

MRID No. 443322-53

**DATA EVALUATION RECORD  
ALGAE OR DIATOM EC<sub>50</sub> TEST  
GUIDELINE 123-2 (TIER II)**

1. **CHEMICAL:** S-dimethenamid PC Code No.: 120051

2. **TEST MATERIAL:** SAN 1289H Technical  
91.1% as S-dimethenamid

3. **CITATION:**

Authors: James R. Hoberg  
Title: SAN 1289H Technical - Toxicity to the  
Freshwater Green Alga, *Selenastrum  
capricornutum*

Study Completion Date: January 20, 1997

Laboratory: Springborn Laboratories, Inc.,  
Wareham, MA

Sponsor: Sandoz Agro, Inc., Des Plaines, IL

Laboratory Report ID: 96-11-6778

DP Barcode: D238350, D238356

MRID No.: 443322-53

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

**Signature:** *Karl Bullock*

**Date:** 10/24/97

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

**Signature:** *P. Kosalwat*

**Date:** 10/24/97

5. **APPROVED BY:**

**Signature:** *Jocanne J. Edwards*  
*Jan A. Bailey*

**Date:** 12/3/97  
11/4/98

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. The 5-day EC<sub>50</sub> and NOEC for *S. capricornutum* exposed to SAN 1289H Technical were 0.014 and 0.0021 ppm ai, respectively.



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

**B. Test System**

Guideline Criteria	Reported Information
<b><u>Solvent</u></b>	None
<b><u>Temperature</u></b> Skeletonema: 20°C Others: 24-25°C	25°C
<b><u>Light Intensity</u></b> Anabaena: 2.0 KLux (±15%) Others: 4.0-5.0 KLux (±15%)	4.0-4.5 KLux
<b><u>Photoperiod</u></b> Skeletonema: 14 h light, 10 h dark or 16 h light, 8 h dark Others: Continuous	Continuous

Guideline Criteria	Reported Information
<b>pH</b> Skeletonema: approx. 8.0 Others: approx. 7.5	Initial: 7.3-7.6 Final: 7.7-10.0

**C. Test Design**

Guideline Criteria	Reported Information
<b>Dose range</b> 2X or 3X progression	2X
<b>Doses</b> at least 5	0.0016, 0.0030, 0.0063, 0.013, 0.025, and 0.05 mg ai/L
<b>Controls</b> negative and/or solvent	Negative control
<b>Replicates per dose</b> 3 or more	3
<b>Duration of test</b> 120 hours	120 hours
<b>Daily observations were made?</b>	Yes
<b>Method of Observations</b>	Cellular counts
<b>Maximum Labeled Rate</b>	1.25 lb ai/A (0.92 ppm ai)

**12. REPORTED RESULTS:**

Guideline Criteria	Reported Information
<b>Initial and 120 h cell densities were measured?</b>	Yes
<b>Control cell count at 120 hr <math>\geq</math>2X initial count?</b>	Yes
<b>Initial chemical concentrations measured? (Optional)</b>	Yes
<b>Raw data included?</b>	Yes

Dose Response

Mean measured concentration (mg ai/L)	Avg. Cell Density ( $\times 10^4$ cells/mL)	Inhibition (%)	Final pH
Control	243	-	9.7
0.0013	181	25	9.9
0.0021	237	2.3	9.7
0.0054	198	18	10.0
0.0096	167	31	8.4
0.021	66	73	8.0
0.044	1.8	99	7.7

Other Significant Results: Signs of toxicity were limited to the observance of bloated cells, which were reported in the two highest test concentrations. At test termination, a 3.0-mL subsample of the highest treatment level (0.044 ppm ai) was removed and diluted to 50 mL with AAP medium, resulting in a nominal concentration of 0.0030 ppm ai, to determine growth recovery. After 8 days, cell density of *Selenastrum capricornutum* increased from  $0.11 \times 10^4$  cells/mL to  $101 \times 10^4$  cells/mL, indicating that SAN 1289H technical has an algistatic, rather than algicidal effect at a mean measured concentration of 0.044 ppm ai.

Statistical Results for Cell Density:

Statistical Method: Linear regression analysis for  $EC_{50}$  and William's test for NOEC

$EC_{50}$ : 0.017 ppm ai  
Probit Slope: N/A

95% C.I.: 0.0041-0.030 ppm ai  
NOEC: 0.0021 ppm ai

**13. VERIFICATION OF STATISTICAL RESULTS:**

Statistical Method: Non-linear regression analysis for  $EC_{50}$  and Williams' test for NOEC

$EC_{50}$ : 0.014 ppm ai  
Probit Slope: N/A

95% C.I.: 0.012-0.018 ppm ai  
NOEC: 0.0021 ppm ai

- 14. REVIEWER'S COMMENTS:** This study is scientifically sound and fulfills the guideline requirements for an algal toxicity test. The 5-day  $EC_{50}$  and NOEC for *S. capricornutum* exposed to SAN 1289H Technical were 0.014 and 0.0021 ppm ai, respectively. This study can be categorized as **Core**.

KARL BULLOCK SAN 1289H SELENASTRUM 10-22-97

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CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
.044	100	99	99	0
.021	100	73	73	0
9.600001E-03		100	31	31
.0054	100	18	18	0
.0021	100	2	2	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 1.364564E-02

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS	
4	1.142894E-02	1.193326E-02	1.071443E-02	1.334166E-02

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
4	.0213391	1	5.950737E-02

SLOPE = 3.015949  
95 PERCENT CONFIDENCE LIMITS = 2.575382 AND 3.456516

LC50 = 1.214082E-02  
95 PERCENT CONFIDENCE LIMITS = .0108682 AND 1.358012E-02

LC10 = 4.604189E-03  
95 PERCENT CONFIDENCE LIMITS = 3.771288E-03 AND 5.39534E-03

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S-dimethenamid - Selenastrum capricornutum  
 File: 44332253 Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	control	3	243.000	243.000	243.000
2	0.0013	3	182.667	182.667	210.000
3	0.0021	3	237.333	237.333	210.000
4	0.0054	3	198.000	198.000	198.000
5	0.0096	3	167.000	167.000	167.000
6	0.021	3	66.000	66.000	66.000
7	0.044	3	1.867	1.867	1.867

S-dimethenamid - Selenastrum capricornutum  
 File: 44332253 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
control	243.000				
0.0013	210.000	1.564		1.76	k= 1, v=14
0.0021	210.000	1.564		1.85	k= 2, v=14
0.0054	198.000	2.133	*	1.88	k= 3, v=14
0.0096	167.000	3.603	*	1.89	k= 4, v=14
0.021	66.000	8.391	*	1.90	k= 5, v=14
0.044	1.867	11.432	*	1.91	k= 6, v=14

s = 25.834

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

S-dimethenamid - Selenastrum  
14:07 Wednesday, October 22, 1997

OBS	CONC	LOG_CONC	Y1	Y2	Y3	Y4	Y5	Y6
1	0.0000	.	229	240.0	260.0	.	.	.
2	0.0021	-2.67778	239	228.0	245.0	.	.	.
3	0.0054	-2.26761	144	237.0	213.0	.	.	.
4	0.0096	-2.01773	167	198.0	136.0	.	.	.
5	0.0210	-1.67778	77	86.0	35.0	.	.	.
6	0.0440	-1.35655	3	1.3	.	.	.	.

S-dimethenamid - Selenastrum  
MODEL: COUNT = CO \* PROBLOG ((LOG\_EC50 - LOG\_CONC) / SIGMA)  
WEIGHTED REGRESSION  
14:07 Wednesday, October 22, 1997

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

Iter	LOG_EC50	SIGMA	CO	Weighted SS
0	-1.976000	0.332000	243.000000	97.606205
1	-1.862862	0.262488	232.348132	84.538725
2	-1.850139	0.247162	229.952136	84.039383
3	-1.844638	0.241791	228.901073	83.919943
4	-1.842375	0.239654	228.470295	83.898646
5	-1.841431	0.238774	228.290179	83.894562
6	-1.841035	0.238408	228.214565	83.893695
7	-1.840869	0.238254	228.182771	83.893480
8	-1.840799	0.238189	228.169393	83.893416
9	-1.840769	0.238162	228.163762	83.893394
10	-1.840757	0.238151	228.161392	83.893385
11	-1.840751	0.238146	228.160394	83.893382
12	-1.840749	0.238144	228.159974	83.893381
13	-1.840748	0.238143	228.159798	83.893380

NOTE: Convergence criterion met.

Non-Linear Least Squares Summary Statistics Dependent Variable COUNT

Source	DF	Weighted SS	Weighted MS
Regression	3	2739.600000	913.200000
Residual	15	83.893380	5.592892
Uncorrected Total	18	2823.493380	
(Corrected Total)	17	2451.3869425	

Parameter	Estimate	Asymptotic Std. Error	Asymptotic 95 % Confidence Interval	
			Lower	Upper
LOG_EC50	-1.8407483	0.041498299	-1.92919953	-1.75229714
SIGMA	0.2381431	0.033622079	0.16647960	0.30980658
CO	228.1597977	12.893684330	200.67766456	255.64193081

Asymptotic Correlation Matrix

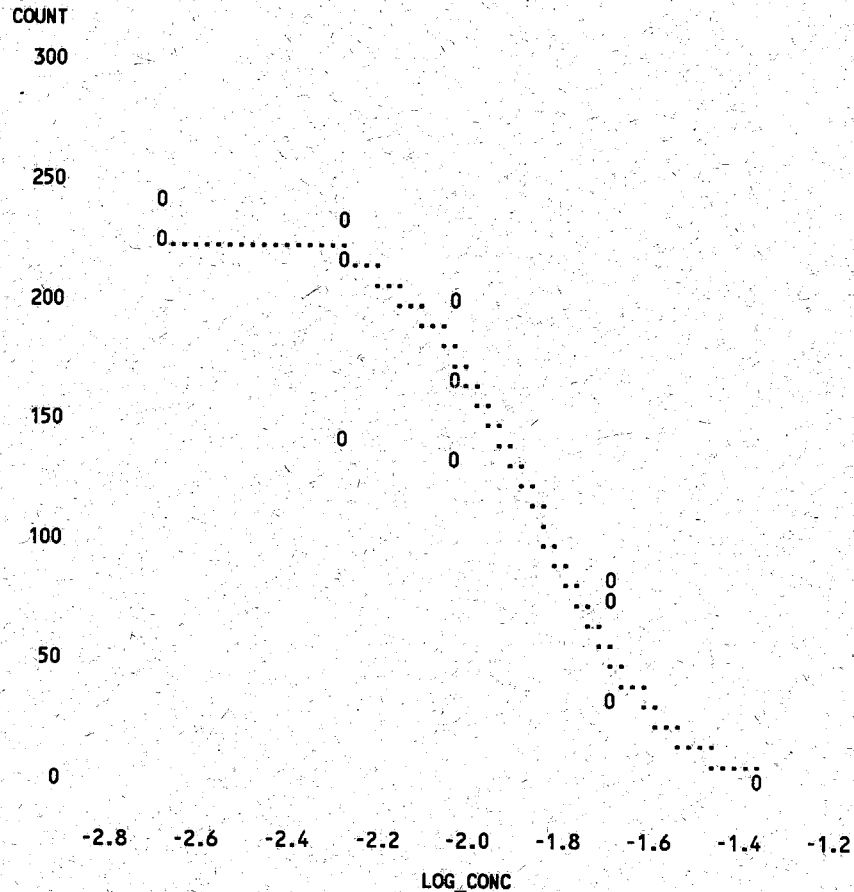
Corr	LOG_EC50	SIGMA	CO
LOG_EC50	1	-0.687232185	-0.600147134
SIGMA	-0.687232185	1	0.4297750251
CO	-0.600147134	0.4297750251	1

S-dimethenamid - Selenastrum  
MODEL: COUNT = CO \* PROBLOG ((LOG\_EC50 - LOG\_CONC) / SIGMA)  
SUMMARY OF NONLINEAR REGRESSION  
14:07 Wednesday, October 22, 1997

OBS	CONC	LOG_EC50	SIGMA	CO	RESID_SS	EC50
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1 0 -1.84075 0.23814 228.160 83.8934 0.014430  
S-dimethenamid - Selenastrum  
MODEL: COUNT = CO \* PROBLOG ((LOG\_EC50 - LOG\_CONC) / SIGMA)  
14:07 Wednesday, October 22, 1997

Plot of COUNT\*LOG\_CONC. Symbol used is 'O'.  
Plot of PRED\*LOG\_CONC. Symbol used is '.'.



NOTE: 1349 obs had missing values. 1277 obs hidden.  
S-dimethenamid - Selenastrum  
COMPARISON OF MEANS FOR NOEL DETERMINATION  
TEST IF TREATMENT IS LESS THAN CONTROL  
14:07 Wednesday, October 22, 1997

General Linear Models Procedure  
Class Level Information

Class	Levels	Values
DOSE	6	0 0.021 0.044 0.0021 0.0054 0.0096

Number of observations in data set = 36

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

S-dimethenamid - Selenastrum 6  
 COMPARISON OF MEANS FOR NOEL DETERMINATION  
 TEST IF TREATMENT IS LESS THAN CONTROL  
 14:07 Wednesday, October 22, 1997

General Linear Models Procedure

Dependent Variable: RESPONSE

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	5	143518.6667	28703.7333	39.54	0.0001
Error	12	8710.5933	725.8828		
Corrected Total	17	152229.2600			

R-Square            C.V.            Root MSE            RESPONSE Mean  
 0.942780            17.70185            26.94221            152.2000

Source	DF	Type I SS	Mean Square	F Value	Pr > F
DOSE	5	143518.6667	28703.7333	39.54	0.0001
Source	DF	Type III SS	Mean Square	F Value	Pr > F
DOSE	5	143518.6667	28703.7333	39.54	0.0001

S-dimethenamid - Selenastrum 7  
 COMPARISON OF MEANS FOR NOEL DETERMINATION  
 TEST IF TREATMENT IS LESS THAN CONTROL  
 14:07 Wednesday, October 22, 1997

General Linear Models Procedure

Level of DOSE	N	Mean	SD
0	3	243.000000	15.7162336
0.021	3	66.000000	27.2213152
0.044	3	1.866667	0.9814955
0.021	3	237.333333	8.6216781
0.0054	3	198.000000	48.2804308
0.0096	3	167.000000	31.0000000

S-dimethenamid - Selenastrum 8  
 COMPARISON OF MEANS FOR NOEL DETERMINATION  
 TEST IF TREATMENT IS LESS THAN CONTROL  
 14:07 Wednesday, October 22, 1997

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= 725.8828  
 Critical Value of Dunnett's T= 2.502  
 Minimum Significant Difference= 55.043

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

DOSE Comparison	Simultaneous Lower Confidence Limit	Difference Between Means	Simultaneous Upper Confidence Limit
0.0021 - 0	-60.71	-5.67	49.38
0.0054 - 0	-100.04	-45.00	10.04
0.0096 - 0	-131.04	-76.00	-20.96 ***
0.021 - 0	-232.04	-177.00	-121.96 ***
0.044 - 0	-296.18	-241.13	-186.09 ***