Data Evaluation Report on the Acute Toxicity of AE 0317309 Technical (Pyrasulfotole) to Algae, *Skeletonema costatum*

PMRA Submission Number 2006-2445 EPA MRID Number 468017-40

Data Requirement:	PMRA DATA CODE	9.8.3
	EPA DP Barcode	D328639
	OECD Data Point	IIA 8.4
	EPA MRID	468017-40
	EPA Guideline	850.5400 (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:** 5/16/06

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

Primary Reviewer: Melissa Panger **Date:** 9/29/06
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., C.S. Banman and C.V. Lam. 2004. Toxicity of AE 0317309 Technical to the Saltwater Diatom *Skeletonema costatum*. Unpublished study performed by Bayer CropScience, Research and Development Department, Stilwell, KS. Laboratory report number EBAAIX007 (A9883601). Study sponsored by Bayer CropScience, Research Triangle Park, NC. Study completed February 4, 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

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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.

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PMRA Submission Number 2006-2445 EPA MRID Number 468017-40

EXECUTIVE SUMMARY:

In a 96-hour acute toxicity study, cultures of *Skeletonema costatum* (SK-88) were exposed to AE 0317309 Technical at mean-measured concentrations of <0.51 (<LOQ; negative control), 2.53, 6.40, 16.35, 41.10 and 99.50 mg a.i./L under static conditions. The NOAEC and EC_{50}/IC_{50} values based on biomass, the most sensitive endpoint, were 2.53 and 8.3 mg a.i./L, respectively. The % growth inhibition, based on cell density (cells/ml x 10^4), in the treated algal culture as compared to the control ranged from 3 to 100%. The % growth inhibition, based on cumulative biomass, in the treated algal culture as compared to the control ranged from 3 to 99%.

The following abnormalities were noted: inhibition of cell density, biomass and growth rate.

This toxicity study is classified as **ACCEPTABLE**, is scientifically sound and does satisfy the guideline requirement for a nonvascular aquatic plant toxicity study with the saltwater diatom, *Skeletonema costatum*.

Results Synopsis

Test Organism: *Skeletonema costatum* (SK-88)
Test Type (Flow-through, Static, Static Renewal): Static

Cell density; reviewer-determined:

EC_{05}:	4.4 mg a.i./L	95% C.I.: 0.55-35 mg a.i./L
EC_{50}:	9.4 mg a.i./L	95% C.I.: 3.5-26 mg a.i./L
NOAEC:	6.4 mg a.i./L	
Probit Slope:	4.93±3.74	

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

EC_{05}:	15.33 mg a.i./L	95% C.I.: 0.000708-1438.47 mg a.i./L
EC_{50}:	17.87 mg a.i./L	95% C.I.: 0.0124-80.56 mg a.i./L
NOAEC:	6.4 mg a.i./L	
Probit Slope:	Not reported	

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

EC_{05}:	2.6 mg a.i./L	95% C.I.: 0.52-13 mg a.i./L
EC_{50}:	8.3 mg a.i./L	95% C.I.: 3.8-18 mg a.i./L
NOAEC:	2.53 mg a.i./L	
Probit Slope:	3.26±1.26	

Endpoint(s) Affected: Cell density, growth rate and biomass.
Most sensitive endpoint: Biomass

I. MATERIALS AND METHODS

GUIDELINE FOLLOWED: This study was based on guidelines outline in ASTM Standard Guide for Conducting Static 96-h Toxicity Tests with Microalgae, ASTM Standard E1218; OECD Test Guideline 201, Alga Growth Inhibition Test; 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 850.5400 *draft*, Algal Toxicity, Tiers 1 and 2. The following deviations were noted:

1. The physiochemical properties of the test material were not reported.
2. The pretest health of the test organisms was not reported.
3. The recommended pH for this species is 8.1±0.1. The reported pH of the test solutions was lower than recommended at 0-hours (7.4-8.1) and exceeded the recommended range at 96-hours (7.9-8.8).
4. The results of a periodic screening analysis of the dilution water and/or nutrient medium were not reported.
5. The photoperiod during the definitive test (16L:8D) provided longer light exposure than recommended (14L:10D).
6. The salinity (26-28 ppt) was lower than recommended by EPA (30-35 ppt).

The deviations did not affect 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 0- and 96-hours. Mean recoveries were 99-103% 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 conditions.

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: *Skeletonema costatum*

EPA requires a nonvascular species: For tier I testing, only one species, S. capricornutum, to be tested; for tier II testing, S. costatum, A. flos-aquae, S. capricorntum, and a freshwater diatom is tested.

OECD suggests the following species are considered suitable: S. capricornutum, S. subspicatus, and C. vulgaris. If other species are used, the strain should be reported

Strain: SK-88
Source: In-house laboratory cultures
Age of inoculum: 3-day old
Method of cultivation: Based on methods of Stein (1973); the culture area was maintained on a 16-hour light/8-hour dark light cycle at approximately 20[B]C

B. STUDY DESIGN:

1. Experimental Conditions

a. Range-finding study: A preliminary range-finding test was conducted with nominal concentrations of 0 (negative control), 0.001, 0.1, 1.0, 10.0 and 100 mg a.i./L. Inhibition of cell density compared to the negative control was 5, -9, -13, 40 and 99%, respectively.

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 Enriched Saltwater Media; same as test. Not reported	*EPA recommends two week acclimation period.* *OECD recommends an amount of algae suitable for the inoculation of test cultures and incubated under the conditions of the test and used when still exponentially growing, normally after an incubation period of about 3 days. When the algal cultures contain deformed or abnormal cells, they must be discarded.*
<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	96-hours	*EPA requires: 96-120 hours* *OECD: 72 hours*
<u>Test vessel</u> Material: *(glass/stainless steel)*	Glass	

Parameter	Details	Remarks *Criteria*
Size: Fill volume:	250 mL 100 mL	*OECD recommends 250 ml conical flasks are suitable when the volume of the test solution is 100 ml or use a culturing apparatus.*
Details of growth medium name pH at test initiation: pH at test termination: Chelator used: Carbon source: Salinity (**for marine algae**):	 7.4-8.1 7.9-8.8 Disodium EDTA $NaHCO_3$ 26-28 ppt	The recommended pH for this species is 8.1±0.1. The reported pH of the test solutions was lower than recommended at 0-hours (7.4-8.1) and exceeded the recommended range at 96-hours (7.9-8.8). *OECD recommends the medium pH after equilibration with air is ~8 with less than .001 mmol/l of chelator if used.* *EPA recommends 20X-AAP and chelating agents (e.g. EDTA) 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)	Enriched Saltwater Medium. Detailed composition was provided silicate levels were modified to maintain log phase growth in the controls for the duration of the exposure.	

Parameter	Details	Remarks *Criteria*
Dilution water source/type: pH: salinity (**for marine algae**): water pretreatment (if any): Total Organic Carbon: particulate matter: metals: pesticides: chlorine:	 Distilled Water 8.0 26-28ppt Cold-filter sterilization Not reported Not reported Not reported Not reported Not reported	*EPA pH: Skeletonema costatum= ~8.0 Others = ~7.5 from beginning to end of the test. EPA salinity: 30-35 ppt. EPA is against the use of dechlorinated water.* *OECD: pH is measured at beginning of the test and at 72 hours, it should not normally deviate by more than one unit during the test.*
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.	
Aeration or agitation	Agitation (approx. 100 revs./min.)	
Initial cells density	10,000 cells/mL (for each replicate)	*EPA requires an initial number of 3,000 - 10,000 cells/mL. For Anabaena flos-aquae, cell counts on day 2 are not required.* *OECD recommends that the initial cell concentration be approximately 10,000 cells/ml for S. capricornutum and S. subspicatus. When other species are used the biomass should be comparable.*

Parameter	Details	Remarks *Criteria*
Number of replicates Control: Solvent control: Treatments:	 3 N/A 3	A solvent control was not used. *EPA requires a negative and/or solvent control with 3 or more replicates per doses. Navicula sp.tests should be conducted with four replicate.* *OECD preferably three replicates at each test concentration and ideally twice that number of controls. When a vehicle is used to solubilize the test substance, additional controls containing the vehicle at the highest concentration used in the test.*
Test concentrations Nominal: Measured:	 0 (negative control), 2.56, 6.40, 16, 40 and 100 mg a.i./L <0.51 (<LOQ, negative control), 2.53, 6.40, 16.35, 41.10 and 99.50 mg a.i./L).	Mean measured values were 99 – 103% of nominal. *EPA requires at least 5 test concentrations, with each at least 60% of the next higher one.* *OECD recommends at least five concentrations arranged in a geometric series, with the lowest concentration tested should have no observed effect on the growth of the algae. The highest concentration tested should inhibit growth by at least 50% relatively to the control and, preferably, stop growth completely.*
Solvent (type, percentage, if used)	N/A	
Method and interval of analytical verification	Test solutions were analyzed for the presence of AE 0317309 Technical at 0- and 96-hours using HPLC.	

Parameter	Details	Remarks *Criteria*
Test conditions Temperature: Photoperiod: Light intensity and quality:	 19.1-19.9°C 16L:8D 4.3 klux	*EPA temperature: Skeletonema: 20EC, Others: 24-25EC; EPA photoperiod: S. costatum 14 hr light/ 10 hr dark, Others: Continuous; EPA light: Anabaena: 2.0 Klux (±15%), Others: 4 - 5 Klux (±15%)* *OECD recommended the temperature in the range of 21 to25°C maintained at ± 2°C and continuous uniform illumination provided at approximately 8000 Lux measured with a spherical collector.*
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 including the growth inhibition/other toxicity symptoms	Cell density, biomass and growth rate.	*EPA recommends the growth of the algae expressed as the cell count per mL, biomass per volume, or degree of growth as determined by spectrophotometric means.*

Parameters	Details	Remarks Criteria
Measurement technique for cell density and other end points	Cell density was determined using a light microscope and an Improved Nebauer hemocytometer. Growth rate was determined by comparing the change in cell density from Day 0 to Day 4. The cumulative biomass, was determined by plotting the daily cell density and determining the area under the curve.	*EPA recommends the measurement technique of cell counts or chlorophyll a* *OECD recommends the electronic particle counter, microscope with counting chamber, fluorimeter, spectrophotometer, and colorimeter. (note: in order to provide useful measurements at low cell concentrations when using a spectrophotometer, it may be necessary to use cuvettes with a light path of at least 4 cm).*
Observation intervals	Every 24-hours	*EPA and OECD: every 24 hours.*
Other observations, if any	None	
Indicate whether there was an exponential growth in the control	Yes. Cell density in the control increased by a factor of 76.5 by test termination.	*EPA requires control cell count at termination to be 2X initial count or by a factor of at least 16 during the test.* *OECD: cell concentration in control cultures should have increased by a factor of at least 16 within three days.*
Were raw data included?	Yes	

II. RESULTS and DISCUSSION:

A. INHIBITORY EFFECTS:

At test termination, cell density percent reductions were 3, 6, 93, 99 and 100% when compared to the negative control at the mean-measured 2.53, 6.4, 16.4, 41 and 100 mg a.i./L treatment levels, respectively. Biomass was reduced 3, 19, 91, 98 and 99% and mean growth rate was reduced 1, 2, 61, 103 and 162%, at the mean-measured 2.53, 6.4, 16.4, 41 and 100 mg a.i./L treatment levels, respectively. The study author-reported EC_{50} values for cell density, biomass and growth rate were 10.4, 9.2 and 15.7 mg a.i./L, respectively.

There was not a major change in pH during the study. There were no compound related phytotoxic effects.

Table 3: Effect of AE 0317309 Technical on algal growth the marine diatom, *Skeletonema costatum*.

Treatment Measured and (nominal) concentration (mg a.i./L)	Initial cell density	Cell density at			
		24 hours	48 hours	96-hours	
				cell count ($x10^4$)	% inhibition
Negative control	10,000	3.8	13.0	76.5	--
2.53 (2.56)	10,000	3.2	10.9	74.1	3
6.40 (6.40)	10,000	2.7	8.3	71.7	6
16.35 (16)	10,000	3.6	2.9	5.6	93
41.10 (40)	10,000	2.9	1.4	0.9	99
99.50 (100)	10,000	1.9	1.2	0.0	100
Reference chemical (if used)	N/A	N/A	N/A	N/A	N/A

Table 4: Effect of AE 0317309 Technical on algal growth the marine diatom, *Skeletonema costatum*

Treatment measured and (nominal) concentrations (mg a.i./L)	Initial cell density	Mean Growth Rate		Mean Area Under the Growth Curve	
		0-96 hours	Percent Inhibition	0-96 hours	Percent Inhibition
Negative control	10,000	0.04518	--	2270.4	--
2.53 (2.56)	10,000	0.04484	1	2211.2	3
6.40 (6.40)	10,000	0.04448	2	1830.8	19
16.35 (16)	10,000	0.01785	61	203.2	91
41.10 (40)	10,000	-0.00151	103	55.6	98
99.50 (100)	10,000	-0.02801	162	13.2	99

B. REPORTED STATISTICS:

Statistical analysis was performed for the endpoints of cell density, growth rate and cumulative biomass (area under the growth curve). Statistical analysis of the raw or transformed 96-hour cumulative biomass data passed the criteria for normality and homogeneity of variance. Therefore, parametric analyses were conducted on this endpoint. Statistical analysis of the raw and transformed data for all other 96-hour analyzed endpoints did not pass the criteria for homogeneity of variance. Therefore, a non-parametric analysis was performed for these endpoints. The 96-hour

EC_{25} and EC_{50} values were determined using regression analysis. All analyses were conducted using PC-based computer programs (SAS version 8).

Table 5: Statistical endpoint values reported by study authors.*

Statistical Endpoint	Cell Density	Growth Rate	Biomass
NOAEC or EC_{05} (mg a.i./L)	6.4[a]	6.4[a]	6.4[a]
EC_{50} (mg a.i./L)	10.4	15.7	9.2
IC_{50} or EC_{50} (mg a.i./L) (95% C.I.)	9.6-11.2	12.7-18.8	8.5-9.8
Other (IC_{25}/EC_{25})	8.5 (7.6-9.4)	14.1 (11.3-16.8)	6.9 (6.3-7.6)
Reference chemical, if used NOAEC IC_{50}/EC_{50}	N/A	N/A	N/A

[a] Represents the NOAEC value

Toxicity values based on mean-measured concentrations.

C. VERIFICATION OF STATISTICAL RESULTS:

Statistical Method(s): Replicate data for cell density, biomass (area under the growth curve), and growth rate were tested for normality and homogeneity. 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. Nuthatch is unable to handle negative numbers; therefore, for growth rate, the ECx values were determined using the Bruce and Versteeg (1992) method using SAS 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.

Cell density:

EC_{05}:	4.4 mg a.i./L	95% C.I.: 0.55-35 mg a.i./L
EC_{50}:	9.4 mg a.i./L	95% C.I.: 3.5-26 mg a.i./L
NOAEC:	6.4 mg a.i./L	
Probit Slope:	4.93±3.74	

Growth rate (0-96 hours):

EC_{05}:	15.33 mg a.i./L	95% C.I.: 0.000708-1438.47 mg a.i./L
EC_{50}:	17.87 mg a.i./L	95% C.I.: 0.0124-80.56 mg a.i./L
NOAEC:	6.4 mg a.i./L	
Probit Slope:	Not determined	

Area under the growth curve (biomass, 0-96 hours):

EC_{05}:	2.6 mg a.i./L	95% C.I.: 0.52-13 mg a.i./L
EC_{50}:	8.3 mg a.i./L	95% C.I.: 3.8-18 mg a.i./L
NOAEC:	2.53 mg a.i./L	
Probit Slope:	3.26±1.26	

Endpoint(s) Affected: Cell density, growth rate and biomass.
Most sensitive endpoint: Biomass

D. STUDY DEFICIENCIES:

There were no study deficiencies.

E. REVIEWER'S COMMENTS:

The reviewer's toxicity values are similar to, but more conservative than those of the study author for cell density and biomass endpoints and the reviewer's analysis provided EC_{05} estimates and slope values. The reviewer's toxicity values are reported for growth rate, cell density, and biomass in the Executive Summary and Conclusions section of this DER.

The reviewer's NOAEC value for cell density was determined visually. The observed reductions in cell density were 3, 6, 93, 99 and 100%, relative to the negative control, at the mean-measured 2.53, 6.40, 16.35, 41.10 and 99.5 mg a.i./L treatment levels, respectively. However, the reviewer's analysis did not detect a difference at the 16.4 mg a.i./L treatment level. The reviewer felt that the 93% reduction in cell density at this treatment level was biologically significant; therefore, the reviewer visually determined the NOAEC value to be 6.40 mg a.i./L.

The in-life portion of the definitive algal toxicity test was conducted between June 2 and June 6, 2003.

F. CONCLUSIONS:

This study is scientifically sound and is classified as **ACCEPTABLE**. Biomass was the most sensitive endpoint with EC_{50} and NOAEC values of 8.3 and 2.53 mg a.i./L, respectively.

Cell density; reviewer-determined:

EC_{05}:	4.4 mg a.i./L	95% C.I.: 0.55-35 mg a.i./L
EC_{50}:	9.4 mg a.i./L	95% C.I.: 3.5-26 mg a.i./L
NOAEC:	6.4 mg a.i./L	
Probit Slope:	4.93±3.74	

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

EC_{05}:	15.33 mg a.i./L	95% C.I.: 0.000708-1438.47 mg a.i./L
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NOAEC:	6.4 mg a.i./L	
Probit Slope:	Not determined	

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

EC_{05}:	2.6 mg a.i./L	95% C.I.: 0.52-13 mg a.i./L
EC_{50}:	8.3 mg a.i./L	95% C.I.: 3.8-18 mg a.i./L
NOAEC:	2.53 mg a.i./L	
Probit Slope:	3.26±1.26	

Endpoint(s) Affected: Cell density, growth rate and biomass.
Most sensitive endpoint: Biomass

III. REFERENCES:

American Public Health Association (APHA). 1989. Standard Methods for the Examination of Water and Wastewater, 17th Edition. Washington, D.C.

American Society for Testing and Materials (ASTM). 1997. Standard Guide for Conducting Static 96-h Toxicity Tests with Microalgae. ASTM Standard E1218, Philadelphia, PA.

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APPENDIX 1. OUTPUT OF REVIEWER'S STATISTICAL VERIFICATION:

Marine diatom, cell density, mg a.i./L; 96-hours
File: 1740cd 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	76.533	76.533	46.000
2	2.53	74.133	74.133	41.500
3	6.40	71.700	71.700	38.500
4	16.35	5.600	5.600	24.000
5	41.10	0.900	0.900	15.000
6	99.5	0.033	0.033	6.000

Calculated H Value = 15.049 Critical H Value Table = 11.070
Since Calc H > Crit H REJECT Ho:All groups are equal.

Marine diatom, cell density, mg a.i./L; 96-hours
File: 1740cd Transform: NO TRANSFORMATION

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

GROUP	IDENTIFICATION	TRANSFORMED MEAN	ORIGINAL MEAN	GROUP 6	5	4	3	2	1
6	99.5	0.033	0.033	\					
5	41.10	0.900	0.900	.	\				
4	16.35	5.600	5.600	.	.	\			
3	6.40	71.700	71.700	.	.	.	\		
2	2.53	74.133	74.133	.	.	.	.	\	
1	neg control	76.533	76.533	*	.	.	.	.	\

* = significant difference (p=0.05) . = no significant difference
Table q value (0.05,6) = 2.936 SE = 4.354

Estimates of EC%

Parameter	Estimate	95% Bounds Lower	95% Bounds Upper	Std.Err.	Lower Bound /Estimate
EC5	4.4	0.55	35.	0.42	0.13
EC10	5.2	0.84	32.	0.37	0.16
EC25	6.9	1.7	28.	0.29	0.24
EC50	9.4	3.5	26.	0.20	0.37

Slope = 4.93 Std.Err. = 3.74

Goodness of fit: p = 0.12 based on DF= 3.0 12.

1740CD : Marine diatom, cell density, mg a.i./L; 96-hours

Observed vs. Predicted Treatment Group Means

Dose	#Reps.	Obs.	Pred.	Obs.	Pred.	%Change

		Mean	Mean	-Pred.	%Control	
0.00	3.00	76.5	78.6	-2.04	100.	0.00
2.53	3.00	74.1	78.4	-4.25	99.8	0.244
6.40	3.00	71.7	62.5	9.15	79.6	20.4
16.4	3.00	5.60	9.33	-3.73	11.9	88.1
41.1	3.00	0.900	0.0631	0.837	0.0803	99.9
99.5	3.00	0.0333	1.75e-05	0.0333	2.23e-05	100.

Marine diatom, biomass, mg a.i./L; 96-hours
File: 1740cb Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	18644356.560	3728871.312	216.618
Within (Error)	12	206568.960	17214.080	
Total	17	18850925.520		

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

Marine diatom, biomass, mg a.i./L; 96-hours
File: 1740cb 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	2270.400	2270.400		
2	2.53	2211.200	2211.200	0.553	
3	6.40	1830.800	1830.800	4.104	*
4	16.35	203.200	203.200	19.297	*
5	41.10	55.600	55.600	20.675	*
6	99.50	13.200	13.200	21.070	*

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

Marine diatom, biomass, mg a.i./L; 96-hours
File: 1740cb 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	2.53	3	267.816	11.8	59.200
3	6.40	3	267.816	11.8	439.600
4	16.35	3	267.816	11.8	2067.200
5	41.10	3	267.816	11.8	2214.800

6	99.50	3	267.816	11.8	2257.200

Marine diatom, biomass, mg a.i./L; 96-hours
File: 1740cb 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	2270.400	2270.400	2270.400
2	2.53	3	2211.200	2211.200	2211.200
3	6.40	3	1830.800	1830.800	1830.800
4	16.35	3	203.200	203.200	203.200
5	41.10	3	55.600	55.600	55.600
6	99.50	3	13.200	13.200	13.200

Marine diatom, biomass, mg a.i./L; 96-hours
File: 1740cb 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	2270.400				
2.53	2211.200	0.553		1.78	k= 1, v=12
6.40	1830.800	4.104	*	1.87	k= 2, v=12
16.35	203.200	19.297	*	1.90	k= 3, v=12
41.10	55.600	20.675	*	1.92	k= 4, v=12
99.50	13.200	21.070	*	1.93	k= 5, v=12

s = 131.202
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	2.6	0.52	13.	0.33	0.20
EC10	3.4	0.82	14.	0.29	0.24
EC25	5.2	1.7	15.	0.22	0.34
EC50	8.3	3.8	18.	0.16	0.46

Slope = 3.26 Std.Err. = 1.26

Goodness of fit: p = 0.38 based on DF= 3.0 12.

1740CB : Marine diatom, biomass, mg a.i./L; 96-hours

Observed vs. Predicted Treatment Group Means

Dose	#Reps.	Obs. Mean	Pred. Mean	Obs. -Pred.	Pred. %Control	%Change

0.00	3.00	2.27e+03	2.37e+03	-99.8	100.	0.00
2.53	3.00	2.21e+03	2.26e+03	-49.3	95.4	4.63
6.40	3.00	1.83e+03	1.53e+03	305.	64.4	35.6
16.4	3.00	203.	400.	-196.	16.9	83.1
41.1	3.00	55.6	27.9	27.7	1.18	98.8
99.5	3.00	13.2	0.519	12.7	0.0219	100.

Marine diatom, growth rate (x 1000), mg a.i./L; 96-hrs
File: 1740gr Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	13866.191	2773.238	562.751
Within (Error)	12	59.131	4.928	
Total	17	13925.322		

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

Marine diatom, growth rate (x 1000), mg a.i./L; 96-hrs
File: 1740gr 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	45.183	45.183		
2	2.53	44.840	44.840	0.189	
3	6.40	44.483	44.483	0.386	
4	16.35	17.853	17.853	15.078	*
5	41.10	-1.507	-1.507	25.759	*
6	99.50	-28.017	-28.017	40.385	*

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

Marine diatom, growth rate (x 1000), mg a.i./L; 96-hrs
File: 1740gr 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	2.53	3	4.531	10.0	0.343
3	6.40	3	4.531	10.0	0.700
4	16.35	3	4.531	10.0	27.330
5	41.10	3	4.531	10.0	46.690
6	99.50	3	4.531	10.0	73.200

Marine diatom, growth rate (x 1000), mg a.i./L; 96-hrs
File: 1740gr 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	45.183	45.183	45.183
2	2.53	3	44.840	44.840	44.840
3	6.40	3	44.483	44.483	44.483
4	16.35	3	17.853	17.853	17.853
5	41.10	3	-1.507	-1.507	-1.507
6	99.50	3	-28.017	-28.017	-28.017

Marine diatom, growth rate (x 1000), mg a.i./L; 96-hrs
File: 1740gr 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	45.183				
2.53	44.840	0.189		1.78	k= 1, v=12
6.40	44.483	0.386		1.87	k= 2, v=12
16.35	17.853	15.079	*	1.90	k= 3, v=12
41.10	-1.507	25.760	*	1.92	k= 4, v=12
99.50	-28.017	40.387	*	1.93	k= 5, v=12

s = 2.220
Note: df used for table values are approximate when v > 20.

Marine diatom, biomass, mg a.i./L; 96-hours
6
3
3
3
3
3
3
neg control
2403.6
2194.8
2212.8
2.53
2358
2012.4
2263.2
6.40
1680
1711.2
2101.2
16.35
190.8
238.8
180
41.10
79.2
57.6
30
99.50
55.2
-12
-3.6

Marine diatom, cell density, mg a.i./L; 96-hours
6
3
3
3
3
3
3
neg control
74.3
76.3
79
2.53
78.3
69.3
74.8
6.40
74.8
65
75.3
16.35
6.3
5.9
4.6
41.10
1.2
0.6
0.9
99.5
0
0
0.1

Marine diatom, growth rate (x 1000), mg a.i./L; 96-hrs
6
3
3
3
3
3
3
neg control
44.88
45.15
45.52
2.53
45.42
44.15
44.95
6.40
44.95
43.48
45.02
16.35
19.17
18.49
15.90
41.10
1.90
-5.32
-1.10
99.50
-30.03
-30.03
-23.99