

US EPA ARCHIVE DOCUMENT

4-6-98

DATA EVALUATION RECORD
ALGAE OR DIATOM EC₅₀ TEST
GUIDELINE 122-2 OR 123-2 (TIER I OR II)

1. CHEMICAL: SAN 1269H (Diflufenzopyr+Dicamba) PC Code No:005107

2. TEST MATERIAL: The test substance, SAN 1269H, is a formulated product consisting of the active ingredients Dicamba sodium salt and SAN 836H (Diflufenzopyr sodium salt).
Purity: 50% (wt. as acid equivalents) as Dicamba; 20% (wt. as acid equivalents) as Diflufenzopyr.

3. CITATION
Author: Hoberg, James
Title: SAN 1269H- Toxicity to the Freshwater Blue-Green, Alga, *Anabaena flos-aquae*
Study Completion Date: May 14, 1996
Laboratory: Springborn Laboratories, Inc.
Sponsor: Sandoz Agro, Inc.
Laboratory Report ID: 96-6-6553
DP Barcode:D238406
MRID No.:443074-50

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: 5 Days
Type of Concentrations: Mean measured

7. CONCLUSIONS:

Results Synopsis
EC₅₀: > 0.26 ppm A.E. (acid equivalents) 95% C.I.: N/A
NOEL: 0.0059 ppm A.E. Slope: N/A

8. ADEQUACY OF THE STUDY

- A. Classification: Core
- B. Rationale: The study met guideline criteria.

C. Repairability: N/A

9. GUIDELINE DEVIATIONS

The light intensity range for the test conditions reported (1.076-3.228 K lux) in the study fall outside of *The Hazard Evaluation Division Standard Evaluation Procedure for Non-Target Plants: Growth and Reproduction of Aquatic Plants-Tiers 1 and 2* (SEP) recommended light intensity of 2 K lux.

10. SUBMISSION PURPOSE:

Registration under Section 3

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	10000 cells/ml
<u>Nutrients</u> Standard formula, e.g. 20XAAP	AAP Medium

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B. Test System

Guideline Criteria	Reported Information
<u>Solvent</u>	N/A
<u>Temperature</u> Skeletonema: 20°C Others: 24-25°C	24°C
<u>Light Intensity</u> Anabaena: 2.2 K lux (+15%) Others: 4.3 K lux (+15%)	1.076-3.228 K Lux
<u>Photoperiod</u> Skeletonema: 14 h light, 10 h dark or 16 h light, 8 h dark Others: Continuous	Continuous
<u>pH</u> Skeletonema: approx. 8.0 Others: approx. 7.5	Ranged from 7.2 to 8.4

C. Test Design

Guideline Criteria	Reported Information
<u>Dose range</u> 2X or 3X progression	2X progression
<u>Doses</u> at least 5	6 doses
<u>Controls</u> negative and/or solvent	negative control
<u>Replicates per dose</u> 3 or more (4 or more for Navicula)	3
<u>Duration of test</u> 120 hours	120 hours
<u>Daily observations were made?</u>	Yes

Guideline Criteria	Reported Information
Method of Observations	Cellular counts
Maximum Labeled Rate	0.35 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
Raw data included?	Yes

Dose Response

Dose (Mean Measured) (ppm A.E.) ¹	Cell Density (x 10 ⁴ cells/ml)	% Inhibition	120-Hour pH
Control	181	N/A	8.4
0.0059	172	4.7	8.4
0.011	165	8.7	8.4
0.025	162	11	8.4
0.046	146	19	8.4
0.12	141	22	8.4
0.26	139	23	8.3

1 The nominal concentrations used in the study were 0.0095, 0.019, 0.038, 0.075, 0.15 and 0.30 ppm A.E.. The mean measured concentrations of SAN 836h were ranged from 84% to 93% of the nominal concentrations. The mean measured concentrations for Dicamba ranged from 44 to 87% of the nominal fortified levels.

Statistical Results

Statistical Method: Williams Test (NOEC) and Observation (EC₅₀)

EC₅₀: > 0.26 ppm A.E. 95% C.I.: N/A

Slope: N/A NOEC: 0.0059 mg A.E./L

13. Verification of Statistical Results

Statistical Method: Observation (EC₅₀) and Williams Test (NOEC)

EC₅₀: > 0.26 ppm A.E. 95% C.I.: N/A

Slope: N/A NOEC: 0.0059 ppm A.E.

14. REVIEWER'S COMMENTS:

The study was scientifically sound and met guideline requirements. There was a deviation from the guidelines (See Guideline Deviations), but this deviation did not significantly impair the results of the study.

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 1269H.

Mean Measured Concentration (mg A.E./L)	OBSERVATION INTERVAL (DAYS)					Percent Reduction
	Day 1	Day 2	Day 3	Day 4	Day 5	
Control A	2	4	45	120	160	
B	3	7	25	121	182	
C	4	7	46	120	200	
Mean(SD) ^a	3(1)	6(2)	39(12)	120(1)	181(20)	NA ^b
0.0059 A	4	7	48	115	171	
B	2	4	47	106	169	
C	3	5	43	120	176	
Mean(SD) ^a	3(1)	5(2)	46(3)	114(7)	172(4)	4.7
0.011 A	2	3	45	107	162	
B	2	4	43	109	167	
C	3	4	46	103	166	
Mean(SD) ^a	2(1)	4(1)	44(2)	107(3)	165(2) ^c	8.7
0.025 A	2	3	42	91	167	
B	3	5	43	102	158	
C	1	3	44	98	160	
Mean(SD) ^a	2(1)	3(1)	43(1)	97(6)	162(5) ^c	11
0.046 A	2	2	43	80	144	
B	1	2	41	79	148	
C	1	3	38	87	146	
Mean(SD) ^a	1(<1)	2(1)	40(2)	82(4)	146(2) ^c	19
0.12 A	2	0	34	68	142	
B	0	0	32	73	138	
C	1	2	39	65	143	
Mean(SD) ^a	1(1)	1(1)	35(4)	69(4)	141(3) ^c	22
0.26 A	0	0	36	64	140	
B	1	3	33	58	137	
C	0	0	35	62	141	
Mean(SD) ^a	<1(1)	1(1)	35(1)	61(3)	139(2) ^c	23

- ^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 Statistically reduced as compared to the control based on Williams' Test.

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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	180.667	180.667	180.667
2	0.0059	3	172.000	172.000	172.000
3	0.011	3	165.000	165.000	165.000
4	0.025	3	161.667	161.667	161.667
5	0.046	3	146.000	146.000	146.000
6	0.12	3	141.000	141.000	141.000
7	0.26	3	139.333	139.333	139.333

Anabaena 1269h tox

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WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. MEAN	SIG WILLIAMS	TABLE P=.05	DEGREES OF WILLIAMS	FREEDOM
control	180.667					
0.0059	172.000	1.311		1.76	k= 1, v=14	
0.011	165.000	2.370	*	1.85	k= 2, v=14	
0.025	161.667	2.874	*	1.88	k= 3, v=14	
0.046	146.000	5.243	*	1.89	k= 4, v=14	
0.12	141.000	5.999	*	1.90	k= 5, v=14	
0.26	139.333	6.252	*	1.91	k= 6, v=14	

s = 8.098

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

Anabaena 1269h tox

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