6-13-00 2

MRID No. 449017-09

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

1. **CHEMICAL:** Mesotrione PC Code No.: 122990

2. **TEST MATERIAL:** 2-amino-4-methylsulfonylbenzoic acid (AMBA)
A metabolite of mesotrione - 99% purity

3. **CITATION:**

Authors:	D.V. Smyth, S.E. Magor, and N. Shillabeer
Title:	R044276 (AMBA): Toxicity to the Green Alga *Selenastrum capricornutum*
Study Completion Date:	April 9, 1998
Laboratory:	Brixham Environmental Laboratory, Devon, England
Sponsor:	ZENECA Ag Products, Wilmington, DE
Laboratory Report ID:	BL6354/B
MRID No.:	449017-09
DP Barcode:	D259964

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

Signature: [signature] **Date:** 12/21/99

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

Signature: [signature] **Date:** 12/21/99

5. **APPROVED BY:** James Goodyear, USEPA

Signature: [signature] **Date:** 6/13/00

6. **STUDY PARAMETERS:**

Definitive Test Duration: 72 hours
Type of Concentrations: Nominal

7. **CONCLUSIONS:** This study is scientifically sound but does not fulfill the guideline requirements for an algal toxicity test using *Selenastrum capricornutum*.

Results Synopsis

EC_{50}: 8.9 mg/L 95% C.I.: 5.6 - 14 mg/L
Probit Slope: N/A NOEC: 3.7 mg/L

8. **ADEQUACY OF THE STUDY:**

A. Classification: Supplemental.

B. Rationale: The test was only conducted for 72 hours.

C. Repairability: No.

9. **GUIDELINE DEVIATIONS:** The test length (72 hours) was less then recommended (96 hours).

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	Freshwater algal medium

B. Test System

Guideline Criteria	Reported Information
Solvent	None
Temperature Skeletonema: 20°C Others: 24-25°C	24 ±1°C
Light Intensity Anabaena: 2.0 KLux (±15%) Others: 4.0-5.0 KLux (±15%)	8.0 KLux
Photoperiod Skeletonema: 14 h light, 10 h dark or 16 h light, 8 h dark Others: Continuous	Continuous lighting

Guideline Criteria	Reported Information
pH Skeletonema: approx. 8.0 Others: approx. 7.5	 Initial: 3.7 - 7.5 Final: 3.7 - 9.0

C. Test Design

Guideline Criteria	Reported Information
Dose range 2X or 3X progression	 2.2X
Doses at least 5	0.73, 1.6, 3.5, 7.7, 17, 37, 82, and 180 mg/L
Controls negative and/or solvent	 Medium and blank controls
Replicates per dose 3 or more	Control - 6 replicates Treatments - 3 replicates
Duration of test 120 hours	 72 hours
Daily observations were made?	Yes
Method of Observations	Electronic cell counts
Maximum Labeled Rate	N/A

12. **REPORTED RESULTS:**

Guideline Criteria	Reported Information
Initial and terminal cell densities were measured?	 Yes
Control cell count at termination ≥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

Measured Concentrations

Toxicant Concentration (mg/L)				
Nominal	0 hour	72 hour	Mean	Percent of Nominal
Control	<LOD	<LOD	<LOD	N/A
0.73	0.75	0.75	0.75	103
1.6	1.6	1.6	1.6	100
3.5	3.7	3.8	3.8	109
7.7	7.9	8.0	8.0	104
17	17	17	17	100
37	37	38	38	103
82	82	81	82	100
180	180	180	180	100

Note: Limit of determination (LOD) = 0.044 mg/L

Dose Response

Initial Measured Concentration (mg/L)	72-hr. Average Cell Density (x 10^4 cells/mL)	Inhibition (%)	Final pH
Control	232	N/A	8.4-9.0
0.75	251	-8*	9.0
1.6	219	6	8.1
3.7	189	19	7.6
7.9	144	38	7.3-7.4
17	41	82	6.8
37	29	88	6.3
82	1	100	4.2
180	0	100	3.7

*Negative value indicates growth stimulation.

Other Significant Results: No signs of toxicity were reported.

Statistical Results

Statistical Methods: Results were based on both area under the growth curve and growth rate, using nominal concentrations. Probit analysis was used for EC_{50} estimation and Dunnett's test was used for NOEC determination. The more sensitive response was that of area under the growth curve (results reported below).

EC_{50}: 9.4 mg/L	95% C.I.: 5.3 - 16 mg/L
Probit Slope: not reported	NOEC: 7.7 mg/L

13. VERIFICATION OF STATISTICAL RESULTS:

Statistical Method: Nonlinear regression was used for EC_{50} estimation and Williams' test was used for NOEC determination. The analyses were based on cell density and initial measured concentrations.

EC_{50}: 8.9 mg/L	95% C.I.: 5.6 - 14 mg/L
Probit Slope: not reported	NOEC: 3.7 mg/L

14. REVIEWER'S COMMENTS: This study is scientifically sound but does not fulfill the guideline requirements for an algal toxicity test since it was conducted for only 72 hours. Based on mean measured concentrations, the 72-hour EC_{50} was 8.9 mg/L. The NOEC was determined to be 3.7 mg/L. This study can be categorized as **Supplemental**.

Selenastrum cell density - 72 h
File: sel Transform: LOG BASE 10(Y)

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN ($x10^4$)	TRANSFORMED MEAN	ISOTONIZED MEAN
1	Control	6	232.167	2.362	2.374
2	0.75 ppm	3	251.000	2.398	2.374
3	1.6 ppm	3	219.000	2.331	2.331
4	3.7 ppm	3	188.667	2.265	2.265
5	7.9 ppm	3	144.367	2.120	2.120
6	17 ppm	3	40.967	1.606	1.606
7	37 ppm	3	28.833	1.460	1.460
8	82 ppm	3	0.900	-0.077	-0.077
9	180 ppm	3	0.200	-0.741	-0.741

Selenastrum cell density - 72 h
File: sel 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
Control	2.374				
0.75 ppm	2.374	0.123		1.72	k= 1, v=21
1.6 ppm	2.331	0.315		1.80	k= 2, v=21
3.7 ppm	2.265	1.000		1.83	k= 3, v=21
7.9 ppm	2.120	2.501	*	1.84	k= 4, v=21
17 ppm	1.606	7.812	*	1.85	k= 5, v=21
37 ppm	1.460	9.325	*	1.85	k= 6, v=21
82 ppm	-0.077	25.213	*	1.85	k= 7, v=21
180 ppm	-0.741	32.075	*	1.86	k= 8, v=21

s = 0.137
Note: df used for table values are approximate when v > 20.

selenastrum cell density 1
09:02 Tuesday, December 21, 1999

OBS	CONC	LOG_CONC	Y1	Y2	Y3	Y4	Y5	Y6
1	0.0	.	184.0	219.0	228.0	268	277	217
2	1.6	0.20412	231.0	161.0	265.0	.	.	.
3	3.7	0.56820	150.0	167.0	249.0	.	.	.
4	7.9	0.89763	128.0	226.0	79.1	.	.	.
5	17.0	1.23045	50.2	39.5	33.2	.	.	.
6	37.0	1.56820	29.7	28.2	28.6	.	.	.

selenastrum cell density 2
MODEL: COUNT = CO * PROBNORM ((LOG_EC50 - LOG_CONC) / SIGMA)
WEIGHTED REGRESSION
09:02 Tuesday, December 21, 1999

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

Iter	LOG_EC50	SIGMA	CO	Weighted SS
0	0.957000	0.456000	232.200000	210.892275
1	0.946863	0.464543	234.028232	211.602017
2	0.947746	0.463500	233.905909	211.504539
3	0.947645	0.463610	233.920375	211.515358
4	0.947656	0.463598	233.918820	211.514196
5	0.947655	0.463599	233.918988	211.514322
6	0.947655	0.463599	233.918969	211.514308
7	0.947655	0.463599	233.918971	211.514310

NOTE: Convergence criterion met.

Non-Linear Least Squares Summary Statistics Dependent Variable COUNT

Source	DF	Weighted SS	Weighted MS
Regression	3	3258.5000000	1086.1666667
Residual	18	211.5143097	11.7507950
Uncorrected Total	21	3470.0143097	
(Corrected Total)	20	1760.2982645	

Parameter	Estimate	Asymptotic Std. Error	Asymptotic 95 % Confidence Interval Lower	Upper
LOG_EC50	0.9476549	0.093650814	0.75090309	1.14440661
SIGMA	0.4635992	0.091574804	0.27120895	0.65598946
CO	233.9189714	19.354497489	193.25694976	274.58099311

Asymptotic Correlation Matrix

Corr	LOG_EC50	SIGMA	CO
LOG_EC50	1	-0.671526021	-0.687805129
SIGMA	-0.671526021	1	0.4712360804
CO	-0.687805129	0.4712360804	1

selenastrum cell density 3
MODEL: COUNT = CO * PROBNORM ((LOG_EC50 - LOG_CONC) / SIGMA)
SUMMARY OF NONLINEAR REGRESSION
09:02 Tuesday, December 21, 1999

OBS	CONC	LOG_EC50	SIGMA	CO	RESID_SS	EC50
1	0	0.94765	0.46360	233.919	211.514	8.86451

selenastrum cell density 4
MODEL: YOUNG = CO * PROBNORM ((LOG_EC25 - LOG_CONC) / SIGMA - 0.67449)
WEIGHTED REGRESSION
09:02 Tuesday, December 21, 1999

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

Iter	LOG_EC25	SIGMA	CO	Weighted SS
0	0.653000	0.456000	232.200000	209.989431
1	0.633306	0.464734	234.041291	211.611299
2	0.635149	0.463482	233.903551	211.502764
3	0.634942	0.463612	233.920628	211.515547
4	0.634964	0.463598	233.918792	211.514176
5	0.634962	0.463599	233.918991	211.514324
6	0.634962	0.463599	233.918969	211.514308
7	0.634962	0.463599	233.918971	211.514310

NOTE: Convergence criterion met.

Non-Linear Least Squares Summary Statistics Dependent Variable COUNT

Source	DF	Weighted SS	Weighted MS
Regression	3	3258.5000000	1086.1666667
Residual	18	211.5143097	11.7507950
Uncorrected Total	21	3470.0143097	
(Corrected Total)	20	1760.2982643	

Parameter	Estimate	Asymptotic Std. Error	Asymptotic 95 % Confidence Interval Lower	Upper
LOG_EC25	0.6349618	0.142668794	0.33522779	0.93469586
SIGMA	0.4635992	0.091574804	0.27120895	0.65598946
CO	233.9189715	19.354497496	193.25694978	274.58099315

Asymptotic Correlation Matrix

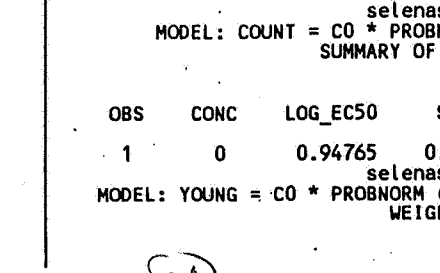
Corr	LOG_EC25	SIGMA	CO
LOG_EC25	1	-0.873738712	-0.655504346
SIGMA	-0.873738712	1	0.4712360804
CO	-0.655504346	0.4712360804	1

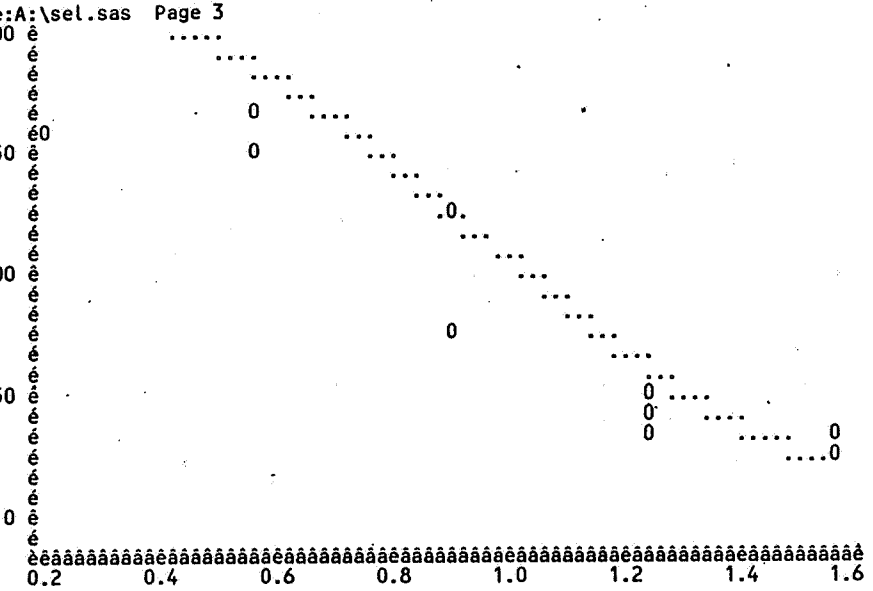
selenastrum cell density 5
MODEL: YOUNG = CO * PROBNORM ((LOG_EC25 - LOG_CONC) / SIGMA - 0.67449)
SUMMARY OF NONLINEAR REGRESSION
09:02 Tuesday, December 21, 1999

OBS	CONC	LOG_EC25	SIGMA	CO	RESID_SS	EC25
1	0	0.63496	0.46360	233.919	211.514	4.31481

selenastrum cell density 6
MODEL: YOUNG = CO * PROBNORM ((LOG_EC25 - LOG_CONC) / SIGMA - 0.67449)
09:02 Tuesday, December 21, 1999

Plot of COUNT*LOG_CONC. Symbol used is '0'.
Plot of PRED*LOG_CONC. Symbol used is '.'.





NOTE: 1392 obs had missing values. 1306 obs hidden.

General Linear Models Procedure
Class Level Information

Class	Levels	Values
DOSE	6	0 17 37 1.6 3.7 7.9

Number of observations in data set = 36

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

General Linear Models Procedure

Dependent Variable: RESPONSE

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	5	138520.0067	27704.0013	14.53	0.0001
Error	15	28604.0400	1906.9360		
Corrected Total	20	167124.0467			

R-Square	C.V.	Root MSE	RESPONSE Mean
0.828845	28.14295	43.66848	155.1667

Source	DF	Type I SS	Mean Square	F Value	Pr > F
DOSE	5	138520.0067	27704.0013	14.53	0.0001

Source	DF	Type III SS	Mean Square	F Value	Pr > F
DOSE	5	138520.0067	27704.0013	14.53	0.0001

General Linear Models Procedure

Level of DOSE	N	RESPONSE Mean	RESPONSE SD
0	6	232.166667	34.7414258
17	3	40.966667	8.5943780
37	3	28.833333	0.7767453
1.6	3	219.000000	53.0282943
3.7	3	188.666667	52.9370696
7.9	3	144.366667	74.8051023

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= 15 MSE= 1906.936
Critical Value of Dunnett's T= 2.506

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

DOSE Comparison	Simultaneous Lower Confidence Limit	Difference Between Means	Simultaneous Upper Confidence Limit	
1.6 - 0	-90.55	-13.17	64.22	
3.7 - 0	-120.89	-43.50	33.89	
7.9 - 0	-165.19	-87.80	-10.41	***
17 - 0	-268.59	-191.20	-113.81	***
37 - 0	-280.72	-203.33	-125.95	***