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 Marine Diatom, *Skeletonema costatum*
Study Completion Date:	January 20, 1997
Laboratory:	Springborn Laboratories, Inc., Wareham, MA
Sponsor:	Sandoz Agro, Inc., Des Plaines, IL
Laboratory Report ID:	96-12-6815
DP Barcode:	D238350, D238356
MRID No.:	443322-56

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:** 10/27/97

5. **APPROVED BY:**

Signature: Joanne S. Edwards Jon 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 a marine diatom toxicity test. The 5-day EC_{50} and NOEC for *Skeletonema costatum* exposed to SAN 1289H Technical were determined to be 0.13 and 0.030 ppm ai, respectively.

4.5

2013097

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*	*Skeletonema costatum*
Initial Number of Cells 3,000 - 10,000 cells/mL	10,000 cells/mL
Nutrients Standard formula, e.g. 20XAAP	AES medium

B. Test System

Guideline Criteria	Reported Information
Solvent	None
Temperature Skeletonema: 20°C Others: 24-25°C	20-21°C
Light Intensity Anabaena: 2.0 KLux (±15%) Others: 4.0-5.0 KLux (±15%)	4.3-4.6 KLux

Guideline Criteria	Reported Information
Photoperiod Skeletonema: 14 h light, 10 h dark or 16 h light, 8 h dark Others: Continuous	16 h light, 8 h dark
pH Skeletonema: approx. 8.0 Others: approx. 7.5	Initial: 8.1-8.2 Final: 8.2-9.0

C. Test Design

Guideline Criteria	Reported Information
Dose range 2X or 3X progression	2X
Doses at least 5	0.013, 0.031, 0.065, 0.13, 0.25, and 0.50 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	108	-	9.0
0.013	106	2.2	8.9
0.030	103	4.6	8.9
0.048	82	24	8.6
0.11	69	36	8.4
0.22	26	76	8.4
0.45	12	89	8.2

Other Significant Results: None.

Statistical Results for Cell Density:

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

EC_{50}: 0.12 ppm ai 95% C.I.: 0.054-0.26 ppm ai
Probit Slope: N/A NOEC: 0.030 ppm ai

13. VERIFICATION OF STATISTICAL RESULTS:

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

EC_{50}: 0.13 ppm ai	95% C.I.: 0.11-0.15 ppm ai
Probit Slope: N/A	NOEC: 0.030 ppm ai

14. REVIEWER'S COMMENTS: This study is scientifically sound and fulfills the guideline requirements for a toxicity test with a marine diatom. The 5-day EC_{50} and NOEC for *Skeletonema costatum* exposed to SAN 1289H Technical were determined to be 0.13 and 0.030 ppm ai, respectively. This study can be categorized as **Core**.

KARL BULLOCK SAN 1289H SKELETONEMA 10-23-97

****

CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
.45	100	89	89	0
.22	100	76	76	0
.11	100	36	36	0
.048	100	24	24	0
.03	100	5	5	0
.013	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 .1394108

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS	
4	.019446	.1231262	.1086698	.1402441

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
3	1.822709E-02	1	5.811233E-02

SLOPE = 2.245167

95 PERCENT CONFIDENCE LIMITS = 1.942052 AND 2.548282

LC50 = .1250474

95 PERCENT CONFIDENCE LIMITS = .1098587 AND .1431876

LC10 = 3.399499E-02

95 PERCENT CONFIDENCE LIMITS = 2.713344E-02 AND 4.082368E-02

****

S-dimethenamid - Skeletonema
File: 44332256 Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	control	3	108.333	108.333	108.333
2	0.013	3	106.000	106.000	106.000
3	0.030	3	103.333	103.333	103.333
4	0.048	3	82.333	82.333	82.333
5	0.11	3	69.000	69.000	69.000
6	0.22	3	26.000	26.000	26.000
7	0.45	3	11.667	11.667	11.667

S-dimethenamid - Skeletonema
File: 44332256 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	108.333				
0.013	106.000	0.823		1.76	k= 1, v=14
0.030	103.333	1.764		1.85	k= 2, v=14
0.048	82.333	9.174	*	1.88	k= 3, v=14
0.11	69.000	13.879	*	1.89	k= 4, v=14
0.22	26.000	29.052	*	1.90	k= 5, v=14
0.45	11.667	34.109	*	1.91	k= 6, v=14

s = 3.471
Note: df used for table values are approximate when v > 20.

S-dimethenamid - Skeletonema 1
11:40 Thursday, October 23, 1997

OBS	CONC	LOG_CONC	Y1	Y2	Y3	Y4	Y5	Y6
1	0.000	.	108	106	111	.	.	.
2	0.013	-1.88606	102	109	107	.	.	.
3	0.030	-1.52288	100	103	107	.	.	.
4	0.048	-1.31876	79	86	82	.	.	.
5	0.110	-0.95861	75	68	64	.	.	.
6	0.220	-0.65758	29	24	25	.	.	.
7	0.450	-0.34679	12	13	10	.	.	.

S-dimethenamid - Skeletonema 2
MODEL: COUNT = C0 * PROBNORM ((LOG_EC50 - LOG_CONC) / SIGMA)
WEIGHTED REGRESSION
11:40 Thursday, October 23, 1997

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

Iter	LOG_EC50	SIGMA	C0	Weighted SS
0	-0.903000	0.445000	108.000000	12.340093
1	-0.891758	0.422106	107.377719	11.898078
2	-0.891522	0.421805	107.366044	11.895993
3	-0.891512	0.421792	107.365203	11.895931
4	-0.891511	0.421792	107.365167	11.895929
5	-0.891511	0.421792	107.365165	11.895928

NOTE: Convergence criterion met.

Non-Linear Least Squares Summary Statistics Dependent Variable COUNT

Source	DF	Weighted SS	Weighted MS
Regression	3	1520.0000000	506.6666667
Residual	18	11.8959285	0.6608849
Uncorrected Total	21	1531.8959285	
(Corrected Total)	20	722.9888338	

Parameter	Estimate	Asymptotic Std. Error	Asymptotic 95 % Confidence Interval Lower	Upper
LOG_EC50	-0.8915113	0.0320949560	-0.95893982	-0.82408271
SIGMA	0.4217915	0.0312675478	0.35610126	0.48748175
C0	107.3651654	3.3071291747	100.41719076	114.31314009

Asymptotic Correlation Matrix

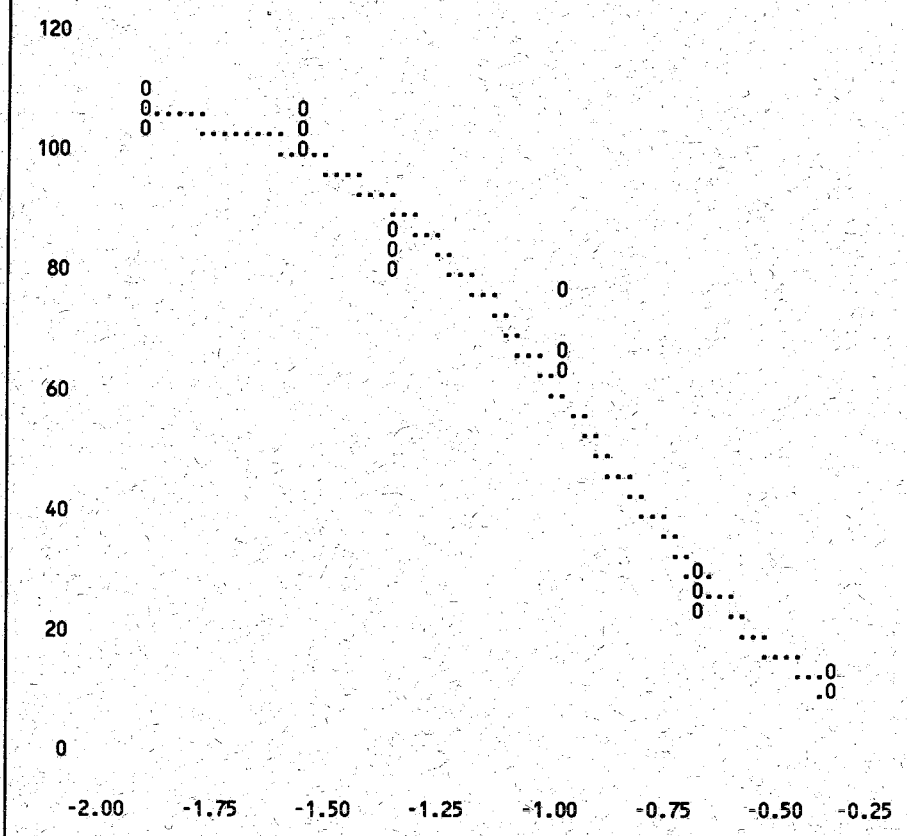
Corr	LOG_EC50	SIGMA	C0
LOG_EC50	1	-0.708563985	-0.734200978
SIGMA	-0.708563985	1	0.5568570191
C0	-0.734200978	0.5568570191	1

S-dimethenamid - Skeletonema 3
MODEL: COUNT = C0 * PROBNORM ((LOG_EC50 - LOG_CONC) / SIGMA)
SUMMARY OF NONLINEAR REGRESSION
11:40 Thursday, October 23, 1997

OBS	CONC	LOG_EC50	SIGMA	C0	RESID_SS	EC50
1	0	-0.89151	0.42179	107.365	11.8959	0.12838

S-dimethenamid - Skeletonema 4
MODEL: COUNT = C0 * PROBNORM ((LOG_EC50 - LOG_CONC) / SIGMA)
11:40 Thursday, October 23, 1997

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



NOTE: 1570 obs had missing values. 1491 obs hidden.
S-dimethenamid - Skeletonema 5
COMPARISON OF MEANS FOR NOEL DETERMINATION
TEST IF TREATMENT IS LESS THAN CONTROL
11:40 Thursday, October 23, 1997

General Linear Models Procedure
Class Level Information

Class	Levels	Values
DOSE	7	0 0.03 0.11 0.22 0.45 0.013 0.048

Number of observations in data set = 42

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

S-dimethenamid - Skeletonema 6
COMPARISON OF MEANS FOR NOEL DETERMINATION
TEST IF TREATMENT IS LESS THAN CONTROL

11:40 Thursday, October 23, 1997

General Linear Models Procedure

Dependent Variable: RESPONSE

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	6	27986.28571	4664.38095	387.16	0.0001
Error	14	168.66667	12.04762		
Corrected Total	20	28154.95238			

R-Square	C.V.	Root MSE	RESPONSE Mean
0.994009	4.795416	3.470968	72.38095

Source	DF	Type I SS	Mean Square	F Value	Pr > F
DOSE	6	27986.28571	4664.38095	387.16	0.0001

Source	DF	Type III SS	Mean Square	F Value	Pr > F
DOSE	6	27986.28571	4664.38095	387.16	0.0001

S-dimethenamid - Skeletonema 7
COMPARISON OF MEANS FOR NOEL DETERMINATION
TEST IF TREATMENT IS LESS THAN CONTROL
11:40 Thursday, October 23, 1997

General Linear Models Procedure

Level of DOSE	N	RESPONSE Mean	RESPONSE SD
0	3	108.333333	2.51661148
0.03	3	103.333333	3.51188458
0.11	3	69.000000	5.56776436
0.22	3	26.000000	2.64575131
0.45	3	11.666667	1.52752523
0.013	3	106.000000	3.60555128
0.048	3	82.333333	3.51188458

S-dimethenamid - Skeletonema 8
COMPARISON OF MEANS FOR NOEL DETERMINATION
TEST IF TREATMENT IS LESS THAN CONTROL
11:40 Thursday, October 23, 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= 14 MSE= 12.04762
Critical Value of Dunnett's T= 2.532
Minimum Significant Difference= 7.1763

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

DOSE Comparison	Simultaneous Lower Confidence Limit	Difference Between Means	Simultaneous Upper Confidence Limit	
0.013 - 0	-9.510	-2.333	4.843	
0.03 - 0	-12.176	-5.000	2.176	
0.048 - 0	-33.176	-26.000	-18.824	***
0.11 - 0	-46.510	-39.333	-32.157	***
0.22 - 0	-89.510	-82.333	-75.157	***
0.45 - 0	-103.843	-96.667	-89.490	***