DATA EVALUATION RECORD
ALGAE OR DIATOM EC$_{50}$ 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: _[signature]_   **Date:** 11/5/98

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

   Signature: _[signature]_ P. Kosalwat   **Date:** 11/5/98

5. **APPROVED BY:** _[illegible]_

   Signature: _[signature]_ Jim A. Bailey   **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$_{50}$: 14 ppm ai   95% C.I.: 10 - 20 ppm ai
   NOEC: 10 ppm ai   Probit Slope: N/A

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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
Species *Skeletonema costatum* *Anabaena flos-aquae* *Selenastrum capricornutum* *Navicula pelliculosa*	*Selenastrum capricornutum*
Initial Number of Cells 10,000 - 20,000 cells/ml	3,000 cells/ml
Nutrients Standard formula, e.g. 20XAAP	Standard algal medium

   B. **Test System**

Guideline Criteria	Reported Information
Solvent	None
Temperature Skeletonema: 20°C Others: 24-25°C	24.1-24.2°C
Light Intensity Anabaena: 2.0 KLux (±15%) Others: 4.0-5.0 KLux (±15%)	5.0 KLux

Photoperiod
Skeletonema:
  14 h light, 10 h dark or
  16 h light, 8 h dark
Others: Continuous
	Continuous

Guideline Criteria	Reported Information

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
	Initial: 3.5 - 7.5
	Final: 3.6 - 8.9

C. Test Design

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

12. REPORTED RESULTS:

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

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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 ($x\ 10^4$ 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_{50}: 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_{50}. Results are based on mean measured concentrations.

EC_{50}: 14 ppm ai   95% C.I.: 10 - 20 ppm ai
Probit Slope: N/A   NOEC: 10 ppm ai

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14. <u>**REVIEWER'S COMMENTS**</u>: This study is scientifically sound and fulfills the guideline requirements for an algal toxicity test. The 120-hour EC_{50} 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

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Mossler   glyphosate acid   Selenastrum capricornutum   10-21-98
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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.

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