

DATA EVALUATION RECORD  
ALGAE OR DIATOM EC<sub>50</sub> TEST  
GUIDELINE 123-2 (TIER II)

2/17/1999

1. CHEMICAL: Glyphosate acid PC Code No.: 103601

2. TEST MATERIAL: Glyphosate acid Purity: 95.6%

3. CITATION:

Authors: D.V. Smyth, S.J. Kent, D.S. Morris, D.J. Morgan, and S.E. Magor

Title: Glyphosate Acid: Acute Toxicity to the Green Alga (*Selenastrum capricornutum*)

Study Completion Date: August 12, 1995

Laboratory: Brixham Environmental Laboratory,  
Brixham, Devon, UK

Sponsor: ZENECA Ag Products, Wilmington, DE

Laboratory Project ID: BL5550/B

MRID No.: 443206-37

DP Barcode: None reported

4. REVIEWED BY: Mark Mossler, M.S., Toxicologist,  
Golder Associates Inc.

Signature:

Date: 11/5/98

APPROVED BY: Pim Kosalwat, Ph.D., Senior Scientist,  
Golder Associates Inc.

Signature:

Date: 11/5/98

5. APPROVED BY:

Signature:

Date: 1-8-99  
1/22/99  
2/17/99

6. STUDY PARAMETERS:

Definitive Test Duration: 120 hours

Type of Concentrations: Mean measured

7. CONCLUSIONS: This study is scientifically sound and fulfills the guideline requirements for an algal toxicity test.

Results Synopsis:

EC<sub>50</sub>: 14 ppm ai  
NOEC: 10 ppm ai

95% C.I.: 10 - 20 ppm ai  
Probit Slope: N/A

A. Classification: Core

B. Rationale: N/A

C. Repairability: N/A

9. GUIDELINE DEVIATIONS: The maximum label rate was not reported.10. SUBMISSION PURPOSE: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>Selenastrum capricornutum</i>
<u>Initial Number of Cells</u> 10,000 - 20,000 cells/ml	3,000 cells/ml
<u>Nutrients</u> Standard formula, e.g. 20XAAP	Standard algal medium

B. Test System

Guideline Criteria	Reported Information
<u>Solvent</u>	None
<u>Temperature</u> <i>Skeletonema</i> : 20°C Others: 24-25°C	24.1-24.2°C
<u>Light Intensity</u> <i>Anabaena</i> : 2.0 KLux (±15%) Others: 4.0-5.0 KLux (±15%)	5.0 KLux
<u>Photoperiod</u> <i>Skeletonema</i> : 14 h light, 10 h dark or 16 h light, 8 h dark Others: Continuous	Continuous

Guideline Criteria	Reported Information
<b><u>Photoperiod</u></b> Skeletonema: 14 h light, 10 h dark or 16 h light, 8 h dark Others: Continuous	Continuous
<b><u>pH</u></b> Skeletonema: approx. 8.0 Others: approx. 7.5	Initial: 3.5 - 7.5 Final: 3.6 - 8.9

**C. Test Design**

Guideline Criteria	Reported Information
<b><u>Dose range</u></b> 2X or 3X progression	≈2X
<b><u>Doses</u></b> at least 5	Control, 5.6, 10, 18, 32, 56, and 100 ppm active ingredient (ai)
<b><u>Controls</u></b> negative and/or solvent	Negative control group
<b><u>Replicates per dose</u></b> 3 or more	6 replicates for the control group and 3 replicates for the treatment groups
<b><u>Duration of test</u></b> 120 hours	120 hours
<b>Daily observations were made?</b>	Yes
<b><u>Method of Observations</u></b>	Cellular counts
<b><u>Maximum Labeled Rate</u></b>	Not reported

**12. REPORTED RESULTS:**

Guideline Criteria	Reported Information
<b>Initial and 120 h cell densities were measured?</b>	Yes
<b>Control cell count at 120 hr ≥2X initial count?</b>	Yes

Guideline Criteria	Reported Information
Initial chemical concentrations measured? (Optional)	Samples were collected at initiation and termination and analyzed by HPLC.
Raw data included?	Yes

Dose Response

Mean Measured Concentration (ppm ai)	120-hr Avg. Cell Density ( $\times 10^4$ cells/ml)	% Inhibition	120-Hour pH
Control	567	N/A	8.5
5.6	605	0	8.5-8.9
10	568	0	8.2-8.5
20	4.2	99	6.3-6.4
33	0.5	100	5.1
58	0.1	100	4.1
100	0.2	100	3.6

Other Significant Results: None noted.

Statistical Results

Statistical Methods: Data were analyzed with respect to area under the growth curve and growth rate. Probit analysis coupled with analysis of variance and Dunnett's test were used to analyze the more sensitive data (area under the growth curve). Results are based on nominal concentrations.

EC<sub>50</sub>: 17 ppm ai                      95% C.I.: 13 - 22 ppm ai  
 Probit Slope: not reported        NOEC: 10 ppm ai

**13. VERIFICATION OF STATISTICAL RESULTS:**

Statistical Method: Williams' test was used for mean separation. Binomial probability was used to estimate the EC<sub>50</sub>. Results are based on mean measured concentrations.

EC<sub>50</sub>: 14 ppm ai                      95% C.I.: 10 - 20 ppm ai  
 Probit Slope: N/A                  NOEC: 10 ppm ai

14. REVIEWER'S COMMENTS: This study is scientifically sound and fulfills the guideline requirements for an algal toxicity test. The 120-hour EC<sub>50</sub> and NOEC for *S. capricornutum* exposed to glyphosate acid were 14 and 10 ppm ai, respectively. This study is categorized as **Core**.

Day 5 frond counts

File: sel Transform: NO TRANSFORM

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	Control	6	566.833	566.833	579.556
2	5.6 ppm ai	3	605.000	605.000	579.556
3	10 ppm ai	3	567.667	567.667	567.667
4	20 ppm ai	3	4.200	4.200	4.200
5	33 ppm ai	3	0.467	0.467	0.467
6	58 ppm ai	3	0.167	0.167	0.183
7	100 ppm ai	3	0.200	0.200	0.183

Day 5 frond counts

File: sel 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	579.556				
5.6 ppm ai	579.556	1.367		1.74	k= 1, v=17
10 ppm ai	567.667	0.090		1.82	k= 2, v=17
20 ppm ai	4.200	60.463	*	1.85	k= 3, v=17
33 ppm ai	0.467	60.864	*	1.87	k= 4, v=17
58 ppm ai	0.183	60.894	*	1.87	k= 5, v=17
100 ppm ai	0.183	60.894	*	1.88	k= 6, v=17

s = 13.160

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

*NOEC = 10 ppm ai*

Mossler glyphosate acid Selenastrum capricornutum 10-21-98

\*\*\*\*\*

CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
33	100	100	100	0
20	100	99	99	0
10	100	0	0	0

BECAUSE THE NUMBER OF ORGANISMS USED WAS SO LARGE, THE 95 PERCENT = 10-20 ppm  
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 14.39136

WHEN THERE ARE LESS THAN TWO CONCENTRATIONS AT WHICH THE  
PERCENT DEAD IS BETWEEN 0 AND 100, NEITHER THE MOVING AVERAGE  
NOR THE PROBIT METHOD CAN GIVE ANY STATISTICALLY SOUND RESULTS.

\*\*\*\*\*