

DATA EVALUATION RECORD
ALGAE OR DIATOM EC₅₀ TEST
GUIDELINE 123-2 (TIER II)

1. **CHEMICAL:** Mesotrione **PC Code No.:** 122990
2. **TEST MATERIAL:** ZA1296 **Purity:** 95.1%
3. **CITATION:**
Authors: N. Shillabeer, S.J. Kent, and D.V. Smyth
Title: ZA1296: Toxicity to the Green Alga
Selenastrum capricornutum
Study Completion Date: August 27, 1997 = K12 CHMER 1A
Laboratory: Brixham Environmental Laboratory, Brixham
Devon, UK
Sponsor: ZENECA Ag Products, Wilmington, DE
Laboratory Report ID: BL6113/B
DP Barcode: D245475
MRID No.: 445051-24

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

Signature: *Karl Bullock* **Date:** 7/30/98

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

Signature: *Max Feken* **Date:** 7/30/98

5. **APPROVED BY:**

Signature: *Max Feken* **Date:** 6/13/00

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

EC₅₀: 1.9 ppm 95% C.I.: 1.5 - 2.4 ppm

Probit Slope: N/A NOEC: 0.82 ppm

8. ADEQUACY OF THE STUDY:**A. Classification:** Core.**B. Rationale:** N/A.**C. Repairability:** N/A.**9. GUIDELINE DEVIATIONS:** None.**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>Selenastrum capricornutum</i>
<u>Initial Number of Cells</u> 3,000 - 10,000 cells/mL	3,000 cells/mL
<u>Nutrients</u> Standard formula, e.g. 20XAAP	ATCC 22662

B. Test System

Guideline Criteria	Reported Information
<u>Solvent</u>	None
<u>Temperature</u> <i>Skeletonema</i> : 20°C Others: 24-25°C	24.1-24.2°C
<u>Light Intensity</u> <i>Anabaena</i> : 2.0 KLux (±15%) Others: 4.0-5.0 KLux (±15%)	4.82 KLux
<u>Photoperiod</u> <i>Skeletonema</i> : 14 h light, 10 h dark or 16 h light, 8 h dark Others: Continuous	Continuous

Guideline Criteria	Reported Information
pH Skeletonema: approx. 8.0 Others: approx. 7.5	Initial: 6.2 - 7.4 Final: 7.1 - 10.0

C. Test Design

Guideline Criteria	Reported Information
<u>Dose range</u> 2X or 3X progression	2X
<u>Doses</u> at least 5	0.38, 0.75, 1.5, 3.0, 6.0, 12, 24 and 48 mg/L, not corrected for percent active ingredient
<u>Controls</u> negative and/or solvent	Negative control
<u>Replicates per dose</u> 3 or more	3 (6 in the control)
<u>Duration of test</u> 120 hours	120 hours
Daily observations were made?	Yes
<u>Method of Observations</u>	Cellular counts
<u>Maximum Labeled Rate</u>	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
Initial chemical concentrations measured? (Optional)	Yes, samples collected at test initiation and termination were analyzed by HPLC.
Raw data included?	Yes

Dose Response

Toxicant concentration (mg/L)		120-hr. Avg. Cell Density (x 10 ⁴ cells/mL)	Inhibition (%)	Final pH
Nominal	Mean Measured			
Negative Control	<0.036	496	-	10.0
0.38	0.39	486	2	9.9
0.75	0.82	478	4	10.0
1.5	1.6	304	39	8.5
3.0	3.0	129	74	8.2
6.0	6.1	53.4	89	7.9
12	13	7.64	98	7.7
24	24	3.31	99	7.5
48	49	1.61	100	7.2

Other Significant Results: No signs of test material toxicity were observed. After test solution preparation, a pale yellow coloration was noted in the 12, 24, and 48 mg/L nominal treatments. All other test solutions were clear and colorless. Area under the growth curve was the most sensitive endpoint measured in this study.

Statistical Results for Cell Density:

Statistical Method: Linear regression analysis for EC₅₀ and Dunnett's test for NOEC, using the area under the growth curve and nominal concentrations

EC₅₀: 3.5 mg/L

95% C.I.: 1.9 - 6.1 mg/L

Probit Slope: N/A

NOEC: 0.75 mg/L

13. VERIFICATION OF STATISTICAL RESULTS:

Statistical Method: Non-linear regression analysis for EC₅₀ and Williams' test for NOEC, using algal biomass and mean measured concentrations

EC₅₀: 1.9 ppm

95% C.I.: 1.5 - 2.4 ppm

Probit Slope: N/A

NOEC: 0.82 ppm

14. **REVIEWER'S COMMENTS:** This study is scientifically sound and fulfills the guideline requirements for an algal toxicity test. Based on mean measured concentrations, the 120-hour EC₅₀ and NOEC for *S. capricornutum* exposed to ZA1296 were 1.4 and 0.82 ppm, respectively. This study can be categorized as **Core**.

ZAI296 Toxicity to Selenastrum
16:18 Friday, July 17, 1998

OBS	CONC	LOG_CONC	Y1	Y2	Y3	Y4	Y5	Y6
1	0.00	487.00	497.00	539.00	504	468	482	
2	0.39	506.00	477.00	474.00				
3	0.82	-0.40894	489.00	470.00				
4	1.60	0.20412	208.00	284.00				
5	3.00	0.47712	171.00	183.00				
6	6.10	0.78533	53.00	42.40				
7	13.00	1.11394	7.49	11.70				
8	24.00	1.38021	4.14	3.61				
9	49.00	1.69020	1.65	1.55				

MODEL: COUNT = CO * PROBNOORM ((LOG_EC50 - LOG_CONC) / SIGMA)
WEIGHTED REGRESSION 16:18 Friday, July 17, 1998

Non-Linear Least Squares Iterative Phase

Iter	Dependent Variable	COUNT	Method: Gauss-Newton	Weighted SS
0	LOG_EC50	496.000000		572.317859
1	SIGMA	0.344760		515.956214
2	LOG_EC50	0.410055		314.546284
3	SIGMA	0.392816		309.048770
4	LOG_EC50	0.398228		310.438554
5	SIGMA	0.395381		310.109696
6	LOG_EC50	0.395582		310.188241
7	SIGMA	0.395534		310.189525
8	LOG_EC50	0.395545		310.173987
9	SIGMA	0.395542		310.172923
10	LOG_EC50	0.395543		310.173177
11	SIGMA	0.395543		310.173116
12	LOG_EC50	0.395543		310.173131
13	SIGMA	0.395543		310.173127
14	LOG_EC50	0.395543		310.173128

NOTE: Convergence criterion met.

Non-Linear Least Squares Summary Statistics

Source	DF	Weighted SS	Dependent Variable	Weighted MS
Regression	3	7363.7800000	LOG_EC50	2454.5933333
Residual	27	310.1731283	SIGMA	11.4878936
Uncorrected Total	30	7673.9531283		
(Corrected Total)	29	7479.6275425		

Parameter	Estimate	Asymptotic Std. Error	Asymptotic 95 % Confidence Interval
LOG_EC50	0.2805740	0.051277507	Lower 0.17536209 Upper 0.38578596
SIGMA	0.3955430	0.039469233	Lower 0.31455942 Upper 0.47652652
CO	512.4271887	27.053857496	Lower 456.91768969 Upper 567.93668778

Asymptotic Correlation Matrix

Corr	LOG_EC50	SIGMA	CO
LOG_EC50	1		
SIGMA	-0.68519478	1	
CO	-0.663409436	0.4442508936	1

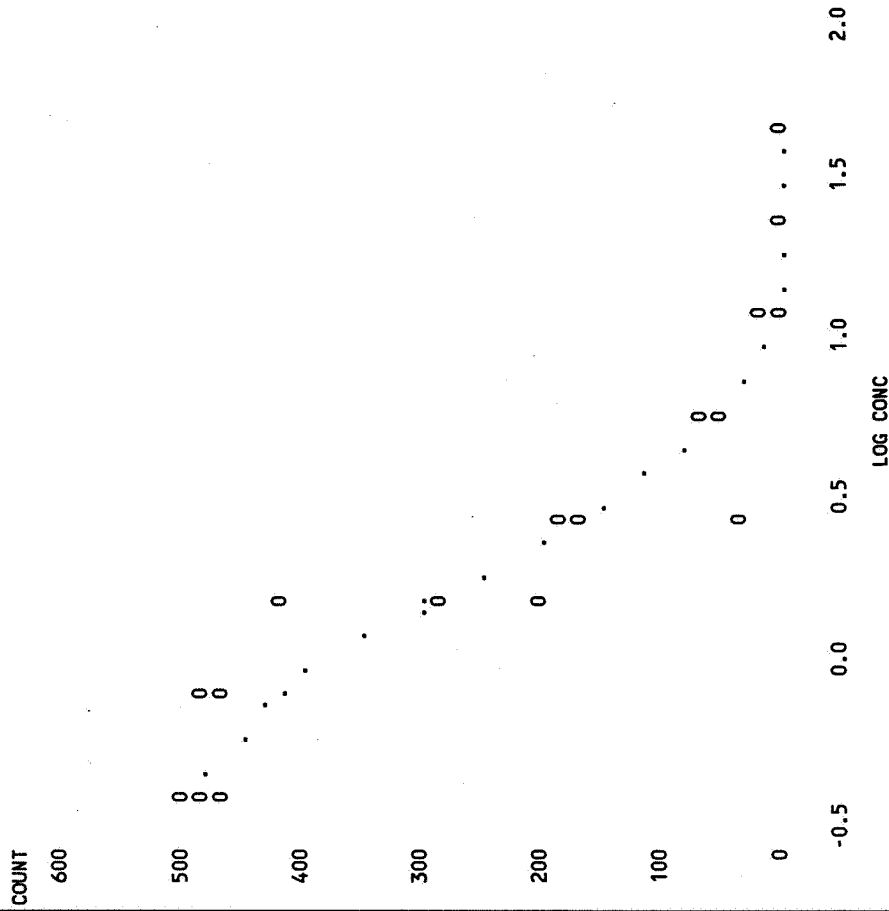
MODEL: COUNT = CO * PROBNOORM ((LOG_EC50 - LOG_CONC) / SIGMA)
SUMMARY OF NONLINEAR REGRESSION 16:18 Friday, July 17, 1998

$$EC_{50} = 1.9 (1.5 - 2.4)$$

OBS	CONC	LOG_EC50	SIGMA	CO	RESID_SS	EC50
1	0	0.28057	0.39554	512.427	310.173	1.90798

MODEL: COUNT = CO * PROBNOORM ((LOG_EC50 - LOG_CONC) / SIGMA)
ZAI296 Toxicity to Selenastrum
16:18 Friday, July 17, 1998

Plot of COUNT*LOG_CONC. Symbol used is 'O'.
Plot of PRED*LOG_CONC. Symbol used is 'O'.



NOTE: 57 obs had missing values. 57 obs hidden.
ZAI296 Toxicity to Selenastrum
COMPARISON OF MEANS FOR NOEL DETERMINATION
TEST IF TREATMENT IS LESS THAN CONTROL
16:18 Friday, July 17, 1998

General Linear Models Procedure
Class Level Information

Class	Levels	Values
DOSE	9	0 3 13 24 49 1.6 6.1 0.39 0.82

Number of observations in data set = 54

NOTE: Due to missing values, only 30 observations can be used in this analysis.

ZA1296 Toxicity to Selenastrum
COMPARISON OF MEANS FOR NOEL DETERMINATION
TEST IF TREATMENT IS LESS THAN CONTROL
16:18 Friday, July 17, 1998

General Linear Models Procedure

Dependent Variable: RESPONSE					
Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	8	1398321.060	174790.133	89.01	0.0001
Error	21	41236.869	1963.660		
Corrected Total	29	1439557.929			

R-Square	C.V.	Root MSE	RESPONSE Mean
0.971354	18.05318	44.31321	245.4593

Source	DF	Type I SS	Mean Square	F Value	Pr > F
DOSE	8	1398321.060	174790.133	89.01	0.0001
Source	DF	Type III SS	Mean Square	F Value	Pr > F
DOSE	8	1398321.060	174790.133	89.01	0.0001

ZA1296 Toxicity to Selenastrum
COMPARISON OF MEANS FOR NOEL DETERMINATION
TEST IF TREATMENT IS LESS THAN CONTROL
16:18 Friday, July 17, 1998

General Linear Models Procedure

Level of DOSE	N	Mean	SD
0	6	496.16667	24.391939
3	3	128.66667	83.930527
13	3	7.640000	3.987117
24	3	3.310000	1.013854
49	3	1.610000	0.052915
1.6	3	304.000000	107.405773
6.1	3	53.366667	11.154521
0.39	3	485.666667	17.672955
0.82	3	478.000000	9.848858

ZA1296 Toxicity to Selenastrum
COMPARISON OF MEANS FOR NOEL DETERMINATION
TEST IF TREATMENT IS LESS THAN CONTROL
16:18 Friday, July 17, 1998

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= 21 MSE= 1963.66
Critical Value of Dunnett's T= 2.625

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

DOSE Comparison	Simultaneous		Difference Between Means		Simultaneous	
	Lower Confidence Limit	Upper Confidence Limit			Lower Confidence Limit	Upper Confidence Limit
0.39 - 0	-92.76	71.76	-10.50		-92.76	71.76
0.82 - 0	-100.43	64.09	-18.17		-100.43	64.09
1.6 - 0	-274.43	-109.91	-192.17	***	-274.43	-109.91
3 - 0	-449.76	-285.24	-367.50	***	-449.76	-285.24
6.1 - 0	-525.06	-360.54	-442.80	***	-525.06	-360.54
13 - 0	-570.79	-488.53	-488.53	***	-570.79	-488.53
24 - 0	-575.12	-492.86	-492.86	***	-575.12	-492.86
49 - 0	-576.82	-494.56	-494.56	***	-576.82	-494.56

ZA1296: Toxicity to Selenastrum - Algal Density
 File: 44505124 Transform: NO TRANSFORM

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	Control	6	496.167	496.167	496.167
2	0.39	3	485.667	485.667	485.667
3	0.82	3	478.000	478.000	478.000
4	1.6	3	304.000	304.000	304.000
5	3.0	3	128.667	128.667	128.667
6	6.1	3	53.367	53.367	53.367
7	13	3	7.640	7.640	7.640
8	24	3	3.310	3.310	3.310
9	49	3	1.610	1.610	1.610

ZA1296: Toxicity to Selenastrum - Algal Density
 File: 44505124 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
Control	496.167				
0.39	485.667	0.335		1.72	k= 1, v=21
0.82	478.000	0.580		1.80	k= 2, v=21
1.6	304.000	6.133	*	1.83	k= 3, v=21
3.0	128.667	11.728	*	1.84	k= 4, v=21
6.1	53.367	14.132	*	1.85	k= 5, v=21
13	7.640	15.591	*	1.85	k= 6, v=21
24	3.310	15.729	*	1.85	k= 7, v=21
49	1.610	15.783	*	1.86	k= 8, v=21

s = 44.313

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