DATA EVALUATION RECORD
ALGAE OR DIATOM EC_{50} TEST
GUIDELINE 123-2 (TIER II)

1. **CHEMICAL:** Cloransulam-methyl (DE-565) PC Code No.: 129116

2. **TEST MATERIAL:** XDE-565 (Firstrate®) Purity: 82.2%

3. **CITATION:**

Authors:	H.D. Kirk, J.M. Hugo, and L.G. McFadden
Title:	Phytotoxicological Evaluation of XDE-565 (NAF-75 Formulation) with a Freshwater Green Algae, *Selenastrum capricornutum*
Study Completion Date:	September 15, 1997
Laboratory:	The Dow Chemical Company, Midland, MI
Sponsor:	DowElanco, Indianapolis, IN
Laboratory Report ID:	971123
DP Barcode:	D252903
MRID No.:	447445-17

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

Signature: Mark A. Mossler **Date:** 4/6/99

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

Signature: P. Kosalwat **Date:** 4/6/99

5. **APPROVED BY:**

Signature: M. E. Ellis **Date:** 4/15/99

6. **STUDY PARAMETERS:**

Definitive Test Duration: 96 hours
Type of Concentrations: Initial measured

7. **CONCLUSIONS:** This study is scientifically sound and fulfills the guideline requirements for an algal toxicity test. The 96-hour EC_{50} and NOEC for *S. capricornutum* exposed to the NAF-75 formulation of XDE-565 were 2.7 and 0.9 ppb ai, respectively.

8. **ADEQUACY OF THE STUDY:**

A. Classification: Core for a formulated product.

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
Species *Skeletonema costatum* *Anabaena flos-aquae* *Selenastrum capricornutum* *Navicula pelliculosa*	*Selenastrum capricornutum*
Initial Number of Cells 3,000 - 10,000 cells/mL	10,000 cells/mL
Nutrients Standard formula, e.g. 20XAAP	Algal assay medium

B. **Test System**

Guideline Criteria	Reported Information
Solvent	DMF
Temperature Skeletonema: 20°C Others: 24-25°C	24.7 - 25.2°C
Light Intensity Anabaena: 2.0 KLux (±15%) Others: 4.0-5.0 KLux (±15%)	3.4-5.3 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	Range of 7.6-8.7

C. Test Design

Guideline Criteria	Reported Information
Dose range 2X or 3X progression	2X
Doses Must be tested at the maximum label rate	0.5, 1.0, 2.0, 4.0, 8.0, and 16 ppb active ingredient (ai)
Controls Negative and/or solvent	Negative and solvent (100 µL/L) control groups
Replicates per dose 3 or more	3
Duration of test 120 or 96 hours	96 hours
Daily observations were made?	Yes
Method of Observations	Cellular counts
Maximum Labeled Rate	0.0548 lb ai per acre (from MRID No. 447445-16)

12. REPORTED RESULTS:

Guideline Criteria	Reported Information
Initial and 96 h cell densities were measured?	Yes
Control cell count at 96 hr ≥2X initial count?	Yes
Initial chemical concentrations measured? (Optional) Percent of nominal, Procedural recovery, Limit of quantitation (LOQ)	Samples analyzed by HPLC 39-98%, Procedural recovery of 104%, LOQ = 0.12 ppb
Raw data included?	Yes

Analytical Results

Concentration (ppb ai)	Measured concentrations (ppb ai)	
Nominal	Hour of Study	
	0	96
Control	<LOQ	<LOQ
Solvent Control	<LOQ	<LOQ
0.50	0.49	0.26
1.0	0.94	0.42
2.0	1.7	0.79
4.0	3.6	2.0
8.0	6.9	4.0
16.0	14.1	7.5

Dose Response

Nominal Concentration (ppb ai)	Initial measured concentration (ppb ai)	Day 4 Avg. Cell Density (x 10^4 cells/mL)	% Inhibition*	pH
Control	<LOQ	117.7	N/A	8.2
Sol. Con.	<LOQ	133.0	N/A	8.1
0.50	0.49	131.5	1	8.7
1.0	0.94	119.2	10	8.4
2.0	1.7	95.4	28	8.1
4.0	3.6	47.5	64	7.8
8.0	6.9	25.5	81	7.7
16.0	14.1	11.2	92	7.6

*Comparison to the solvent control

Other Significant Results: Results from a recovery test conducted for five days after exposure indicated that the test material was algistatic, rather than algicidal.

Statistical Methods: linear regression and Dunnett's test

EC_{50}: 2.9 ppb ai	95% C.I.: 1.5 - 5.7 ppb ai
Probit Slope: N/A	NOEC: 0.9 ppb ai

13. VERIFICATION OF STATISTICAL RESULTS:

Statistical Methods: Probit analysis was used to initially estimate the EC_{50} value. The response was then remodeled using non-linear regression. Williams' test was used to determine the NOEC.

EC_{50}: 2.7 ppb ai	95% C.I.: 2.1 - 3.4 ppb ai
Probit Slope: N/A	NOEC: 0.9 ppb ai

14. REVIEWER'S COMMENTS: This study is scientifically sound and fulfills the guideline requirements for an algal toxicity test. The 96-hour EC_{50} and NOEC for *S. capricornutum* exposed to the NAF-75 formulation of XDE-565 were 2.7 and 0.9 ppb ai, respectively. This study can be categorized as **Core for a formulated product.**

Selenastrum cell density

Estimated EC Values and Confidence Limits

Point	Conc.	Lower 95% Confidence	Upper Limits
EC 1.00	0.2998	0.1927	0.4177
EC 5.00	0.5860	0.4211	0.7536
EC10.00	0.8377	0.6371	1.0351
EC15.00	1.0662	0.8409	1.2847
EC50.00	2.9550	2.5932	3.3565
EC85.00	8.1897	6.8879	10.1806
EC90.00	10.4234	8.5603	13.4201
EC95.00	14.9004	11.7707	20.2807
E[illegible]9.00	29.1249	21.2595	44.2685

$EC_{25} = 1.5$ ppb ai

Selenastrum cell density
File: sel 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	1330452.667	1330452.667	1330452.667
2	0.5 ppb ai	3	1314968.333	1314968.333	1314968.333
3	0.9 ppb ai	3	1192313.000	1192313.000	1192313.000
4	1.7 ppb ai	3	953778.333	953778.333	953778.333
5	3.6 ppb ai	3	474528.333	474528.333	474528.333
6	6.9 ppb ai	3	255356.333	255356.333	255356.333
7	14.1 ppb ai	3	111692.333	111692.333	111692.333

Selenastrum cell density
File: sel 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.	1330452.667				
0.5 ppb ai	1314968.333	0.159		1.76	k= 1, v=14
0.9 ppb ai	1192313.000	1.421		1.85	k= 2, v=14
1.7 ppb ai	953778.333	3.874	*	1.88	k= 3, v=14
3.6 ppb ai	474528.333	8.802	*	1.89	k= 4, v=14
6.9 ppb ai	255356.333	11.056	*	1.90	k= 5, v=14
14.1 ppb ai	111692.333	12.534	*	1.91	k= 6, v=14

s = 119093.224
Note: df used for table values are approximate when v > 20.

NOEC = 0.9 ppb ai

selenastrum cell density 1
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OBS	CONC	LOG_CONC	Y1	Y2	Y3	Y4	Y5	Y6
1	0.00	.	1155615	1490959	1344784	.	.	.
2	0.94	-0.02687	1054963	1268061	1253915	.	.	.
3	1.70	0.23045	855033	950751	1055551	.	.	.
4	3.60	0.55630	474005	361404	588176	.	.	.
5	6.90	0.83885	242059	268187	255823	.	.	.
6	14.10	1.14922	104447	109553	121077	.	.	.

selenastrum cell density 2
MODEL: COUNT = C0 * PROBNORM ((LOG_EC50 - LOG_CONC) / SIGMA)
WEIGHTED REGRESSION
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Non-Linear Least Squares Iterative Phase
Dependent Variable COUNT Method: Gauss-Newton

Iter	LOG_EC50	SIGMA	C0	Weighted SS
0	0.471000	0.427000	1330453	233062
1	0.419398	0.487634	1384594	199605
2	0.428626	0.478594	1372350	199221
3	0.426890	0.480148	1374361	199220
4	0.427194	0.479872	1374006	199218
5	0.427140	0.479920	1374069	199218
6	0.427149	0.479912	1374058	199218
7	0.427148	0.479913	1374060	199218
8	0.427148	0.479913	1374059	199218
9	0.427148	0.479913	1374060	199218

NOTE: Convergence criterion met.

Non-Linear Least Squares Summary Statistics Dependent Variable COUNT

Source	DF	Weighted SS	Weighted MS
Regression	3	12954363.000	4318121.000
Residual	15	199218.130	13281.209
Uncorrected Total	18	13153581.130	
(Corrected Total)	17	7314686.413	

Parameter	Estimate	Asymptotic Std. Error	Asymptotic 95 % Confidence Interval Lower	Upper
LOG_EC50	0.427	0.049008	0.3227	0.5316
SIGMA	0.480	0.040398	0.3938	0.5660
C0	1374059.516	73831.288756	1216692.4470	1531426.5842

Asymptotic Correlation Matrix

Corr	LOG_EC50	SIGMA	C0
LOG_EC50	1	-0.731516174	-0.801596954
SIGMA	-0.731516174	1	0.5533885914
C0	-0.801596954	0.5533885914	1

selenastrum cell density 3
MODEL: COUNT = C0 * PROBNORM ((LOG_EC50 - LOG_CONC) / SIGMA)
SUMMARY OF NONLINEAR REGRESSION
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OBS	CONC	LOG_EC50	SIGMA	C0	RESID_SS	EC50
1	0	0.42715	0.47991	1374059.52	199218.13	2.67392

selenastrum cell density 4
MODEL: YOUNG = C0 * PROBNORM ((LOG_EC25 - LOG_CONC) / SIGMA - 0.67449)
WEIGHTED REGRESSION

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

Iter	LOG_EC25	SIGMA	C0	Weighted SS
0	0.184000	0.427000	1330453	232615
1	0.090500	0.487671	1384568	199601
2	0.105825	0.478591	1372346	199221
3	0.103034	0.480149	1374362	199220
4	0.103525	0.479871	1374006	199218
5	0.103438	0.479921	1374069	199218
6	0.103454	0.479912	1374058	199218
7	0.103451	0.479913	1374060	199218
8	0.103451	0.479913	1374059	199218
9	0.103451	0.479913	1374060	199218

NOTE: Convergence criterion met.

Non-Linear Least Squares Summary Statistics Dependent Variable COUNT

Source	DF	Weighted SS	Weighted MS
Regression	3	12954363.000	4318121.000
Residual	15	199218.130	13281.209
Uncorrected Total	18	13153581.130	
(Corrected Total)	17	7314686.413	

Parameter	Estimate	Asymptotic Std. Error	Asymptotic 95 % Confidence Interval Lower	Upper
LOG_EC25	0.103	0.071401	-0.0487	0.2556
SIGMA	0.480	0.040398	0.3938	0.5660
C0	1374059.516	73831.288757	1216692.4471	1531426.5843

Asymptotic Correlation Matrix

Corr	LOG_EC25	SIGMA	C0
LOG_EC25	1	-0.883730737	-0.76139436
SIGMA	-0.883730737	1	0.5533885914
C0	-0.76139436	0.5533885914	1

selenastrum cell density 5
MODEL: YOUNG = C0 * PROBNORM ((LOG_EC25 - LOG_CONC) / SIGMA - 0.67449)
SUMMARY OF NONLINEAR REGRESSION
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OBS	CONC	LOG_EC25	SIGMA	C0	RESID_SS	EC25
1	0	0.10345	0.47991	1374059.52	199218.13	1.26897

selenastrum cell density 6
MODEL: YOUNG = C0 * PROBNORM ((LOG_EC25 - LOG_CONC) / SIGMA - 0.67449)
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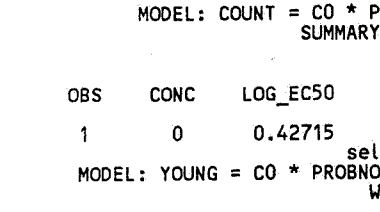
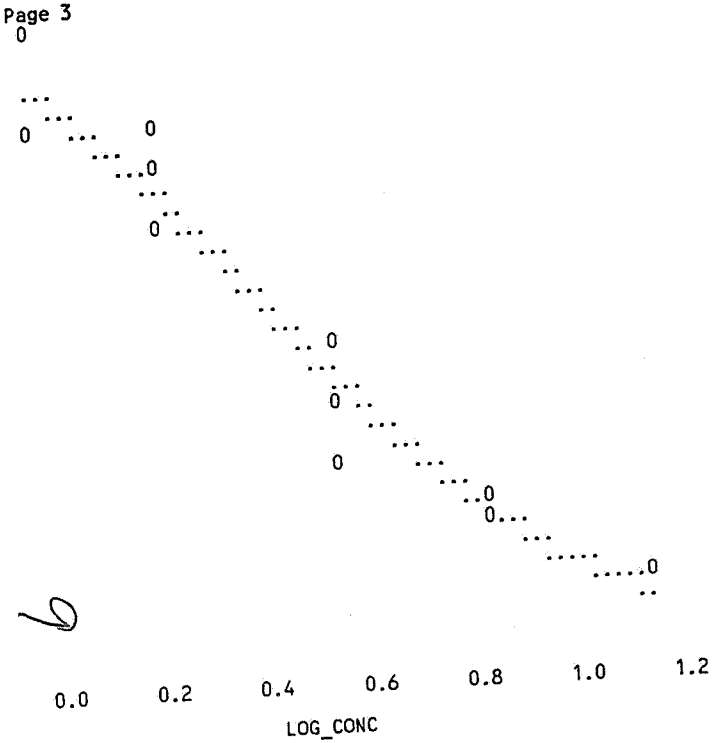
Plot of COUNT*LOG_CONC. Symbol used is '0'.
Plot of PRED*LOG_CONC. Symbol used is '.'.





204 obs had missing values. 1132 obs hidden.

selenastrum cell density
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	6	0 1.7 3.6 6.9 0.94 14.1

Number of observations in data set = 36

Due to missing values, only 18 observations can be used in this analysis.

selenastrum cell density
COMPARISON OF MEANS FOR NOEL DETERMINATION
TEST IF TREATMENT IS LESS THAN CONTROL
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General Linear Models Procedure

dent Variable: RESPONSE

	DF	Sum of Squares	Mean Square	F Value	Pr > F
e	5	3.88972E+12	7.77943E+11	71.13	0.0001

Error	12	1.31251E+11	1.09376E+10	
Corrected Total	17	4.02097E+12		

R-Square	C.V.	Root MSE	RESPONSE Mean
0.967358	14.53172	104582.9	719686.8

Source	DF	Type I SS	Mean Square	F Value	Pr > F
DOSE	5	3.88972E+12	7.77943E+11	71.13	0.0001

Source	DF	Type III SS	Mean Square	F Value	Pr > F
DOSE	5	3.88972E+12	7.77943E+11	71.13	0.0001

selenastrum cell density
COMPARISON OF MEANS FOR NOEL DETERMINATION
TEST IF TREATMENT IS LESS THAN CONTROL
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General Linear Models Procedure

Level of DOSE	N	RESPONSE Mean	RESPONSE SD
0	3	1330452.67	168130.723
1.7	3	953778.33	100293.273
3.6	3	474528.33	113386.906
6.9	3	255356.33	13070.250
0.94	3	1192313.00	119158.693
14.1	3	111692.33	8518.908

selenastrum cell density
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= 12 MSE= 1.094E10
Critical Value of Dunnett's T= 2.502
Minimum Significant Difference= 213663

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

DOSE Comparison	Simultaneous Lower Confidence Limit	Difference Between Means	Simultaneous Upper Confidence Limit	
0.94 - 0	-351803	-138140	75523	
1.7 - 0	-590337	-376674	-163011	***
3.6 - 0	-1069587	-855924	-642261	***
6.9 - 0	-1288759	-1075096	-861433	***
14.1 - 0	-1432423	-1218760	-1005097	***