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Data Evaluation Report on the acute toxicity of Prothioconazole on the Saltwater Algae, *Skeletonema costatum*

PMRA Submission #: 2004-0843 EPA MRID #: 46246110

Data Requirement:

PMRA DATA CODE	9.8.3
EPA DP Barcode	D303488
OECD Data Point	IIA 8.4.1
EPA MRID	46246110
EPA Guideline	123-2 (OPPTS 850.5400)

Test material: JAU 6476 Technical **Purity:** 98.2%
Common name: Prothioconazole
Chemical name: IUPAC: 2-[2-(1-Chlorocyclopropyl)-3-(2-chlorophenyl)-2-hydroxypropyl]-2,4-dihydro-3*H*-1,2,4,-triazole-3-thione
CAS name: 2-[2-(1-Chlorocyclopropyl)-3-(2-chlorophenyl)-2-hydroxypropyl]-2,4-dihydro-3*H*-1,2,4,-triazole-3-thione
CAS No.: (1E): 303048-99-9; nonstereo: 178928-70-6
Synonyms: JAU6476

Primary Reviewer: Rebecca Bryan
Staff Scientist, Dynamac Corporation

Signature:
Date: 8/18/04

QC Reviewer: Teri Myers, Ph.D.
Staff Scientist, Dynamac Corporation

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Date: 8/26/04

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Date: 11/3/2005
11/3/05

Company Code: BCZ
Active Code: PRB
Use Site Category: 7, 13, 14
EPA PC Code: 113961

Date Evaluation Completed:

CITATION: Kern, M.E. and De Haan, R.A. 2004. Toxicity of JAU 6476 Technical to the Saltwater Diatom *Skeletonema costatum*. Unpublished study performed by Bayer CropScience, Research and Development Department, Ecotoxicology, Stilwell, Kansas, Laboratory Study No. EBJAX076 (J6883601), and sponsored by Bayer CropScience, RTP, NC. Experimental start date September 23, 2002 and experimental termination date September 27, 2002. The final report issued March 10, 2004.

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PMRA Submission #: 2004-0843 EPA MRID #: 46246110

EXECUTIVE SUMMARY:

In a 96-hour acute toxicity study, cultures of *Skeletonema costatum* were exposed to Prothioconazole (JAU 6476 Technical) under static conditions at nominal concentrations of 0 (negative and solvent controls), 3.10, 7.70, 19.2, 48.0, and 120 µg/L. The 0-hour measured concentrations were <0.5 (< LOQ, controls), 3.00, 7.30, 17.5, 46.8, and 117 ppb a.i.. The 96-hour cell density percent inhibitions were 1, 3, 22, 89, and 99% in the 3.0, 7.3, 17.5, 46.8, and 117.0 ppb a.i. treatment groups, respectively. The area under the growth curve (0 to 96 hours) percent inhibitions were 4, 1, 40, 92, and 100% in the 3.0, 7.3, 17.5, 46.8, and 117.0 ppb a.i. treatment groups, respectively. The growth rate (0 to 96 hours) percent inhibitions were 0, 1, 5, 42, and 99% in the 3.0, 7.3, 17.5, 46.8, and 117.0 ppb a.i. treatment groups, respectively. Biomass (area under the growth curve) was the most sensitive endpoint tested, based on an EC_{50} of 21 ppb a.i.; the NOAEC was 7.3 ppb a.i..

The study is scientifically sound and satisfies the U.S. EPA Guideline §123-2 for an aquatic nonvascular plant study with *Skeletonema costatum*. This study is classified as Acceptable.

Results Synopsis

Test Organism: *Skeletonema costatum*
Test Type: Static

Cell density:
NOAEC: 7.3 ppb a.i.
LOAEC: 17.5 ppb a.i.
EC_{05}: 11 ppb a.i. 95% C.I.: 7.2-15 ppb a.i.
EC_{50}/IC_{50}: 25 ppb a.i. 95% C.I.: 21-30 ppb a.i.
Slope: 4.30±0.541

Growth rate:
NOAEC: 17.5 ppb a.i.
LOAEC: 46.8 ppb a.i.
EC_{05}: 24 ppb a.i. 95% C.I.: 18-32 ppb a.i.
EC_{50}/IC_{50}: 52 ppb a.i. 95% C.I.: 45-60 ppb a.i.
Slope: 4.89±0.572

Plant biomass (area under the growth curve):
NOAEC: 7.3 ppb a.i.
LOAEC: 17.5 ppb a.i.
EC_{05}: 7.7 ppb a.i. 95% C.I.: 5.6-11 ppb a.i.
EC_{50}/IC_{50}: 21 ppb a.i. 95% C.I.: 18-24 ppb a.i.
Slope: 3.88±0.381

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

I. MATERIALS AND METHODS

GUIDELINE FOLLOWED: The test was based on the following guideline: U.S. Environmental Protection Agency Guideline 123-2, Growth and Reproduction of Aquatic Plants (Tier 2). The following deviation from U.S. EPA Guideline, §123-2 was noted:

1. The dilution water characteristics were not reported.

COMPLIANCE: Signed and dated GLP, Quality Assurance and No Data Confidentiality statements were provided. The test was conducted according to the U.S. EPA (40 CFR, Part 160).

A. MATERIALS:

1. Test Material Prothioconazole (JAU 6476 Technical)

Description: Light beige powder

Lot No./Batch No. : 6233/0031

Purity: 98.2%

Stability of Compound Under Test Conditions: The 0 hour measured test concentrations were 91-98% of the nominal concentrations and the 96 hour measured test concentrations were 22-82% of the nominal concentrations.

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

Water solubility: 0.3 g/L in distilled water at 20°C and approximately pH 8.0.

Storage conditions of test chemicals: Stored at 4°C in the dark.

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-75
Source: Originally from University of Texas, Austin, Texas. Current in-house laboratory cultures.
Age of inoculum: 3 days old
Method of cultivation: Enriched Saltwater Nutrient Medium (Table 1, p. 19).

B. STUDY DESIGN:

1. Experimental Conditions

a) Range-finding Study: A preliminary range-finding study was conducted to determine the nominal test concentrations for the definitive test. The test concentrations were 0.001, 0.01, 0.1, and 1 mg a.i./L with negative and solvent controls. The percent growth inhibitions were 0, 14, 81, and 100% in the 0.001, 0.01, 0.1, and 1 mg a.i./L treatment groups, respectively, compared to the controls.

b) Definitive Study

Table 1 . Experimental Parameters

Parameter	Details	Remarks *Criteria*
Acclimation period: culturing media and conditions: (same as test or not) health: (any toxicity observed)	Continuous Enriched Saltwater Nutrient Medium; same as test. The algae was in log phase growth.	*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.*
Test system static/static renewal: renewal rate for static renewal:	Static	
Incubation facility	Environmental chamber	
Duration of the test	96 hours	*EPA requires: 96 - 120 hours* *OECD: 72 hours*
Test vessel material: (glass/polystyrene) size: fill volume:	 Borosilicate glass 250 mL 100 mL	Test vessels covered with inverted glass beakers. *OECD recommends 250 ml conical flasks are suitable when the volume of the test solution is 100 ml or use a culturing apparatus.*

Parameter	Details	Remarks *Criteria*
Details of growth medium name: pH at test initiation: pH at test termination: Chelator used: Carbon source: Salinity **(for marine algae):**	 Enriched Saltwater Nutrient Medium 7.9 8.0-8.8 disodium EDTA N/A 26‰	*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 medium.*
If non-standard nutrient medium was used, detailed composition provided (Yes/No)	N/A	
Dilution water source: type: pH: salinity **(for marine algae):** water pretreatment (if any): Total Organic Carbon: particulate matter: metals: pesticides: chlorine:	 Distilled water Cold-filter sterilized (0.22 µm). 7.9 26‰ None 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	
Aeration or agitation	Agitation, 100 rpm.	*EPA recommends agitation only for Selenastrum at 100 cycles per min and Skeletonema at ~60 cycles per min. Aeration is not recommended.*

Parameter	Details	Remarks *Criteria*
Initial cells density	Approximately 10,000 cells/mL	*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.*
Number of replicates control: solvent control: treated ones:	 3 3 3	*EPA requires a negative and/or solvent control with 3 or more replicates per doses. Navicula sp.tests should be conducted with four replicates.* *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 cultures should be included in the test.*
Test concentrations nominal: measured:	 0 (negative and solvent controls), 3.10, 7.70, 19.2, 48.0, and 120 µg/L Day 0: <0.5 (< LOQ, controls), 3.00, 7.30, 17.5, 46.8, and 117 ppb a.i. Day 4: <0.5 (< LOQ, controls), 0.70, 1.66, 7.85, 22.1, 98.8 ppb a.i.	*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)	Acetone, 0.5 mL/L	

Parameter	Details	Remarks *Criteria*
Method and interval of analytical verification	HPLC/MS; 0 and 96 hours.	
Test conditions temperature: photoperiod: light intensity and quality:	19.3-20.3°C Continuous 4.3 klux	*EPA temperature: Skeletonema: 20°C, Others: 24-25°C; 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	
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 count (area under the growth curve and growth rates were calculated).	*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 counts using a microscope and a hemocytometer.	*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 exponential growth in the control	Yes, dilution water control and solvent control cell densities at test termination were 195-219X greater than the dilution water control and solvent control cell densities at test initiation.	*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:

The 96-hour cell density percent inhibitions were 1, 3, 22, 89, and 99% in the 3.0, 7.3, 17.5, 46.8, and 117.0 ppb a.i. treatment groups, respectively. The area under the growth curve (0 to 96 hours) percent inhibitions were 4, 1, 40, 92, and 100% in the 3.0, 7.3, 17.5, 46.8, and 117.0 ppb a.i. treatment groups, respectively. The growth rate (0 to 96 hours) percent inhibitions were 0, 1, 5, 42, and 99% in the 3.0, 7.3, 17.5, 46.8, and 117.0 ppb a.i. treatment groups, respectively.

Table 3: Effect of Prothioconazole on Algae (*Skeletonema costatum*)

Treatment day 0 measured and nominal concentrations [a] (ppb a.i.)	Initial cell density (cells/mL)	Mean Cell density (cells/mL) at		
		24 hours	96 hours	
			cell count	% inhibition[b]
Dilution water control	10,000	65,000	1,954,000[c]	--
Solvent control	10,000	56,000	2,193,000	--
3.00 (3.10)	10,000	56,000	2,078,000	1
7.30 (7.70)	10,000	56,000	2,035,000	3
17.5 (19.2)	10,000	47,000	1,644,000	22
46.8 (48.0)	10,000	24,000	239,000	89*
117 (120)	10,000	12,000	11,000	99*
Reference chemical (if used)	N/A	N/A	N/A	N/A

[a] Nominal test concentrations are in parentheses.
[b] The percent inhibitions were calculated by comparison of the treatment groups to the pooled control.
[C] Replicate A was not included in calculations due to reduced growth.
* Statistically significant from the pooled control (Dunnett's one tailed test).

Table 4: Effect of Prothioconazole on Algae (*Skeletonema costatum*)

Treatment day 0 measured and Concentrations [a] (ppb a.i.)	Initial cell density (cells/mL)	Mean Growth Rate per day	% inhibition (Mean Growth Rate per day)[b]	Mean Area Under Growth Curve	% inhibition (Mean Area Under Growth Curve)[b]
Dilution water control	10,000	--	--	--	--
Solvent control	10,000	--	--	--	--
Pooled control	--	0.05549	--	6744.0	--
3.00 (3.10)	10,000	0.05536	0	6504.0	4
7.30 (7.70)	10,000	0.05532	1	6672.0	1
17.5 (19.2)	10,000	0.05310	5	4033.2	40*
46.8 (48.0)	10,000	0.03232	42*	556.8	92*
117 (120)	10,000	0.00068	99*	-2.8	100*
Reference chemical (if used)	N/A	N/A	N/A	N/A	N/A

[a] Nominal test concentrations are in parentheses.
[b] The percent inhibitions were calculated by comparison of the treatment groups to the pooled control.
* Statistically significant from the pooled control (Dunnett's one tailed test).

Table 5: Statistical endpoint values.

Statistical Endpoint	Biomass	Growth rate	Cell density
NOAEC (ppb a.i.)	7.3	17.5	17.5
IC_{50} or EC_{50} (ppb a.i.) (and 95% C.I.)	20.1 (19.0-21.2)	49.9 (45.5-54.2)	25.6 (23.6-27.6)
IC_{25}/EC_{25} (ppb a.i.) (and 95% C.I.)	13.8 (12.7-14.9)	40.1 (33.7-46.8)	18.5 (16.5-20.5)
Reference chemical, if used NOAEC IC_{25}/EC_{25}	N/A	N/A	N/A

N/A = Not applicable.

B. REPORTED STATISTICS:

Statistical Method: The formulas for growth rate and area under the growth curve (biomass) are found on page 49. The 96-hour growth data was analyzed using a t-test for the controls (pooled controls used for all comparisons), Shapiro-Wilks test for normality, and Levene's test for homogeneity of variances. The statistical analyses included ANOVA followed by the Dunnett's Test. The NOAEC and LOAEC were determined from analyzed data. The EC values were determined using the logistic regression model. The parametric analyses were conducted for biomass and nonparametric analyses were conducted for cell density and growth rates. The analyses were conducted using the computer program SAS version 8.2. All statistical calculations were performed using the day 0 measured concentrations.

Cell density:
NOAEC: 17.5 ppb a.i.
LOAEC: 46.8 ppb a.i.
EC_{50}/IC_{50}: 25.6 ppb a.i. 95% C.I.: 23.6-27.6ppb a.i.
Slope: N/A

Growth rates:
NOAEC: 17.5 ppb a.i.
LOAEC: 46.8 µg a.i./
EC_{50}/IC_{50}: 49.9 ppb a.i. 95% C.I.: 45.5-54.2 ppb a.i.
Slope: N/A

Plant biomass (area under the growth curve):
NOAEC: 7.3 ppb a.i.
LOAEC: 17.5 ppb a.i.
EC_{50}/IC_{50}: 20.1 ppb a.i. 95% C.I.: 19.0-21.2 ppb a.i.

Slope: N/A

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

C. VERIFICATION OF STATISTICAL RESULTS:

Statistical Method: Area under the growth curve (biomass) and growth rate data satisfied the assumptions of ANOVA (i.e., normality and homogeneity of variances); cell density data were square-root transformed to satisfy these assumptions The NOAEC and LOAEC values were determined using ANOVA, followed by William's multiple comparison test. For all endpoints, the solvent control was compared to the nutrient control using a Student's t-test and, upon finding no significant differences, the control groups were pooled for comparison to the treatment groups. The analyses described above were conducted using TOXSTAT statistical software and the day 0 measured concentrations were used for all calculations. The EC_{05} and EC_{50} values were determined using the Probit method via Nuthatch statistical software.

Cell density:
NOAEC: 7.3 ppb a.i.
LOAEC: 17.5 ppb a.i.
EC_{05}: 11 ppb a.i. 95% C.I.: 7.2-15 ppb a.i.
EC_{50}/IC_{50}: 25 ppb a.i. 95% C.I.: 21-30 ppb a.i.
Slope: 4.30±0.541

Growth rate:
NOAEC: 17.5 ppb a.i.
LOAEC: 46.8 ppb a.i.
EC_{05}: 24 ppb a.i. 95% C.I.: 18-32 ppb a.i.
EC_{50}/IC_{50}: 52 ppb a.i. 95% C.I.: 45-60 ppb a.i.
Slope: 4.89±0.572

Plant biomass (area under the growth curve):
NOAEC: 7.3 ppb a.i.
LOAEC: 17.5 ppb a.i.
EC_{05}: 7.7 ppb a.i. 95% C.I.: 5.6-11 ppb a.i.
EC_{50}/IC_{50}: 21 ppb a.i. 95% C.I.: 18-24 ppb a.i.
Slope: 3.88±0.381

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

D. STUDY DEFICIENCIES:

There were no significant study deficiencies.

E. REVIEWER'S COMMENTS:

The reviewer's conclusions were similar to the study authors'; biomass was the most sensitive endpoint. Because the reviewer's toxicity values for all endpoints were associated with slope estimates, they are reported in the Executive Summary and Conclusions sections.

F. CONCLUSIONS: This study is scientifically sound, fulfills U.S. EPA guideline §123-2, and is classified as Acceptable. Biomass (area under the growth curve) was the most sensitive endpoint tested, based on an EC_{50} of 21 ppb a.i.; the NOAEC was 7.3 ppb a.i..

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Slope: 3.88±0.381

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

III. REFERENCES:

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USEPA. 1994. Pesticide Reregistration Rejection Rate Analysis. Ecological Effects. EPA 738-R-94-035: p 161.

APPENDIX I. OUTPUT OF REVIEWER'S STATISTICAL VERIFICATION:

cell density
File: 6110cd Transform: SQUARE ROOT(Y)

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	544.510	108.902	100.186
Within (Error)	14	15.217	1.087	
Total	19	559.727		

Critical F value = 2.96 (0.05,5,14)
Since F > Critical F REJECT Ho:All groups equal

cell density
File: 6110cd Transform: SQUARE ROOT(Y)

BONFERRONI T-TEST - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	GRPS 1&2 POOLED	14.458	209.720		
2	3.0	14.336	207.800	0.161	
3	7.3	14.250	203.533	0.274	
4	17.5	12.808	164.367	2.168	
5	46.8	4.810	23.933	12.671	*
6	117.4	1.056	1.167	17.602	*

Bonferroni T table value = 2.63 (1 Tailed Value, P=0.05, df=14,5)

cell density
File: 6110cd Transform: SQUARE ROOT(Y)

BONFERRONI T-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	GRPS 1&2 POOLED	5			
2	3.0	3	53.800	25.7	1.920
3	7.3	3	53.800	25.7	6.187
4	17.5	3	53.800	25.7	45.353
5	46.8	3	53.800	25.7	185.787
6	117.4	3	53.800	25.7	208.553

cell density
File: 6110cd Transform: SQUARE ROOT(Y)

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	GRPS 1&2 POOLED	5	209.720	14.458	14.458
2	3.0	3	207.800	14.336	14.336
3	7.3	3	203.533	14.250	14.250
4	17.5	3	164.367	12.808	12.808
5	46.8	3	23.933	4.810	4.810
6	117.4	3	1.167	1.056	1.056

cell density
File: 6110cd Transform: SQUARE ROOT(Y)

WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
GRPS 1&2 POOLED	14.458				
3.0	14.336	0.161		1.76	k= 1, v=14
7.3	14.250	0.274		1.85	k= 2, v=14
17.5	12.808	2.168	*	1.88	k= 3, v=14
46.8	4.810	12.672	*	1.89	k= 4, v=14
117.4	1.056	17.603	*	1.90	k= 5, v=14

s = 1.043
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	11.	7.2	15.	0.078	0.68
EC10	13.	9.2	18.	0.068	0.72
EC25	18.	14.	23.	0.052	0.78
EC50	25.	21.	30.	0.037	0.84

Slope = 4.30 Std.Err. = 0.541

Goodness of fit: p = 0.75 based on DF= 3.0 14.

6110CD : cell density

Observed vs. Predicted Treatment Group Means

Dose	#Reps.	Obs. Mean	Pred. Mean	Obs. -Pred.	Pred. %Control	%Change
0.00	5.00	210.	209.	0.605	100.	0.00
3.00	3.00	208.	209.	-1.31	100.	0.00327
7.30	3.00	204.	207.	-3.52	99.0	0.985
17.5	3.00	164.	158.	5.87	75.8	24.2
46.8	3.00	23.9	26.7	-2.79	12.8	87.2
117.	3.00	1.17	0.452	0.715	0.216	99.8

biomass
File: 6110b Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	159057164.353	31811432.871	59.683
Within (Error)	14	7462152.959	533010.926	
Total	19	166519317.312		

Critical F value = 2.96 (0.05,5,14)
Since F > Critical F REJECT Ho:All groups equal

biomass
File: 6110b Transform: NO TRANSFORMATION

BONFERRONI T-TEST - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	GRPS 1&2 POOLED	6744.000	6744.000		
2	3.0	6504.000	6504.000	0.450	
3	7.3	6672.000	6672.000	0.135	
4	17.5	4033.200	4033.200	5.084	*
5	46.8	556.800	556.800	11.605	*
6	117.4	-2.800	-2.800	12.654	*

Bonferroni T table value = 2.63 (1 Tailed Value, P=0.05, df=14,5)

biomass
File: 6110b Transform: NO TRANSFORMATION

BONFERRONI T-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	GRPS 1&2 POOLED	5			
2	3.0	3	1399.577	20.8	240.000
3	7.3	3	1399.577	20.8	72.000
4	17.5	3	1399.577	20.8	2710.800
5	46.8	3	1399.577	20.8	6187.200
6	117.4	3	1399.577	20.8	6746.800

biomass
File: 6110b Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	GRPS 1&2 POOLED	5	6744.000	6744.000	6744.000
2	3.0	3	6504.000	6504.000	6588.000

3	7.3	3	6672.000	6672.000	6588.000
4	17.5	3	4033.200	4033.200	4033.200
5	46.8	3	556.800	556.800	556.800
6	117.4	3	-2.800	-2.800	-2.800

biomass
File: 6110b 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
GRPS 1&2 POOLED	6744.000				
3.0	6588.000	0.293		1.76	k= 1, v=14
7.3	6588.000	0.293		1.85	k= 2, v=14
17.5	4033.200	5.084	*	1.88	k= 3, v=14
46.8	556.800	11.605	*	1.89	k= 4, v=14
117.4	-2.800	12.654	*	1.90	k= 5, v=14

s = 730.076
Note: df used for table values are approximate when v > 20.

Estimates of EC%

Parameter	Estimate	95% Bounds Lower	Upper	Std.Err.	Lower Bound /Estimate
EC5	7.7	5.6	11.	0.067	0.72
EC10	9.6	7.2	13.	0.058	0.75
EC25	14.	11.	17.	0.045	0.80
EC50	21.	18.	24.	0.032	0.86

Slope = 3.88 Std.Err. = 0.381

Goodness of fit: p = 0.92 based on DF= 3.0 14.

6110BN : biomass

Observed vs. Predicted Treatment Group Means

Dose	#Reps.	Obs. Mean	Pred. Mean	Obs. -Pred.	Pred. %Control	%Change
0.00	5.00	6.74e+03	6.72e+03	21.0	100.	0.00
3.00	3.00	6.50e+03	6.72e+03	-215.	99.9	0.0596
7.30	3.00	6.67e+03	6.45e+03	222.	95.9	4.07
17.5	3.00	4.03e+03	4.08e+03	-45.0	60.7	39.3
46.8	3.00	557.	557.	-0.138	8.28	91.7
117.	3.00	14.0	11.2	2.79	0.167	99.8

growth rate
File: 6110g Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	7726.402	1545.280	160.399

Within (Error)	14	134.883	9.634
Total	19	7861.285	

Critical F value = 2.96 (0.05,5,14)
Since F > Critical F REJECT Ho:All groups equal

growth rate
File: 6110g Transform: NO TRANSFORMATION

BONFERRONI T-TEST - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	GRPS 1&2 POOLED	55.616	55.616		
2	3.0	55.353	55.353	0.116	
3	7.3	55.327	55.327	0.128	
4	17.5	53.107	53.107	1.107	
5	46.8	32.323	32.323	10.276	*
6	117.4	0.683	0.683	24.234	*

Bonferroni T table value = 2.63 (1 Tailed Value, P=0.05, df=14,5)

growth rate
File: 6110g Transform: NO TRANSFORMATION

BONFERRONI T-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	GRPS 1&2 POOLED	5			
2	3.0	3	5.950	10.7	0.263
3	7.3	3	5.950	10.7	0.289
4	17.5	3	5.950	10.7	2.509
5	46.8	3	5.950	10.7	23.293
6	117.4	3	5.950	10.7	54.933

growth rate
File: 6110g Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	GRPS 1&2 POOLED	5	55.616	55.616	55.616
2	3.0	3	55.353	55.353	55.353
3	7.3	3	55.327	55.327	55.327
4	17.5	3	53.107	53.107	53.107
5	46.8	3	32.323	32.323	32.323
6	117.4	3	0.683	0.683	0.683

growth rate
File: 6110g 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
GRPS 1&2 POOLED	55.616				
3.0	55.353	0.116		1.76	k= 1, v=14
7.3	55.327	0.128		1.85	k= 2, v=14
17.5	53.107	1.107		1.88	k= 3, v=14
46.8	32.323	10.276	*	1.89	k= 4, v=14
117.4	0.683	24.234	*	1.90	k= 5, v=14

s = 3.104
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	24.	18.	32.	0.060	0.75
EC10	28.	22.	37.	0.053	0.77
EC25	38.	31.	46.	0.040	0.82
EC50	52.	45.	60.	0.029	0.87

Slope = 4.89 Std.Err. = 0.572

Goodness of fit: p = 0.99 based on DF= 3.0 14.

6110GN : growth rate

Observed vs. Predicted Treatment Group Means

Dose	#Reps.	Obs. Mean	Pred. Mean	Obs. -Pred.	Pred. %Control	%Change
0.00	5.00	55.6	55.1	0.508	100.	0.00
3.00	3.00	55.4	55.1	0.245	100.	7.26e-08
7.30	3.00	55.3	55.1	0.219	100.	0.00159
17.5	3.00	53.1	54.5	-1.41	98.9	1.06
46.8	3.00	32.3	32.2	0.125	58.4	41.6
117.	3.00	2.23	2.25	-0.0201	4.08	95.9

EAD Assessment of USEPA DER

Reviewer: Émilie Larivière (#1269); PMRA Date: November 3, 2005

PMRA Submission Number: 2004-0843

Study Type: Acute Toxicity to Marine Algae (saltwater diatom, *Skeletonema costatum*)

Kern, M.E. and De Haan, R.A. 2004. Toxicity of JAU 6476 Technical to the Saltwater Diatom *Skeletonema costatum*. Unpublished study performed by Bayer CropScience, Research and Development Department, Ecotoxicology, Stilwell, Kansas, Laboratory Study No. EBJAX076 (J6883601), and sponsored by Bayer CropScience, RTP, NC. Experimental start date September 23, 2002 and experimental termination date September 27, 2002. The final report issued March 10, 2004.

PMRA DATA CODE: 9.8.3
EPA DP Barcode: D303488
OECD Data Point: IIA 8.4.1
EPA MRID: 46246110
EPA Guideline: 123-2 (OPPTS 850.5400)

Reviewing Agency: US EPA

EAD Executive Summary:

In a 96-hour acute toxicity study, cultures of *Skeletonema costatum* were exposed to Prothioconazole (JAU 6476 Technical, purity 98.2%) under static conditions at nominal concentrations of 0 (negative and solvent controls), 3.10, 7.70, 19.2, 48.0, and 120 µg/L. The 0-hour measured concentrations were <0.5 (< LOQ, controls), 3.00, 7.30, 17.5, 46.8, and 117 µg a.i./L (91-98% of nominal); the 96-hour measured concentrations were <0.5 (< LOQ, controls), 0.70, 1.66, 7.85, 22.1, and 98.8 µg a.i./L (22-82% of nominal). Mean measured concentrations were <0.5 (< LOQ, controls), 1.85, 4.48, 12.68, 34.45 and 107.9 µg a.i./L. The 0-hour measured concentrations were used for toxicity estimates by the EPA reviewer, but the EAD reviewer felt that using mean measured concentrations were a better estimate of exposure, as concentrations in all but one treatments declined significantly by day 4. The study was conducted following U.S. EPA Guideline 123-2 and in compliance with U.S. EPA (40 CFR, Part 160). The 96-hour cell density percent inhibitions were 1, 3, 22, 89, and 99% in the mean measured 1.85, 4.48, 12.68, 34.45 and 107.9 µg a.i./L treatment groups, respectively. The area under the growth curve (0 to 96 hours) percent inhibitions were 4, 1, 40, 92, and 100% in the 1.85, 4.48, 12.68, 34.45 and 107.9 µg a.i./L treatment groups, respectively. The growth rate (0 to 96 hours) percent inhibitions were 0, 1, 5, 42, and 99% in the 1.85, 4.48, 12.68, 34.45 and 107.9 µg a.i./L treatment

groups, respectively. The NOEC for cell density, biomass (area under the growth curve) and growth rate were 4.48, 4.48 and 12.68 µg a.i./L, respectively, based on mean measured concentrations. The EC_{50}/IC_{50} (and corresponding 95% confidence intervals) were 21.9 µg a.i./L (19.2-23.0 µg a.i./L), 44.9 µg a.i./L (33.5-51.4 µg a.i./L) and 15.7 µg a.i./L (13.5-17.8 µg a.i./L) for cell density, biomass and growth rate, respectively.

Results Synopsis

Test Organism: *Skeletonema costatum*
Test Type: Static

Cell density (EAD reviewer, mean-measured concentrations):
NOEC: 4.48 µg a.i./L (7.3 µg a.i./L, day 0 measured concentrations)
LOEC: 12.68 µg a.i./L (17.5 µg a.i./L, day 0 measured concentrations)
EC_{50}/IC_{50}: 21.9 µg a.i./L 95% C.I.: 19.2-23.0 µg a.i./L
Slope: N/A

Growth rate (EAD reviewer, mean-measured concentrations):
NOEC: 12.68 µg a.i./L (17.5 µg a.i./L, day 0 measured concentrations)
LOEC: 34.45 µg a.i./L (46.8 µg a.i./L, day 0 measured concentrations)
EC_{50}/IC_{50}: 44.9 µg a.i./L 95% C.I.: 33.5-51.4 µg a.i./L
Slope: N/A

Plant biomass (area under the growth curve) (EAD reviewer, mean-measured concentrations):
NOEC: 4.48 µg a.i./L (7.3 µg a.i./L, day 0 measured concentrations)
LOEC: 12.68 µg a.i./L (17.5 µg a.i./L, day 0 measured concentrations)
EC_{50}/IC_{50}: 15.7 µg a.i./L 95% C.I.: 13.5-17.8 µg a.i./L
Slope: N/A

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

EAD Comments:

1. The appropriate PMRA information (PMRA Submission Number, PMRA Data Code, PMRA company code, PMRA active ingredient code, PMRA use site category, OECD data point, name of PMRA secondary reviewer) was added to the EPA-DER as well as information on the chemical name (CAS name and synonym) available from the PMRA Chemistry review.

2. The EPA reviewer followed the U.S. EPA policy of using the day 0 measured concentrations when levels decline below 70% of nominal. However, the EAD reviewer felt that using the mean

measured concentrations to determine the EC_{50}/IC_{50} in this study was a better estimate of exposure, as concentrations after 4 days were significantly lower than on day 0 in all but one treatment vessel. Using higher day 0 values could underestimate exposure and the differing results may affect the outcome of risk assessments. Mean measured concentrations were <0.5(LOQ, controls), 1.85, 4.48, 12.68, 34.45 and 107.9 µg a.i./L. Other algae studies with prothioconazole show declines in concentrations in the lowest treatment levels after 4 days of exposure. The recovery in treatments with higher concentrations was often above 90% in other studies, which would indicate that the decline in concentration is not due to instability of the chemical. This difference is perhaps due to binding or absorption of the chemical or problems with detection, more observed at the lower concentrations. The concentrations used in this study are lower than in most of the other algae studies submitted for prothioconazole.

3. The EAD reviewer agrees with the NOECs reported by the EPA reviewer for the three endpoints. After a review of the data and of the results, the EAD reviewer did not feel that redoing the statistical analyses to verify the NOECs was necessary.

4. The EAD reviewer recalculated the 96-hour EC_{50}/IC_{50} for cell density, cumulative biomass as well as growth rate using linear interpolation (ICp, US EPA, 1993), based on the mean measured concentrations. The EC_{50}/IC_{50} values were outside the range of values reported by the EPA reviewer. The results of the EAD reviewer will be reported in the EAD Executive Summary.

Study Acceptability: The study is scientifically sound and satisfies the data requirements for an aquatic nonvascular plant study with *Skeletonema costatum*. This study is classified as Acceptable.

Statistical output of the EAD reviewer

Cell Density

Conc. ID		1	2	3	4	5	6
Conc. Tested		0	1.85	4.48	12.68	34.45	107.9
Response	1	174.8	152.3	177.8	166.1	12.6	0.8
Response	2	216	255.8	207.8	181.5	29.8	0.8
Response	3	201.8	215.3	225	145.5	29.4	1.9
Response	4	207					
Response	5	249					

```
*** Inhibition Concentration Percentage Estimate ***
Toxicant/Effluent: Prothioconazole Cell density Mean meas.
Test Start Date:    Test Ending Date:
Test Species: Skeletonema costatum
Test Duration:        96 hours
DATA FILE: SkedenpM.icp
OUTPUT FILE: SkedenpM.i50
```

Conc. ID	Number Replicates	Concentration ug ai/L	Response Means	Std. Dev.	Pooled Response Means
1	5	0.000	209.720	26.795	209.720
2	3	1.850	207.800	52.156	207.800
3	3	4.480	203.533	23.888	203.533
4	3	12.680	164.367	18.062	164.367
5	3	34.450	23.933	9.817	23.933
6	3	107.900	1.167	0.635	1.167

```
The Linear Interpolation Estimate:    21.9047   Entered P Value: 50
Number of Resamplings:   80
The Bootstrap Estimates Mean:  21.3328 Standard Deviation:     1.0959
Original Confidence Limits:   Lower:    19.1721 Upper:    22.9786
Expanded Confidence Limits:   Lower:    17.8058 Upper:    23.5156
Resampling time in Seconds:     0.00  Random_Seed: 77586304
```

Growth Rate

Conc. ID		1	2	3	4	5	6
Conc. Tested		0	1.85	4.48	12.68	34.45	107.9
Response	1	0.05379	0.05235	0.05397	0.05326	0.02639	-0.00232
Response	2	0.05599	0.05775	0.05559	0.05418	0.03536	-0.00232
Response	3	0.05528	0.05596	0.05642	0.05188	0.03522	0.00669
Response	4	0.05555					
Response	5	0.05747					

```
*** Inhibition Concentration Percentage Estimate ***
Toxicant/Effluent: Prothioconazole growth rate mean meas.
```

```
Test Start Date:     Test Ending Date:
Test Species: Skeletonema costatum
Test Duration:         96 hours
DATA FILE: SkegropM.icp
OUTPUT FILE: SkegropM.i50
```

Conc. ID	Number Replicates	Concentration ug ai/L	Response Means	Std. Dev.	Pooled Response Means
1	5	0.000	0.056	0.001	0.056
2	3	1.850	0.055	0.003	0.055
3	3	4.480	0.055	0.001	0.055
4	3	12.680	0.053	0.001	0.053
5	3	34.450	0.032	0.005	0.032
6	3	107.900	0.001	0.005	0.001

```
The Linear Interpolation Estimate:    44.9320   Entered P Value: 50
Number of Resamplings:   80
The Bootstrap Estimates Mean:  44.7877 Standard Deviation:     4.7826
Original Confidence Limits:   Lower:    33.5351 Upper:    51.3617
Expanded Confidence Limits:   Lower:    27.8366 Upper:    54.5765
Resampling time in Seconds:     0.00  Random_Seed: -1222482304
```

Biomass (Area under the growth curve)

Conc. ID		1	2	3	4	5	6
Conc. Tested		0	1.85	4.48	12.68	34.45	107.9
Response	1	6957.6	5331.6	5820.0	3682.8	304.8	-31.2
Response	2	6200.4	7154.4	7264.8	4359.6	708	-19.2
Response	3	7322.4	7026.0	6931.2	4057.2	657.6	42
Response	4	7924.8					
Response	5	7106.4					

```
*** Inhibition Concentration Percentage Estimate ***
Toxicant/Effluent: Prothioconazole biomass mean measured
Test Start Date:     Test Ending Date:
Test Species: Skeletonema costatum
Test Duration:         96 hours
DATA FILE: SkebiopM.icp
OUTPUT FILE: SkebiopM.i50
```

Conc. ID	Number Replicates	Concentration ug ai/L	Response Means	Std. Dev.	Pooled Response Means
1	5	0.000	7102.320	624.365	7102.320
2	3	1.850	6504.000	1017.356	6588.000
3	3	4.480	6672.000	756.472	6588.000
4	3	12.680	4033.200	339.038	4033.200
5	3	34.450	556.800	219.689	556.800
6	3	107.900	-2.800	39.259	0.000

```
The Linear Interpolation Estimate:    15.6986   Entered P Value: 50
```

```
---------------------------------------------------------------------
Number of Resamplings:   80
The Bootstrap Estimates Mean:  15.6846 Standard Deviation:     1.2115
Original Confidence Limits:   Lower:    13.5463 Upper:    17.7889
Expanded Confidence Limits:   Lower:    12.4701 Upper:    18.8341
Resampling time in Seconds:     0.00  Random_Seed: -372727040
```