

US EPA ARCHIVE DOCUMENT

Data Evaluation Report on the acute toxicity of Glyphosate Acid on the Aquatic Plant, *Lemna gibba*

PMRA Submission #: {.....}

EPA MRID#: 45773101

Data Requirement:

PMRA Data Code:	{.....}
EPA DP Barcode:	D306494
OECD Data Point:	{.....}
EPA MRID:	45773101
EPA Guideline:	123-2

Test material: Glyphosate Acid **Purity:** 96.8%
Common name: Glyphosate Acid
Chemical name: IUPAC: Not reported
 CAS name: Not reported
 CAS No.: Not reported
 Synonyms: Not reported

Primary Reviewer: Rebecca Bryan
Staff Scientist, Dynamac Corporation

Signature: 
Date: 11/10/04

QC Reviewer: Teri Myers
Staff Scientist, Dynamac Corporation

Signature: 
Date: 11/30/04

Primary Reviewer: Anita Pease
{EPA/OECD/PMRA}

Date: {.....} 
4/14/05

Secondary Reviewer(s): {.....}
{EPA/OECD/PMRA}

Date: {.....}

Company Code	{.....}	[For PMRA]
Active Code	{.....}	[For PMRA]
EPA PC Code	417300	

Date Evaluation Completed: {dd-mmm-yyyy}

CITATION: R.L. Boeri and T.J. Ward. 2002. Glyphosate Acid: Toxicity to the Duckweed, *Lemna sp.* Unpublished study performed by T.R Wilbury Laboratories, Inc., Marblehead, MA, Laboratory Study No. 2066-LH, and sponsored by (I.Pi.Ci.) Industria Prodotti Chimici, S.p.A., Novate Milanese, Italy. Experimental start date September 28, 2001 and experimental termination date May 23, 2002. The final report issued July 9, 2002.



US EPA ARCHIVE DOCUMENT

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EXECUTIVE SUMMARY:

In a 7-day acute toxicity study, the freshwater aquatic vascular plant duckweed, *Lemna gibba* G3, was exposed to Glyphosate Acid at nominal concentrations of 0 (negative control), 6.5, 13, 25, 50, and 100 ppm. The Day 0 and day 7 measured treatment concentrations ranged from 103-120% and 95-112% of the nominal treatment concentrations, respectively. The mean measured concentrations (average of day 0 and 7) were 7.56, 14.1, 26.4, 49.7, and 106.5 ppm. The percent inhibition of frond number was -12, 15, 53, 79, and 90% at the 7.56, 14.1, 26.4, 49.7, and 106.5 ppm treatment levels, respectively, compared to the control. The percent inhibition of growth rate was 2, 12, 36, 67, and 96% at the 7.56, 14.1, 26.4, 49.7, and 106.5 ppm treatment levels, respectively. The percent inhibition of biomass (area under growth curve) was -5, 11, 57, 82, and 96% at the 7.56, 14.1, 26.4, 49.7, and 106.5 ppm treatment levels, respectively. Biomass was the most sensitive endpoint, with an EC₅₀ of 24 ppm; the NOEC for biomass (and frond number) was 7.56 ppm.

This toxicity study is scientifically sound and satisfies the U.S. EPA Guideline Subdivision J, §123-2 for a Tier II acute aquatic vascular plant study with *Lemna gibba*. As a result, this study is classified as Core.

Results Synopsis

Test Organism: *Lemna gibba* G3
Test Type: Static

Frond Number:

NOEC: 7.56 ppm
LOEC: 14.1 ppm
EC₀₅: 5.3 ppm 95% C.I.: 3.0-9.3 ppm
EC₅₀: 25 ppm 95% C.I.: 19-32 ppm
Slope: 2.44±0.261

Growth Rate:

NOEC: 14.1 ppm
LOEC: 26.4 ppm
EC₀₅: 13 ppm 95% C.I.: 11-17 ppm
EC₅₀: 37 ppm 95% C.I.: 33-41 ppm
Slope: 3.71±0.250

Biomass (Area Under Growth Curve):

NOEC: 7.56 ppm
LOEC: 14.1 ppm
EC₀₅: 6.5 ppm 95% C.I.: 4.7-9.8 ppm
EC₅₀: 24 ppm 95% C.I.: 21-28 ppm
Slope: 2.91±0.209

Most Sensitive Endpoint: Biomass

I. MATERIALS AND METHODS

GUIDELINE FOLLOWED: The test was based on the following guidelines: U.S. Environmental Protection Agency, OPPTS 850.4400, "Aquatic Plant Toxicity Test Using *Lemna* spp., Tiers I and II" (1996). The following deviation from U.S. EPA Guideline 123-2 was noted:

US EPA ARCHIVE DOCUMENT

1. The carbon source and chelator use in the algal nutrient medium preparation were not reported.
2. The residual chlorine concentration of the dilution water was not reported.
3. The number of fronds per replicate (14) was slightly lower than recommended (15).
4. The test was conducted under static conditions, however, the percent recoveries of test solutions after 7 days were 95-112% of nominal concentrations.

No deviations affected the acceptability or validity of the study.

COMPLIANCE: Signed and dated GLP, Quality Assurance and No Data Confidentiality statements were provided. The test was conducted according to the U.S. EPA (FIFRA, 1993) and OECD (1997) Good Laboratory Practice Standards with the exceptions of analytical methods in the protocol and verification of reference substance container weights (p. 3).

A. MATERIALS:

1. Test Material Glyphosate Acid

Description: White powder

Lot No./Batch No. : 5WC 145/1

Purity: 96.8%

Stability of Compound

Under Test Conditions: The day 0 test concentrations were 103-120% of nominal concentrations and the day 7 test concentrations were 95-112% of nominal concentrations (Table 2, p. 18).

(OECD requires water solubility, stability in water and light, pKa, Pow, vapor pressure of test compound)

Storage conditions of test chemicals: Stored at room temperature in the dark.

2. Test organism:

Name: Duckweed, *Lemna gibba* (EPA requires a vascular species: *Lemna gibba*)

Strain, if provided: G3

Source: Laboratory cultures (original supplier: Climate Stress Laboratory, USDA, Beltsville, MD)

Age of inoculum: 11 days old

Method of cultivation: 20X AAP Medium

B. STUDY DESIGN:

a) Range-finding Study: The definitive test treatment concentrations and the duckweed species were based on the results of two range-finding tests. Two species of duckweed, *Lemna minor* and *Lemna gibba*, were used for the concurrent tests. The concentrations for both tests were 0.10, 1.0, 10, 100, and 1000 mg/L. The percent of control growth for the *Lemna gibba* test were 115, 98, 92, 15, and 0% in the 0.10, 1.0, 10, 100, and 1000 mg/L treatment

Data Evaluation Report on the acute toxicity of Glyphosate Acid on the Aquatic Plant, *Lemna gibba*

PMRA Submission #:{.....}

EPA MRID#:45773101

groups, respectively. The percent of control growth for the *Lemna minor* test were 80, 107, 86, 17, and 0% in the 0.10, 1.0, 10, 100, and 1000 mg/L treatment groups, respectively. There were 12 chlorotic fronds in the 1000 mg/L treatment groups of both test species. Based on these results, *Lemna gibba* (approximately equal sensitivity) was selected as the species to be tested during the definitive toxicity test.

b) Definitive Study

Data Evaluation Report on the acute toxicity of Glyphosate Acid on the Aquatic Plant, *Lemna gibba*

PMRA Submission #:{.....}

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Table 1 . Experimental Parameters

Parameter	Details	Remarks
		Criteria
Acclimation period: culturing media and conditions: (same as test or not) health: (any toxicity observed)	At least 14 days 20X-AAP algal media; same as test. Inoculum was actively growing and 11 days old.	
Test system static/static renewal/ renewal rate for static renewal:	Static N/A	<i>EPA expects the test concentrations to be renewed every 3 to 4 days (one renewal for the 7 day test, 3-4 renewals for the 14 day test).</i>
Incubation facility	Incubator	
Duration of the test	7 days	<i>EPA requires a duration of 14 days. Seven day studies will be accepted for review by the Agency.</i>
Test vessel material: (glass/polystyrene) size: fill volume:	Glass Erlenmeyer flasks 500 mL 200 mL	Test vessels covered with inverted glass beakers.
Details of growth medium name: pH at test initiation: pH at test termination: Chelator used: Carbon source:	20X AAP Medium 6.7-7.6 8.4-9.4 Not reported Not reported	<i>EPA recommend the following culture media: Modified hoagland's E+ or 20X-AAP.</i>
If non-standard nutrient medium was used, detailed composition provided (Yes/No)	N/A	
Dilution water source/type: pH: water pretreatment (if any): Total Organic Carbon: particulate matter: metals: pesticides: chlorine:	20X AAP Medium 7.5 ± 0.1 N/A 3.6 mg/L <10 mg/L See Table 1, p. 11 Not detected Not reported	<i>EPA recommends a pH of ~5.0. A solution pH of 7.5 is acceptable if type 20X-AAP nutrient media is used.</i>

Data Evaluation Report on the acute toxicity of Glyphosate Acid on the Aquatic Plant, *Lemna gibba*

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Parameter	Details	Remarks
		Criteria
Indicate how the test material is added to the medium (added directly or used stock solution)	Stock solution	
Aeration or agitation	Agitation, swirled and repositioned 3 times daily.	
Sediment used (for rooted aquatic vascular plants) origin: textural classification (% sand, silt and clay): organic carbon (%): geographic location:	N/A	
Number of replicates control: solvent control: treatments:	3 N/A 3	
Number of plants/replicate	4 plants per replicate	<i>EPA requires 5 plants.</i>
Number of fronds/plant	3-4 fronds per plant (14 total fronds per replicate)	<i>EPA requires 3 fronds per plant.</i>
Test concentrations nominal: measured:	0 (negative control), 6.5, 13, 25, 50, and 100 ppm Day 0: <0.00165 (LOQ; control), 7.82, 15.0, 27.2, 51.7, and 113 ppm	The reviewer-calculated mean-measured concentrations were 7.56, 14.1, 26.4, 49.7, and 106.5 ppm, respectively (based on day 0 and 7 measured concentrations, Table 2, p. 18). <i>EPA requires at least 5 test concentrations with a dose range of 2X or 3X progression.</i>
Solvent (type, percentage, if used)	N/A	
Method and interval of analytical verification	HPLC; Days 0 and 7	
Test conditions temperature: photoperiod: light intensity and quality:	25.2-25.7°C continuous light 5220-5690 lux, cool-white fluorescent	<i>EPA temperature: 25°C</i> <i>EPA photoperiod: continuous</i> <i>EPA light: 5.0 Klux (±15%)</i>

US EPA ARCHIVE DOCUMENT

Data Evaluation Report on the acute toxicity of Glyphosate Acid on the Aquatic Plant, *Lemna gibba*

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Parameter	Details	Remarks
		<i>Criteria</i>
Reference chemical (if used) name: concentrations:	N/A	
Other parameters, if any	None	

US EPA ARCHIVE DOCUMENT

2. Observations:

Table 2: Observation parameters

Parameters	Details	Remarks/Criteria
Parameters measured (eg: number of fronds, plant dry weight or other toxicity symptoms)	Number of fronds, growth rate, area under growth curve, and toxicity symptoms.	
Measurement technique for frond number and other end points	Direct counts.	
Observation intervals	3, 5 and 7 days.	
Other observations, if any	None	
Indicate whether there was an exponential growth in the control	Yes, frond number in the control on day 7 was approximately 11X the control frond number on day 0.	
Were raw data included?	Replicate data provided.	

II. RESULTS and DISCUSSION:

A. INHIBITORY EFFECTS:

The percent inhibition of frond number was -12, 15, 53, 79, and 90% at the 7.82, 15.0, 27.2, 51.7, and 113 ppm treatment levels, respectively, compared to the control. The percent inhibition of growth rate was 2, 12, 36, 67, and 96% at the 7.82, 15.0, 27.2, 51.7, and 113 ppm treatment levels, respectively. The percent inhibition of biomass (area under growth curve) was -5, 11, 57, 82, and 96% at the 7.82, 15.0, 27.2, 51.7, and 113 ppm treatment levels, respectively. The 7-day NOEC based on frond number and growth rate was 7.82 ppm, the lowest treatment group. The 7-day NOEC based on biomass was <7.82 ppm. Chlorotic fronds were observed in the all treatment groups (Table 4, p. 20).

Table 3: Effect of Glyphosate Acid on Frond Number, Growth Rate, and Biomass of Duckweed, *Lemna gibba*.

Treatment Day 0 Measured (Nominal) Concentrations ppm	Initial Frond Number/ Replicate	Mean Frond Number at:				Day 7 Biomass		Day 7 Growth Rate	
		3 days	5 days	7 days	% Inhibition at 7 days	Mean	% Inhibition	Mean	% Inhibition
Negative control (dilution water)	14	36	81	155	---	330	--	0.365	--
7.82 (6.5)	14	34	82	173	-12 ^a	345	-5	0.359	2*
15.0 (13)	14	35	75	132	15*	293	11*	0.320	12*
27.2 (25)	14	26	40	73	53*	141	57*	0.235	36*
51.7 (50)	14	22	24	32	79*	58	82*	0.120	67*
113 (100)	14	17	16	15	90*	13	96*	0.013	96*
Reference chemical (if used)	N/A								

^a A negative % inhibition indicates an increase in frond number or biomass compared to the control group.

* Statistically significant difference (p=0.05) from the control using the Dunnett's test.

Table 4: Statistical endpoint values.

Statistical Endpoint	Frond Number	Growth rate	Biomass
NOEC or EC ₀₅ (ppm)	7.82	7.82	<7.82
LOEC (ppm)	15.0	15.0	7.82
EC ₅₀ /IC ₅₀ (ppm) (95% C.I.)	Not determined	38.1 (34.3-42.2)	24.9 (21.2-29.2)
Reference chemical NOAEC IC ₂₅ /EC ₂₅	N/A	N/A	N/A

B. REPORTED STATISTICS:

The growth rate and area under the growth curve equations are found on page 16. The data was tested for normality (Chi² Test) and homogeneity of variance (Bartlett's test). The NOEC and LOEC values were determined using ANOVA and Dunnett's test. The EC₅₀ was calculated using the weighted least squares non-linear regression method. The computer program TOXSTAT was used to conduct the statistical analyses. All statistical calculations were performed using the initial mean measured concentrations.

FronD Number:

NOEC: 7.82 ppm

LOEC: 15.0 ppm

EC₅₀/IC₅₀: Not reported

95% C.I.: Not reported

Slope: Not reported

Growth Rate:

NOEC: 7.82 ppm

LOEC: 15.0 ppm

EC₅₀/IC₅₀: 38.1 ppm

95% C.I.: 34.3-42.2 ppm

Slope: Not reported

Biomass (Area Under Growth Curve):

NOEC: <7.82 ppm

LOEC: 7.82 ppm

EC₅₀/IC₅₀: 24.9 ppm

95% C.I.: 21.2-29.2 ppm

Slope: Not reported

Most Sensitive Endpoint: Biomass

C. VERIFICATION OF STATISTICAL RESULTS:

After confirming normality and homogeneity of variances, NOEC and LOEC values were determined for day-7 non-chlorotic frond number, growth rate, and biomass using ANOVA and Dunnett's multiple comparison test via TOXSTAT statistical software. The EC₀₅ and EC₅₀ values were determined using the Probit method via Nuthatch statistical software. The reviewer was able to statistically determine/verify EC₀₅, EC₅₀, NOEC and LOEC values for the biomass and growth rate endpoints. All toxicity values were determined using the average of the initial and final measured treatment concentrations.

FronD Number:

NOEC: 7.56 ppm

LOEC: 14.1 ppm

EC₀₅: 5.3 ppm

95% C.I.: 3.0-9.3 ppm

EC₅₀: 25 ppm

95% C.I.: 19-32 ppm

Slope: 2.44±0.261

Growth Rate:

NOEC: 14.1 ppm

LOEC: 26.4 ppm

EC₀₅: 13 ppm

95% C.I.: 11-17 ppm

EC₅₀: 37 ppm

95% C.I.: 33-41 ppm

Slope: 3.71±0.250

Biomass (Area Under Growth Curve):

NOEC: 7.56 ppm
LOEC: 14.1 ppm
EC₀₅: 6.5 ppm 95% C.I.: 4.7-9.8 ppm
EC₅₀: 24 ppm 95% C.I.: 21-28 ppm
Slope: 2.91±0.209

Most Sensitive Endpoint: Biomass

D. STUDY DEFICIENCIES:

The deviations did not affect the acceptability or the validity of the study.

E. REVIEWER'S COMMENTS:

The reviewer's conclusions were similar to those of the study authors; both concluded that biomass was the most sensitive endpoint. The reviewer's analysis provided EC₀₅ and slope values and the reviewer determined toxicity values for frond number (the study authors did not). Differences between the reviewer's and study authors' toxicity estimates are likely due to the fact that the reviewer based these estimates on the mean measured concentrations (initial and final), while the study authors based them on the initial measured concentrations only. The reviewer's estimates are reported in the Executive Summary and Conclusions sections.

The NOEC and LOEC values for growth rate and biomass differ from the study author's. The study author-reported NOEC values were greater than the reported LOEC values, which appears to be an error in the study report (pages 8 and 17).

F. CONCLUSIONS:

This toxicity study is scientifically sound and satisfies the U.S. EPA Guideline Subdivision J, §123-2 for an aquatic vascular plant study with *Lemna gibba*. As a result, this study is classified as Core. Biomass was the most sensitive endpoint, with an EC₅₀ of 24 ppm.

Frond Number:

NOEC: 7.56 ppm
LOEC: 14.1 ppm
EC₀₅: 5.3 ppm 95% C.I.: 3.0-9.3 ppm
EC₅₀: 25 ppm 95% C.I.: 19-32 ppm
Slope: 2.44±0.261

Growth Rate:

NOEC: 14.1 ppm
LOEC: 26.4 ppm
EC₀₅: 13 ppm 95% C.I.: 11-17 ppm
EC₅₀: 37 ppm 95% C.I.: 33-41 ppm
Slope: 3.71±0.250

Biomass (Area Under Growth Curve):

NOEC: 7.56 ppm

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LOEC: 14.1 ppm
EC₀₅: 6.5 ppm 95% C.I.: 4.7-9.8 ppm
EC₅₀: 24 ppm 95% C.I.: 21-28 ppm
Slope: 2.91±0.209 Most Sensitive Endpoint: Biomass

III. REFERENCES:

- ASTM. 1991. Standard Practice for Conducting Static Toxicity Tests with *Lemna gibba* G3. Designation E 1415-91.
- Bruce, R.D. and D.J. Versteeg, 1992. A Statistical Procedure for Modeling Continuous Data. Environmental Toxicology and Chemistry. Volume 11. No. 10, pp. 1485-1494.
- Gulley, D.D., A.M. Boelter, and H.L. Bergman. 1990. TOXSTAT Release 3.3. Fish Physiology and Toxicology Laboratory, University of Wyoming, Laramie, Wyoming.
- OECD. 1997. OECD Principles of Good Laboratory Practice. [C(97)186/Final].
- OPPTS. 1996. U.S. EPA Prevention, Pesticides, and Toxic Substances. Ecological Effects Test Guidelines. OPPTS 850.4400. Aquatic Plant Toxicity Test Using *Lemna* spp., Tiers I and II. Public Draft. EPA 712-C-96-156. April 1996.
- U.S. EPA. 1989. Pesticide Assessment Guidelines. Subdivision J. 123-2: Growth and Reproduction of Aquatic Plants-Tier 2. Ecological Effects Branch, Hazard Evaluation Division, Office of Pesticide Programs, Washington, D.C.
- U.S. EPA. 1993. 40 CFR Part 160. Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA); Good Laboratory Practice Standards.

APPENDIX I. OUTPUT OF REVIEWER'S STATISTICAL RESULTS:

frond number
 File: 3101fn Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	65390.000	13078.000	110.363
Within (Error)	12	1422.000	118.500	
Total	17	66812.000		

Critical F value = 3.11 (0.05,5,12)
 Since F > Critical F REJECT Ho:All groups equal

frond number
 File: 3101fn Transform: NO TRANSFORMATION

DUNNETTS TEST - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	control	154.667	154.667		
2	7.56	173.333	173.333	-2.100	
3	14.1	131.667	131.667	2.588	*
4	26.4	72.667	72.667	9.226	*
5	49.7	32.333	32.333	13.764	*
6	106.5	15.333	15.333	15.676	*

Dunnett table value = 2.50 (1 Tailed Value, P=0.05, df=12,5)

frond number
 File: 3101fn Transform: NO TRANSFORMATION

DUNNETTS TEST - TABLE 2 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	control	3			
2	7.56	3	22.220	14.4	-18.667
3	14.1	3	22.220	14.4	23.000
4	26.4	3	22.220	14.4	82.000
5	49.7	3	22.220	14.4	122.333
6	106.5	3	22.220	14.4	139.333

frond number
 File: 3101fn Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

Data Evaluation Report on the acute toxicity of Glyphosate Acid on the Aquatic Plant, *Lemna gibba*
 PMRA Submission #:{.....} EPA MRID#:45773101

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	control	3	154.667	154.667	164.000
2	7.56	3	173.333	173.333	164.000
3	14.1	3	131.667	131.667	131.667
4	26.4	3	72.667	72.667	72.667
5	49.7	3	32.333	32.333	32.333
6	106.5	3	15.333	15.333	15.333

frond number
 File: 3101fn Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
control	164.000				
7.56	164.000	1.050		1.78	k= 1, v=12
14.1	131.667	2.588	*	1.87	k= 2, v=12
26.4	72.667	9.226	*	1.90	k= 3, v=12
49.7	32.333	13.764	*	1.92	k= 4, v=12
106.5	15.333	15.676	*	1.93	k= 5, v=12

s = 10.886

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

Estimates of EC%

Parameter	Estimate	95% Bounds		Std.Err.	Lower Bound /Estimate
		Lower	Upper		
EC5	5.3	3.0	9.3	0.12	0.57
EC10	7.4	4.5	12.	0.10	0.61
EC25	13.	9.0	19.	0.076	0.69
EC50	25.	19.	32.	0.052	0.77

Slope = 2.44 Std.Err. = 0.261

!!!Poor fit: p = 0.0017 based on DF= 3.0 12.

3101FN : frond number

Observed vs. Predicted Treatment Group Means

Dose	#Reps.	Obs. Mean	Pred. Mean	Obs. -Pred.	Pred. %Control	%Change
0.00	3.00	155.	171.	-16.4	100.	0.00
7.56	3.00	173.	153.	20.0	89.6	10.4
14.1	3.00	132.	124.	7.47	72.6	27.4
26.4	3.00	72.7	81.2	-8.56	47.5	52.5
49.7	3.00	32.3	39.6	-7.31	23.2	76.8
107.	3.00	15.3	10.6	4.77	6.18	93.8

!!!Warning: EC5 not bracketed by doses evaluated.

!!!Warning: EC10 not bracketed by doses evaluated.

Data Evaluation Report on the acute toxicity of Glyphosate Acid on the Aquatic Plant, *Lemna gibba*
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growth rate
 File: 3101g Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	291675.333	58335.067	225.911
Within (Error)	12	3098.667	258.222	
Total	17	294774.000		

Critical F value = 3.11 (0.05,5,12)
 Since F > Critical F REJECT Ho:All groups equal

growth rate
 File: 3101g Transform: NO TRANSFORMATION

DUNNETTS TEST - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	control	343.333	343.333		
2	7.56	358.667	358.667	-1.169	
3	14.1	320.000	320.000	1.778	
4	26.4	235.333	235.333	8.231	*
5	49.7	117.667	117.667	17.200	*
6	106.5	13.000	13.000	25.177	*

Dunnett table value = 2.50 (1 Tailed Value, P=0.05, df=12,5)

growth rate
 File: 3101g Transform: NO TRANSFORMATION

DUNNETTS TEST - TABLE 2 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	control	3			
2	7.56	3	32.801	9.6	-15.333
3	14.1	3	32.801	9.6	23.333
4	26.4	3	32.801	9.6	108.000
5	49.7	3	32.801	9.6	225.667
6	106.5	3	32.801	9.6	330.333

growth rate
 File: 3101g Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
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US EPA ARCHIVE DOCUMENT

Data Evaluation Report on the acute toxicity of Glyphosate Acid on the Aquatic Plant, *Lemna gibba*
 PMRA Submission #:{.....} EPA MRID#:45773101

1	control	3	343.333	343.333	351.000
2	7.56	3	358.667	358.667	351.000
3	14.1	3	320.000	320.000	320.000
4	26.4	3	235.333	235.333	235.333
5	49.7	3	117.667	117.667	117.667
6	106.5	3	13.000	13.000	13.000

growth rate
 File: 3101g Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
control	351.000				
7.56	351.000	0.584		1.78	k= 1, v=12
14.1	320.000	1.778		1.87	k= 2, v=12
26.4	235.333	8.231	*	1.90	k= 3, v=12
49.7	117.667	17.200	*	1.92	k= 4, v=12
106.5	13.000	25.177	*	1.93	k= 5, v=12

s = 16.069
 Note: df. used for table values are approximate when v > 20.

Estimates of EC%

Parameter	Estimate	95% Bounds		Std.Err.	Lower Bound /Estimate
		Lower	Upper		
EC5	13.	11.	17.	0.047	0.80
EC10	17.	14.	20.	0.040	0.82
EC25	24.	21.	28.	0.031	0.86
EC50	37.	33.	41.	0.021	0.90

Slope = 3.71 Std.Err. = 0.250

Goodness of fit: p = 0.44 based on DF= 3.0 12.

3101G : growth rate

Observed vs. Predicted Treatment Group Means

Dose	#Reps.	Obs. Mean	Pred. Mean	Obs. -Pred.	Pred. %Control	%Change
0.00	3.00	343.	347.	-4.14	100.	0.00
7.56	3.00	359.	346.	13.1	99.5	0.543
14.1	3.00	320.	326.	-6.17	93.9	6.13
26.4	3.00	235.	244.	-9.09	70.3	29.7
49.7	3.00	118.	109.	8.49	31.4	68.6
107.	3.00	13.0	15.1	-2.14	4.36	95.6

biomass
 File: 3101b Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
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Between	5	313227.333	62645.467	312.101
Within (Error)	12	2408.667	200.722	
Total	17	315636.000		

Critical F value = 3.11 (0.05,5,12)
 Since F > Critical F REJECT Ho:All groups equal

biomass
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DUNNETTS TEST - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	control	331.333	331.333		
2	7.56	344.667	344.667	-1.153	
3	14.1	291.000	291.000	3.487	*
4	26.4	140.000	140.000	16.540	*
5	49.7	58.333	58.333	23.600	*
6	106.5	14.667	14.667	27.375	*

Dunnett table value = 2.50 (1 Tailed Value, P=0.05, df=12,5)

biomass
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DUNNETTS TEST - TABLE 2 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	control	3			
2	7.56	3	28.920	8.7	-13.333
3	14.1	3	28.920	8.7	40.333
4	26.4	3	28.920	8.7	191.333
5	49.7	3	28.920	8.7	273.000
6	106.5	3	28.920	8.7	316.667

biomass
 File: 3101b Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	control	3	331.333	331.333	338.000
2	7.56	3	344.667	344.667	338.000
3	14.1	3	291.000	291.000	291.000
4	26.4	3	140.000	140.000	140.000
5	49.7	3	58.333	58.333	58.333
6	106.5	3	14.667	14.667	14.667

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biomass
 File: 3101b Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
control	338.000				
7.56	338.000	0.576		1.78	k= 1, v=12
14.1	291.000	3.487	*	1.87	k= 2, v=12
26.4	140.000	16.540	*	1.90	k= 3, v=12
49.7	58.333	23.600	*	1.92	k= 4, v=12
106.5	14.667	27.375	*	1.93	k= 5, v=12

s = 14.168
 Note: df used for table values are approximate when v > 20.

Estimates of EC%

Parameter	Estimate	95% Bounds		Std.Err.	Lower Bound /Estimate
		Lower	Upper		
EC5	6.5	4.7	9.0	0.066	0.72
EC10	8.7	6.5	12.	0.058	0.75
EC25	14.	11.	17.	0.044	0.80
EC50	24.	21.	28.	0.031	0.86

Slope = 2.91 Std.Err. = 0.209

!!!Poor fit: p = 0.0012 based on DF= 3.0 12.

3101B : biomass

Observed vs. Predicted Treatment Group Means

Dose	#Reps.	Obs. Mean	Pred. Mean	Obs. -Pred.	Pred. %Control	%Change
0.00	3.00	331.	354.	-22.9	100.	0.00
7.56	3.00	345.	329.	16.2	92.7	7.26
14.1	3.00	291.	265.	26.1	74.8	25.2
26.4	3.00	140.	159.	-19.3	45.0	55.0
49.7	3.00	58.3	62.7	-4.35	17.7	82.3
107.	3.00	14.7	10.4	4.30	2.93	97.1

!!!Warning: EC5 not bracketed by doses evaluated.