

4-6-98

DATA EVALUATION RECORD
ALGAE OR DIATOM EC₅₀ 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, Robert

Title: SAN 836H-Toxicity to the Freshwater Blue-Green ALGA, *Anabaena flos-aquae*

Study Completion Date: February 22, 1995

Laboratory: Springborn Laboratories, Inc

Sponsor: Sandoz Agro, Inc.

Laboratory Report ID: 95-4-5776

DP Barcode: D238406

MRID No.: 443074-23

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: *Anabaena flos-aquae*

Definitive Test Duration: 120 hours

Type of Concentrations: Mean Measured

7. CONCLUSIONS:

Results Synopsis

EC₅₀: 0.15 ppm A.E.

95% C.I.: 0.12-0.20 ppm A.E.

NOEL: 0.014 ppm A.E.

Slope: N/A

8. ADEQUACY OF THE STUDY

A. Classification: Core.

B. Rationale: The study met guidelines.

C. Repairability: N/A

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9. GUIDELINE DEVIATIONS

N/A

10. SUBMISSION PURPOSE:

Section 3. Registration

11. MATERIALS AND METHODS

A. Test Organisms

Guideline Criteria	Reported Information
<u>Species</u> <i>Skeletonema costatum</i> <i>Anabaena flos-aquae</i> <i>Selenastrum capricornutum</i> <i>Navicula pelliculosa</i>	<i>Anabaena flos-aquae</i>
<u>Initial Number of Cells</u> 3,000 - 10,000 cells/ml	10,000 cells/ml
<u>Nutrients</u> Standard formula, e.g. 20XAAP	Algal Assay Medium prepared with sterile, deionized water.

B. Test System

Guideline Criteria	Reported Information
<u>Solvent</u>	N/A
<u>Temperature</u> <i>Skeletonema</i> : 20°C Others: 24-25°C	24±°C
<u>Light Intensity</u> <i>Anabaena</i> : 2.2 K lux (±15%) Others: 4.3 K lux (±15%)	1.1-3.3 K Lux
<u>Photoperiod</u> <i>Skeletonema</i> : 14 h light, 10 h dark or 16 h light, 8 h dark Others: Continuous	Continuous
<u>pH</u> <i>Skeletonema</i> : approx. 8.0 Others: approx. 7.5	The pH ranged from 7.3 to 7.5 at test initiation and from 8.0 to 9.4 at test termination.

C. Test Design

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Guideline Criteria	Reported Information
<u>Dose range</u> 2X or 3X progression	2X progression
<u>Doses</u> at least 5	Mean measured (ppm A.E.): Negative control, 0.0065, 0.014, 0.028, 0.052, 0.099, 0.18. Nominal (ppm A.E.): Negative control, 0.0063, 0.013, 0.025, 0.050, 0.10 and 0.20.
<u>Controls</u> negative and/or solvent	negative
<u>Replicates per dose</u> 3 or more (4 or more for Navicula)	3
<u>Duration of test</u> 120 hours	120 hours
Daily observations were made?	Yes
<u>Method of Observations</u>	Cellular counts
<u>Maximum Labeled Rate</u>	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 $\geq$ 2X initial count?	Yes
Initial chemical concentrations measured? (Optional)	Yes

Guideline Criteria	Reported Information
Raw data included?	Yes

Dose Response

Mean Measured ¹ Dose (ppm A.E.)	Cell Density (x 10 ⁴ cells/ml)	% Inhibition	120-Hour pH
Control	104	N/A	9.4
0.0065	104	0.077	9.4
0.014	100	3.9	9.2
0.028	87	16	9.0
0.052	77	26	8.5
0.099	65	37	8.0
0.18	48	54	8.0

¹ The nominal concentrations were as follows (ppm A.E.): 0.0063, 0.013, 0.025, 0.050, and 0.10. The mean measured test concentrations ranged from 91% to 110% of the nominal concentrations.

Other Significant Results:Statistical Results

Statistical Method: Williams' Test (for NOEC). The EC₅₀ values were determined by linear regression of response vs. exposure concentration over the range of test concentrations where a clear exposure-response relationship was observed.

EC₅₀: 0.19 ppm A.E.

95% C.I.: 0.090-0.43 ppm A.E.

Slope: N/A

NOEC: 0.014 ppm A.E.

13. Verification of Statistical Results

Statistical Method: Williams (for NOEC); Probit Method (for EC₅₀)

EC₅₀: 0.15 ppm A.E.

95% C.I.: 0.12-0.20 ppm A.E.

DP Barcode: D238406

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Slope: N/A

NOEC: 0.014 ppm A.E.

14. REVIEWER'S COMMENTS:

The study was scientifically sound and meets guideline criteria of the *Hazard Evaluation Division Standard Evaluation Procedure for Non-Target Plants: Growth and Reproduction of Aquatic Plants- Tiers 1 and 2.*

Table 4. Cell density ($\times 10^4$ cells/mL) of *Anabaena flos-aquae* after 1, 2, 3, 4 and 5 days of exposure to SAN 836H.

Mean Measured Concentration (mg A.E./L)		OBSERVATION INTERVAL (DAYS)					Percent Reduction
		Day 1	Day 2	Day 3	Day 4	Day 5	
Control	A	8	21	33	51	108	NA ^a
	B	6	25	36	53	102	
	C	5	24	32	49	103	
	Mean(SD) ^a	6(1)	23(2)	33(2)	51(2)	104(3)	
0.0065	A	7	24	32	53	103	0.077
	B	7	27	38	56	105	
	C	8	20	32	55	104	
	Mean(SD) ^a	7(1)	24(3)	34(3)	55(2)	104(1)	
0.014	A	6	21	31	48	97	3.9
	B	6	22	33	35	101	
	C	5	20	29	34	103	
	Mean(SD) ^a	6(<1)	21(1)	31(2)	39(8)	100(3)	
0.028	A	7	19	31	23	94	18
	B	8	18	32	28	85	
	C	6	19	33	32	83	
	Mean(SD) ^a	7(1)	18(1)	32(1)	28(5)	87(6) ^d	
0.052	A	7	16	26	34	86	26
	B	5	13	24	43	74	
	C	5	14	23	24	72	
	Mean(SD) ^a	5(1)	14(2)	25(2)	33(10)	77(8) ^d	
0.099	A	4	8	19	26	69	37
	B	4	10	12	24	66	
	C	3	8	13	23	61	
	Mean(SD) ^a	4(<1) ^c	9(1) ^c	14(4) ^c	24(2) ^e	65(4) ^{cd}	
0.18	A	1	5	12	21	44	54
	B	3	4	7	14	50	
	C	3	3	6	17	48	
	Mean(SD) ^a	2(1) ^e	4(1) ^e	9(3) ^c	17(4) ^e	48(3) ^{cd}	

- ^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 Statistically ($p \leq 0.05$) reduced as compared to the control based on Williams' Test.

SAN 836 ANABEANA TOXICITY
 File: 836BEANA Transform: NO TRANSFORM

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	ORIGINAL N	MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	CONTROL	3	104.333	104.333	104.333
2	0.0065	3	104.000	104.000	104.000
3	0.014	3	100.333	100.333	100.333
4	0.028	3	90.667	90.667	90.667
5	0.052	3	77.333	77.333	77.333
6	0.099	3	65.333	65.333	65.333
7	0.18	3	47.333	47.333	47.333

SAN 836 ANABEANA TOXICITY
 File: 836BEANA Transform: NO TRANSFORM

WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
CONTROL	104.333				
0.0065	104.000	0.095	1.76	k= 1, v=14	
0.014	100.333	1.146	1.85	k= 2, v=14	
0.028	90.667	3.914	*	1.88	k= 3, v=14
0.052	77.333	7.733	*	1.89	k= 4, v=14
0.099	65.333	11.170	*	1.90	k= 5, v=14
0.18	47.333	16.325	*	1.91	k= 6, v=14

s = 4.276

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

Fred Jenkins SAN 836H Anabeana toxicity

CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
.18	100	54	54	0
.099	100	37	37	0
.052	100	26	26	0
.028	100	16	16	0
.014	100	4	4	0
.0065	100	1	1	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 .1565527

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS
1	.6626327	.1565526	.1212378 .3780448

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H
4	.7219644	3.843667E-02

GOODNESS OF FIT PROBABILITY 1

SLOPE = 1.55792
95 PERCENT CONFIDENCE LIMITS = 1.252486 AND 1.863355

LC50 = .1494752
95 PERCENT CONFIDENCE LIMITS = .1193847 AND .2010013

LC10 = 2.287599E-02
95 PERCENT CONFIDENCE LIMITS = 1.672678E-02 AND 2.891912E-02

Fred Jenkins SAN 836H ANABEANA TOXICITY

CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
.18	100	54	54	0
.099	100	37	37	0
.052	100	26	26	0
.028	100	16	16	0
.014	100	4	4	0
.0065	100	1	1	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 .1565527

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