Data Evaluation Report on the Acute Toxicity of AE 0317309 Technical (Pyrasulfotole) to Aquatic Vascular Plants, *Lemna gibba* G3

PMRA Submission Number 2006-2445 EPA MRID Number 468017-36

Data Requirement:	PMRA DATA CODE	9.8.5
	EPA DP Barcode	D328639
	OECD Data Point	IIA 8.6
	EPA MRID	468017-36
	EPA Guideline	850.4400 (123-2)

Test material: AE 0317309 Technical **Purity:** 95.4%
Common name: Pyrasulfotole
Chemical name: IUPAC: (5-Hydroxy-1,3-dimethyl-1*H*-pyrazol-4-yl)[2-(methylsulfonyl)-4-(trifluoromethyl) phenyl] methanone
CAS name: Not reported
CAS No.: 365400-11-9
Synonyms: None reported

Primary Reviewer: John Marton **Signature:**
Staff Scientist, Cambridge Environmental, Inc. **Date:** 5/18/06

Secondary Reviewer: Teri S. Myers **Signature:**
Senior Scientist, Cambridge Environmental, Inc. **Date:** 5/23/06

Primary Reviewer: Melissa Panger **Date:** 8/11/06
EPA

Secondary Reviewer: J.D. Whall (Officer No. 1268) **Date:** 11/14/06
PMRA

Secondary Reviewer(s): David McAdam **Date:** 6 Nov 2006
Australian Government Department of the Environment and Heritage (DEH)

Reference/Submission No.: {.....}

Company Code	BCZ
Active Code	PSA
Use Site Category:	13, 14
EPA PC Code	000692

Date Evaluation Completed: 11-28-2006

CITATION: Kern, M.E., Banman, C.S. and C.V. Lam. 2004. Toxicity of AE 0317309 Technical to Duckweed (*Lemna gibba* G3) Under Static Conditions. Unpublished study performed by Bayer CropScience, Research and Development Department, Stilwell, KS. Laboratory report number EBAIX009 (A9883701). Study sponsored by Bayer CropScience, Research Triangle Park, NC. Study completed January 22, 2004.

DISCLAIMER: This document provides guidance for EPA and PMRA reviewers on how to complete a data evaluation record after reviewing a scientific study concerning the acute toxicity of a pesticide to aquatic nonvascular plants. It is not intended to prescribe conditions to any external party for conducting this study nor to establish absolute criteria regarding the assessment of whether the study is scientifically sound and whether the study satisfies any applicable data requirements. Reviewers are expected to review and to determine for each study, on a case-by-case basis, whether it is scientifically sound and provides sufficient information to satisfy applicable data

requirements. Studies that fail to meet any of the conditions may be accepted, if appropriate; similarly, studies that meet all of the conditions may be rejected, if appropriate. In sum, the reviewer is to take into account the totality of factors related to the test methodology and results in determining the acceptability of the study.

Data Evaluation Report on the Acute Toxicity of AE 0317309 Technical (Pyrasulfotole) to Aquatic Vascular Plants, *Lemna gibba* G3

PMRA Submission Number 2006-2445 EPA MRID Number 468017-36

EXECUTIVE SUMMARY:

In a 7-day acute toxicity study, the freshwater floating aquatic vascular plants duckweed (*Lemna gibba* G3) were exposed to AE 0317309 Technical at mean-measured concentrations of <0.96 (<LOQ; negative control), 4.14, 9.57, 18.6, 33.1, 74.2 and 153 µg a.i./L under static conditions. The NOAEC and EC_{50}/IC_{50} values based on frond dry weight, the most sensitive endpoint, were 9.57 and 28 µg a.i./L, respectively. The % growth inhibition, based on frond counts, in the treated culture as compared to the control ranged from -2 to 69%. The % growth inhibition, based on area under the growth curve (biomass) in the treated culture as compared to the control ranged from -1 to 75%. The % growth inhibition, based on growth rate in the treated culture as compared to the control ranged from 0 to 55%. The % growth inhibition, based on dry weight in the treated culture as compared to the control ranged from 0 to 77%.

On Day 3, brown fronds were observed in the mean-measured 33.1, 74.2 and 152.5 µg a.i./L treatment levels; a reduction in frond size was also noted at these treatment levels. Transparent fronds were observed at the mean-measured 74.2 µg a.i./L treatment level. On Day 5, reductions in frond size were observed at the mean-measured 18.6-153 µg a.i./L treatment levels, brown fronds were observed at the mean-measured 33.1-153 µg a.i./L treatment levels and transparent fronds were observed at the mean-measured 153 µg a.i./L treatment level. On Day 7, reductions in frond size were observed at the mean-measured 18.6-153 µg a.i./L treatment levels and brown and transparent fronds were observed at the mean-measured 33.1-153 µg a.i./L treatment levels.

This toxicity study is classified as **ACCEPTABLE**, is deemed scientifically sound, and does satisfy the guideline requirement for a vascular aquatic plant toxicity study with duckweed (*Lemna gibba*).

Results Synopsis

Test Organism: Duckweed (*Lemna gibba*)
Test Type (Flow-through, Static, Static Renewal): Static

Frond Number; reviewer-determined:

EC_{05}:	<4.14 µg a.i./L	95% C.I.: N/A
EC_{50}:	40 µg a.i./L	95% C.I.: 24-68 µg a.i./L
NOAEC:	4.14 µg a.i./L	
Probit Slope:	1.13±0.177	

Growth rate (0-96 hours) ; reviewer-determined:

EC_{05}:	<4.14 µg a.i./L	95% C.I.: N/A
EC_{50}:	98 µg a.i./L	95% C.I.: 71-130 µg a.i./L
NOAEC:	9.57 µg a.i./L	
Probit Slope:	1.05±0.136	

Area under the growth curve (biomass, 0-96 hours); study author-determined:

EC_{05}:	Not reported	95% C.I.: Not reported
EC_{50}:	42.4 µg a.i./L	95% C.I.: Not reported
NOAEC:	18.6 µg a.i./L	
Probit Slope:	Not reported	

Frond dry weight (96 hours) ; reviewer-determined:

EC_{05}:	<4.14 µg a.i./L	95% C.I.: N/A
EC_{50}:	28 µg a.i./L	95% C.I.: 17-45 µg a.i./L
NOAEC:	9.57 µg a.i./L	
Probit Slope:	1.29±0.173	

Endpoint(s) Affected: Frond number, growth rate, cumulative biomass, and frond dry weight.
Most sensitive endpoint: Frond dry weight

I. MATERIALS AND METHODS

GUIDELINE FOLLOWED: This study was based on guidelines outline in ASTM Standard Guide for Conducting Static Toxicity Tests with *Lemna gibba* G3, ASTM Standard E1415; USEPA Pesticide Assessment Guidelines, Subdivision J, Hazard Evaluation, Non-Target Plants, EPA-540/9/82-020; USEPA Short-Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms; USEPA Standard Evaluation Procedure, Non-Target Plants, Growth and Reproduction of Aquatic Plants, Tiers 1 and 2, EPA-540/9-86-134; USEPA Pesticide Reregistration Rejection Rate Analysis, EPA738-R94-035; and USEPA OPPTS 850.4400 *draft*, Aquatic Plant Toxicity Test Using *Lemna* spp., Tiers 1 and 2. The following deviations were noted:

1. The physiochemical properties of the test material were not reported.
2. The reported pH of the test solutions at Day 0 (7.8-7.9) and at Day 7 (8.6-8.7) exceeded the recommended values 7.5±0.1.
3. The results of a periodic screening analysis of the dilution water were not reported.
4. Fewer plants per replicate (3) were used than are recommended (5), making the total number of fronds per treatment (13) less than recommended (15).
5. Cool-light instead of warm-light fluorescent lighting was used.
6. Colonies were not transferred to test solutions on day 3 and 5 (EPA recommends renewal at least once during 7-day test).
7. Measured concentrations on day 7 ranged from 74 to 94 % of initial concentration (EPA recommends 80% of initial concentration); the only concentration that fell below the 80% of initial conc. on day 7 was the 4.69 conc. (the lowest conc.).

The deviations did not impact the acceptability of the study.

COMPLIANCE: Signed and dated Data Confidentiality, GLP and Quality Assurance statements were provided. This study was conducted in compliance with 40 CFR Part 160.

A. MATERIALS:

1. Test material AE 0317309 Technical

Description: Light Brown Powder

Lot No./Batch No. : Op. 1-4

Purity: 95.4%

Stability of compound under test conditions: Analytical verification of the test material was conducted at Days 0 and 7. Mean recoveries were 88-102% of nominal

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

Storage conditions of test chemicals: Stored under ambient laboratory condition

Physicochemical properties of AE 0317309 Technical.

Parameter	Value	Comment
Molecular weight	362.3 g/mol	
Water Solubility (g/L) at 20°C	4.2 at pH 4 69.1 at pH 7 49.0 at pH 9	Very soluble
Vapor Pressure/Volatility	2.7×10^{-7} Pa at 20°C 6.8×10^{-7} Pa at 25°C	Non-volatile
UV Absorption	water $\lambda_{max} = 264$ 0.1M HCl $\lambda_{max} = 241$ 0.1M NaOH $\lambda_{max} = 216$	Not likely to undergo photolysis.
Pka	4.2 ± 0.15	
log K_{ow} at 23°C	0.276 at pH 4 -1.362 at pH 7 -1.58 at pH 9	Not likely to bioaccumulate
Stability of compound at room temperature, if provided		No significant degradation over 12 months at ambient temperatures.

Data obtained from pyrasulfatole chemistry review of Submission 2006-2445.

2. Test organism:

Name: Duckweed, *Lemna gibba* G3. *EPA requires a vascular species: Lemna gibba.*
Strain, if provided: Not reported
Source: In-house laboratory culture
Age of inoculum: 7-Days
Method of cultivation: Grown under test conditions (20xAAP) in an environmental chamber

B. STUDY DESIGN:

1. Experimental Conditions

a. Range-finding study: Definitive concentrations were set based upon a range-finding test conducted from August 12 to August 19, 2002. Nominal test concentrations were 0 (negative control), 0.001, 0.01, 0.1, 1.0 and 10.0 mg a.i./L. Percent inhibitions for frond counts were -1, -3, 80, 83 and 83% and percent inhibitions for plant dry weights were -3, -14, 88, 88 and 88%, respectively, at the nominal 0.001, 0.01, 0.1, 1.0 and 10.0 mg a.i./L treatment levels.

b. Definitive Study

Table 1: Experimental Parameters

Parameter	Details	Remarks *Criteria*
Acclimation period: Culturing media and conditions: (same as test or not) Health: (any mortality observed)	Continuous 20xAAP, same as test Density was documented to verify that the batch culture was in log phase growth at study initiation.	
<u>Test system</u> Static/static renewal Renewal rate for static renewal	Static N/A	*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).*
Incubation facility	Environmental chamber	
Duration of the test	7-Days	*EPA requires a duration of 14 days. Seven day studies will be accepted for review by the Agency.*
<u>Test vessel</u> Material: *(glass/stainless steel)* Size: Fill volume:	 Glass 650 mL 260 mL	The test vessels were round with a diameter of 125 mm and a height of 65 mm; test solution depth was 25 mm.

Parameter	Details	Remarks *Criteria*
Details of growth medium name pH at test initiation: pH at test termination: Chelator used: Carbon source:	20xAAP 7.8-7.9 8.6-8.7 Yes $NaHCO_3$	*EPA recommends the following culture media: Modified Hoagland's E+ or 20X-AAP. Chelating agents (e.g. EDTA) are recommended in the nutrient medium for optimum cell growth. Lower concentrations of chelating agents (down to one-third of the normal concentration recommended for AAP medium) may be used in the nutrient medium used for test solution preparation if it is suspected that the chelator will interact with the test material. ASTM reference, E1415-91and D 3978-80 (reapproved 1987).*
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:	 Distilled water Adjusted to a pH of 7.5 Cold filter sterilized Not reported Not reported Not reported Not reported Not reported	*EPA recommends a pH of ~5.0. A solution pH of 7.5 is acceptable if type 20X-AAP nutrient media is used.*
Indicate how the test material is added to the medium (added directly or used stock solution)	Stock solutions were prepared. The highest concentration was prepared first and then serially diluted to obtain stock solutions for all other treatment levels.	

Parameter	Details	Remarks *Criteria*
Aeration or agitation	Not reported	
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	A solvent control was not used.
Number of plants/replicate	3 plants/rep	*EPA requires 5 plants.*
Number of fronds/plant	13 fronds/rep	*EPA requires 3 fronds per plant.*
Test concentrations Nominal: Measured:	 0 (negative control), 4.69, 9.38, 18.75, 37.5, 75.0 and 150 µg a.i./L <0.96 (<LOQ; negative control), 4.14, 9.57, 18.6, 33.1, 74.2 and 153 µg a.i./L	*EPA requires at least 5 test concentrations with a dose range of 2X or 3X progression.*
Solvent (type, percentage, if used)	N/A; a solvent was not used	
Method and interval of analytical verification	Test solutions were analyzed for the presence of AE 0317309 Technical at Days 0 and 7 using HPLC.	
Test conditions Temperature: Photoperiod: Light intensity and quality:	 24.3-24.8°C Continuous light 5.0 klux	

Parameter	Details	Remarks *Criteria*
Reference chemical (if used) name: concentrations:	 N/A N/A	A reference chemical was not used.
Other parameters, if any	None	

2. Observations:

Table 2: Observation parameters

Parameters	Details	Remarks/Criteria
Parameters measured (e.g.,: number of fronds, plant dry weight or other toxicity symptoms)	Frond counts, cumulative biomass, growth rate, and frond dry weight.	
Measurement technique for frond number and other end points	Frond number was determined by direct frond counts on Days 0, 3, 5 and 7. Growth rate was determined by comparing the change in frond number from Day 0 to Day 7. Cumulative biomass was determined by plotting the periodic frond counts from Day 0 to Day 7. Frond dry weight was determined on Day 7.	
Observation intervals	Days 0, 3, 5 and 7	
Other observations, if any	None	
Indicate whether there was an exponential growth in the control	Yes. Frond count in the control increased by a factor of 8.46 by test termination.	
Were raw data included?	Yes	

II. RESULTS and DISCUSSION:

A. INHIBITORY EFFECTS:

By test termination, percent reductions of frond counts were -2, 12, 35, 55, 58 and 69% when compared to the negative control at the mean-measured 4.14, 9.57, 18.6, 33.1, 74.2 and 153 µg a.i./L treatment levels, respectively. Percent reductions in cumulative biomass were -1, 11, 22, 53, 66 and 75% and percent reductions in growth rate were 0, 6, 21, 38, 41 and 55%, respectively. Percent reductions in frond dry weights were 0, 13, 37, 61, 70 and 77% All reductions were linear and appeared to be dose-dependent. The EC_{50} values for frond count, cumulative biomass, growth rate, and frond dry weights were 44.9, 42.4, 110, 30.2 and µg a.i./L, respectively.

On Day 3, brown fronds were observed in the mean-measured 33.1, 74.2 and 152.5 µg a.i./L treatment levels; a reduction in frond size was also noted at these treatment levels. Transparent fronds were observed at the mean-measured 74.2 µg a.i./L treatment level. On Day 5, reductions in frond size were observed at the mean-measured 18.6-153 µg a.i./L treatment levels, brown fronds were observed at the mean-measured 33.1-153 µg a.i./L treatment levels and transparent fronds were observed at the mean-measured 153 µg a.i./L treatment level. On Day 7, reductions in frond size were observed at the mean-measured 18.6-153 µg a.i./L treatment levels and brown and transparent fronds were observed at the mean-measured 33.1-153 µg a.i./L treatment levels.

Table 3: Effect of AE 0317309 Technical on frond number of Duckweed (*Lemna gibba* G3).

Treatment measured and (nominal) concentration (µg a.i./L)	Initial Mean frond number/test solution	frond number at			
		Day 3	Day 5	x_n days	
				frond number	% inhibition
Negative control	13	41	64	110	--
4.14 (4.69)	13	40	66	113	-2
9.75 (9.38)	13	38	59	97	12
18.6 (18.75)	13	38	57	71	35
33.1 (37.5)	13	27	40	49	55
74.2 (75.0)	13	22	30	46	58
153 (150)	13	19	29	34	69
Reference chemical (if used)	N/A	N/A	N/A	N/A	N/A

Table 4: Effect of AE 0317309 Technical on Duckweed (*Lemna gibba* G3).

Treatment measured and (nominal) concentrations (µg a.i./L)	Mean Growth Rate		Mean Area Under the Growth Curve		Frond Dry Weight (g)	
	Day 0-7	Percent Inhibition	Day 0-7	Percent Inhibition	Day 7	Percent Inhibition
Negative control	0.01271	--	6456	--	0.0107	--

4.14 (4.69)	0.01274	0	6520	-1	0.0105	0
9.75 (9.38)	0.01197	6	5752	11	0.0091	13
18.6 (18.75)	0.01010	21	5040	22	0.0066	37
33.1 (37.5)	0.00789	38	3036	53	0.0041	61
74.2 (75.0)	0.00755	41	2192	66	0.0032	70
153 (150)	0.00566	55	1628	75	0.0024	77

B. REPORTED STATISTICS:

The reviewers had no objections to the study authors' statistical analyses. The study author's statistical methods (p. 13 of the study report) are appended to this DER.

Table 5: Statistical endpoint values reported by study authors.

Statistical Endpoint	Frond No.	Growth Rate	Cumulative Biomass	Frond Dry Weight
NOAEC or EC_{05} (µg a.i./L)	9.57[a]	9.57[a]	18.6[a]	9.57[a]
LOAEC (µg a.i./L)	18.6	18.6	33.1	18.6
IC_{50} or EC_{50} (µg a.i./L) (95% C.I.)	44.9	110	42.4	30.2
Other (IC_{25}/EC_{25})	11.3	24.8	14.7	9.6
Reference chemical NOAEC IC_{50}/EC_{50}	N/A	N/A	N/A	N/A

[a] Represents NOAEC values

C. VERIFICATION OF STATISTICAL RESULTS:

Statistical Method(s): Replicate data for frond number, frond dry weight, and growth rate were tested for normality and homogeneity. The reviewers used the relative growth rate calculation to verify results for this parameter; however, replicate data were not provided nor calculated for cumulative biomass. If these assumptions of ANOVA were met, the NOAEC value was determined using the parametric Dunnett's and William's Test. If the assumptions were not met, the NOAEC value was determined using the non-parametric Kruskal-Wallis Test. All NOAEC values were determined using Toxstat Statistical Software. ECx values (with 95% C.I.) and probit slopes were determined using probit analyses via Nuthatch Statistical Software. All toxicity values were determined using the 96-hour mean-measured concentrations. The replicate values for growth rate were multiplied by 1000 to avoid mean values of 0

Frond Number:

EC_{05}:	<4.14 µg a.i./L	95% C.I.: N/A
EC_{50}:	40 µg a.i./L	95% C.I.: 24-68 µg a.i./L
NOAEC:	4.14 µg a.i./L	
Probit Slope:	1.13±0.177	

Growth rate (0-96 hours):

EC_{05}:	<4.14 µg a.i./L	95% C.I.: N/A
EC_{50}:	98 µg a.i./L	95% C.I.: 71-130 µg a.i./L
NOAEC:	9.57 µg a.i./L	
Probit Slope:	1.05±0.136	

Frond dry weight:

EC_{05}:	<4.14 µg a.i./L	95% C.I.: N/A
EC_{50}:	28 µg a.i./L	95% C.I.: 17-45 µg a.i./L
NOAEC:	9.57 µg a.i./L	
Probit Slope:	1.29±0.173	

Endpoint(s) Affected: Frond number, growth rate and frond dry weight.
Most sensitive endpoint: Frond dry weight

D. STUDY DEFICIENCIES:

There were no study deficiencies.

E. REVIEWERS' COMMENTS:

The reviewers' results were similar to the study authors', but were slightly more conservative and included the EC_{05} values, 95% C.I., and probit slopes. The study authors did not include the 95% C.I. associated with the reported EC_{50} values; therefore, the reviewers' results are reported in the Executive Summary and Conclusions sections of this DER for all but the cumulative biomass endpoint (which was not statistically verified by the reviewers because replicate data were not provided).

The reviewers' analysis for frond number showed that these data did not satisfy the assumptions of ANOVA (normality and homogeneity), so this endpoint was analyzed using a non-parametric test. The reviewers' analysis did not detect significant differences at the mean-measured 9.57-74.2 µg a.i./L treatment levels, despite the 12-58% reductions at these levels. Therefore, the reviewers visually determined the NOAEC value to be 4.14 µg a.i./L based on the -2% reduction at this level, relative to the negative control.

The in-life portion of the definitive toxicity test with Duckweed was conducted from May 16 to May 23, 2003.

F. CONCLUSIONS:

This study is scientifically and is classified as **ACCEPTABLE**. Frond dry weight was the most sensitive endpoint with NOAEC and EC_{50} values of 9.57 and 28 µg a.i./L, respectively.

Frond Number; reviewer-determined:
EC_{05}: <4.14 µg a.i./L 95% C.I.: N/A
EC_{50}: 40 µg a.i./L 95% C.I.: 24-68 µg a.i./L
NOAEC: 4.14 µg a.i./L
Probit Slope: 1.13±0.177

Growth rate (0-96 hours) ; reviewer-determined:
EC_{05}: <4.14 µg a.i./L 95% C.I.: N/A
EC_{50}: 98 µg a.i./L 95% C.I.: 71-130 µg a.i./L
NOAEC: 9.57 µg a.i./L
Probit Slope: 1.05±0.136

Area under the growth curve (biomass, 0-96 hours); study author-determined:
EC_{05}: Not reported 95% C.I.: Not reported
EC_{50}: 42.4 µg a.i./L 95% C.I.: Not reported
NOAEC: 18.6 µg a.i./L
Probit Slope: Not reported

Frond dry weight (96 hours) ; reviewer-determined:
EC_{05}: <4.14 µg a.i./L 95% C.I.: N/A
EC_{50}: 28 µg a.i./L 95% C.I.: 17-45 µg a.i./L
NOAEC: 9.57 µg a.i./L
Probit Slope: 1.29±0.173

Endpoint(s) Affected: Frond number, growth rate, cumulative biomass, and frond dry weight.
Most sensitive endpoint: Frond dry weight

III. REFERENCES:

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USEPA. 1996. Series 850- Ecological Effects Test Guidelines (*draft*), OPPTS Number 850.4400: Aquatic Plant Toxicity Test Using *Lemna* spp., Tiers I and II.

APPENDIX I. OUTPUT OF REVIEWER'S STATISTICAL VERIFICATION:

Frond number, ug a.i./L; Day 7
File: 1736fn Transform: NO TRANSFORMATION

KRUSKAL-WALLIS ANOVA BY RANKS - TABLE 1 OF 2

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	RANK SUM
1	neg control	110.000	110.000	54.000
2	4.14	112.667	112.667	54.000
3	9.57	97.333	97.333	45.000
4	18.6	71.000	71.000	33.000
5	33.1	49.000	49.000	22.000
6	74.2	46.333	46.333	17.000
7	153	33.667	33.667	6.000

Calculated H Value = 18.531 Critical H Value Table = 12.590
Since Calc H > Crit H REJECT Ho:All groups are equal.

Frond number, ug a.i./L; Day 7
File: 1736fn Transform: NO TRANSFORMATION

DUNNS MULTIPLE COMPARISON - KRUSKAL-WALLIS - TABLE 2 OF 2

GROUP	IDENTIFICATION	TRANSFORMED MEAN	ORIGINAL MEAN	GROUP 0 0 0 0 0 0 0 7 6 5 4 3 1 2
7	153	33.667	33.667	\
6	74.2	46.333	46.333	. \
5	33.1	49.000	49.000	. . \
4	18.6	71.000	71.000	. . . \
3	9.57	97.333	97.333	. . . . \
1	neg control	110.000	110.000	* . . . . \
2	4.14	112.667	112.667	* . . . . . \

* = significant difference (p=0.05) . = no significant difference
Table q value (0.05,7) = 3.038 SE = 5.056

Estimates of EC%

Parameter	Estimate	95% Bounds Lower	95% Bounds Upper	Std.Err.	Lower Bound /Estimate
EC5	1.4	0.33	6.1	0.30	0.23
EC10	3.0	0.86	10.	0.26	0.29
EC25	10.	4.3	24.	0.18	0.42
EC50	40.	24.	68.	0.11	0.59

Slope = 1.13 Std.Err. = 0.177

!!!Poor fit: p = 0.022 based on DF= 4.0 14.

1736FN : Frond number, ug a.i./L; Day 7

Observed vs. Predicted Treatment Group Means

Dose	#Reps.	Obs. Mean	Pred. Mean	Obs. -Pred.	Pred. %Control	%Change
0.00	3.00	110.	117.	-6.89	100.	0.00
4.14	3.00	113.	101.	11.2	86.8	13.2
9.57	3.00	97.3	88.8	8.56	75.9	24.1
18.6	3.00	71.0	75.7	-4.66	64.7	35.3
33.1	3.00	49.0	62.9	-13.9	53.8	46.2
74.2	3.00	46.3	44.6	1.76	38.1	61.9
153.	3.00	33.7	29.9	3.81	25.5	74.5

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

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

Mean biomass (mg), ug a.i./L Day 7
File: 1736fb Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	6	223.940	37.323	32.768
Within (Error)	14	15.947	1.139	
Total	20	239.887		

Critical F value = 2.85 (0.05,6,14)
Since F > Critical F REJECT Ho:All groups equal

Mean biomass (mg), ug a.i./L Day 7
File: 1736fb 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	neg control	10.700	10.700		
2	4.14	10.600	10.600	0.115	
3	9.57	9.100	9.100	1.836	
4	18.6	6.600	6.600	4.705	*
5	33.1	4.067	4.067	7.612	*
6	74.2	3.200	3.200	8.607	*
7	153	2.400	2.400	9.525	*

Dunnett table value = 2.53 (1 Tailed Value, P=0.05, df=14,6)

Mean biomass (mg), ug a.i./L Day 7
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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	neg control	3			
2	4.14	3	2.205	20.6	0.100
3	9.57	3	2.205	20.6	1.600
4	18.6	3	2.205	20.6	4.100
5	33.1	3	2.205	20.6	6.633
6	74.2	3	2.205	20.6	7.500
7	153	3	2.205	20.6	8.300

Mean biomass (mg), ug a.i./L Day 7
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WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	neg control	3	10.700	10.700	10.700
2	4.14	3	10.600	10.600	10.600
3	9.57	3	9.100	9.100	9.100
4	18.6	3	6.600	6.600	6.600
5	33.1	3	4.067	4.067	4.067
6	74.2	3	3.200	3.200	3.200
7	153	3	2.400	2.400	2.400

Mean biomass (mg), ug a.i./L Day 7
File: 1736fb 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
neg control	10.700				
4.14	10.600	0.115		1.76	k= 1, v=14
9.57	9.100	1.836		1.85	k= 2, v=14
18.6	6.600	4.705	*	1.88	k= 3, v=14
33.1	4.067	7.612	*	1.89	k= 4, v=14
74.2	3.200	8.607	*	1.90	k= 5, v=14
153	2.400	9.525	*	1.91	k= 6, v=14

s = 1.067
Note: df used for table values are approximate when v > 20.

Estimates of EC%

Parameter	Estimate	95% Bounds Lower	95% Bounds Upper	Std.Err.	Lower Bound /Estimate
EC5	1.5	0.45	5.0	0.25	0.30
EC10	2.8	1.0	8.0	0.21	0.36
EC25	8.4	4.0	18.	0.15	0.47
EC50	28.	17.	45.	0.098	0.62

Slope = 1.29 Std.Err. = 0.173

!!!Poor fit: p = 0.042 based on DF= 4.0 14.

1736FB : Mean biomass (mg), ug a.i./L Day 7

Observed vs. Predicted Treatment Group Means

Dose	#Reps.	Obs. Mean	Pred. Mean	Obs. -Pred.	Pred. %Control	%Change
0.00	3.00	10.7	11.4	-0.669	100.	0.00
4.14	3.00	10.6	9.76	0.844	85.8	14.2
9.57	3.00	9.10	8.26	0.838	72.7	27.3
18.6	3.00	6.60	6.72	-0.123	59.1	40.9
33.1	3.00	4.07	5.27	-1.20	46.4	53.6
74.2	3.00	3.20	3.34	-0.137	29.4	70.6
153.	3.00	2.40	1.95	0.450	17.2	82.8

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

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

Mean growth rate, ug a.i./L; Day 7
File: 1736gr Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	6	143.095	23.849	56.648
Within (Error)	14	5.892	0.421	
Total	20	148.987		

Critical F value = 2.85 (0.05,6,14)
Since F > Critical F REJECT Ho:All groups equal

Mean growth rate, ug a.i./L; Day 7
File: 1736gr 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	neg control	12.710	12.710		
2	4.14	12.743	12.743	-0.063	
3	9.57	11.963	11.963	1.409	
4	18.6	10.100	10.100	4.927	*
5	33.1	7.900	7.900	9.079	*
6	74.2	7.553	7.553	9.734	*
7	153	5.660	5.660	13.307	*

Dunnett table value = 2.53 (1 Tailed Value, P=0.05, df=14,6)

Mean growth rate, ug a.i./L; Day 7

File: 1736gr 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	neg control	3			
2	4.14	3	1.340	10.5	-0.033
3	9.57	3	1.340	10.5	0.747
4	18.6	3	1.340	10.5	2.610
5	33.1	3	1.340	10.5	4.810
6	74.2	3	1.340	10.5	5.157
7	153	3	1.340	10.5	7.050

Mean growth rate, ug a.i./L; Day 7
File: 1736gr Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	neg control	3	12.710	12.710	12.727
2	4.14	3	12.743	12.743	12.727
3	9.57	3	11.963	11.963	11.963
4	18.6	3	10.100	10.100	10.100
5	33.1	3	7.900	7.900	7.900
6	74.2	3	7.553	7.553	7.553
7	153	3	5.660	5.660	5.660

Mean growth rate, ug a.i./L; Day 7
File: 1736gr 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
neg control	12.727				
4.14	12.727	0.031		1.76	k= 1, v=14
9.57	11.963	1.410		1.85	k= 2, v=14
18.6	10.100	4.928	*	1.88	k= 3, v=14
33.1	7.900	9.081	*	1.89	k= 4, v=14
74.2	7.553	9.735	*	1.90	k= 5, v=14
153	5.660	13.310	*	1.91	k= 6, v=14

s = 0.649
Note: df used for table values are approximate when v > 20.

Estimates of EC%

Parameter	Estimate	95% Bounds Lower	95% Bounds Upper	Std.Err.	Lower Bound /Estimate
EC5	2.7	0.88	8.0	0.23	0.33
EC10	5.9	2.4	14.	0.19	0.41

EC25	22.	13.	39.	0.12	0.57
EC50	98.	71.	1.3E+02	0.066	0.73

Slope = 1.05 Std.Err. = 0.136

!!!Poor fit: p = 0.010 based on DF= 4.0 14.

1736GR : Mean growth rate, ug a.i./L; Day 7

Observed vs. Predicted Treatment Group Means

Dose	#Reps.	Obs. Mean	Pred. Mean	Obs. -Pred.	Pred. %Control	%Change
0.00	3.00	12.7	13.2	-0.455	100.	0.00
4.14	3.00	12.7	12.2	0.562	92.5	7.47
9.57	3.00	12.0	11.3	0.704	85.5	14.5
18.6	3.00	10.1	10.2	-0.105	77.5	22.5
33.1	3.00	7.90	9.07	-1.17	68.9	31.1
74.2	3.00	7.55	7.24	0.317	55.0	45.0
153.	3.00	5.66	5.51	0.148	41.9	58.1

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

Mean biomass (mg), ug a.i./L Day 7
7
3
3
3
3
3
3
3
neg control
8.8
11.9
11.4
4.14
12.0
11.4
8.4
9.57
9.8
8.2
9.3
18.6
7
6.2
6.6
33.1
4.6
3.4
4.2
74.2
3.4
3.3
2.9
153
2.5
1.9
2.8

Frond number, ug a.i./L; Day 7
7
3
3
3
3
3
3
3
neg control
108
113
109
4.14
136
117
85
9.57
105
88
99
18.6
75
67
71
33.1
51
51
45
74.2
44
51
44
153
33
33
35

Mean growth rate, ug a.i./L; Day 7
7
3
3
3
3
3
3
neg control
12.60
12.87
12.66
4.14
13.97
13.08
11.18
9.57
12.43
11.38
12.08
18.6
10.43
9.76
10.11
33.1
8.17
8.14
7.39
74.2
7.26
8.14
7.26
153
5.54
5.54
5.90

Growth Rates

Mean-Measured Concentraton (µg a.i./L)	Day 0 Frond Count	Day 7 Frond Count
0		
Rep 1	13	108
Rep 2	13	113
Rep 3	13	109
Mean	*13*	*110*
4.14		
Rep 1	13	136
Rep 2	13	117
Rep 3	13	85
Mean	*13*	*113*
9.57		
Rep 1	13	105
Rep 2	13	88
Rep 3	13	99
Mean	*13*	*97*
18.6		
Rep 1	13	75
Rep 2	13	67
Rep 3	13	71
Mean	*13*	*71*
33.1		
Rep 1	13	51
Rep 2	13	51
Rep 3	13	45
Mean	*13*	*49*
74.2		
Rep 1	13	44
Rep 2	13	51
Rep 3	13	44
Mean	*13*	*46*
153		
Rep 1	13	33
Rep 2	13	33
Rep 3	13	35
Mean	*13*	*34*

Growth Rate

0.01260
0.01287
0.01266
0.01271

0.01397
0.01308
0.01118
0.01274

0.01243
0.01138
0.01208
0.01197

0.01043
0.00976
0.01011
0.01010

0.00814
0.00814
0.00739
0.00789

0.00726
0.00814
0.00726
0.00755

0.00554
0.00554
0.00590
0.00566