

MRID No. 443206-40

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

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

2. **TEST MATERIAL:** Glyphosate acid Purity: 95.6%

3. **CITATION:**

Author: D.V. Smyth, S.J. Kent, D.S. Morris, J.M. Shearing, and N. Shillabeer

Title: Glyphosate Acid: Toxicity to the Marine Alga (*Skeletonema costatum*)

Study Completion Date: February 10, 1996

Laboratory: Brixham Environmental Laboratory, ZENECA Limited, Brixham, UK

Sponsor: ZENECA Ag Products, Wilmington, DE

Laboratory Report ID: BL5684/B

DP Barcode: Not reported

MRID No.: 443206-40

4. **REVIEWED BY:** Karl Bullock, M.S., Environmental Scientist, Golder Associates Inc.

Signature: *Karl Bullock* Date: 11/4/98

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

Signature: *P. Kosalwat* Date: 11/4/98

5. **APPROVED BY:**

Signature: *John G. Bailey* Date: 12-21-98 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

EC₅₀: 12 ppm

95% C.I.: 7.6 - 19 ppm

Probit Slope: N/A

NOEC: 1.8 ppm



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A. Classification: Core.

B. Rationale: N/A.

C. Repairability: N/A.

9. GUIDELINE DEVIATIONS:

1. The light intensity (4.34 KLux) was greater than recommended (2.5 KLux $\pm$ 15%).

2. The maximum labeled 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>Skeletonema costatum</i>
<u>Initial Number of Cells</u> 10,000 - 20,000 cells/mL	10,000 cells/mL
<u>Nutrients</u> Standard formula, e.g. 20XAAP	Culture medium described by Walsh and Alexander, 1980.

B. Test System

Guideline Criteria	Reported Information
<u>Solvent</u>	None.
<u>Temperature</u> <i>Skeletonema</i> : 20°C Others: 24-25°C	Daily range: 20.0 - 20.1°C Hourly range: 20 $\pm$ 1°C
<u>Light Intensity</u> <i>Anabaena</i> : 2.0 KLux ($\pm$ 15%) Others: 4.0-5.0 KLux ($\pm$ 15%)	4.34 KLux

Guideline Criteria	Reported Information
<u>Photoperiod</u> Skeletonema: 14 h light, 10 h dark or 16 h light, 8 h dark Others: Continuous	16 h light, 8 h dark
<u>pH</u> Skeletonema: approx. 8.0 Others: approx. 7.5	Initial: 7.1 - 8.1 Final: 8.1 - 8.8

C. Test Design

Guideline Criteria	Reported Information
<u>Dose range</u> 2X or 3X progression	1.8X
<u>Doses</u> at least 5	1.0, 1.8, 3.2, 5.6, 10, 18, 32, and 56 mg/L
<u>Controls</u> negative and/or solvent	Negative control
<u>Replicates per dose</u> 3 or more	6 replicates in the control, 3 replicates per treatment
<u>Duration of test</u> 120 hours	120 hours
Daily observations were made?	Yes
<u>Method of Observations</u>	Electronic particle Coulter counter (cell density measurement)
<u>Maximum Labeled Rate</u>	Not reported

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

Guideline Criteria	Reported Information
Initial chemical concentrations measured? (Optional)	Yes, chemical concentrations were measured at test initiation and termination using HPLC. Mean measured concentrations ranged from 94 to 106% of nominal.
Raw data included?	Yes

Dose Response

Concentration (mg/L)		Avg. Cell Density (x 10 ⁴ cells/mL) ^a	Inhibition (%) ^b	Final pH
Nominal	Mean Measured			
Control	<0.023	83.71	-	8.8
1.0	0.99	82.14	1.9	8.7
1.8	1.8	78.35	6.4	8.6
3.2	3.0	79.66	4.8	8.6
5.6	5.6	80.57	3.8	8.5
10	9.8	93.65	-11.9	8.5
18	19	4.89	94.2 ^c	8.2
32	32	1.81	97.8 ^c	8.1
56	59	1.33	98.4 ^c	8.1

^a Calculated using the inoculum (Day 0) particle density determined by Coulter counter = 1.308 cells/particle.

^b Compared to the control. Negative sign indicates stimulation.

^c Significantly reduced when compared to the control ($p < 0.05$).

Statistical Results for Area Under the Growth Curve:
 Statistical Method: Linear regression analysis for EC₅₀ and Dunnett's test for NOEC using nominal concentrations

EC₅₀: 12 mg/L

95% C.I.: 7.6 - 19 mg/L

Probit Slope: N/A

NOEC: 1.8 mg/L

13. VERIFICATION OF STATISTICAL RESULTS:

Statistical Method: Moving average method for EC₅₀ and Dunnett's test for NOEC using mean measured concentrations. Statistical analysis performed on reduction in cell density.

EC₅₀: 14.0 ppm

95% C.I.: 12.5 - 15.7 ppm

Probit Slope: N/A

NOEC: 1.8 ppm

- 14. REVIEWER'S COMMENTS:** This study is scientifically sound and fulfills the guideline requirements for an algal toxicity test. Based on nominal concentrations, the 120-hour EC₅₀ and NOEC for *S. costatum* exposed to Glyphosate acid were 12 and 1.8 ppm, respectively. This study can be categorized as **Core.**

Karl Bullock Glyphosate Acid Skeletonema 10-22-98

CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
59	100	98	98	0
32	100	98	98	0
19	100	94	94	0
9.8	100	0	0	0
5.6	100	4	4	0
3	100	5	5	0
1.8	100	6	6	0
.99	100	2	2	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 14.40328

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS
5	1.920028E-02	13.99959	12.46901 15.70574

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
7	1.149766	50.50513	0

A PROBABILITY OF 0 MEANS THAT IT IS LESS THAN 0.001.

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

SLOPE = 3.135606
95 PERCENT CONFIDENCE LIMITS = -.226614 AND 6.497826

LC50 = 12.32908
95 PERCENT CONFIDENCE LIMITS = 0 AND +INFINITY

LC10 = 4.851828
95 PERCENT CONFIDENCE LIMITS = 0 AND 11.4582

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 Glyphosate Acid: Toxicity to Skeletonema
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OBS	CONC	LOG_CONC	Y1	Y2	Y3	Y4	Y5	Y6
1	0.00	.	67.30	66.00	63.10	64.9	64.6	58
2	0.99	-0.00436	59.00	59.90	69.50	.	.	.
3	1.80	0.25527	56.90	60.40	62.40	.	.	.
4	3.00	0.47712	58.20	58.80	65.80	.	.	.
5	5.60	0.74819	63.30	64.30	57.10	.	.	.
6	9.80	0.99123	70.50	71.90	72.30	.	.	.
7	19.00	1.27875	4.71	3.12	3.39	.	.	.
8	32.00	1.50515	1.65	1.09	1.40	.	.	.
9	59.00	1.77085	1.01	1.07	0.98	.	.	.

Glyphosate Acid: Toxicity to Skeletonema
 MODEL: COUNT = CO * PROBNORM ((LOG_EC50 - LOG_CONC) / SIGMA)
 WEIGHTED REGRESSION

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Non-Linear Least Squares Iterative Phase
 Dependent Variable COUNT Method: Gauss-Newton

Iter	LOG_EC50	SIGMA	CO	Weighted SS
0	1.158400	0.318900	64.000000	120.182145
1	1.158555	0.179079	63.433802	204.563286
2	1.120958	0.184843	64.823465	269.337461
3	1.141255	0.164223	64.155981	812.232880
4	1.114102	0.188028	65.059014	252.484965
5	1.146051	0.160376	63.977736	1037.326548
6	1.106962	0.194808	65.273342	201.888100
7	1.152834	0.155259	63.720743	1464.380591
8	1.095995	0.205578	65.585038	155.430432
9	1.162372	0.148690	63.343221	2346.761021
10	1.078829	0.223312	66.032287	124.313356
11	1.175654	0.141034	62.787035	4116.659768
12	1.053634	0.251810	66.593681	119.038678
13	1.193052	0.134033	62.006284	6239.422325
14	1.029670	0.285830	66.941014	133.011939
15	1.210147	0.129865	61.196643	6519.319425
16	1.027257	0.302223	66.721269	138.338725
17	1.215403	0.128819	60.982996	6377.120908
18	1.030219	0.303770	66.579911	137.429850
19	1.214801	0.129011	61.031083	6321.701128
20	1.030109	0.303284	66.594179	137.323448
21	1.214708	0.129027	61.033493	6327.440972
22	1.030035	0.303281	66.596927	137.356624
23	1.214733	0.129019	61.031946	6328.987260
24	1.030043	0.303297	66.596317	137.358330
25	1.214735	0.129019	61.031949	6328.696996
26	1.030045	0.303297	66.596258	137.357096
27	1.214734	0.129020	61.032001	6328.661777
28	1.030044	0.303296	66.596282	137.357111
29	1.214734	0.129020	61.031998	6328.673751
30	1.030044	0.303296	66.596283	137.357152
31	1.214734	0.129020	61.031997	6328.674233
32	1.030044	0.303296	66.596282	137.357149
33	1.214734	0.129020	61.031997	6328.673797
34	1.030044	0.303296	66.596282	137.357148
35	1.214734	0.129020	61.031997	6328.673807
36	1.030044	0.303296	66.596282	137.357148
37	1.214734	0.129020	61.031997	6328.673821
38	1.030044	0.303296	66.596282	137.357148
39	1.214734	0.129020	61.031997	6328.673820

Glyphosate Acid: Toxicity to Skeletonema
 MODEL: COUNT = CO * PROBNORM ((LOG_EC50 - LOG_CONC) / SIGMA)
 WEIGHTED REGRESSION

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Non-Linear Least Squares Iterative Phase
 Dependent Variable COUNT Method: Gauss-Newton

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Iter	LOG_EC50	SIGMA	CO	Weighted SS
40	1.030044	0.303296	66.596282	137.357148
41	1.214734	0.129020	61.031997	6328.673819
42	1.030044	0.303296	66.596282	137.357148
43	1.214734	0.129020	61.031997	6328.673819
44	1.030044	0.303296	66.596282	137.357148
45	1.214734	0.129020	61.031997	6328.673819
46	1.030044	0.303296	66.596282	137.357148
47	1.214734	0.129020	61.031997	6328.673819
48	1.030044	0.303296	66.596282	137.357148
49	1.214734	0.129020	61.031997	6328.673819
50	1.030044	0.303296	66.596282	137.357148
51	1.214734	0.129020	61.031997	6328.673819
52	1.030044	0.303296	66.596282	137.357148
53	1.214734	0.129020	61.031997	6328.673819
54	1.030044	0.303296	66.596282	137.357148
55	1.214734	0.129020	61.031997	6328.673819
56	1.030044	0.303296	66.596282	137.357148
57	1.214734	0.129020	61.031997	6328.673819
58	1.030044	0.303296	66.596282	137.357148
59	1.214734	0.129020	61.031997	6328.673819
60	1.030044	0.303296	66.596282	137.357148
61	1.214734	0.129020	61.031997	6328.673819
62	1.030044	0.303296	66.596282	137.357148
63	1.214734	0.129020	61.031997	6328.673819
64	1.030044	0.303296	66.596282	137.357148
65	1.214734	0.129020	61.031997	6328.673819
66	1.030044	0.303296	66.596282	137.357148
67	1.214734	0.129020	61.031997	6328.673819
68	1.030044	0.303296	66.596282	137.357148
69	1.214734	0.129020	61.031997	6328.673819
70	1.030044	0.303296	66.596282	137.357148
71	1.214734	0.129020	61.031997	6328.673819
72	1.030044	0.303296	66.596282	137.357148
73	1.214734	0.129020	61.031997	6328.673819
74	1.030044	0.303296	66.596282	137.357148
75	1.214734	0.129020	61.031997	6328.673819
76	1.030044	0.303296	66.596282	137.357148
77	1.214734	0.129020	61.031997	6328.673819
78	1.030044	0.303296	66.596282	137.357148
79	1.214734	0.129020	61.031997	6328.673819

Glyphosate Acid: Toxicity to Skeletonema
 MODEL: COUNT = CO * PROBNORM ((LOG_EC50 - LOG_CONC) / SIGMA)
 WEIGHTED REGRESSION

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Non-Linear Least Squares Iterative Phase
 Dependent Variable COUNT Method: Gauss-Newton

Iter	LOG_EC50	SIGMA	CO	Weighted SS
80	1.030044	0.303296	66.596282	137.357148
81	1.214734	0.129020	61.031997	6328.673819
82	1.030044	0.303296	66.596282	137.357148
83	1.214734	0.129020	61.031997	6328.673819
84	1.030044	0.303296	66.596282	137.357148
85	1.214734	0.129020	61.031997	6328.673819
86	1.030044	0.303296	66.596282	137.357148
87	1.214734	0.129020	61.031997	6328.673819
88	1.030044	0.303296	66.596282	137.357148
89	1.214734	0.129020	61.031997	6328.673819
90	1.030044	0.303296	66.596282	137.357148
91	1.214734	0.129020	61.031997	6328.673819
92	1.030044	0.303296	66.596282	137.357148
93	1.214734	0.129020	61.031997	6328.673819
94	1.030044	0.303296	66.596282	137.357148
95	1.214734	0.129020	61.031997	6328.673819
96	1.030044	0.303296	66.596282	137.357148
97	1.214734	0.129020	61.031997	6328.673819
98	1.030044	0.303296	66.596282	137.357148
99	1.214734	0.129020	61.031997	6328.673819

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 100 1.030044 0.303296 66.596282 137.357148
 WARNING: PROC NLIN failed to converge.

Non-Linear Least Squares Summary Statistics

Source	DF	Weighted SS	Weighted MS
Regression	3	1385.2788713	461.7596238
Residual	27	137.3571484	5.0873018
Uncorrected Total	30	1522.6360197	
(Corrected Total)	29	1390.4308566	

WARNING: PROC NLIN failed to converge.

Parameter	Estimate	Asymptotic Std. Error	Asymptotic 95 % Confidence Interval Lower Upper
LOG_EC50	1.03004437	0	1.030044372 1.030044372
SIGMA	0.30329613	0	0.303296133 0.303296133
CO	66.59628175	0	66.596281752 66.596281752

Glyphosate Acid: Toxicity to Skeletonema
 MODEL: COUNT = CO * PROBNORM ((LOG_EC50 - LOG_CONC) / SIGMA)
 WEIGHTED REGRESSION
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Asymptotic Correlation Matrix

Corr	LOG_EC50	SIGMA	CO
LOG_EC50	1	-0.677885177	-0.558690287
SIGMA	-0.677885177	1	0.3800307645
CO	-0.558690287	0.3800307645	1

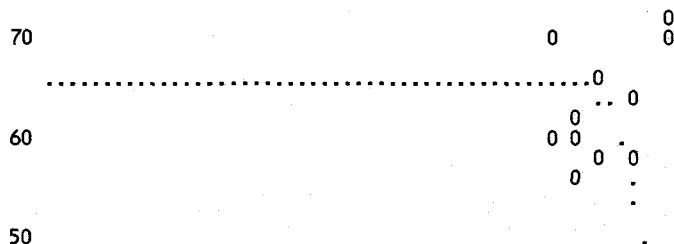
Glyphosate Acid: Toxicity to Skeletonema
 MODEL: COUNT = CO * PROBNORM ((LOG_EC50 - LOG_CONC) / SIGMA)
 SUMMARY OF NONLINEAR REGRESSION
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OBS	CONC	LOG_EC50	SIGMA	CO	RESID_SS	EC50
1	0	1.03004	0.30330	66.5963	137.357	10.7163

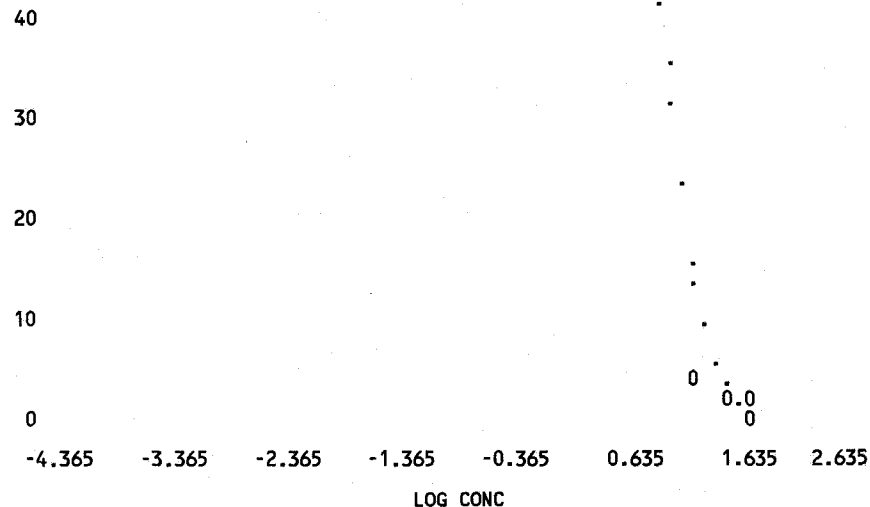
Glyphosate Acid: Toxicity to Skeletonema
 MODEL: COUNT = CO * PROBNORM ((LOG_EC50 - LOG_CONC) / SIGMA)
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Plot of COUNT*LOG_CONC. Symbol used is 'O'.
 Plot of PRED*LOG_CONC. Symbol used is '.'.

COUNT
80



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NOTE: 98 obs had missing values. 55 obs hidden.
 Glyphosate Acid: Toxicity to Skeletonema
 COMPARISON OF MEANS FOR NOEL DETERMINATION
 TEST IF TREATMENT IS LESS THAN CONTROL
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General Linear Models Procedure Class Level Information

Class	Levels	Values
DOSE	9	0 3 19 32 59 1.8 5.6 9.8 0.99

Number of observations in data set = 54

NOTE: Due to missing values, only 30 observations can be used in this analysis.

Glyphosate Acid: Toxicity to Skeletonema
 COMPARISON OF MEANS FOR NOEL DETERMINATION
 TEST IF TREATMENT IS LESS THAN CONTROL
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General Linear Models Procedure

Dependent Variable: RESPONSE

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	8	24098.86005	3012.35751	307.63	0.0001
Error	21	205.63773	9.79227		
Corrected Total	29	24304.49779			

R-Square	C.V.	Root MSE	RESPONSE Mean
0.991539	6.940443	3.129261	45.08733

Source	DF	Type I SS	Mean Square	F Value	Pr > F
DOSE	8	24098.86005	3012.35751	307.63	0.0001
Source	DF	Type III SS	Mean Square	F Value	Pr > F
DOSE	8	24098.86005	3012.35751	307.63	0.0001

Glyphosate Acid: Toxicity to Skeletonema 10
 COMPARISON OF MEANS FOR NOEL DETERMINATION
 TEST IF TREATMENT IS LESS THAN CONTROL
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General Linear Models Procedure

Level of DOSE	N	Mean	SD
0	6	63.9833333	3.25171749
3	3	60.9333333	4.22532050
19	3	3.7400000	0.85082313
32	3	1.3800000	0.28053520
59	3	1.0200000	0.04582576
1.8	3	59.9000000	2.78388218
5.6	3	61.5666667	3.90042733
9.8	3	71.5666667	0.94516313
0.99	3	62.8000000	5.81979381

Glyphosate Acid: Toxicity to Skeletonema 11
 COMPARISON OF MEANS FOR NOEL DETERMINATION
 TEST IF TREATMENT IS LESS THAN CONTROL
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General Linear Models Procedure

Dunnett's One-tailed T tests for variable: RESPONSE

NOTE: This tests controls the type I experimentwise error for
 comparisons of all treatments against a control.

Alpha= 0.05 Confidence= 0.95 df= 21 MSE= 9.792273
 Critical Value of Dunnett's T= 2.625

Comparisons significant at the 0.05 level are indicated by '***'.

DOSE Comparison	Simultaneous Lower Confidence Limit	Difference Between Means	Simultaneous Upper Confidence Limit	
9.8 - 0	1.774	7.583	13.392	
0.99 - 0	-6.992	-1.183	4.626	
5.6 - 0	-8.226	-2.417	3.392	
3 - 0	-8.859	-3.050	2.759	
1.8 - 0	-9.892	-4.083	1.726	
19 - 0	-66.052	-60.243	-54.434	***
32 - 0	-68.412	-62.603	-56.794	***
59 - 0	-68.772	-62.963	-57.154	***