

MRID No. 443206-39

**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, N. Shillabeer, D.S. Morris,
and S.J. Wallace

Title: Glyphosate Acid: Toxicity to Blue-Green
Alga (*Anabaena flos-aquae*)

Study Completion Date: March 9, 1996

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

Sponsor: ZENECA Ag Products, Wilmington, DE

Laboratory Report ID: BL5698/B

DP Barcode: Not reported

MRID No.: 443206-39

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. Laird

Signature:

Jim G. Bailey

Date: 12-7-98
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: 15 ppm

95% C.I.: 9.7 - 27 ppm

Probit Slope: N/A

NOEC: 12 ppm

8. ADEQUACY OF THE STUDY:**A. Classification:** Core.**B. Rationale:** N/A.**C. Repairability:** N/A.**9. GUIDELINE DEVIATIONS:**1. The light intensity (3.6 KLux) was greater than recommended (2.5 KLux $\pm$ 15%).

2. The maximum labeled rate was not reported.

3. The initial number of algal cells (20.5×10^3) was greater than recommended ($3.0 - 10.0 \times 10^3$).**10. SUBMISSION PURPOSE:****11. MATERIALS AND METHODS:****A. Test Organisms**

Guideline Criteria	Reported Information
Species <i>Skeletonema costatum</i> <i>Anabaena flos-aquae</i> <i>Selenastrum capricornutum</i> <i>Navicula pelliculosa</i>	<i>Anabaena flos-aquae</i>
Initial Number of Cells 10,000 - 20,000 cells/mL	20,500 cells/mL
Nutrients Standard formula, e.g. 20XAAP	Culture medium described by Miller et al, 1978.

B. Test System

Guideline Criteria	Reported Information
Solvent	None.
Temperature <i>Skeletonema</i> : 20°C Others: 24-25°C	Daily range: 24.1 - 24.2°C Hourly range: 24 $\pm$ 1°C

Guideline Criteria	Reported Information
<u>Light Intensity</u> Anabaena: 2.0 KLux ($\pm 15\%$) Others: 4.0-5.0 KLux ($\pm 15\%$)	3.6 KLux
<u>Photoperiod</u> Skeletonema: 14 h light, 10 h dark or 16 h light, 8 h dark Others: Continuous	Continuous
<u>pH</u> Skeletonema: approx. 8.0 Others: approx. 7.5	Initial: 3.5 - 7.2 Final: 3.6 - 8.2

C. Test Design

Guideline Criteria	Reported Information
<u>Dose range</u> 2X or 3X progression	2X
<u>Doses</u> at least 5	0.75, 1.5, 3.0, 6.0, 12, 24, 48, and 96 mg/L
<u>Controls</u> negative and/or solvent	Negative control
<u>Replicates per dose</u> 3 or more	6 replicates for control and 3 replicates for each treatment
<u>Duration of test</u> 120 hours	120 hours
Daily observations were made?	Yes
<u>Method of Observations</u>	Spectrophotometric absorbance
<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 98 to 110% of nominal.
Raw data included?	Yes

Dose Response

Concentration (mg/L)		Avg. Cell Density ($\times 10^4$ cells/mL) ^a	Inhibition (%) ^b	Final pH
Nominal	Mean Measured			
Control	<0.0021	609.9	-	8.1
0.75	0.75	627.8	-2.9	8.1
1.5	1.5	609.9	0	8.1
3.0	3.1	615.0	-0.8	8.1
6.0	6.1	626.0	-2.6	7.9
12	13	606.5	0.6	7.8
24	24	7.2	98.8 ^c	5.9
48	47	<4.1	99.4 ^c	4.2
96	110	<4.1	99.4 ^c	3.6

^a Calculated using the inoculum (Day 0) cell absorbance = 0.008 (2.05×10^4 cells/mL).

^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: 15 mg/L
Probit Slope: N/A

95% C.I.: 9.7 - 27 mg/L
NOEC: 12 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 was performed on cell density reduction.

EC₅₀: 19.82 ppm

95% C.I.: 17.47 - 22.70 ppm

Probit Slope: N/A

NOEC: 12 ppm

14. REVIEWER'S COMMENTS: This study is scientifically sound and fulfills the guideline requirements for an algal toxicity test. It appears that acidity of the test solutions at 24, 48, and 96 mg/L may have been an adverse effect growth factor. Based on nominal concentrations, the 120-hour EC₅₀ and NOEC for *A. flos-aquae* exposed to Glyphosate acid were 15 and 12 ppm, respectively. This study can be categorized as **Core**.

ENTER YOUR NAME. Karl Bullock Glyphosate Acid Anabaena 10-22-98

CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
110	100	99	99	0
47	100	99	99	0
24	100	99	99	0
13	100	1	1	0
6.1	100	0	0	0
3.1	100	0	0	0
1.5	100	0	0	0
.75	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 17.66352

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS
5	1.831628E-02	19.82483	17.46701 22.69618

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
16	572356	9787182	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 = 8.081079
95 PERCENT CONFIDENCE LIMITS = -6105.596 AND 6121.759

LC50 = 18.37792
95 PERCENT CONFIDENCE LIMITS = 0 AND +INFINITY

LC10 = 12.79791
95 PERCENT CONFIDENCE LIMITS = 0 AND +INFINITY

Glyphosate Acid: Toxicity to Anabaena 12
15:13 Thursday, October 22, 1998

OBS	CONC	LOG_CONC	Y1	Y2	Y3	Y4	Y5	Y6
1	0.00	.	2.580	2.600	2.190	2.07	2.47	2.37
2	0.75	-0.12494	2.410	2.540	2.400	.	.	.
3	1.50	0.17609	2.580	2.190	2.370	.	.	.
4	3.00	0.47712	2.350	2.580	2.270	.	.	.
5	6.00	0.77815	2.460	2.540	2.330	.	.	.
6	12.00	1.07918	2.440	2.280	2.380	.	.	.
7	24.00	1.38021	0.031	0.026	0.027	.	.	.
8	48.00	1.68124	0.016	0.016	0.016	.	.	.
9	96.00	1.98227	0.016	0.016	0.016	.	.	.

Glyphosate Acid: Toxicity to Anabaena 13
MODEL: COUNT = CO * PROBNORM ((LOG_EC50 - LOG_CONC) / SIGMA)
WEIGHTED REGRESSION

15:13 Thursday, October 22, 1998

Non-Linear Least Squares Iterative Phase

Iter	LOG_EC50	SIGMA	CO	Weighted SS
0	1.297200	0.123700	2.380000	21116.630216
1	1.018932	0.408844	2.582102	6.436310
2	1.306132	0.130222	2.284434	3237.699774
3	1.080954	0.337728	2.533539	4.767805
4	1.267135	0.132348	2.350280	9999.381651
5	1.072540	0.289907	2.560083	4.624740
6	1.264850	0.119124	2.350170	380541
7	0.945092	0.407604	2.651863	8.257899
8	1.333808	0.129854	2.220158	1171.253554
9	1.130692	0.308547	2.486867	3.891931
10	1.240846	0.142203	2.390705	3475.248949
11	1.131140	0.210697	2.515788	13.790384
12	1.226719	0.119839	2.406971	2210678
13	1.067533	0.246354	2.579800	6.897399
14	1.259423	0.112407	2.354866	5132427
15	0.843641	0.476961	2.720380	11.704483
16	1.385859	0.145059	2.108045	22.669997
17	1.166923	0.263771	2.451120	3.242499
18	1.219836	0.144895	2.419059	4457.028018
19	1.151460	0.183206	2.497487	108.524648
20	1.208083	0.129974	2.431330	245111
21	1.134288	0.190601	2.518598	72.813082
22	1.217572	0.123913	2.418240	937445
23	1.104469	0.214859	2.547908	16.570889
24	1.238135	0.114681	2.388879	7429353
25	0.991216	0.312532	2.637006	7.018877
26	1.295991	0.123060	2.285845	27429.363322
27	0.996672	0.431342	2.589280	7.073767
28	1.322093	0.127248	2.258266	3204.523792
29	1.100460	0.343265	2.510200	4.592171
30	1.258740	0.139784	2.364535	2867.824693
31	1.111650	0.241697	2.530440	4.976104
32	1.242089	0.117150	2.385568	2433751
33	1.000115	0.313784	2.627943	6.654532
34	1.293810	0.121585	2.291980	44890.923382
35	0.971819	0.456766	2.605740	7.762007
36	1.339597	0.124689	2.227826	2709.751515
37	1.135002	0.325886	2.479995	4.070405
38	1.240020	0.149520	2.392082	932.418215
39	1.146070	0.196882	2.501006	30.419047

Glyphosate Acid: Toxicity to Anabaena 14
MODEL: COUNT = CO * PROBNORM ((LOG_EC50 - LOG_CONC) / SIGMA)
WEIGHTED REGRESSION

15:13 Thursday, October 22, 1998

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

Iter	LOG_EC50	SIGMA	CO	Weighted SS
40	1.276036	0.125261	2.421450	665790
41	1.111506	0.209369	2.541199	21.585956
42	1.233659	0.116160	2.395559	5533975
43	1.022073	0.284648	2.615292	6.320612
44	1.281387	0.117435	2.314800	276727
45	0.881272	0.519979	2.672541	10.245829
46	1.392717	0.129474	2.129232	335.651094
47	1.201262	0.253241	2.431690	3.018862
48	1.201946	0.162709	2.440697	389.865345
49	1.178556	0.156771	2.467748	2112.418597
50	1.183704	0.149436	2.461614	6859.926177
51	1.176140	0.155872	2.470845	2682.240458
52	1.183958	0.149154	2.461261	7187.097609
53	1.175798	0.156162	2.471262	2577.400988
54	1.184284	0.148875	2.460860	7506.755973
55	1.175425	0.156482	2.471716	2466.924210
56	1.184639	0.148571	2.460422	7873.431603
57	1.175015	0.156834	2.472216	2351.511303
58	1.185028	0.148239	2.459943	8296.937567
59	1.174562	0.157222	2.472767	2231.196242
60	1.185454	0.147875	2.459417	8789.903398
61	1.174060	0.157652	2.473377	2106.049102
62	1.185922	0.147476	2.458839	9368.902263
63	1.173501	0.158130	2.474055	1976.180599
64	1.186439	0.147036	2.458202	10056.096645
65	1.172877	0.158663	2.474812	1841.747685
66	1.187011	0.146549	2.457495	10881.764836
67	1.172175	0.159263	2.475662	1702.960407
68	1.187648	0.146009	2.456707	11888.304878
69	1.171380	0.159941	2.476623	1560.091560
70	1.188362	0.145405	2.455823	13136.807388
71	1.170472	0.160715	2.477719	1413.491133
72	1.189169	0.144725	2.454824	14718.298818
73	1.169423	0.161606	2.478981	1263.607963
74	1.190089	0.143953	2.453684	16773.932171
75	1.168195	0.162647	2.480454	1111.024829
76	1.191151	0.143066	2.452365	19533.394763
77	1.166734	0.163883	2.482201	956.515480
78	1.192396	0.142033	2.450815	23393.280217
79	1.164957	0.165382	2.484316	801.140919

Glyphosate Acid: Toxicity to Anabaena 15
MODEL: COUNT = CO * PROBNORM ((LOG_EC50 - LOG_CONC) / SIGMA)
WEIGHTED REGRESSION

15:13 Thursday, October 22, 1998

Non-Linear Least Squares Iterative Phase

Iter	LOG_EC50	SIGMA	CO	Weighted SS
80	1.193887	0.140808	2.448955	29091.482283
81	1.162732	0.167250	2.486945	646.416088
82	1.195721	0.139321	2.446660	38140.387168
83	1.159839	0.169667	2.490334	494.607364
84	1.198062	0.137457	2.443715	54069.859284
85	1.155868	0.172964	2.494926	349.273370
86	1.201212	0.135017	2.439723	86771.911519
87	1.149965	0.177829	2.501617	216.254434
88	1.205796	0.131625	2.433842	172704
89	1.140017	0.185956	2.512512	105.318055
90	1.213332	0.126512	2.423948	520767
91	1.119218	0.202845	2.533836	31.438862
92	1.228343	0.118324	2.403237	3419634
93	1.054351	0.256620	2.590762	6.397602
94	1.265883	0.113240	2.343504	2609541
95	0.825715	0.515780	2.724123	12.112939
96	1.404726	0.142466	2.084453	19.231245
97	1.175302	0.264856	2.441096	3.196733
98	1.215770	0.149937	2.424237	1990.780496
99	1.160694	0.174425	2.487602	251.203921

Non-Linear Least Squares Summary Statistics Dependent Variable COUNT

Source	DF	Weighted SS	Weighted MS
Regression	3	50.586000	16.862000
Residual	27	77986.829074	2888.401077
Uncorrected Total	30	78037.415074	
(Corrected Total)	29	50.625546	

WARNING: PROC NLIN failed to converge.

Parameter	Estimate	Asymptotic Std. Error	Asymptotic 95 % Confidence Interval Lower Upper
LOG_EC50	1.200451095	0	1.2004510947 1.2004510947
SIGMA	0.135567618	0	0.1355676177 0.1355676177
CO	2.441002003	0	2.4410020033 2.4410020033

Glyphosate Acid: Toxicity to Anabaena
 MODEL: COUNT = CO * PROBNORM ((LOG_EC50 - LOG_CONC) / SIGMA)
 WEIGHTED REGRESSION
 15:13 Thursday, October 22, 1998

Asymptotic Correlation Matrix

Corr	LOG_EC50	SIGMA	CO
LOG_EC50	1	-0.687514534	-0.366386584
SIGMA	-0.687514534	1	0.2605513018
CO	-0.366386584	0.2605513018	1

Glyphosate Acid: Toxicity to Anabaena
 MODEL: COUNT = CO * PROBNORM ((LOG_EC50 - LOG_CONC) / SIGMA)
 SUMMARY OF NONLINEAR REGRESSION
 15:13 Thursday, October 22, 1998

OBS	CONC	LOG_EC50	SIGMA	CO	RESID_SS	EC50
1	0	1.20045	0.13557	2.44100	77986.83	15.8654

Glyphosate Acid: Toxicity to Anabaena
 MODEL: COUNT = CO * PROBNORM ((LOG_EC50 - LOG_CONC) / SIGMA)
 15:13 Thursday, October 22, 1998

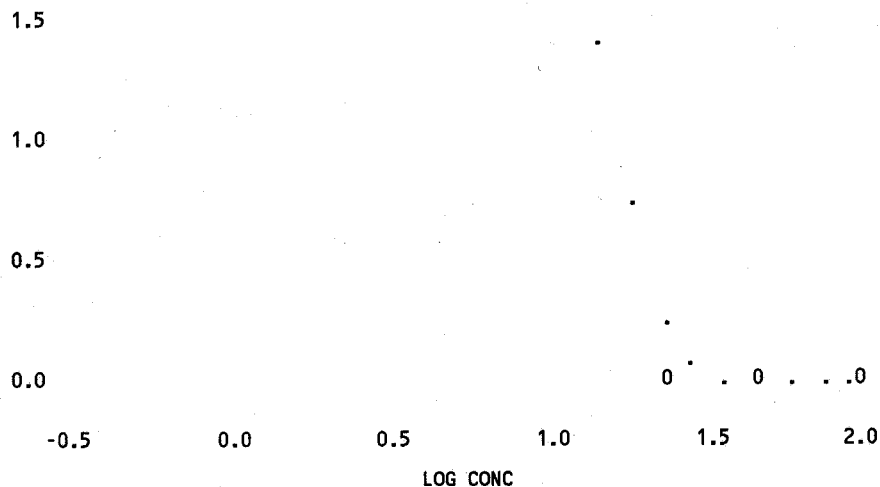
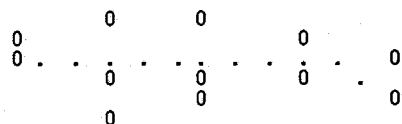
Plot of COUNT*LOG_CONC. Symbol used is '0'.
 Plot of PRED*LOG_CONC. Symbol used is '.'.

COUNT

3.0

2.5

2.0



NOTE: 58 obs had missing values. 59 obs hidden.
 Glyphosate Acid: Toxicity to Anabaena
 COMPARISON OF MEANS FOR NOEL DETERMINATION
 TEST IF TREATMENT IS LESS THAN CONTROL
 15:13 Thursday, October 22, 1998

General Linear Models Procedure
 Class Level Information

Class	Levels	Values
DOSE	9	0 3 6 12 24 48 96 1.5 0.75

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 Anabaena
 COMPARISON OF MEANS FOR NOEL DETERMINATION
 TEST IF TREATMENT IS LESS THAN CONTROL
 15:13 Thursday, October 22, 1998

General Linear Models Procedure

Dependent Variable: RESPONSE

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	8	35.70607467	4.46325933	231.69	0.0001
Error	21	0.40454733	0.01926416		
Corrected Total	29	36.11062200			

R-Square	C.V.	Root MSE	RESPONSE Mean
0.988797	8.232229	0.138795	1.686000

Source	DF	Type I SS	Mean Square	F Value	Pr > F
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DOSE	8	35.70607467	4.46325933	231.69	0.0001
Source	DF	Type III SS	Mean Square	F Value	Pr > F
DOSE	8	35.70607467	4.46325933	231.69	0.0001

Glyphosate Acid: Toxicity to Anabaena 21
 COMPARISON OF MEANS FOR NOEL DETERMINATION
 TEST IF TREATMENT IS LESS THAN CONTROL
 15:13 Thursday, October 22, 1998

General Linear Models Procedure

Level of DOSE	N	Mean	SD
0	6	2.38000000	0.21391587
3	3	2.40000000	0.16093477
6	3	2.44333333	0.10598742
12	3	2.36666667	0.08082904
24	3	0.02800000	0.00264575
48	3	0.01600000	0.00000000
96	3	0.01600000	0.00000000
1.5	3	2.38000000	0.19519221
0.75	3	2.45000000	0.07810250

Glyphosate Acid: Toxicity to Anabaena 22
 COMPARISON OF MEANS FOR NOEL DETERMINATION
 TEST IF TREATMENT IS LESS THAN CONTROL
 15:13 Thursday, October 22, 1998

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= 0.019264
 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	
0.75 - 0	-0.18765	0.07000	0.32765	
6 - 0	-0.19431	0.06333	0.32098	
3 - 0	-0.23765	0.02000	0.27765	
1.5 - 0	-0.25765	-0.00000	0.25765	
12 - 0	-0.27098	-0.01333	0.24431	
24 - 0	-2.60965	-2.35200	-2.09435	***
48 - 0	-2.62165	-2.36400	-2.10635	***
96 - 0	-2.62165	-2.36400	-2.10635	***