

8-11-95

DATA EVALUATION RECORD
ALGAE EC₅₀ TEST
GUIDELINE 123-2 (TIER II)

1. CHEMICAL: Sulfentrazone (129081)
2. TEST MATERIAL: Sulfentrazone technical; 94.2%
3. CITATION:

Authors: Ward, T.J., P.L. Kowalski, and R.L. Boeri
Title: Acute toxicity of sulfentrazone technical
to the freshwater alga, *Anabaena flos-aquae*
Date: 1995
Laboratory: T.R. Wilbury Laboratories, Inc.,
Marblehead, MA
Lab. Report #: 617-FM
Sponsor: FMC Corporation, Princeton, NJ
MRID No.: 436510-06

4. REVIEWED BY:

William Erickson
Biologist
EEB/EFED/EPA

Signature: *W. Erickson*

Date: 8/09/95

5. APPROVED BY:

Harry Craven
Section Head 4
EEB/EFED/EPA

Signature: *Harry Craven*

Date: 8/11/95

6. STUDY PARAMETERS/RESULTS SYNOPSIS:

Definitive Test Duration: 120 hours
Type of Concentrations: Mean measured
EC₅₀: 32.8 mg/l
95% C.I.: 29.0-36.6 mg/l
NOEC: <15.9

7. CONCLUSIONS: The study is scientifically sound but does not fulfill the guideline requirement for Tier 2 aquatic plant toxicity testing with a blue-green alga.
 8. ADEQUACY OF THE STUDY: Supplemental.
 9. MAJOR GUIDELINE DEVIATIONS: An NOEC was not determined.
 10. SUBMISSION PURPOSE: New chemical.
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11. MATERIALS AND METHODS:

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	3000 cells/ml
<u>Nutrients</u> Standard formula, e.g. 20XAAP	sterile enriched media

Test System:

Guideline Criteria	Reported Information
<u>Solvent</u>	DMF
<u>Temperature</u> <i>Skeletonema</i> : 20°C Others: 24-25°C	24 ± 2°C
<u>Light Intensity</u> <i>Anabaena</i> : 2.0 Lux (±15%) Others: 4.0-5.0 Lux (±15%)	2300 lux
<u>Photoperiod</u> <i>Skeletonema</i> : 14-16 hr light Others: continuous	continuous
<u>pH</u> <i>Skeletonema</i> : approx. 8.0 Others: approx. 7.5	7.5

Test Design:

Guideline Criteria	Reported Information
<u>Dose range</u> 2X or 3X progression	2X
<u>Doses</u> at least 5	5

Guideline Criteria	Reported Information
<u>Controls</u> negative and/or solvent	negative and solvent
<u>Replicates per dose</u> 3 or more	3
<u>Duration of test</u> 120 hours	120 hours
<u>Daily observations were made?</u>	cell counts at 72, 96, and 120 h
<u>Method of Observations</u>	hemacytometer/microscope
<u>Maximum Labeled Rate</u>	n/a

12. REPORTED RESULTS:

Guideline Criteria	Reported Information
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 (mg ai/L)	Cell density ($\times 10^4$ cells/ml)	% inhibition	120-hr pH
control	388.8	-	9.0
solvent control	410.3	-	8.9
15.9	348.7	15	8.8
30.6	180.6	56	8.6
65.4	97.2	76	7.8
142	4.8	99	6.8
250	<1.2	100	6.3

Other Findings: Some insoluble material was observed at the highest test concentration (250 mg/l); therefore, these media were sonicated for 5 minutes.

Statistical Results:

Methods: Nonlinear regression (EC_{50})
Dunnett's test (NOEC)

EC_{50} : 32.9 mg/l
95% C.I.: 27.6-39.2 mg/l
NOEC: 15.9 mg/l

13. VERIFICATION OF STATISTICAL RESULTS: (results attached)

Methods: Probit Analysis (EC_{50})
Williams Test (NOEC)

EC_{50} : 32.8 mg/l
95% C.I.: 29.0-36.6 mg/l
NOEC: <15.9 mg/l

14. REVIEWER'S COMMENTS: The study is scientifically sound but does not fulfill the guideline requirement for Tier 2 aquatic plant toxicity testing with a freshwater alga (*Anabaena flos-aquae*). An NOEC was not determined.

W. ERICKSON SULFENTRAZONE ANABAENA TOXICITY

CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
250	100	100	100	0
142	100	99	99	0
65.4	100	76	76	0
30.6	100	56	56	0
15.9	100	15	15	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 28.01916

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS	
3	1.718435E-02		32.82865	29.08091

36.65802

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H
3	.1770729	2.616699

GOODNESS OF FIT PROBABILITY
4.921341E-02

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

SLOPE = 3.080249
95 PERCENT CONFIDENCE LIMITS = 1.784079 AND 4.376418

LC50 = 31.94295
95 PERCENT CONFIDENCE LIMITS = 22.26566 AND 43.25351

LC10 = 12.36149
95 PERCENT CONFIDENCE LIMITS = 5.150584 AND 18.61046

ANABAENA CELL GROWTH DATA

SUMMARY STATISTICS

TABLE 1 of 2

GRP	IDENTIFICATION	N	MIN	MAX	MEAN
1	SOLVENT CONTROL	33483000.0004560000.0004102666.667			
2	15.9	33405000.0003630000.0003486666.667			
3	30.6	31680000.0002055000.0001806000.000			
4	65.4	3 885000.0001130000.000 971666.667			
5	142	3 16000.000 78000.000 48000.000			
6	250	3 11000.000 13000.000 11666.667			

SUMMARY STATISTICS

TABLE 2 of 2

GRP	IDENTIFICATION	VARIANCE	SD	SEM
1	SOLVENT CONTROL	309746333333.500	556548.590	321323.478
2	15.9	15.915508333333.328	124532.459	71898.849
3	30.6	30.646503000000.000	215645.542	124503.012
4	65.4	65.418858333333.344	137325.647	79284.999
5	142	964000000.000	31048.349	17925.773
6	250	1333333.333	1154.701	666.667

WILLIAMS TEST (Isotonic regression model)

TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	SOLVENT CONTROL	3	4102666.667	4102666.667	4102666.667
2	15.9	3	3486666.667	3486666.667	3486666.667
3	30.6	3	1806000.000	1806000.000	1806000.000
4	65.4	3	971666.667	971666.667	971666.667
5	142	3	48000.000	48000.000	48000.000
6	250	3	11666.667	11666.667	11666.667

WILLIAMS TEST (Isotonic regression model)

TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
SOLVENT CONTROL	4102666.667				
15.9	3486666.667	2.953	*	1.78	k= 1, v=12
30.6	1806000.000	11.011	*	1.87	k= 2, v=12
65.4	971666.667	15.010	*	1.90	k= 3, v=12
142	48000.000	19.439	*	1.92	k= 4, v=12
250	11666.667	19.613	*	1.93	k= 5, v=12

s = 255467.328

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