

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 Acid Purity: >97%
(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 Acid Exposed Bluegreen
Alga, *Anabaena flos-aquae*

Study Completion Date: July 15, 1998

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


Sponsor: Dow AgroSciences, LLC, Indianapolis, IN

Laboratory Report ID: 981078

DP Barcode: D252903

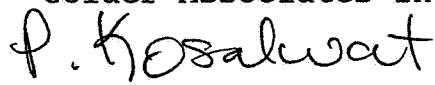
MRID No.: 447445-06

4. REVIEWED BY: Max Feken, M.S., Environmental Toxicologist,
Golder Associates Inc.

Signature: 

Date: 4/2/99

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

Signature: 

Date: 4/2/99

5. APPROVED BY:

Signature: 

Date: 4/15/99

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 aquatic plant toxicity
test.

Results Synopsis

EC₅₀: 33.6 ppm

95% C.I.: 23.7 - 47.6 ppm

NOEC: 26.5 ppm

Probit Slope: N/A

8. ADEQUACY OF THE STUDY:

A. Classification: Core

B. Rationale: N/A

C. Repairability: N/A

9. GUIDELINE DEVIATIONS:

1. Each test vessel contained approximately 22,000 cells/mL at the start of the test. The guidelines recommend an initial cell count of 3,000 to 10,000 cells/mL.
2. The pH of the two highest test concentrations were lower than recommended as a result of the acidic nature of the test substance. Algal growth inhibition was highest at these two concentrations and was, more than likely, directly related to the low pH of the test solutions.

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	22,000 cells/mL
<u>Nutrients</u> Standard formula, e.g. 20XAAP	Algal Assay Medium

B. Test System

Guideline Criteria	Reported Information
Solvent	DMF (0.5 mL/L)
Temperature Skeletonema: 20°C Others: 24-25°C	23.0 - 24.1°C
Light Intensity Anabaena: 2.0 Klux (±15%) Others: 4.0-5.0 Klux (±15%)	1.7 - 2.3 Klux
Photoperiod Skeletonema: 14 h light, 10 h dark or 16 h light, 8 h dark Others: Continuous	Continuous
pH Skeletonema: approx. 8.0 Others: approx. 7.5	Without alga: 3.6 - 7.1 With alga: 3.6 - 8.8

C. Test Design

Guideline Criteria	Reported Information
Dose range 2X or 3X progression	2X
Doses at least 5	Six doses: 3.13, 6.25, 12.5, 25, 50, and 100 mg/L
Controls negative and/or solvent	Negative and solvent control
Replicates per dose 3 or more	3
Duration of test 120 hours	120 hours
Daily observations were made?	Yes
Method of Observations	Cellular counts
Maximum Labeled Rate	Not reported

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)	Yes
Raw data included?	Yes

Measured Concentrations

Nominal Conc. (mg/L)	Measured Concentration (mg/L)			
	0-Hour	120-Hour	Mean	% Nominal
Control	<0.8	<0.8	N/A	N/A
Solvent Control	<0.8	<0.8	N/A	N/A
3.13	3.05	3.72	3.39	108
6.25	6.18	7.52	6.85	110
12.5	12.6	13.9	13.3	106
25.0	26.2	26.7	26.5	106
50.0	54.8	53.6	54.2	108
100	110	110	110	110

Dose Response

Mean Measured Concentration (mg/L)	Avg. Cell Density ($\times 10^3$ cells/ml)	% reduction ^a	pH range
Control	560	--	8.0 - 8.6
Solvent Cont.	587	--	8.4 - 8.5
3.39	635	-8 ^b	8.6 - 8.7
6.85	629	-7	8.6 - 8.7
13.3	556	5	8.7 - 8.8
26.5	555	6	8.5 - 8.7
54.2	13	98	4.2 - 4.4
110	12	98	3.6 - 3.7

^aCompared to the solvent control

^bNegative value indicates stimulation of growth

Other Significant Results: None

Statistical Results

Statistical Method: Least squares linear regression was used to estimate the EC₅₀ and Dunnett's test was used for mean comparisons versus the pooled control. Results based on the initial measured concentrations.

EC₅₀: 54.4 mg/L 95% C.I.: 12.8 - 96.0 mg/L

Probit Slope: Not reported NOEC: 26.2 mg/L

13. VERIFICATION OF STATISTICAL RESULTS:

Statistical Method: Weighted nonlinear model (PROC NLIN) and Williams' test for mean comparisons. Results based on mean measured concentrations.

EC₅₀: 33.6 ppm 95% C.I.: 23.7 - 47.6 ppm

Probit Slope: N/A NOEC: 26.5 ppm

14. REVIEWER'S COMMENTS: This study is scientifically sound and fulfills the guideline requirements for an aquatic plant toxicity test. Based on mean measured concentrations, the 120-hour EC₅₀ for *Anabaena flos-aquae* exposed to 5-hydroxy-DE-565 Acid was 33.6 ppm. The NOEC was 26.5 ppm. This study is categorized as **Core**.

5-OH-DE-565 ACID - ANABAENA

File: 44744506

Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model)

TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	SOLVENT	3	587459.000	587459.000	617006.333
2	3.39	3	635056.333	635056.333	617006.333
3	6.85	3	628503.667	628503.667	617006.333
4	13.3	3	556445.333	556445.333	556445.333
5	26.5	3	555083.333	555083.333	555083.333
6	54.2	3	13097.667	13097.667	13097.667
7	110	3	11786.000	11786.000	11786.000

5-OH-DE-565 ACID - ANABAENA

File: 44744506

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
SOLVENT	617006.333				
3.39	617006.333	0.593		1.76	k= 1, v=14
6.85	617006.333	0.593		1.85	k= 2, v=14
13.3	556445.333	0.622		1.88	k= 3, v=14
26.5	555083.333	0.649		1.89	k= 4, v=14
54.2	13097.667	11.518	*	1.90	k= 5, v=14
110	11786.000	11.544	*	1.91	k= 6, v=14

s = 61075.285

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

NOEC = 26.5

MAX FEKEN 5-OH-DE-565 ACID ANABAENA 03-23-99

CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
54.2	100	98	98	0
26.5	100	6	6	0
13.3	100	5	5	0
6.85	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 36.755

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS	
2	1.376584E-02	35.72419	33.11781	38.85127

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT	PROBABILITY
8	8.668607	50.31772	0	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 = 6.726607
95 PERCENT CONFIDENCE LIMITS = -13.07821 AND 26.53142

LC50 = 34.29237
95 PERCENT CONFIDENCE LIMITS = 0 AND +INFINITY

LC10 = 22.20216
95 PERCENT CONFIDENCE LIMITS = 0 AND +INFINITY

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OBS	CONC	LOG_CONC	Y1	Y2	Y3	Y4	Y5	Y6
1	0.00		467569	702000	592808			
2	3.39	0.53020	624306	644811	636052			
3	6.85	0.83569	575889	575706	733916			
4	13.50	1.12585	607349	569644	492343			
5	26.50	1.42325	571769	532162	561319			
6	54.20	1.73400	17377	8818	13098			
7	110.00	2.04139	15474	14801	5083			

MODEL: COUNT = CO * PROBLOGM ((LOG_EC50 - LOG_CONC) / SIGMA)
 WEIGHTED REGRESSION

Non-Linear Least Squares Iterative Phase
 Dependent Variable COUNT Method: Gauss-Newton Weighted SS

Iter	LOG_EC50	SIGMA	CO	Weighted SS
0	1.535000	0.149000	587459	2637144
1	1.518365	0.164914	621250	1256159
2	1.510251	0.154481	616176	1841343
3	1.523410	0.160726	619442	1444930
4	1.528235	0.156727	617393	1680956
5	1.525271	0.159173	618660	1529618
6	1.527128	0.157636	617870	1622040
7	1.525979	0.158585	618360	1563896
8	1.526696	0.157992	618055	1599793
9	1.526251	0.158360	618245	1577372
10	1.526528	0.158131	618126	1591275
11	1.526356	0.158273	618200	1582616
12	1.526463	0.158185	618154	1587994
13	1.526396	0.158240	618183	1584648
14	1.526438	0.158205	618165	1586728
15	1.526412	0.158227	618176	1585434
16	1.526428	0.158215	618169	1586238
17	1.526418	0.158222	618173	1585738
18	1.526424	0.158217	618171	1586049
19	1.526420	0.158220	618172	1585856
20	1.526423	0.158218	618171	1585976
21	1.526421	0.158219	618172	1585901
22	1.526422	0.158218	618172	1585948
23	1.526422	0.158219	618172	1585919
24	1.526422	0.158218	618172	1585937
25	1.526422	0.158219	618172	1585925
26	1.526422	0.158218	618172	1585932
27	1.526422	0.158219	618172	1585928
28	1.526422	0.158219	618172	1585931
29	1.526422	0.158219	618172	1585930
30	1.526422	0.158219	618172	1585929
31	1.526422	0.158219	618172	1585930
32	1.526422	0.158219	618172	1585930
33	1.526422	0.158219	618172	1585930
34	1.526422	0.158219	618172	1585930
35	1.526422	0.158219	618172	1585930
36	1.526422	0.158219	618172	1585930
37	1.526422	0.158219	618172	1585930
38	1.526422	0.158219	618172	1585930
39	1.526422	0.158219	618172	1585930

NOTE: Convergence criterion met.
 5-OH-DE-565 ACID - ANABAENA
 MODEL: COUNT = CO * PROBLOGM ((LOG_EC50 - LOG_CONC) / SIGMA)
 WEIGHTED REGRESSION

Non-Linear Least Squares Summary Statistics

Source	DF	Weighted SS	Dependent Variable COUNT	Weighted MS
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Regression Residual	3	8962294.000	2987431.333
Uncorrected Total	18	1585929.718	88107.207
(Corrected Total)	20	8967312.887	

Parameter	Estimate	Asymptotic Std. Error	Confidence Interval Lower	Confidence Interval Upper	Asymptotic 95 %
LOG_EC50	1.5264	0.071934	1.37530	1.67755	
SIGMA	0.1582	0.055901	0.04078	0.27566	
CO	618171.7136	68356.613161	474560.74731	761782.67983	

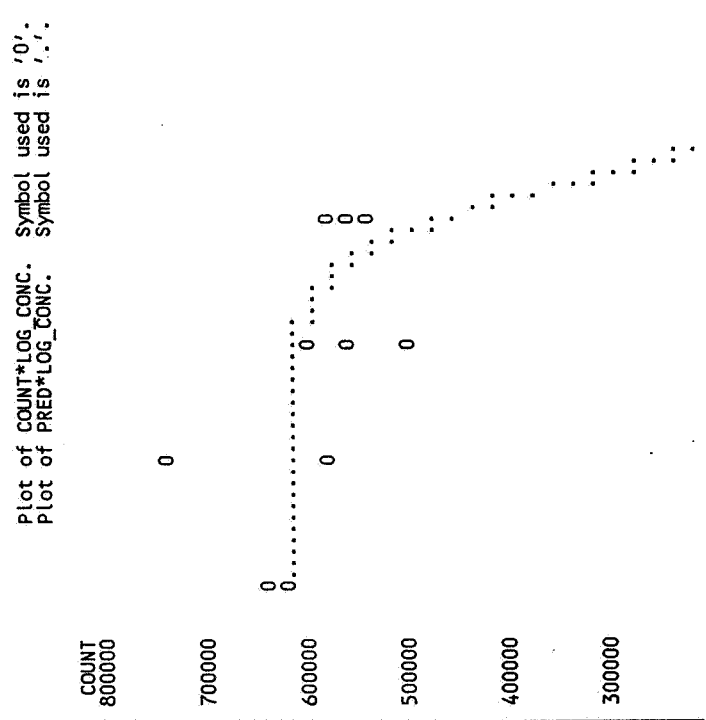
Asymptotic Correlation Matrix

Corr	LOG_EC50	SIGMA	CO
LOG_EC50	1		
SIGMA	-0.645567149	1	
CO	-0.442587179	0.2943884729	1

MODEL: COUNT = CO * PROBLOGM ((LOG_EC50 - LOG_CONC) / SIGMA)
 SUMMARY OF NONLINEAR REGRESSION
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OBS	CONC	LOG_EC50	SIGMA	CO	RESID_SS	EC50
1	0	1.52642	0.15822	618171.71	1585929.72	33.6064

MODEL: COUNT = CO * PROBLOGM ((LOG_EC50 - LOG_CONC) / SIGMA)
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30000

0
0.50 0.75 1.00 1.25 1.50 1.75 2.00 2.25
LOG_CONC
FE: 1541 obs had missing values. 1469 obs hidden.
5-OH-DE-565 ACID - ANABAENA
COMPARISON OF MEANS FOR NOEL DETERMINATION
TEST IF TREATMENT IS LESS THAN CONTROL
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General Linear Models Procedure
Class Level Information

Class Levels Values
DOSE 7 0 110 13.3 26.5 3.39 54.2 6.85

Number of observations in data set = 42

FE: Due to missing values, only 21 observations can be used in this analysis.

5-OH-DE-565 ACID - ANABAENA
COMPARISON OF MEANS FOR NOEL DETERMINATION
TEST IF TREATMENT IS LESS THAN CONTROL
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General Linear Models Procedure

Dependent Variable: RESPONSE
R-Square 0.965456
C.V. 14.31086
Root MSE 61075.28
Type I SS 14.31086
Type III SS 14.31086
Mean Square 2.43259E+11
F Value 65.21
Pr > F 0.0001

DF 6
Sum of Squares 1.45955E+12
Mean Square 2.43259E+11
F Value 65.21
Pr > F 0.0001

5-OH-DE-565 ACID - ANABAENA

General Linear Models Procedure

Level of DOSE	N	Mean	SD
0	3	587459.000	117307.000
110	3	11786.000	5814.713
13.3	3	556445.333	58628.053
26.5	3	555083.333	20526.599
3.39	3	635056.333	10288.696
54.2	3	13097.667	4279.500
6.85	3	628503.667	91289.804

5-OH-DE-565 ACID - ANABAENA
COMPARISON OF MEANS FOR NOEL DETERMINATION
TEST IF TREATMENT IS LESS THAN CONTROL
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General Linear Models Procedure

Dunnett's One-tailed T tests for variable: RESPONSE

NOTE: This tests controls the type I experimentwise error for comparisons of all treatments against a control.

Alpha= 0.05 Confidence= 0.95 df= 14 MSE= 3.7302E9
Critical Value of Dunnett's T= 2.532
Minimum Significant Difference= 126275

Comparisons significant at the 0.05 level are indicated by ****.

DOSE Comparison	Simultaneous Confidence Limit		Difference Between Means	Simultaneous Confidence Limit	
	Lower Limit	Upper Limit		Lower Limit	Upper Limit
3.39 - 0	-78678	47597	47597	173873	
6.85 - 0	-85231	41045	41045	167320	
13.3 - 0	-157289	-31014	-31014	95262	
26.5 - 0	-158651	-32376	-32376	93900	***
54.2 - 0	-700637	-574361	-574361	-448086	***
110 - 0	-701948	-575673	-575673	-449598	***