

US EPA ARCHIVE DOCUMENT

MRID No. 443322-57

**DATA EVALUATION RECORD
AQUATIC PLANT EC₅₀ TEST
GUIDELINE 123-2 (TIER II)**

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

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

3. **CITATION:**

Authors: James R. Hoberg

Title: SAN 1289H Technical - Toxicity to
Duckweed, *Lemna gibba*

Study Completion Date: January 20, 1997

Laboratory: Springborn Laboratories, Inc.,
Wareham, MA

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

Laboratory Report ID: 96-11-6787

DP Barcode: D238350, D238356

MRID No.: 443322-57

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: *James R. Hoberg*

Date: 12/14/97

6. **STUDY PARAMETERS:** *James R. Bullock*

Definitive Test Duration: 14 days

Type of Concentrations: Initial measured

7. **CONCLUSIONS:** This study is scientifically sound and fulfills the guideline requirements for an aquatic plant toxicity test. Based on frond density, the 14-day EC₅₀ and NOEC for *L. gibba* exposed to SAN 1289H were 0.013 and 0.0073 ppm ai, respectively. Based on frond biomass, the 14-day EC₅₀ and NOEC for *L. gibba* exposed to SAN 1289H were 0.0089 and 0.0012 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:

10. SUBMISSION PURPOSE:

11. MATERIALS AND METHODS:

A. Test Organisms

Guideline Criteria	Reported Information
<u>Species</u> <i>Lemna gibba</i>	<i>Lemna gibba</i>
<u>Number of Plants/Fronds</u> 5 plants, 3 fronds each	5 plants, 3 fronds each
<u>Nutrients</u> Standard formula, e.g. 20XAAP	Hoagland's medium

B. Test System

Guideline Criteria	Reported Information
<u>Solvent</u>	None
<u>Temperature</u> 25°C	24-25°C
<u>Light Intensity</u> 5.0 KLux ($\pm 15\%$)	4.5-5.4 KLux
<u>Photoperiod</u> Continuous	Continuous
<u>Test System</u> Static or Renewal	Renewal (every 3 days)
<u>pH</u> Approx. 5.0	Initial: 4.9 Final: 5.3-6.6

C. Test Design

Guideline Criteria	Reported Information
<u>Dose range</u> 2X or 3X progression	3X
<u>Doses</u> at least 5	0.0010, 0.0030, 0.0089, 0.027, 0.081 ppm ai
<u>Controls</u> negative and/or solvent	Negative control
<u>Replicates per dose</u> 3 or more	3
<u>Duration of test</u> 14 days	14 days
Daily observations were made?	Counts and observations made every two or three days
<u>Method of Observations</u>	Number of fronds
<u>Maximum Labeled Rate</u>	1.25 lb ai/A (0.92 ppm ai)

12. REPORTED RESULTS:

Guideline Criteria	Reported Information
Initial and 14 day frond numbers were measured?	Yes
Control frond at 14 days \geq 2X initial count?	Yes
Initial chemical concentrations measured? (Optional)	Yes
Raw data included?	Yes

KARL BULLOCK SAN 1289H DUCKWEED 10-23-97

CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
.074	100	96	96	0
.026	100	90	90	0
.0073	100	8	8	0
.0032	100	0	0	0
.0012	100	5	5	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 1.409405E-02

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS	
3	1.116096E-02	1.567512E-02	1.385113E-02	1.777627E-02

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
5	2.638926	48.43482	0

A PROBABILITY OF 0 MEANS THAT IT IS LESS THAN 0.001.

SINCE THE PROBABILITY IS LESS THAN 0.05, RESULTS CALCULATED USING THE PROBIT METHOD PROBABLY SHOULD NOT BE USED.

SLOPE = 2.710873
95 PERCENT CONFIDENCE LIMITS = -1.692878 AND 7.114625

LC50 = 1.391994E-02
95 PERCENT CONFIDENCE LIMITS = 0 AND +INFINITY

LC10 = 4.733186E-03
95 PERCENT CONFIDENCE LIMITS = 0 AND +INFINITY

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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	840.667	840.667	849.000
2	0.0012	3	799.333	799.333	849.000
3	0.0032	3	907.000	907.000	849.000
4	0.0073	3	772.333	772.333	772.333
5	0.026	3	86.333	86.333	86.333
6	0.074	3	34.667	34.667	34.667

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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	849.000				
0.0012	849.000	0.138		1.78	k= 1, v=12
0.0032	849.000	0.138		1.87	k= 2, v=12
0.0073	772.333	1.132		1.90	k= 3, v=12
0.026	86.333	12.498	*	1.92	k= 4, v=12
0.074	34.667	13.354	*	1.93	k= 5, v=12

s = 73.922

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

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Chi-square test for normality: actual and expected frequencies

INTERVAL	<-1.5	-1.5 to <-0.5	-0.5 to 0.5	>0.5 to 1.5	>1.5
EXPECTED	1.206	4.356	6.876	4.356	1.206
OBSERVED	0	7	5	6	0

Calculated Chi-Square goodness of fit test statistic = 5.1491

Table Chi-Square value (alpha = 0.01) = 13.277

Data PASS normality test. Continue analysis.

Dose Response

Initial measured concentration (mg ai/L)	Avg. Frond Number	% Inhibition	14-day pH
Control	841	-	6.6
0.0012	799	4.9	6.5
0.0032	907	0(-7.9)	6.5
0.0073	772	8.1	6.4
0.026	86	90	5.6
0.074	35	96	5.3

Other Significant Results: Signs of toxicity included curled fronds, chlorotic fronds, fronds with less root formation in comparison to the control, and fronds which were smaller in comparison to the control. Some or all of these signs were present in the four highest concentrations tested.

Statistical Results:

Statistical Method: Linear regression analysis for EC₅₀ and Williams' test for NOEC

Frond Density:

EC₅₀: 0.016 ppm ai
Probit Slope: N/A

95% C.I.: 0.0055-0.048 ppm ai
NOEC: 0.0073 ppm ai

Frond Biomass:

EC₅₀: 0.0089 ppm ai
Probit Slope: N/A

95% C.I.: 0.0025-0.032 ppm ai
NOEC: 0.0012 ppm ai

13. VERIFICATION OF STATISTICAL RESULTS:

Statistical Method: Non-linear regression analysis for EC₅₀ and Williams' test for NOEC using frond density

EC₅₀: 0.013 ppm ai
Probit Slope: N/A

95% C.I.: 0.009-0.018 ppm ai
NOEC: 0.0073 ppm ai

- 14. REVIEWER'S COMMENTS:** This study is scientifically sound and fulfills the guideline requirements for an aquatic plant toxicity test. Based on frond density, the 14-day EC₅₀ and NOEC for *L. gibba* exposed to SAN 1289H Technical were 0.013 and 0.0073 ppm ai, respectively. This study can be categorized as Core.

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Hartley's test for homogeneity of variance

Calculated H statistic (max Var/min Var) = 11149.75

Closest, conservative, Table H statistic = 1362.0 (alpha = 0.01)

Used for Table H ==> R (# groups) = 6, df (# reps-1) = 2
Actual values ==> R (# groups) = 6, df (# avg reps-1) = 2.00

Data FAIL homogeneity test. Try another transformation.

NOTE: This test requires equal replicate sizes. If they are unequal but do not differ greatly, Hartley's test may still be used as an approximate test (average df are used).

S-dimethenamid - Duckweed

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Chi-square test for normality: actual and expected frequencies

INTERVAL	<-1.5	-1.5 to <-0.5	-0.5 to 0.5	>0.5 to 1.5	>1.5
EXPECTED	1.206	4.356	6.876	4.356	1.206
OBSERVED	0	7	5	6	0

Calculated Chi-Square goodness of fit test statistic = 5.1491

Table Chi-Square value (alpha = 0.01) = 13.277

Data PASS normality test. Continue analysis.

S-dimethenamid - Duckweed

File: 44332257 Transform: LOG BASE 10(Y)

Hartley's test for homogeneity of variance

Calculated H statistic (max Var/min Var) = 35.86

Closest, conservative, Table H statistic = 1362.0 (alpha = 0.01)

Used for Table H ==> R (# groups) = 6, df (# reps-1) = 2
Actual values ==> R (# groups) = 6, df (# avg reps-1) = 2.00

Data PASS homogeneity test. Continue analysis.

NOTE: This test requires equal replicate sizes. If they are unequal but do not differ greatly, Hartley's test may still be used as an approximate test (average df are used).

S-dimethenamid - Duckweed

File: 44332257

Transform: LOG BASE 10(Y)

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	control	3	840.667	2.922	2.927
2	0.0012	3	799.333	2.902	2.927
3	0.0032	3	907.000	2.957	2.927
4	0.0073	3	772.333	2.884	2.884
5	0.026	3	86.333	1.931	1.931
6	0.074	3	34.667	1.540	1.540

S-dimethenamid - Duckweed

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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	2.927				
0.0012	2.927	0.107		1.78	k= 1, v=12
0.0032	2.927	0.107		1.87	k= 2, v=12
0.0073	2.884	0.880		1.90	k= 3, v=12
0.026	1.931	22.673	*	1.92	k= 4, v=12
0.074	1.540	31.611	*	1.93	k= 5, v=12

s = 0.054

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

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OBS	CONC	LOG_CONC	Y1	Y2	Y3	Y4	Y5	Y6
1	0.0000		744	948	830	.	.	.
2	0.0012	-2.92082	850	757	791	.	.	.
3	0.0032	-2.49485	920	970	831	.	.	.
4	0.0073	-2.13668	803	876	638	.	.	.
5	0.0260	-1.58503	98	68	93	.	.	.
6	0.0740	-1.13077	34	36	34	.	.	.

S-dimethenamid - Duckweed
MODEL: COUNT = CO * PROBORN ((LOG_EC50 - LOG_CONC) / SIGMA)
WEIGHTED REGRESSION
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Non-Linear Least Squares Iterative Phase

Iter	Dependent Variable	LOG_EC50	SIGMA	CO	Weighted SS
0	LOG_EC50	-1.856000	0.369000	804.000000	471.322380
1		-1.893317	0.340543	876.918555	430.174681
2		-1.893552	0.341246	878.253150	427.878901
3		-1.893121	0.340893	878.040424	428.257756
4		-1.893409	0.341132	878.182965	427.995434
5		-1.893215	0.340970	878.086912	428.171944
6		-1.893346	0.341079	878.151688	428.052825
7		-1.893258	0.341006	878.108030	428.133073
8		-1.893317	0.341055	878.137467	428.078949
9		-1.893277	0.341022	878.117624	428.115425
10		-1.893304	0.341044	878.131002	428.090829
11		-1.893286	0.341029	878.121984	428.107408
12		-1.893298	0.341040	878.128064	428.096230
13		-1.893290	0.341033	878.123965	428.103765
14		-1.893295	0.341037	878.126728	428.098685
15		-1.893292	0.341034	878.124865	428.102110
16		-1.893294	0.341036	878.126121	428.099801
17		-1.893292	0.341035	878.125275	428.101358
18		-1.893294	0.341036	878.125845	428.100308
19		-1.893293	0.341035	878.125461	428.101016
20		-1.893293	0.341036	878.125720	428.100539
21		-1.893293	0.341035	878.125545	428.100860
22		-1.893293	0.341036	878.125663	428.100644
23		-1.893293	0.341035	878.125583	428.100790
24		-1.893293	0.341035	878.125637	428.100691
25		-1.893293	0.341035	878.125601	428.100758
26		-1.893293	0.341035	878.125625	428.100713
27		-1.893293	0.341035	878.125609	428.100743
28		-1.893293	0.341035	878.125620	428.100723
29		-1.893293	0.341035	878.125612	428.100736
30		-1.893293	0.341035	878.125617	428.100727
31		-1.893293	0.341035	878.125614	428.100733
32		-1.893293	0.341035	878.125616	428.100729

NOTE: Convergence criterion met.

Non-Linear Least Squares Summary Statistics

Source	DF	Weighted SS	Weighted MS
Regression	3	10321.000000	3440.333333
Residual	15	428.100729	28.540049
Uncorrected Total	18	10749.100729	
(Corrected Total)	17	8965.892867	

S-dimethenamid - Duckweed
MODEL: COUNT = CO * PROBORN ((LOG_EC50 - LOG_CONC) / SIGMA)
WEIGHTED REGRESSION
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Parameter	Estimate	Asymptotic Std. Error	Asymptotic 95 % Confidence Interval	
			Lower	Upper
LOG_EC50	-1.8932931	0.073037107	-2.04896741	-1.7376188
SIGMA	0.3410354	0.055675766	0.22236580	0.4597051
CO	878.1256164	58.084496180	754.32191408	1001.9293187

Asymptotic Correlation Matrix

Corr	LOG_EC50	SIGMA	CO
LOG_EC50	1	-0.711312842	-0.605846613
SIGMA	-0.711312842	1	0.4524644872
CO	-0.605846613	0.4524644872	1

S-dimethenamid - Duckweed
MODEL: COUNT = CO * PROBORN ((LOG_EC50 - LOG_CONC) / SIGMA)

SUMMARY OF NONLINEAR REGRESSION

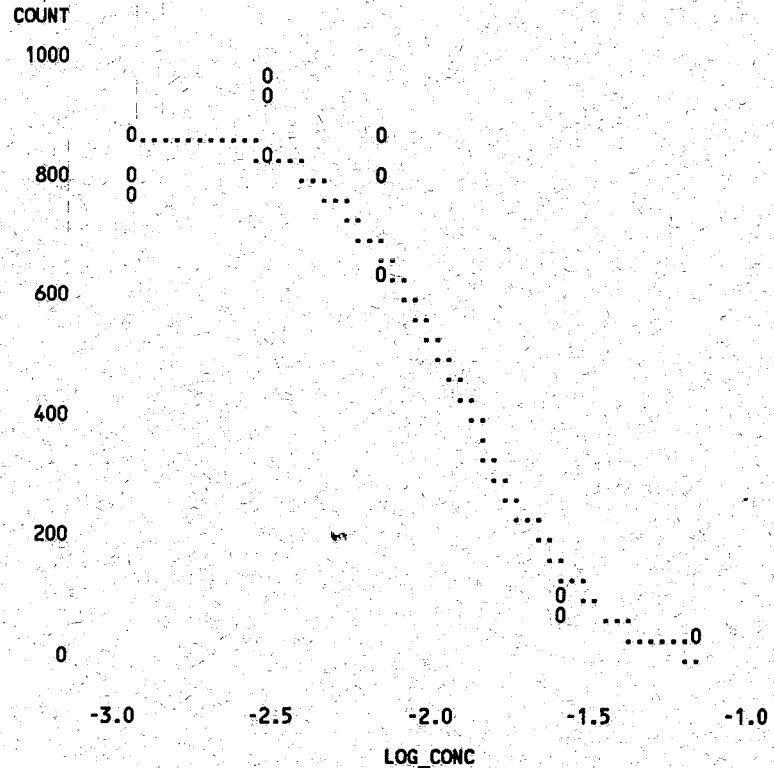
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OBS	CONC	LOG_EC50	SIGMA	CO	RESID_SS	EC50
1	0	-1.89329	0.34104	878.126	428.101	0.012785

S-dimethenamid - Duckweed
MODEL: COUNT = CO * PROBORN ((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: 1818 obs had missing values. 1750 obs hidden.

S-dimethenamid - Duckweed
 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	6	0 0.026 0.074 0.0012 0.0032 0.0073

Number of observations in data set = 36

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

S-dimethenamid - Duckweed
 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	5	2402424.944	480484.989	87.93	0.0001
Error	12	65573.333	5464.444		
Corrected Total	17	2467998.278			

R-Square	C.V.	Root MSE	RESPONSE Mean
0.973431	12.89210	73.92188	573.3889

Source	DF	Type I SS	Mean Square	F Value	Pr > F
DOSE	5	2402424.944	480484.989	87.93	0.0001
Source	DF	Type III SS	Mean Square	F Value	Pr > F
DOSE	5	2402424.944	480484.989	87.93	0.0001

S-dimethenamid - Duckweed
 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	Mean	SD
0	3	840.666667	102.417446
0.026	3	86.333333	16.072751
0.074	3	34.666667	1.154701
0.0012	3	799.333333	47.056703
0.0032	3	907.000000	70.405966
0.0073	3	772.333333	121.927574

S-dimethenamid - Duckweed

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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= 12 MSE= 5464.444
 Critical Value of Dunnett's T= 2.502
 Minimum Significant Difference= 151.02

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

DOSE Comparison	Simultaneous Lower Confidence Limit	Difference Between Means	Simultaneous Upper Confidence Limit	
0.0032 - 0	-84.69	66.33	217.36	
0.0012 - 0	-192.36	-41.33	109.69	
0.0073 - 0	-219.36	-68.33	82.69	
0.026 - 0	-905.36	-754.33	-603.31	***
0.074 - 0	-957.02	-806.00	-654.98	***