DATA EVALUATION RECORD
ALGAE OR DIATOM EC_{50} 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 Blue-green Alga, *Anabaena flos-aquae*
Study Completion Date: January 20, 1997
Laboratory: Springborn Laboratories, Inc., Wareham, MA
Sponsor: Sandoz Agro, Inc., Des Plaines, IL
Laboratory Report ID: 96-12-6798
DP Barcode: D238350, D238356
MRID No.: 443322-55

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: Joanne L. Edwards for A. B. 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 a freshwater blue-green alga toxicity test. The 5-day EC_{50} and NOEC for *Anabaena flos-aquae* exposed to SAN 1289H Technical were 0.37 and 0.028 ppm ai, respectively.

4.5

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

B. Test System

Guideline Criteria	Reported Information
Solvent	None
Temperature Skeletonema: 20°C Others: 24-25°C	25°C
Light Intensity Anabaena: 2.0 KLux (±15%) Others: 4.0-5.0 KLux (±15%)	2.2-2.4 KLux

Guideline Criteria	Reported Information
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	Initial: 7.3-7.5 Final: 8.3-8.9

C. **Test Design**

Guideline Criteria	Reported Information
Dose range 2X or 3X progression	2X
Doses at least 5	0.031, 0.065, 0.13, 0.25, 0.50, and 1.0 mg ai/L
Controls negative and/or solvent	Negative 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	1.25 lb ai/A (0.92 ppm ai)

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
Raw data included?	Yes

Dose Response

Mean measured concentration (mg ai/L)	Avg. Cell Density (x 10^4 cells/mL)	Inhibition (%)	Final pH
Control	94	-	8.9
0.028	93	1.5	8.8
0.049	85	11	8.8
0.11	78	17	8.8
0.26	58	38	8.5
0.41	40	58	8.5
0.86	31	68	8.3

Other Significant Results: None.

Statistical Results for Cell Density:

Statistical Method: Linear regression analysis for EC_{50} and Williams' test for NOEC

EC_{50}: 0.38 ppm ai 95% C.I.: 0.18-0.83 ppm ai
Probit Slope: N/A NOEC: 0.028 ppm ai

13. **VERIFICATION OF STATISTICAL RESULTS:**

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

EC_{50}: 0.37 ppm ai	95% C.I.: 0.30-0.47 ppm ai
Probit Slope: N/A	NOEC: 0.028 ppm ai

14. **REVIEWER'S COMMENTS:** This study is scientifically sound and fulfills the guideline requirements for a toxicity test with a freshwater blue-green alga. The 5-day EC_{50} and NOEC for *Anabaena flos-aquae* exposed to SAN 1289H Technical were 0.37 and 0.028 ppm ai, respectively. This study can be categorized as **Core**.

KARL BULLOCK SAN 1289H ANABAENA FLOS-AQUAE 10-23-97

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CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
.86	100	68	68	0
.41	100	58	58	0
.26	100	38	38	0
.11	100	17	17	0
.049	100	11	11	0
.028	100	2	2	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 .3419129

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS	
3	9.094541E-02	.3718415	.2964575	.470002

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
3	2.722726E-02	1	.3357095

SLOPE = 1.581446

95 PERCENT CONFIDENCE LIMITS = 1.320496 AND 1.842395

LC50 = .3823605

95 PERCENT CONFIDENCE LIMITS = .3195658 AND .4707696

LC10 = 6.017422E-02

95 PERCENT CONFIDENCE LIMITS = 4.356523E-02 AND 7.711684E-02

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S-dimethenamid - Anabaena
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WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	control	3	94.667	94.667	94.667
2	0.028	3	93.333	93.333	93.333
3	0.049	3	84.333	84.333	84.333
4	0.11	3	78.333	78.333	78.333
5	0.26	3	58.333	58.333	58.333
6	0.41	3	40.000	40.000	40.000
7	0.86	3	31.000	31.000	31.000

S-dimethenamid - Anabaena
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WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
control	94.667				
0.028	93.333	0.298		1.76	k= 1, v=14
0.049	84.333	2.307	*	1.85	k= 2, v=14
0.11	78.333	3.646	*	1.88	k= 3, v=14
0.26	58.333	8.112	*	1.89	k= 4, v=14
0.41	40.000	12.204	*	1.90	k= 5, v=14
0.86	31.000	14.214	*	1.91	k= 6, v=14

s = 5.486
Note: df used for table values are approximate when v > 20.

S-dimethenamid - Anabaena 1
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OBS	CONC	LOG_CONC	Y1	Y2	Y3	Y4	Y5	Y6
1	0.000	.	87	101	96	.	.	.
2	0.028	-1.55284	103	92	85	.	.	.
3	0.049	-1.30980	88	83	82	.	.	.
4	0.110	-0.95861	78	82	75	.	.	.
5	0.260	-0.58503	63	59	53	.	.	.
6	0.410	-0.38722	37	43	40	.	.	.
7	0.860	-0.06550	32	26	35	.	.	.

S-dimethenamid - Anabaena 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	-0.41800	0.632000	94.000000	10.602389
1	-0.430912	0.693625	96.315253	9.863267
2	-0.428874	0.688593	96.156458	9.864049
3	-0.429167	0.689229	96.179373	9.863631
4	-0.429131	0.689148	96.176507	9.863683
5	-0.429135	0.689158	96.176871	9.863677
6	-0.429135	0.689157	96.176825	9.863678
7	-0.429135	0.689157	96.176831	9.863677
8	-0.429135	0.689157	96.176830	9.863677

NOTE: Convergence criterion met.

Non-Linear Least Squares Summary Statistics Dependent Variable COUNT

Source	DF	Weighted SS	Weighted MS
Regression	3	1440.0000000	480.0000000
Residual	18	9.8636774	0.5479821
Uncorrected Total	21	1449.8636774	
(Corrected Total)	20	227.0956624	

Parameter	Estimate	Asymptotic Std. Error	Asymptotic 95 % Confidence Interval Lower	Upper
LOG_EC50	-0.42913458	0.0478923198	-0.529751950	-0.32851722
SIGMA	0.68915716	0.0750659155	0.531450564	0.84686375
C0	96.17682984	3.5493113664	88.720052635	103.63360705

Asymptotic Correlation Matrix

Corr	LOG_EC50	SIGMA	C0
LOG_EC50	1	-0.550776983	-0.811256671
SIGMA	-0.550776983	1	0.6553897732
C0	-0.811256671	0.6553897732	1

S-dimethenamid - Anabaena 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	-0.42913	0.68916	96.1768	9.86368	0.37228

S-dimethenamid - Anabaena 4
MODEL: COUNT = C0 * PROBNORM ((LOG_EC50 - LOG_CONC) / SIGMA)
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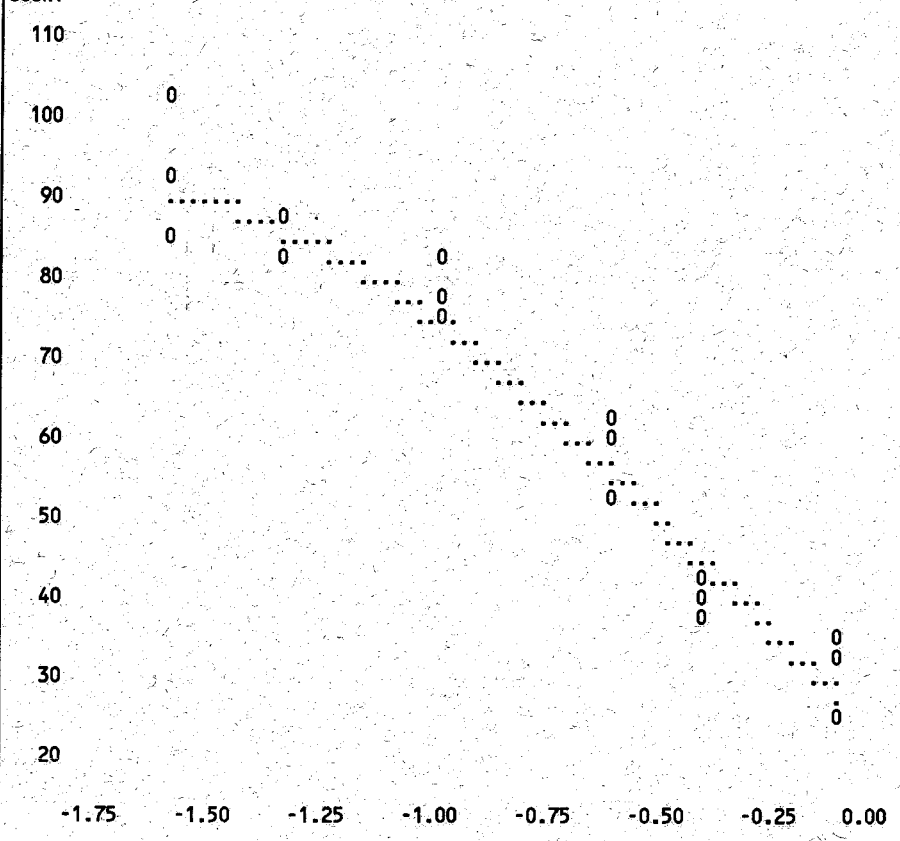
Plot of COUNT*LOG_CONC. Symbol used is '0'.
Plot of PRED*LOG_CONC. Symbol used is '.'.



NOTE: 1518 obs had missing values. 1442 obs hidden.

S-dimethenamid - Anabaena 5
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 0.11 0.26 0.41 0.86 0.028 0.049

Number of observations in data set = 42

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

S-dimethenamid - Anabaena 6
COMPARISON OF MEANS FOR NOEL DETERMINATION
TEST IF TREATMENT IS LESS THAN CONTROL
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General Linear Models Procedure

Dependent Variable: RESPONSE

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	6	11911.80952	1985.30159	65.97	0.0001
Error	14	421.33333	30.09524		
Corrected Total	20	12333.14286			

R-Square	C.V.	Root MSE	RESPONSE Mean
0.965837	8.000289	5.485913	68.57143

Source	DF	Type I SS	Mean Square	F Value	Pr > F
DOSE	6	11911.80952	1985.30159	65.97	0.0001

Source	DF	Type III SS	Mean Square	F Value	Pr > F
DOSE	6	11911.80952	1985.30159	65.97	0.0001

S-dimethenamid - Anabaena 7
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	94.6666667	7.09459888
0.11	3	78.3333333	3.51188458
0.26	3	58.3333333	5.03322296
0.41	3	40.0000000	3.00000000
0.86	3	31.0000000	4.58257569
0.028	3	93.3333333	9.07377173
0.049	3	84.3333333	3.21455025

S-dimethenamid - Anabaena 8
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= 30.09524
Critical Value of Dunnett's T= 2.532
Minimum Significant Difference= 11.342

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

DOSE Comparison	Simultaneous Lower Confidence Limit	Difference Between Means	Simultaneous Upper Confidence Limit	
0.028 - 0	-12.676	-1.333	10.009	
0.049 - 0	-21.676	-10.333	1.009	
0.11 - 0	-27.676	-16.333	-4.991	***
0.26 - 0	-47.676	-36.333	-24.991	***
0.41 - 0	-66.009	-54.667	-43.324	***
0.86 - 0	-75.009	-63.667	-52.324	***