3	8.7 ppm	3	1767209.333	6.247	6.191
4	18.0 ppm	3	1709738.000	6.230	6.191
5	36.3 ppm	3	1021610.333	5.992	5.992
6	70.4 ppm	3	13993.667	4.140	4.140
7	133 ppm	3	10667.333	4.021	4.021

Navicula cell density

File: nav Transform: LOG BASE 10(Y)

WILLIAMS TEST (Isotonic regression model)

TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
Sol. Con.	6.191				
4.1 ppm	6.191	1.286		1.76	k= 1, v=14
8.7 ppm	6.191	1.286		1.85	k= 2, v=14
18.0 ppm	6.191	1.286		1.88	k= 3, v=14
36.3 ppm	5.992	1.556		1.89	k= 4, v=14
70.4 ppm	4.140	27.983	*	1.90	k= 5, v=14
133 ppm	4.021	29.682	*	1.91	k= 6, v=14

s = 0.086

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

NOEC = 36.3 ppm

6.

Mossler 5-hydroxy DE565 Navicula pelliculosa 3-18-99

CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
70.4	100	99	99	0
36.3	100	20	20	0
18	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 44.97638

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS
2	9.743828E-03	42.69599	40.23586 45.46019

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
6	6.084578E-02	1	.9567501

SLOPE = 11.01629
95 PERCENT CONFIDENCE LIMITS = 8.29891 AND 13.73368

LC50 = 43.28386
95 PERCENT CONFIDENCE LIMITS = 40.95134 AND 46.29675

LC10 = 33.19267
95 PERCENT CONFIDENCE LIMITS = 30.52492 AND 35.29055
