

MRID No. 443206-41

DATA EVALUATION RECORD  
ALGAE OR DIATOM EC<sub>50</sub> 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, P.A. Johnson, and N. Shillabeer

Title: Glyphosate Acid: Toxicity to the Freshwater Diatom (*Navicula pelliculosa*)

Study Completion Date: February 3, 1996

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

Sponsor: ZENECA Ag Products, Wilmington, DE

Laboratory Report ID: BL5673/B

DP Barcode: Not reported

MRID No.: 443206-41

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:** C.E.Z.

**Signature:**

*J.C. Z...*  
*J.M. Bailey*

**Date:** 12-22-98  
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**

EC<sub>50</sub>: 22.4 ppm

95% C.I.: 20.0 - 25.0 ppm

Probit Slope: N/A

NOEC: 19 ppm



A. Classification: Core.

B. Rationale: N/A.

C. Repairability: N/A.

9. **GUIDELINE DEVIATIONS:** The maximum labeled rate was not reported.

10. **SUBMISSION PURPOSE:**

11. **MATERIALS AND METHODS:**

A. Test Organisms

Guideline Criteria	Reported Information
<u><b>Species</b></u> <i>Skeletonema costatum</i> <i>Anabaena flos-aquae</i> <i>Selenastrum capricornutum</i> <i>Navicula pelliculosa</i>	<i>Navicula pelliculosa</i>
<u><b>Initial Number of Cells</b></u> 10,000 cells/mL	3,000 cells/mL
<u><b>Nutrients</b></u> Standard formula, e.g. 20XAAP	Culture medium described by Miller et al, 1978.

B. Test System

Guideline Criteria	Reported Information
<u><b>Solvent</b></u>	None.
<u><b>Temperature</b></u> Skeletonema: 20°C Others: 24-25°C	Daily range: 24.0 - 24.1°C Hourly range: 24 ± 1°C
<u><b>Light Intensity</b></u> Anabaena: 2.0 KLux (±15%) Others: 4.0-5.0 KLux (±15%)	4.56 KLux
<u><b>Photoperiod</b></u> Skeletonema: 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: 3.7 - 8.3 Final: 3.7 - 8.7

**C. Test Design**

Guideline Criteria	Reported Information
<b><u>Dose range</u></b> 2X or 3X progression	1.8X
<b><u>Doses</u></b> at least 5	1.8, 3.2, 5.6, 10, 18, 32, 56, and 100 mg/L
<b><u>Controls</u></b> negative and/or solvent	Negative control
<b><u>Replicates per dose</u></b> 3 or more	6 replicates in the control, 3 replicates per treatment
<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>	Electronic particle Coulter counter (cell density)
<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 <math>\geq</math> 2X initial count?</b>	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 106 to 111% of nominal.
Raw data included?	Yes

Dose Response

Concentration (mg/L)		Avg. Cell Density ( $\times 10^4$ cells/mL)	Inhibition (%) <sup>a</sup>	Final pH
Nominal	Mean Measured			
Control	<0.0021	170	-	8.0
1.8	1.9	197	-16	8.1
3.2	3.4	156	8.2	8.1
5.6	6.2	166	2.4	8.0
10	11	160	5.9	8.1
18	19	187	-10	8.6
32	35	0.237	99.9 <sup>b</sup>	6.2
56	61	0.212	99.9 <sup>b</sup>	4.7
100	110	0.147	99.9 <sup>b</sup>	3.7

<sup>a</sup> Compared to the control. Negative sign indicates stimulation.

<sup>b</sup> Significantly reduced when compared to the control ( $p < 0.05$ ).

Statistical Results for Area Under the Growth Curve:

Statistical Method: Linear regression analysis for EC<sub>50</sub> and Dunnett's test for NOEC using nominal concentrations

EC<sub>50</sub>: 17 mg/L

95% C.I.: 13 - 24 mg/L

Probit Slope: N/A

NOEC: 18 mg/L

**Note:** According to the authors, "For both areas under the growth curve and growth rates, there was a large difference

in dose response between the nominal 18 and 32 mg/L test concentrations. Following transformation to a probability scale this large difference led to a skewed data set. This effect resulted in  $E_bC_{50}$  and  $E_rC_{50}$  values of 17 mg/L, which were below the 18 mg/L nominal test concentration in which there were no significant effects. The EC50 results represent "worse-case" values."

**13. VERIFICATION OF STATISTICAL RESULTS:**

Statistical Method: Moving average method for  $EC_{50}$  and Dunnett's test for NOEC using mean measured concentrations. Statistical analysis performed on reduction of cell density.

$EC_{50}$ : 22.4 ppm

95% C.I.: 20.0 - 25.0 ppm

Probit Slope: N/A

NOEC: 19 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  $EC_{50}$  and NOEC for *N. pelliculosa* exposed to Glyphosate acid were 22.4 and 19 ppm, respectively. This study can be categorized as **Core**.

Karl Bullock Glyphosate Acid Navicula 10-23-98

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CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
110	100	100	100	0
61	100	100	100	0
35	100	100	100	0
19	100	0	0	0
11	100	6	6	0
6.2	100	2	2	0
3.4	100	8	8	0
1.9	100	0	0	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 25.78759

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS
6	1.270002E-02	22.36186	20.06553 25.01188

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
8	2.805588	109.0431	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 = 3.754079  
95 PERCENT CONFIDENCE LIMITS = -2.533962 AND 10.04212

LC50 = 22.10446  
95 PERCENT CONFIDENCE LIMITS = 0 AND +INFINITY

LC10 = 10.14335  
95 PERCENT CONFIDENCE LIMITS = 0 AND +INFINITY

\*\*\*\*\*

Glyphosate Acid: Toxicity to Navicula 10  
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OBS	CONC	LOG_CONC	Y1	Y2	Y3	Y4	Y5	Y6
1	0.0	.	155.000	213.000	165.000	184	154	150
2	1.9	0.27875	209.000	198.000	185.000	.	.	.
3	3.4	0.53148	154.000	159.000	155.000	.	.	.
4	6.2	0.79239	163.000	171.000	164.000	.	.	.
5	11.0	1.04139	170.000	155.000	156.000	.	.	.
6	19.0	1.27875	196.000	203.000	163.000	.	.	.
7	35.0	1.54407	0.310	0.173	0.227	.	.	.
8	61.0	1.78533	0.224	0.243	0.170	.	.	.
9	110.0	2.04139	0.173	0.122	0.147	.	.	.

Glyphosate Acid: Toxicity to Navicula 11  
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	1.349500	0.266380	170.000000	400.380813
1	1.434401	0.099986	170.214425	613333
2	1.420151	0.088163	171.774530	421660623
3	1.408471	0.082918	172.739453	33625825806
4	1.400833	0.079073	173.238982	1726736619338
5	1.381263	0.080075	174.446751	3429563378946
6	1.381266	0.080073	174.446461	3429569085144
7	1.381266	0.080073	174.446456	3585.019619
8	1.418289	0.050829	170.749008	3137918537
9	-1.051043	1.048706	195.471210	75738.492819
10	-0.464878	6.004180	165.878317	2977.447382
11	0.873051	1.936027	166.129509	1982.968933
12	1.344732	0.453184	166.442146	651.530479
13	1.446101	0.099726	168.212467	331573
14	1.395409	0.090284	174.064282	912181478
15	1.399197	0.083441	173.594339	54704822627
16	1.399626	0.079719	173.456880	862283743707
17	1.399049	0.078620	173.455902	1724577210530
18	1.399112	0.078230	173.440782	3449455094788
19	1.398802	0.078097	173.453273	3449206685483
20	1.398730	0.077978	173.453536	3449201465816
21	1.398727	0.077960	173.453119	3449209751436
22	1.398726	0.077957	173.453059	3449210939608
23	1.398726	0.077956	173.453029	3449211543318
24	1.398726	0.077955	173.453014	3449211847581
25	1.398726	0.077955	173.453012	3449211885765
26	1.398726	0.077955	173.453011	3449211904866

NOTE: Convergence criterion met.

## Non-Linear Least Squares Summary Statistics Dependent Variable COUNT

Source	DF	Weighted SS	Weighted MS
Regression	3	3621.07910156	1207.02636719
Residual	27	3449211904866	127748589069
Uncorrected Total	30	3449211908487	
(Corrected Total)	29	67542007307.9	

Parameter	Estimate	Asymptotic Std. Error	Asymptotic 95 % Confidence Interval Lower Upper
LOG_EC50	1.3987263	5911.2458	-12127.3816 12130.1791
SIGMA	0.0779553	3169.0057	-6502.1342 6502.2901
CO	173.4530107	1109507.8912	-2276331.0062 2276677.9123

Glyphosate Acid: Toxicity to Navicula 12  
MODEL: COUNT = CO \* PROBNORM ((LOG\_EC50 - LOG\_CONC) / SIGMA)  
WEIGHTED REGRESSION  
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## Asymptotic Correlation Matrix

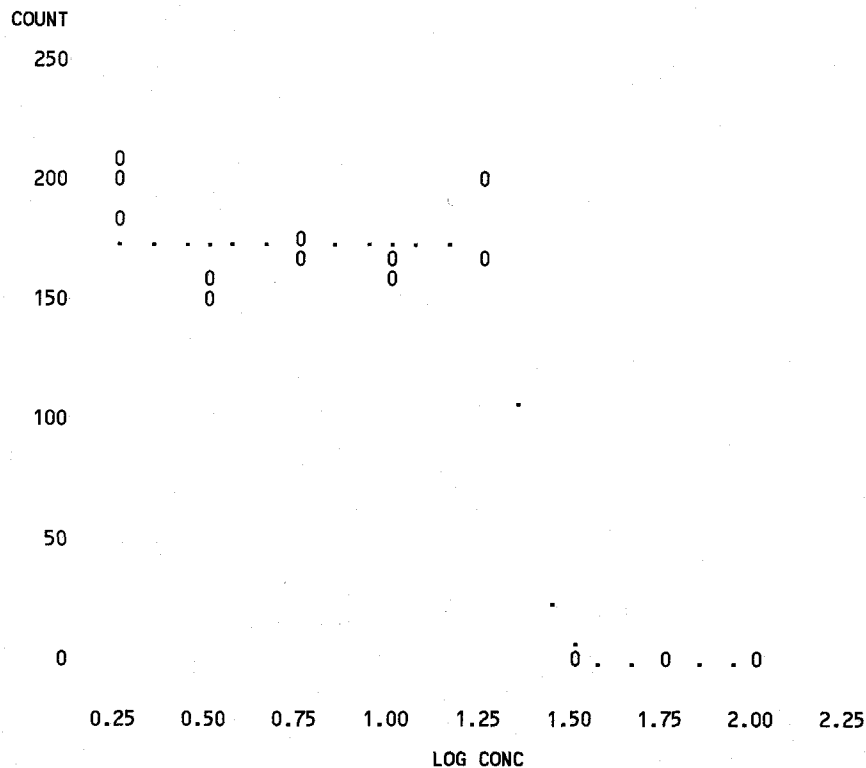
Corr	LOG_EC50	SIGMA	CO
LOG_EC50	1	-0.864248687	-0.371990703
SIGMA	-0.864248687	1	0.3346681565
CO	-0.371990703	0.3346681565	1

Glyphosate Acid: Toxicity to Navicula 13  
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	1.39873	0.077955	173.453	3.4492E12	25.0453

Glyphosate Acid: Toxicity to Navicula 14  
MODEL: COUNT = CO \* PROBNORM ((LOG\_EC50 - LOG\_CONC) / SIGMA)  
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Plot of COUNT\*LOG\_CONC. Symbol used is '0'.  
Plot of PRED\*LOG\_CONC. Symbol used is '.'.



NOTE: 54 obs had missing values. 58 obs hidden.  
Glyphosate Acid: Toxicity to Navicula  
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 11 19 35 61 1.9 110 3.4 6.2

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 Navicula  
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	190911.3112	23863.9139	115.34	0.0001
Error	21	4344.8470	206.8975		
Corrected Total	29	195256.1582			

R-Square	C.V.	Root MSE	RESPONSE Mean
0.977748	11.90792	14.38393	120.7930

Source	DF	Type I SS	Mean Square	F Value	Pr > F
DOSE	8	190911.3112	23863.9139	115.34	0.0001

Source	DF	Type III SS	Mean Square	F Value	Pr > F
DOSE	8	190911.3112	23863.9139	115.34	0.0001

Glyphosate Acid: Toxicity to Navicula  
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	170.166667	24.2933461
11	3	160.333333	8.3864971
19	3	187.333333	21.3619600
35	3	0.236667	0.0690097
61	3	0.212333	0.0378726
1.9	3	197.333333	12.0138809
110	3	0.147333	0.0255016
3.4	3	156.000000	2.6457513
6.2	3	166.000000	4.3588989

Glyphosate Acid: Toxicity to Navicula

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= 206.8975  
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	
1.9 - 0	0.466	27.167	53.868	
19 - 0	-9.534	17.167	43.868	
6.2 - 0	-30.868	-4.167	22.534	
11 - 0	-36.534	-9.833	16.868	
3.4 - 0	-40.868	-14.167	12.534	
35 - 0	-196.631	-169.930	-143.229	***
61 - 0	-196.655	-169.954	-143.253	***
110 - 0	-196.720	-170.019	-143.318	***