4-6-98
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DATA EVALUATION RECORD
ALGAE OR DIATOM EC_{50} TEST
GUIDELINE 122-2 OR 123-2 (TIER I OR II)

1. **CHEMICAL: SAN 836H** PC Code No.: 005107

2. **TEST MATERIAL: SAN 836H** Purity: 99.47%

3. **CITATION**

Authors: Hoberg, James
Title: SAN 836H - Toxicity To The Marine Diatom, *Skeletonema costatum*
Study Completion Date: 23 to 28 March 1995
Laboratory: Springborn Laboratories, Inc.
Sponsor: Sandoz Agro, Inc.
Laboratory Report ID: 95-4-5817
DP Barcode: D238406 kw
MRID No.: 443074-26

4. **REVIEWED BY:** Fred Jenkins, Aquatic Biologist, ERBII, EFED

Signature: Fred Jenkins **Date:** 4/6/98

5. **APPROVED BY:** Mike Davy, Agronomist, ERBII, EFED

Signature: Michael Davy **Date:** 4-6-98

6. **STUDY PARAMETERS**

Scientific Name of Test Organism: *Skeletonema costatum*
Definitive Test Duration: 120 hours
Type of Concentrations: Mean measured

7. **CONCLUSIONS:**

Results Synopsis

EC_{50}: 0.12 ppm A.E. 95% C.I.: 0.09-0.16 ppm A.E.
NOEL: 0.0064 ppm A.E. Slope: ~~N/A~~ 1.39

8. **ADEQUACY OF THE STUDY**

A. Classification: Core

B. Rationale: The study met guidelines of *The Hazard Evaluation Division Standard Evaluation Procedure for Non-Target Plants: Growth and Reproduction of Aquatic Plants-Tiers 1 and 2* (SEP).

C. Repairability: N/A

9. **GUIDELINE DEVIATIONS**

N/A

10. **SUBMISSION PURPOSE:**
Section 3 Registration.

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	10000 cells/ml
Nutrients Standard formula, e.g. 20XAAP	Artificially Enriched Seawater prepared with sterile, filtered natural seawater.

B. Test System

Guideline Criteria	Reported Information
Solvent	N/A
Temperature Skeletonema: 20°C Others: 24-25°C	20±°C

Guideline Criteria	Reported Information
Light Intensity Anabaena: 2.2 K lux (±15%) Others: 4.3 K lux (±15%)	4.3-5.4 K Lux
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	Test initiation pH: 8.1 Throughout test range: 8.5 to 9.7.

C. Test Design

Guideline Criteria	Reported Information
Dose range 2X or 3X progression	2X progression
Doses at least 5	Mean measured (ppm A.E.): 0.0064, 0.013, 0.024, 0.043, 0.092, and 0.19 Nominal concentrations (ppm A.E.): 0.0063, 0.013, 0.025, 0.050, 0.10 and 0.20
Controls negative and/or solvent	negative
Replicates per dose 3 or more (4 or more for Navicula)	3
Duration of test 120 hours	120 hours
Daily observations were made?	Yes
Method of Observations	Cellular counts
Maximum Labeled Rate	0.20 lb A.E./acre

12. REPORTED RESULTS

Guideline Criteria	Reported Information
Quality assurance and GLP compliance statements were included in the report?	Yes
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 Dose[1] (ppm A.E.)	Cell Density (x 10^4 cells/ml)	% Inhibition	120-Hour pH
Control	106	N/A	9.2
0.0064	104	2	9.1
0.013	98	8	8.8
0.024	87	18	8.7
0.043	76	28	8.5
0.092	56	47	8.5
0.18	49	54	8.5

[1]The nominal concentrations were as follows (ppm A.E.): 0.0063, 0.013, 0.025, 0.050, 0.10, and 0.20. The mean measured test concentrations ranged from 87 to 100% of nominal concentration.

Other Significant Results:

Statistical Results

Statistical Method: The Williams test was used to determine the NOEC. The EC50 values and their 95% confidence limits were determined by linear regression of response vs. mean measured exposure concentration over the range of test concentration where a clear exposure-response relationship was observed.

EC_{50}: 0.14 ppm A.E. 95% C.I.: 0.075-0.270 ppm

Slope: N/A NOEC: 0.0064 ppm A.E.

13. Verification of Statistical Results

Statistical Method: Williams Method (For NOEC); Probit Method (For EC_{50})

EC_{50}: 0.12 ppm 95% C.I.: 0.098-0.16 ppm

Slope: NOEC: 0.0064 ppm A.E.

14. REVIEWER'S COMMENTS:

The study was scientifically sound and follows the guidelines of the SEP.

Table 4. **Cell densities (x 10^4 cells/mL) of *Skeletonema costatum* after 1, 2, 3, 4 and 5 days of exposure to SAN 836H**

Mean Measured Concentration (mg A.E./L)		Cell Density (x 10^4 cells/mL) Day 1	Day 2	Day 3	Day 4	Day 5	Day 5 Percent Inhibition
Control	A	1	5	47	70	103	
	B	1	5	48	71	111	
	C	1	5	46	71	103	
	Mean(SD)[a]	1(<1)	5(<1)	47(1)	71(1)	106(5)	NA[b]
0.0064	A	1	5	46	70	102	
	B	<1	4	42	76	103	
	C	1	4	47	74	106	
	Mean(SD)[a]	1(<1)	4(1)	45(2)	73(3)	104(2)	2.0
0.013	A	1	4	38	76	101	
	B	1	4	42	69	96	
	C	1	4	40	59	96	
	Mean(SD)[a]	1(<1)	4(<1)	40(2)	68(9)	98(3)*	7.8
0.024	A	1	3	36	57	89	
	B	1	2	37	51	79	
	C	1	3	35	59	93	
	Mean(SD)[a]	1(<1)	3(1)	36(1)	55(4)	87(7)*	18
0.043	A	<1	2	24	49	80	
	B	1	3	24	56	71	
	C	1	4	25	45	77	
	Mean(SD)[a]	<1(<1)	3(1)	24(1)	50(6)	76(5)*	28
0.092	A	1	2	16	48	50	
	B	1	2	15	41	58	
	C	1	3	13	46	59	
	Mean(SD)[a]	1(0)	2(<1)	15(2)[c]	45(3)[c]	56(5)[cde]*	47
0.18	A	0	1	12	43	49	
	B	<1	1	13	47	46	
	C	0	1	12	36	51	
	Mean(SD)[a]	<1(<1)[c]	1(<1)[c]	12(1)[cd]	42(6)[cd]	49(2)[cde]*	54

[a] Mean and standard deviation (SD) were calculated from original raw data (Appendix V), not from the rounded values presented in this table.
[b] NA = Not applicable.
[c] Cell fragments were observed.
[d] Thin cell walls were observed.
* Significantly ($p \leq 0.05$) reduced as compared to the control, based on Williams' Test.

Diatom 836h toxicity
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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	105.667	105.667	105.667
2	0.0064	3	103.667	103.667	103.667
3	0.013	3	97.667	97.667	97.667
4	0.024	3	87.000	87.000	87.000
5	0.043	3	76.000	76.000	76.000
6	0.092	3	55.667	55.667	55.667
7	0.18	3	48.667	48.667	48.667

Diatom 836h toxicity
File: dia836 Transform: NO TRANSFORM

WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG	TABLE P=.05 WILLIAMS	DEGREES OF FREEDOM
control	105.667				
0.0064	103.667	0.552		1.76	k= 1, v=14
0.013	97.667	2.209	*	1.85	k= 2, v=14
0.024	87.000	5.155	*	1.88	k= 3, v=14
0.043	76.000	8.193	*	1.89	k= 4, v=14
0.092	55.667	13.809	*	1.90	k= 5, v=14
0.18	48.667	15.742	*	1.91	k= 6, v=14

s = 4.435
Note: df used for table values are approximate when v > 20.

Fred Jenkins SAN 836H Diatom toxicity

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CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
.18	100	54	54	0
.092	100	47	47	0
.043	100	28	28	0
.024	100	18	18	0
.013	100	8	8	0
.0064	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 .1226527

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS
2	.2720809	.1252815	9.066341E-02 .2217411

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
4	3.676159E-02	1	.5565916

SLOPE = 1.394697

95 PERCENT CONFIDENCE LIMITS = 1.127287 AND 1.662106

LC50 = .1234262

95 PERCENT CONFIDENCE LIMITS = 9.761587E-02 AND .1672991

LC10 = 1.516404E-02

95 PERCENT CONFIDENCE LIMITS = 1.064229E-02 AND 1.973739E-02

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Fred Jenkins SAN 836H DIATOM TOXICITY

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CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
.18	100	54	54	0
.092	100	47	47	0
.043	100	28	28	0
.024	100	18	18	0
.013	100	8	8	0
.0064	100	2	2	0

THE BINOMIAL TEST SHOWS THAT .092 AND .18 CAN BE USED AS STATISTICALLY SOUND CONSERVATIVE 95 PERCENT CONFIDENCE LIMITS, BECAUSE THE ACTUAL CONFIDENCE LEVEL ASSOCIATED WITH THESE LIMITS IS GREATER THAN 95 PERCENT.

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AN APPROXIMATE LC50 FOR THIS SET OF DATA IS .1226527